Mathematical modelling to restore circulating IGF-1 concentrations in children with Crohn's disease-induced growth failure: a pharmacokinetic study

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ARTICLE SUMMARY

Article focus

One third of children with Crohn's disease have growth retardation.

Children with active inflammation have an insensitivity to growth hormone, resulting in low circulating IGF-1.

There is no agreed growth therapy for children whose inflammation is intractable to treatment, because very high and sustained IGF-1 concentrations are a risk factor for colon cancer in adults with acromegaly.

Key messages

IGF-1 can be restored to the normal range by subcutaneous injections.

The endogenous synthesis of IGF-1 depends on Crohn's disease activity; and variations in both determine the circulating IGF-1 concentrations.

A new mathematical model which incorporates disease activity (in addition to weight and age) will allow paediatric gastroenterologists to calculate doses that keep the circulating IGF-1 in the upper normal range.

Strengths and Limitations

Using the mathematical model, IGF-1 can be prescribed in doses that do not increase the risk of cancer.

For the first time, long term IGF-1 treatment can be studied in children to determine if it enhances growth, and this is a critical step in offering a therapy (much demanded by patients) for growth retardation in children with Crohn's disease.

A limitation of the study is that long term studies over several years will still be required.

ABSTRACT

Objectives: Children with Crohn's disease grow poorly, and inflammation depresses the response of IGF-1 to growth hormone. Correcting the inflammation normalizes growth velocity; however, removing inflammation cannot be achieved in all children. Our lack of understanding of IGF-1 kinetics has hampered its use, particularly as high IGF-1 concentrations over long periods may predispose to colon cancer. We hypothesized that mathematical modelling of IGF-1 would define dosing regimes that return IGF-1 concentrations into the normal range, without reaching values that risk cancer.

Design: Pharmacokinetic intervention study

Setting: Tertiary paediatric gastroenterology unit

Participants: 8 children (M:F; 4:4) entered the study. All completed: 6 South Asian British; 2 White British. Inclusion criteria: Children over 10 years with active Crohn's disease (CRP >10mg/l or ESR >25mm/h) and height velocity <-2 SD score. Exclusion criteria: closed epiphyses; corticosteroids within 3 months; neoplasia or known hypersensitivity to rhIGF-1.

Interventions: Subcutaneous recombinant human IGF-1 (rhIGF-1) (120µg/kg) per dose over two admissions: the first as a single dose, and the second as twice daily doses over 5 days.

Primary outcome: Significant increase in circulating IGF-1

Secondary outcomes: Incidence of side effects of IGF-1. A mathematical model of circulating IGF-1 (A_c) was developed to include parameters of: endogenous synthesis (K_{syn}); exogenous uptake (K_a) from the subcutaneously dose (A_s): and IGF-1 clearance: where $dA_c/dt= K_{syn}- K_{out}\cdot A_c + K_a\cdot A_s$.

Results: Subcutaneous IGF-1 increased concentrations, which were maintained on twice daily doses. In covariate analysis, disease activity reduced K_{syn} ($p<0.001$). Optimal dosing was derived from least

squares regression fitted to a dataset of 384 Crohn’s patients, with model parameters assigned by simulation.

Conclusions: By using age, weight and disease activity scaling in IGF-1 dosing, 94% of children will have normalized IGF-1 concentrations below +2.5 standard deviations of the normal population mean, a level not associated with cancer risk.

Abstract word count: 296

INTRODUCTION

A quarter of cases of Crohn’s disease now present in children and adolescents under 18 years [1, 2]; and its incidence in childhood is increasing [3]. Around a third of children experience linear growth retardation, caused in part by undernutrition and in part by the direct effects of inflammation on growth [4].

Growth in childhood is regulated by growth hormone (GH), secreted from the pituitary, which stimulates the production of insulin-like growth factor-1 (IGF-1) by the liver and growth plates of bones [5]. Children with active Crohn’s disease have low circulating IGF-1 and increased circulating cytokines [6, 7]. Treatment of the inflammation, for example by an enteral diet, results in a reduction in cytokines like IL-6 and an increase in IGF-1 within 3 days [7]. Thus, the optimum treatment for improving growth is to eliminate the inflammation. Nevertheless, some children’s inflammation remains intractable to treatment despite the best efforts of clinicians. For this group, there is currently no agreed therapy to enhance growth.

Rats with TNBS-induced colonic inflammation grow poorly [8]; and this is also associated with increased cytokines IL-6 and TNF, and a low circulating IGF-1 [9]. Exogenous IGF-1 given to the rats with colitis enhanced growth [8]. In transgenic mice, without inflammation, high levels of circulating IL-6, generated by a widely acting promoter, depressed both IGF-1 and growth [10]. Thus the link between inflammatory cytokines, IGF-1 and poor growth is strong [11]. There is a functional insensitivity to GH in children with Crohn’s disease. Recombinant IGF-1 is now recognized therapy for children with GH insensitivity syndrome (GHIS) due to genetic defects of the GH receptor or IGF-1 gene [12]; we, therefore, hypothesized that IGF-1 concentrations in children with active Crohn’s disease and poor linear growth could be restored by administering rhIGF-1.

Restoring IGF-1 in children with Crohn's disease who may have some endogenous production is not straightforward, because high levels of IGF-1 may be harmful. For rhIGF-1 to be therapeutically useful, its circulating levels over the long term should be returned to normal values by replacement, and not given in excess. Patients with acromegaly who have very high concentrations of GH and IGF-1, maintained over decades, have a doubling in the incidence of colon cancer [13]. This could, in theory, be compounded in children with Crohn's disease, as inflammation is also a risk factor for intestinal cancer.

A mathematical model for administration of rhIGF-1, based on detailed pharmacokinetics would resolve these difficulties, as dosing could be tailored to achieve circulating concentrations that remain within the normal range. We therefore undertook a careful study of subcutaneous rhIGF-1 administration in children with Crohn's disease-induced growth retardation. We developed a model which used physiological parameterisation, disease activity to predict endogenous IGF-1 synthesis, and body weight to scale the volume of distribution. Parameters of protein loss, such as protein-losing enteropathy, were also considered. The model was used to recommend dosing which allows children to be treated with rhIGF-1 without its concentration rising above the normal range.

METHODS

Patient Selection

Following ethical approval from the East London & City Research Ethics Committee (ELREC; reference number 07/H0705/77) and regulatory approval from the UK Medicines and Healthcare products Regulatory Agency (MHRA) (Eudract number: 2007-004269-16), written informed consent was obtained from the parents and patients attending the paediatric inflammatory bowel disease clinic at Barts and The London Hospital for Children. Inclusion criteria were: age ≥ 10 years; known Crohn's disease diagnosed by endoscopic, histological and radiological methods [14]; a height

velocity measured over at least a 6 month period of <-2 Standard Deviation Score (SDS) according to the patient's age and gender; and evidence of active inflammation as demonstrated by either an ESR > 25mm/hr and/or a CRP > 10mg/l. Exclusion criteria were: corticosteroid use in the preceding 3 months; active or suspected neoplasia; known hypersensitivity to exogenous rhIGF-1 (Increlex, Ipsen UK); and the presence of closed epiphyses.

All patients had a full history and physical examination, including an accurate recording of height, weight and assessment of pubertal status. In addition, they also had an electrocardiogram and faecal alpha-1-antitrypsin levels (g/l) measured.

Study Design

Exogenous rhIGF-1 was administered by subcutaneous injection at doses of 120μg/kg over two admissions. Admission 1 investigated the effects of a single dose of rhIGF-1 over a 24 hour inpatient stay. Baseline blood screens included full blood count (FBC), electrolytes, inflammatory markers (CRP and ESR), baseline IGF-1, IGFBP-3, acid labile subunit (ALS) and blood sugar. The Pediatric Crohn's Disease Activity Index (PCDAI) was calculated [15, 16]

Each patient was then given a single subcutaneous injection of rhIGF-1 at a dose of 120μg/kg. Serial venous blood samples were drawn at the following time points: 0, 1, 2, 3, 4, 6, 12, 17 and 24 hours. Blood sugars and vital signs were checked regularly. Children ate and drank freely, and continued to receive their prescribed, non-corticosteroid, treatment for Crohn's disease.

Following a wash out of at least 3 months, subjects were readmitted for admission 2, investigating the effects of repeated doses of rhIGF-1. On this occasion 6 doses of rhIGF-1 were administered over a 5 day trial period, with doses given at: 0, 12, 72, 84, 96 and 108 hours. The injection sites were rotated according to the patient's wishes. Serial blood samples for further IGF-1 , IGFBP-3, acid

labile subunit (ALS) and blood sugar were collected during the 5 days at 0, 1, 2, 3, 4, 6, 12, 17, 24, 48, 72, 96 and 120 hours. Additional samples were also taken for clinical reasons when there was a possibility of hypoglycaemia. If sufficient sample was collected, IGF-1 levels were also measured. In addition, vital signs, FBC, electrolytes, and inflammatory markers were measured daily. Patients were kept as inpatients during the days that the rhIGF-1 was administered, but were allowed home during the two days in which they received no injections. Patients were discharged home on day 6.

Assays and samples

All samples were stored at -20°C until analysis. Plasma glucose was determined immediately after blood sampling (Beckman Instruments, Palo Alto, CA).

IGF-1, IGFBP-3.

IGF-1 and IGFBP-3 levels were measured by competitive binding radioimmunoassay (Esoterix Inc Laboratory Services). The sensitivity of the assay for IGF-1 was 15ng/ml and the intra-assay coefficient of variation averaged 14.1%. The sensitivity of the assay for IGFBP-3 was 0.3mg/L and the intra-assay coefficient of variation averaged 13%.

ALS

ALS levels were measured by radioimmunoassay (ALS-RIA) using purified human ALS as tracer. The intra-assay coefficient of variation ranged from 8.0-17.4%

Mathematical model development

Measured IGF-1 concentrations were fitted for all individuals simultaneously using the non-linear mixed effects modelling software NONMEM version 7.1[17]. Model building was undertaken using the first-order conditional estimation method with interaction (FOCEI). A turnover model was used according to the following differential equations:

$$\frac{dA_c}{dt} = K_{syn} - K_{out} \cdot A_c + K_a \cdot A_s$$ Equation 1

$$\frac{dA_s}{dt} = -K_a \cdot A_s$$ Equation 2

Where: A_c is the amount of circulating IGF-1 at time t; A_s is the amount in the subcutaneous tissue following a dose, with initial conditions adjusted each time a dose is administered; K_{syn} is a zero order production rate of endogenous IGF-1 in $\mu g.hr^{-1}$; K_{out} is a first order elimination rate constant in hr^{-1}; and K_a is a first order absorption rate constant describing exogenous IGF-1 appearance following a subcutaneous dose in hr^{-1}. At steady state prior to exogenous dose, circulating IGF-1 is given by the ratio of K_{syn} to K_{out}.

In addition to K_{syn} and K_a, the fixed effect parameters IGF-1 clearance (C_L) in $L.hr^{-1}$ and distribution volume (V_D) in L were estimated, K_{out} being given by the ratio of C_L to V_D. Between subject variability was tested for all parameters, and residual variability was described using a heteroscedastic model. Allometric weight scaling was applied to C_L and V_D *a priori* using linear scaling for V_D and weight raised to the power 0.75 for C_L[18] , K_{syn} was further scaled with linear weight. Tested covariates were CRP, ESR, PCDAI score and age.

Model development used the NONMEM objective function value (OFV); parameter estimate precision was derived through a non-parametric bootstrap [19] and graphical assessment of basic goodness-of-fit plots and model simulations was undertaken. The OFV is proportional to -2 times the log-likelihood of the data given the parameter estimates, and a decrease in OFV of 3.84 with one degree of freedom gives a significantly improved fit with a probability $p < 0.05$. A utility function

based on the final model was used to predict average concentration (C_{ave}) with a dosing regimen derived from the integral of the IGF-1 against the time curve divided by time. Maximum utility was defined as C_{ave} of +0.75 SDS with linear penalty for deviations above and below this target. Dose was optimisied to give a model-derived steady-state C_{ave}, by the following model:

$$Target = SDS_i + \varepsilon_i \qquad \text{Equation 3}$$

$$Utility = \sum_{i=1}^{n} (Target - SDS_i)^2 \qquad \text{Equation 4}$$

Where Target is the SDS aim (in this case +0.75); SDS_i is the individual predicted SDS derived from model predicted C_{AVE}; ε_i denotes individual deviations from the target. Utility minimised ε_i by least squares regression.

Demographic details for 384 subjects aged 8 to 14 years from the clinic database were each assigned a random PCDAI score (from a uniform distribution 0-48) and also individual model parameters. The utility function was maximised with either fixed dosing, dosing based on weight, weight and age, or weight, age and PCDAI score. Differences in concentrations were explored with 12 and 24 hourly dosing regimens.

RESULTS

rhIGF-1 therapy in children with Crohn's disease

Children with active Crohn's disease, and whose growth was inhibited were recruited into the study (Table 1). Impairment of height velocity is characteristic of many children with active Crohn's disease [4, 20-22], and all 8 subjects recruited into the study (median age 12.97) had a height velocity that was more than two standard deviations below the mean (i.e. a standard deviation score

[SDS] of <-2), as an entry criterion. The median height velocity SDS was -3.35, indicating extreme growth failure.

Table 1: Patient characteristics at recruitment into the trial. Tanner stage: P: pubic hair, B: breast stage, G: genitalia . Medications: 5 –ASA: 5-aminosalicylic acid, AZA: azathioprine, IFX: infliximab, Adalum = adalumimab. Montreal classification: L1: ileal, L2: colonic, L3: ileocolonic, B1: non-stricturing, non-penetrating, B2: stricturing, B3: penetrating

	Patients							
	LN01	LN02	LN03	LN04	LN05	LN06	LN07	LN08
Gender	F	F	M	M	F	F	M	M
Age	13.11	11.5	14.23	10.67	14.82	12.7	12.82	14.66
Ethnicity	Caucasian	Asian	Asian	Asian	Asian	Asian	African	Caucasian
Tanner Stage	P1 B1	P1 B1	P3 G3	P1 G1	P2 B2	P2 B2	P2 G2	P1 G1
HV SDS at recruitment	-2.11	-2.11	-4.18	-2.83	-2.27	-3.87	-4.43	-4.88
HV SDS at time of trial	-2.11	-2.14	-1.84	0.36	1.69	3.55	-4.43	-4.88
PCDAI	12.5	20	17.5	10	47.5	15	12.5	10
Medication	5-ASA	5-ASA	5-ASA AZA IFX	5-ASA AZA Adalum	5-ASA AZA Adalum	5-ASA AZA IFX	5-ASA AZA	5-ASA
Faecal A1AT (g/l)	3.34	0.82	0.88	0.49	2.63	2.28	0.08	0.45
Montreal Classification								
Disease Location	L1	L2	L3	L3	L1	L3	L1	L1
Behaviour	B1	B1	B3	B3	B2	B1	B2	B1
Upper	N	N	Y	N	Y	Y	N	N
Perianal	N	N	Y	Y	N	N	N	N

All the subjects completed both parts of the study. The rhIGF-1 was generally well tolerated. Although subcutaneous rhIGF-1 has been associated with hypoglycaemia in children treated for GHIS [12], only one patient (LN01) had a single asymptomatic hypoglycaemic episode (blood sugar <

3.5mmol/l). This was corrected with oral glucose. This occurred following the 12 hour dosage of rhIGF-1 during admission 2 in a child who had not eaten prior to the drug administration. No further episodes of hypoglycaemia occurred. There were no other adverse effects.

Determinants of circulating IGF-1

As previously demonstrated, children with Crohn's disease-induced growth failure had depressed circulating IGF-1 concentrations. The severity of disease in any one child varies over time, and this was reflected in the variation of IGF-1 between the first and second admissions, 3 or more months apart. (Figure 1a). Despite the variation both between and within patients, a single subcutaneous bolus of rhIGF-1 significantly increased IGF-1 in the circulation (Figure 1). In some cases where the baseline IGF-1 was less depressed, the post IGF-1 level was increased to above normal (Figure 1a). The concentrations of IGF-1 returned to baseline over the following 24 hours (Figure 1b).

IGF-1 is maintained in the circulation by IGF binding proteins, the most abundant being IGFBP-3 [23]. A major characteristic of Crohn's disease is protein-losing enteropathy, resulting in proteins being lost into the lumen of the intestine [24, 25]. We aimed therefore to ascertain if the degree of protein-losing enteropathy blunted the ability of subcutaneous rhIGF-1 to achieve significant peak concentrations, or if it directly affected IGF binding proteins. Protein losing enteropathy was quantified by the concentrations of alpha-1- antitrypsin in stool [26]. Using this measure, protein losing enteropathy had no effect on the change in IGF-1 induced by a subcutaneous injection (Figure 2a)

IGFBP-3 concentrations are depressed in children with chronic IGF-1 deficiency [27], and all children in the study had low IGFBP-3 (Figure 2b). We wished to determine, however, if IGFBP-3 concentrations depended on the degree of protein losing enteropathy, as this would indicate that protein losing enteropathy may affect IGF-1 pharmacokinetics. However, no association was observed.

In the second admission, the subjects received twice daily doses, to determine if IGF-1 concentrations would be enhanced over a longer period. The initial injection increased the IGF-1 concentrations into the normal range (Figure 1a). Increases over a sustained period were successfully achieved (Figure 1c), and as expected multiple daily dosing led to lower variability between peak and trough concentrations. Taking the area under the curve divided by time as the average IGF-1 concentration, it became clear that on 120µg/kg, some children had an average IGF-1 SDS of greater than +2.5, while other children had appropriate concentrations. Increased circulating IGF-1 in acromegaly has been linked with an increased risk of colon cancer [13, 28]. Fortunately, we have a clear understanding of how IGF-1 concentrations relate to colon cancer risk in acromegaly [13]. These studies show that the increased cancer risk was seen in individuals whose circulating IGF-1 was +2.5 SDS or greater. Using our data, we developed a model from which the dose-concentration relationship could be derived. We then investigated the covariates on which dosing could be individualised. This allowed us to correct circulating IGF-1 without causing excessively high concentrations: ensuring that our dosing regimens resulted in concentrations that were below +2.5 SDS, we developed a utility model to predict the IGF-1 SDS achieved on regular dosing.

Mathematical modelling of IGF-1 links endogenous production to disease severity

A turnover model which accounted for both endogenous production and exogenous dosing of IGF-1 was fitted to the data (see methods for mathematical formulae) using non-linear mixed effects analysis. Four fixed effects were estimated, namely endogenous synthesis rate (K_{SYN}), exogenous absorption rate through the skin following subcutaneous injection (K_a), systemic clearance (C_L), and distribution volume V_D. Parameter level random effects (inter-subject variability) were included on K_{SYN} and C_L. (Table 2) A further level of random effects (inter-occasion variability) also provided significantly improved fit when added to K_{SYN}. Following a limited covariate analysis, the PCDAI score was found to significantly improve fit when added to K_{SYN}, with K_{SYN} falling with increasing disease severity (Figure 3a). The effect of disease severity as measured by PCDAI was tested for C_L and K_{SYN} with significant improvement ($p<0.001$) being shown for K_{SYN}. Furthermore, adding the PCDAI score as a covariate to K_{SYN} allowed inter-occasion variability to be removed without detriment to the model fit (no change in log-likelihood). Adding PCDAI to K_{SYN} also decreased inter-individual variability. In short, disease severity was a major driver of IGF-1 synthesis rate both between patients, and in the same patient on different occasions.

Table 2: Parameter estimates from the model including non-parametric 95%CI from a bootstrap of 714 successful runs. IIV (inter-individual variability).

Parameter	Estimate	Bootstrap median	Bootstrap 2.5th percentile	Bootstrap 97.5th percentile
C_L (L/h/70kg)	1.61	1.60	1.36	1.82
V_D (L/70kg)	2.42	2.41	1.78	3.10
K_{SYN} (µg/h)	433	423	352	490
Ka (h^{-1})	0.10	0.10	0.083	0.12
Coefficient of PCDAI on K_{SYN}	-6.57	-6.41	-8.19	-4.89
IIV on CL (%CV)	10.38	9.46	3.49	15.1
IIV on K_{SYN} (%CV)	24.61	24.10	9.98	33.58
Residual error proportional (%CV)	9.81	9.69	7.85	11.04
Residual error, additive (µg/L)	28.32	25.93	13.29	42.78

There was good agreement between model predictions and observed concentrations (Figure 3b), and trendless distribution of standardised residuals, indicating that the parametric modelling assumptions were satisfied (Figure 3c, d and e). The median of the simulated data was not significantly different to the median of the observed data (Figure 3f). Superimposing model predictions at a population and individual level on the observed data showed that model predictions captured the dynamic behaviour of the system (Figure 4).

Dosing to maintain circulating rhIGF-1 concentrations in the target range

We undertook mathematical modelling to quantify the relationship between endogenous IGF-1 production and exogenous dosing, and to understand how IGF-1 average concentrations (area under the curve/time) can be adjusted. Using the knowledge that in acromegaly there is no increased risk of cancer in patients whose IGF-1 is below +2.5 SDS [13], we defined the optimal IGF-1 concentration range as being between 0 and +2.5 SDS. This range would improve growth without elevating cancer risk. Different possible dose scaling systems were then explored to maximise the probability of an individual having IGF-1 levels in the target range. By individualising dose by weight, age (because different aged children have different target circulating IGF-1 levels), and PCDAI score, 94% of children were predicted to be corrected to less than +2.5 SDS (median +0.5 SDS; Figure 5). We can therefore suggest a dosing scheme that limits the risk of malignancy to that of the normal patient popultion. The dosing schedule recommended from the utility function is:

Age 10 to <12 years, 26 µg/kg plus 1 µg/kg /PCDAI point; Age 12 to <14 years, 48 µg/kg plus 1.4 µg/kg /PCDAI point.

DISCUSSION

Impaired growth has been a recognised feature of children with Crohn's disease for 50 years [29]. In 1986, low IGF-1 was associated with poor growth in these children [30]; and over the following decade it was realized that the depressed concentration was a direct consequence of inflammation [8, 10], and not merely the result of poor energy intake or undernutrition. The best strategy to increase IGF-1 and therefore to improve growth is the resolution of inflammation. Treatments which reverse the inflammation, but do not actively suppress growth, such as surgery [31], anti-TNF [32, 33] and enteral diets [6, 34] have all been shown to improve linear growth.

Nevertheless, there are patients with Crohn's disease whose inflammation cannot be reversed. In this group, we need a distinct strategy to accelerate growth. Both hGH [35, 36] and rhIGF-1 have been suggested. Because patients with Crohn's disease have a functional insensitivity to GH, the present study examined the possibility that rhIGF-1 would be efficacious in improving IGF-1 concentrations, as it does in children with growth hormone insensitivity syndrome (GHIS). However, there are significant problems in applying our knowledge of rhIGF-1 usage in GHIS to children with Crohn's disease. First, children present at a much later age in Crohn's disease than they do in GHIS, which is caused by a genetic mutation (approximately 8-15 years as opposed to less than 5 years old). The compartments of the IGF-1 system including several IGF binding proteins, cannot be assumed to distribute as they do in younger children, particularly if the relative volumes of these compartments change at the time of puberty. Second, both IGF-1 and the IGF binding proteins are circulating proteins. Proteins are lost into the intestine through protein losing enteropathy (PLE), where inflammation reduces the ability of the intestinal vasculature to maintain them in the capillary lumen [37-39]. The PLE could, in theory, alter the kinetics of IGF-1. Finally, very high AND sustained circulating IGF-1 concentrations are associated with an increase in colon cancer in middle aged and older patients with acromegaly. A modelling approach to kinetics and dosing was necessary to avert causing high average IGF-1 concentrations.

Previous studies on rhIGF-1 pharmacokinetics [40, 41] have mainly focussed on its use in malnourished adult patients with renal failure, where it has been used to maintain protein balance. These reports undertook purely descriptive analyses such as Area Under the Concentration-Time Curve (AUC) and comparing healthy volunteer parameters with patients, rather than modelling the data. Furthermore, they did not account for endogenous IGF-1 production. If we were to use our data to recommend a dose, a more sophisticated approach was required. In this study, we fitted a structural model that simplified the system without losing biological interpretation of the parameters. Mixed effects modelling allowed for the addition of inter-individual and inter-occasion variability in model parameters, in addition to residual variability. We were, therefore, able simultaneously to fit the model to all patients and investigate covariate-parameter relationships. Instead of exploring dose recommendations by simulation, we aimed to optimise the dose based on the target SDS; and we minimised deviations from this using least-squares regression [42] . The advantage of this approach, over testing competing dosing regimens by simulation, is that once the optimal target is defined, the optimal dose needed to reach that target is determined in a single step. Testing covariates for dose scaling is also simple with this method. Furthermore, by using a large dataset of hypothetical patients with real Crohn's Disease demographics, we were able to show how scaling dose by age, weight and PCDAI score could adequately and safely correct IGF-1 (Figure 5).

The goodness-of-fit plots (Figures 3c-e) show that the model predictions are an unbiased description of the data and simulated data that is not significantly different to the observations (Figures 3b and 4). Furthermore, covariate analysis showed that the disease severity (PCDAI score) significantly reduced endogenous synthesis (K_{syn}) rather than affecting IGF-1 clearance. Variations in IGF-1 synthesis distinguish patients with Crohn's disease from children with GHIS.

A possible criticism of using PCDAI to quantify disease in this context is that it includes growth as a marker of inflammation, and it could be suggested that the dependence of K_{syn} on PCDAI might be due to the height velocity element of the index. Recently, the determinants of the disease activity index have been separately analyzed for predictive value in defined populations of children with Crohn's disease [43]. Linear growth was discovered not to be discriminatory. This report developed a new index, the weighted Pediatric Crohn's Disease Activity Index (wPCDAI), based on these observations, which does not include growth. We repeated our covariate modelling with wPCDAI and showed that adding it to K_{syn} reduced parameter variability and significantly improved model fit, in the same way as the standard PCDAI had. Thus, the relationship between K_{syn} and disease activity was due to objective measures of inflammation, and not to the growth component of the PCDAI calculation.

Patients and their support groups have long requested more research into therapies that directly improve growth in children whose inflammation cannot be controlled. The present study shows that twice daily rhIGF-1 can enhance average circulating IGF-1 concentrations into the upper normal range. The use of a utility function from mathematical modelling allows paediatricians to give rhIGF-1 without their entering an unphysiological high range, hence preventing the increased the risk of cancer.

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Competing interests: None

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Contributorship: IRS conceived and helped in its design the study; obtained the funding, ethics, MHRA approval and drafted initial manuscript; JFS developed the mathematical modelling; AVR consented the patients; arranged admission and undertook all the investigation; SK oversaw the clinical care of the patients while admitted for research investigation. MOS helped design the study and provided paediatric endocrinology expertise. All authors contributed to the final manuscript.

Data Sharing: Anomymised original data will be given to research academics at a recognised university departments in line with the policies of Queen Mary, University of London. Requests should be made to Professor Sanderson. However, there are no data in addition to those published, except as the individual numercal results that are displayed as figures.

Patient identifiable data cannot be shared, as this is a condition of the research ethics committee approval

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Legends to figures:

Figure 1 Subcutaneous rhIGF-1 increases the circulating concentrations of IGF-1 in children with growth failure induced by Crohn's disease. (a) IGF-1 standard deviation scores (SDS) below the normal range in both first [Mean -1.78 (SD 1.37)] and second admissions [Mean -2.34 (SD 0.75)] are significantly increased a single injection of rhIGF1 ($p<0.0005$ and <0.0001, respectively). (b) An injection reaches a peak within 4 hours and returns to low levels within 24 hours. (c) Twice daily injections of rhIGF-1 increase circulating IGF-1 over a sustained period. rhIGF-1 was given on day 1 and the circulating concentrations allowed to fall, before giving twice daily injections on days 4 and 5.

Figure 2 Protein losing enteropathy did not alter IFG-1 or IGFBP-3. (a) Variations in protein-losing enteropathy (as measured by faecal alpha 1 anti-trypsin) did not correlate with changes in IGF-1 concentrations achieved on giving rhIGF-1 ($p=0.703$). (b) Variations in protein-losing enteropathy did not correlate with IGFBP-3 concentrations

Figure 3. IGF-1 in a mathematical model. (a) Increasing disease activity (PCDAI) significantly ($p<0.001$) diminishes the estimates of K_{syn} in the covariate model building. (b) Population model predictions versus observed concentrations are unbiased, indicating good structural model fit. (c) Individual model predicted concentrations are in agreement with observed concentrations. (d) Conditional weighted residuals (CWRES), which are a form of standardised residuals expected to follow the Normal Independent Distribution (NID; 0,1) lie within -2 and +2 standard deviations and do not change with model predictions, indicating good structural model fit. (e) Similarly the QQ plot of CWRES indicate that the assumption of normality of residuals is met; (f) Median observed IGF-1 concentrations (solid line) similar to median simulated (dashed line) and observed median lies within 95% confidence interval of the model simulations (grey shaded area).

Figure 4. Population level (blue line) and individual (red line) model predictions are similar to observed data (black open circles) in both (a) single dose and (b) repeated doses in each part of the study.

Figure 5. Incorporation of a disease activity index into dose calculations allows an accurate prediction of circulating IGF-1. Utility function results showing the effect of increasing sophistication on dose scaling method. Scaling by weight, age group and PCDAI score limits average IGF-1 concentrations in 94% of children to less than +2.5 SDS.

Figure 1a

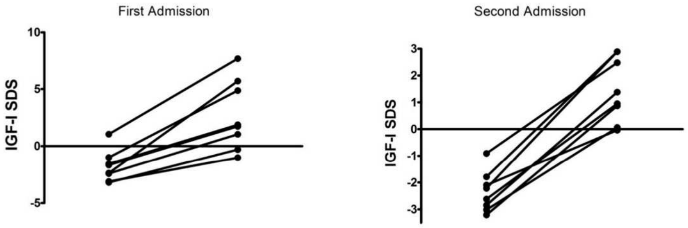

Figure 1b

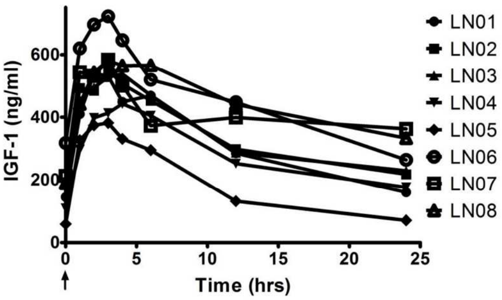

Figure 1c

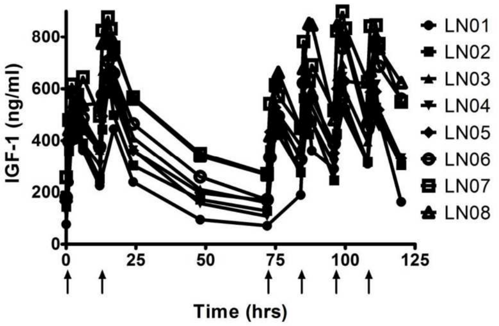

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Figure 2a

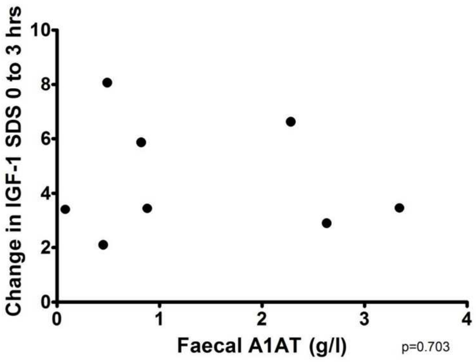
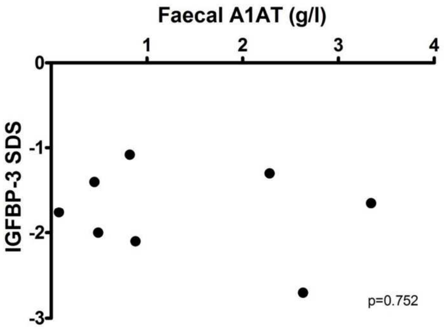


Figure 2b



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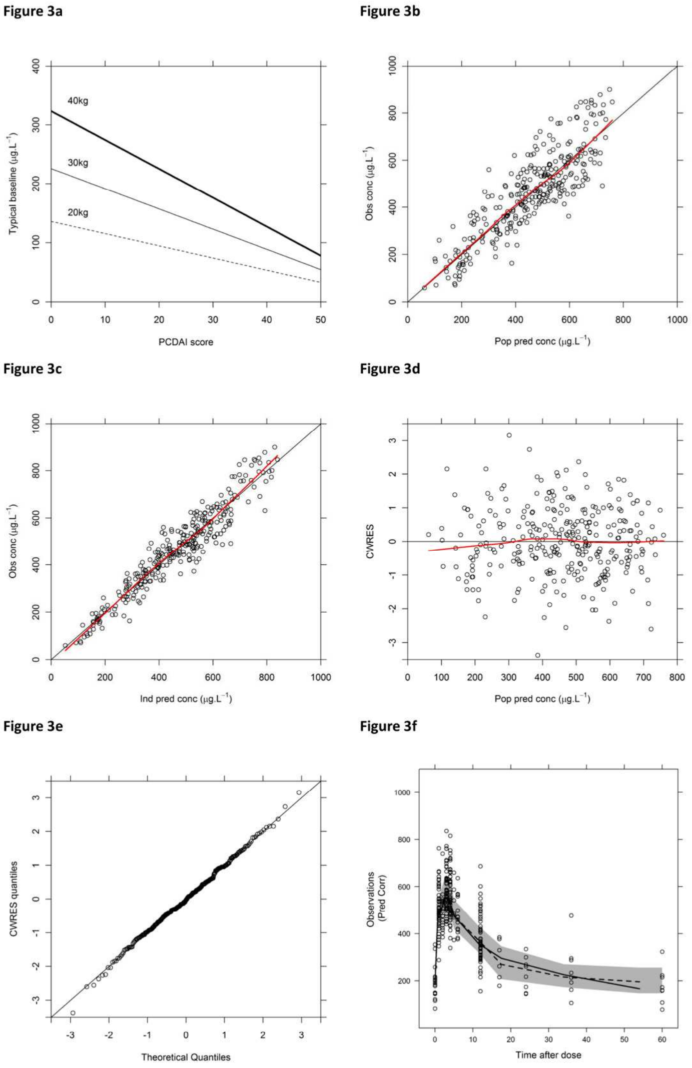

IGF-1 in a mathematical model. (a) Increasing disease activity (PCDAI) significantly (p<0.001) diminishes the estimates of Ksyn in the covariate model building. (b) Population model predictions versus observed concentrations are unbiased, indicating good structural model fit. (c) Individual model predicted concentrations are in agreement with observed concentrations. (d) Conditional weighted residuals (CWRES), which are a form of standardised residuals expected to follow the Normal Independent Distribution (NID; 0,1) lie within -2 and +2 standard deviations and do not change with model predictions, indicating good structural model fit. (e) Similarly the QQ plot of CWRES indicate that the assumption of normality of residuals is met; (f) Median observed IGF-1 concentrations (solid line) similar to median simulated (dashed line) and observed median lies within 95% confidence interval of the model simulations (grey shaded area).
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Figure 4a

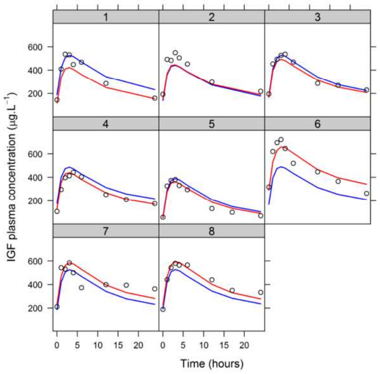
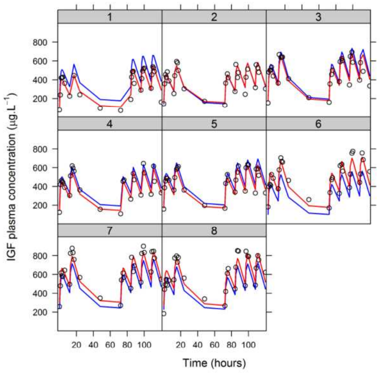


Figure 4b



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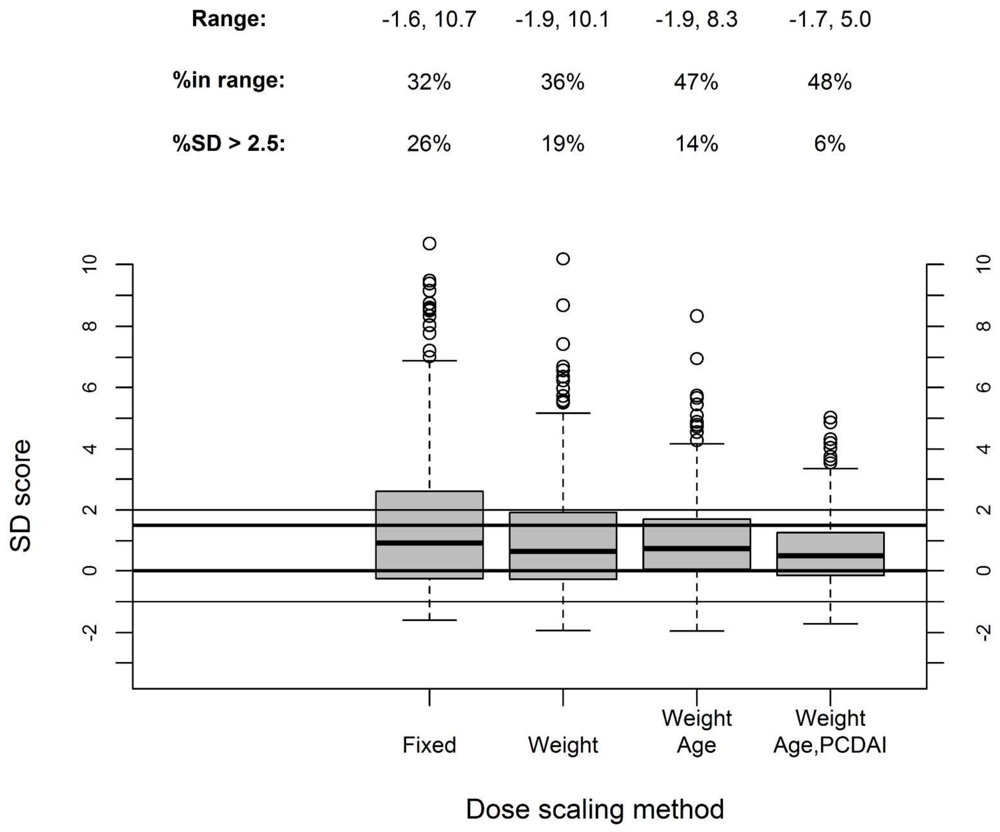

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152x152mm (600 x 600 DPI)

Research Checklist

Pharmacokinetic studies of recombinant human insulin-like growth factor I (rhIGF-I) in children with Crohn's disease- induced growth retardation

- **Approved by the East London Research Ethics Committee (ELREC):**

  ELREC Reference: 07/H0705/77

- **Approved by Medicine and Healthcare products Regulatory Agency (MHRA)**

MHRA reference:	21313/0012/001-0001
Eudract Number	2007-004269-16
Product:	Increlex 10mg/ml solution for injection
Protocol number:	IGFI-1

- **R&D approved and Sponsored by Queen Mary, University of London**

  ReDA reference: 005251

- **Adopted by Medicine for Children Research Network (MCRN)**

- **Registered with the UK Clinical Research Network (UKCRN):**

  ID 4293

Mathematical modelling to restore circulating IGF-1 concentrations in children with Crohn's disease-induced growth failure: a pharmacokinetic study

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1. Mathematical modelling to restore circulating IGF-1 concentrations in children with Crohn's disease-induced growth failure: a pharmacokinetic study

Running head: Restoring IGF-1 in children with Crohn's disease

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4. Key words: insulin-like growth factor-1; adolescent; inflammatory bowel disease; pharmacokinetics; height

5. Word count 2370

ARTICLE SUMMARY

Article focus

One third of children with Crohn's disease have growth retardation.

Children with active inflammation have an insensitivity to growth hormone, resulting in low circulating IGF-1.

There is no agreed growth therapy for children whose inflammation is intractable to treatment, because very high and sustained IGF-1 concentrations are a risk factor for colon cancer in adults with acromegaly.

Key messages

IGF-1 can be restored to the normal range by subcutaneous injections.

The endogenous synthesis of IGF-1 depends on Crohn's disease activity; and variations in both determine the circulating IGF-1 concentrations.

A new mathematical model which incorporates disease activity (in addition to weight and age) will allow paediatric gastroenterologists to calculate doses that keep the circulating IGF-1 in the upper normal range.

Strengths and Limitations

Using the mathematical model, IGF-1 can be prescribed in doses that do not increase the risk of cancer.

For the first time, long term IGF-1 treatment can be studied in children to determine if it enhances growth, and this is a critical step in offering a therapy (much demanded by patients) for growth retardation in children with Crohn's disease.

A limitation of the study is that long term studies over several years will still be required on a large group of children.

ABSTRACT

Objectives: Children with Crohn's disease grow poorly, and inflammation depresses the response of IGF-1 to growth hormone. Correcting the inflammation normalizes growth velocity; however, removing inflammation cannot be achieved in all children. Our lack of understanding of IGF-1 kinetics has hampered its use, particularly as high IGF-1 concentrations over long periods may predispose to colon cancer. We hypothesized that mathematical modelling of IGF-1 would define dosing regimes that return IGF-1 concentrations into the normal range, without reaching values that risk cancer.

Design: Pharmacokinetic intervention study

Setting: Tertiary paediatric gastroenterology unit

Participants: 8 children (M:F; 4:4) entered the study. All completed: 5 South Asian British; 2 White British; 1 African British. Inclusion criteria: Children over 10 years with active Crohn's disease (CRP >10mg/l or ESR >25mm/h) and height velocity <-2 SD score. Exclusion criteria: closed epiphyses; corticosteroids within 3 months; neoplasia or known hypersensitivity to rhIGF-1.

Interventions: Subcutaneous recombinant human IGF-1 (rhIGF-1) (120µg/kg) per dose over two admissions: the first as a single dose, and the second as twice daily doses over 5 days.

Primary outcome: Significant increase in circulating IGF-1

Secondary outcomes: Incidence of side effects of IGF-1. A mathematical model of circulating IGF-1 (A_c) was developed to include parameters of: endogenous synthesis (K_{syn}); exogenous uptake (K_a) from the subcutaneous dose (A_s): and IGF-1 clearance: where $dA_c/dt = K_{syn} - K_{out} \cdot A_c + K_a \cdot A_s$.

Results: Subcutaneous IGF-1 increased concentrations, which were maintained on twice daily doses. In covariate analysis, disease activity reduced K_{syn} ($p<0.001$). Optimal dosing was derived from least

squares regression fitted to a dataset of 384 Crohn's patients, with model parameters assigned by simulation.

Conclusions: By using age, weight and disease activity scaling in IGF-1 dosing, over 95.% of children will have normalized IGF-1 concentrations below +2.5 standard deviations of the normal population mean, a level not associated with cancer risk.

Abstract word count: 299

INTRODUCTION

A quarter of cases of Crohn’s disease now present in children and adolescents under 18 years [1, 2]; and its incidence in childhood is increasing [3]. Around a third of children experience linear growth retardation, caused in part by undernutrition and in part by the direct effects of inflammation on growth [4].

Growth in childhood is regulated by growth hormone (GH), secreted from the pituitary, which stimulates the production of insulin-like growth factor-1 (IGF-1) by the liver and growth plates of bones [5]. Children with active Crohn’s disease have low circulating IGF-1 and increased circulating cytokines [6, 7]. Treatment of the inflammation, for example by an enteral diet, results in a reduction in cytokines like IL-6 and an increase in IGF-1 within 3 days [7]. Thus, the optimum treatment for improving growth is to eliminate the inflammation. Nevertheless, some children’s inflammation remains intractable to treatment despite the best efforts of clinicians. For this group, there is currently no agreed therapy to enhance growth.

Rats with TNBS-induced colonic inflammation grow poorly [8]; and this is also associated with increased cytokines IL-6 and TNF, and a low circulating IGF-1 [9]. Exogenous IGF-1 given to the rats with colitis enhanced growth [8]. In transgenic mice, without inflammation, high levels of circulating IL-6, generated by a widely acting promoter, depressed both IGF-1 and growth [10]. Thus the link between inflammatory cytokines, IGF-1 and poor growth is strong [11]. There is a functional insensitivity to GH in children with Crohn’s disease. Recombinant IGF-1 is now recognized therapy for children with GH insensitivity syndrome (GHIS) due to genetic defects of the GH receptor or IGF-1 gene [12]; we, therefore, hypothesized that IGF-1 concentrations in children with active Crohn’s disease and poor linear growth could be restored by administering rhIGF-1.

Restoring IGF-1 in children with Crohn's disease who may have some endogenous production is not straightforward, because high levels of IGF-1 may be harmful. For rhIGF-1 to be therapeutically useful, its circulating levels over the long term should be returned to normal values by replacement, and not given in excess. Patients with acromegaly who have very high concentrations of GH and IGF-1, maintained over decades, have a doubling in the incidence of colon cancer [13]. This could, in theory, be compounded in children with Crohn's disease, as inflammation is also a risk factor for intestinal cancer.

A mathematical model for administration of rhIGF-1, based on detailed pharmacokinetics would resolve these difficulties, as dosing could be tailored to achieve circulating concentrations that remain within the normal range. We therefore undertook a careful study of subcutaneous rhIGF-1 administration in children with Crohn's disease-induced growth retardation. We developed a model which used physiological parameterisation, disease activity to predict endogenous IGF-1 synthesis, and body weight to scale the volume of distribution. Parameters of protein loss, such as protein-losing enteropathy, were also considered. The model was used to recommend dosing which allows children to be treated with rhIGF-1 without its concentration rising above the normal range.

METHODS

Patient Selection

Following ethical approval from the East London & City Research Ethics Committee (ELREC; reference number 07/H0705/77) and regulatory approval from the UK Medicines and Healthcare products Regulatory Agency (MHRA) (Eudract number: 2007-004269-16), written informed consent was obtained from the parents and patients attending the paediatric inflammatory bowel disease clinic at Barts and The London Hospital for Children. Inclusion criteria were: age ≥ 10 years; known Crohn's disease diagnosed by endoscopic, histological and radiological methods [14]; a height

velocity measured over at least a 6 month period of <-2 Standard Deviation Score (SDS) according to the patient's age and gender; and evidence of active inflammation as demonstrated by either an ESR > 25mm/hr and/or a CRP > 10mg/l. Exclusion criteria were: corticosteroid use in the preceding 3 months; active or suspected neoplasia; known hypersensitivity to exogenous rhIGF-1 (Increlex, Ipsen UK); and the presence of closed epiphyses.

All patients had a full history and physical examination, including an accurate recording of height, weight and assessment of pubertal status. In addition, they also had an electrocardiogram and faecal alpha-1-antitrypsin levels (g/l) measured.

Study Design

Exogenous rhIGF-1 was administered by subcutaneous injection at doses of 120μg/kg over two admissions. Admission 1 investigated the effects of a single dose of rhIGF-1 over a 24 hour inpatient stay. Baseline blood screens included full blood count (FBC), electrolytes, inflammatory markers (CRP and ESR), baseline IGF-1, IGFBP-3, acid labile subunit (ALS) and blood sugar. The Pediatric Crohn's Disease Activity Index (PCDAI) was calculated [15, 16]

Each patient was then given a single subcutaneous injection of rhIGF-1 at a dose of 120μg/kg. Serial venous blood samples were drawn at the following time points: 0, 1, 2, 3, 4, 6, 12, 17 and 24 hours. Blood sugars and vital signs were checked regularly. Children ate and drank freely, and continued to receive their prescribed, non-corticosteroid, treatment for Crohn's disease.

Following a wash out of at least 3 months, subjects were readmitted for admission 2, investigating the effects of repeated doses of rhIGF-1. On this occasion 6 doses of rhIGF-1 were administered over a 5 day trial period, with doses given at: 0, 12, 72, 84, 96 and 108 hours. The injection sites were rotated according to the patient's wishes. Serial blood samples for further IGF-1 , IGFBP-3, acid

labile subunit (ALS) and blood sugar were collected during the 5 days at 0, 1, 2, 3, 4, 6, 12, 17, 24, 48, 72, 96 and 120 hours. Additional samples were also taken for clinical reasons when there was a possibility of hypoglycaemia. If sufficient sample was collected, IGF-1 levels were also measured. In addition, vital signs, FBC, electrolytes, and inflammatory markers were measured daily. Patients were kept as inpatients during the days that the rhIGF-1 was administered, but were allowed home during the two days in which they received no injections. Patients were discharged home on day 6.

Assays and samples

All samples were stored at -20°C until analysis. Plasma glucose was determined immediately after blood sampling (Beckman Instruments, Palo Alto, CA).

IGF-1, IGFBP-3.

IGF-1 and IGFBP-3 levels were measured by competitive binding radioimmunoassay (Esoterix Inc Laboratory Services). The sensitivity of the assay for IGF-1 was 15ng/ml and the intra-assay coefficient of variation averaged 14.1%. The sensitivity of the assay for IGFBP-3 was 0.3mg/L and the intra-assay coefficient of variation averaged 13%.

ALS

ALS levels were measured by radioimmunoassay (ALS-RIA) using purified human ALS as tracer. The intra-assay coefficient of variation ranged from 8.0-17.4%

Mathematical model development

Measured IGF-1 concentrations were fitted for all individuals simultaneously using the non-linear mixed effects modelling software NONMEM version 7.1[17]. Model building was undertaken using the first-order conditional estimation method with interaction (FOCEI). A turnover model was used according to the following differential equations:

$$\frac{dA_c}{dt} = K_{syn} - K_{out} \cdot A_c + K_a \cdot A_s \qquad \text{Equation 1}$$

$$\frac{dA_s}{dt} = -K_a \cdot A_s \qquad \text{Equation 2}$$

Where: A_c is the amount of circulating IGF-1 at time t; A_s is the amount in the subcutaneous tissue following a dose, with initial conditions adjusted each time a dose is administered; K_{syn} is a zero order production rate of endogenous IGF-1 in $\mu g.hr^{-1}$; K_{out} is a first order elimination rate constant in hr^{-1}; and K_a is a first order absorption rate constant describing exogenous IGF-1 appearance following a subcutaneous dose in hr^{-1}. At steady state prior to exogenous dose, circulating IGF-1 is given by the ratio of K_{syn} to K_{out}.

In addition to K_{syn} and K_a, the fixed effect parameters IGF-1 clearance (C_L) in $L.hr^{-1}$ and distribution volume (V_D) in L were estimated, K_{out} being given by the ratio of C_L to V_D. Between subject variability was tested for all parameters, and residual variability was described using a heteroscedastic model. Allometric weight scaling was applied to C_L and V_D *a priori* using linear scaling for V_D and weight raised to the power 0.75 for C_L[18] , K_{syn} was further scaled with linear weight. Tested covariates were CRP, ESR, PCDAI score and age.

Model development used the NONMEM objective function value (OFV); parameter estimate precision was derived through a non-parametric bootstrap [19] and graphical assessment of basic goodness-of-fit plots and model simulations was undertaken. The OFV is proportional to -2 times the log-likelihood of the data given the parameter estimates, and a decrease in OFV of 3.84 with one degree of freedom gives a significantly improved fit with a probability $p < 0.05$. A utility function

based on the final model was used to predict average concentration (C_{ave}) with a dosing regimen derived from the integral of the IGF-1 against the time curve divided by time. Maximum utility was defined as C_{ave} of +0.50 SDS with linear penalty for deviations above and below this target. Dose was optimisied to give a model-derived steady-state C_{ave}, by the following model:

$$Target = SDS_i + \varepsilon_i$$ Equation 3

$$Utility = \sum_{i=1}^{n} (Target - SDS_i)^2$$ Equation 4

Where Target is the SDS aim (in this case +0.50); SDS_i is the individual predicted SDS derived from model predicted C_{AVE}; ε_i denotes individual deviations from the target. Utility minimised ε_i by least squares regression.

Demographic details for 384 subjects aged 8 to 14 years from the clinic database were each assigned a random PCDAI score (from a uniform distribution 0-48) and also individual model parameters. The utility function was maximised with either fixed dosing, dosing based on weight, weight and age, or weight, age and PCDAI score. Differences in concentrations were explored with 12 and 24 hourly dosing regimens.

Calculation of Sample Size

The size was derived from the number of children needed to show a significant increase in IGF-1, based on the increases in IGF-1 seen in a cohort of children with treated with IGF-1 for Growth Hormone Insensitivity Syndrome (GHIS). This showed that in 18 children, the concentration before drug was 330 ng/ml (SEM 20 ng/ml); and the concentration after 4 hours was 535 ng/ml (SEM 20 ng/ml). Therefore, to detect a difference in concentration of 205 (535-330) before drug and after 4

hrs, and SD of 84.85 (calculated from Standard Error of the Mean of 20 with sample size of 18), with 80% power at the 5% level of significance four patients are required.

RESULTS

rhIGF-1 therapy in children with Crohn's disease

Children with active Crohn's disease, and whose growth was inhibited were recruited into the study (Table 1). Impairment of height velocity is characteristic of many children with active Crohn's disease [4, 20-22], and all 8 subjects recruited into the study (median age 12.97) had a height

Table 1: Patient characteristics at recruitment into the trial. Tanner stage: P: pubic hair, B: breast stage, G: genitalia . Medications: 5 –ASA: 5-aminosalicylic acid, AZA: azathioprine, IFX: infliximab, Adalum = adalumimab. Montreal classification: L1: ileal, L2: colonic, L3: ileocolonic, B1: non-stricturing, non-penetrating, B2: stricturing, B3: penetrating

	Patients							
	LN01	LN02	LN03	LN04	LN05	LN06	LN07	LN08
Gender	F	F	M	M	F	F	M	M
Age	13.11	11.5	14.23	10.67	14.82	12.7	12.82	14.66
Ethnicity	Caucasian	Asian	Asian	Asian	Asian	Asian	African	Caucasian
Tanner Stage	P1 B1	P1 B1	P3 G3	P1 G1	P2 B2	P2 B2	P2 G2	P1 G1
HV SDS at recruitment	-2.11	-2.11	-4.18	-2.83	-2.27	-3.87	-4.43	-4.88
HV SDS at time of trial	-2.11	-2.14	-1.84	0.36	1.69	3.55	-4.43	-4.88
PCDAI	12.5	20	17.5	10	47.5	15	12.5	10
Medication	5-ASA	5-ASA	5-ASA AZA IFX	5-ASA AZA Adalum	5-ASA AZA Adalum	5-ASA AZA IFX	5-ASA AZA	5-ASA
Faecal A1AT (g/l)	3.34	0.82	0.88	0.49	2.63	2.28	0.08	0.45
Montreal Classification								
Disease Location	L1	L2	L3	L3	L1	L3	L1	L1
Behaviour	B1	B1	B3	B3	B2	B1	B2	B1
Upper	N	N	Y	N	Y	Y	N	N
Perianal	N	N	Y	Y	N	N	N	N

velocity that was more than two standard deviations below the mean (i.e. a standard deviation score [SDS] of <-2), as an entry criterion. The median height velocity SDS was -3.35, indicating extreme growth failure.

All the subjects completed both parts of the study. The rhIGF-1 was generally well tolerated. Although subcutaneous rhIGF-1 has been associated with hypoglycaemia in children treated for GHIS [12], only one patient (LN01) had a single asymptomatic hypoglycaemic episode (blood sugar < 3.5mmol/l). This was corrected with oral glucose. This occurred following the 12 hour dosage of rhIGF-1 during admission 2 in a child who had not eaten prior to the drug administration. No further episodes of hypoglycaemia occurred. There were no other adverse effects.

Determinants of circulating IGF-1

As previously demonstrated, children with Crohn's disease-induced growth failure had depressed circulating IGF-1 concentrations. The severity of disease in any one child varies over time, and this was reflected in the variation of IGF-1 between the first and second admissions, 3 or more months apart. (Figure 1a). Despite the variation both between and within patients, a single subcutaneous bolus of rhIGF-1 significantly increased IGF-1 in the circulation (Figure 1). In some cases where the baseline IGF-1 was less depressed, the post IGF-1 level was increased to above normal (Figure 1a). The concentrations of IGF-1 returned to baseline over the following 24 hours (Figure 1b).

IGF-1 is maintained in the circulation by IGF binding proteins, the most abundant being IGFBP-3 [23]. A major characteristic of Crohn's disease is protein-losing enteropathy, resulting in proteins being lost into the lumen of the intestine [24, 25]. We aimed therefore to ascertain if the degree of protein-losing enteropathy blunted the ability of subcutaneous rhIGF-1 to achieve significant peak concentrations, or if it directly affected IGF binding proteins. Protein losing enteropathy was quantified by the concentrations of alpha-1- antitrypsin in stool [26]. Using this measure, protein

losing enteropathy had no effect on the change in IGF-1 induced by a subcutaneous injection (Figure 2a)

IGFBP-3 concentrations are depressed in children with chronic IGF-1 deficiency [27], and all children in the study had low IGFBP-3 (Figure 2b). We wished to determine, however, if IGFBP-3 concentrations depended on the degree of protein losing enteropathy, as this would indicate that protein losing enteropathy may affect IGF-1 pharmacokinetics. However, no association was observed.

In the second admission, the subjects received twice daily doses, to determine if IGF-1 concentrations would be enhanced over a longer period. The initial injection increased the IGF-1 concentrations into the normal range (Figure 1a). Increases over a sustained period were successfully achieved (Figure 1c), and as expected multiple daily dosing led to lower variability between peak and trough concentrations. Taking the area under the curve divided by time as the average IGF-1 concentration, it became clear that on 120µg/kg, some children had an average IGF-1 SDS of greater than +2.5, while other children had appropriate concentrations. Increased circulating IGF-1 in acromegaly has been linked with an increased risk of colon cancer [13, 28]. Fortunately, we have a clear understanding of how IGF-1 concentrations relate to colon cancer risk in acromegaly [13]. These studies show that the increased cancer risk was seen in individuals whose circulating IGF-1 was +2.5 SDS or greater. Using our data, we developed a model from which the dose-concentration relationship could be derived. We then investigated the covariates on which dosing could be individualised. This allowed us to correct circulating IGF-1 without causing excessively high concentrations: ensuring that our dosing regimens resulted in concentrations that were below +2.5 SDS, we developed a utility model to predict the IGF-1 SDS achieved on regular dosing.

Mathematical modelling of IGF-1 links endogenous production to disease severity

A turnover model which accounted for both endogenous production and exogenous dosing of IGF-1 was fitted to the data (see methods for mathematical formulae) using non-linear mixed effects analysis. Four fixed effects were estimated, namely endogenous synthesis rate (K_{SYN}), exogenous absorption rate through the skin following subcutaneous injection (K_a), systemic clearance (C_L), and distribution volume V_D. Parameter level random effects (inter-subject variability) were included on K_{SYN} and C_L . (Table 2) A further level of random effects (inter-occasion variability) also provided significantly improved fit when added to K_{SYN}. Following a limited covariate analysis, the PCDAI score was found to significantly improve fit when added to K_{SYN}, with K_{SYN} falling with increasing disease severity (Figure 3a). The effect of disease severity as measured by PCDAI was tested for C_L and K_{SYN} with significant improvement ($p<0.001$) being shown for K_{SYN}. Furthermore, adding the PCDAI score as a covariate to K_{SYN} allowed inter-occasion variability to be removed without detriment to the model fit (no change in log-likelihood). Adding PCDAI to K_{SYN} also decreased inter-individual variability. In short, disease severity was a major driver of IGF-1 synthesis rate both between patients, and in the same patient on different occasions.

Table 2: Parameter estimates from the model including non-parametric 95%CI from a bootstrap of 714 successful runs. IIV (inter-individual variability).

Parameter	Estimate	Bootstrap median	Bootstrap 2.5th percentile	Bootstrap 97.5th percentile
C_L (L/h/70kg)	1.61	1.60	1.36	1.82
V_D (L/70kg)	2.42	2.41	1.78	3.10
K_{SYN} (µg/h)	433	423	352	490
Ka (h^{-1})	0.10	0.10	0.083	0.12
Coefficient of PCDAI on K_{SYN}	-6.57	-6.41	-8.19	-4.89
IIV on CL (%CV)	10.38	9.46	3.49	15.1
IIV on K_{SYN} (%CV)	24.61	24.10	9.98	33.58
Residual error proportional (%CV)	9.81	9.69	7.85	11.04
Residual error, additive (µg/L)	28.32	25.93	13.29	42.78

There was good agreement between model predictions and observed concentrations (Figure 3b), and trendless distribution of standardised residuals, indicating that the parametric modelling assumptions were satisfied (Figure 3c, d and e). The median of the simulated data was not significantly different to the median of the observed data (Figure 3f). Superimposing model predictions at a population and individual level on the observed data showed that model predictions captured the dynamic behaviour of the system (Figure 4).

Dosing to maintain circulating rhIGF-1 concentrations in the target range

We undertook mathematical modelling to quantify the relationship between endogenous IGF-1 production and exogenous dosing, and to understand how IGF-1 average concentrations (area under the curve/time) can be adjusted. Using the knowledge that in acromegaly there is no increased risk of cancer in patients whose IGF-1 is below +2.5 SDS [13], we defined the optimal IGF-1 concentration range as being between 0 and +2.0 SDS. This range would improve growth without elevating cancer risk. Different possible dose scaling systems were then explored to maximise the probability of an individual having IGF-1 levels in the target range. By individualising dose by weight, age (because different aged children have different target circulating IGF-1 levels), and PCDAI score, 92.7% of children were predicted to be corrected to less than or equal to +2.0 SDS (95.3% below +2.5 SDS; Figure 5). We can therefore suggest a dosing scheme that limits the risk of malignancy to that of the normal patient population. The dosing schedule recommended from the utility function is:

Age 10 to <12 years, 21 µg/kg plus 1 µg/kg /PCDAI point; Age 12 to <14 years, 41 µg/kg plus 1.4 µg/kg /PCDAI point.

DISCUSSION

Impaired growth has been a recognised feature of children with Crohn's disease for 50 years [29]. In 1986, low IGF-1 was associated with poor growth in these children [30]; and over the following decade it was realized that the depressed concentration was a direct consequence of inflammation [8, 10], and not merely the result of poor energy intake or undernutrition. The best strategy to increase IGF-1 and therefore to improve growth is the resolution of inflammation. Treatments which reverse the inflammation, but do not actively suppress growth, such as surgery [31], anti-TNF [32, 33] and enteral diets [6, 34] have all been shown to improve linear growth.

Nevertheless, there are patients with Crohn's disease whose inflammation cannot be reversed. In this group, we need a distinct strategy to accelerate growth. Both hGH [35, 36] and rhIGF-1 have been suggested. Because patients with Crohn's disease have a functional insensitivity to GH, the present study examined the possibility that rhIGF-1 would be efficacious in improving IGF-1 concentrations, as it does in children with growth hormone insensitivity syndrome (GHIS). However, there are significant problems in applying our knowledge of rhIGF-1 usage in GHIS to children with Crohn's disease. First, children present at a much later age in Crohn's disease than they do in GHIS, which is caused by a genetic mutation (approximately 8-15 years as opposed to less than 5 years old). The compartments of the IGF-1 system including several IGF binding proteins, cannot be assumed to distribute as they do in younger children, particularly if the relative volumes of these compartments change at the time of puberty. Second, both IGF-1 and the IGF binding proteins are circulating proteins. Proteins are lost into the intestine through protein losing enteropathy (PLE), where inflammation reduces the ability of the intestinal vasculature to maintain them in the capillary lumen [37-39]. The PLE could, in theory, alter the kinetics of IGF-1. Finally, very high and sustained circulating IGF-1 concentrations are associated with an increase in colon cancer in middle aged and older patients with acromegaly. A modelling approach to kinetics and dosing was necessary to avert causing high average IGF-1 concentrations.

Several of the children enrolled in the study had delayed puberty (Table 1). Although this is commonly observed in Crohn's disease, the study could be criticized for not taking this into account when calculating IGF-1 SDSs, as circulating IGF-1 increases in puberty. We do not have normative data for circulating IGF-1 against pubertal stage. However, delay in puberty is closely correlated with a delay in bone age, as measured on a wrist X-ray. We therefore recalculated each child's IGF-1 SDS against their bone age before and after the initial injection of rhIGF-1 (data not shown). However, this made little difference to the results obtained when comparing this data to that based on chronological age (Figure 1a).

Previous studies on rhIGF-1 pharmacokinetics [40, 41] have mainly focussed on its use in malnourished adult patients with renal failure, where it has been used to maintain protein balance. These reports undertook purely descriptive analyses such as Area Under the Concentration-Time Curve (AUC) and comparing healthy volunteer parameters with patients, rather than modelling the data. Furthermore, they did not account for endogenous IGF-1 production. If we were to use our data to recommend a dose, a more sophisticated approach was required. In this study, we fitted a structural model that simplified the system without losing biological interpretation of the parameters. Mixed effects modelling allowed for the addition of inter-individual and inter-occasion variability in model parameters, in addition to residual variability. We were, therefore, able simultaneously to fit the model to all patients and investigate covariate-parameter relationships. Instead of exploring dose recommendations by simulation, we aimed to optimise the dose based on the target SDS; and we minimised deviations from this using least-squares regression [42] . The advantage of this approach, over testing competing dosing regimens by simulation, is that once the optimal target is defined, the optimal dose needed to reach that target is determined in a single step. Testing covariates for dose scaling is also simple with this method. Furthermore, by using a large dataset of hypothetical patients with real Crohn's Disease demographics, we were able to show how scaling dose by age, weight and PCDAI score could adequately and safely correct IGF-1 (Figure 5).

The goodness-of-fit plots (Figures 3b-e and Figure 4) show that the model predictions are an unbiased description of the data and simulated data that is not significantly different to the observations (Figure 3f). Furthermore, covariate analysis showed that the disease severity (PCDAI score) significantly reduced endogenous synthesis (K_{syn}) rather than affecting IGF-1 clearance. Variations in IGF-1 synthesis distinguish patients with Crohn's disease from children with GHIS.

A possible criticism of using PCDAI to quantify disease in this context is that it includes growth as a marker of inflammation, and it could be suggested that the dependence of K_{syn} on PCDAI might be due to the height velocity element of the index. Recently, the determinants of the disease activity index have been separately analyzed for predictive value in defined populations of children with Crohn's disease [43]. Linear growth was discovered not to be discriminatory. This report developed a new index, the weighted Pediatric Crohn's Disease Activity Index (wPCDAI), based on these observations, which does not include growth. We repeated our covariate modelling with wPCDAI and showed that adding it to K_{syn} reduced parameter variability and significantly improved model fit, in the same way as the standard PCDAI had. Thus, the relationship between K_{syn} and disease activity was due to objective measures of inflammation, and not to the growth component of the PCDAI calculation.

This report focused on the effects of IGF-1. As mentioned above, hGH may also be of benefit on the linear growth of children with Crohn's disease [36]. It was not the purpose of the present study to examine which therapy would be more efficacious; however, future studies comparing IGF-1 to hGH in a controlled randomized multicentre study are planned. This trial will also give us the opportunity to test the dosing regimen derived from this model presented in the present report on a wide sample of children.

Patients and their support groups have long requested more research into therapies that directly improve growth in children whose inflammation cannot be controlled. The present study shows that twice daily rhIGF-1 can enhance average circulating IGF-1 concentrations into the upper normal range. The use of a utility function from mathematical modelling allows paediatricians to give rhIGF-1 without their entering an unphysiological high range, hence preventing the increased the risk of cancer.

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Competing interests: None

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Contributorship: IRS conceived the study and helped in its design; obtained the funding, ethics, MHRA approval and drafted initial manuscript; JFS developed the mathematical modelling; AVR consented the patients; arranged admission and undertook all the investigation; SK oversaw the clinical care of the patients while admitted for research investigation. MOS helped design the study and provided paediatric endocrinology expertise. All authors contributed to the final manuscript.

Data Sharing: Anomymised original data will be given to research academics at a recognised university departments in line with the policies of Queen Mary, University of London. Requests should be made to Professor Sanderson. However, there are no data in addition to those published, except as the individual numerical results that are displayed as

figures.

Patient identifiable data cannot be shared, as this is a condition of the research ethics committee approval

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Legends to figures:

Figure 1 Subcutaneous rhIGF-1 increases the circulating concentrations of IGF-1 in children with growth failure induced by Crohn's disease. (a) IGF-1 standard deviation scores (SDS) below the normal range in both first [Mean -1.78 (SD 1.37)] and second admissions [Mean -2.34 (SD 0.75)] are significantly increased a single injection of rhIGF1 ($p<0.0005$ and <0.0001, respectively). (b) An injection reaches a peak within 4 hours and returns to low levels within 24 hours. (c) Twice daily injections of rhIGF-1 increase circulating IGF-1 over a sustained period. rhIGF-1 was given on day 1 and the circulating concentrations allowed to fall, before giving twice daily injections on days 4 and 5.

Figure 2 Protein losing enteropathy did not alter IGF-1 or IGFBP-3. (a) Variations in protein-losing enteropathy (as measured by faecal alpha 1 anti-trypsin) did not correlate with changes in IGF-1 concentrations achieved on giving rhIGF-1 ($p=0.703$). (b) Variations in protein-losing enteropathy did not correlate with IGFBP-3 concentrations

Figure 3. IGF-1 in a mathematical model. (a) Increasing disease activity (PCDAI) significantly ($p<0.001$) diminishes the estimates of K_{syn} in the covariate model building. (b) Population model predictions versus observed concentrations are unbiased, indicating good structural model fit. (c) Individual model predicted concentrations are in agreement with observed concentrations. (d) Conditional weighted residuals (CWRES), which are a form of standardised residuals expected to follow the Normal Independent Distribution (NID; 0,1) lie within -2 and +2 standard deviations and do not change with model predictions, indicating good structural model fit. (e) Similarly the QQ plot of CWRES indicate that the assumption of normality of residuals is met; (f) Median observed IGF-1 concentrations (solid line) similar to median simulated (dashed line) and observed median lies within 95% confidence interval of the model simulations (grey shaded area).

Figure 4. Population level (blue line) and individual (red line) model predictions are similar to observed data (black open circles) in both (a) single dose and (b) repeated doses in each part of the study.

Figure 5. Incorporation of a disease activity index into dose calculations allows an accurate prediction of circulating IGF-1. Utility function results showing the effect of increasing sophistication on dose scaling method. Scaling by weight, age group and PCDAI score limits average IGF-1 concentrations in 93% of children to less than +2 SDS. Solid lines are the target range of 0 to +2 SDS, dashed lines are for reference -2SDS and +2.5 SDS.

1. Mathematical modelling to restore circulating IGF-1 concentrations in children with Crohn's disease-induced growth failure: a pharmacokinetic study

Running head: Restoring IGF-1 in children with Crohn's disease

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ARTICLE SUMMARY

Article focus

One third of children with Crohn's disease have growth retardation.

Children with active inflammation have an insensitivity to growth hormone, resulting in low circulating IGF-1.

There is no agreed growth therapy for children whose inflammation is intractable to treatment, because very high and sustained IGF-1 concentrations are a risk factor for colon cancer in adults with acromegaly.

Key messages

IGF-1 can be restored to the normal range by subcutaneous injections.

The endogenous synthesis of IGF-1 depends on Crohn's disease activity; and variations in both determine the circulating IGF-1 concentrations.

A new mathematical model which incorporates disease activity (in addition to weight and age) will allow paediatric gastroenterologists to calculate doses that keep the circulating IGF-1 in the upper normal range.

Strengths and Limitations

Using the mathematical model, IGF-1 can be prescribed in doses that do not increase the risk of cancer.

For the first time, long term IGF-1 treatment can be studied in children to determine if it enhances growth, and this is a critical step in offering a therapy (much demanded by patients) for growth retardation in children with Crohn's disease.

A limitation of the study is that long term studies over several years will still be required on a large group of children.

ABSTRACT

Objectives: Children with Crohn's disease grow poorly, and inflammation depresses the response of IGF-1 to growth hormone. Correcting the inflammation normalizes growth velocity; however, removing inflammation cannot be achieved in all children. Our lack of understanding of IGF-1 kinetics has hampered its use, particularly as high IGF-1 concentrations over long periods may predispose to colon cancer. We hypothesized that mathematical modelling of IGF-1 would define dosing regimes that return IGF-1 concentrations into the normal range, without reaching values that risk cancer.

Design: Pharmacokinetic intervention study

Setting: Tertiary paediatric gastroenterology unit

Participants: 8 children (M:F; 4:4) entered the study. All completed: 5 South Asian British; 2 White British; 1 African British. Inclusion criteria: Children over 10 years with active Crohn's disease (CRP >10mg/l or ESR >25mm/h) and height velocity <-2 SD score. Exclusion criteria: closed epiphyses; corticosteroids within 3 months; neoplasia or known hypersensitivity to rhIGF-1.

Interventions: Subcutaneous recombinant human IGF-1 (rhIGF-1) (120µg/kg) per dose over two admissions: the first as a single dose, and the second as twice daily doses over 5 days.

Primary outcome: Significant increase in circulating IGF-1

Secondary outcomes: Incidence of side effects of IGF-1. A mathematical model of circulating IGF-1 (A_c) was developed to include parameters of: endogenous synthesis (K_{syn}); exogenous uptake (K_a) from the subcutaneous~~ly~~ dose (A_s): and IGF-1 clearance: where $dA_c/dt= K_{syn}- K_{out}\cdot A_c + K_a\cdot A_s$.

Results: Subcutaneous IGF-1 increased concentrations, which were maintained on twice daily doses. In covariate analysis, disease activity reduced K_{syn} ($p<0.001$). Optimal dosing was derived from least

squares regression fitted to a dataset of 384 Crohn’s patients, with model parameters assigned by simulation.

Conclusions: By using age, weight and disease activity scaling in IGF-1 dosing, over 95.~~4~~% of children will have normalized IGF-1 concentrations below +2.5 standard deviations of the normal population mean, a level not associated with cancer risk.

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INTRODUCTION

A quarter of cases of Crohn's disease now present in children and adolescents under 18 years [1, 2]; and its incidence in childhood is increasing [3]. Around a third of children experience linear growth retardation, caused in part by undernutrition and in part by the direct effects of inflammation on growth [4].

Growth in childhood is regulated by growth hormone (GH), secreted from the pituitary, which stimulates the production of insulin-like growth factor-1 (IGF-1) by the liver and growth plates of bones [5]. Children with active Crohn's disease have low circulating IGF-1 and increased circulating cytokines [6, 7]. Treatment of the inflammation, for example by an enteral diet, results in a reduction in cytokines like IL-6 and an increase in IGF-1 within 3 days [7]. Thus, the optimum treatment for improving growth is to eliminate the inflammation. Nevertheless, some children's inflammation remains intractable to treatment despite the best efforts of clinicians. For this group, there is currently no agreed therapy to enhance growth.

Rats with TNBS-induced colonic inflammation grow poorly [8]; and this is also associated with increased cytokines IL-6 and TNF, and a low circulating IGF-1 [9]. Exogenous IGF-1 given to the rats with colitis enhanced growth [8]. In transgenic mice, without inflammation, high levels of circulating IL-6, generated by a widely acting promoter, depressed both IGF-1 and growth [10]. Thus the link between inflammatory cytokines, IGF-1 and poor growth is strong [11]. There is a functional insensitivity to GH in children with Crohn's disease. Recombinant IGF-1 is now recognized therapy for children with GH insensitivity syndrome (GHIS) due to genetic defects of the GH receptor or IGF-1 gene [12]; we, therefore, hypothesized that IGF-1 concentrations in children with active Crohn's disease and poor linear growth could be restored by administering rhIGF-1.

Restoring IGF-1 in children with Crohn's disease who may have some endogenous production is not straightforward, because high levels of IGF-1 may be harmful. For rhIGF-1 to be therapeutically useful, its circulating levels over the long term should be returned to normal values by replacement, and not given in excess. Patients with acromegaly who have very high concentrations of GH and IGF-1, maintained over decades, have a doubling in the incidence of colon cancer [13]. This could, in theory, be compounded in children with Crohn's disease, as inflammation is also a risk factor for intestinal cancer.

A mathematical model for administration of rhIGF-1, based on detailed pharmacokinetics would resolve these difficulties, as dosing could be tailored to achieve circulating concentrations that remain within the normal range. We therefore undertook a careful study of subcutaneous rhIGF-1 administration in children with Crohn's disease-induced growth retardation. We developed a model which used physiological parameterisation, disease activity to predict endogenous IGF-1 synthesis, and body weight to scale the volume of distribution. Parameters of protein loss, such as protein-losing enteropathy, were also considered. The model was used to recommend dosing which allows children to be treated with rhIGF-1 without its concentration rising above the normal range.

METHODS

Patient Selection

Following ethical approval from the East London & City Research Ethics Committee (ELREC; reference number 07/H0705/77) and regulatory approval from the UK Medicines and Healthcare products Regulatory Agency (MHRA) (Eudract number: 2007-004269-16), written informed consent was obtained from the parents and patients attending the paediatric inflammatory bowel disease clinic at Barts and The London Hospital for Children. Inclusion criteria were: age ≥ 10 years; known Crohn's disease diagnosed by endoscopic, histological and radiological methods [14]; a height

velocity measured over at least a 6 month period of <-2 Standard Deviation Score (SDS) according to the patient’s age and gender; and evidence of active inflammation as demonstrated by either an ESR > 25mm/hr and/or a CRP > 10mg/l. Exclusion criteria were: corticosteroid use in the preceding 3 months; active or suspected neoplasia; known hypersensitivity to exogenous rhIGF-1 (Increlex, Ipsen UK); and the presence of closed epiphyses.

All patients had a full history and physical examination, including an accurate recording of height, weight and assessment of pubertal status. In addition, they also had an electrocardiogram and faecal alpha-1-antitrypsin levels (g/l) measured.

Study Design

Exogenous rhIGF-1 was administered by subcutaneous injection at doses of 120µg/kg over two admissions. Admission 1 investigated the effects of a single dose of rhIGF-1 over a 24 hour inpatient stay. Baseline blood screens included full blood count (FBC), electrolytes, inflammatory markers (CRP and ESR), baseline IGF-1, IGFBP-3, acid labile subunit (ALS) and blood sugar. The Pediatric Crohn’s Disease Activity Index (PCDAI) was calculated [15, 16]

Each patient was then given a single subcutaneous injection of rhIGF-1 at a dose of 120µg/kg. Serial venous blood samples were drawn at the following time points: 0, 1, 2, 3, 4, 6, 12, 17 and 24 hours. Blood sugars and vital signs were checked regularly. Children ate and drank freely, and continued to receive their prescribed, non-corticosteroid, treatment for Crohn’s disease.

Following a wash out of at least 3 months, subjects were readmitted for admission 2, investigating the effects of repeated doses of rhIGF-1. On this occasion 6 doses of rhIGF-1 were administered over a 5 day trial period, with doses given at: 0, 12, 72, 84, 96 and 108 hours. The injection sites were rotated according to the patient’s wishes. Serial blood samples for further IGF-1 , IGFBP-3, acid

labile subunit (ALS) and blood sugar were collected during the 5 days at 0, 1, 2, 3, 4, 6, 12, 17, 24, 48, 72, 96 and 120 hours. Additional samples were also taken for clinical reasons when there was a possibility of hypoglycaemia. If sufficient sample was collected, IGF-1 levels were also measured. In addition, vital signs, FBC, electrolytes, and inflammatory markers were measured daily. Patients were kept as inpatients during the days that the rhIGF-1 was administered, but were allowed home during the two days in which they received no injections. Patients were discharged home on day 6.

Assays and samples

All samples were stored at -20°C until analysis. Plasma glucose was determined immediately after blood sampling (Beckman Instruments, Palo Alto, CA).

IGF-1, IGFBP-3.

IGF-1 and IGFBP-3 levels were measured by competitive binding radioimmunoassay (Esoterix Inc Laboratory Services). The sensitivity of the assay for IGF-1 was 15ng/ml and the intra-assay coefficient of variation averaged 14.1%. The sensitivity of the assay for IGFBP-3 was 0.3mg/L and the intra-assay coefficient of variation averaged 13%.

ALS

ALS levels were measured by radioimmunoassay (ALS-RIA) using purified human ALS as tracer. The intra-assay coefficient of variation ranged from 8.0-17.4%

Mathematical model development

Measured IGF-1 concentrations were fitted for all individuals simultaneously using the non-linear mixed effects modelling software NONMEM version 7.1[17]. Model building was undertaken using the first-order conditional estimation method with interaction (FOCEI). A turnover model was used according to the following differential equations:

$$\frac{dA_c}{dt} = K_{syn} - K_{out} \cdot A_c + K_a \cdot A_s$$ Equation 1

$$\frac{dA_s}{dt} = - K_a \cdot A_s$$ Equation 2

Where: A_c is the amount of circulating IGF-1 at time t; A_s is the amount in the subcutaneous tissue following a dose, with initial conditions adjusted each time a dose is administered; K_{syn} is a zero order production rate of endogenous IGF-1 in µg.hr^{-1}; K_{out} is a first order elimination rate constant in hr^{-1}; and K_a is a first order absorption rate constant describing exogenous IGF-1 appearance following a subcutaneous dose in hr^{-1}. At steady state prior to exogenous dose, circulating IGF-1 is given by the ratio of K_{syn} to K_{out}.

In addition to K_{syn} and K_a, the fixed effect parameters IGF-1 clearance (C_L) in L.hr^{-1} and distribution volume (V_D) in L were estimated, K_{out} being given by the ratio of C_L to V_D. Between subject variability was tested for all parameters, and residual variability was described using a heteroscedastic model. Allometric weight scaling was applied to C_L and V_D *a priori* using linear scaling for V_D and weight raised to the power 0.75 for C_L[18] , K_{syn} was further scaled with linear weight. Tested covariates were CRP, ESR, PCDAI score and age.

Model development used the NONMEM objective function value (OFV); parameter estimate precision was derived through a non-parametric bootstrap [19] and graphical assessment of basic goodness-of-fit plots and model simulations was undertaken. The OFV is proportional to -2 times the log-likelihood of the data given the parameter estimates, and a decrease in OFV of 3.84 with one degree of freedom gives a significantly improved fit with a probability $p < 0.05$. A utility function based on the final model was used to predict average concentration (C_{ave}) with a dosing regimen derived from the integral of the IGF-1 against the time curve divided by time. Maximum utility was defined as C_{ave} of +0.50~~75~~ SDS with linear penalty for deviations above and below this target. Dose was optimisied to give a model-derived steady-state C_{ave}, by the following model:

$$Target = SDS_i + \varepsilon_i$$ Equation 3

$$Utility = \sum_{i=1}^{n}(Target - SDS_i)^2$$ Equation 4

Where Target is the SDS aim (in this case +0.50~~75~~); SDS_i is the individual predicted SDS derived from model predicted C_{AVE}; ε_i denotes individual deviations from the target. Utility minimised ε_i by least squares regression.

Demographic details for 384 subjects aged 8 to 14 years from the clinic database were each assigned a random PCDAI score (from a uniform distribution 0-48) and also individual model parameters. The utility function was maximised with either fixed dosing, dosing based on weight, weight and age, or weight, age and PCDAI score. Differences in concentrations were explored with 12 and 24 hourly dosing regimens.

Calculation of Sample Size

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The size was derived from the number of children needed to show a significant increase in IGF-1, based on the increases in IGF-1 seen in a cohort of children with treated with IGF-1 for Growth Hormone Insensitivity Syndrome (GHIS). This showed that in 18 children, the concentration before drug was 330 ng/ml (SEM 20 ng/ml); and the concentration after 4 hours was 535 ng/ml (SEM 20 ng/ml). Therefore, to detect a difference in concentration of 205 (535-330) before drug and after 4 hrs, and SD of 84.85 (calculated from Standard Error of the Mean of 20 with sample size of 18), with 80% power at the 5% level of significance four patients are required.

RESULTS

rhIGF-1 therapy in children with Crohn's disease

Children with active Crohn's disease, and whose growth was inhibited were recruited into the study (Table 1). Impairment of height velocity is characteristic of many children with active Crohn's disease [4, 20-22], and all 8 subjects recruited into the study (median age 12.97) had a height

Table 1: Patient characteristics at recruitment into the trial. Tanner stage: P: pubic hair, B: breast stage, G: genitalia . Medications: 5 –ASA: 5-aminosalicylic acid, AZA: azathioprine, IFX: infliximab, Adalum = adalumimab. Montreal classification: L1: ileal, L2: colonic, L3: ileocolonic, B1: non-stricturing, non-penetrating, B2: stricturing, B3: penetrating

	Patients							
	LN01	LN02	LN03	LN04	LN05	LN06	LN07	LN08
Gender	F	F	M	M	F	F	M	M
Age	13.11	11.5	14.23	10.67	14.82	12.7	12.82	14.66
Ethnicity	Caucasian	Asian	Asian	Asian	Asian	Asian	African	Caucasian
Tanner Stage	P1 B1	P1 B1	P3 G3	P1 G1	P2 B2	P2 B2	P2 G2	P1 G1
HV SDS at recruitment	-2.11	-2.11	-4.18	-2.83	-2.27	-3.87	-4.43	-4.88
HV SDS at time of trial	-2.11	-2.14	-1.84	0.36	1.69	3.55	-4.43	-4.88
PCDAI	12.5	20	17.5	10	47.5	15	12.5	10
Medication	5-ASA	5-ASA	5-ASA AZA IFX	5-ASA AZA Adalum	5-ASA AZA Adalum	5-ASA AZA IFX	5-ASA AZA	5-ASA
Faecal A1AT (g/l)	3.34	0.82	0.88	0.49	2.63	2.28	0.08	0.45
Montreal Classification								
Disease Location	L1	L2	L3	L3	L1	L3	L1	L1
Behaviour	B1	B1	B3	B3	B2	B1	B2	B1
Upper	N	N	Y	N	Y	Y	N	N
Perianal	N	N	Y	Y	N	N	N	N

velocity that was more than two standard deviations below the mean (i.e. a standard deviation score [SDS] of <-2), as an entry criterion. The median height velocity SDS was -3.35, indicating extreme growth failure.

All the subjects completed both parts of the study. The rhIGF-1 was generally well tolerated. Although subcutaneous rhIGF-1 has been associated with hypoglycaemia in children treated for GHIS [12], only one patient (LN01) had a single asymptomatic hypoglycaemic episode (blood sugar < 3.5mmol/l). This was corrected with oral glucose. This occurred following the 12 hour dosage of rhIGF-1 during admission 2 in a child who had not eaten prior to the drug administration. No further episodes of hypoglycaemia occurred. There were no other adverse effects.

Determinants of circulating IGF-1

As previously demonstrated, children with Crohn's disease-induced growth failure had depressed circulating IGF-1 concentrations. The severity of disease in any one child varies over time, and this was reflected in the variation of IGF-1 between the first and second admissions, 3 or more months apart. (Figure 1a). Despite the variation both between and within patients, a single subcutaneous bolus of rhIGF-1 significantly increased IGF-1 in the circulation (Figure 1). In some cases where the baseline IGF-1 was less depressed, the post IGF-1 level was increased to above normal (Figure 1a). The concentrations of IGF-1 returned to baseline over the following 24 hours (Figure 1b).

IGF-1 is maintained in the circulation by IGF binding proteins, the most abundant being IGFBP-3 [23]. A major characteristic of Crohn's disease is protein-losing enteropathy, resulting in proteins being lost into the lumen of the intestine [24, 25]. We aimed therefore to ascertain if the degree of protein-losing enteropathy blunted the ability of subcutaneous rhIGF-1 to achieve significant peak concentrations, or if it directly affected IGF binding proteins. Protein losing enteropathy was quantified by the concentrations of alpha-1- antitrypsin in stool [26]. Using this measure, protein

losing enteropathy had no effect on the change in IGF-1 induced by a subcutaneous injection (Figure 2a)

IGFBP-3 concentrations are depressed in children with chronic IGF-1 deficiency [27], and all children in the study had low IGFBP-3 (Figure 2b). We wished to determine, however, if IGFBP-3 concentrations depended on the degree of protein losing enteropathy, as this would indicate that protein losing enteropathy may affect IGF-1 pharmacokinetics. However, no association was observed.

In the second admission, the subjects received twice daily doses, to determine if IGF-1 concentrations would be enhanced over a longer period. The initial injection increased the IGF-1 concentrations into the normal range (Figure 1a). Increases over a sustained period were successfully achieved (Figure 1c), and as expected multiple daily dosing led to lower variability between peak and trough concentrations. Taking the area under the curve divided by time as the average IGF-1 concentration, it became clear that on 120μg/kg, some children had an average IGF-1 SDS of greater than +2.5, while other children had appropriate concentrations. Increased circulating IGF-1 in acromegaly has been linked with an increased risk of colon cancer [13, 28]. Fortunately, we have a clear understanding of how IGF-1 concentrations relate to colon cancer risk in acromegaly [13]. These studies show that the increased cancer risk was seen in individuals whose circulating IGF-1 was +2.5 SDS or greater. Using our data, we developed a model from which the dose-concentration relationship could be derived. We then investigated the covariates on which dosing could be individualised. This allowed us to correct circulating IGF-1 without causing excessively high concentrations: ensuring that our dosing regimens resulted in concentrations that were below +2.5 SDS, we developed a utility model to predict the IGF-1 SDS achieved on regular dosing.

Mathematical modelling of IGF-1 links endogenous production to disease severity

A turnover model which accounted for both endogenous production and exogenous dosing of IGF-1 was fitted to the data (see methods for mathematical formulae) using non-linear mixed effects analysis. Four fixed effects were estimated, namely endogenous synthesis rate (K_{SYN}), exogenous absorption rate through the skin following subcutaneous injection (K_a), systemic clearance (C_L), and distribution volume V_D. Parameter level random effects (inter-subject variability) were included on K_{SYN} and C_L. (Table 2) A further level of random effects (inter-occasion variability) also provided significantly improved fit when added to K_{SYN}. Following a limited covariate analysis, the PCDAI score was found to significantly improve fit when added to K_{SYN}, with K_{SYN} falling with increasing disease severity (Figure 3a). The effect of disease severity as measured by PCDAI was tested for C_L and K_{SYN} with significant improvement ($p<0.001$) being shown for K_{SYN}. Furthermore, adding the PCDAI score as a covariate to K_{SYN} allowed inter-occasion variability to be removed without detriment to the model fit (no change in log-likelihood). Adding PCDAI to K_{SYN} also decreased inter-individual variability. In short, disease severity was a major driver of IGF-1 synthesis rate both between patients, and in the same patient on different occasions.

Table 2: Parameter estimates from the model including non-parametric 95%CI from a bootstrap of 714 successful runs. IIV (inter-individual variability).

Parameter	Estimate	Bootstrap median	Bootstrap 2.5th percentile	Bootstrap 97.5th percentile
C_L (L/h/70kg)	1.61	1.60	1.36	1.82
V_D (L/70kg)	2.42	2.41	1.78	3.10
K_{SYN} (µg/h)	433	423	352	490
Ka (h^{-1})	0.10	0.10	0.083	0.12
Coefficient of PCDAI on K_{SYN}	-6.57	-6.41	-8.19	-4.89
IIV on CL (%CV)	10.38	9.46	3.49	15.1
IIV on K_{SYN} (%CV)	24.61	24.10	9.98	33.58
Residual error proportional (%CV)	9.81	9.69	7.85	11.04
Residual error, additive (µg/L)	28.32	25.93	13.29	42.78

There was good agreement between model predictions and observed concentrations (Figure 3b), and trendless distribution of standardised residuals, indicating that the parametric modelling assumptions were satisfied (Figure 3c, d and e). The median of the simulated data was not significantly different to the median of the observed data (Figure 3f). Superimposing model predictions at a population and individual level on the observed data showed that model predictions captured the dynamic behaviour of the system (Figure 4).

Dosing to maintain circulating rhIGF-1 concentrations in the target range

We undertook mathematical modelling to quantify the relationship between endogenous IGF-1 production and exogenous dosing, and to understand how IGF-1 average concentrations (area under the curve/time) can be adjusted. Using the knowledge that in acromegaly there is no increased risk of cancer in patients whose IGF-1 is below +2.5 SDS [13], we defined the optimal IGF-1 concentration range as being between 0 and +2.0~~5~~ SDS. This range would improve growth without elevating cancer risk. Different possible dose scaling systems were then explored to maximise the probability of an individual having IGF-1 levels in the target range. By individualising dose by weight, age (because different aged children have different target circulating IGF-1 levels), and PCDAI score, 92.7~~9~~3~~4~~% of children were predicted to be corrected to less than or equal to +2.0~~5~~ SDS (95.3% below +2.5 SDS~~median +0.5 SDS~~; Figure 5). We can therefore suggest a dosing scheme that limits the risk of malignancy to that of the normal patient population. The dosing schedule recommended from the utility function is:

Age 10 to <12 years, 21 µg/kg plus 1 µg/kg /PCDAI point; Age 12 to <14 years, 41 µg/kg plus 1.4 µg/kg /PCDAI point.~~Age 10 to <12 years, 26 µg/kg plus 1 µg/kg /PCDAI point; Age 12 to <14 years, 48 µg/kg plus 1.4 µg/kg /PCDAI point.~~

DISCUSSION

Impaired growth has been a recognised feature of children with Crohn’s disease for 50 years [29]. In 1986, low IGF-1 was associated with poor growth in these children [30]; and over the following decade it was realized that the depressed concentration was a direct consequence of inflammation [8, 10], and not merely the result of poor energy intake or undernutrition. The best strategy to increase IGF-1 and therefore to improve growth is the resolution of inflammation. Treatments which reverse the inflammation, but do not actively suppress growth, such as surgery [31], anti-TNF [32, 33] and enteral diets [6, 34] have all been shown to improve linear growth.

Nevertheless, there are patients with Crohn’s disease whose inflammation cannot be reversed. In this group, we need a distinct strategy to accelerate growth. Both hGH [35, 36] and rhIGF-1 have been suggested. Because patients with Crohn’s disease have a functional insensitivity to GH, the present study examined the possibility that rhIGF-1 would be efficacious in improving IGF-1 concentrations, as it does in children with growth hormone insensitivity syndrome (GHIS). However, there are significant problems in applying our knowledge of rhIGF-1 usage in GHIS to children with Crohn’s disease. First, children present at a much later age in Crohn’s disease than they do in GHIS, which is caused by a genetic mutation (approximately 8-15 years as opposed to less than 5 years old). The compartments of the IGF-1 system including several IGF binding proteins, cannot be assumed to distribute as they do in younger children, particularly if the relative volumes of these compartments change at the time of puberty. Second, both IGF-1 and the IGF binding proteins are circulating proteins. Proteins are lost into the intestine through protein losing enteropathy (PLE), where inflammation reduces the ability of the intestinal vasculature to maintain them in the capillary lumen [37-39]. The PLE could, in theory, alter the kinetics of IGF-1. Finally, very high ~~AND~~ and sustained circulating IGF-1 concentrations are associated with an increase in colon cancer in middle aged and older patients with acromegaly. A modelling approach to kinetics and dosing was necessary to avert causing high average IGF-1 concentrations.

Several of the children enrolled in the study had delayed puberty (Table 1). Although this is commonly observed in Crohn's disease, the study could be criticized for not taking this into account when calculating IGF-1 SDSs, as circulating IGF-1 increases in puberty. We do not have normative data for circulating IGF-1 against pubertal stage. However, delay in puberty is closely correlated with a delay in bone age, as measured on a wrist X-ray. We therefore recalculated each child's IGF-1 SDS against their bone age before and after the initial injection of rhIGF-1 (data not shown). However, this made little difference to the results obtained when comparing this data to that based on chronological age (Figure 1a).

Previous studies on rhIGF-1 pharmacokinetics [40, 41] have mainly focussed on its use in malnourished adult patients with renal failure, where it has been used to maintain protein balance. These reports undertook purely descriptive analyses such as Area Under the Concentration-Time Curve (AUC) and comparing healthy volunteer parameters with patients, rather than modelling the data. Furthermore, they did not account for endogenous IGF-1 production. If we were to use our data to recommend a dose, a more sophisticated approach was required. In this study, we fitted a structural model that simplified the system without losing biological interpretation of the parameters. Mixed effects modelling allowed for the addition of inter-individual and inter-occasion variability in model parameters, in addition to residual variability. We were, therefore, able simultaneously to fit the model to all patients and investigate covariate-parameter relationships. Instead of exploring dose recommendations by simulation, we aimed to optimise the dose based on the target SDS; and we minimised deviations from this using least-squares regression [42] . The advantage of this approach, over testing competing dosing regimens by simulation, is that once the optimal target is defined, the optimal dose needed to reach that target is determined in a single step. Testing covariates for dose scaling is also simple with this method. Furthermore, by using a large dataset of hypothetical patients with real Crohn's Disease demographics, we were able to show how scaling dose by age, weight and PCDAI score could adequately and safely correct IGF-1 (Figure 5).

The goodness-of-fit plots (Figures 3b-e and Figure 4) show that the model predictions are an unbiased description of the data and simulated data that is not significantly different to the observations (Figure 3f). Furthermore, covariate analysis showed that the disease severity (PCDAI score) significantly reduced endogenous synthesis (K_{syn}) rather than affecting IGF-1 clearance. Variations in IGF-1 synthesis distinguish patients with Crohn's disease from children with GHIS.

A possible criticism of using PCDAI to quantify disease in this context is that it includes growth as a marker of inflammation, and it could be suggested that the dependence of K_{syn} on PCDAI might be due to the height velocity element of the index. Recently, the determinants of the disease activity index have been separately analyzed for predictive value in defined populations of children with Crohn's disease [43]. Linear growth was discovered not to be discriminatory. This report developed a new index, the weighted Pediatric Crohn's Disease Activity Index (wPCDAI), based on these observations, which does not include growth. We repeated our covariate modelling with wPCDAI and showed that adding it to K_{syn} reduced parameter variability and significantly improved model fit, in the same way as the standard PCDAI had. Thus, the relationship between K_{syn} and disease activity was due to objective measures of inflammation, and not to the growth component of the PCDAI calculation.

This report focused on the effects of IGF-1. As mentioned above, hGH may also be of benefit on the linear growth of children with Crohn's disease [36]. It was not the purpose of the present study to examine which therapy would be more efficacious; however, future studies comparing IGF-1 to hGH in a controlled randomized multicentre study are planned. This trial will also give us the opportunity to test the dosing regimen derived from this model presented in the present report on a wide sample of children.

Patients and their support groups have long requested more research into therapies that directly improve growth in children whose inflammation cannot be controlled. The present study shows that twice daily rhIGF-1 can enhance average circulating IGF-1 concentrations into the upper normal range. The use of a utility function from mathematical modelling allows paediatricians to give rhIGF-1 without their entering an unphysiological high range, hence preventing the increased the risk of cancer.

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Competing interests: None

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Contributorship: IRS conceived the study and helped in its design; obtained the funding, ethics, MHRA approval and drafted initial manuscript; JFS developed the mathematical modelling; AVR consented the patients; arranged admission and undertook all the investigation; SK oversaw the clinical care of the patients while admitted for research investigation. MOS helped design the study and provided paediatric endocrinology expertise. All authors contributed to the final manuscript.

Data Sharing: Anomymised original data will be given to research academics at a recognised university departments in line with the policies of Queen Mary, University of London. Requests should be made to Professor Sanderson. However, there are no data in addition to those published, except as the individual numerical results that are displayed as

figures.

Patient identifiable data cannot be shared, as this is a condition of the research ethics committee approval

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Legends to figures:

Figure 1 Subcutaneous rhIGF-1 increases the circulating concentrations of IGF-1 in children with growth failure induced by Crohn’s disease. (a) IGF-1 standard deviation scores (SDS) below the normal range in both first [Mean -1.78 (SD 1.37)] and second admissions [Mean -2.34 (SD 0.75)] are significantly increased a single injection of rhIGF1 ($p<0.0005$ and <0.0001, respectively). (b) An injection reaches a peak within 4 hours and returns to low levels within 24 hours. (c) Twice daily injections of rhIGF-1 increase circulating IGF-1 over a sustained period. rhIGF-1 was given on day 1 and the circulating concentrations allowed to fall, before giving twice daily injections on days 4 and 5.

Figure 2 Protein losing enteropathy did not alter IGF-1 or IGFBP-3. (a) Variations in protein-losing enteropathy (as measured by faecal alpha 1 anti-trypsin) did not correlate with changes in IGF-1 concentrations achieved on giving rhIGF-1 ($p=0.703$). (b) Variations in protein-losing enteropathy did not correlate with IGFBP-3 concentrations

Figure 3. IGF-1 in a mathematical model. (a) Increasing disease activity (PCDAI) significantly ($p<0.001$) diminishes the estimates of K_{syn} in the covariate model building. (b) Population model predictions versus observed concentrations are unbiased, indicating good structural model fit. (c) Individual model predicted concentrations are in agreement with observed concentrations. (d) Conditional weighted residuals (CWRES), which are a form of standardised residuals expected to follow the Normal Independent Distribution (NID; 0,1) lie within -2 and +2 standard deviations and do not change with model predictions, indicating good structural model fit. (e) Similarly the QQ plot of CWRES indicate that the assumption of normality of residuals is met; (f) Median observed IGF-1 concentrations (solid line) similar to median simulated (dashed line) and observed median lies within 95% confidence interval of the model simulations (grey shaded area).

Figure 4. Population level (blue line) and individual (red line) model predictions are similar to observed data (black open circles) in both (a) single dose and (b) repeated doses in each part of the study.

Figure 5. Incorporation of a disease activity index into dose calculations allows an accurate prediction of circulating IGF-1. ~~Utility function results showing the effect of increasing sophistication on dose scaling method. Scaling by weight, age group and PCDAI score limits average IGF-1 concentrations in 94% of children to less than +2.5 SDS.~~ Utility function results showing the effect of increasing sophistication on dose scaling method. Scaling by weight, age group and PCDAI score limits average IGF-1 concentrations in 93% of children to less than +2 SDS. Solid lines are the target range of 0 to +2 SDS, dashed lines are for reference -2SDS and +2.5 SDS.

Figure 1a

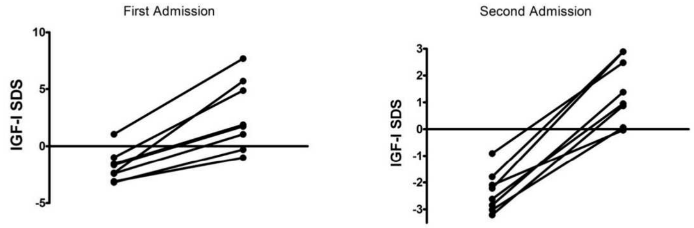

Figure 1b

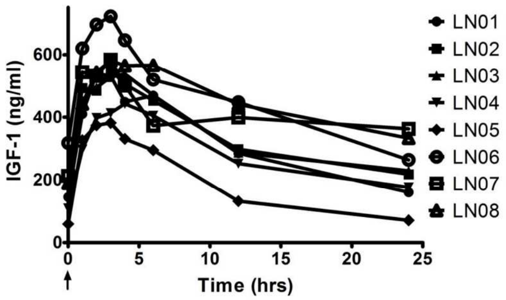

Figure 1c

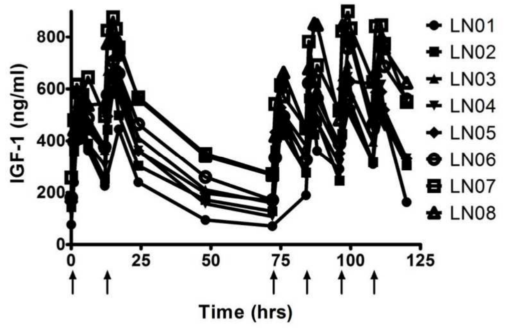

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Figure 2a

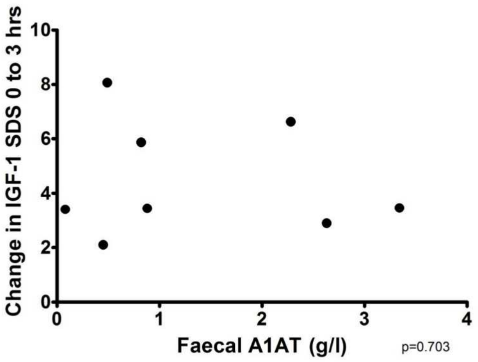
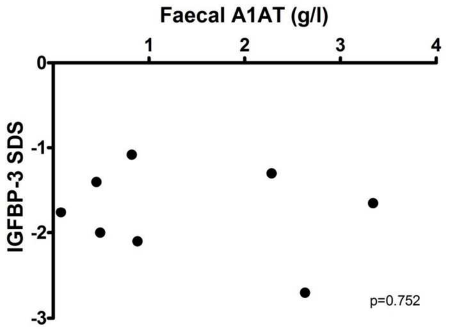


Figure 2b



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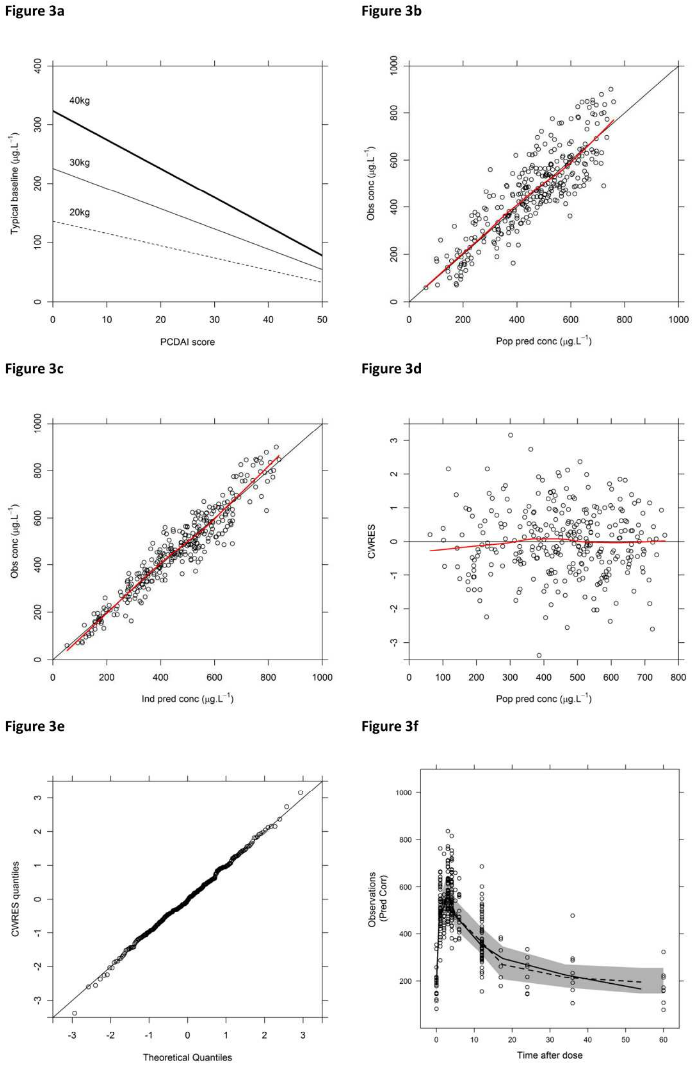

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Figure 4a

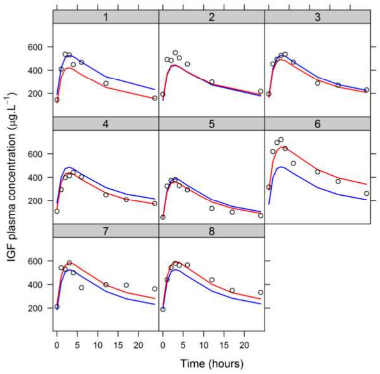
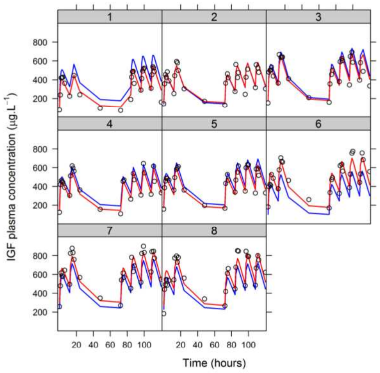


Figure 4b



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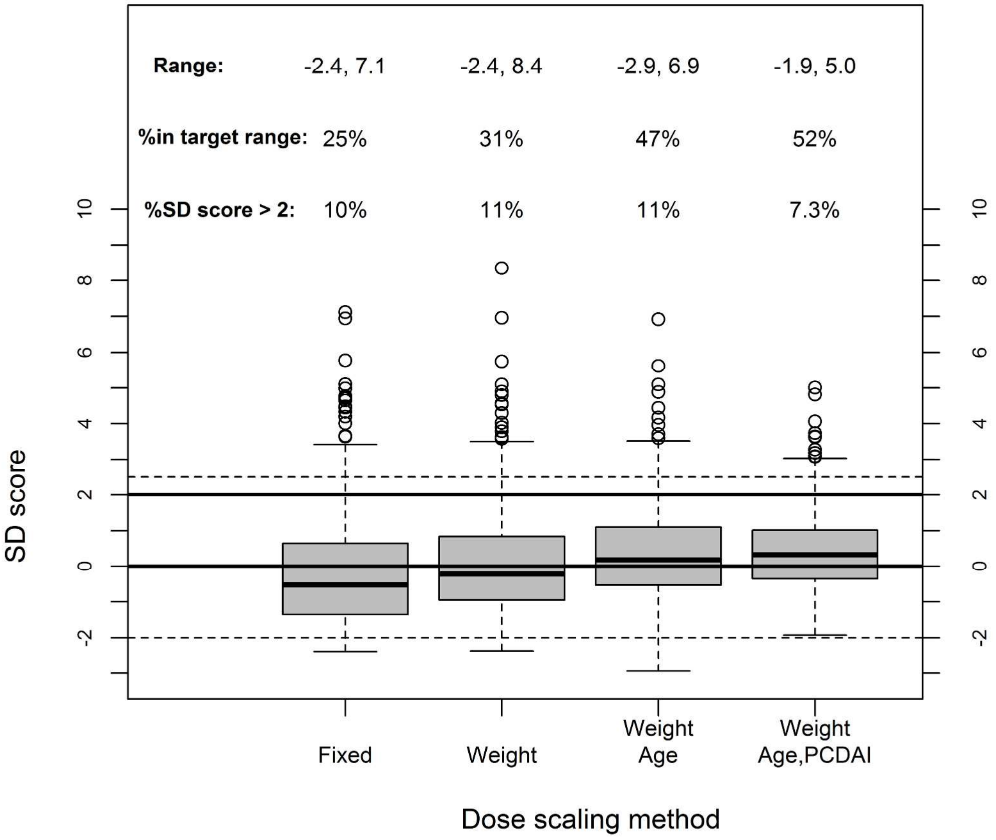

Incorporation of a disease activity index into dose calculations allows an accurate prediction of circulating IGF-1. Utility function results showing the effect of increasing sophistication on dose scaling method. Scaling by weight, age group and PCDAI score limits average IGF-1 concentrations in 93% of children to less than +2 SDS. Solid lines are the target range of 0 to +2 SDS, dashed lines are for reference -2SDS and +2.5 SDS.
152x152mm (600 x 600 DPI)

Research Checklist

Pharmacokinetic studies of recombinant human insulin-like growth factor I (rhIGF-I) in children with Crohn's disease- induced growth retardation

- **Approved by the East London Research Ethics Committee (ELREC):**

  ELREC Reference: 07/H0705/77

- **Approved by Medicine and Healthcare products Regulatory Agency (MHRA)**

MHRA reference:	21313/0012/001-0001
Eudract Number	2007-004269-16
Product:	Increlex 10mg/ml solution for injection
Protocol number:	IGFI-1

- **R&D approved and Sponsored by Queen Mary, University of London**

  ReDA reference: 005251

- **Adopted by Medicine for Children Research Network (MCRN)**

- **Registered with the UK Clinical Research Network (UKCRN):**

  ID 4293

Mathematical modelling to restore circulating IGF-1 concentrations in children with Crohn's disease-induced growth failure: a pharmacokinetic study

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1. Mathematical modelling to restore circulating IGF-1 concentrations in children with Crohn's disease-induced growth failure: a pharmacokinetic study

Running head: Restoring IGF-1 in children with Crohn's disease

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4. Key words: insulin-like growth factor-1; adolescent; inflammatory bowel disease; pharmacokinetics; height

5. Word count 2584

ARTICLE SUMMARY

Article focus

One third of children with Crohn's disease have growth retardation.

Children with active inflammation have an insensitivity to growth hormone, resulting in low circulating IGF-1.

There is no agreed growth therapy for children whose inflammation is intractable to treatment, because very high and sustained IGF-1 concentrations are a risk factor for colon cancer in adults with acromegaly.

Key messages

IGF-1 can be restored to the normal range by subcutaneous injections.

The endogenous synthesis of IGF-1 depends on Crohn's disease activity; and variations in both determine the circulating IGF-1 concentrations.

A new mathematical model which incorporates disease activity (in addition to weight and age) will allow paediatric gastroenterologists to calculate doses that keep the circulating IGF-1 in the upper normal range.

Strengths and Limitations

Using the mathematical model, IGF-1 can be prescribed in doses that do not increase the risk of cancer.

For the first time, long term IGF-1 treatment can be studied in children to determine if it enhances growth, and this is a critical step in offering a therapy (much demanded by patients) for growth retardation in children with Crohn's disease.

A limitation of the study is that long term studies over several years will still be required on a large group of children.

ABSTRACT

Objectives: Children with Crohn's disease grow poorly, and inflammation depresses the response of IGF-1 to growth hormone. Correcting the inflammation normalizes growth velocity; however, removing inflammation cannot be achieved in all children. Our lack of understanding of IGF-1 kinetics has hampered its use, particularly as high IGF-1 concentrations over long periods may predispose to colon cancer. We hypothesized that mathematical modelling of IGF-1 would define dosing regimes that return IGF-1 concentrations into the normal range, without reaching values that risk cancer.

Design: Pharmacokinetic intervention study

Setting: Tertiary paediatric gastroenterology unit

Participants: 8 children (M:F; 4:4) entered the study. All completed: 5 South Asian British; 2 White British; 1 African British. Inclusion criteria: Children over 10 years with active Crohn's disease (CRP >10mg/l or ESR >25mm/h) and height velocity <-2 SD score. Exclusion criteria: closed epiphyses; corticosteroids within 3 months; neoplasia or known hypersensitivity to rhIGF-1.

Interventions: Subcutaneous recombinant human IGF-1 (rhIGF-1) (120μg/kg) per dose over two admissions: the first as a single dose, and the second as twice daily doses over 5 days.

Primary outcome: Significant increase in circulating IGF-1

Secondary outcomes: Incidence of side effects of IGF-1. A mathematical model of circulating IGF-1 (A_c) was developed to include parameters of: endogenous synthesis (K_{syn}); exogenous uptake (K_a) from the subcutaneous dose (A_s): and IGF-1 clearance: where $dA_c/dt= K_{syn}- K_{out}\cdot A_c + K_a\cdot A_s$.

Results: Subcutaneous IGF-1 increased concentrations, which were maintained on twice daily doses. In covariate analysis, disease activity reduced K_{syn} (p<0.001). Optimal dosing was derived from least

squares regression fitted to a dataset of 384 Crohn’s patients, with model parameters assigned by simulation.

Conclusions: By using age, weight and disease activity scaling in IGF-1 dosing, over 95.% of children will have normalized IGF-1 concentrations below +2.5 standard deviations of the normal population mean, a level not associated with cancer risk.

Abstract word count: 299

INTRODUCTION

A quarter of cases of Crohn's disease now present in children and adolescents under 18 years [1, 2]; and its incidence in childhood is increasing [3]. Around a third of children experience linear growth retardation, caused in part by undernutrition and in part by the direct effects of inflammation on growth [4].

Growth in childhood is regulated by growth hormone (GH), secreted from the pituitary, which stimulates the production of insulin-like growth factor-1 (IGF-1) by the liver and growth plates of bones [5]. Children with active Crohn's disease have low circulating IGF-1 and increased circulating cytokines [6, 7]. Treatment of the inflammation, for example by an enteral diet, results in a reduction in cytokines like IL-6 and an increase in IGF-1 within 3 days [7]. Thus, the optimum treatment for improving growth is to eliminate the inflammation. Nevertheless, some children's inflammation remains intractable to treatment despite the best efforts of clinicians. For this group, there is currently no agreed therapy to enhance growth.

Rats with TNBS-induced colonic inflammation grow poorly [8]; and this is also associated with increased cytokines IL-6 and TNF, and a low circulating IGF-1 [9]. Exogenous IGF-1 given to the rats with colitis enhanced growth [8]. In transgenic mice, without inflammation, high levels of circulating IL-6, generated by a widely acting promoter, depressed both IGF-1 and growth [10]. Thus the link between inflammatory cytokines, IGF-1 and poor growth is strong [11]. There is a functional insensitivity to GH in children with Crohn's disease. Recombinant IGF-1 is now recognized therapy for children with GH insensitivity syndrome (GHIS) due to genetic defects of the GH receptor or IGF-1 gene [12]; we, therefore, hypothesized that IGF-1 concentrations in children with active Crohn's disease and poor linear growth could be restored by administering rhIGF-1.

Restoring IGF-1 in children with Crohn's disease who may have some endogenous production is not straightforward, because high levels of IGF-1 may be harmful. For rhIGF-1 to be therapeutically useful, its circulating levels over the long term should be returned to normal values by replacement, and not given in excess. Patients with acromegaly who have very high concentrations of GH and IGF-1, maintained over decades, have a doubling in the incidence of colon cancer [13]. This could, in theory, be compounded in children with Crohn's disease, as inflammation is also a risk factor for intestinal cancer.

A mathematical model for administration of rhIGF-1, based on detailed pharmacokinetics would resolve these difficulties, as dosing could be tailored to achieve circulating concentrations that remain within the normal range. We therefore undertook a careful study of subcutaneous rhIGF-1 administration in children with Crohn's disease-induced growth retardation. We developed a model which used physiological parameterisation, disease activity to predict endogenous IGF-1 synthesis, and body weight to scale the volume of distribution. Parameters of protein loss, such as protein-losing enteropathy, were also considered. The model was used to recommend dosing which allows children to be treated with rhIGF-1 without its concentration rising above the normal range.

METHODS

Patient Selection

Following ethical approval from the East London & City Research Ethics Committee (ELREC; reference number 07/H0705/77) and regulatory approval from the UK Medicines and Healthcare products Regulatory Agency (MHRA) (Eudract number: 2007-004269-16), written informed consent was obtained from the parents and patients attending the paediatric inflammatory bowel disease clinic at Barts and The London Hospital for Children. Inclusion criteria were: age ≥ 10 years; known Crohn's disease diagnosed by endoscopic, histological and radiological methods [14]; a height

velocity measured over at least a 6 month period of <-2 Standard Deviation Score (SDS) according to the patient's age and gender; and evidence of active inflammation as demonstrated by either an ESR > 25mm/hr and/or a CRP > 10mg/l. Exclusion criteria were: corticosteroid use in the preceding 3 months; active or suspected neoplasia; known hypersensitivity to exogenous rhIGF-1 (Increlex, Ipsen UK); and the presence of closed epiphyses.

All patients had a full history and physical examination, including an accurate recording of height, weight and assessment of pubertal status. In addition, they also had an electrocardiogram and faecal alpha-1-antitrypsin levels (g/l) measured.

Study Design

Exogenous rhIGF-1 was administered by subcutaneous injection at doses of 120µg/kg over two admissions. Admission 1 investigated the effects of a single dose of rhIGF-1 over a 24 hour inpatient stay. Baseline blood screens included full blood count (FBC), electrolytes, inflammatory markers (CRP and ESR), baseline IGF-1, IGFBP-3, acid labile subunit (ALS) and blood sugar. The Pediatric Crohn's Disease Activity Index (PCDAI) was calculated [15, 16]

Each patient was then given a single subcutaneous injection of rhIGF-1 at a dose of 120µg/kg. Serial venous blood samples were drawn at the following time points: 0, 1, 2, 3, 4, 6, 12, 17 and 24 hours. Blood sugars and vital signs were checked regularly. Children ate and drank freely, and continued to receive their prescribed, non-corticosteroid, treatment for Crohn's disease.

Following a wash out of at least 3 months, subjects were readmitted for admission 2, investigating the effects of repeated doses of rhIGF-1. On this occasion 6 doses of rhIGF-1 were administered over a 5 day trial period, with doses given at: 0, 12, 72, 84, 96 and 108 hours. The injection sites were rotated according to the patient's wishes. Serial blood samples for further IGF-1 , IGFBP-3, acid

labile subunit (ALS) and blood sugar were collected during the 5 days at 0, 1, 2, 3, 4, 6, 12, 17, 24, 48, 72, 96 and 120 hours. Additional samples were also taken for clinical reasons when there was a possibility of hypoglycaemia. If sufficient sample was collected, IGF-1 levels were also measured. In addition, vital signs, FBC, electrolytes, and inflammatory markers were measured daily. Patients were kept as inpatients during the days that the rhIGF-1 was administered, but were allowed home during the two days in which they received no injections. Patients were discharged home on day 6.

Assays and samples

All samples were stored at -20°C until analysis. Plasma glucose was determined immediately after blood sampling (Beckman Instruments, Palo Alto, CA).

IGF-1, IGFBP-3.

IGF-1 and IGFBP-3 levels were measured by competitive binding radioimmunoassay (Esoterix Inc Laboratory Services). The sensitivity of the assay for IGF-1 was 15ng/ml and the intra-assay coefficient of variation averaged 14.1%. The sensitivity of the assay for IGFBP-3 was 0.3mg/L and the intra-assay coefficient of variation averaged 13%.

ALS

ALS levels were measured by radioimmunoassay (ALS-RIA) using purified human ALS as tracer. The intra-assay coefficient of variation ranged from 8.0-17.4%

Mathematical model development

Measured IGF-1 concentrations were fitted for all individuals simultaneously using the non-linear mixed effects modelling software NONMEM version 7.1[17]. Model building was undertaken using the first-order conditional estimation method with interaction (FOCEI). A turnover model was used according to the following differential equations:

$$\frac{dA_c}{dt} = K_{syn} - K_{out} \cdot A_c + K_a \cdot A_s$$ Equation 1

$$\frac{dA_s}{dt} = - K_a \cdot A_s$$ Equation 2

Where: A_c is the amount of circulating IGF-1 at time t; A_s is the amount in the subcutaneous tissue following a dose, with initial conditions adjusted each time a dose is administered; K_{syn} is a zero order production rate of endogenous IGF-1 in µg.hr^{-1}; K_{out} is a first order elimination rate constant in hr^{-1}; and K_a is a first order absorption rate constant describing exogenous IGF-1 appearance following a subcutaneous dose in hr^{-1}. At steady state prior to exogenous dose, circulating IGF-1 is given by the ratio of K_{syn} to K_{out}.

In addition to K_{syn} and K_a, the fixed effect parameters IGF-1 clearance (C_L) in L.hr^{-1} and distribution volume (V_D) in L were estimated, K_{out} being given by the ratio of C_L to V_D. Between subject variability was tested for all parameters, and residual variability was described using a heteroscedastic model. Allometric weight scaling was applied to C_L and V_D *a priori* using linear scaling for V_D and weight raised to the power 0.75 for C_L[18] , K_{syn} was further scaled with linear weight. Tested covariates were CRP, ESR, PCDAI score and age.

Model development used the NONMEM objective function value (OFV); parameter estimate precision was derived through a non-parametric bootstrap [19] and graphical assessment of basic goodness-of-fit plots and model simulations was undertaken. The OFV is proportional to -2 times the log-likelihood of the data given the parameter estimates, and a decrease in OFV of 3.84 with one degree of freedom gives a significantly improved fit with a probability $p < 0.05$. A utility function

based on the final model was used to predict average concentration (C_{ave}) with a dosing regimen derived from the integral of the IGF-1 against the time curve divided by time. Maximum utility was defined as C_{ave} of +0.50 SDS with linear penalty for deviations above and below this target. Dose was optimisied to give a model-derived steady-state C_{ave}, by the following model:

$$Target = SDS_i + \varepsilon_i$$ Equation 3

$$Utility = \sum_{i=1}^{n} (Target - SDS_i)^2$$ Equation 4

Where Target is the SDS aim (in this case +0.50); SDS_i is the individual predicted SDS derived from model predicted C_{AVE}; ε_i denotes individual deviations from the target. Utility minimised ε_i by least squares regression.

Demographic details for 384 subjects aged 8 to 14 years from the clinic database were each assigned a random PCDAI score (from a uniform distribution 0-48) and also individual model parameters. The utility function was maximised with either fixed dosing, dosing based on weight, weight and age, or weight, age and PCDAI score. Differences in concentrations were explored with 12 and 24 hourly dosing regimens.

Calculation of Sample Size

The size was derived from the number of children needed to show a significant increase in IGF-1, based on the increases in IGF-1 seen in a cohort of children with treated with IGF-1 for Growth Hormone Insensitivity Syndrome (GHIS). This showed that in 18 children, the concentration before drug was 330 ng/ml (SEM 20 ng/ml); and the concentration after 4 hours was 535 ng/ml (SEM 20 ng/ml). Therefore, to detect a difference in concentration of 205 (535-330) before drug and after 4

hrs, and SD of 84.85 (calculated from Standard Error of the Mean of 20 with sample size of 18), with 80% power at the 5% level of significance four patients are required.

RESULTS

rhIGF-1 therapy in children with Crohn's disease

Children with active Crohn's disease, and whose growth was inhibited were recruited into the study (Table 1). Impairment of height velocity is characteristic of many children with active Crohn's disease [4, 20-22], and all 8 subjects recruited into the study (median age 12.97) had a height

Table 1: Patient characteristics at recruitment into the trial. Tanner stage: P: pubic hair, B: breast stage, G: genitalia . Medications: 5 –ASA: 5-aminosalicylic acid, AZA: azathioprine, IFX: infliximab, Adalum = adalumimab. Montreal classification: L1: ileal, L2: colonic, L3: ileocolonic, B1: non-stricturing, non-penetrating, B2: stricturing, B3: penetrating

	Patients							
	LN01	LN02	LN03	LN04	LN05	LN06	LN07	LN08
Gender	F	F	M	M	F	F	M	M
Age	13.11	11.5	14.23	10.67	14.82	12.7	12.82	14.66
Ethnicity	Caucasian	Asian	Asian	Asian	Asian	Asian	African	Caucasian
Tanner Stage	P1 B1	P1 B1	P3 G3	P1 G1	P2 B2	P2 B2	P2 G2	P1 G1
HV SDS at recruitment	-2.11	-2.11	-4.18	-2.83	-2.27	-3.87	-4.43	-4.88
HV SDS at time of trial	-2.11	-2.14	-1.84	0.36	1.69	3.55	-4.43	-4.88
PCDAI	12.5	20	17.5	10	47.5	15	12.5	10
Medication	5-ASA	5-ASA	5-ASA AZA IFX	5-ASA AZA Adalum	5-ASA AZA Adalum	5-ASA AZA IFX	5-ASA AZA	5-ASA
Faecal A1AT (g/l)	3.34	0.82	0.88	0.49	2.63	2.28	0.08	0.45
Montreal Classification								
Disease Location	L1	L2	L3	L3	L1	L3	L1	L1
Behaviour	B1	B1	B3	B3	B2	B1	B2	B1
Upper	N	N	Y	N	Y	Y	N	N
Perianal	N	N	Y	Y	N	N	N	N

velocity that was more than two standard deviations below the mean (i.e. a standard deviation score [SDS] of <-2), as an entry criterion. The median height velocity SDS was -3.35, indicating extreme growth failure.

All the subjects completed both parts of the study. The rhIGF-1 was generally well tolerated. Although subcutaneous rhIGF-1 has been associated with hypoglycaemia in children treated for GHIS [12], only one patient (LN01) had a single asymptomatic hypoglycaemic episode (blood sugar < 3.5mmol/l). This was corrected with oral glucose. This occurred following the 12 hour dosage of rhIGF-1 during admission 2 in a child who had not eaten prior to the drug administration. No further episodes of hypoglycaemia occurred. There were no other adverse effects.

Determinants of circulating IGF-1

As previously demonstrated, children with Crohn’s disease-induced growth failure had depressed circulating IGF-1 concentrations. The severity of disease in any one child varies over time, and this was reflected in the variation of IGF-1 between the first and second admissions, 3 or more months apart. (Figure 1a). Despite the variation both between and within patients, a single subcutaneous bolus of rhIGF-1 significantly increased IGF-1 in the circulation (Figure 1). In some cases where the baseline IGF-1 was less depressed, the post IGF-1 level was increased to above normal (Figure 1a). The concentrations of IGF-1 returned to baseline over the following 24 hours (Figure 1b).

IGF-1 is maintained in the circulation by IGF binding proteins, the most abundant being IGFBP-3 [23]. A major characteristic of Crohn’s disease is protein-losing enteropathy, resulting in proteins being lost into the lumen of the intestine [24, 25]. We aimed therefore to ascertain if the degree of protein-losing enteropathy blunted the ability of subcutaneous rhIGF-1 to achieve significant peak concentrations, or if it directly affected IGF binding proteins. Protein losing enteropathy was quantified by the concentrations of alpha-1- antitrypsin in stool [26]. Using this measure, protein

losing enteropathy had no effect on the change in IGF-1 induced by a subcutaneous injection (Figure 2a)

IGFBP-3 concentrations are depressed in children with chronic IGF-1 deficiency [27], and all children in the study had low IGFBP-3 (Figure 2b). We wished to determine, however, if IGFBP-3 concentrations depended on the degree of protein losing enteropathy, as this would indicate that protein losing enteropathy may affect IGF-1 pharmacokinetics. However, no association was observed.

In the second admission, the subjects received twice daily doses, to determine if IGF-1 concentrations would be enhanced over a longer period. The initial injection increased the IGF-1 concentrations into the normal range (Figure 1a). Increases over a sustained period were successfully achieved (Figure 1c), and as expected multiple daily dosing led to lower variability between peak and trough concentrations. Taking the area under the curve divided by time as the average IGF-1 concentration, it became clear that on 120µg/kg, some children had an average IGF-1 SDS of greater than +2.5, while other children had appropriate concentrations. Increased circulating IGF-1 in acromegaly has been linked with an increased risk of colon cancer [13, 28]. Fortunately, we have a clear understanding of how IGF-1 concentrations relate to colon cancer risk in acromegaly [13]. These studies show that the increased cancer risk was seen in individuals whose circulating IGF-1 was +2.5 SDS or greater. Using our data, we developed a model from which the dose-concentration relationship could be derived. We then investigated the covariates on which dosing could be individualised. This allowed us to correct circulating IGF-1 without causing excessively high concentrations: ensuring that our dosing regimens resulted in concentrations that were below +2.5 SDS, we developed a utility model to predict the IGF-1 SDS achieved on regular dosing.

Mathematical modelling of IGF-1 links endogenous production to disease severity

A turnover model which accounted for both endogenous production and exogenous dosing of IGF-1 was fitted to the data (see methods for mathematical formulae) using non-linear mixed effects analysis. Four fixed effects were estimated, namely endogenous synthesis rate (K_{SYN}), exogenous absorption rate through the skin following subcutaneous injection (K_a), systemic clearance (C_L), and distribution volume V_D. Parameter level random effects (inter-subject variability) were included on K_{SYN} and C_L. (Table 2) A further level of random effects (inter-occasion variability) also provided significantly improved fit when added to K_{SYN}. Following a limited covariate analysis, the PCDAI score was found to significantly improve fit when added to K_{SYN}, with K_{SYN} falling with increasing disease severity (Figure 3a). The effect of disease severity as measured by PCDAI was tested for C_L and K_{SYN} with significant improvement ($p<0.001$) being shown for K_{SYN}. Furthermore, adding the PCDAI score as a covariate to K_{SYN} allowed inter-occasion variability to be removed without detriment to the model fit (no change in log-likelihood). Adding PCDAI to K_{SYN} also decreased inter-individual variability. In short, disease severity was a major driver of IGF-1 synthesis rate both between patients, and in the same patient on different occasions.

Table 2: Parameter estimates from the model including non-parametric 95%CI from a bootstrap of 714 successful runs. IIV (inter-individual variability).

Parameter	Estimate	Bootstrap median	Bootstrap 2.5th percentile	Bootstrap 97.5th percentile
C_L (L/h/70kg)	1.61	1.60	1.36	1.82
V_D (L/70kg)	2.42	2.41	1.78	3.10
K_{SYN} (µg/h)	433	423	352	490
Ka (h^{-1})	0.10	0.10	0.083	0.12
Coefficient of PCDAI on K_{SYN}	-6.57	-6.41	-8.19	-4.89
IIV on CL (%CV)	10.38	9.46	3.49	15.1
IIV on K_{SYN} (%CV)	24.61	24.10	9.98	33.58
Residual error proportional (%CV)	9.81	9.69	7.85	11.04
Residual error, additive (µg/L)	28.32	25.93	13.29	42.78

There was good agreement between model predictions and observed concentrations (Figure 3b), and trendless distribution of standardised residuals, indicating that the parametric modelling assumptions were satisfied (Figure 3c, d and e). The median of the simulated data was not significantly different to the median of the observed data (Figure 3f). Superimposing model predictions at a population and individual level on the observed data showed that model predictions captured the dynamic behaviour of the system (Figure 4).

Dosing to maintain circulating rhIGF-1 concentrations in the target range

We undertook mathematical modelling to quantify the relationship between endogenous IGF-1 production and exogenous dosing, and to understand how IGF-1 average concentrations (area under the curve/time) can be adjusted. Using the knowledge that in acromegaly there is no increased risk of cancer in patients whose IGF-1 is below +2.5 SDS [13], we defined the optimal IGF-1 concentration range as being between 0 and +2.0 SDS. This range would improve growth without elevating cancer risk. Different possible dose scaling systems were then explored to maximise the probability of an individual having IGF-1 levels in the target range. By individualising dose by weight, age (because different aged children have different target circulating IGF-1 levels), and PCDAI score, 92.7% of children were predicted to be corrected to less than or equal to +2.0 SDS (95.3% below +2.5 SDS; Figure 5). We can therefore suggest a dosing scheme that limits the risk of malignancy to that of the normal patient population. The dosing schedule recommended from the utility function is:

Age 10 to <12 years, 21 µg/kg plus 1 µg/kg /PCDAI point; Age 12 to <14 years, 41 µg/kg plus 1.4 µg/kg /PCDAI point.

DISCUSSION

Impaired growth has been a recognised feature of children with Crohn’s disease for 50 years [29]. In 1986, low IGF-1 was associated with poor growth in these children [30]; and over the following decade it was realized that the depressed concentration was a direct consequence of inflammation [8, 10], and not merely the result of poor energy intake or undernutrition. The best strategy to increase IGF-1 and therefore to improve growth is the resolution of inflammation. Treatments which reverse the inflammation, but do not actively suppress growth, such as surgery [31], anti-TNF [32, 33] and enteral diets [6, 34] have all been shown to improve linear growth.

Nevertheless, there are patients with Crohn’s disease whose inflammation cannot be reversed. In this group, we need a distinct strategy to accelerate growth. Both hGH [35, 36] and rhIGF-1 have been suggested. Because patients with Crohn’s disease have a functional insensitivity to GH, the present study examined the possibility that rhIGF-1 would be efficacious in improving IGF-1 concentrations, as it does in children with growth hormone insensitivity syndrome (GHIS). However, there are significant problems in applying our knowledge of rhIGF-1 usage in GHIS to children with Crohn’s disease. First, children present at a much later age in Crohn’s disease than they do in GHIS, which is caused by a genetic mutation (approximately 8-15 years as opposed to less than 5 years old). The compartments of the IGF-1 system including several IGF binding proteins, cannot be assumed to distribute as they do in younger children, particularly if the relative volumes of these compartments change at the time of puberty. Second, both IGF-1 and the IGF binding proteins are circulating proteins. Proteins are lost into the intestine through protein losing enteropathy (PLE), where inflammation reduces the ability of the intestinal vasculature to maintain them in the capillary lumen [37-39]. The PLE could, in theory, alter the kinetics of IGF-1. Finally, very high and sustained circulating IGF-1 concentrations are associated with an increase in colon cancer in middle aged and older patients with acromegaly. A modelling approach to kinetics and dosing was necessary to avert causing high average IGF-1 concentrations.

The reduction in IGFBP-3 raises the question as to whether giving rhIGF-1 to children with a reduced IGFBP-3 may increase free IGF-1 concentrations. Since we did not have access to an assay for free IGF-1, our data are not totally informative on this issue. However, they will not have risen to unsafe levels from saturating the IGF-1 binding capacity. The concentrations of IGFBP-3 are not greatly depressed in the affected children. Even in the most severe case, the IGFBP-3 is within two standard deviations of normal. In addition, IGF-1 is over 95% bound to IGF binding proteins, with an excess of binding capacity. In general when analysing pharmacokinetic data, changes in protein binding do not affect free concentrations but may affect free fraction. This means that for two individuals with the same total concentration, the free concentration maybe elevated in the one with lower binding protein. However, two individuals with different IGFBP-3 concentrations given rhIGF-1 will not achieve the same total concentrations, because as IGF-1 undergoes first-order elimination, higher free concentrations will be more rapidly eliminated due to homeostasis. IGFBP-3 was not a significant covariate for volume of distribution in our model, on analyzing our small dataset. Indeed, our model predicts the majority of patients will have total concentrations just above the normal range.

Several of the children enrolled in the study had delayed puberty (Table 1). Although this is commonly observed in Crohn's disease, the study could be criticized for not taking this into account when calculating IGF-1 SDSs, as circulating IGF-1 increases in puberty. We do not have normative data for circulating IGF-1 against pubertal stage. However, delay in puberty is closely correlated with a delay in bone age, as measured on a wrist X-ray. We therefore recalculated each child's IGF-1 SDS against their bone age before and after the initial injection of rhIGF-1 (data not shown). However, this made little difference to the results obtained when comparing this data to that based on chronological age (Figure 1a).

Previous studies on rhIGF-1 pharmacokinetics [40, 41] have mainly focussed on its use in malnourished adult patients with renal failure, where it has been used to maintain protein balance.

These reports undertook purely descriptive analyses such as Area Under the Concentration-Time Curve (AUC) and comparing healthy volunteer parameters with patients, rather than modelling the data. Furthermore, they did not account for endogenous IGF-1 production. If we were to use our data to recommend a dose, a more sophisticated approach was required. In this study, we fitted a structural model that simplified the system without losing biological interpretation of the parameters. Mixed effects modelling allowed for the addition of inter-individual and inter-occasion variability in model parameters, in addition to residual variability. We were, therefore, able simultaneously to fit the model to all patients and investigate covariate-parameter relationships. Instead of exploring dose recommendations by simulation, we aimed to optimise the dose based on the target SDS; and we minimised deviations from this using least-squares regression [42] . The advantage of this approach, over testing competing dosing regimens by simulation, is that once the optimal target is defined, the optimal dose needed to reach that target is determined in a single step. Testing covariates for dose scaling is also simple with this method. Furthermore, by using a large dataset of hypothetical patients with real Crohn's Disease demographics, we were able to show how scaling dose by age, weight and PCDAI score could adequately and safely correct IGF-1 (Figure 5).

The goodness-of-fit plots (Figures 3b-e and Figure 4) show that the model predictions are an unbiased description of the data and simulated data that is not significantly different to the observations (Figure 3f). Furthermore, covariate analysis showed that the disease severity (PCDAI score) significantly reduced endogenous synthesis (K_{syn}) rather than affecting IGF-1 clearance. Variations in IGF-1 synthesis distinguish patients with Crohn's disease from children with GHIS.

A possible criticism of using PCDAI to quantify disease in this context is that it includes growth as a marker of inflammation, and it could be suggested that the dependence of K_{syn} on PCDAI might be due to the height velocity element of the index. Recently, the determinants of the disease activity index have been separately analyzed for predictive value in defined populations of children with Crohn's disease [43]. Linear growth was discovered not to be discriminatory. This report developed a new index, the weighted Pediatric Crohn's Disease Activity Index (wPCDAI), based on these observations, which does not include growth. We repeated our covariate modelling with wPCDAI and showed that adding it to K_{syn} reduced parameter variability and significantly improved model fit, in the same way as the standard PCDAI had. Thus, the relationship between K_{syn} and disease activity was due to objective measures of inflammation, and not to the growth component of the PCDAI calculation.

This report focused on the effects of IGF-1. As mentioned above, hGH may also be of benefit on the linear growth of children with Crohn's disease [36]. It was not the purpose of the present study to examine which therapy would be more efficacious; however, future studies comparing IGF-1 to hGH in a controlled randomized multicentre study are planned. This trial will also give us the opportunity to test the dosing regimen derived from this model presented in the present report on a wide sample of children.

Patients and their support groups have long requested more research into therapies that directly improve growth in children whose inflammation cannot be controlled. The present study shows that twice daily rhIGF-1 can enhance average circulating IGF-1 concentrations into the upper normal range. The use of a utility function from mathematical modelling allows paediatricians to give rhIGF-1 without their entering an unphysiological high range, hence preventing the increased the risk of cancer.

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Competing interests: None

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Contributorship: IRS conceived the study and helped in its design; obtained the funding, ethics, MHRA approval and drafted initial manuscript; JFS developed the mathematical modelling; AVR consented the patients; arranged admission and undertook all the investigation; SK oversaw the clinical care of the patients while admitted for research investigation. MOS helped design the study and provided paediatric endocrinology expertise. All authors contributed to the final manuscript.

Data Sharing: Anomymised original data will be given to research academics at a recognised university departments in line with the policies of Queen Mary, University of London. Requests should be made to Professor Sanderson. However, there are no data in addition to those published, except as the individual numerical results that are displayed as

figures.

Patient identifiable data cannot be shared, as this is a condition of the research ethics committee approval

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Legends to figures:

Figure 1 Subcutaneous rhIGF-1 increases the circulating concentrations of IGF-1 in children with growth failure induced by Crohn's disease. (a) IGF-1 standard deviation scores (SDS) below the normal range in both first [Mean -1.78 (SD 1.37)] and second admissions [Mean -2.34 (SD 0.75)] are significantly increased a single injection of rhIGF1 ($p<0.0005$ and <0.0001, respectively). (b) An injection reaches a peak within 4 hours and returns to low levels within 24 hours. (c) Twice daily injections of rhIGF-1 increase circulating IGF-1 over a sustained period. rhIGF-1 was given on day 1 and the circulating concentrations allowed to fall, before giving twice daily injections on days 4 and 5.

Figure 2 Protein losing enteropathy did not alter IGF-1 or IGFBP-3. (a) Variations in protein-losing enteropathy (as measured by faecal alpha 1 anti-trypsin) did not correlate with changes in IGF-1 concentrations achieved on giving rhIGF-1 ($p=0.703$). (b) Variations in protein-losing enteropathy did not correlate with IGFBP-3 concentrations

Figure 3. IGF-1 in a mathematical model. (a) Increasing disease activity (PCDAI) significantly ($p<0.001$) diminishes the estimates of K_{syn} in the covariate model building. (b) Population model predictions versus observed concentrations are unbiased, indicating good structural model fit. (c) Individual model predicted concentrations are in agreement with observed concentrations. (d) Conditional weighted residuals (CWRES), which are a form of standardised residuals expected to follow the Normal Independent Distribution (NID; 0,1) lie within -2 and +2 standard deviations and do not change with model predictions, indicating good structural model fit. (e) Similarly the QQ plot of CWRES indicate that the assumption of normality of residuals is met; (f) Median observed IGF-1 concentrations (solid line) similar to median simulated (dashed line) and observed median lies within 95% confidence interval of the model simulations (grey shaded area).

Figure 4. Population level (blue line) and individual (red line) model predictions are similar to observed data (black open circles) in both (a) single dose and (b) repeated doses in each part of the study.

Figure 5. Incorporation of a disease activity index into dose calculations allows an accurate prediction of circulating IGF-1. Utility function results showing the effect of increasing sophistication on dose scaling method. Scaling by weight, age group and PCDAI score limits average IGF-1 concentrations in 93% of children to less than +2 SDS. Solid lines are the target range of 0 to +2 SDS, dashed lines are for reference -2SDS and +2.5 SDS.

1. Mathematical modelling to restore circulating IGF-1 concentrations in children with Crohn's disease-induced growth failure: a pharmacokinetic study

Running head: Restoring IGF-1 in children with Crohn's disease

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ARTICLE SUMMARY

Article focus

One third of children with Crohn’s disease have growth retardation.

Children with active inflammation have an insensitivity to growth hormone, resulting in low circulating IGF-1.

There is no agreed growth therapy for children whose inflammation is intractable to treatment, because very high and sustained IGF-1 concentrations are a risk factor for colon cancer in adults with acromegaly.

Key messages

IGF-1 can be restored to the normal range by subcutaneous injections.

The endogenous synthesis of IGF-1 depends on Crohn’s disease activity; and variations in both determine the circulating IGF-1 concentrations.

A new mathematical model which incorporates disease activity (in addition to weight and age) will allow paediatric gastroenterologists to calculate doses that keep the circulating IGF-1 in the upper normal range.

Strengths and Limitations

Using the mathematical model, IGF-1 can be prescribed in doses that do not increase the risk of cancer.

For the first time, long term IGF-1 treatment can be studied in children to determine if it enhances growth, and this is a critical step in offering a therapy (much demanded by patients) for growth retardation in children with Crohn’s disease.

A limitation of the study is that long term studies over several years will still be required on a large group of children.

ABSTRACT

Objectives: Children with Crohn's disease grow poorly, and inflammation depresses the response of IGF-1 to growth hormone. Correcting the inflammation normalizes growth velocity; however, removing inflammation cannot be achieved in all children. Our lack of understanding of IGF-1 kinetics has hampered its use, particularly as high IGF-1 concentrations over long periods may predispose to colon cancer. We hypothesized that mathematical modelling of IGF-1 would define dosing regimes that return IGF-1 concentrations into the normal range, without reaching values that risk cancer.

Design: Pharmacokinetic intervention study

Setting: Tertiary paediatric gastroenterology unit

Participants: 8 children (M:F; 4:4) entered the study. All completed: 5 South Asian British; 2 White British; 1 African British. Inclusion criteria: Children over 10 years with active Crohn's disease (CRP >10mg/l or ESR >25mm/h) and height velocity <-2 SD score. Exclusion criteria: closed epiphyses; corticosteroids within 3 months; neoplasia or known hypersensitivity to rhIGF-1.

Interventions: Subcutaneous recombinant human IGF-1 (rhIGF-1) (120µg/kg) per dose over two admissions: the first as a single dose, and the second as twice daily doses over 5 days.

Primary outcome: Significant increase in circulating IGF-1

Secondary outcomes: Incidence of side effects of IGF-1. A mathematical model of circulating IGF-1 (A_c) was developed to include parameters of: endogenous synthesis (K_{syn}); exogenous uptake (K_a) from the subcutaneous dose (A_s): and IGF-1 clearance: where $dA_c/dt = K_{syn} - K_{out} \cdot A_c + K_a \cdot A_s$.

Results: Subcutaneous IGF-1 increased concentrations, which were maintained on twice daily doses. In covariate analysis, disease activity reduced K_{syn} (p<0.001). Optimal dosing was derived from least

squares regression fitted to a dataset of 384 Crohn’s patients, with model parameters assigned by simulation.

Conclusions: By using age, weight and disease activity scaling in IGF-1 dosing, over 95.% of children will have normalized IGF-1 concentrations below +2.5 standard deviations of the normal population mean, a level not associated with cancer risk.

Abstract word count: 299

INTRODUCTION

A quarter of cases of Crohn's disease now present in children and adolescents under 18 years [1, 2]; and its incidence in childhood is increasing [3]. Around a third of children experience linear growth retardation, caused in part by undernutrition and in part by the direct effects of inflammation on growth [4].

Growth in childhood is regulated by growth hormone (GH), secreted from the pituitary, which stimulates the production of insulin-like growth factor-1 (IGF-1) by the liver and growth plates of bones [5]. Children with active Crohn's disease have low circulating IGF-1 and increased circulating cytokines [6, 7]. Treatment of the inflammation, for example by an enteral diet, results in a reduction in cytokines like IL-6 and an increase in IGF-1 within 3 days [7]. Thus, the optimum treatment for improving growth is to eliminate the inflammation. Nevertheless, some children's inflammation remains intractable to treatment despite the best efforts of clinicians. For this group, there is currently no agreed therapy to enhance growth.

Rats with TNBS-induced colonic inflammation grow poorly [8]; and this is also associated with increased cytokines IL-6 and TNF, and a low circulating IGF-1 [9]. Exogenous IGF-1 given to the rats with colitis enhanced growth [8]. In transgenic mice, without inflammation, high levels of circulating IL-6, generated by a widely acting promoter, depressed both IGF-1 and growth [10]. Thus the link between inflammatory cytokines, IGF-1 and poor growth is strong [11]. There is a functional insensitivity to GH in children with Crohn's disease. Recombinant IGF-1 is now recognized therapy for children with GH insensitivity syndrome (GHIS) due to genetic defects of the GH receptor or IGF-1 gene [12]; we, therefore, hypothesized that IGF-1 concentrations in children with active Crohn's disease and poor linear growth could be restored by administering rhIGF-1.

Restoring IGF-1 in children with Crohn's disease who may have some endogenous production is not straightforward, because high levels of IGF-1 may be harmful. For rhIGF-1 to be therapeutically useful, its circulating levels over the long term should be returned to normal values by replacement, and not given in excess. Patients with acromegaly who have very high concentrations of GH and IGF-1, maintained over decades, have a doubling in the incidence of colon cancer [13]. This could, in theory, be compounded in children with Crohn's disease, as inflammation is also a risk factor for intestinal cancer.

A mathematical model for administration of rhIGF-1, based on detailed pharmacokinetics would resolve these difficulties, as dosing could be tailored to achieve circulating concentrations that remain within the normal range. We therefore undertook a careful study of subcutaneous rhIGF-1 administration in children with Crohn's disease-induced growth retardation. We developed a model which used physiological parameterisation, disease activity to predict endogenous IGF-1 synthesis, and body weight to scale the volume of distribution. Parameters of protein loss, such as protein-losing enteropathy, were also considered. The model was used to recommend dosing which allows children to be treated with rhIGF-1 without its concentration rising above the normal range.

METHODS

Patient Selection

Following ethical approval from the East London & City Research Ethics Committee (ELREC; reference number 07/H0705/77) and regulatory approval from the UK Medicines and Healthcare products Regulatory Agency (MHRA) (Eudract number: 2007-004269-16), written informed consent was obtained from the parents and patients attending the paediatric inflammatory bowel disease clinic at Barts and The London Hospital for Children. Inclusion criteria were: age ≥ 10 years; known Crohn's disease diagnosed by endoscopic, histological and radiological methods [14]; a height

velocity measured over at least a 6 month period of <-2 Standard Deviation Score (SDS) according to the patient's age and gender; and evidence of active inflammation as demonstrated by either an ESR > 25mm/hr and/or a CRP > 10mg/l. Exclusion criteria were: corticosteroid use in the preceding 3 months; active or suspected neoplasia; known hypersensitivity to exogenous rhIGF-1 (Increlex, Ipsen UK); and the presence of closed epiphyses.

All patients had a full history and physical examination, including an accurate recording of height, weight and assessment of pubertal status. In addition, they also had an electrocardiogram and faecal alpha-1-antitrypsin levels (g/l) measured.

Study Design

Exogenous rhIGF-1 was administered by subcutaneous injection at doses of 120µg/kg over two admissions. Admission 1 investigated the effects of a single dose of rhIGF-1 over a 24 hour inpatient stay. Baseline blood screens included full blood count (FBC), electrolytes, inflammatory markers (CRP and ESR), baseline IGF-1, IGFBP-3, acid labile subunit (ALS) and blood sugar. The Pediatric Crohn's Disease Activity Index (PCDAI) was calculated [15, 16]

Each patient was then given a single subcutaneous injection of rhIGF-1 at a dose of 120µg/kg. Serial venous blood samples were drawn at the following time points: 0, 1, 2, 3, 4, 6, 12, 17 and 24 hours. Blood sugars and vital signs were checked regularly. Children ate and drank freely, and continued to receive their prescribed, non-corticosteroid, treatment for Crohn's disease.

Following a wash out of at least 3 months, subjects were readmitted for admission 2, investigating the effects of repeated doses of rhIGF-1. On this occasion 6 doses of rhIGF-1 were administered over a 5 day trial period, with doses given at: 0, 12, 72, 84, 96 and 108 hours. The injection sites were rotated according to the patient's wishes. Serial blood samples for further IGF-1 , IGFBP-3, acid

labile subunit (ALS) and blood sugar were collected during the 5 days at 0, 1, 2, 3, 4, 6, 12, 17, 24, 48, 72, 96 and 120 hours. Additional samples were also taken for clinical reasons when there was a possibility of hypoglycaemia. If sufficient sample was collected, IGF-1 levels were also measured. In addition, vital signs, FBC, electrolytes, and inflammatory markers were measured daily. Patients were kept as inpatients during the days that the rhIGF-1 was administered, but were allowed home during the two days in which they received no injections. Patients were discharged home on day 6.

Assays and samples

All samples were stored at -20°C until analysis. Plasma glucose was determined immediately after blood sampling (Beckman Instruments, Palo Alto, CA).

IGF-1, IGFBP-3.

IGF-1 and IGFBP-3 levels were measured by competitive binding radioimmunoassay (Esoterix Inc Laboratory Services). The sensitivity of the assay for IGF-1 was 15ng/ml and the intra-assay coefficient of variation averaged 14.1%. The sensitivity of the assay for IGFBP-3 was 0.3mg/L and the intra-assay coefficient of variation averaged 13%.

ALS

ALS levels were measured by radioimmunoassay (ALS-RIA) using purified human ALS as tracer. The intra-assay coefficient of variation ranged from 8.0-17.4%

Mathematical model development

Measured IGF-1 concentrations were fitted for all individuals simultaneously using the non-linear mixed effects modelling software NONMEM version 7.1[17]. Model building was undertaken using the first-order conditional estimation method with interaction (FOCEI). A turnover model was used according to the following differential equations:

$$\frac{dA_c}{dt} = K_{syn} - K_{out} \cdot A_c + K_a \cdot A_s \qquad \text{Equation 1}$$

$$\frac{dA_s}{dt} = -\,K_a \cdot A_s \qquad \text{Equation 2}$$

Where: A_c is the amount of circulating IGF-1 at time t; A_s is the amount in the subcutaneous tissue following a dose, with initial conditions adjusted each time a dose is administered; K_{syn} is a zero order production rate of endogenous IGF-1 in $\mu g.hr^{-1}$; K_{out} is a first order elimination rate constant in hr^{-1}; and K_a is a first order absorption rate constant describing exogenous IGF-1 appearance following a subcutaneous dose in hr^{-1}. At steady state prior to exogenous dose, circulating IGF-1 is given by the ratio of K_{syn} to K_{out}.

In addition to K_{syn} and K_a, the fixed effect parameters IGF-1 clearance (C_L) in $L.hr^{-1}$ and distribution volume (V_D) in L were estimated, K_{out} being given by the ratio of C_L to V_D. Between subject variability was tested for all parameters, and residual variability was described using a heteroscedastic model. Allometric weight scaling was applied to C_L and V_D *a priori* using linear scaling for V_D and weight raised to the power 0.75 for C_L[18] , K_{syn} was further scaled with linear weight. Tested covariates were CRP, ESR, PCDAI score and age.

Model development used the NONMEM objective function value (OFV); parameter estimate precision was derived through a non-parametric bootstrap [19] and graphical assessment of basic goodness-of-fit plots and model simulations was undertaken. The OFV is proportional to -2 times the log-likelihood of the data given the parameter estimates, and a decrease in OFV of 3.84 with one degree of freedom gives a significantly improved fit with a probability $p < 0.05$. A utility function

based on the final model was used to predict average concentration (C_{ave}) with a dosing regimen derived from the integral of the IGF-1 against the time curve divided by time. Maximum utility was defined as C_{ave} of +0.50 SDS with linear penalty for deviations above and below this target. Dose was optimisied to give a model-derived steady-state C_{ave}, by the following model:

$$Target = SDS_i + \varepsilon_i$$ Equation 3

$$Utility = \sum_{i=1}^{n} (Target - SDS_i)^2$$ Equation 4

Where Target is the SDS aim (in this case +0.50); SDS_i is the individual predicted SDS derived from model predicted C_{AVE}; ε_i denotes individual deviations from the target. Utility minimised ε_i by least squares regression.

Demographic details for 384 subjects aged 8 to 14 years from the clinic database were each assigned a random PCDAI score (from a uniform distribution 0-48) and also individual model parameters. The utility function was maximised with either fixed dosing, dosing based on weight, weight and age, or weight, age and PCDAI score. Differences in concentrations were explored with 12 and 24 hourly dosing regimens.

Calculation of Sample Size

The size was derived from the number of children needed to show a significant increase in IGF-1, based on the increases in IGF-1 seen in a cohort of children with treated with IGF-1 for Growth Hormone Insensitivity Syndrome (GHIS). This showed that in 18 children, the concentration before drug was 330 ng/ml (SEM 20 ng/ml); and the concentration after 4 hours was 535 ng/ml (SEM 20 ng/ml). Therefore, to detect a difference in concentration of 205 (535-330) before drug and after 4

hrs, and SD of 84.85 (calculated from Standard Error of the Mean of 20 with sample size of 18), with 80% power at the 5% level of significance four patients are required.

RESULTS

rhIGF-1 therapy in children with Crohn's disease

Children with active Crohn's disease, and whose growth was inhibited were recruited into the study (Table 1). Impairment of height velocity is characteristic of many children with active Crohn's disease [4, 20-22], and all 8 subjects recruited into the study (median age 12.97) had a height

Table 1: Patient characteristics at recruitment into the trial. Tanner stage: P: pubic hair, B: breast stage, G: genitalia . Medications: 5 –ASA: 5-aminosalicylic acid, AZA: azathioprine, IFX: infliximab, Adalum = adalumimab. Montreal classification: L1: ileal, L2: colonic, L3: ileocolonic, B1: non-stricturing, non-penetrating, B2: stricturing, B3: penetrating

	Patients							
	LN01	LN02	LN03	LN04	LN05	LN06	LN07	LN08
Gender	F	F	M	M	F	F	M	M
Age	13.11	11.5	14.23	10.67	14.82	12.7	12.82	14.66
Ethnicity	Caucasian	Asian	Asian	Asian	Asian	Asian	African	Caucasian
Tanner Stage	P1 B1	P1 B1	P3 G3	P1 G1	P2 B2	P2 B2	P2 G2	P1 G1
HV SDS at recruitment	-2.11	-2.11	-4.18	-2.83	-2.27	-3.87	-4.43	-4.88
HV SDS at time of trial	-2.11	-2.14	-1.84	0.36	1.69	3.55	-4.43	-4.88
PCDAI	12.5	20	17.5	10	47.5	15	12.5	10
Medication	5-ASA	5-ASA	5-ASA AZA IFX	5-ASA AZA Adalum	5-ASA AZA Adalum	5-ASA AZA IFX	5-ASA AZA	5-ASA
Faecal A1AT (g/l)	3.34	0.82	0.88	0.49	2.63	2.28	0.08	0.45
Montreal Classification								
Disease Location	L1	L2	L3	L3	L1	L3	L1	L1
Behaviour	B1	B1	B3	B3	B2	B1	B2	B1
Upper	N	N	Y	N	Y	Y	N	N
Perianal	N	N	Y	Y	N	N	N	N

velocity that was more than two standard deviations below the mean (i.e. a standard deviation score [SDS] of <-2), as an entry criterion. The median height velocity SDS was -3.35, indicating extreme growth failure.

All the subjects completed both parts of the study. The rhIGF-1 was generally well tolerated. Although subcutaneous rhIGF-1 has been associated with hypoglycaemia in children treated for GHIS [12], only one patient (LN01) had a single asymptomatic hypoglycaemic episode (blood sugar < 3.5mmol/l). This was corrected with oral glucose. This occurred following the 12 hour dosage of rhIGF-1 during admission 2 in a child who had not eaten prior to the drug administration. No further episodes of hypoglycaemia occurred. There were no other adverse effects.

Determinants of circulating IGF-1

As previously demonstrated, children with Crohn's disease-induced growth failure had depressed circulating IGF-1 concentrations. The severity of disease in any one child varies over time, and this was reflected in the variation of IGF-1 between the first and second admissions, 3 or more months apart. (Figure 1a). Despite the variation both between and within patients, a single subcutaneous bolus of rhIGF-1 significantly increased IGF-1 in the circulation (Figure 1). In some cases where the baseline IGF-1 was less depressed, the post IGF-1 level was increased to above normal (Figure 1a). The concentrations of IGF-1 returned to baseline over the following 24 hours (Figure 1b).

IGF-1 is maintained in the circulation by IGF binding proteins, the most abundant being IGFBP-3 [23]. A major characteristic of Crohn's disease is protein-losing enteropathy, resulting in proteins being lost into the lumen of the intestine [24, 25]. We aimed therefore to ascertain if the degree of protein-losing enteropathy blunted the ability of subcutaneous rhIGF-1 to achieve significant peak concentrations, or if it directly affected IGF binding proteins. Protein losing enteropathy was quantified by the concentrations of alpha-1- antitrypsin in stool [26]. Using this measure, protein

losing enteropathy had no effect on the change in IGF-1 induced by a subcutaneous injection (Figure 2a)

IGFBP-3 concentrations are depressed in children with chronic IGF-1 deficiency [27], and all children in the study had low IGFBP-3 (Figure 2b). We wished to determine, however, if IGFBP-3 concentrations depended on the degree of protein losing enteropathy, as this would indicate that protein losing enteropathy may affect IGF-1 pharmacokinetics. However, no association was observed.

In the second admission, the subjects received twice daily doses, to determine if IGF-1 concentrations would be enhanced over a longer period. The initial injection increased the IGF-1 concentrations into the normal range (Figure 1a). Increases over a sustained period were successfully achieved (Figure 1c), and as expected multiple daily dosing led to lower variability between peak and trough concentrations. Taking the area under the curve divided by time as the average IGF-1 concentration, it became clear that on 120µg/kg, some children had an average IGF-1 SDS of greater than +2.5, while other children had appropriate concentrations. Increased circulating IGF-1 in acromegaly has been linked with an increased risk of colon cancer [13, 28]. Fortunately, we have a clear understanding of how IGF-1 concentrations relate to colon cancer risk in acromegaly [13]. These studies show that the increased cancer risk was seen in individuals whose circulating IGF-1 was +2.5 SDS or greater. Using our data, we developed a model from which the dose-concentration relationship could be derived. We then investigated the covariates on which dosing could be individualised. This allowed us to correct circulating IGF-1 without causing excessively high concentrations: ensuring that our dosing regimens resulted in concentrations that were below +2.5 SDS, we developed a utility model to predict the IGF-1 SDS achieved on regular dosing.

Mathematical modelling of IGF-1 links endogenous production to disease severity

A turnover model which accounted for both endogenous production and exogenous dosing of IGF-1 was fitted to the data (see methods for mathematical formulae) using non-linear mixed effects analysis. Four fixed effects were estimated, namely endogenous synthesis rate (K_{SYN}), exogenous absorption rate through the skin following subcutaneous injection (K_a), systemic clearance (C_L), and distribution volume V_D. Parameter level random effects (inter-subject variability) were included on K_{SYN} and C_L . (Table 2) A further level of random effects (inter-occasion variability) also provided significantly improved fit when added to K_{SYN}. Following a limited covariate analysis, the PCDAI score was found to significantly improve fit when added to K_{SYN}, with K_{SYN} falling with increasing disease severity (Figure 3a). The effect of disease severity as measured by PCDAI was tested for C_L and K_{SYN} with significant improvement ($p<0.001$) being shown for K_{SYN}. Furthermore, adding the PCDAI score as a covariate to K_{SYN} allowed inter-occasion variability to be removed without detriment to the model fit (no change in log-likelihood). Adding PCDAI to K_{SYN} also decreased inter-individual variability. In short, disease severity was a major driver of IGF-1 synthesis rate both between patients, and in the same patient on different occasions.

Table 2: Parameter estimates from the model including non-parametric 95%CI from a bootstrap of 714 successful runs. IIV (inter-individual variability).

Parameter	Estimate	Bootstrap median	Bootstrap 2.5th percentile	Bootstrap 97.5th percentile
C_L (L/h/70kg)	1.61	1.60	1.36	1.82
V_D (L/70kg)	2.42	2.41	1.78	3.10
K_{SYN} (µg/h)	433	423	352	490
Ka (h^{-1})	0.10	0.10	0.083	0.12
Coefficient of PCDAI on K_{SYN}	-6.57	-6.41	-8.19	-4.89
IIV on CL (%CV)	10.38	9.46	3.49	15.1
IIV on K_{SYN} (%CV)	24.61	24.10	9.98	33.58
Residual error proportional (%CV)	9.81	9.69	7.85	11.04
Residual error, additive (µg/L)	28.32	25.93	13.29	42.78

There was good agreement between model predictions and observed concentrations (Figure 3b), and trendless distribution of standardised residuals, indicating that the parametric modelling assumptions were satisfied (Figure 3c, d and e). The median of the simulated data was not significantly different to the median of the observed data (Figure 3f). Superimposing model predictions at a population and individual level on the observed data showed that model predictions captured the dynamic behaviour of the system (Figure 4).

Dosing to maintain circulating rhIGF-1 concentrations in the target range

We undertook mathematical modelling to quantify the relationship between endogenous IGF-1 production and exogenous dosing, and to understand how IGF-1 average concentrations (area under the curve/time) can be adjusted. Using the knowledge that in acromegaly there is no increased risk of cancer in patients whose IGF-1 is below +2.5 SDS [13], we defined the optimal IGF-1 concentration range as being between 0 and +2.0 SDS. This range would improve growth without elevating cancer risk. Different possible dose scaling systems were then explored to maximise the probability of an individual having IGF-1 levels in the target range. By individualising dose by weight, age (because different aged children have different target circulating IGF-1 levels), and PCDAI score, 92.7% of children were predicted to be corrected to less than or equal to +2.0 SDS (95.3% below +2.5 SDS; Figure 5). We can therefore suggest a dosing scheme that limits the risk of malignancy to that of the normal patient population. The dosing schedule recommended from the utility function is:

Age 10 to <12 years, 21 μg/kg plus 1 μg/kg /PCDAI point; Age 12 to <14 years, 41 μg/kg plus 1.4 μg/kg /PCDAI point.

DISCUSSION

Impaired growth has been a recognised feature of children with Crohn's disease for 50 years [29]. In 1986, low IGF-1 was associated with poor growth in these children [30]; and over the following decade it was realized that the depressed concentration was a direct consequence of inflammation [8, 10], and not merely the result of poor energy intake or undernutrition. The best strategy to increase IGF-1 and therefore to improve growth is the resolution of inflammation. Treatments which reverse the inflammation, but do not actively suppress growth, such as surgery [31], anti-TNF [32, 33] and enteral diets [6, 34] have all been shown to improve linear growth.

Nevertheless, there are patients with Crohn's disease whose inflammation cannot be reversed. In this group, we need a distinct strategy to accelerate growth. Both hGH [35, 36] and rhIGF-1 have been suggested. Because patients with Crohn's disease have a functional insensitivity to GH, the present study examined the possibility that rhIGF-1 would be efficacious in improving IGF-1 concentrations, as it does in children with growth hormone insensitivity syndrome (GHIS). However, there are significant problems in applying our knowledge of rhIGF-1 usage in GHIS to children with Crohn's disease. First, children present at a much later age in Crohn's disease than they do in GHIS, which is caused by a genetic mutation (approximately 8-15 years as opposed to less than 5 years old). The compartments of the IGF-1 system including several IGF binding proteins, cannot be assumed to distribute as they do in younger children, particularly if the relative volumes of these compartments change at the time of puberty. Second, both IGF-1 and the IGF binding proteins are circulating proteins. Proteins are lost into the intestine through protein losing enteropathy (PLE), where inflammation reduces the ability of the intestinal vasculature to maintain them in the capillary lumen [37-39]. The PLE could, in theory, alter the kinetics of IGF-1. Finally, very high and sustained circulating IGF-1 concentrations are associated with an increase in colon cancer in middle aged and older patients with acromegaly. A modelling approach to kinetics and dosing was necessary to avert causing high average IGF-1 concentrations.

The reduction in IGFBP-3 raises the question as to whether giving rhIGF-1 to children with a reduced IGFBP-3 may increase free IGF-1 concentrations. Since we did not have access to an assay for free IGF-1, our data are not totally informative on this issue. However, they will not have risen to unsafe levels from saturating the IGF-1 binding capacity. The concentrations of IGFBP-3 are not greatly depressed in the affected children. Even in the most severe case, the IGFBP-3 is within two standard deviations of normal. In addition, IGF-1 is over 95% bound to IGF binding proteins, with an excess of binding capacity. In general when analysing pharmacokinetic data, changes in protein binding do not affect free concentrations but may affect free fraction. This means that for two individuals with the same total concentration, the free concentration maybe elevated in the one with lower binding protein. However, two individuals with different IGFBP-3 concentrations given rhIGF-1 will not achieve the same total concentrations, because as IGF-1 undergoes first-order elimination, higher free concentrations will be more rapidly eliminated due to homeostasis. IGFBP-3 was not a significant covariate for volume of distribution in our model, on analyzing our small dataset. Indeed, our model predicts the majority of patients will have total concentrations just above the normal range.

Several of the children enrolled in the study had delayed puberty (Table 1). Although this is commonly observed in Crohn's disease, the study could be criticized for not taking this into account when calculating IGF-1 SDSs, as circulating IGF-1 increases in puberty. We do not have normative data for circulating IGF-1 against pubertal stage. However, delay in puberty is closely correlated with a delay in bone age, as measured on a wrist X-ray. We therefore recalculated each child's IGF-1 SDS against their bone age before and after the initial injection of rhIGF-1 (data not shown). However, this made little difference to the results obtained when comparing this data to that based on chronological age (Figure 1a).

Previous studies on rhIGF-1 pharmacokinetics [40, 41] have mainly focussed on its use in malnourished adult patients with renal failure, where it has been used to maintain protein balance.

These reports undertook purely descriptive analyses such as Area Under the Concentration-Time Curve (AUC) and comparing healthy volunteer parameters with patients, rather than modelling the data. Furthermore, they did not account for endogenous IGF-1 production. If we were to use our data to recommend a dose, a more sophisticated approach was required. In this study, we fitted a structural model that simplified the system without losing biological interpretation of the parameters. Mixed effects modelling allowed for the addition of inter-individual and inter-occasion variability in model parameters, in addition to residual variability. We were, therefore, able simultaneously to fit the model to all patients and investigate covariate-parameter relationships. Instead of exploring dose recommendations by simulation, we aimed to optimise the dose based on the target SDS; and we minimised deviations from this using least-squares regression [42] . The advantage of this approach, over testing competing dosing regimens by simulation, is that once the optimal target is defined, the optimal dose needed to reach that target is determined in a single step. Testing covariates for dose scaling is also simple with this method. Furthermore, by using a large dataset of hypothetical patients with real Crohn's Disease demographics, we were able to show how scaling dose by age, weight and PCDAI score could adequately and safely correct IGF-1 (Figure 5).

The goodness-of-fit plots (Figures 3b-e and Figure 4) show that the model predictions are an unbiased description of the data and simulated data that is not significantly different to the observations (Figure 3f). Furthermore, covariate analysis showed that the disease severity (PCDAI score) significantly reduced endogenous synthesis (K_{syn}) rather than affecting IGF-1 clearance. Variations in IGF-1 synthesis distinguish patients with Crohn's disease from children with GHIS.

A possible criticism of using PCDAI to quantify disease in this context is that it includes growth as a marker of inflammation, and it could be suggested that the dependence of K_{syn} on PCDAI might be due to the height velocity element of the index. Recently, the determinants of the disease activity index have been separately analyzed for predictive value in defined populations of children with Crohn's disease [43]. Linear growth was discovered not to be discriminatory. This report developed a new index, the weighted Pediatric Crohn's Disease Activity Index (wPCDAI), based on these observations, which does not include growth. We repeated our covariate modelling with wPCDAI and showed that adding it to K_{syn} reduced parameter variability and significantly improved model fit, in the same way as the standard PCDAI had. Thus, the relationship between K_{syn} and disease activity was due to objective measures of inflammation, and not to the growth component of the PCDAI calculation.

This report focused on the effects of IGF-1. As mentioned above, hGH may also be of benefit on the linear growth of children with Crohn's disease [36]. It was not the purpose of the present study to examine which therapy would be more efficacious; however, future studies comparing IGF-1 to hGH in a controlled randomized multicentre study are planned. This trial will also give us the opportunity to test the dosing regimen derived from this model presented in the present report on a wide sample of children.

Patients and their support groups have long requested more research into therapies that directly improve growth in children whose inflammation cannot be controlled. The present study shows that twice daily rhIGF-1 can enhance average circulating IGF-1 concentrations into the upper normal range. The use of a utility function from mathematical modelling allows paediatricians to give rhIGF-1 without their entering an unphysiological high range, hence preventing the increased the risk of cancer.

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Competing interests: None

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Contributorship: IRS conceived the study and helped in its design; obtained the funding, ethics, MHRA approval and drafted initial manuscript; JFS developed the mathematical modelling; AVR consented the patients; arranged admission and undertook all the investigation; SK oversaw the clinical care of the patients while admitted for research investigation. MOS helped design the study and provided paediatric endocrinology expertise. All authors contributed to the final manuscript.

Data Sharing: Anomymised original data will be given to research academics at a recognised university departments in line with the policies of Queen Mary, University of London. Requests should be made to Professor Sanderson. However, there are no data in addition to those published, except as the individual numerical results that are displayed as

figures.

Patient identifiable data cannot be shared, as this is a condition of the research ethics committee approval

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Legends to figures:

Figure 1 Subcutaneous rhIGF-1 increases the circulating concentrations of IGF-1 in children with growth failure induced by Crohn's disease. (a) IGF-1 standard deviation scores (SDS) below the normal range in both first [Mean -1.78 (SD 1.37)] and second admissions [Mean -2.34 (SD 0.75)] are significantly increased a single injection of rhIGF1 ($p<0.0005$ and <0.0001, respectively). (b) An injection reaches a peak within 4 hours and returns to low levels within 24 hours. (c) Twice daily injections of rhIGF-1 increase circulating IGF-1 over a sustained period. rhIGF-1 was given on day 1 and the circulating concentrations allowed to fall, before giving twice daily injections on days 4 and 5.

Figure 2 Protein losing enteropathy did not alter IGF-1 or IGFBP-3. (a) Variations in protein-losing enteropathy (as measured by faecal alpha 1 anti-trypsin) did not correlate with changes in IGF-1 concentrations achieved on giving rhIGF-1 ($p=0.703$). (b) Variations in protein-losing enteropathy did not correlate with IGFBP-3 concentrations

Figure 3. IGF-1 in a mathematical model. (a) Increasing disease activity (PCDAI) significantly ($p<0.001$) diminishes the estimates of K_{syn} in the covariate model building. (b) Population model predictions versus observed concentrations are unbiased, indicating good structural model fit. (c) Individual model predicted concentrations are in agreement with observed concentrations. (d) Conditional weighted residuals (CWRES), which are a form of standardised residuals expected to follow the Normal Independent Distribution (NID; 0,1) lie within -2 and +2 standard deviations and do not change with model predictions, indicating good structural model fit. (e) Similarly the QQ plot of CWRES indicate that the assumption of normality of residuals is met; (f) Median observed IGF-1 concentrations (solid line) similar to median simulated (dashed line) and observed median lies within 95% confidence interval of the model simulations (grey shaded area).

Figure 4. Population level (blue line) and individual (red line) model predictions are similar to observed data (black open circles) in both (a) single dose and (b) repeated doses in each part of the study.

Figure 5. Incorporation of a disease activity index into dose calculations allows an accurate prediction of circulating IGF-1. Utility function results showing the effect of increasing sophistication on dose scaling method. Scaling by weight, age group and PCDAI score limits average IGF-1 concentrations in 93% of children to less than +2 SDS. Solid lines are the target range of 0 to +2 SDS, dashed lines are for reference -2SDS and +2.5 SDS.

Figure 1a

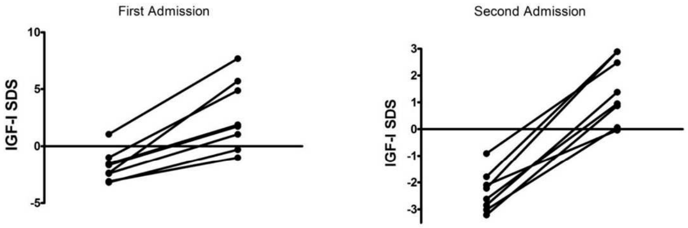

Figure 1b

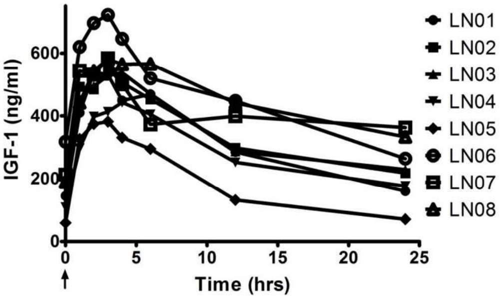

Figure 1c

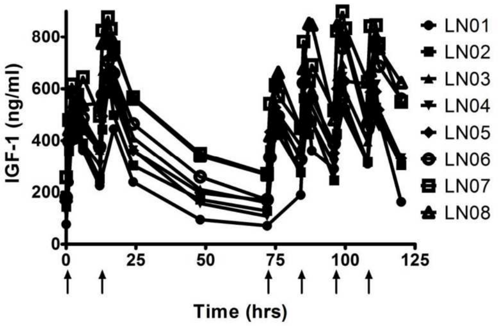

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Figure 2a

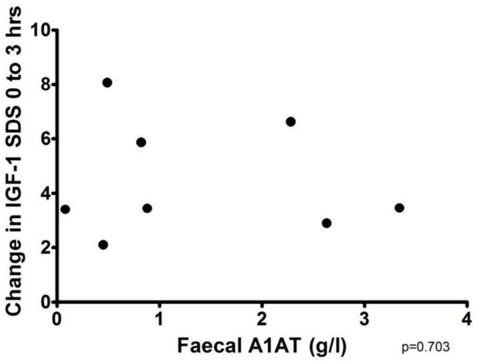
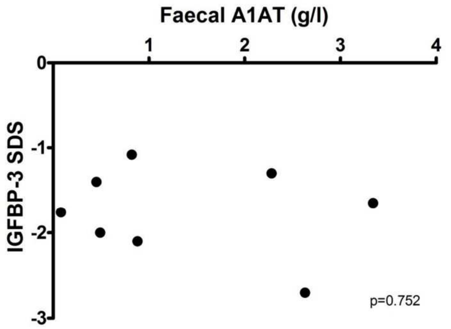


Figure 2b



Protein losing enteropathy did not alter IFG-1 or IGFBP-3. (a) Variations in protein-losing enteropathy (as measured by faecal alpha 1 anti-trypsin) did not correlate with changes in IGF-1 concentrations achieved on giving rhIGF-1 (p=0.703). (b) Variations in protein-losing enteropathy did not correlate with IGFBP-3 concentrations
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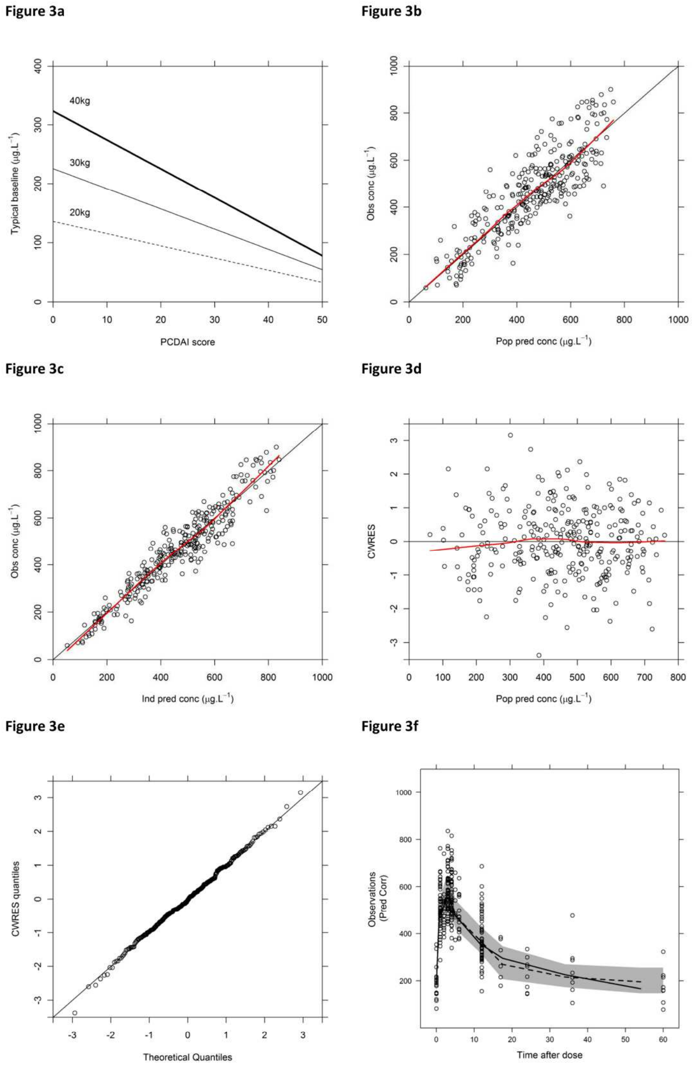


Figure 3a
Figure 3b
Figure 3c
Figure 3d
Figure 3e
Figure 3f

IGF-1 in a mathematical model. (a) Increasing disease activity (PCDAI) significantly ($p<0.001$) diminishes the estimates of Ksyn in the covariate model building. (b) Population model predictions versus observed concentrations are unbiased, indicating good structural model fit. (c) Individual model predicted concentrations are in agreement with observed concentrations. (d) Conditional weighted residuals (CWRES), which are a form of standardised residuals expected to follow the Normal Independent Distribution (NID; 0,1) lie within -2 and +2 standard deviations and do not change with model predictions, indicating good structural model fit. (e) Similarly the QQ plot of CWRES indicate that the assumption of normality of residuals is met; (f) Median observed IGF-1 concentrations (solid line) similar to median simulated (dashed line) and observed median lies within 95% confidence interval of the model simulations (grey shaded area).
165x233mm (300 x 300 DPI)

Figure 4a

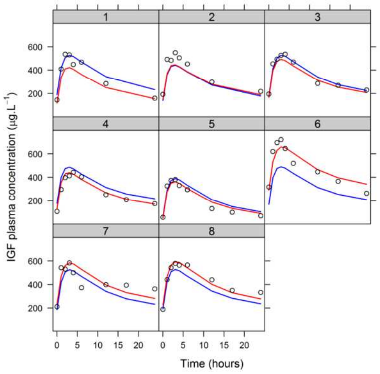
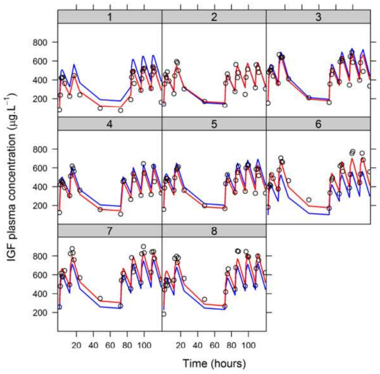


Figure 4b



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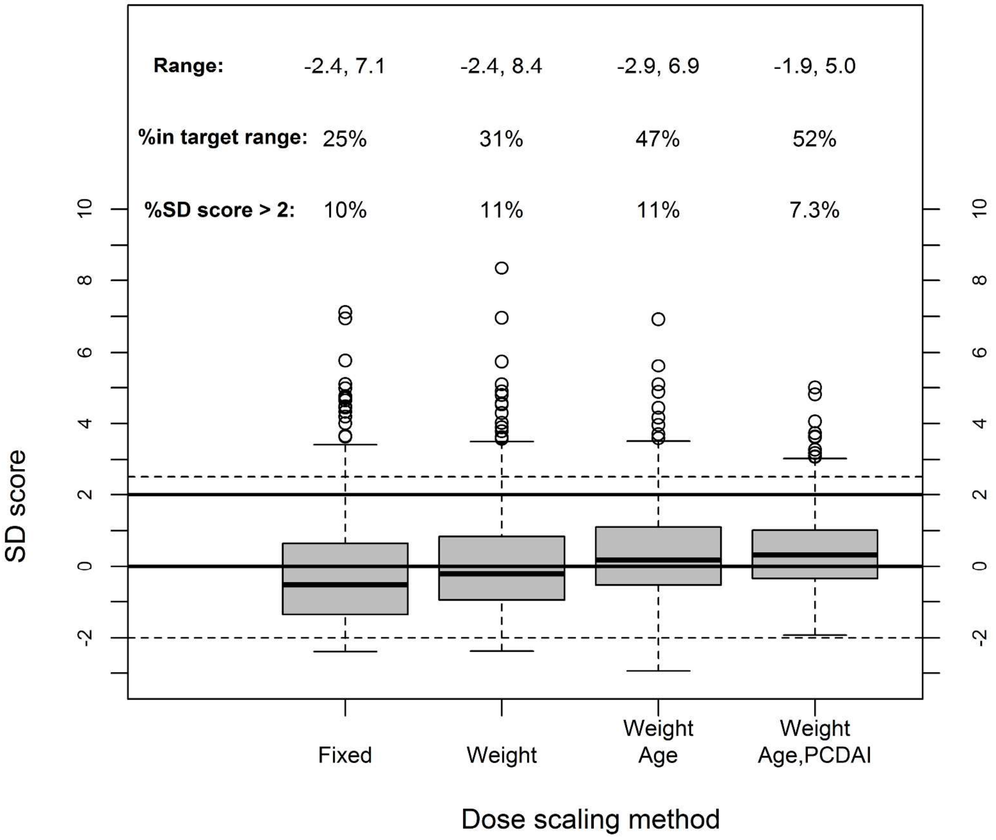
165x233mm (300 x 300 DPI)



Incorporation of a disease activity index into dose calculations allows an accurate prediction of circulating IGF-1. Utility function results showing the effect of increasing sophistication on dose scaling method. Scaling by weight, age group and PCDAI score limits average IGF-1 concentrations in 93% of children to less than +2 SDS. Solid lines are the target range of 0 to +2 SDS, dashed lines are for reference -2SDS and +2.5 SDS.
152x152mm (600 x 600 DPI)

Research Checklist

Pharmacokinetic studies of recombinant human insulin-like growth factor I (rhIGF-I) in children with Crohn's disease- induced growth retardation

- **Approved by the East London Research Ethics Committee (ELREC):**

  ELREC Reference: 07/H0705/77

- **Approved by Medicine and Healthcare products Regulatory Agency (MHRA)**

MHRA reference:	21313/0012/001-0001
Eudract Number	2007-004269-16
Product:	Increlex 10mg/ml solution for injection
Protocol number:	IGFI-1

- **R&D approved and Sponsored by Queen Mary, University of London**

  ReDA reference: 005251

- **Adopted by Medicine for Children Research Network (MCRN)**

- **Registered with the UK Clinical Research Network (UKCRN):**

  ID 4293