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Study Protocol

SCISSOR – Spinal Cord Injury Study on Small molecule derived Rho-inhibition: A Clinical Study Protocol

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ABSTRACT

Introduction The approved analgesic and anti-inflammatory drugs ibuprofen and indometacin target the COX-pathway unselectively. Moreover, they block the small GTPase RhoA, a key enzyme within a downstream cascade of extra-neuronal signals that impede axonal sprouting after axonal damage. Preclinical studies in spinal cord injury (SCI) imply improved motor recovery after ibuprofen/indometacin-mediated Rho-inhibition. This has been re-assessed by a meta-analysis of the underlying experimental evidence, which indicates an overall effect size of 20.2% regarding motor outcome achieved after ibuprofen/indometacin treatment compared to vehicle controls. Additionally, anti-inflammatory actions of ibuprofen/indometacin probably limit the development of sickness behavior, non-neurogenic systemic inflammatory response syndrome (SIRS), neuropathic pain, and heterotopic ossifications after SCI. Consequently, 'small molecule' mediated Rho-inhibition after acute SCI warrants clinical investigation.

Methods and analysis We report the protocol of an investigator initiated clinical pilot trial on high-dose ibuprofen treatment after acute traumatic, motor complete SCI. The open-label study has the primary safety endpoint: occurrence of serious adverse events, primarily gastroduodenal bleedings. Secondary endpoints are pharmacokinetics, feasibility and preliminary effects on neurological recovery, neuropathic pain and heterotopic ossifications. Underlying experimental evidence was challenged by systematic review of preclinical studies on ibuprofen/indometacin effects on motor recovery after SCI. The primary safety analysis is based on the incidence of severe gastrointestinal bleedings. Additional analyses will be mainly descriptive and casuistic.

Ethics and dissemination The clinical trial protocol was approved by the responsible German state Ethics Board, and the Federal Institute for Drugs and Medical Devices. The study complies with the Helsinki Declaration, the principles of Good Clinical Practice and all further applicable regulations. The current safety and pharmacokinetics trial informs the planning of a subsequent randomized controlled trial. Regardless to the result of the primary and secondary outcome assessments the clinical trial will be reported as publication in a peer-reviewed journal.

Registration ClinicalTrials.gov identifier: NCT02096913.

(300 words)

Keywords: ibuprofen, neuroprotection, plasticity, neuropathic pain, heterotopic ossifications

INTRODUCTION

At present, the effective pharmacological treatment of acute traumatic spinal cord injury (SCI) is an unmet medical need.[1] The current opportunities for restitution of neurological function after SCI are limited to early surgical decompression, stabilization, and physiotherapy. Neuroprotective or plasticity enhancing therapies are under investigation in preclinical studies and early-phase clinical trials. As yet, however, none of these approaches could be translated into clinical routine.[2-4]

A major reason for the poor prognosis of central nervous system (CNS) injury is the incapacity of axons to re-grow within the CNS. Molecular barriers preventing axonal regeneration after SCI are situated in the environment of the injured axon i.e. in the scar tissue and myelin.[5 6] Those molecules, such as chondroitin sulfate proteoglycans (CSPGs), Nogo-A, myelin-associated glycoprotein (MAG), oligodendrocyte-myelin glycoprotein (OMgp), Ephrins, and RGMa are up-regulated after CNS injury and interfere with a repertoire of cognate receptors on the axon membrane as reviewed elsewhere.[6 7] Signals from those receptors are mediated downstream to RhoA. The small GTPase RhoA is a key molecule in a pathway which, once activated, leads to the collapse of axonal growth cones and consequently to failure of axonal plasticity or regeneration.[8]

Therefore, the Rho-pathway constitutes a target for treatments aiming to overcome molecular obstacles to a restoration of neuronal connectivity and subsequent functional recovery. The inhibition of Rho or the downstream located Rho-associated coiled kinase (ROCK) has been demonstrated to foster axonal sprouting or plasticity,[9-22] to have neuroprotective effects,[9 10 12 13 19 23 24], and to enhance neurological recovery[9 10 12 15 17-19 21-23 25] after acute SCI (Figure 1). These findings are backed up with evidence from other experimental CNS-injury conditions as reviewed elsewhere.[7 26] The reported effects of various Rho/ROCK-blocking approaches on open field motor recovery after experimental SCI have been re-assessed by a systematic review and meta-analysis including correction for publication bias.[27] Specific Rho-inhibition mediated by the clostridium botulinum derived enzyme C3-transferase, also referred to as BA-210 or Cethrin™,[28] has been studied in a recently completed Phase I/IIa clinical trial. The investigators concluded that topically applied BA-210 is safe and is associated with favorable neurological outcome.[29] However, a confirmatory Phase III trial has not yet been conducted.

Over the last decade, upcoming evidence has assigned a subset of non-steroid anti-inflammatory drugs (NSAIDs) to the group of unspecific Rho-inhibitors. The FDA-approved NSAIDs ibuprofen,[10 16 18 20 30 31], indometacin,[18 30] and suldinac sulfide,[30] were shown to inhibit Rho-activation independently of their 'classical' mode of action as inhibitors of Cyclooxygenases (COX). It was subsequently demonstrated that ibuprofen treatment

enhances axonal sprouting,[10 16 18], including that of human model neurons,[20] and improves neurological recovery.[10 18] It is noteworthy that ibuprofen-mediated Rho-inhibition involves Peroxisome Proliferator Activated Receptor γ (PPARγ) activation.[16] It remains unclear, however, what the exact mechanism for this activation is and whether co-factors are required for PPARγ-associated Rho-inhibition, because other PPARγ activators such as rosiglitazone also inhibit Rho,[16 32], while other NSAIDs such as Naproxen activate PPARγ strongly[33] but do not cause Rho-inhibition.[10 16 30]

Other pharmacological targets of ibuprofen – namely PPARγ-activation,[16 33 34], COX-1/2-inhibition,[35] and NF-κb-inhibition,[36] promise a concomitant limitation of secondary damage by anti-inflammatory actions, but might also modify the effects of Rho-blockade (Figure 1). In more detail, PPARγ activation reduces the cellular,[37] and soluble inflammatory response,[38] that is suggested to alter tissue pathology after SCI as reviewed by McTigue.[39] In the context of experimental systemic inflammation COX-1, which reveals sustained upregulation in the spinal cord after SCI,[40] promotes sickness behaviour.[41] COX related pathways also exert immune modulation in terms of immune depression,[42] and impaired host defense.[42 43] Those effects might aggravate the maladaptive immune response after SCI,[44 45] that is associated with increased susceptibility to infections, which are a risk factor for poorer neurological outcome after SCI.[46] Furthermore, NF-κB, which is activated after SCI,[47] contributes in neurodegenerative disease to microglia-induced loss of motor neurons.[48] Together, anti-phlogistic actions of ibuprofen are likely to reduce neurodegeneration driven by CNS-inflammation,[48 49], which is triggered through the COX and/or NF-κB related systemic inflammatory response syndrome,[42 50], or infections.[42 43] Besides, NF-κB,[51] and COX-metabolites such as prostaglandin E_2,[52] are linked to the induction of neuropathic pain. Thus, NSAIDs might be effective in preventing SCI-specific sequelae such as neuropathic pain,[51 53-56], as well as inflammation-related neurogenic heterotopic ossifications (Figure 1).[57-59]

Ibuprofen is recommended primarily to improve neurological function through the enhanced plasticity conferred by its Rho-inhibiting properties. The combination of Rho-inhibition with anti-inflammatory actions of ibuprofen might, however, dissolve conflicting aspects of anti-inflammatory therapies after axonal injury. It has been demonstrated that secondary axonal damage is reduced when inflammation has been limited, but this occurs at the expense of the regenerative capacity of the spared axons.[60] In this context, an increased blockade of axonal re-growth capacity as a side effect of anti-inflammatory neuroprotective therapy could be prevented by concurrent Rho-inhibition (Figure 1).

In vitro sprouting responses under ibuprofen,[10 16 18 20], or indometacin,[18] treatment in the presence of inhibitory matrix components such as myelin or CSPGs are well reproducible. However, in vivo evidence provided by some groups for promoting effects of

'small molecule' Rho-inhibitors on axonal sprouting,[10 18], or on neurological recovery,[10 18 61 62], have not or have only partially been confirmed by others.[31 63] Reasons for the variability in the results could be multiple. One possible reason would be differences in the experimental design, such as in timing of the experiments, the animal model applied, the route of drug delivery, and assessment tools. On the other hand the variability could be a product of chance due to small sample sizes, which is a general problem in preclinical studies.[64] One approach to address the variability of preclinical studies is to subject them to meta-analysis.[64]

This work includes a systematic review and meta-analysis of experiments reporting the effect of Rho-inhibiting NSAIDs on neurobehavioral recovery after SCI. The published preclinical evidence and its positive predictive value, as representing the justification of the current clinical investigation, were challenged by the meta-analysis. The study protocol of the first clinical trial on high-dose ibuprofen as a Rho-inhibitor after acute SCI is addressing safety, feasibility and pharmacokinetics. Additionally, the study explores preliminary efficacy including aspects of repurposing ibuprofen,[65] as a compound with multiple pharmacological targets for the treatment of SCI.

METHODS AND ANALYSIS

Ibuprofen is a drug that has been FDA-approved and is available worldwide for decades. However, in the context of traumatic injuries its use is generally restricted to short-term low-dose administration as an analgesic. The mid-term high-dose application of ibuprofen, as a Rho-inhibiting and anti-inflammatory treatment after SCI, is not an approved indication and information on its tolerability is not available for the population of acute SCI patients. This is relevant because critically injured SCI patients require treatment in an intensive care unit, which is a risk factor for gastric ulcers,[66] and particularly SCI patients with cervical and high thoracic levels might be at risk for damage to the gastric mucosa due to a disturbance of autonomous innervation.[67] Furthermore, pharmacological data on CNS-permeability are available for non-trauma patients but little is known about pharmacokinetics of orally administered ibuprofen after SCI. Therefore, the SCISSOR-study primarily addresses safety, feasibility and pharmacokinetics under the clinical condition of acute traumatic SCI. Secondary objectives are neurological recovery and SCI-specific complications.

Assessment of underlying evidence

In order to re-asses the preclinical evidence regarding Rho-inhibiting NSAIDs and to justify the risks and efforts of the clinical trial, a systematic review was performed. Six publications,[10 18 31 61-63], containing 11 single experiments with a total of n=255 animals (Table 1) were included for meta-analysis after stepwise study selection (Figure 2).

Table 1: Preclinical study characteristics.

ID	Publication	Drug	Species	n	Dose (Duration)	Motor score	Injury level	Follow -up	Type of Injury	Application
1	Redondo-Castro, E	Ibu	Rats	16	60 mg/kg/d (42d)	BBB	T8	42d	Contusion	s.c.
2	Sharp, K	Ibu	Rats	73	60 mg/kg/d (28d)	BBB	T6/7	42d	Hemisection	s.c.
3	Wang, X	Ibu	Mice	46	35-70 mg/kg/d (28d)	BMS	T8	35d	Transection	s.c.
4	Guth, L	Indo	Rats	12	0.2 mg/d (21d)	Tarlov	T8	21d	Compression	i.p.
5	Wang, X	Ibu	Rats	47	70 mg/kg/d (28d)	BBB	T7	49d	Contusion	s.c.
6 7	Fu, Q	Ibu	Rats	19 12	60 mg/kg/d (28d) 60 mg/kg/d (28d)	BBB	T6/7	42d	Hemisection Contusion	s.c.
8 9 10 11	Pantovic, R	Indo	Rabbits	6 6 6 6 *6*	0.1 mg/kg/d (9d) 0.3 mg/kg/d (9d) 1.0 mg/kg/d (9d) 3.0 mg/kg/d (9d) *vehicle*	Tarlov	L2	9d	Contusion	i.v.
Abbreviations: Ibu=Ibuprofen, Indo= Indometacin, n= number of animals, d= days, s.c.= subcutaneous, i.p.= intraperitoneal, i.v.= intravenous										

Preclinical study characteristics were extracted for each publication and functional outcome was measured for each experiment in order to perform the meta-analysis. The method and statistical approach is described in greater detail elsewhere.[27 68] In brief, we used a

random effects weighted mean difference meta-analysis to calculate an overall estimate of effect size between treated and untreated (control) animals based on the final time point of the assessment of functional recovery. A random effects meta-regression was used to determine how much heterogeneity can be explained by study design characteristics using STATA13 with a significance level of $p<0.05$. We checked for possible publication bias using trim and fill method for funnel plots and Egger regression in STATA13.

The effect size in the open field motor testing of treatments with ibuprofen or indometacin after experimental SCI was 20.2% (95% CI, 10.8 – 29.6) in the overall analysis (Figure 3) and varied in the single experiments from -33.2% (-79,2 – 12.8) to 44.9% (19,5 – 70.4). Meta-regression analyses to identify of subgroup effects regarding the administered drug, the behavioral assessment tool, the SCI model, the route of drug delivery or the study quality revealed no statistically significant proportion of between-study heterogeneity for any of the stratifications. Likewise the tests to detect possible publication bias implied no missing experiment, although statistical significance should not be expected given the study's small overall size.[69]

Nevertheless, the design of the studies on ibuprofen was different from those on indometacin treatment in terms of the neurobehavioral scales, the animal models, and the route of drug delivery (Table 1). The ibuprofen treated animals had all been assessed with the recent Basso Beattie Bresnahan (BBB) score,[70] or the Basso Mouse Scale (BMS),[71] whereas modifications of the outdated Tarlov score,[72] were applied for indometacin treated animals. Furthermore, the ibuprofen treated groups underwent contusion, transection, and hemisection models in contrast to the exclusive use of compression or contusion experiments in indometacin treated groups, which received the drug intravenously or intraperitoneally compared to subcutaneous administration in the ibuprofen groups. Therefore, differences in effect size between the two investigated compounds require careful interpretation and do not allow conclusions on differences in their potential therapeutic efficacy.

Among the 'small molecule' Rho inhibitors, ibuprofen is the most feasible for clinical investigation in the indication of acute traumatic SCI due to its greater quantity and the higher quality of its preclinical data. All studies on ibuprofen revealed Rho-inhibition in vivo within the spinal cord after systemic drug administration,[10 18 31], and comprise experimental models applicable for translational research,[73] as well as recent behavioral scores.[70 71]

Study design

The SCISSOR-study is designed as a prospective non-randomized open label phase I study, as this is a preferred design for tolerability and pharmacokinetic investigations.[74 75]

Study participants are enrolled consecutively in two treatment cohorts characterized by the duration of therapy as further detailed below and illustrated in Figure 4.

Setting

The initiating sponsor and coordinator of the trial is the Department of Experimental Neurology, Clinical and Experimental Spinal Cord Injury Research (Neuroparaplegiology) at Charité University Hospital, Campus Mitte, Berlin, Germany. Data management and statistics are performed by the Department of Clinical Epidemiology and Applied Biostatistics at Eberhard-Karls-University Tübingen, Germany. The recruiting trial center is the Treatment Center for Spinal Cord Injuries at the Trauma Hospital Berlin, Germany. The study investigators are physicians trained and experienced in the management and assessment of patients with acute and chronic SCI.

The reference center for laboratory safety parameters is the Central Laboratory at the trial center, Trauma Hospital Berlin, Germany. The central laboratory is regularly certified for clinical diagnostics. The Department of Pharmaceutical and Medicinal Chemistry, Institute of Pharmacy, Eberhard-Karls-University Tübingen, Germany will perform the measurement of ibuprofen concentrations in plasma and CSF using High Performance Liquid Chromatography - Mass Spectrometry (HPLC-MS). The *Labor Berlin - Charité Vivantes GmbH*, a certified laboratory for clinical and research diagnostics, will run the nephelometric protein measurements in serum and CSF for quantification of post-SCI Blood-Spinal Cord Barrier breakdown.

Intervention

The study medication is ibuprofen in the galenic preparation of water-soluble lysine salt. Ibuprofen lysine salt is absorbed faster, leading to earlier peaks of plasma concentrations compared to the free acid.[76] The brand name of the study medication is Dolormin® extra. Ibuprofen is applied as tablets administered orally for 4 weeks in cohort I or 12 weeks in cohort II, respectively (Figure 4). The daily dose of 2400mg is administered as three single doses of 800 mg. In the case of swallowing disorders, which occur in 16% of tetraplegic acute SCI patients,[77] it is recommended that the tablets be disaggregated in water and the medication administered via stomach tube.

During the first 4 weeks of treatment, the proton pump inhibitor pantoprazole is used as a concomitant medication in a dosage 40mg/d. This reduces the risk of damage to the gastrointestinal mucosa and, after individual risk-benefit assessment, can be reduced to 20mg/d for the following treatment period (applicable to cohort II).

Dose estimation

Ibuprofen doses of 60 to 70 mg/kg/d have been used in preclinical trials.[10 18 31 63] To estimate the pharmacologically active dose (PAD) in humans, we applied a conversion model which is feasible for systemically administered active substances of a small molecular size, provided that further pharmacologic properties of the compound have been taken into account.[78] The human equivalent dose (HED), converted from the PAD in rats, is about 11.3 mg/kg/d (rat PAD of 70 mg/kg/d / 6.2 = HED 11,3 mg/kg/d). The binding capacity for ibuprofen in vitro is higher in human albumin than it is in rat albumin. At identical concentrations, the free bioactive ibuprofen fraction in human albumin solution is lower by a factor of about 3.[79 80] We therefore multiplied the HED by that factor to achieve an estimate of comparable bioactive concentration of 34 mg/kg. Assuming an average body weight of 70 kg, the daily dose of ibuprofen in this trial was therefore set at 2400 mg/d regardless of individual body weight. This is within the FDA-approved range of up to 3200 mg/d for adults.

Outcome measures

The primary endpoint of the study is the safety of high-dose ibuprofen application after SCI as measured by the occurrence of serious adverse events (SAE) related to the study medication. In particular, severe gastroduodenal bleeding attributable to the study medication is the primary safety parameter (Table 2).

Table 2: Clinical trial outcome measures

	Parameter	**Assessments/Measures**	**Timing** (see also Figure 5)	**Safety issue**
Primary Endpoint	Gastroduodenal bleeding	Serious adverse event (SAE) report	Continuous observation	yes
Secondary Endpoints	Adverse events	Adverse event monitoring, SAE/SUSAR (serious unexpected suspected adverse reaction) report	Continuous observation	yes
	Spasticity	Modified Ashworth Scale, anti-spastic medication	Follow-up 1 & 2	yes
	Neuropathic pain	Neuropathic Pain Scale, pain medication	Baseline, Follow-up 1 & 2	yes
	Severity of SCI	ASIA impairment scale	Baseline, Follow-up 1 & 2	no
	Motor function	Upper and lower extremity motor score	Baseline, Follow-up 1 & 2	no
	Sensory function	Pin prick, Light touch	Baseline, Follow-up 1 & 2	no
	Lesion height	Motor and sensory level, zone of partial preservation, if applicable	Baseline, Follow-up 1 & 2	no
	Ibuprofen levels	Blood & Cerebrospinal fluid (CSF) collection	Pharmacokinetics 1, 2, & *3**	no
	Serum/CSF protein levels	Blood & CSF collection	Pharmacokinetics 1, 2, & *3**	no
	Heterotopic	Ultrasound of the hip joints, Magnetic	Baseline,	yes

	ossifications	resonance imaging, if applicable	Follow-up 1 & 2	
Other Endpoints	Laboratory abnormalities	Blood & urine collection	Safety 1, 2, 3 & *4**	yes
	Cardiac arrhythmia	Electrocardiography	Baseline, Follow-up 1 & 2	yes
	Deep vein thrombosis	Ultrasound of pelvic & lower extremity veins	Baseline, Follow-up 1 & 2	yes
	Circulatory disturbance	Blood pressure & heart rate	Baseline, Safety 1, 2	yes
	Clinical observation	Epigastric pain / Pain projected to the shoulder tip	Baseline, Safety 1, 2, & *3**	yes
	Feasibility of recruitment	Screening protocol	Screening	no
Differences between the cohorts are based on the course of an extended intervention. In cohort II additional pharmacokinetic and safety assessments are scheduled (indicated by asterisks).				

Secondary endpoints are all further adverse events (AE) including SAE and suspected unexpected serious adverse reactions (SUSAR). Clinical, laboratory and technical safety examinations facilitate the detection of AEs that can be expected and as well as the assessment of their causality (Table 2). In addition, the sensitive measurement of neuropathic pain and spasticity is also relevant for safety reasons, since the course of those very frequent SCI-specific sequelae might be altered by plasticity-enhancing therapies. The Neuropathic Pain Scale,[81 82], and the Modified Ashworth Scale,[83] are therefore applied for assessment of pain and muscle tone, respectively. The pharmacological laboratory endpoints are ibuprofen levels in plasma and CSF as measured at the time of expected peak levels.[84] The neurological examination is performed according to the International Standards for Neurological Classification of Spinal Cord Injury (ISNCSCI) definitions of 2011.[85] The ISNCSCI comprises the ASIA impairment scale (AIS) as a measure for completeness and severity of SCI, the ASIA motor scores for upper and lower extremity motor function, the ASIA sensory scores for residual pin prick and light touch sensation, the motor and sensory neurological level, as well as the zone of partial preservation, if applicable. Optional elements such as non-key muscles for determination of the AIS are not applied in this study.[85] Neurogenic heterotopic ossifications constitute a further clinical endpoint. These will be identified with an ultrasound screening of the hip joints,[86] followed by MRI if heterotopic ossifications are suspected (Table 2).

Enrolment

In the study center we expect to be screening about 40-60 SCI admitted patients per year, about 6-8 of whom are expected to meet eligibility criteria. The investigators will evaluate patient eligibility as soon as possible after admission to the trial center. The investigators will conduct an interview with each patient to verify the inclusion and exclusion criteria as related to individual medical history as well as to inform the patient about the trial and its potential risks and benefits. Prior to inclusion, written informed consent will be

obtained from the patient, and a written announcement of recruitment will be sent out to the sponsor by the investigators.

Eligibility criteria

The inclusion and exclusion criteria (Table 3) were chosen with regard to scientific, ethical, and practical considerations specific to SCI.[87] Included in the trial up to day 21 post-injury are patients with an acute traumatic motor complete SCI, classified as AIS A or AIS B, and with a neurological level of Th4 to C4. Only in this group of patients is a realistic assessment of neurogenic gastrointestinal bleedings possible, because this classification is most likely to be associated with an autonomic complete lesion,[88] that in the acute stage can cause damage to the gastroduodenal mucosa.[67] The imbalance between the altered sympathetic outflow through the splanchnic nerve and the intact parasympathetic innervation through the vagus nerve,[67] may increase the 'baseline' risk posed by the general post-traumatic and ventilation-triggered stress response.[66]

In order to limit risk to patients, the exclusion criteria comprise all absolute contraindications of the study medication according to the summary of product characteristics. The exclusion criteria also include drug interactions or other conditions mandating precaution. To ensure reliable assessment of safety and preliminary efficacy, patients with concomitant injury to the CNS, pre-existing neurological diseases, or severe psychiatric disorders are excluded from the trial. Other exclusion criteria assure the adherence to legal requirements (Table 3).

Table 3: Clinical trial eligibility criteria

Inclusion criteria
• Acute SCI of the cervical spine due to trauma • Time frame of 4-21 days post-trauma • Motor complete injury AIS (ASIA impairment scale) A and B • Neurological level of the lesion C4-Th4 • No participation in a different clinical trial according to German Medicinal Products Act one month before and during participation in the current trial • The patient has been informed and his/her written consent has been obtained • Age: 18 to 65 years • For women of reproductive age: Negative pregnancy test and highly effective contraception (defined as Pearl Index < 1) or sexual abstinence during participation in the trial.
Exclusion criteria
• Multifocal lesions of the spinal cord • Accompanying TBI with visible structural lesions including intracranial hemorrhage on diagnostic images • Significant accompanying injury to the peripheral nervous system, particularly plexus lesions

- Acute or chronic systemic diseases accompanied by neurological deficits or that have caused permanent neurological deficits which may overlay or hinder the registration of sensomotor functions (e.g. multiple sclerosis, Guillain-Barré syndrome, HIV infection, Lues etc.)
- Malignant neoplasms, except if these are in complete remission.
- Mental diseases or dementia which, in the investigator's opinion, limit the patient's cooperation in respect of the intake of the study medication and/or significantly hinder the registration of follow-up parameters
- Hemophilia
- History of myocardial infarction or stroke
- Current and persistent misuse of illegal drugs or alcohol
- Hypothermia below 35 C°
- Pregnancy and lactation
- All further contraindications to the study medication, including other ingredients of the pharmaceutical form according to the Summary of Product Characteristics
- Known hypersensitivity to the active substance contained in the concomitant medication Pantoprazole or one of the components of the drug.
- Intake of ibuprofen or intake of other active substances from the group of NSAIDs; (Non-steroid anti-inflammatory drugs: e.g., diclofenac, indometacin) or the intake of NSAIDs in maximum recommended daily doses during one week prior to enrolment in the trial
- Simultaneous intake of salicylates, particularly acetylsalicylic acid
- Simultaneous intake of oral anticoagulants, or heparinisation in therapeutic dosage
- Simultaneous intake of systemic glucocorticoids
- Unwilling to consent to storage and transfer of pseudonymized medical data for the purpose of the clinical trial
- Admitted to an institution by a court or official order

Individual timeline

Patient evaluation and inclusion will be performed within 21 days after SCI. The CRF at baseline comprises the eligibility criteria, the assessment of injury date and time, medical history, concomitant injury and surgical interventions. Furthermore, the clinical, laboratory, and technical safety parameters as well as the ISNCSCI are assessed at baseline (Figure 4, Table 2).

Since the intervention in cohort II is of longer duration, more frequent safety and pharmacokinetic assessments will be performed in this arm during the intervention and follow-up (Figure 4). In addition to the continuous monitoring of AEs, safety data comprise laboratory measures and clinical observations will be collected in tightly scheduled safety assessments. Further safety issues such as spasticity and neuropathic pain are part of the follow-up documentation (Figure 4) that also includes the neurological endpoints and possible confounders such as co-medications or infections.[46]

Overall Duration

A recruitment period of 24 months is scheduled. Each patient will be followed-up to 24 weeks post-trauma. After completion of recruitment and follow-up, a further six-month period is planned for clearing the database, the statistical evaluation and preparation of the trial report. The trial was activated in June 2013, but not recruiting. After trial registration and completion the recruiting center's initiation, enrolment started in April 2014. Expected enrolment completion date is the second quarter of 2016. Publication of the trial report is scheduled for the year 2017.

Sample size estimation

The sample size of 12 patients and the analysis strategy are justified by the fact that – given that the number of gastrointestinal ulcerations/bleedings after SCI is 3,5% in the first month as reported by Kewalramani 1979[67] – the probability for the occurrence of more than one event is 6,1%. Consequently, observation of more than one event provides evidence of safety problems of Ibuprofen in the indication of acute SCI and probably limits its use in subsequent phases of the clinical trial. The occurrence of further bleedings in months two and three or during follow-up calls for the same consequences. Nevertheless, based on the abovementioned frequency of gastrointestinal ulcerations, the probability for the occurrence of an event is low (0.7% probability) in a sample of 12 SCI patients.[67] However, the upper bound of the confidence interval for the probability of an event is 38,5%; for zero events it is 26.5%. This mandates implementing additional safety criteria if subsequent study phases are considered, and a placebo control should be taken into consideration. In our pilot study a comparison with patients receiving placebo would have a clear lack of statistical power, so no placebo group is scheduled.

Data management

Data are collected on a paper Case Report Form (pCRF). All patient data are managed with a six-digit pseudonym. At the study office the trial coordinators check the pCRFs for completeness and consistency. Implausible or missing data may be corrected or added after consulting the investigator at the trial site through the sponsor (Queries). The corrected documents will be archived together with the completed CRFs. Data are entered twice to allow double-check for correctness and are stored electronically in a database. Access to the database is restricted, and regular data backups are performed.

Sample handling

Peripheral blood and urine samples collected for laboratory safety measures are analyzed immediately after sample collection at the central laboratory of the trial-center, and

the results are available for the study investigators at once. This facilitates the timely recognition of AEs.

Blood and CSF samples for pharmacological and protein analyses are collected under sterile conditions. Those samples are labeled with the six-digit pseudonym and any personal information of the participants is removed. All samples are processed for storage as soon as possible, at the latest within 8 hours of withdrawal by centrifugation at 3000 g for 10 minutes. Serum, heparin plasma and CSF supernatants are stored at -80°C, with central temperature control up to subsequent batch analysis.

Statistical analysis

The analysis will be based on the safety population, as this is a pilot study for safety and feasibility designed to enable planning of a subsequent study. The primary analysis is based on the incidence of severe gastrointestinal bleedings. If more than one event is observed in the study population (n =12) the principle investigator/sponsor on recommendation of the independent Data-Monitoring-Safety-Board will perform a new risk-benefit assessment and will decide the interruption or early termination of the trial. Additional safety analyses, mainly descriptive and casuistic, will be performed. The descriptive analysis will be according to the scale and distribution of the data, using frequencies and means, medians, quartiles and ranges. Linear regression will be used as appropriate.

Quality Assurance

Adherence to i) the recruitment rate, ii) the selection criteria iii) the treatment in accordance with the protocol, and iv) the investigation time points is regarded as a quality indicator for the course of the trial. An independent monitor is responsible for reviewing study progress, verifying adherence to the protocol, compliance to ICH/GCP and national regulations, and furthermore for handling any problems that arise. The monitor will visit the clinical study sites on a regular basis, first after start of enrollment, then after completion of recruitment into cohort I, and finally at study completion.

Key study data will be checked in all patients. This pertains to patients' demographical data, signed informed consent, adherence to inclusion and exclusion criteria, documentation on primary objectives, and adverse events. Source data verification will be performed for approximately 25% of the data. Any unclear and/or incomplete data will elicit increased in-depth monitoring.

Data Monitoring and Safety Board

An independent Data Monitoring and Safety Board (DMSB) addresses patient safety and performs risk/benefit assessments to ensure that for the patients there is no unavoidable risk

or harm. According to its operating procedures, the DMSB reviews accumulating data from the trial to fulfill the safety monitoring. Additionally, the DMSB will assess trial progress, study integrity, and design aspects. The DMSB provides the sponsor with recommendations regarding study modification, continuation or termination. The DMSB consists of three members: a biostatistician, a neurologist, and an internist, all of whom have practical experience in the work of a DMSB. The DMSB will perform an interim review for safety reasons when the entire cohort I has completed week 4 follow up and after completion of enrollment and, if necessary, upon request of the sponsor and/or principal investigator.

Stopping rules

The discontinuation criteria defined for premature drop-out of a patient from the trial include cases of emergency or circumstances associated with increased risk for the participant, as well as a patient's individual wish (Table 4).

Table 4: Clinical trial stopping rules

Premature drop-out of a patient
• Gastrointestinal ulceration with or without hemorrhage and/or perforation
• A drop in hemoglobin levels below 5 mmol/l consistent after receiving more than 8 red blood cell concentrates
• Acute renal failure, defined as an increase in creatinine levels by more than 50% of the baseline value and/or oliguria (urine volume <500 ml/d) persisting for several days after exclusion of extra renal causes
• Any hypersensitivity reaction that the investigator attributes to the trial medication
• Neurological progression of SCI with ascending paralysis with a loss of more than 2 motor levels
• Cerebrovascular hemorrhage
• Myocardial infarction or stroke
• Any new injury to the spine affecting the spinal cord
• The additional intake of more than 1200 mg/d ibuprofen for more than 1 week or the intake of maximal daily doses of other NSAIDs (Non-steroid anti-inflammatory drugs) for more than 2 weeks during the intervention.
• The patient's personal wish
• Any other situation which, according to the investigator, would be such that further participation in the clinical not be in the best interests of the patient
• The onset of pregnancy
• Later occurrence of exclusion criteria.

Decisions on the discontinuation of the entire trial will be taken, if the risk-benefit assessment demonstrates unjustifiable risks and toxicities or new scientific conclusions during the clinical trial could compromise the safety of the study participants. The decision-

making body consists of the sponsor and principal investigator and acts, if appropriate, also upon recommendation of the DMSB.

ETHICS AND DISSEMINATION

The study protocol was approved by the German state Ethics Board located at the *Landesamt für Gesundheit und Soziales (LaGeSo),* Berlin, Germany (13/0127-EK13) and the Federal Institute for Drugs and Medical Devices *(BfArM)*. Participants will be informed about the trial and its anticipated risks and benefits, orally and in written form, using patient information sheets. Patients' written informed consent will be obtained prior to inclusion. This study complies with the Helsinki Declaration, the principles of Good Clinical Practice (GCP), the German Medicinal Products Act (AMG), and the Personal Data Protection Act. The study with the full official title "The Rho-Inhibitor Ibuprofen for the Treatment of Acute Spinal Cord Injury: Investigation of Safety, Feasibility and Pharmacokinetics" has been registered in the ClinicalTrials.gov database (NCT02096913).

Risk benefit assessment

In a large number of patients, traumatic SCI signifies a severe lifelong physical disability. A standard treatment to promote neuronal plasticity after SCI is not yet available. Based on preclinical investigations in established animal models, a better recovery of neurological function in cases of acute SCI is anticipated from making use of 'small molecule' Rho-inhibition. The systematic review of preclinical data revealed 11 eligible studies on effects of Rho-inhibiting NSAIDs with motor function as behavioral endpoint. These studies were conducted in six laboratories and used four different SCI models in three rodent species. The meta-analysis demonstrated an overall effect size of 20.2%. This is backed up by pervious analyses including studies on specific Rho/ROCK-inhibitors that have demonstrated overall effect sizes of 21% or 15% after correction for publication bias, respectively.[27] Ibuprofen is an established, globally approved drug available for clinical investigation of its ability to improve neurological function by Rho-inhibition. Furthermore, preventive treatments for inflammation-triggered SCI-specific complications in terms of neuropathic pain[51-56] and neurogenic heterotopic ossifications after SCI,[57-59], are not well established. Favorable effects on these threatening sequelae can be anticipated from ibuprofen treatment by the reduction of COX- and NFκB-mediated inflammation in the CNS and the peripheral soft tissue.

The appraised benefits of the intervention have to be weighed against its potential risks, some of which may be serious. Gastrointestinal ulcers accompanied by hemorrhage or by perforation are the most prominent side effect of NSAIDs. According to FDA estimates from 1987, gastrointestinal hemorrhage due to peptic ulcers or perforation occurred in 1-2% of patients under sustained three-month intake of NSAIDs.[89] The factors that increase the risk of gastrointestinal hemorrhage are: advanced age, high daily doses, a medical history of ulcers, simultaneous intake of systemic corticosteroids and the intake of anticoagulants.[90]

Within the group of NSAIDs, ibuprofen has a comparatively low gastrointestinal toxicity.[90] A recent Cochrane database review summarized results from recent clinical trials on long-term high-dose ibuprofen administered to reduce respiratory complications in cystic fibrosis. The studies showed a positive overall benefit-risk profile.[91] However, a clinical data base analysis comparing 1365 ibuprofen treated patients with 8960 controls demonstrated a low overall risk but a higher annual incidence of gastrointestinal bleeding in the ibuprofen group of 0.37% vs. 0.14%.[92] In the acute phase, acute injury to the cervical and upper thoracic segments of the spinal cord is probably an additional risk factor for gastroduodenal ulceration,[67] which is why the gastrointestinal safety of ibuprofen treatment in the context of SCI is the primary endpoint of this trial.

Under normal conditions acute renal failure due to NSAIDs is a rare but serious adverse reaction. The risk for acute renal failure increases in critically ill patients with a volume deficiency, myocardial insufficiency, or pre-existing renal insufficiency; the same holds true for simultaneous administration of other nephrotoxic substances such as aminoglycosides, angiotensin-converting-enzyme inhibitors, and Angiotensin II receptor antagonists.[93] Acute renal failure caused by NSAIDs such as ibuprofen, a substance with a short half-life and rapid achievement of effective levels, commonly manifests within a few days. After early diagnosis and discontinuation of the treatment, renal function usually returns to normal within one week. Only if renal failure is not diagnosed in time may the condition progress rapidly to dependence on dialysis. Compared to other NSAIDs, an intermediate level of nephrotoxicity is reported for ibuprofen.[94] Acute SCI is generally not associated with a disturbance of renal function. However, due to the traumatic etiology of paraplegia, renal function may be transiently limited in some cases due to a volume deficiency or rhabdomyolysis. In those cases renal side effects of ibuprofen might be observed more frequently.

In order to limit the anticipated risks in the Ibuprofen-SCI-Safety trial, its exclusion criteria comprise known risk factors such as age > 65 years, relevant co-morbidities, history of critical events, particularly peptic ulcerations, as well as drug-interactions. In addition, the trial will be conducted under in-hospital conditions of acute care and rehabilitation. In-hospital monitoring and carefully scheduled laboratory investigations allow for early awareness of AE and their immediate medical treatment.

Limitations

Limitations of the clinical trial protocol are its small sample size, the lack of a placebo control group and a relatively wide timeframe for inclusion. This design, chosen with regard to the primary safety endpoint and feasibility of the pharmacokinetic issues, restricts efficacy evaluation. The time-frame of inclusion extended until day 21 after SCI was incorporated for ethical reasons in order to enable the patients giving informed to consent before start of the

intervention. However, a late start of intervention might diminish therapeutic efficacy because recovery-promoting effects of Rho-inhibiton,[22] as well as anti-inflammatory effects of ibuprofen,[95] depend on the timing of the intervention, and an early start of treatment seems favorable.

The meta-analysis of published preclinical experiments is limited by the relative low number of studies specific to ibuprofen/Indometacin mediated Rho-inhibition, and they thus hardly enable meta-regression or adjustment for publication bias. Still, our analysis is in line with a larger previous meta-analysis that also includes studies on specific Rho/ROCK-inhibitors that demonstrated relevant effect sizes after correction for publication bias.[27] A limitation of the single in vivo experiments on ibuprofen is that they lack dose response curves, and all research groups have applied the drug in comparable dosages. Administration of even higher doses would still be within FDA approved range for application in humans and might have larger effects. Confirmative preclinical analyses should therefore also consider dose-response curves to show functional recovery.

Possible consequences

The explorative safety evaluation, feasibility aspects of recruitment and treatment regime in the acute phase after SCI are of interest for the planning of a subsequent randomized controlled trial (RCT) in a larger sample. Of particular relevance in the clinical trial are treatment timing and the CNS availability of the systemically delivered compound behind the blood spinal cord barrier after acute SCI. An interim bed to bench-side translation based on the clinical pharmacological data and preliminary efficacy endpoints could be valuable for adjustment of the treatment schedule before embarking on a RCT. Regardless to the result of the primary and secondary outcome assessments the clinical trial will be reported as publication in a peer-reviewed journal.

Improved neurological recovery anticipated after SCI, which is proposed as the main objective of a subsequent RCT might lead to an improvement of aspects of daily living, even if the recovery has affected only two segments of the spinal cord. For example, regaining more than one neurological motor level can be considered as a notable difference with influence on physical independence,[96] and long-term survival.[97] Prevention of SCI related complications might contribute additionally to improved quality of life.

Authors' contributions

MAK, TL, PM, SMS, JMS designed the trial protocol. TL, RW, PM, SL, CB, RS, GJJ, SK, AE, UD, AN reviewed the trial protocol. MAK, RW did the systematic review. RW performed the meta-analysis. MAK, RW wrote the manuscript. All authors critically revised the

manuscript for important intellectual content. All authors have read and approved the final version of the manuscript.

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Competing interests

We declare to have no conflicts of interest.

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Figure Legends

Figure 1: Pharmacological targets of ibuprofen. Intracellular signaling cascades converge at the GTPase RhoA, which is activated after SCI by myelin and scar associated proteins (for review,[5 7 28]). Downstream to Rho, the activated ROCK inhibits axonal re-growth, promotes neurodegeneration, contributes to the development of neuropathic pain and tissue loss, and impedes neurorestoration and functional recovery (reviewed by Watzlawick et al.[27]). This pathway can be blocked by the ROCK-inhibitors Y-27632 and Fasudil or the specific Rho-inhibitors $P21^{CIP1/WAF1}$, C3 transferase[27], and by the R(-) and S(-) enantiomers of ibuprofen,[10 16 18 20 30 31], as the most convincing Rho-inhibitor among individual drugs from the group of NSAIDs. Ibuprofen mediated Rho-inhibition depends on the upregulation of PPARγ.[16] Treatment with PPARγ agonists was demonstrated to have anti-inflammatory effects[37 38] and to protect tissue and thereby motor function in other CNS injury conditions (reviewed by McTigue,[39]). It is not yet clear whether the inhibition of NF-κB as a further target of R(-)/S(+) ibuprofen,[36] is independent of PPARγ. Notably, PPARγ inhibits gene expression by antagonizing the activities of the pro-inflammatory transcription factors NF-κ□B.[37] Another pathway, mainly operated by the S-enantiomer of ibuprofen, is the inhibition of COX 1/2 and consequently of the prostaglandin E_2 production, which activates NF-κB or counter-regulates it at very high concentrations.[52] COX 1/2 or NF-κB are associated with inflammation-induced neuropathic pain,[51 52], neurodegeneration,[48] sickness behavior,[41] and the systemic inflammatory response syndrome.[42 50] Systemic inflammation contributes to neurogenic heterotopic ossificastions.[57]

Taken together, Rho-blocking NSAIDs have the potential to decrease the systemic and acute CNS inflammatory response by targeting at least two separate pathways, PPARγ and COX 1/2. The suspected side effect of neuroprotective anti-inflammatory therapy, that is, that it further limits the regeneration capacity of spared axons,[60] is suggested to be abrogated by Rho-Inhibition. Abbreviations: CNS = central nervous system; COX = cyclooxygenase; NSAIDs = non-steroid anti-inflammatory drugs; PPARγ = peroxisome proliferator-activated receptor γ; NF-κB = nuclear factor-κB; ROCK = Rho-associated coiled kinase; SCI = spinal cord injury.

Figure 2: Systematic review preclinical study selection chart. To identify animal studies reporting the effect of ibuprofen or indometacin treatment for neurobehavioral recovery after SCI the following search term was used for PubMed, EMBASE, and ISI Web of science (search conducted May 18, 2015): (Ibuprofen OR Indometacin OR NSAID OR nonsteroidal anti-inflammatory drugs) AND (spinal cord injury OR hemisection OR contusion

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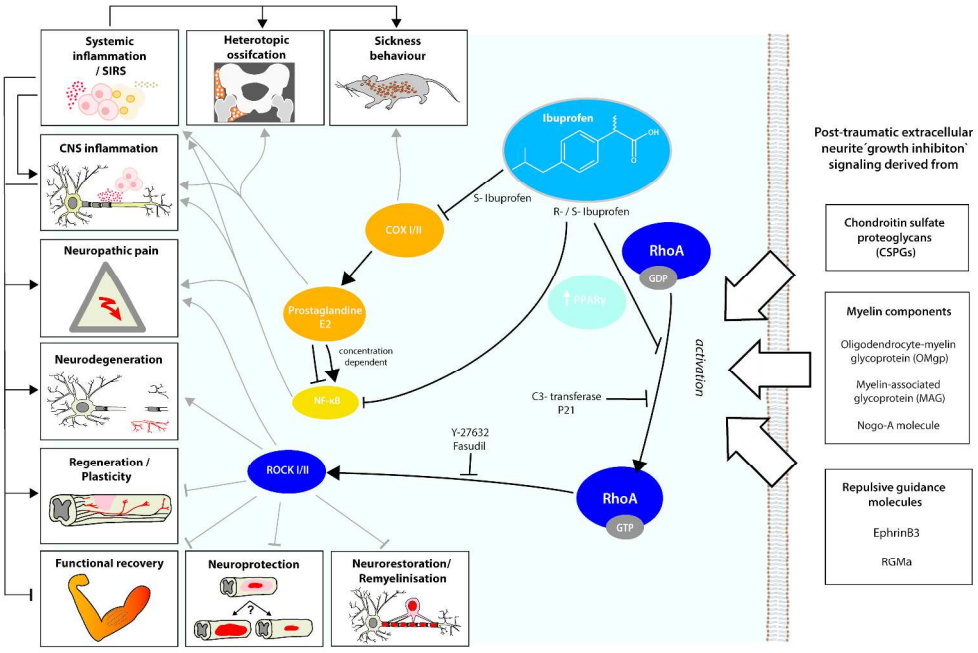
Figure 3: Meta analysis of preclinical effects on motor recovery. Improvement in neurobehavioral score ranked by effect size. The overall number of included animals was n=255 (median n=12, range: 8 – 73). Black dots represent studies using Ibuprofen; white dots show Indometacin studies. The horizontal bar represents the 95% CI of the effect size (ES). Details on the design of the included studies are summarized in Table 1.

Figure 4: Longitudinal clinical trial design. Diagram of frequency and scope of trial procedures. The evaluation for eligibility should start as early as possible after acute SCI. The baseline will be obtained at the day of the inclusion from day 4 and latest at day 21 post-trauma, in any case as early as possible. Start of the study medication is directly after the baseline assessment. The duration of the intervention is 4 weeks for cohort I, and 12 weeks for cohort II. Frequent safety laboratory measurements are performed. Samples for pharmacokinetic measurements are collected two times in cohort I and three times in cohort II. The follow-up visits for determination of secondary endpoints are performed at week 4 (± 3 days) and after the end of intervention at week 24 (± 14 days). Final safety laboratory measurements will be performed 4 weeks after the end of the study medication. Abbreviations: SCI = spinal cord injury.



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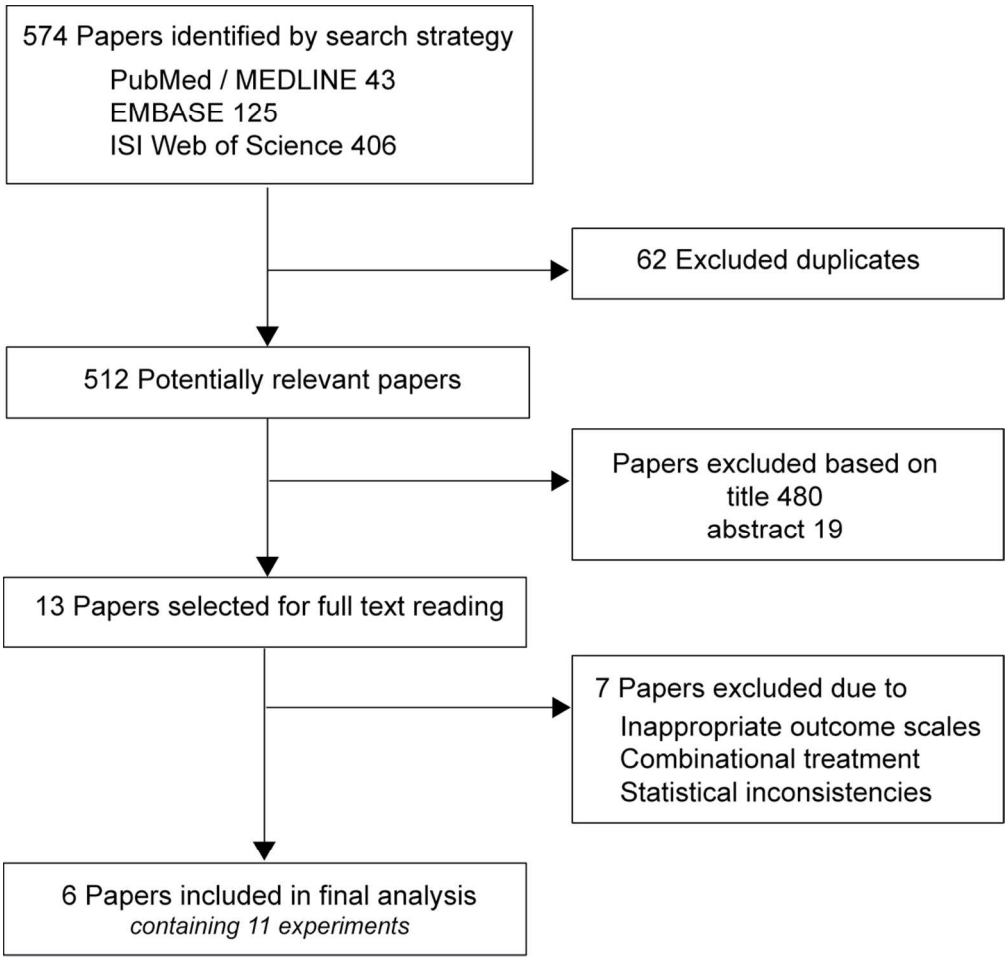

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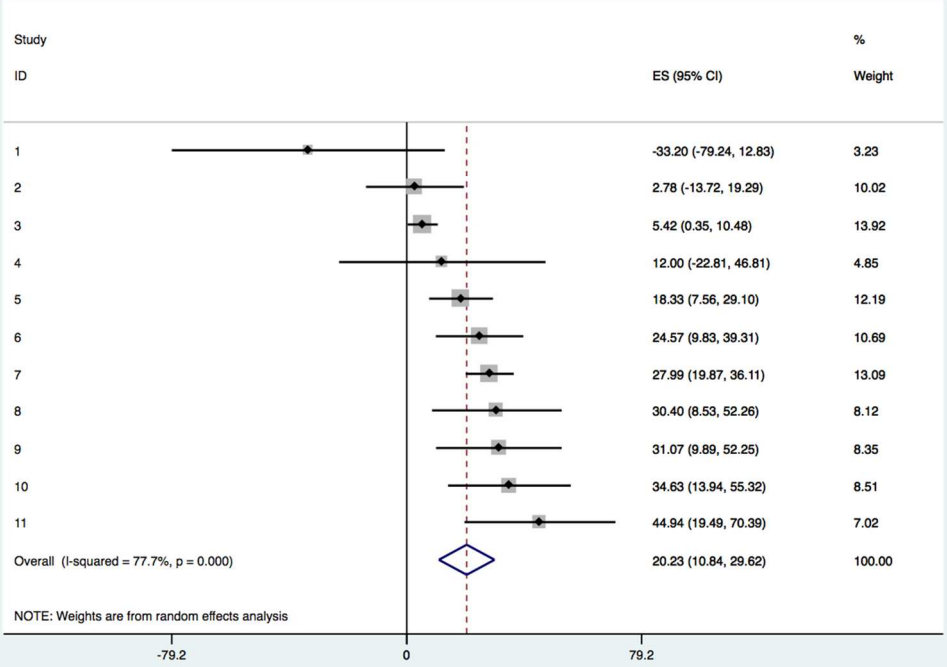

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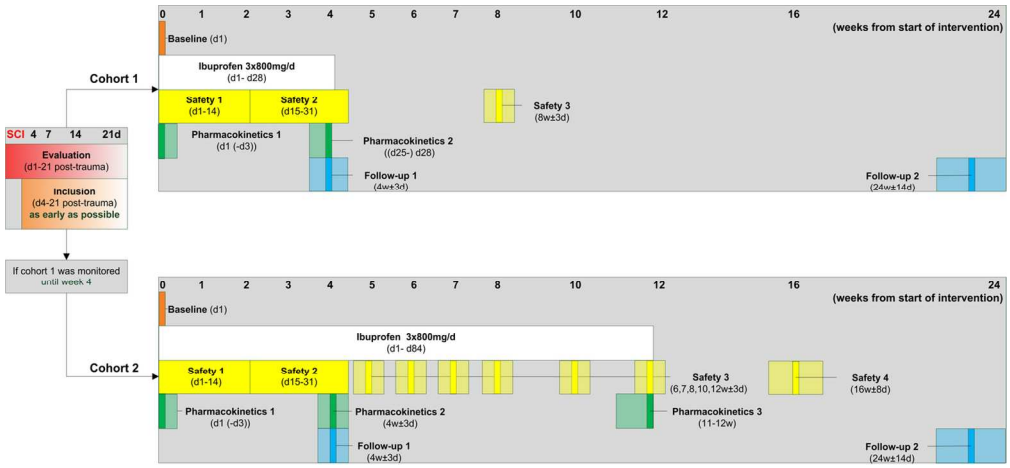

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SCISSOR – Spinal Cord Injury Study on Small molecule derived Rho-inhibition: A Clinical Study Protocol

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Primary Subject Heading:	Neurology
Secondary Subject Heading:	Pharmacology and therapeutics, Research methods
Keywords:	ibuprofen, Neuroprotection, Plasticity, Neuropathic pain, Heterotopic ossifications

Study Protocol

SCISSOR – Spinal Cord Injury Study on Small molecule derived Rho-inhibition: A Clinical Study Protocol

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ABSTRACT

Introduction The approved analgesic and anti-inflammatory drugs ibuprofen and indometacin block the small GTPase RhoA, a key enzyme that impedes axonal sprouting after axonal damage. Dosages required to block the Rho-Pathway in a central nervous system effective manner are higher compared with orthodox cyclooxygenase-blocking effects. Preclinical studies in spinal cord injury (SCI) imply improved motor recovery after ibuprofen/indometacin-mediated Rho-inhibition. This has been re-assessed by a meta-analysis of the underlying experimental evidence, which indicates an overall effect size of 20.2% regarding motor outcome achieved after ibuprofen/indometacin treatment compared to vehicle controls. In addition, ibuprofen/indometacin may also limit sickness behavior, non-neurogenic systemic inflammatory response syndrome (SIRS), neuropathic pain, and heterotopic ossifications after SCI. Consequently, 'small molecule' mediated Rho-inhibition after acute SCI warrants clinical investigation.

Methods and analysis Protocol of an investigator initiated clinical open-label pilot trial on high-dose ibuprofen treatment after acute traumatic, motor complete SCI. A sample of n=12 patients will be enrolled in two cohorts treated with 2400mg/d ibuprofen for 4 or 12 weeks, respectively. The primary safety endpoint is: occurrence of serious adverse events, primarily gastroduodenal bleedings. Secondary endpoints are pharmacokinetics, feasibility, and preliminary effects on neurological recovery, neuropathic pain and heterotopic ossifications. The primary safety analysis is based on the incidence of severe gastrointestinal bleedings. Additional analyses will be mainly descriptive and casuistic.

Ethics and dissemination The clinical trial protocol was approved by the responsible German state Ethics Board, and the Federal Institute for Drugs and Medical Devices. The study complies with the Helsinki Declaration, the principles of Good Clinical Practice and all further applicable regulations. This safety and pharmacokinetics trial informs the planning of a subsequent randomized controlled trial. Regardless to the result of the primary and secondary outcome assessments the clinical trial will be reported as publication in a peer-reviewed journal.

Registration ClinicalTrials.gov identifier: NCT02096913.

(299 words)

Keywords: ibuprofen, neuroprotection, plasticity, neuropathic pain, heterotopic ossifications

STRENGTHS AND LIMITATIONS

- The SCISSOR study is the first clinical trial on high-dose application of the globally approved NSAID ibuprofen as a “small molecule” Rho-Inhibitor after acute traumatic spinal cord injury (SCI) within a concept of drug repurposing.
- Preclinical evidence for recovery-enhancing effects of ibuprofen-mediated Rho-inhibition after SCI has been challenged by systematic review and meta-analysis.
- Limitations of this pilot study inherent to a phase I trial are small sample size, the lack of a placebo control group, and a relatively wide timeframe for inclusion.
- The results of the SCISSOR trial might inform an interim bed to bench-side translation and subsequent randomized controlled trials.

INTRODUCTION

At present, the effective pharmacological treatment of acute traumatic spinal cord injury (SCI) is an unmet medical need.[1] The current opportunities for restitution of neurological function after SCI are limited to early surgical decompression, stabilization, intensive care, rehabilitation, and the prevention or therapy of SCI specific sequelae.[2] Neuroprotective or plasticity enhancing therapies are under investigation in preclinical studies and early-phase clinical trials. As yet, however, none of these approaches could be translated into clinical routine.[2-4]

A major reason for the poor prognosis of central nervous system (CNS) injury is the incapacity of axons to re-grow within the CNS. Molecular barriers preventing axonal regeneration after SCI are situated in the environment of the injured axon i.e. in the scar tissue and myelin or myelin debris.[5 6] Those molecules, such as chondroitin sulfate proteoglycans (CSPGs), Nogo-A, myelin-associated glycoprotein (MAG), oligodendrocyte-myelin glycoprotein (OMgp), Ephrins, and RGMa are up-regulated after CNS injury and interfere with a repertoire of cognate receptors on the axon membrane as reviewed elsewhere.[6 7] Signals from those receptors are mediated downstream to RhoA. The small GTPase RhoA is a key molecule in a pathway which, once activated, leads to the collapse of axonal growth cones and consequently to failure of axonal plasticity or regeneration.[8] Furthermore, myelin debris inhibits the differentiation of oligodendrocyte precursor cells partially dependent on RhoA-associated pathways[9] and thus may prevent remyelination of spared axons.

Therefore, the Rho-pathway constitutes a target for treatments aiming to overcome molecular obstacles to a restoration of neuronal connectivity and subsequent functional recovery. The inhibition of Rho or the downstream located Rho-associated coiled kinase (ROCK) has been demonstrated to foster axonal sprouting or plasticity,[10-23] to have neuroprotective effects,[10 11 13 14 20 24 25], to promote oligodendrocyte precursor cell differentiation[9] or remyelination,[25] and to enhance neurological recovery[10 11 13 16 18-20 22-24 26] after acute SCI (Figure 1). These findings are backed up with evidence from other experimental CNS-injury conditions as reviewed elsewhere.[7 27] The reported effects of various Rho/ROCK-blocking approaches on open field motor recovery after experimental SCI have been re-assessed by a systematic review and meta-analysis including correction for publication bias.[28] Specific Rho-inhibition mediated by the clostridium botulinum derived enzyme C3-transferase, also referred to as BA-210 or Cethrin™,[29] has been studied in a recently completed Phase I/IIa clinical trial. The investigators concluded that topically applied BA-210 is safe and is associated with favorable neurological outcome.[30] However, a confirmatory Phase III trial has not yet been conducted.

Over the last decade, upcoming evidence has assigned a subset of non-steroid anti-

inflammatory drugs (NSAIDs) to the group of unspecific Rho-inhibitors. The FDA-approved NSAIDs ibuprofen,[11 17 19 21 31 32], indometacin,[19 31] and suldinac sulfide,[31] were shown to inhibit Rho-activation independently of their 'classical' mode of action as inhibitors of Cyclooxygenases (COX). It was subsequently demonstrated that ibuprofen treatment enhances axonal sprouting,[11 17 19], including that of human model neurons,[21] and improves neurological recovery.[11 19] It is noteworthy that ibuprofen-mediated Rho-inhibition involves Peroxisome Proliferator Activated Receptor γ (PPARγ) activation.[17] It remains unclear, however, what the exact mechanism for this activation is and whether co-factors are required for PPARγ-associated Rho-inhibition, because other PPARγ activators such as rosiglitazone also inhibit Rho,[17 33], while other NSAIDs such as Naproxen activate PPARγ strongly[34] but do not cause Rho-inhibition.[11 17 31]

Importantly, ibuprofen dose regimes currently applied in the clinical setting are sub-therapeutic as being likely unable to block the Rho-pathway in the CNS compartment sufficiently. Moreover, NSAIDs are usually applied in a later phase after SCI. Thus, retrospective analysis studying the effect of lower dose NSAID as being applied at present cannot address the hypothesis sufficiently whether ibuprofen mediated Rho-inhibition may elicit improved neurological recovery when being applied in sufficient dosage and appropriate time frame.[35]

Other pharmacological targets of ibuprofen – namely PPARγ-activation,[17 34 36], COX-1/2-inhibition,[37] and NF-κb-inhibition,[38] promise a concomitant limitation of secondary damage by anti-inflammatory actions, but might also modify the effects of Rho-blockade (Figure 1). In more detail, PPARγ activation reduces the cellular,[39] and soluble inflammatory response,[40] that is suggested to alter tissue pathology after SCI as reviewed by McTigue.[41] In the context of experimental systemic inflammation COX-1, which reveals sustained upregulation in the spinal cord after SCI,[42] promotes sickness behaviour.[43] COX related pathways also exert immune modulation in terms of immune depression,[44] and impaired host defense.[44 45] Those effects might aggravate the maladaptive immune response after SCI,[46 47] that is associated with increased susceptibility to infections, which are a risk factor for poorer neurological outcome after SCI.[48] Furthermore, NF-κB, which is activated after SCI,[49] contributes in neurodegenerative disease to microglia-induced loss of motor neurons.[50] Together, anti-phlogistic actions of ibuprofen are likely to reduce neurodegeneration driven by CNS-inflammation,[50 51], which is triggered through the COX and/or NF-κB related systemic inflammatory response syndrome,[44 52], or infections.[44 45] Besides, NF-κB,[53] and COX-metabolites such as prostaglandin $E_{2,}$[54] are linked to the induction of neuropathic pain. Thus, NSAIDs might be effective in preventing SCI-specific sequelae such as neuropathic pain,[53 55-58], as well as inflammation-related neurogenic heterotopic ossifications (Figure 1).[59-61]

Ibuprofen is recommended primarily to improve neurological function through the enhanced plasticity conferred by its Rho-inhibiting properties. The combination of Rho-inhibition with anti-inflammatory actions of ibuprofen might, however, dissolve conflicting aspects of anti-inflammatory therapies after axonal injury. It has been demonstrated that secondary axonal damage is reduced when inflammation has been limited, but this occurs at the expense of the regenerative capacity of the spared axons.[62] In this context, an increased blockade of axonal re-growth capacity as a side effect of anti-inflammatory neuroprotective therapy could be prevented by concurrent Rho-inhibition (Figure 1).

In vitro sprouting responses under ibuprofen,[11 17 19 21], or indometacin,[19] treatment in the presence of myelin or inhibitory matrix components such as CSPGs are well reproducible. However, in vivo evidence provided by some groups for promoting effects of Rho-inhibition on axonal sprouting,[11 19], or on neurological recovery,[11 19 63 64], have not or have only partially been confirmed by others.[32 65] Reasons for the variability in the results could be multiple. One possible reason would be differences in the experimental design, such as in timing of the experiments, the animal model applied, the route of drug delivery, and assessment tools. On the other hand the variability could be a product of chance due to small sample sizes, which is a general problem in preclinical studies.[66] One approach to address the variability of preclinical studies is to subject them to meta-analysis.[66]

This work includes a systematic review and meta-analysis of experiments reporting the effect of Rho-inhibiting NSAIDs on neurobehavioral recovery after SCI. The published preclinical evidence and its positive predictive value, as representing the justification of the current clinical investigation, were challenged by the meta-analysis. The study protocol of the first clinical trial on high-dose ibuprofen as a Rho-inhibitor after acute SCI is addressing safety, feasibility and pharmacokinetics. Additionally, the study explores preliminary efficacy including aspects of repurposing ibuprofen,[67] as a compound with multiple pharmacological targets for the treatment of SCI.

METHODS AND ANALYSIS

Ibuprofen is a drug that has been FDA-approved and is available worldwide for decades. However, in the context of traumatic injuries its use is generally restricted to short-term low-dose administration as an analgesic. The mid-term high-dose application of ibuprofen, as a Rho-inhibiting and anti-inflammatory treatment after SCI, is not an approved indication and information on its tolerability is not available for the population of acute SCI patients. This is relevant because critically injured SCI patients require treatment in an intensive care unit, which is a risk factor for gastric ulcers,[68] and particularly SCI patients with cervical and high thoracic levels might be at risk for damage to the gastric mucosa due to a disturbance of autonomous innervation.[69] Furthermore, pharmacological data on CNS-permeability are available for non-trauma patients but little is known about pharmacokinetics of orally administered ibuprofen after SCI. Therefore, the SCISSOR-study primarily addresses safety, feasibility and pharmacokinetics under the clinical condition of acute traumatic SCI. Secondary objectives are neurological recovery and SCI-specific complications.

Assessment of underlying evidence

In order to re-asses the preclinical evidence regarding Rho-inhibiting NSAIDs and to justify the risks and efforts of the clinical trial, a systematic review was performed. Six publications,[11 19 32 63-65], containing 11 single experiments with a total of n=255 animals (Table 1) were included for meta-analysis after stepwise study selection (Figure 2).

Table 1: Preclinical study characteristics.

ID	Publication	Drug	Species	n	Dose (Duration)	Motor score	Injury level	Follow -up	Type of Injury	Appli-cation
1	Redondo-Castro, E	Ibu	Rats	16	60 mg/kg/d (42d)	BBB	T8	42d	Contusion	s.c.
2	Sharp, K	Ibu	Rats	73	60 mg/kg/d (28d)	BBB	T6/7	42d	Hemisection	s.c.
3	Wang, X	Ibu	Mice	46	35-70 mg/kg/d (28d)	BMS	T8	35d	Transection	s.c.
4	Guth, L	Indo	Rats	12	0.2 mg/d (21d)	Tarlov	T8	21d	Compression	i.p.
5	Wang, X	Ibu	Rats	47	70 mg/kg/d (28d)	BBB	T7	49d	Contusion	s.c.
6 7	Fu, Q	Ibu	Rats	19 12	60 mg/kg/d (28d) 60 mg/kg/d (28d)	BBB	T6/7	42d	Hemisection Contusion	s.c.
8 9 10 11	Pantovic, R	Indo	Rabbits	6 6 6 6 *6*	0.1 mg/kg/d (9d) 0.3 mg/kg/d (9d) 1.0 mg/kg/d (9d) 3.0 mg/kg/d (9d) *vehicle*	Tarlov	L2	9d	Contusion	i.v.
Abbreviations: Ibu=Ibuprofen, Indo= Indometacin, n= number of animals, d= days, s.c.= subcutaneous, i.p.= intraperitoneal, i.v.= intravenous										

Preclinical study characteristics were extracted for each publication and functional outcome was measured for each experiment in order to perform the meta-analysis. The method and statistical approach is described in greater detail elsewhere.[28 70] In brief, we used a random

effects weighted mean difference meta-analysis to calculate an overall estimate of effect size between treated and untreated (control) animals based on the final time point of the assessment of functional recovery. A random effects meta-regression was used to determine how much heterogeneity can be explained by study design characteristics using STATA13 with a significance level of $p<0.05$. We checked for possible publication bias using trim and fill method for funnel plots and Egger regression in STATA13.

The effect size in the open field motor testing of treatments with ibuprofen or indometacin after experimental SCI was 20.2% (95% CI, 10.8 – 29.6) in the overall analysis (Figure 3) and varied in the single experiments from -33.2% (-79,2 – 12.8) to 44.9% (19,5 – 70.4). Meta-regression analyses to identify of subgroup effects regarding the administered drug, the behavioral assessment tool, the SCI model, the route of drug delivery or the study quality revealed no statistically significant proportion of between-study heterogeneity for any of the stratifications. Likewise the tests to detect possible publication bias implied no missing experiment, although statistical significance should not be expected given the study's small overall size.[71]

Nevertheless, the design of the studies on ibuprofen was different from those on indometacin treatment in terms of the neurobehavioral scales, the animal models, and the route of drug delivery (Table 1). The ibuprofen treated animals had all been assessed with the recent Basso Beattie Bresnahan (BBB) score,[72] or the Basso Mouse Scale (BMS),[73] whereas modifications of the outdated Tarlov score,[74] were applied for indometacin treated animals. Furthermore, the ibuprofen treated groups underwent contusion, transection, and hemisection models in contrast to the exclusive use of compression or contusion experiments in indometacin treated groups, which received the drug intravenously or intraperitoneally compared to subcutaneous administration in the ibuprofen groups. Therefore, differences in effect size between the two investigated compounds require careful interpretation and do not allow conclusions on differences in their potential therapeutic efficacy.

Among the 'small molecule' Rho inhibitors, ibuprofen is the most feasible for clinical investigation in the indication of acute traumatic SCI due to its greater quantity and the higher quality of its preclinical data. All studies on ibuprofen revealed Rho-inhibition in vivo within the spinal cord after systemic drug administration,[11 19 32], and comprise experimental models applicable for translational research,[75] as well as recent behavioral scores.[72 73]

Study design

The SCISSOR-study is designed as a prospective non-randomized open label phase I study, as this is a well established design for tolerability and pharmacokinetic

investigations.[76 77] Study participants are enrolled consecutively in two treatment cohorts characterized by the duration of therapy as further detailed below and illustrated in Figure 4.

Setting

The initiating sponsor and coordinator of the trial is the Department of Experimental Neurology, Clinical and Experimental Spinal Cord Injury Research (Neuroparaplegiology) at Charité University Hospital, Campus Mitte, Berlin, Germany, represented by Prof. Jan Schwab. For contact information see correspondence address. Data management and statistics are performed by the Department of Clinical Epidemiology and Applied Biostatistics at Eberhard-Karls-University Tübingen, Germany. The recruiting trial center is the Treatment Center for Spinal Cord Injuries at the Trauma Hospital Berlin, Germany. The study investigators are physicians trained and experienced in the management and assessment of patients with acute and chronic SCI.

The reference center for laboratory safety parameters is the Central Laboratory at the trial center, Trauma Hospital Berlin, Germany. The central laboratory is regularly certified for clinical diagnostics. The Department of Pharmaceutical and Medicinal Chemistry, Institute of Pharmacy, Eberhard-Karls-University Tübingen, Germany will perform the measurement of ibuprofen concentrations in plasma and CSF using High Performance Liquid Chromatography - Mass Spectrometry (HPLC-MS). The *Labor Berlin - Charité Vivantes GmbH*, a certified laboratory for clinical and research diagnostics, will run the nephelometric protein measurements in serum and CSF for quantification of post-SCI Blood-Spinal Cord Barrier breakdown.

Intervention

The study medication is ibuprofen in the galenic preparation of water-soluble lysine salt. Ibuprofen lysine salt is absorbed faster, leading to earlier peaks of plasma concentrations compared to the free acid.[78] The brand name of the study medication is Dolormin® extra. Ibuprofen is applied as tablets administered orally for 4 weeks in cohort I or 12 weeks in cohort II, respectively (Figure 4). The daily dose of 2400mg is administered as three single doses of 800 mg. In the case of swallowing disorders, which occur in 16% of tetraplegic acute SCI patients,[79] it is recommended that the tablets be disaggregated in water and the medication administered via stomach tube.

The proton pump inhibitor pantoprazole is used as a concomitant medication in a dosage 40mg/d. This reduces the risk of damage to the gastrointestinal mucosa. After 4 weeks of treatment and individual risk-benefit assessment, the dosage of pantoprazole may be reduced to 20mg/d during the following weeks of treatment (applicable to cohort II).

Dose estimation

Ibuprofen doses of 60 to 70 mg/kg/d have been used in preclinical trials.[11 19 32 65] To estimate the pharmacologically active dose (PAD) in humans, we applied a conversion model which is feasible for systemically administered active substances of a small molecular size, provided that further pharmacologic properties of the compound have been taken into account.[80] The human equivalent dose (HED), converted from the PAD in rats, is about 11.3 mg/kg/d (rat PAD of 70 mg/kg/d / 6.2 = HED 11,3 mg/kg/d). The binding capacity for ibuprofen in vitro is higher in human albumin than it is in rat albumin. At identical concentrations, the free bioactive ibuprofen fraction in human albumin solution is lower by a factor of about 3.[81 82] We therefore multiplied the HED by that factor to achieve an estimate of comparable bioactive concentrations. Assuming an average body weight of 70 kg, the estimated PAD in humans is 34 mg/kg regardless of individual body weight. The daily dose of ibuprofen in this trial was therefore set at 2400 mg/d. This is within the FDA-approved range of up to 3200 mg/d for adults.

Outcome measures

The primary endpoint of the study is the safety of high-dose ibuprofen application after SCI as measured by the occurrence of serious adverse events (SAE) related to the study medication. In particular, severe gastroduodenal bleeding attributable to the study medication is the primary safety parameter (Table 2). SAE definitions are in accordance with the ICH-GCP guidelines[83]. All other adverse events (AE) that do not fulfill these definitions are documented on AE documentation sheets and type, severity, relatedness, treatment, and outcome are recorded.

Secondary endpoints are all further AE including SAE and suspected unexpected serious adverse reactions (SUSAR). Clinical, laboratory and technical safety examinations facilitate the detection of AEs that can be expected and as well as the assessment of their causality (Table 2). In addition, the sensitive measurement of neuropathic pain and spasticity is also relevant for safety reasons, since the course of those very frequent SCI-specific sequelae might be altered by plasticity-enhancing therapies. The Neuropathic Pain Scale,[84 85], and the Modified Ashworth Scale,[86] are therefore applied for assessment of pain and muscle tone, respectively. The pharmacological laboratory endpoints are ibuprofen levels in plasma and CSF as measured at the time of expected peak levels.[87] The neurological examination is performed according to the International Standards for Neurological Classification of Spinal Cord Injury (ISNCSCI) definitions of 2011.[88] The ISNCSCI comprises the ASIA impairment scale (AIS) as a measure for completeness and severity of SCI, the ASIA motor scores for upper and lower extremity motor function, the ASIA sensory scores for residual pin prick and light touch sensation, the motor and sensory neurological level, as well

as the zone of partial preservation, if applicable. Optional elements such as non-key muscles for determination of the AIS are not applied in this study.[88] Neurogenic heterotopic ossifications constitute a further clinical endpoint. These will be identified with an ultrasound screening of the hip joints,[89] followed by MRI if heterotopic ossifications are suspected (Table 2).

Table 2: Clinical trial outcome measures

	Parameter	**Assessments/Measures**	**Timing** (see also Figure 5)	**Safety issue**
Primary Endpoint	Gastroduodenal bleeding	Serious adverse event (SAE) report	Continuous observation	yes
Secondary Endpoints	Adverse events	Adverse event monitoring, SAE/SUSAR (serious unexpected suspected adverse reaction) report	Continuous observation	yes
	Spasticity	Modified Ashworth Scale, anti-spastic medication	Follow-up 1 & 2	yes
	Neuropathic pain	Neuropathic Pain Scale, pain medication	Baseline, Follow-up 1 & 2	yes
	Severity of SCI	ASIA impairment scale	Baseline, Follow-up 1 & 2	no
	Motor function	Upper and lower extremity motor score	Baseline, Follow-up 1 & 2	no
	Sensory function	Pin prick, Light touch	Baseline, Follow-up 1 & 2	no
	Lesion height	Motor and sensory level, zone of partial preservation, if applicable	Baseline, Follow-up 1 & 2	no
	Ibuprofen levels	Blood & Cerebrospinal fluid (CSF) collection	Pharmacokinetics 1, 2, & *3**	no
	Serum/CSF protein levels	Blood & CSF collection	Pharmacokinetics 1, 2, & *3**	no
	Heterotopic ossifications	Ultrasound of the hip joints, Magnetic resonance imaging, if applicable	Baseline, Follow-up 1 & 2	yes
Other Endpoints	Laboratory abnormalities	Blood & urine collection	Safety 1, 2, 3 & *4**	yes
	Cardiac arrhythmia	Electrocardiography	Baseline, Follow-up 1 & 2	yes
	Deep vein thrombosis	Ultrasound of pelvic & lower extremity veins	Baseline, Follow-up 1 & 2	yes
	Circulatory disturbance	Blood pressure & heart rate	Baseline, Safety 1, 2	yes
	Clinical observation	Epigastric pain / Pain projected to the shoulder tip	Baseline, Safety 1, 2, & *3**	yes
	Feasibility of recruitment	Screening protocol	Screening	no
Differences between the cohorts are based on the course of an extended intervention. In cohort II additional pharmacokinetic and safety assessments are scheduled (indicated by asterisks).				

Data on adverse effects of perioperative NSAIDs on bone healing in terms of pseudoarthrosis after spinal fusion have been discussed in the past.[90] These data, however, are based different types of NSAIDs and from retrospective cohort studies, the results of which are sometimes conflicting.[91 92] In this study all spinal surgeries during the follow-up period will be documented. In combination with data from routinely performed spinal imaging

procedures relevant impairment of bone healing can be detected and would be documented as SAE.

Enrolment

In the study center we expect to be screening about 40-60 SCI admitted patients per year, about 6-8 of whom are expected to meet eligibility criteria. The investigators will evaluate patient eligibility as soon as possible after admission to the trial center. The investigators will conduct an interview with each patient to verify the inclusion and exclusion criteria as related to individual medical history as well as to inform the patient about the trial and its potential risks and benefits. Prior to inclusion, written informed consent will be obtained from the patient. If the patient is willing to consent but is unable to sign, a witness independent from the trial team must confirm the verbal informed consent by providing his/her signature. A written announcement of recruitment will be sent out to the sponsor by the investigators.

Eligibility criteria

The inclusion and exclusion criteria (Table 3) were chosen with regard to scientific, ethical, and practical considerations specific to SCI.[93] Inclusion in the trial is possible from day four up to day 21 post-injury, but should be performed as soon as possible mainly dependent of the patient's ability to give his/her informed consent. Further inclusion criteria are acute traumatic motor complete SCI, classified as AIS A or AIS B, and a neurological level of Th4 to C4. Only in this group of patients is a realistic assessment of neurogenic gastrointestinal bleedings possible, because this classification is most likely to be associated with an autonomic complete lesion,[94] that in the acute stage can cause damage to the gastroduodenal mucosa.[69] The imbalance between the altered sympathetic outflow through the splanchnic nerve and the intact parasympathetic innervation through the vagus nerve,[69] may increase the 'baseline' risk posed by the general post-traumatic and ventilation-triggered stress response.[68]

In order to limit risk to patients, the exclusion criteria comprise all absolute contraindications of the study medication according to the summary of product characteristics. The exclusion criteria also include drug interactions or other conditions mandating precaution. To ensure reliable assessment of safety and preliminary efficacy, patients with concomitant injury to the CNS, pre-existing neurological diseases, or severe psychiatric disorders are excluded from the trial. Other exclusion criteria assure the adherence to legal requirements (Table 3).

Table 3: Clinical trial eligibility criteria

Inclusion criteria
• Acute SCI of the cervical spine due to trauma • Time frame of 4-21 days post-trauma • Motor complete injury AIS (ASIA impairment scale) A and B • Neurological level of the lesion C4-Th4 • No participation in a different clinical trial according to German Medicinal Products Act one month before and during participation in the current trial • The patient has been informed and his/her written consent has been obtained • Age: 18 to 65 years • For women of reproductive age: Negative pregnancy test and highly effective contraception (defined as Pearl Index < 1) or sexual abstinence during participation in the trial.
Exclusion criteria
• Multifocal lesions of the spinal cord • Penetrating spinal cord injury • Accompanying TBI with visible structural lesions including intracranial hemorrhage on diagnostic images • Significant accompanying injury to the peripheral nervous system, particularly plexus lesions • Acute or chronic systemic diseases accompanied by neurological deficits or that have caused permanent neurological deficits which may overlay or hinder the registration of sensomotor functions (e.g. multiple sclerosis, Guillain-Barré syndrome, HIV infection, Lues etc.) • Malignant neoplasms, except if these are in complete remission. • Mental diseases or dementia which, in the investigator's opinion, limit the patient's cooperation in respect of the intake of the study medication and/or significantly hinder the registration of follow-up parameters • Hemophilia • History of myocardial infarction or stroke • Current and persistent misuse of illegal drugs or alcohol • Hypothermia below 35 C° • Pregnancy and lactation • All further contraindications to the study medication, including other ingredients of the pharmaceutical form according to the Summary of Product Characteristics ○ known hypersensitivity to the active substance ibuprofen or one of the ingredients of the drug ○ known reactions by way of bronchospasm, asthma, rhinitis or urticaria after the intake of acetylsalicylic acid or other NSAIDs in the past, ○ unexplained hematopoietic disorders, ○ peptic ulcers or hemorrhagia: either at the present time or occurred repetitively in the past (at least 2 different episodes of proven ulceration or hemorrhage), ○ gastrointestinal hemorrhage or perforation in the patient's medical history in connection with previous treatment with NSAIDs, ○ cerebrovascular or other active hemorrhage, ○ severe disturbance of liver function (with coagulation disorder due to reduced protein synthesis) ○ severe renal function disorder (defined as chronic renal insufficiency, including post-kidney transplantation or acute renal failure, defined as elevated creatinine values and/or oliguria for several days with a limited GFR) ○ severe myocardial insufficiency (NYHA grade III-IV) ○ severe dehydration (caused by vomitus, diarrhea or insufficient volume resuscitation). • Known hypersensitivity to the active substance contained in the concomitant medication Pantoprazole or one of the components of the drug. • Intake of ibuprofen or intake of other active substances from the group of NSAIDs; (Non-steroid anti-inflammatory drugs: e.g., diclofenac, indometacin) or the intake of NSAIDs in maximum recommended daily doses during more than one week prior to enrolment in the trial • Simultaneous intake of salicylates, particularly acetylsalicylic acid • Simultaneous intake of oral anticoagulants, or heparinisation in therapeutic dosage • Simultaneous intake of systemic glucocorticoids • Unwilling to consent to storage and transfer of pseudonymized medical data for the purpose of the clinical trial • Admitted to an institution by a court or official order

Individual timeline

Patient evaluation and inclusion will be performed within 21 days after SCI. The CRF at baseline comprises the eligibility criteria, the assessment of injury date and time, medical history, concomitant injury and surgical interventions. Furthermore, the clinical, laboratory, and technical safety parameters as well as the ISNCSCI are assessed at baseline (Figure 4, Table 2).

Since the intervention in cohort II is of longer duration, more frequent safety and pharmacokinetic assessments will be performed in this arm during the intervention and follow-up (Figure 4). In addition to the continuous monitoring of AEs, safety data comprise laboratory measures and clinical observations will be collected in tightly scheduled safety assessments up to 24 weeks after inclusion. This timeframe seems reasonable for the recognition of the major safety endpoints. Further safety issues such as spasticity and neuropathic pain are part of the follow-up documentation (Figure 4) that also includes the neurological endpoints and possible confounders such as co-medications or infections.[48]

Overall Duration

A recruitment period of 24 months is scheduled. Each patient will be followed-up to 24 weeks post-trauma. After completion of recruitment and follow-up, a further six-month period is planned for clearing the database, the statistical evaluation and preparation of the trial report. The trial was activated in June 2013, but not recruiting. After trial registration and completion the recruiting center's initiation, enrolment started in April 2014. Expected enrolment completion date is the second quarter of 2016. Publication of the trial report is scheduled for the year 2017.

Sample size estimation

The sample size of 12 patients and the analysis strategy are justified by the fact that – given that the number of gastrointestinal ulcerations/bleedings after SCI is 3,5% in the first month as reported by Kewalramani 1979[69] – the probability for the occurrence of more than one event is 6,1%. Consequently, observation of more than one event provides evidence of safety problems of Ibuprofen in the indication of acute SCI and probably limits its use in subsequent phases of the clinical trial. The occurrence of further bleedings in months two and three or during follow-up calls for the same consequences. Nevertheless, based on the abovementioned frequency of gastrointestinal ulcerations, the probability for the occurrence of an event is low (0.7% probability) in a sample of 12 SCI patients.[69] However, the upper bound of the confidence interval for the probability of an event is 38,5%; for zero events it is 26.5%. This mandates implementing additional safety criteria if subsequent study phases are considered, and a placebo control should be taken into consideration. In our pilot study a

comparison with patients receiving placebo would have a clear lack of statistical power, so no placebo group is scheduled.

Data management

All study documents including personal identifiers are stored at the recruiting trial centre in locked file cabinets in a room with restricted access. Data are collected on a paper Case Report Form (pCRF) and CRFs and all patient data are managed with a six-digit pseudonym. At the sponsor's study office the trial coordinators check the pCRFs for completeness and consistency. Implausible or missing data may be corrected or added after consulting the investigator at the trial site through the sponsor (Queries). The corrected documents will be archived together with the completed CRFs. Data are entered twice to allow double-check for correctness and are stored electronically in a database (Oracle). Access to the database is restricted, and regular data backups are performed. The principal investigator / sponsor and the trial statistician will have full access to the dataset.

Sample handling

Peripheral blood and urine samples collected for laboratory safety measures are analyzed immediately after sample collection at the central laboratory of the trial-center, and the results are available for the study investigators at once. This facilitates the timely recognition of AEs.

Blood and CSF samples for pharmacological and protein analyses are collected under sterile conditions. Those samples are labeled with the six-digit pseudonym and any personal information of the participants is removed. All samples are processed for storage as soon as possible, at the latest within 8 hours of withdrawal by centrifugation at 3000 g for 10 minutes. Serum, heparin plasma and CSF supernatants are stored at the sponsor's institution at -80°C, with central temperature control up to subsequent batch analysis.

Statistical analysis

The analysis will be based on the safety population, as this is a pilot study for safety and feasibility designed to enable planning of a subsequent study. The primary analysis is based on the incidence of severe gastrointestinal bleedings. If more than one event is observed in the study population (n =12) the principle investigator/sponsor on recommendation of the independent Data-Monitoring-Safety-Board will perform a new risk-benefit assessment and will decide the interruption or early termination of the trial. Additional safety analyses, mainly descriptive and casuistic, will be performed. The descriptive analysis will be according to the scale and distribution of the data, using frequencies and means, medians, quartiles and ranges. Linear regression will be used as appropriate.

Quality Assurance

Adherence to i) the recruitment rate, ii) the selection criteria iii) the treatment in accordance with the protocol, and iv) the investigation time points is regarded as a quality indicator for the course of the trial. An independent monitor is responsible for reviewing study progress, verifying adherence to the protocol, compliance to ICH/GCP and national regulations, and furthermore for handling any problems that arise. The monitor will visit the clinical study sites on a regular basis, first after start of enrollment, then after completion of recruitment into cohort I, and finally at study completion.

Key study data will be checked in all patients. This pertains to patients' demographical data, signed informed consent, adherence to inclusion and exclusion criteria, documentation on primary objectives, and adverse events. Source data verification will be performed for approximately 25% of the data. Any unclear and/or incomplete data will elicit increased in-depth monitoring.

Data Monitoring and Safety Board

An independent Data Monitoring and Safety Board (DMSB) addresses patient safety and performs risk/benefit assessments to ensure that for the patients there is no unavoidable risk or harm. All DMSB members reviewed the trial protocol prior to study activation in order to ensure the implementation of safety endpoints and procedures necessary to fulfill the DMSB's assignment. According with its operating procedures, the DMSB reviews accumulating data from the trial to fulfill the safety monitoring. Additionally, the DMSB will assess trial progress, study integrity, and design aspects. The DMSB provides the sponsor with recommendations regarding study modification, continuation or termination. The DMSB consists of three members: a biostatistician, a neurologist, and an internist, all of whom have practical experience in the work of a DMSB. The DMSB will perform an interim review for safety reasons when the entire cohort I has completed week 4 follow up and after completion of enrollment and, if necessary, upon request of the sponsor and/or principal investigator.

Stopping rules

The discontinuation criteria defined for premature drop-out of a patient from the trial include cases of emergency or circumstances associated with increased risk for the participant, as well as a patient's individual wish (Table 4). Patients who have dropped out of the trial prematurely should be examined from the time of discontinuation of treatment according to the scheduled program, provided the patient has given his/her consent to such examination. At least the final examination should be performed as far as possible.

Decisions on the discontinuation of the entire trial will be taken, if the risk-benefit assessment demonstrates unjustifiable risks and toxicities or new scientific conclusions during the clinical trial could compromise the safety of the study participants. The decision-making body consists of the sponsor and principal investigator and acts, if appropriate, also upon recommendation of the DMSB.

Table 4: Clinical trial stopping rules

Premature drop-out of a patient
• Gastrointestinal ulceration with or without hemorrhage and/or perforation • A drop in hemoglobin levels below 5 mmol/l consistent after receiving more than 8 red blood cell concentrates • Acute renal failure, defined as an increase in creatinine levels by more than 50% of the baseline value and/or oliguria (urine volume <500 ml/d) persisting for several days after exclusion of extra renal causes • Any hypersensitivity reaction that the investigator attributes to the trial medication • Neurological progression of SCI with ascending paralysis with a loss of more than 2 motor levels • Cerebrovascular hemorrhage • Myocardial infarction or stroke • Any new injury to the spine affecting the spinal cord • The additional intake of more than 1200 mg/d ibuprofen for more than 1 week or the intake of maximal daily doses of other NSAIDs (Non-steroid anti-inflammatory drugs) for more than 2 weeks during the intervention. • The patient's personal wish • Any other situation which, according to the investigator, would be such that further participation in the clinical not be in the best interests of the patient • The onset of pregnancy • Later occurrence of exclusion criteria.

ETHICS AND DISSEMINATION

The study protocol (protocol version 1.2, date 06.05.2013) was approved by the Ethics Board of the *Landesamt für Gesundheit und Soziales (LaGeSo),* Berlin, Germany (13/0127-EK13) and the Federal Institute for Drugs and Medical Devices *(BfArM)*. The protocol amendment (version 2.0, date 12.08.2015) was related to changes of the Summary of Product Characteristics of the study medication ibuprofen and on a recent advice of the European Medicines Agency (EMA)[95]. Two further exclusion criteria i) "severe dehydration" and ii) "history of myocardial infarction or stroke" were added to the protocol. The abovementioned regulatory authorities approved the amendment.

Participants will be informed about the trial and its anticipated risks and benefits, orally and in written form, using patient information sheets. Patients' written informed consent will be obtained prior to inclusion. This study complies with the Helsinki Declaration, the principles of Good Clinical Practice (GCP), the German Medicinal Products Act (AMG) and the Personal Data Protection Act. The study with the full official title "The Rho-Inhibitor Ibuprofen for the Treatment of Acute Spinal Cord Injury: Investigation of Safety, Feasibility and Pharmacokinetics" has been registered in the ClinicalTrials.gov database (NCT02096913). The registration data is summarized in table 5.

Table 5

Data category	Information
Primary registry and trial identifying number	ClinicalTrials.gov NCT02096913
Date of registration in primary registry	24.03.2014
Secondary identifying numbers	2011-000584-28
Sources of monetary or material support	Charité Universitätsmedizin Berlin, Else Kröner Fresenius Foundation
Primary sponsor	Charitè Universitätsmedizin Berlin, Prof. Jan M. Schwab MD, PhD
Contact for public queries	Prof. Jan M. Schwab MD, PhD (jan.schwab@charite.de) Marcel A. Kopp MD (marcel.kopp@charite.de)
Contact for scientific queries	Prof. Jan M. Schwab MD, PhD (jan.schwab@charite.de) Marcel A. Kopp MD (marcel.kopp@charite.de)
Public title	Safety Study of Ibuprofen to Treat Acute Traumatic Spinal Cord Injury
Scientific title	The Rho-Inhibitor Ibuprofen for the Treatment of Acute Spinal Cord Injury: Investigation of Safety, Feasibility and Pharmacokinetics
Countries of recruitment	Germany
Health conditions or problem studied	Spinal Cord Injury
Interventions	Ibuprofen (Dolormin extra), 2400mg/d (400mg 2-2-2) applied orally for 4 weeks (Arm I; n=6) or 12 weeks (Arm II, n=6).
Key inclusion criteria	Acute traumatic SCI; neurologic level C4-T4; AIS A or B; inclusion at day 4 -21 post-injury; no participation in another clinical trial; written consent; age 18-65 years; no pregnancy of female participants during trial conduction

Key exclusion criteria	Multifocal lesions; penetrating injury; traumatic brain injury (TBI) with visible structural lesions; accompanying injury to the peripheral nervous system (plexus lesions); acute or chronic diseases causing/including neurological deficits; malignant neoplasms; significant mental disease or dementia; hemophilia; history of myocardial infarction / stroke; drug abuse; hypothermia below 35°C; pregnancy/lactation; contraindications/hypersensitivity to study medication; current intake of ibuprofen or other NSAIDs or previous intake of maximum doses one week prior to enrolment; intake of salicylates, systemic glucocorticoids, oral anticoagulants or therapeutic heparinisation; no consent to storage and transfer of trial-based data; admittance to institution by court or official order
Study type	Interventional; Phase 1; open-label
Study activation	20.06.2013
First patient in	07.04.2014
Target sample size	12
Recruitment status	Recruiting
Primary outcomes	Severe gastroduodenal bleedings
Key secondary outcomes	Spasticity; neuropathic pain; ASIA impairment scale; ISNCSCI/ASIA motor and sensory score; documentation of adverse events; plasma and cerebrospinal fluid ibuprofen level; heterotopic ossifications

Risk benefit assessment

In a large number of patients, traumatic SCI signifies a severe lifelong physical disability. A standard treatment to promote neuronal plasticity after SCI is not yet available. Based on preclinical investigations in established animal models, a better recovery of neurological function in cases of acute SCI is anticipated from making use of 'small molecule' Rho-inhibition. The systematic review of preclinical data revealed 11 eligible studies on effects of Rho-inhibiting NSAIDs with motor function as behavioral endpoint. These studies were conducted in six laboratories and used four different SCI models in three rodent species. The meta-analysis demonstrated an overall effect size of 20.2%. This is backed up by pervious analyses including studies on specific Rho/ROCK-inhibitors that have demonstrated overall effect sizes of 21% or 15% after correction for publication bias, respectively.[28] Ibuprofen is an established, globally approved drug available for clinical investigation of its ability to improve neurological function by Rho-inhibition. Furthermore, preventive treatments for inflammation-triggered SCI-specific complications in terms of neuropathic pain[53-58] and neurogenic heterotopic ossifications after SCI,[59-61], are not well established. Favorable effects on these threatening sequelae can be anticipated from ibuprofen treatment by the reduction of COX- and NFκB-mediated inflammation in the CNS and the peripheral soft tissue.

The appraised benefits of the intervention have to be weighed against its potential risks, some of which may be serious. Gastrointestinal ulcers accompanied by hemorrhage or by perforation are the most prominent side effect of NSAIDs. According to FDA estimates from 1987, gastrointestinal hemorrhage due to peptic ulcers or perforation occurred in 1-2% of patients under sustained three-month intake of NSAIDs.[96] The factors that increase the risk of gastrointestinal hemorrhage are: advanced age, high daily doses, a medical history of

ulcers, simultaneous intake of systemic corticosteroids and the intake of anticoagulants.[97] Within the group of NSAIDs, ibuprofen has a comparatively low gastrointestinal toxicity.[97] A recent Cochrane database review summarized results from recent clinical trials on long-term high-dose ibuprofen administered to reduce respiratory complications in cystic fibrosis. The studies showed a positive overall benefit-risk profile.[98] However, a clinical data base analysis comparing 1365 ibuprofen treated patients with 8960 controls demonstrated a low overall risk but a higher annual incidence of gastrointestinal bleeding in the ibuprofen group of 0.37% vs. 0.14%.[99] In the acute phase, acute injury to the cervical and upper thoracic segments of the spinal cord is probably an additional risk factor for gastroduodenal ulceration,[69] which is why the gastrointestinal safety of ibuprofen treatment in the context of SCI is the primary endpoint of this trial.

Under normal conditions acute renal failure due to NSAIDs is a rare but serious adverse reaction. The risk for acute renal failure increases in critically ill patients with a volume deficiency, myocardial insufficiency, or pre-existing renal insufficiency; the same holds true for simultaneous administration of other nephrotoxic substances such as aminoglycosides, angiotensin-converting-enzyme inhibitors, and Angiotensin II receptor antagonists.[100] Acute renal failure caused by NSAIDs such as ibuprofen, a substance with a short half-life and rapid achievement of effective levels, commonly manifests within a few days. After early diagnosis and discontinuation of the treatment, renal function usually returns to normal within one week. Only if renal failure is not diagnosed in time may the condition progress rapidly to dependence on dialysis. Compared to other NSAIDs, an intermediate level of nephrotoxicity is reported for ibuprofen.[101] Acute SCI is generally not associated with a disturbance of renal function. However, due to the traumatic etiology of paraplegia, renal function may be transiently limited in some cases due to a volume deficiency or rhabdomyolysis. In those cases renal side effects of ibuprofen might be observed more frequently.

In order to limit the anticipated risks in the Ibuprofen-SCI-Safety trial, its exclusion criteria comprise known risk factors such as age > 65 years, relevant co-morbidities, history of critical events, particularly peptic ulcerations, as well as drug-interactions. In addition, the trial will be conducted under in-hospital conditions of acute care and rehabilitation. In-hospital monitoring and carefully scheduled laboratory investigations allow for early awareness of AE and their immediate medical treatment. In case that a patient suffers harm from his trial participation, compensation will be covered by a clinical trial specific insurance of the sponsor's institution. After completion of the trial the patients will receive further treatment according to the general principles of long-term rehabilitation of SCI and therapy of related secondary complications.

Limitations

Limitations of the clinical trial protocol are its small sample size, the lack of a placebo control group and a relatively wide timeframe for inclusion. This design, chosen with regard to the primary safety endpoint and feasibility of the pharmacokinetic issues, restricts efficacy evaluation. The time-frame of inclusion extended until day 21 after SCI was incorporated for ethical reasons in order to enable the patients giving informed to consent before start of the intervention. However, a late start of intervention might diminish therapeutic efficacy because recovery-promoting effects of Rho-inhibiton,[23] as well as anti-inflammatory effects of ibuprofen,[102] depend on the timing of the intervention, and an early start of treatment seems favorable.

The meta-analysis of published preclinical experiments is limited by the relative low number of studies specific to ibuprofen/Indometacin mediated Rho-inhibition, and they thus hardly enable meta-regression or adjustment for publication bias. Still, our analysis is in line with a larger previous meta-analysis that also includes studies on specific Rho/ROCK-inhibitors that demonstrated relevant effect sizes after correction for publication bias.[28] A limitation of the single in vivo experiments on ibuprofen is that they lack dose response curves, and all research groups have applied the drug in comparable dosages. Administration of even higher doses would still be within FDA approved range for application in humans and might have larger effects. Confirmative preclinical analyses should therefore also consider dose-response curves to show functional recovery.

Possible consequences

The explorative safety evaluation, feasibility aspects of recruitment and treatment regime in the acute phase after SCI are of interest for the planning of a subsequent randomized controlled trial (RCT) in a larger sample. Of particular relevance in the clinical trial are treatment timing and the CNS availability of the systemically delivered compound behind the blood spinal cord barrier after acute SCI. An interim bed to bench-side translation based on the clinical pharmacological data and preliminary efficacy endpoints could be valuable for adjustment of the treatment schedule before embarking on a RCT.

Improved neurological recovery anticipated after SCI, which is proposed as the main objective of a subsequent RCT might lead to an improvement of aspects of daily living, even if the recovery has affected only two segments of the spinal cord. For example, regaining more than one neurological motor level can be considered as a notable difference with influence on physical independence,[103] and long-term survival.[104] Prevention of SCI related complications might contribute additionally to improved quality of life.

Regardless to the result of the primary and secondary outcome assessments the clinical trial will be reported as publication in a peer-reviewed journal compliant with reporting and authorship criteria according to the principles of Good Scientific Practice.

Authors' contributions

MAK, TL, PM, SMS, JMS designed the trial protocol. TL, RW, PM, SL, CB, RS, GJJ, SK, AE, UD, AN reviewed the trial protocol. MAK, RW did the systematic review. RW performed the meta-analysis. MAK, RW wrote the manuscript. All authors critically revised the manuscript for important intellectual content. All authors have read and approved the final version of the manuscript.

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Competing interests

We declare to have no conflicts of interest.

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Figure Legends

Figure 1: Pharmacological targets of ibuprofen. Intracellular signaling cascades converge at the GTPase RhoA, which is activated after SCI by myelin and scar associated proteins (for review,[5 7 29]). Downstream to Rho, the activated ROCK inhibits axonal re-growth, promotes neurodegeneration, contributes to the development of neuropathic pain and tissue loss, and impedes neurorestoration and functional recovery (reviewed by Watzlawick et al.[28]). This pathway can be blocked by the ROCK-inhibitors Y-27632 and Fasudil or the specific Rho-inhibitors P21$^{CIP1/WAF1}$, C3 transferase[28], and by the R(-) and S(-) enantiomers of ibuprofen,[11 17 19 21 31 32], as the most convincing Rho-inhibitor among individual drugs from the group of NSAIDs. Ibuprofen mediated Rho-inhibition depends on the upregulation of PPARγ.[17] Treatment with PPARγ agonists was demonstrated to have anti-inflammatory effects[39 40] and to protect tissue and thereby motor function in other CNS injury conditions (reviewed by McTigue,[41]). It is not yet clear whether the inhibition of NF-κB as a further target of R(-)/S(+) ibuprofen,[38] is independent of PPARγ. Notably, PPARγ inhibits gene expression by antagonizing the activities of the pro-inflammatory transcription factors NF-κ□B.[39] Another pathway, mainly operated by the S-enantiomer of ibuprofen, is the inhibition of COX 1/2 and consequently of the prostaglandin E_2 production, which activates NF-κB or counter-regulates it at very high concentrations.[54] COX 1/2 or NF-κB are associated with inflammation-induced neuropathic pain,[53 54], neurodegeneration,[50] sickness behavior,[43] and the systemic inflammatory response syndrome.[44 52] Systemic inflammation contributes to neurogenic heterotopic ossificastions.[59]

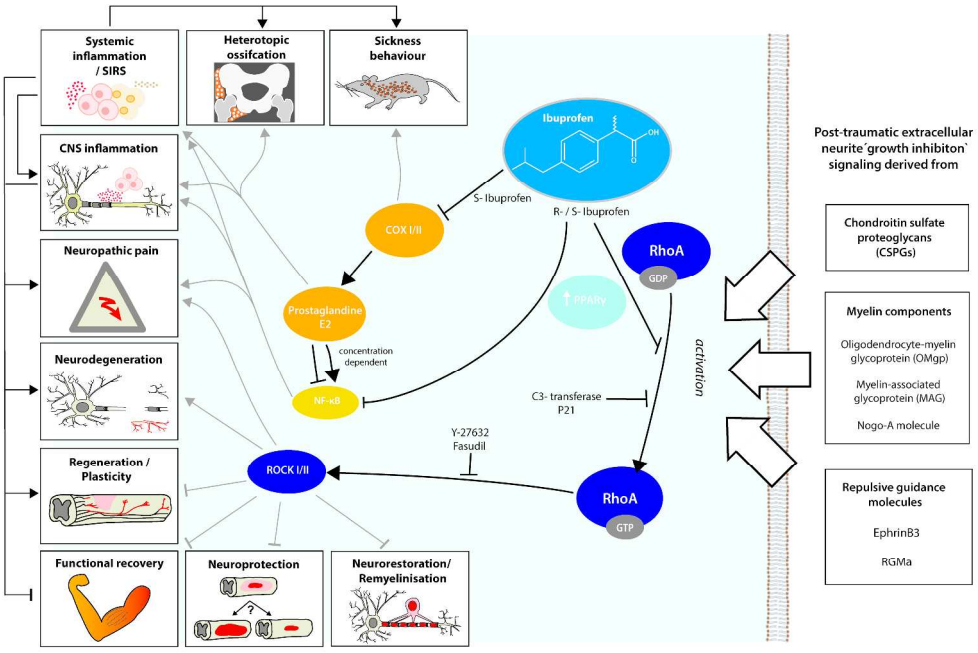
Taken together, Rho-blocking NSAIDs have the potential to decrease the systemic and acute CNS inflammatory response by targeting at least two separate pathways, PPARγ and COX 1/2. The suspected side effect of neuroprotective anti-inflammatory therapy, that is, that it further limits the regeneration capacity of spared axons,[62] is suggested to be abrogated by Rho-Inhibition. Abbreviations: CNS = central nervous system; COX = cyclooxygenase; NSAIDs = non-steroid anti-inflammatory drugs; PPARγ = peroxisome proliferator-activated receptor γ; NF-κB = nuclear factor-κB; ROCK = Rho-associated coiled kinase; SCI = spinal cord injury.

Figure 2: Systematic review preclinical study selection chart. To identify animal studies reporting the effect of ibuprofen or indometacin treatment for neurobehavioral recovery after SCI the following search term was used for PubMed, EMBASE, and ISI Web of science (search conducted May 18, 2015): (Ibuprofen OR Indometacin OR NSAID OR nonsteroidal anti-inflammatory drugs) AND (spinal cord injury OR hemisection OR contusion

OR dorsal column injury OR transection OR corticospinal tract injury OR compression OR spinal cord lesion). Search results were limited to animals. Studies were included if they reported the effects of ibuprofen or indometacin in animal models after various types of SCI. We included SCI experiments comparing functional motor outcome between a group of animals receiving treatment and a control group receiving no treatment (sham group). Non-traumatic models of SCI were excluded, as well as studies reporting only combined treatments. Studies had to report the number of animals for each group, the mean effect size and its variance. Studies were excluded due to inappropriate outcome scales, combination of treatments and statistical inconsistencies.

Figure 3: Meta analysis of preclinical effects on motor recovery. Improvement in neurobehavioral score ranked by effect size. The overall number of included animals was n=255 (median n=12, range: 8 – 73). Black dots represent studies using Ibuprofen; white dots show Indometacin studies. The horizontal bar represents the 95% CI of the effect size (ES). Details on the design of the included studies are summarized in Table 1.

Figure 4: Longitudinal clinical trial design. Diagram of frequency and scope of trial procedures. The evaluation for eligibility should start as early as possible after acute SCI. The baseline will be obtained at the day of the inclusion from day 4 and latest at day 21 post-trauma, in any case as early as possible. Start of the study medication is directly after the baseline assessment. The duration of the intervention is 4 weeks for cohort I, and 12 weeks for cohort II. Frequent safety laboratory measurements are performed. Samples for pharmacokinetic measurements are collected two times in cohort I and three times in cohort II. The follow-up visits for determination of secondary endpoints are performed at week 4 (± 3 days) and after the end of intervention at week 24 (± 14 days). Final safety laboratory measurements will be performed 4 weeks after the end of the study medication. Abbreviations: SCI = spinal cord injury.



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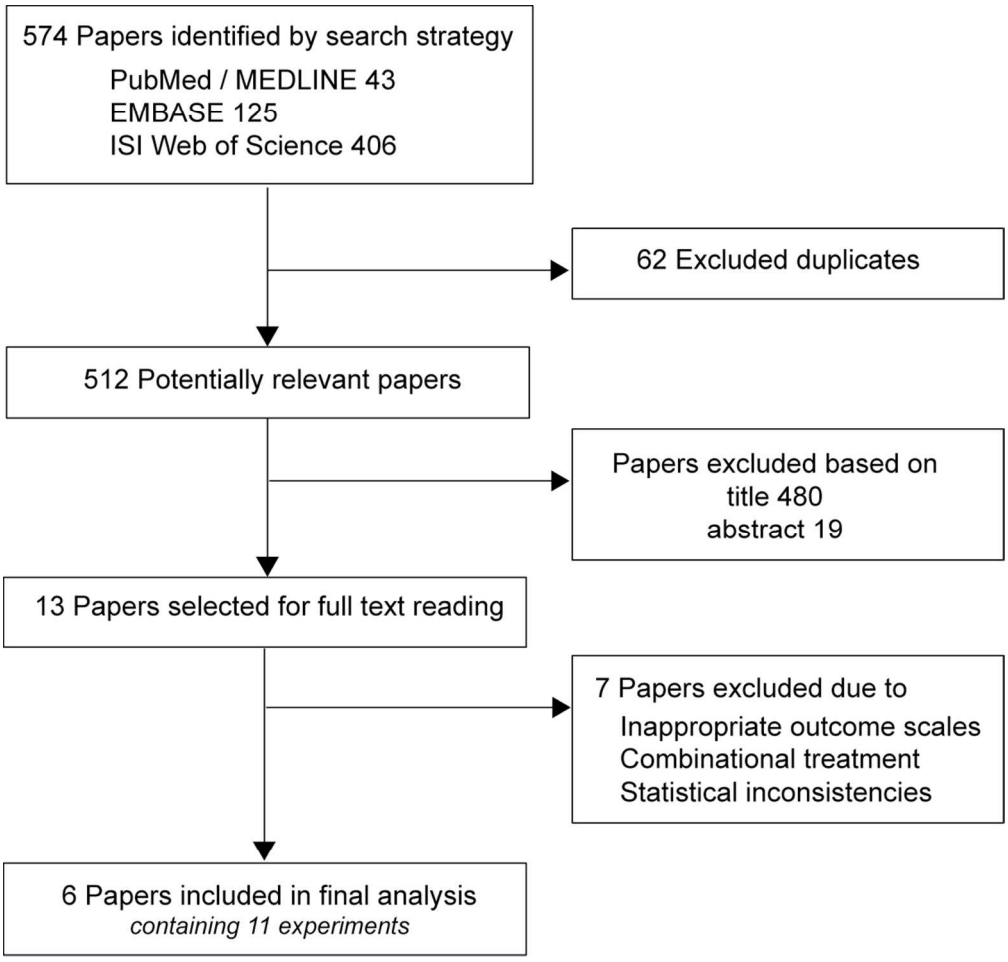

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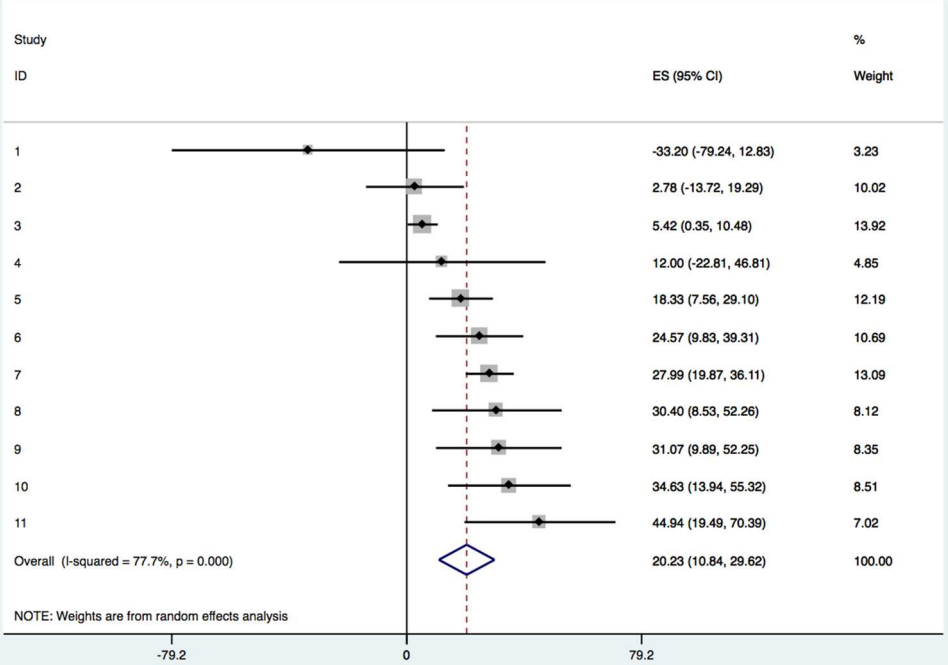

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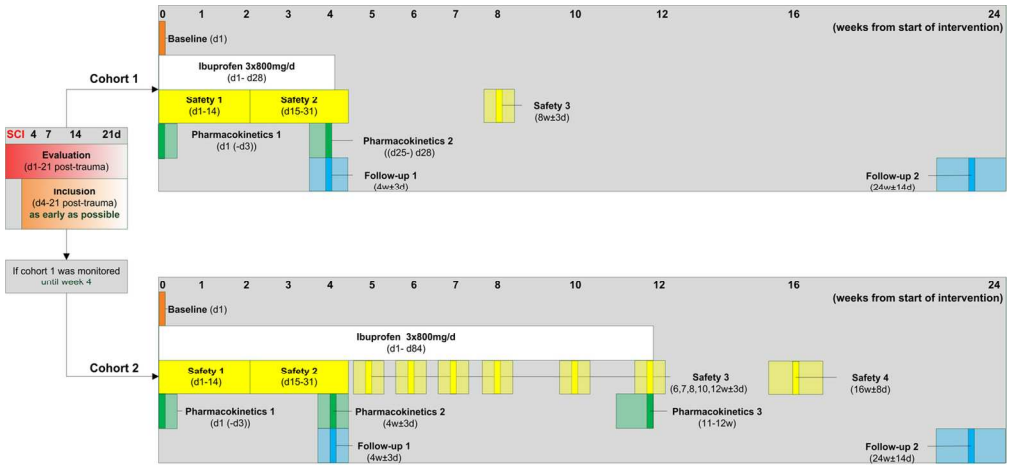

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135x62mm (300 x 300 DPI)

SPIRIT
STANDARD PROTOCOL ITEMS: RECOMMENDATIONS FOR INTERVENTIONAL TRIALS

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	Item No	Description	Addressed on page number
Administrative information			
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	2
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry	3
	2b	All items from the World Health Organization Trial Registration Data Set	19 to 20
Protocol version	3	Date and version identifier	19
Funding	4	Sources and types of financial, material, and other support	24
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	2 and 24
	5b	Name and contact information for the trial sponsor	10
	5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	24
	5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	10

Introduction			
Background and rationale	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	5 to 9
	6b	Explanation for choice of comparators	n.a.
Objectives	7	Specific objectives or hypotheses	7
Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory)	9 and 10
Methods: Participants, interventions, and outcomes			
Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	10
Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	13 to 14
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	10
	11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease)	17 to 18
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	11 and 17
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	14 and 18
Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	11 to 13
Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	15 and 36

Sample size	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	15 to 16_____
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	13_____
Methods: Assignment of interventions (for controlled trials)			
Allocation:			
Sequence generation	16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	n.a._____
Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	n.a._____
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	n.a._____
Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	n.a._____
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	n.a._____
Methods: Data collection, management, and analysis			
Data collection methods	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	11 and 16_____
	18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	17_____

Data management	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	16
Statistical methods	20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	16
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	16
	20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	16
Methods: Monitoring			
Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	17
	21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	17 to 18
Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	11
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	n.a.
Ethics and dissemination			
Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	19
Protocol amendments	25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators)	19

Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	13_____
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	n.a._____
Confidentiality	27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	16_____
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	24_____
Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	16_____
Ancillary and post-trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	21 _____
Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	22 to 23_____
	31b	Authorship eligibility guidelines and any intended use of professional writers	23_____
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	n.a._____ _
Appendices			
Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	Attached as separate files
Biological specimens	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	10 and 16 _

*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "Attribution-NonCommercial-NoDerivs 3.0 Unported" license.