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TITLE PAGE

Non-steroidal anti-inflammatory drug (NSAID) related inhibition of aldosterone glucuronidation and arterial dysfunction in patients with rheumatoid arthritis: a cross-sectional proof of concept study

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ABSTRACT

Objective:
Patients with rheumatoid arthritis (RA) are at increased risk of cardiovascular (CV) disease and are also commonly prescribed non-selective non-steroidal anti-inflammatory drugs (ns-NSAIDS). New *in vitro* evidence suggests that this increased CV risk may be mediated through aldosterone glucuronidation inhibition (AGI), which differs between NSAIDS (diclofenac > naproxen > indomethacin > ibuprofen). Our aim was to explore the association between ns-NSAID-related-AGI and arterial dysfunction.

Methods:
The extent (augmentation index, AIX%) and timing (reflected wave transit time, RWT, msec) of aortic wave reflection (measured using radial applanation pulse wave analysis, PWA, SphygmoCor device) were assessed on a single occasion in 114 consecutive RA patients without overt CV disease aged 40-65 years. A 'higher AIX%' and 'lower RWT' indicate arterial dysfunction. Assessment included fasting blood sample, patient questionnaire and medical record review. Multivariate analysis was used to adjust for age, sex, mean blood pressure, smoking, cumulative erythrocyte sedimentation rate (ESR-years) and Stanford disability score.

Results:
We identified 60 patients taking ns-NSAIDS and 25 non-users. Using a ns-NSAID with the highest AGI was associated with a higher AIX% (and lower RWT) versus treatment with a ns-NSAID with the lowest AGI (diclofenac AIX% 32.3, RWT 132.7 msec; versus ibuprofen AIX% 23.8, RWT 150.9 msec): adjusted mean differences AIX% 6.5 (95%CI 1.0 to 11.9; p=0.02); RWT –14.2 milliseconds (95%CI –22.2 to –6.3; p=0.001). Indomethacin and naproxen demonstrated an intermediate level of arterial dysfunction, although both were more similar to diclofenac than they were to ibuprofen.

Conclusions:
ns-NSAID-related AGI is associated with arterial dysfunction in patients with RA These findings provide a potentially novel insight into the CV toxicity of commonly used ns-NSAIDS. The study findings are limited by the small number of patients involved and require further replication in a much larger study.

KEY WORDS: Arterial dysfunction; Pulse wave analysis; Non-steroidal anti-inflammatory drugs; Rheumatoid arthritis; Aldosterone.

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ARTICLE SUMMARY

Article focus

Aldosterone glucuronidation inhibition (AGI) potentiates the adverse cardiovascular effects of aldosterone. Recently published in vivo research suggests that such inhibition differs between non-selective non-steroidal anti-inflammatory drugs (ns-NSAIDS); with a ranked order of diclofenac > naproxen > indomethacin > ibuprofen. No previous studies have assessed the relationship between ns-NSAID-related AGI and arterial dysfunction in chronic users.

Key messages

In patients with rheumatoid arthritis (RA) we found that chronic use of an ns-NSAID with a greater AGI (diclofenac) was greater arterial dysfunction, assessed using pulse wave analysis (PWA), compared to use of a ns-NSAID with the lower AGI (ibuprofen). This was independent of other cardiovascular and rheumatological factors. It suggests that AGI may play an important role in the CV toxicity of ns-NSAIDS commonly used in routine clinical practice.

Strengths and limitations of this study

A single research nurse assessed RA patients recruited from a consecutive series of patients attending hospital rheumatology clinic who were similar to patients receiving outpatient care elsewhere in the UK. Our multivariate analysis explained a high proportion of the variability in arterial dysfunction among chronic ns-NSAID users and we adjusted for several important cardiovascular and rheumatological factors known to be independently associated with arterial function. The small number of patients taking each NSAID means that the confidence intervals around our findings are wide and the observational cross-sectional design of our study means that we cannot assess causation, nor exclude residual confounding as an explanation for our findings.

INTRODUCTION

Non-steroidal anti-inflammatory (NSAID) are among the most commonly prescribed drugs in clinical practice. Their relative safety has attracted considerable interest, particularly in relation to their association with adverse cardiovascular (CV) events.[1;2] Most of this interest has focused on the role of selective cyclo-oxygenase-2 (COX-2) inhibitors,[3] but non-selective NSAIDS (ns-NSAIDS) also have the potential to increase the risk of adverse CV events. For example, the use of the ns-NSAID diclofenac has been shown to increase the risk of adverse CV events.[2] ns-NSAID use is associated with adverse CV effects including reduction of renal perfusion, electrolyte disturbances (sodium and water retention) and increase in blood pressure. The detrimental effects in renal function are thought to be secondary to a reduced synthesis of vasodilatory prostaglandins such as PGE2. However, ns-NSAIDS have been shown to exert adverse renal effects disproportionate to the level of inhibition of prostaglandin synthesis.[4] This suggests that there might be other yet unknown mechanisms responsible for the potential increase in CV risk associated with ns-NSAID use.[5]

Aldosterone metabolism

Very recent *in vitro* evidence suggests that ns-NSAIDS enhance the action of aldosterone through the inhibition of aldosterone metabolism.[6] Aldosterone is metabolised by 18ß-glucuronidation in both the liver and kidneys in a reaction that is catalyzed by the enzyme UDP-glucuronosyltransferase-2B7. Several ns-NSAIDS have been shown *in vitro* (human kidney cortical microsomes) to inhibit aldosterone 18ß-glucuronidation and individual NSAIDS vary in their ability to inhibit aldosterone glucuronidation.[6] Diclofenac, for example, is a strong inhibitor of glucuronidation, whereas ibuprofen is a weaker inhibitor.[6]

Aldosterone is a mineralocorticoid which plays an important role in the renin-angiotensin-aldosterone system and has generally deleterious effects on the CV system. Higher aldosterone levels are associated with endothelial dysfunction, arterial stiffening, increased arterial wall reflection, myocardial fibrosis and an increase in the risk of CV death.[7-10] Drugs that block the action of aldosterone (such as spironolactone) have been shown to reduce the risk of CV death in patients with heart failure and following myocardial infarction.[11] Aldosterone receptors predominate in the aorta and spironolactone has been shown to improve arterial function assessed using pulse wave analysis (PWA).[7] Consequently the increased risk of adverse CV events in patients taking diclofenac may also be related to the enhanced effects of aldosterone as a consequence of ns-NSAID-related aldosterone glucuronidation inhibition (AGI).

RAAIX study

Patients with rheumatoid arthritis (RA) are known to be at a higher risk of CV death.[12] The RAAIX (RA augmentation index) study was undertaken to assess the relationship between the cumulative inflammatory burden and arterial dysfunction in patients with rheumatoid arthritis (RA).[13;14] RAAIX involved a detailed assessment of both CV and rheumatological features; including the assessment of the use of NSAIDS. Other researchers have subsequently reported the relative level of aldosterone 18ß-glucuronidation inhibition (AGI) for several of the ns-NSAIDS taken by patients in the RAAIX study.[6] In the RAAIX study arterial dysfunction was assessed non-invasively using radial applanation tonometry and pulse wave analysis (PWA).[13;14] PWA is based on the phenomenon of 'arterial wave reflection' which is influenced by pulse wave velocity, endothelial dysfunction, peripheral arterial resistance and left ventricular ejection.[15;16] In each cardiac cycle the outgoing systolic pulse wave is also reflected back towards the heart, predominantly at the level of arterial bifurcations,[17] and returns to the heart during

systole where it augments the central aortic pressure.[15;16] The speed of travel of both outgoing and reflected waves are greater in patients with stiffer arteries; which increases the extent of augmentation (higher AIX%) and reduces the reflected wave transit time (lower RWT).

Study aim

The aim of this exploratory analysis was to assess the association between ns-NSAID-related aldosterone glucuronidation inhibition (AGI) and established markers of arterial dysfunction using data from a previous study of patients with RA.

METHODS

In the original study we recruited patients with a consultant rheumatologist diagnosis of rheumatoid arthritis (RA) by reviewing the medical records of a consecutive series of patients attending hospital-based rheumatology clinics in the city of Aberdeen. We identified patients aged between 40-65 years with RA for more than 6 months duration. We excluded patients with overt arterial disease (angina, prior myocardial infarction, transient ischaemic attack, stroke, arterial revascularisation, intermittent claudication, peripheral arterial disease), atrial fibrillation, heart failure and valvular heart disease. The exclusion of patients with arterial disease was based upon an initial screening patient-questionnaire, resting 12-lead ECG (independently reported by a cardiologist to identify pathological Q-waves, conduction defects, minor Q-waves associated with ST-segment/T-wave anomalies), and a detailed medical record review by a rheumatologist. No participants had a history of recent infection, antibiotic treatment or immunisation within the previous two weeks.

Clinical assessment

Patients attended the Clinical Pharmacology Department at Aberdeen Royal Infirmary (ARI) on a single occasion and underwent assessment by a single clinical research nurse (April-December 2006). Assessment took place in the morning after participants had fasted overnight and abstained from smoking, alcohol and caffeine. Standardised assessment included blood pressure (BP) measurement, pulse wave analysis (PWA), fasting venous blood sample (including erythrocyte sedimentation rate [ESR], rheumatoid factor [RF], and lipid profile). A self-completed patient questionnaire included smoking habit and the Stanford Health Assessment Questionnaire (HAQ).[18] Current medication use was comprehensively reviewed by the research nurse and included use of over-the-counter (without the need for a prescription) NSAIDS. A detailed retrospective review of the medical records using a previously piloted study form, was undertaken by a single rheumatologist blinded to all PWA results and included date of arthritis onset, previous blood test results (erythrocyte sedimentation rate, rheumatoid factor), joint surgery and co-morbidity (including treated hypertension). Our methods have been described in detail elsewhere.[13;14] The nurse remained blind to the patients' previous medical records (which were not made available at assessment) and only reviewed current medication and questionnaire responses (to ensure that all questionnaires items were fully completed), after PWA assessment had been completed.

Pulse wave analysis (PWA)

Patients rested supine in a quiet side-room for at least 10 minutes before undergoing three BP/PWA measurements according to current guidelines.[20] BP was measured at the right brachial artery using an validated automatic oscillometric BP machine (Omron HEM757 IntelliSense BP monitor; Omron Healthcare, Illinois, USA).[21] Pulse wave analysis (PWA) was undertaken using the SphygmoCor device (AtCor Medical, Sydney, Australia) with a hand-held tonometer (Millar, Texas, USA) 'applanated' at the right radial artery. The

'SphygmoCor' PWA device employs a validated 'generalised transfer function' to derive the central aortic pulse waveform from the peripheral waveform.[22] All three PWA recordings were required to have a in-built SphygmoCor quality index score at least 95% (based on average pulse height, pulse height variation and diastolic variation). We have previously demonstrated her high levels of within-observer and between-observer repeatability.[19] The research nurse remained blind to the patients' previous medical history until PWA was completed.

Aldosterone glucuronidation inhibition (AGI)

Aldosterone 18ß-glucuronidation inhibition constants, Ki , derived from *in vitro* studies of human kidney cortical microsomes (HKCM), have been published for 4 of the ns-NSAIDS taken by patients in the RAAIX study (diclofenac 8 µM, naproxen 49 µM, indomethacin 113 µM, ibuprofen 441 µM; a lower Ki indicates greater inhibition).[6] Nabumetone is a close structural analogue of naproxen and the two were combined together in the analysis.

Statistical analysis

Analysis is based on the mean of the three PWA measurements. The principle measures of arterial dysfunction are augmentation index (AIX%) and reflected wave transit time (RWT, msec). Since AIX% varies with heart rate in an individual it was standardised to 75 beats-per-minute.[23] The UK version of the Stanford-HAQ was scored using standard methods without any imputation required for missing data.[18] Cumulative ESR-years were derived from the highest single annual ESR recorded in the medical record during each year of follow-up and calculated using the 'trapezium rule' with linear interpolation when data for a given year was missing.[24] ESR-years reflects both the duration and level of inflammatory burden (e.g. 5 years of arthritis and annual ESR's of 30, 20, 10, 10, 20 mm/hour would equate to approximately 90 ESR-years).

Multiple linear regression (MLR) was used to adjust mean differences in AIX% (and RWT) for variables known to be associated with AIX%, namely: age, sex, mean arterial blood pressure, ever smoked, Stanford HAQ disability score and cumulative ESR-years. Analysis was undertaken using SPSS v 17.We confirmed that the assumptions of linearity, normal distribution and equal variance for MLR were met. 'Goodness to fit' was assessed using the adjusted R^2. The inclusion of additional variables (study ESR, duration of arthritis, fasting cholesterol, smoking pack-years, treated hypertension and current DMARD use) did not alter the adjusted values for AIX and RWT reported, nor improve the goodness to fit of the final regression model.

The study adhered to the principles of the Declaration of Helsinki and was approved by Grampian Research Ethics Committee (study reference: 04/S0801/67). All participants provided informed written consent. The study was funded from a charitable source (NHS Grampian Rheumatology Endowments).

RESULTS

The original study recruited 114 patients. We excluded 9 patients from the analysis who were not currently taking NSAIDS, but had done so within the previous 3 months; and excluded 2 users of infrequently prescribed NSAIDS (ketoprofen and tiaprofenic acid). The characteristics of the remaining 103 patients (82% female) are shown in **Table 1**. No patients were taking aspirin or more than one NSAID concurrently. All NSAID-users had been taking their current NSAID for more than 3 months. Diclofenac was the most commonly used NSAID and was taken by almost a third of patients. One quarter of patients had not taken an NSAID within the previous 3 months (although 88% of these

patients had been prescribed an NSAID in the past). All patients, both NSAID-users and non-users, had previously received DMARD therapy.

Table 1. Characteristics of patients with rheumatoid arthritis

Figures are numbers (%) unless otherwise indicated

	Current NSAID use					
	Yes (n=78)			No (n=25)		
Cardiovascular features						
Female	64	82%		20	80%	
Mean age, years (SD)	53.4	6.9		53.9	5.7	
Mean heart rate, beats per minute (SD)	69.7	10.6		64.5	8.6	
Mean systolic BP, mmHg (SD)	126.2	16.9		124.7	12.1	
Mean diastolic BP, mmHg (SD)	82.9	10.0		81.3	7.6	
Mean pulse pressure, mmHg (SD)	35.6	9.1		36.9	7.4	
Mean arterial BP, mmHg (SD)	99.5	12.2		97.8	8.8	
Mean fasting cholesterol, mmol/L (SD)	5.3	1.2		5.6	1.1	
Mean fasting glucose, mmol/L (SD)	5	0.8		5	0.5	
Mean waist-hip ratio (SD)	0.85	0.08		0.85	0.08	
Ever smoked for 12 months or more	44	56%		17	68%	
Treated hypertension	12	15%		7	28%	
Bendroflumethiazide	10	13%		5	20%	
Atenolol	6	8%		5	20%	
Calcium channel blocker	3	4%		1	4%	
Angiotensin converting enzyme inhibitor	2	3%		3	12%	
Rheumatological features						
Mean age onset arthritis, years (SD)	41.8	10.4		41.4	12.1	
Median duration arthritis, years (IQR)	9	4	15	8	4	16
Median Stanford HAQ disability (IQR)	1.4	0.6	1.9	0.6	0.3	1.3
Median study ESR, mm/h (IQR)	19	8	30	10	6	18
Median cumulative ESR-years (IQR)	206	99	468	93	58	297
Rheumatoid factor positive (>=30 IU/ml)	66	85%		18	72%	
Previous joint surgery	17	22%		4	16%	
Current DMARD therapy	70	90%		24	96%	
Current prednisolone therapy	8	10%		3	12%	
Current NSAID therapy (>3 months)	78	100%		0	0%	
Diclofenac	31	40%		--	--	
Naproxen/ Nabumetone	16	21%		--	--	
Celecoxib/ Etoricoxib	11	14%		--	--	
Ibuprofen	7	9%		--	--	
Meloxicam	7	9%		--	--	
Indomethacin	6	8%		--	--	
Other (ketoprofen, tiaprofemic acid)	2	3%				
Current cytokine therapy	4	5%		1	4%	
Proton pump inhibitor	27	35%		4	16%	
Misoprostol	6	8%		0	0%	
Central arterial function						
Mean augmentation index, AIX% (SD)	31.9	7.9		30.9	8.3	
Mean reflected wave transit time, msec (SD)	135.7	11.4		133.1	12.4	

Blood pressure (BP), disease modifying anti-rheumatic drug (DMARD), erythrocyte sedimentation rate (ESR), Health Assessment Questionnaire (HAQ),non-steroidal anti-inflammatory drug (NSAID), standard deviation (SD), inter-quartile range (IQR)

Table 2. Patient characteristics and use of non-selective non-steroidal anti-inflammatory drugs

Figures are numbers (%) unless otherwise indicated

	Diclofenac (n=31)			Naproxen (n=16)			Indomethacin (n=6)			Ibuprofen (n=7)		
Aldosterone 18ß-glucuronidation inhibition constant (Ki), µM	**Ki = 8**			**Ki = 49**			**Ki = 113**			**Ki = 441**		
Cardiovascular features												
Female	28	90%		12	75%		5	83%		5	71%	
Mean age, years (SD)	54	6.5		55	5.6		57	5.9		50	9.5	
Mean heart rate, beats per minute (SD)	70.7	11.9		73.0	10.5		68.9	4.6		70.0	10.9	
Mean systolic BP, mmHg (SD)	126	17.6		125	20.3		118	14.0		123	13.4	
Mean diastolic BP, mmHg (SD)	83	9.9		82	12.1		75	7.4		84	7.9	
Mean pulse pressure, mmHg (SD)	35.3	10.5		35.3	9.0		34.4	6.1		31.1	8.1	
Mean arterial BP, mmHg (SD)	99	12.2		99	15.3		92	9.8		100	10.3	
Mean fasting cholesterol, mmol/L (SD)	5.4	1.3		5.4	1.5		5.8	0.9		5.1	1.0	
Mean fasting glucose, mmol/L (SD)	4.9	1.0		5.1	0.6		5.0	0.4		4.9	0.5	
Mean waist-hip ratio (SD)	0.85	0.08		0.86	0.09		0.89	0.08		0.88	0.09	
Ever smoked for 12 months or more	17	55%		9	60%		4	67%		3	43%	
Treated hypertension	7	23%		3	19%		2	33%		0	0%	
Rheumatological features												
Mean age onset arthritis, years (SD)	43	11.2		42	8.4		39	7.1		37	11.9	
Median duration arthritis, years (IQR)	9	3	15	10	2	17	19	11	22	11	7	20
Median Stanford HAQ (IQR)	1.4	0.8	2.0	1.6	1.2	1.9	1.5	0.9	1.9	0.6	0.0	1.9
Median ESR-years (IQR)	221	99	526	186	77	435	706	140	825	315	81	829
Rheumatoid factor positive (>=30 IU/ml)	26	84%		13	81%		6	100%		7	100%	
Previous joint surgery	9	29%		4	25%		1	20%		2	29%	
Current DMARD therapy	28	90%		15	94%		5	83%		6	86%	
Central arterial function												
Mean augmentation index, AIX% (SD)	32.3	7.1		34.0	7.3		30.7	3.6		23.8	14.1	
Mean reflected wave transit time, msec (SD)	132.7	7.3		134.5	12.6		136.4	8.7		150.9	18.0	

Blood pressure (BP), disease modifying anti-rheumatic drug (DMARD), erythrocyte sedimentation rate (ESR), Health Assessment Questionnaire (HAQ), standard deviation (SD), inter-quartile range (IQR)

Table 3. Differences in central arterial function associated with the use of non-selective non-steroidal anti-inflammatory drugs

	Unadjusted mean difference				Adjusted mean difference				Summary of model		
		95% CI		P		95% CI		P	R	Adj. R^2	P (ANOVA)
Augmentation index (AIX%)											
Diclofenac	'Reference group'										
Naproxen/ Nabumetone	**1.7**	-2.8	6.1	0.46	**1.7**	-2.0	5.4	0.35	0.70	0.40	0.0003
Indomethacin	**-1.6**	-7.7	4.5	0.59	**-1.0**	-7.8	5.8	0.77	0.65	0.28	0.02
Ibuprofen	**-8.6**	-15.9	-1.2	0.02	**-6.5**	-11.9	-1.0	0.02	0.86	0.67	0.000001
Reflected wave transit (RWT), msec											
Diclofenac	'Reference group'										
Naproxen/ Nabumetone	**1.9**	-3.9	7.7	0.52	**1.3**	-4.5	7.0	0.66	0.54	0.16	0.05
Indomethacin	**3.8**	-3.0	10.6	0.26	**3.5**	-4.8	11.7	0.40	0.56	0.14	0.13
Ibuprofen	**18.2**	9.8	26.6	0.0001	**14.2**	6.3	22.2	0.001	0.82	0.59	0.00001

Adjusted using multiple linear regression (MLR) for age, sex, mean arterial blood pressure, ever smoked, Stanford HAQ score and cumulative ESR-years

Patient characteristics

NSAID-users and non-users were similar for age, sex, waist-hip ratio, fasting lipids, glucose, arthritis duration, age arthritis onset and previous joint surgery **(Table 1).** Based on the Scottish 'ASSIGN' score,[25] NSAID-users had a slightly lower 10 year predicted risk of a CV event compared to non-users (median 6% versus 8%; Mann-Whitney U test, p=0.38).[25] NSAID-users had a higher brachial blood pressure at assessment, lower prevalence of treated hypertension and a lower exposure to tobacco. The level of current inflammation (study ESR), cumulative inflammation (ESR-years) and arthritis related disability (Stanford HAQ) were higher in current NSAID-users. The use of proton pump inhibitors and misoprostol was also higher among current ns-NSAID-users. The current use of prednisolone and DMARD was similar for both groups; the overall use of cytokines was relatively low. The rheumatological and CV features of ns-NSAID-users (with NSAIDS ordered by their level of aldosterone glucuronidation inhibition) are shown in **Table 2**

Differences in arterial function

Increasing levels of ns-NSAID-related AGI were associated with a higher AIX% and a lower RWT (**Figure 1**). Mean differences in arterial function between patients taking diclofenac and the other three ns-NSAIDS (naproxen, indomethacin, ibuprofen) are shown in **Table 3**. Diclofenac was selected as the 'reference group' as it has the highest level of aldosterone glucuronidation inhibition (AGI) and was also the most commonly used ns-NSAID. Compared to the unadjusted comparisons, the adjustment for other CV and rheumatological factors reduced the differences between ns-NSAIDS, but the observed trend of a higher degree of arterial dysfunction (higher AIX% and lower RWT) being associated with increasing levels of ns-NSAID-related AGI persisted. The exception to this pattern was the slightly higher AIX% for naproxen compared to diclofenac. The differences between ibuprofen and diclofenac reached statistical significance (AIX% 6.5, 95%CI 1.0 to 11.9, p=0.02; and RWT –14.2 msecs 95%CI –22.2 to –6.3, p=0.001); although only 7 patients were chronic users of ibuprofen. The differences between diclofenac and the two other nsNSAIDS (indomethacin and naproxen) were not statistically significantly different.

Patients with no ns-NSAID use over the previous 3 months (n=25) had a mean AIX% of 30.9 (SD 8.3) and RWT 133.1 (SD 12.5) msec. On adjusted comparison with current NSAID-users (all NSAIDS listed in **Table 1** combined) AIX% was –1.0 (95%CI –3.9 to 1.9; p=0.49) lower, and RTW 4.3 msec (95%CI –0.6 to 9.2; p=0.09) higher among non-users.

Eleven patients were currently taking a selective COX-2 inhibitor (8 celecoxib; 3 etoricoxib) and had a mean AIX% of 33.6 (6.4) and RWT 132.8 (SD 8.8) msec. On adjusted comparison with diclofenac, mean RWT was similar (adjusted difference –0.7 msec, 95%CI –6.7 to 5.4; p=0.80), whereas AIX% was non-significantly higher for patients taking a selective COX-2 inhibitor (2.9, 95%CI –1.4 to 6.8; p=0.19).

DISCUSSION

In summary we found that the current use of an ns-NSAID with a higher level of aldosterone glucuronidation inhibition (AGI), in patients with RA, appears to be associated with a higher level arterial dysfunction. Arterial dysfunction was highest in patients taking diclofenac and lowest in those taking ibuprofen. These difference in arterial function were not attributable to other important CV and rheumatological features that are known to influence arterial function, since the observed differences remained after adjusting for such features. Consequently differences in AIX and RWT appear to be genuinely related to the use of different ns-NSAIDs. Patients using ns-NSAIDS with intermediate levels of AGI, naproxen and indomethacin, demonstrated intermediate levels of arterial dysfunction.

Comparison with existing literature

A small number of previous studies have assessed arterial dysfunction in patients with RA using pulse wave analysis (PWA).[13;26-29] The difference observed in AIX% in this analysis (of 6.5) between diclofenac and ibuprofen is of a statistically and clinically relevant magnitude. It compares, for example, with a 4.2 point reduction in AIX% associated with 3 months atorvastatin therapy in patients with RA.[28] At coronary angiography a 10-point higher AIX% at baseline is associated with a 27% increased risk of CV events over 4 years follow-up; a 10 millisecond higher RWT is associated with a 15% reduction in CV events.[30] Published research concerning NSAID-related arterial dysfunction is currently limited. Only one previous study has directly assessed the influence of NSAID-use on AIX%. In 12 patients with RA 14-days of therapy with indomethacin (75 mg BD) was associated with a 1.2 (95%CI -2.1 to 4.5) increase in AIX%.[29] In a study of men aged >50 years undergoing community-based screening for abdominal aortic aneurysm in the UK, the use of an NSAID (75% took either ibuprofen, diclofenac or indomethacin) was significantly associated with reduced aortic wall distensibility assessed using M-mode ultrasound.[31]

Study strengths and limitations

The strengths of our study are that a single research nurse undertook high quality PWA in a controlled environment among individuals with RA recruited from a consecutive series of patients attending rheumatology clinic. We measured and adjusted for several important CV and rheumatological factors in our analysis, including factors known to be independently associated with arterial function. PWA has previously been shown to be predictive of CV events in patients after coronary angiography.[30] Our multivariate model explained a high proportion of the variability (60%-70%) in arterial dysfunction between patients taking diclofenac and ibuprofen. The characteristics of our participants are similar to RA patients receiving outpatient care elsewhere in the UK.[32] The study prevalence of treated hypertension is lower than that reported elsewhere and probably relates to the exclusion of patients with overt arterial disease.[33]

The main limitation of our study is its cross-sectional nature which means that we can only assess association rather than direct causation. Since we did not measure serum aldosterone and ns-NSAID levels in these patients with RA we are unable to directly confirm the previous in vitro findings. Our assessment of RA patients included a relatively small number of patients taking each NSAID and only 7 patients were chronic users of ibuprofen. The number of patients taking individual NSAIDS were too small to permit a comparison of association of low/high-dose NSAIDS with arterial dysfunction. The relatively small size of our study restricted the number of potential confounding factors (rheumatological and CV) that could be included in the multivariate analysis without running the risk of over-fitting the data. Although the inclusion of additional variables in the multivariate model, such as treated hypertension, did not improve the goodness to fit, nor substantially alter the adjusted values reported for AIX and RWT. As with all observational studies we cannot excluded the possibility of residual confounding as an explanation for our findings.

Study implications

The finding that ns-NSAID-related AGI appears to be associated with arterial dysfunction in patients with RA provides a novel insight into the CV toxicity of commonly used ns-NSAIDS. Our results are preliminary and require confirmation in larger studies looking at different ns-NSAIDS, serum aldosterone concentrations and surrogate markers of arterial dysfunction. Several important questions remain to be addressed, including whether high/low ns-NSAID dosage is related to arterial dysfunction and if switching ns-NSAIDS

(from high-AGI to low-AGI) improves arterial function. If the adverse CV events associated with ns-NSAID-use are due to AGI, then switching to an alternative 'lower-AGI ns-NSAID' may be an appropriate option for patients heavily dependant on NSAIDS for symptomatic relief.

Acknowledgements
We are grateful to Kathleen Knights for scientific advice concerning NSAID-related inhibition of aldosterone glucuronidation and to all the previous collaborators involved with the original RAAIX study - David J Williams, Alan G Macdonald, Vinod Kumar, Hazel Clark, Neil Scott, John Meecham and David Crosbie.

Competing interests
We have no competing interests to declare.

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The original study was supported by charitable funding from NHS Grampian Rheumatology Endowments. The funders played no role in the analysis or reporting of this study.

Author contributions
MAC - original study conception and design; analysis and interpretation of the data; initial drafting and re-drafting of the article; revising the article critically for important intellectual content; final approval of the version to be published.
AAM - original hypothesis; interpretation of data; revising the article critically for important intellectual content; re-drafting of the article; final approval of the version to be published.

Data-sharing
Consent for data-sharing was not obtained from study participants at the time of recruitment, but the presented data are held in an anonymised dataset. Access to the dataset is available from the corresponding author (at mike.crilly@abdn.ac.uk) in SPSS format for clinical academic researchers interested in undertaking a formally agreed collaborative research project(s). Although the risk of individual patient identification is low any research involving the release of the dataset to other clinical academics would require approval by Grampian Research Ethics Committee.

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FIGURE LEGEND

Figure 1. Use of NSAIDS and central arterial function in patients with rheumatoid arthritis

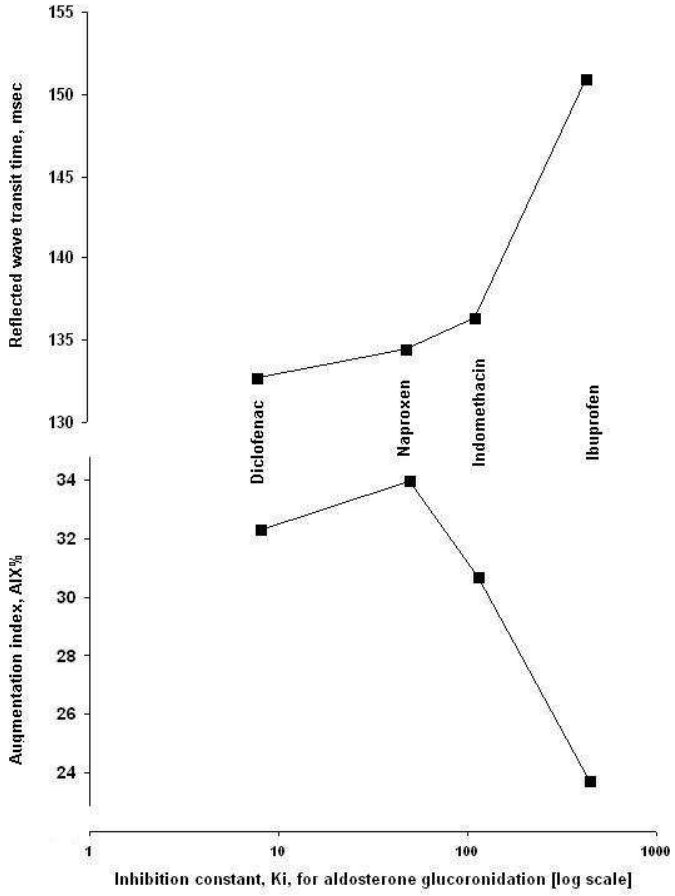

Figure 1. Use of NSAIDS and central arterial function in patients with rheumatoid arthritis
190x254mm (96 x 96 DPI)

STROBE Statement—Checklist of items that should be included in reports of ***cross-sectional studies***

Non-steroidal anti-inflammatory drug (NSAID) related inhibition of aldosterone glucuronidation and arterial dysfunction in patients with rheumatoid arthritis: a cross-sectional proof of concept study.

Michael A Crilly (26 January 2011)

	Item No	Recommendation
Title and abstract	1	(*a*) Indicate study's design in title: **"cross-sectional proof of concept study"**
		(*b*) Abstract: **Structured abstract provided**
Introduction		
Background/rationale	2	Scientific background: **NSAID-related inhibition of aldosterone metabolism is described**
Objectives	3	State specific objectives: **"Aim of this exploratory analysis was to assess the association between ns-NSAID-related aldosterone glucuronidation inhibition (AGI) and established markers of arterial dysfunction using data from a previous study of patients with RA**."
Methods		
Study design	4	Key elements of study design presented early: **"Our methods have been described in detail elsewhere." [ref #13. Crilly MA, Kumar V, Clark HJ, et al .Arterial stiffness and cumulative inflammatory burden in rheumatoid arthritis: a dose-response relationship independent of established cardiovascular risk factors. Rheumatology. 2009;48:1606-1612.] [ref # 14. Crilly MA, Clark HJ, Kumar V, et al. Relationship between arterial stiffness and Stanford Health Assessment Questionnaire disability in rheumatoid arthritis patients without overt arterial disease. J Rheumatol. 2010;37:946-952.]** **"In the original study we recruited patients with a consultant rheumatologist diagnosis of rheumatoid arthritis (RA) by reviewing the medical records of a consecutive series of patients attending hospital-based rheumatology clinics in the city of Aberdeen. We identified patients aged between 40-65 years with RA for more than 6 months duration."**
Setting	5	Setting, locations, and relevant dates: **"In the original study we recruited patients ... by reviewing the medical records of a consecutive series of patients attending hospital-based rheumatology clinics in the city of Aberdeen."** **"Patients attended the Clinical Pharmacology Department at Aberdeen Royal Infirmary (ARI) on a single occasion and underwent assessment by a single clinical research nurse (April-December 2006)."**
Participants	6	(*a*) Eligibility criteria, sources and methods of selection of participants: **"We excluded patients with overt arterial disease (angina, prior myocardial infarction, transient ischaemic attack, stroke, arterial revascularisation, intermittent claudication, peripheral arterial disease), atrial fibrillation, heart failure and valvular heart disease. The exclusion of patients with arterial disease was based upon an initial screening patient-questionnaire, resting 12-lead ECG (independently reported by a cardiologist to identify pathological Q-waves, conduction defects, minor Q-waves associated with ST-segment/T-wave anomalies), and a detailed medical record review by a rheumatologist. No participants had a history of recent infection, antibiotic treatment or immunisation within the previous two weeks."**

Variables	7	Define outcomes, exposures, and potential confounders: **"Standardised assessment included blood pressure (BP) measurement, pulse wave analysis (PWA), fasting venous blood sample (including erythrocyte sedimentation rate [ESR], rheumatoid factor [RF], and lipid profile). A self-completed patient questionnaire included smoking habit and the Stanford Health Assessment Questionnaire (HAQ). Current medication use was comprehensively reviewed by the research nurse and included use of over-the-counter (without the need for a prescription) NSAIDS. A detailed retrospective review of the medical records using a previously piloted study form, was undertaken by a single rheumatologist blinded to all PWA results and included date of arthritis onset, previous blood test results (erythrocyte sedimentation rate, rheumatoid factor), joint surgery and co-morbidity (including treated hypertension)."** Diagnostic criteria: **"Consultant rheumatologist diagnosis of rheumatoid arthritis (RA)"** **"Our methods have been described in detail elsewhere.[ref #13. Rheumatology. 2009;48:1606-1612.]" -- we have previously reported on the proportion of patients with a consultant rheumatologist diagnsosis of rheumatoid arthritis (RA) who meet "American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis.[Arnett FC, et al. Arthritis Rheum 1988 Mar;31(3):315-24].** **[Text from Rheumatology. 2009;48:1606-1612] "Whilst all of our study participants had a clinical diagnosis of RA made by a rheumatologist, only 56% met ACR (4/7) criteria for RA. This may be attributable to a typographical error in our questionnaire (which asked about morning stiffness for 6 months rather than 6 weeks). Whilst 39% of patients reported more than one hour of morning stiffness for more than 6 months in the past, only 18% of patients reported such stiffness over the previous week. Some clinical heterogeneity may exist in our study population of patients with a clinical rheumatological diagnosis of RA, although the inclusion of 'ACR criteria' as a variable in the fully adjusted analysis made no difference to our results. ACR-criteria also 'accumulates' over time. For example, in our study the median duration of arthritis was almost 10 years: some 66% (36/55) of patients with arthritis duration greater than 10 years met ACR-criteria, compared to 48% (28/59) with a shorter duration."**
Data sources/ measurement	8*	Sources of data and details of methods of assessment (measurement): **"Analysis is based on the mean of the three PWA measurements. The principle measures of arterial dysfunction are augmentation index (AIX%) and reflected wave transit time (RWT, msec). Since AIX% varies with heart rate in an individual it was standardised to 75 beats-per-minute."** **"Aldosterone 18ß-glucuronidation inhibition constants, Ki , derived from in vitro studies of human kidney cortical microsomes (HKCM), have been published for 4 of the ns-NSAIDS taken by patients in the RAAIX study (diclofenac 8 µM, naproxen 49 µM, indomethacin 113 µM, ibuprofen 441 µM; a lower Ki indicates greater inhibition). [ref #6: Knights KM, Winner LK, Elliot DJ, et al. Glucuronidation by human liver and kidney microsomes and recombinant UDP-glucuronosyltransferases: inhibition by NSAIDs. Br J Clin Pharmacol 2009;68:402-412.] Nabumetone is a close structural analogue of naproxen and the two were combined together in the analysis."**
Bias	9	Efforts to address potential sources of bias: **"BP was measured at the right brachial artery using an validated automatic oscillometric BP machine (Omron HEM757 IntelliSense BP monitor; Omron Healthcare, Illinois, USA)." [El Assaad MA, et al. Evaluation of two devices for self-measurement of blood pressure according to the international protocol: the Omron M5-I and the Omron 705IT. Blood Press Monit 2003;8:127-133.]**

		"**A detailed retrospective review of the medical records, using a previously piloted study form, was undertaken by a single rheumatologist blinded to all PWA results".** **"The nurse remained blind to the patients' previous medical records (which were not made available at assessment) and only reviewed current medication and questionnaire responses (to ensure that all questionnaires items were fully completed), after PWA assessment had been completed".**
Study size	10	Study size: **"Our methods have been described in detail elsewhere.[ref #13. Rheumatology. 2009;48:1606-1612.]"** **The original study recruited 114 patients.** **[Text from Rheumatology. 2009;48:1606-1612] "A sample size of 110 patients with RA was our intention, in order to ensure that there were 10 subjects for each CV risk factor included in the multi-variable analysis. We included 11 cardiovascular risk factors in our analysis, based on those contributing 90% of the 'population attributable risk' to myocardial infarction in the recent INTERHEART study."**
Quantitative variables	11	Quantitative variables handled: **AIX% and RWT were Normally distributed and summarised as mean (SD).** **"Analysis is based on the mean of the three PWA measurements"** Groupings chosen: **Not applicable**
Statistical methods	12	(*a*) Statistical methods (including control for confounding): **Multiple linear regression (MLR) was used to adjust mean differences in AIX% (and RWT) for variables known to be associated with AIX%, namely: age, sex, mean arterial blood pressure, ever smoked, Stanford HAQ disability score and cumulative ESR-years.**
		(*b*) Subgroups and interactions: **Not applicable**
		(*c*) Missing data: **We had negligible levels of missing data due to the implementation of a rigorous study design and the diligence of our research nurse (and rheumatologist reviewing the medical records). Except for the calculation of 'ESR-years' (as a measure of cumulative inflammatory burden) the level of missing data was minimal.** **"Cumulative ESR-years were derived from the highest single annual ESR recorded in the medical record during each year of follow-up and calculated using the 'trapezium rule' with linear interpolation when data for a given year was missing."** **"Our methods have been described in detail elsewhere. [ref #13. Rheumatology 2009;48:1606-1612.]"** **[Text from Rheumatology. 2009;48:1606-1612] "Medical records were available for 112 patients (complete medical records could not be obtained for two patients) who contributed a total of 1,040 person-years of rheumatoid disease. An annual ESR was available for 77% (797/1,040) of these person-years. Missing ESR data was imputed as previously described. For individual patients the average availability of an annual ESR, for each year since the onset of arthritis, was a median of 93% (IQR 67% to 100%). The availability of an annual ESR did not differ by age, gender, rheumatoid factor, ACR-RA criteria or Stanford disability index (data not shown)."** **Of the 114 patients recruited, 112 had complete data and were included in the fully adjusted regression analysis/model [see Table 3 from ref #13; Rheumatology. 2009;48:1606-1612]**

		(*d*) Analytical methods taking account of sampling strategy: **Not applicable**
		(e) Sensitivity analyses: **"The inclusion of additional variables (study ESR, duration of arthritis, fasting cholesterol, smoking pack-years, treated hypertension and current DMARD use) did not alter the adjusted values for AIX and RWT reported, nor improve the goodness to fit of the final regression model."**
Results		
Participants	13*	(a) Report numbers of individuals at each stage of study: **"The original study recruited 114 patients. We excluded 9 patients from the analysis who were not currently taking NSAIDS, but had done so within the previous 3 months; and excluded 2 users of infrequently prescribed NSAIDS (ketoprofen and tiaprofenic acid)."** **"Our methods have been described in detail elsewhere. [ref #13. Rheumatology 2009;48:1606-1612.]" – we had negligible levels of missing data due to the implementation of a very careful study design and the diligence of our research nurse (and rheumatologist reviewing the medical records). Of the 114 patients recruited 112 had complete data and were included in the fully adjusted regression analysis/model [see Table 3 from ref #13; Rheumatology. 2009;48:1606-1612]** **We have no data available concerning the number of RA patients excluded at the rheumatology clinic level because they were already known to have arterial disease.** **We also have no data concerning the number of eligible RA patients attending rheumatology clinic who declined to be contacted by our research nurse. All of the RA patients who did agreed to being contacted by the research nurse subsequently attended for assessment (N=114) and have been included in this analysis as described.** **No patients were excluded because of technical failure to undertake PWA. Despite initial (pre-study) concerns that it might not be feasible to undertake PWA in some RA patients, due to diseased wrists, this proved not to be the case. The research nurse successfully undertook PWA on all participants.**
		(b) Reasons for non-participation: **Not known/recorded**
		(c) Flow diagram: **None/ not applicable**
Descriptive data	14*	(a) Characteristics of study participants: **Shown in detail in Table 1**
		(b) Indicate number of participants with missing data: **Of the 114 patients recruited, 112 had complete data and were included in the fully adjusted regression analysis/model [see Table 3 from ref #13; Rheumatology. 2009;48:1606-1612]**
Outcome data	15*	Report summary measures: **"Analysis is based on the mean of the three PWA measurements. The principle measures of arterial dysfunction are augmentation index (AIX%) and reflected wave transit time (RWT, msec). Since AIX% varies with heart rate in an individual it was standardised to 75 beats-per-minute."**
Main results	16	(*a*) Unadjusted estimates and confounder-adjusted estimates (95%CI): **Table 3 reports unadjusted and adjusted mean differences in AIX% and RWT (with their related 95%CI's)** Make clear which confounders were adjusted for and why they were included **"Multiple linear regression (MLR) was used to adjust mean differences in AIX% (and RWT) for variables known to be associated with AIX%, namely: age, sex, mean arterial blood pressure, ever smoked, Stanford HAQ disability score and cumulative ESR-years. Analysis was undertaken using SPSS v 17.We confirmed that the assumptions of**

		linearity, normal distribution and equal variance for MLR were met. 'Goodness to fit' was assessed using the adjusted R2. The inclusion of additional variables (study ESR, duration of arthritis, fasting cholesterol, smoking pack-years, treated hypertension and current DMARD use) did not alter the adjusted values for AIX and RWT reported, nor improve the goodness to fit of the final regression model."
		(*b*) Category boundaries (continuous variables categorized): **Not applicable**
		(*c*) Estimates of absolute risk: **Not applicable**
Other analyses	17	Subgroup analysis: **None** Sensitivity analyses: **see STROBE #12e (above)**
Discussion		
Key results	18	Summarise key results with reference to study objectives: **"In summary we found that the current use of an ns-NSAID with a higher level of aldosterone glucuronidation inhibition (AGI), in patients with RA, appears to be associated with a higher level arterial dysfunction. Arterial dysfunction was highest in patients taking diclofenac and lowest in those taking ibuprofen. These difference in arterial function were not attributable to other important CV and rheumatological features that are known to influence arterial function, since the observed differences remained after adjusting for such features. Consequently differences in AIX and RWT appear to be genuinely related to the use of different ns-NSAIDs. Patients using ns-NSAIDS with intermediate levels of AGI, naproxen and indomethacin, demonstrated intermediate levels of arterial dysfunction."**
Limitations	19	Limitations of the study, sources of potential bias or imprecision: **"The main limitation of our study is its cross-sectional nature which means that we can only assess association rather than direct causation. Since we did not measure serum aldosterone and ns-NSAID levels in these patients with RA we are unable to directly confirm the previous in vitro findings. Our assessment of RA patients included a relatively small number of patients taking each NSAID and only 7 patients were chronic users of ibuprofen. The number of patients taking individual NSAIDS were too small to permit a comparison of association of low/high-dose NSAIDS with arterial dysfunction. The relatively small size of our study restricted the number of potential confounding factors (rheumatological and CV) that could be included in the multivariate analysis without running the risk of over-fitting the data. …. As with all observational studies we cannot excluded the possibility of residual confounding as an explanation for our findings."**
Interpretation	20	Cautious overall interpretation of results: **"Our results are preliminary and require confirmation in larger studies looking at different ns-NSAIDS, serum aldosterone concentrations and surrogate markers of arterial dysfunction. Several important questions remain to be addressed, including whether high/low ns-NSAID dosage is related to arterial dysfunction."**
Generalisability	21	Generalisability (external validity) of the study results: **"The characteristics of our participants are similar to RA patients receiving outpatient care elsewhere in the UK. [ref #32. Panoulas VF, Douglas KM, Milionis HJ et al. Prevalence and associations of hypertension and its control in patients with rheumatoid arthritis. Rheumatology 2007;46:1477-1482]"**
Other information		
Funding	22	Source of funding and role of the funders: **"The original study was supported by charitable funding from NHS Grampian Rheumatology Endowments. The funders played no role in the analysis or reporting of this study."**

*Give information separately for exposed and unexposed groups.

Non-steroidal anti-inflammatory drug (NSAID) related inhibition of aldosterone glucuronidation and arterial dysfunction in patients with rheumatoid arthritis: a cross-sectional clinical study

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TITLE PAGE

Non-steroidal anti-inflammatory drug (NSAID) related inhibition of aldosterone glucuronidation and arterial dysfunction in patients with rheumatoid arthritis: a cross-sectional clinical study

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KEY WORDS:
Arterial dysfunction; Pulse wave analysis; Non-steroidal anti-inflammatory drugs; Rheumatoid arthritis; Aldosterone.

WORD COUNT = 3,093
WORD COUNT: ABSTRACT = 292

NUMBER OF TABLES = 3
NUMBER OF FIGURES = 1

ABSTRACT

Objective:
Patients with rheumatoid arthritis (RA) are at increased risk of cardiovascular (CV) disease and are also commonly prescribed non-selective non-steroidal anti-inflammatory drugs (ns-NSAIDS). New *in vitro* evidence suggests that this increased CV risk may be mediated through aldosterone glucuronidation inhibition (AGI), which differs between NSAIDS (diclofenac > naproxen > indomethacin > ibuprofen). Our aim was to explore the association between ns-NSAID-related AGI and arterial dysfunction.

Methods:
The extent (augmentation index, AIX%) and timing (reflected wave transit time, RWT, msec) of aortic wave reflection (measured using radial applanation pulse wave analysis, PWA, SphygmoCor device) were assessed on a single occasion in 114 consecutive RA patients without overt CV disease aged 40-65 years. A 'higher AIX%' and 'lower RWT' indicate arterial dysfunction. Assessment included fasting blood sample, patient questionnaire and medical record review. Multivariate analysis was used to adjust for age, sex, mean blood pressure, smoking, cumulative erythrocyte sedimentation rate (ESR-years) and Stanford disability score.

Results:
We identified 60 patients taking ns-NSAIDS and 25 non-users. Using a ns-NSAID with the highest AGI was associated with a higher AIX% (and lower RWT) versus treatment with a ns-NSAID with the lowest AGI (diclofenac AIX% 32.3, RWT 132.7 msec; versus ibuprofen AIX% 23.8, RWT 150.9 msec): adjusted mean differences AIX% 6.5 (95%CI 1.0 to 11.9; p=0.02); RWT –14.2 milliseconds (95%CI –22.2 to –6.3; p=0.001). Indomethacin demonstrated an intermediate level of arterial dysfunction. In relation to arterial dysfunction both indomethacin and naproxen were more similar to diclofenac than ibuprofen

Conclusions:
ns-NSAID-related AGI is associated with arterial dysfunction in patients with RA These findings provide a potentially novel insight into the CV toxicity of commonly used ns-NSAIDS. The study findings are limited by the small number of patients involved and require further replication in a much larger study.

KEY WORDS: Arterial dysfunction; Pulse wave analysis; Non-steroidal anti-inflammatory drugs; Rheumatoid arthritis; Aldosterone.

ABSTRACT WORD COUNT = 292

ARTICLE SUMMARY

Article focus

Aldosterone glucuronidation inhibition (AGI) potentiates the adverse cardiovascular effects of aldosterone. Recently published *in vivo* research suggests that such inhibition differs between non-selective non-steroidal anti-inflammatory drugs (ns-NSAIDS); with a ranked order of diclofenac > naproxen > indomethacin > ibuprofen. No previous studies have assessed the relationship between ns-NSAID-related AGI and arterial dysfunction in chronic users. This study assessed arterial dysfunction using pulse wave analysis (PWA).

Key messages

In patients with rheumatoid arthritis (RA) we found that chronic use (> 3 months) of diclofenac (high AGI) was associated with greater arterial dysfunction compared to ibuprofen (lower AGI). This association was independent of other cardiovascular and rheumatological factors. Indomethacin (intermediate AGI) was associated with an intermediate level of arterial dysfunction, although naproxen (intermediate AGI) did not fit the anticipated pattern. Our findings support the concept that AGI may play a role in the CV toxicity of some ns-NSAIDS commonly used in routine clinical practice.

Strengths and limitations of this study

A single research nurse assessed RA patients who were recruited from a consecutive series attending hospital rheumatology clinic. We adjusted for several important cardiovascular and rheumatological factors known to be independently associated with arterial function and our multivariate analysis explained a high proportion of the variability in arterial dysfunction among chronic ns-NSAID users. The observational cross-sectional design of our study means that we cannot assess causation, nor exclude residual confounding as an explanation for our findings. The small number of patients taking each NSAID meant that the confidence intervals are wide.

INTRODUCTION

Non-steroidal anti-inflammatory (NSAID) are among the most commonly prescribed drugs in clinical practice. Their relative safety has attracted considerable interest, particularly in relation to their association with adverse cardiovascular (CV) events.[1;2] Most of this interest has focused on the role of selective cyclo-oxygenase-2 (COX-2) inhibitors,[3] but non-selective NSAIDS (ns-NSAIDS) also have the potential to increase the risk of adverse CV events. For example, the use of the ns-NSAID diclofenac has been shown to increase the risk of adverse CV events.[2] ns-NSAID use is associated with adverse CV effects including reduction of renal perfusion, electrolyte disturbances (sodium and water retention) and increase in blood pressure. The detrimental effects in renal function are thought to be secondary to a reduced synthesis of vasodilatory prostaglandins such as PGE2. However, ns-NSAIDS have been shown to exert adverse renal effects disproportionate to the level of inhibition of prostaglandin synthesis.[4] This suggests that there might be other yet unknown mechanisms responsible for the potential increase in CV risk associated with ns-NSAID use.[5]

Aldosterone metabolism

Very recent *in vitro* evidence suggests that ns-NSAIDS enhance the action of aldosterone through the inhibition of aldosterone metabolism.[6] Aldosterone is metabolised by 18ß-glucuronidation in both the liver and kidneys in a reaction that is catalyzed by the enzyme UDP-glucuronosyltransferase-2B7. Several ns-NSAIDS have been shown *in vitro* (human kidney cortical microsomes) to inhibit aldosterone 18ß-glucuronidation and individual NSAIDS vary in their ability to inhibit aldosterone glucuronidation.[6] Diclofenac, for example, is a strong inhibitor of glucuronidation, whereas ibuprofen is a weaker inhibitor.[6]

Aldosterone is a mineralocorticoid which plays an important role in the renin-angiotensin-aldosterone system and has generally deleterious effects on the CV system. Higher aldosterone levels are associated with endothelial dysfunction, arterial stiffening, increased arterial wall reflection, myocardial fibrosis and an increase in the risk of CV death.[7-10] Drugs that block the action of aldosterone (such as spironolactone) have been shown to reduce the risk of CV death in patients with heart failure and following myocardial infarction.[11] Aldosterone receptors predominate in the aorta and spironolactone has been shown to improve arterial function assessed using the technique of pulse wave analysis (PWA).[7] Consequently the increased risk of adverse CV events in patients taking diclofenac may also be related to the enhanced effects of aldosterone as a consequence of ns-NSAID-related aldosterone glucuronidation inhibition (AGI).

RAAIX study

Patients with rheumatoid arthritis (RA) are known to be at a higher risk of CV death.[12] The RAAIX (RA augmentation index) study was undertaken to assess the relationship between the cumulative inflammatory burden and arterial dysfunction in patients with rheumatoid arthritis (RA).[13;14] RAAIX involved a detailed assessment of both CV and rheumatological features; including the assessment of the use of NSAIDS. Other researchers have subsequently reported the relative level of aldosterone 18ß-glucuronidation inhibition (AGI) for several of the ns-NSAIDS taken by patients in the RAAIX study.[6] In the RAAIX study arterial dysfunction was assessed non-invasively using radial applanation tonometry and pulse wave analysis (PWA).[13;14] PWA is based on the phenomenon of 'arterial wave reflection' which is influenced by pulse wave velocity, endothelial dysfunction, peripheral arterial resistance and left ventricular ejection.[15;16] In each cardiac cycle the outgoing systolic pulse wave is also reflected back towards the heart, predominantly at the level of arterial bifurcations,[17] and returns to the heart during

systole where it augments the central aortic pressure.[15;16] The speed of travel of both outgoing and reflected waves are greater in patients with stiffer arteries; which increases the extent of augmentation (higher AIX%) and reduces the reflected wave transit time (lower RWT).

Study aim

The aim of this exploratory analysis was to assess the association between ns-NSAID-related aldosterone glucuronidation inhibition (AGI) and established markers of arterial dysfunction using data from a previous study of patients with RA.

METHODS

In the original study we recruited patients with a consultant rheumatologist diagnosis of rheumatoid arthritis (RA) by reviewing the medical records of a consecutive series of patients attending hospital-based rheumatology clinics in the city of Aberdeen. We identified patients aged between 40-65 years with RA for more than 6 months duration. Whilst all of our study participants had a clinical diagnosis of RA made by a rheumatologist, only 56% (64/114) met 'American College of Rheumatology' criteria (ACR 4/7) for RA. We excluded patients with overt arterial disease (angina, prior myocardial infarction, transient ischaemic attack, stroke, arterial revascularisation, intermittent claudication, peripheral arterial disease), atrial fibrillation, heart failure and valvular heart disease. The exclusion of patients with arterial disease was based upon an initial screening patient-questionnaire, resting 12-lead ECG (independently reported by a cardiologist to identify pathological Q-waves, conduction defects, minor Q-waves associated with ST-segment/T-wave anomalies), and a detailed medical record review by a rheumatologist. No participants had a history of recent infection, antibiotic treatment or immunisation within the previous two weeks.

Clinical assessment

Patients attended the Clinical Pharmacology Department at Aberdeen Royal Infirmary (ARI) on a single occasion and underwent assessment by a single clinical research nurse (April-December 2006). Assessment took place in the morning after participants had fasted overnight and abstained from smoking, alcohol and caffeine. Standardised assessment included blood pressure (BP) measurement, pulse wave analysis (PWA), fasting venous blood sample (including erythrocyte sedimentation rate [ESR], rheumatoid factor [RF], and lipid profile). A self-completed patient questionnaire included smoking habit and the Stanford Health Assessment Questionnaire (HAQ).[18] Current medication use was comprehensively reviewed by the research nurse and included use of over-the-counter (without the need for a prescription) NSAIDS. A detailed retrospective review of the medical records using a previously piloted study form, was undertaken by a single rheumatologist blinded to all PWA results and included date of arthritis onset, previous blood test results (erythrocyte sedimentation rate, rheumatoid factor), joint surgery and co-morbidity (including treated hypertension). Our methods have been described in detail elsewhere.[13;14] The nurse remained blind to the patients' previous medical records (which were not made available at assessment) and only reviewed current medication and questionnaire responses (to ensure that all questionnaires items were fully completed), after PWA assessment had been completed.

Pulse wave analysis (PWA)

Patients rested supine in a quiet side-room for at least 10 minutes before undergoing three BP/PWA measurements according to current guidelines.[20] BP was measured at the right brachial artery using an validated automatic oscillometric BP machine (Omron HEM757 IntelliSense BP monitor; Omron Healthcare, Illinois, USA).[21] Pulse wave analysis (PWA)

was undertaken using the SphygmoCor device (AtCor Medical, Sydney, Australia) with a hand-held tonometer (Millar, Texas, USA) 'applanated' at the right radial artery. The 'SphygmoCor' PWA device employs a validated 'generalised transfer function' to derive the central aortic pulse waveform from the peripheral waveform.[22] All three PWA recordings were required to have a in-built SphygmoCor quality index score at least 95% (based on average pulse height, pulse height variation and diastolic variation). We have previously demonstrated her high levels of within-observer and between-observer repeatability.[19] The research nurse remained blind to the patients' previous medical history until PWA was completed.

Aldosterone glucuronidation inhibition (AGI)

Aldosterone 18ß-glucuronidation inhibition constants, Ki , derived from *in vitro* studies of human kidney cortical microsomes (HKCM), have been published for 4 of the ns-NSAIDS taken by patients in the RAAIX study (diclofenac 8 µM, naproxen 49 µM, indomethacin 113 µM, ibuprofen 441 µM; a lower Ki indicates greater inhibition).[6] Nabumetone is a close structural analogue of naproxen and the two were combined together in the analysis.

Statistical analysis

Analysis is based on the mean of the three PWA measurements. The principle measures of arterial dysfunction are augmentation index (AIX%) and reflected wave transit time (RWT, msec). Since AIX% varies with heart rate in an individual it was standardised to 75 beats-per-minute.[23] The UK version of the Stanford-HAQ was scored using standard methods without any imputation required for missing data.[18] Cumulative ESR-years were derived from the highest single annual ESR recorded in the medical record during each year of follow-up and calculated using the 'trapezium rule' with linear interpolation when data for a given year was missing.[24] ESR-years reflects both the duration and level of inflammatory burden (e.g. 5 years of arthritis and annual ESR's of 30, 20, 10, 10, 20 mm/hour would equate to approximately 90 ESR-years). ESR is routinely measured for almost all RA-patients attending rheumatology out-patient clinic in Aberdeen, where the policy is to review all RA-patients at least annually. Complete medical records were available for 112 patients who contributed a total of 1,040 person-years of rheumatoid disease. An annual ESR was available for 77% (797/1,040) of these person-years and the availability of an annual ESR for each year since the onset of arthritis for individual patients was a median of 93% (IQR 67%–100%). The availability of an annual ESR did not differ by age, gender, rheumatoid factor positivity, RA-criteria (ACR 4/7), or Stanford HAQ disability index (data not shown).

Multiple linear regression (MLR) was used to adjust mean differences in AIX% (and RWT) for variables known to be associated with AIX%, namely: age, sex, mean arterial blood pressure, ever smoked, Stanford HAQ disability score and cumulative ESR-years. Analysis was undertaken using SPSS v 17.We confirmed that the assumptions of linearity, normal distribution and equal variance for MLR were met. 'Goodness to fit' was assessed using the adjusted R^2. The inclusion of additional variables (study ESR, duration of arthritis, RA-criteria, fasting cholesterol, smoking pack-years, treated hypertension and current DMARD use) did not alter the adjusted values for AIX and RWT reported, nor improve the goodness to fit of the final regression model.

The study adhered to the principles of the Declaration of Helsinki and was approved by Grampian Research Ethics Committee (study reference: 04/S0801/67). All participants provided informed written consent. The study was funded from a charitable source (NHS Grampian Rheumatology Endowments).

RESULTS

The original study recruited 114 patients. We excluded 9 patients from the analysis who were not currently taking NSAIDS, but had done so within the previous 3 months; and excluded 2 users of infrequently prescribed NSAIDS (ketoprofen and tiaprofenic acid). The characteristics of the remaining 103 patients (82% female) are shown in **Table 1**.

Table 1. Characteristics of patients with rheumatoid arthritis

Figures are numbers (%) unless otherwise indicated

	Current NSAID use					
	Yes (n=78)			No (n=25)		
Cardiovascular features						
Female	64	82%		20	80%	
Mean age, years (SD)	53.4	6.9		53.9	5.7	
Mean heart rate, beats per minute (SD)	69.7	10.6		64.5	8.6	
Mean systolic BP, mmHg (SD)	126.2	16.9		124.7	12.1	
Mean diastolic BP, mmHg (SD)	82.9	10.0		81.3	7.6	
Mean pulse pressure, mmHg (SD)	35.6	9.1		36.9	7.4	
Mean arterial BP, mmHg (SD)	99.5	12.2		97.8	8.8	
Mean fasting cholesterol, mmol/L (SD)	5.3	1.2		5.6	1.1	
Mean fasting glucose, mmol/L (SD)	5	0.8		5	0.5	
Mean waist-hip ratio (SD)	0.85	0.08		0.85	0.08	
Ever smoked for 12 months or more	44	56%		17	68%	
Treated hypertension	12	15%		7	28%	
Bendroflumethiazide	10	13%		5	20%	
Atenolol	6	8%		5	20%	
Calcium channel blocker	3	4%		1	4%	
Angiotensin converting enzyme inhibitor	2	3%		3	12%	
Rheumatological features						
Mean age onset arthritis, years (SD)	41.8	10.4		41.4	12.1	
Median duration arthritis, years (IQR)	9	4	15	8	4	16
Median Stanford HAQ disability (IQR)	1.4	0.6	1.9	0.6	0.3	1.3
Median study ESR, mm/h (IQR)	19	8	30	10	6	18
Median cumulative ESR-years (IQR)	206	99	468	93	58	297
Rheumatoid factor positive (>=30 IU/ml)	66	85%		18	72%	
Rheumatoid arthritis criteria (ACR 4/7)	47	60%		10	40%	
Previous joint surgery	17	22%		4	16%	
Current DMARD therapy	70	90%		24	96%	
Current prednisolone therapy	8	10%		3	12%	
Current NSAID therapy (>3 months)	78	100%		0	0%	
Diclofenac	31	40%		--	--	
Naproxen/ Nabumetone	16	21%		--	--	
Celecoxib/ Etoricoxib	11	14%		--	--	
Ibuprofen	7	9%		--	--	
Meloxicam	7	9%		--	--	
Indomethacin	6	8%		--	--	
Other (ketoprofen, tiaprofemic acid)	2	3%				
Current cytokine therapy	4	5%		1	4%	
Proton pump inhibitor	27	35%		4	16%	
Misoprostol	6	8%		0	0%	
Central arterial function						
Mean augmentation index, AIX% (SD)	31.9	7.9		30.9	8.3	
Mean reflected wave transit time, msec (SD)	135.7	11.4		133.1	12.4	

Blood pressure (BP), disease modifying anti-rheumatic drug (DMARD), erythrocyte sedimentation rate (ESR), American College of Rheumatology (ACR), Health Assessment Questionnaire (HAQ), non-steroidal anti-inflammatory drug (NSAID), standard deviation (SD), inter-quartile range (IQR)

Table 2. Patient characteristics and use of non-selective non-steroidal anti-inflammatory drugs

Figures are numbers (%) unless otherwise indicated

	Diclofenac (n=31)			Naproxen (n=16)			Indomethacin (n=6)			Ibuprofen (n=7)		
Aldosterone 18ß-glucuronidation inhibition constant (Ki), µM	**Ki = 8**			**Ki = 49**			**Ki = 113**			**Ki = 441**		
Cardiovascular features												
Female	28	90%		12	75%		5	83%		5	71%	
Mean age, years (SD)	54	6.5		55	5.6		57	5.9		50	9.5	
Mean heart rate, beats per minute (SD)	70.7	11.9		73.0	10.5		68.9	4.6		70.0	10.9	
Mean systolic BP, mmHg (SD)	126	17.6		125	20.3		118	14.0		123	13.4	
Mean diastolic BP, mmHg (SD)	83	9.9		82	12.1		75	7.4		84	7.9	
Mean pulse pressure, mmHg (SD)	35.3	10.5		35.3	9.0		34.4	6.1		31.1	8.1	
Mean arterial BP, mmHg (SD)	99	12.2		99	15.3		92	9.8		100	10.3	
Mean fasting cholesterol, mmol/L (SD)	5.4	1.3		5.4	1.5		5.8	0.9		5.1	1.0	
Mean fasting glucose, mmol/L (SD)	4.9	1.0		5.1	0.6		5.0	0.4		4.9	0.5	
Mean waist-hip ratio (SD)	0.85	0.08		0.86	0.09		0.89	0.08		0.88	0.09	
Ever smoked for 12 months or more	17	55%		9	60%		4	67%		3	43%	
Treated hypertension	7	23%		3	19%		2	33%		0	0%	
Rheumatological features												
Mean age onset arthritis, years (SD)	43	11.2		42	8.4		39	7.1		37	11.9	
Median duration arthritis, years (IQR)	9	3	15	10	2	17	19	11	22	11	7	20
Median Stanford HAQ (IQR)	1.4	0.8	2.0	1.6	1.2	1.9	1.5	0.9	1.9	0.6	0.0	1.9
Median ESR-years (IQR)	221	99	526	186	77	435	706	140	825	315	81	829
Rheumatoid factor positive (>=30 IU/ml)	26	84%		13	81%		6	100%		7	100%	
Rheumatoid arthritis criteria (ACR 4/7)	14	45%		11	69%		4	67%		5	71%	
Previous joint surgery	9	29%		4	25%		1	20%		2	29%	
Current DMARD therapy	28	90%		15	94%		5	83%		6	86%	
Central arterial function												
Mean augmentation index, AIX% (SD)	32.3	7.1		34.0	7.3		30.7	3.6		23.8	14.1	
Mean reflected wave transit time, msec (SD)	132.7	7.3		134.5	12.6		136.4	8.7		150.9	18.0	

Blood pressure (BP), disease modifying anti-rheumatic drug (DMARD), erythrocyte sedimentation rate (ESR), American College of Rheumatology (ACR), Health Assessment Questionnaire (HAQ), standard deviation (SD), inter-quartile range (IQR)

Table 3. Differences in central arterial function associated with the use of non-selective non-steroidal anti-inflammatory drugs

	Unadjusted mean difference				Adjusted mean difference				Summary of model		
		95% CI		P		95% CI		P	R	Adj. R^2	P (ANOVA)
Augmentation index (AIX%)											
Diclofenac	'Reference group'										
Naproxen/ Nabumetone	**1.7**	-2.8	6.1	0.46	**1.7**	-2.0	5.4	0.35	0.70	0.40	0.0003
Indomethacin	**-1.6**	-7.7	4.5	0.59	**-1.0**	-7.8	5.8	0.77	0.65	0.28	0.02
Ibuprofen	**-8.6**	-15.9	-1.2	0.02	**-6.5**	-11.9	-1.0	0.02	0.86	0.67	0.000001
Reflected wave transit (RWT), msec											
Diclofenac	'Reference group'										
Naproxen/ Nabumetone	**1.9**	-3.9	7.7	0.52	**1.3**	-4.5	7.0	0.66	0.54	0.16	0.05
Indomethacin	**3.8**	-3.0	10.6	0.26	**3.5**	-4.8	11.7	0.40	0.56	0.14	0.13
Ibuprofen	**18.2**	9.8	26.6	0.0001	**14.2**	6.3	22.2	0.001	0.82	0.59	0.00001

Adjusted using multiple linear regression (MLR) for age, sex, mean arterial blood pressure, ever smoked, Stanford HAQ score and cumulative ESR-years

No patients were taking aspirin or more than one NSAID concurrently. All NSAID-users had been taking their current NSAID for more than 3 months. Diclofenac was the most commonly used NSAID and was taken by almost a third of patients. One quarter of patients had not taken an NSAID within the previous 3 months (although 88% of these patients had been prescribed an NSAID in the past). All patients, both NSAID-users and non-users, had previously received DMARD therapy.

Patient characteristics

NSAID-users and non-users were similar for age, sex, waist-hip ratio, fasting lipids, glucose, arthritis duration, age arthritis onset and previous joint surgery **(Table 1).** Based on the Scottish 'ASSIGN' score,[25] NSAID-users had a slightly lower 10 year predicted risk of a CV event compared to non-users (median 6% versus 8%; Mann-Whitney U test, p=0.38).[25] NSAID-users had a higher brachial blood pressure at assessment, lower prevalence of treated hypertension and a lower exposure to tobacco. The level of current inflammation (study ESR), cumulative inflammation (ESR-years) and arthritis related disability (Stanford HAQ) were higher in current NSAID-users. The use of proton pump inhibitors and misoprostol was also higher among current NSAID-users. The current use of prednisolone and DMARD was similar for both groups; the overall use of cytokines was relatively low. The rheumatological and CV features of ns-NSAID-users (with NSAIDS ordered by their level of aldosterone glucuronidation inhibition) are shown in **Table 2**

Differences in arterial function

Increasing levels of ns-NSAID-related AGI were associated with a higher AIX% and a lower RWT (**Figure 1**). Mean differences in arterial function between patients taking diclofenac and the other three ns-NSAIDS (naproxen, indomethacin, ibuprofen) are shown in **Table 3**. Diclofenac was selected as the 'reference group' as it has the highest level of *in vitro* aldosterone glucuronidation inhibition (AGI) and was also the most commonly used ns-NSAID. Compared to the unadjusted comparisons, the adjustment for other CV and rheumatological factors reduced the differences between ns-NSAIDS, but the observed trend of a higher degree of arterial dysfunction (higher AIX% and lower RWT) being associated with increasing levels of ns-NSAID-related AGI persisted. The exception to this pattern was the slightly higher AIX% for naproxen compared to diclofenac. The differences between ibuprofen and diclofenac reached statistical significance (AIX% 6.5, 95%CI 1.0 to 11.9, p=0.02; and RWT –14.2 msecs 95%CI –22.2 to –6.3, p=0.001); although only 7 patients were chronic users of ibuprofen. The differences between diclofenac and the two other nsNSAIDS (indomethacin and naproxen) were not statistically significantly different.

Patients with no NSAID use over the previous 3 months (n=25) had a mean AIX% of 30.9 (SD 8.3) and RWT 133.1 (SD 12.5) msec. On adjusted comparison with current NSAID-users (all NSAIDS listed in **Table 1** combined) AIX% was –1.0 (95%CI –3.9 to 1.9; p=0.49) lower and RTW 4.3 msec (95%CI –0.6 to 9.2; p=0.09) higher among non-current users. The use indomethacin was associated with a similar level of dysfunction (AIX%) compared to patients not currently taking a NSAID. Interestingly the use of ibuprofen was associated with a lower level of AIX% compared to non-current users (unadjusted mean difference in AIX% 7.1, 95%CI -1.4 to 25.6) .

Eleven patients were currently taking a selective COX-2 inhibitor (8 celecoxib; 3 etoricoxib) and had a mean AIX% of 33.6 (6.4) and RWT 132.8 (SD 8.8) msec. On adjusted comparison with diclofenac, AIX% was non-significantly higher for patients taking a selective COX-2 inhibitor (2.9, 95%CI –1.4 to 6.8; p=0.19), whereas mean RWT was similar (adjusted difference –0.7 msec, 95%CI –6.7 to 5.4; p=0.80),

DISCUSSION

In summary we found that the current use of an ns-NSAID with a higher level of aldosterone glucuronidation inhibition (*in vitro* AGI), in patients with RA, appears to be associated with a higher level arterial dysfunction. Arterial dysfunction was highest in patients taking diclofenac (high AGI) and lowest in those taking ibuprofen (low AGI); difference in arterial function were not attributable to other important CV and rheumatological features that are known to influence arterial function, Patients using indomethacin (intermediate AGI) had an intermediate levels of arterial dysfunction, but we were not able to demonstrate an entirely consistent relationship since naproxen had a higher AIX% than would be anticipated from its relative level of *in vitro* AGI.

Comparison with existing literature

A small number of previous studies have assessed arterial dysfunction in patients with RA using pulse wave analysis (PWA).[13;26-29] The difference observed in AIX% in this analysis (of 6.5) between diclofenac and ibuprofen is of a statistically and clinically relevant magnitude. It compares, for example, with a 4.2 point reduction in AIX% associated with 3 months atorvastatin therapy in patients with RA.[28] At coronary angiography a 10-point higher AIX% at baseline is associated with a 27% increased risk of CV events over 4 years follow-up; a 10 millisecond higher RWT is associated with a 15% reduction in CV events.[30] Published research concerning NSAID-related arterial dysfunction is currently limited. Only one previous study has directly assessed the influence of NSAID-use on AIX%. In 12 patients with RA 14-days of therapy with indomethacin (75 mg BD) was associated with a 1.2 (95%CI -2.1 to 4.5) increase in AIX%.[29] In a study of men aged >50 years undergoing community-based screening for abdominal aortic aneurysm in the UK, the use of an NSAID (75% took either ibuprofen, diclofenac or indomethacin) was significantly associated with reduced aortic wall distensibility assessed using M-mode ultrasound.[31]

Study strengths and limitations

The strengths of our study are that a single research nurse undertook high quality PWA in a controlled environment among individuals with RA recruited from a consecutive series of patients attending rheumatology clinic. Our assessment of the 'cumulative inflammatory burden' (ESR-years) reflects both the level and duration of the inflammatory burden. Only one previous study investigating the relationship between arterial dysfunction and inflammation has attempted to assess 'inflammatory burden', but this was restricted to only the most recent 5 years of rheumatology clinic follow-up.[13] We measured and adjusted for several important CV and rheumatological factors in our analysis, including factors known to be independently associated with arterial function. PWA has previously been shown to be predictive of CV events in patients after coronary angiography.[30] Our multivariate model explained a high proportion of the variability (60%-70%) in arterial dysfunction between patients taking diclofenac and ibuprofen. The characteristics of our participants are similar to RA patients receiving outpatient care elsewhere in the UK.[32] The study prevalence of treated hypertension is lower than that reported elsewhere and probably relates to the exclusion of patients with overt arterial disease.[33]

The main limitation of our study is its cross-sectional nature which means that we can only assess association rather than direct causation. Since we did not measure serum aldosterone and ns-NSAID levels in these patients with RA, we are unable to directly confirm the previous *in vitro* findings. Whilst *in vitro* research is particularly useful for the investigation of biological mechanisms, such laboratory-based findings may not translate exactly to the less controlled situation of patients with rheumatoid arthritis. The analysis reported is based on data from an existing study, rather than from a study specifically

designed to assess the influence of different NSAIDS on arterial dysfunction. Our assessment of RA patients included a relatively small number of patients taking each NSAID and only 7 patients were chronic users of ibuprofen. The number of patients taking individual NSAIDS were too small to permit a comparison of the association of low/high-dose NSAIDS with arterial dysfunction. The relatively small size of our study restricted the number of potential confounding factors (rheumatological and CV) that could be included in the multivariate analysis without running the risk of over-fitting the data. Although the inclusion of additional variables in the multivariate model, such as treated hypertension, did not improve the goodness to fit, nor substantially alter the adjusted values reported for AIX and RWT. As with all observational studies we cannot excluded the possibility of residual confounding as an explanation for our findings.

Study implications

The finding that ns-NSAID-related AGI appears to be associated with arterial dysfunction in patients with RA provides a potentially novel insight into the CV toxicity of commonly used ns-NSAIDS. Our results are preliminary and require confirmation in larger studies looking at different ns-NSAIDS, serum aldosterone concentrations and surrogate markers of arterial dysfunction. Several important questions remain to be addressed, including whether high/low ns-NSAID dosage is related to arterial dysfunction and if switching ns-NSAIDS (from high-AGI to low-AGI) improves arterial function. If the adverse CV events associated with ns-NSAID-use are due to AGI, then switching to an alternative 'lower-AGI ns-NSAID' may be an appropriate option for patients heavily dependant on NSAIDS for symptomatic relief.

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Competing interests

We have no competing interests to declare.

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Author contributions

MAC - original study conception and design; analysis and interpretation of the data; initial drafting and re-drafting of the article; final approval of the version to be published.
AAM - original hypothesis; interpretation of data; revising the article critically for important intellectual content; re-drafting of the article; final approval of the version to be published.

Data-sharing

Consent for data-sharing was not obtained from study participants at the time of recruitment, but the presented data are held in an anonymised dataset. Access to the dataset is available from the corresponding author (at mike.crilly@abdn.ac.uk) in SPSS format for clinical academic researchers interested in undertaking a formally agreed collaborative research project(s). Although the risk of individual patient identification is low

any research involving the release of the dataset to other clinical academics would require approval by Grampian Research Ethics Committee.

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FIGURE LEGEND

Figure 1. Use of NSAIDS and central arterial function in patients with rheumatoid arthritis

STROBE Statement—Checklist of items that should be included in reports of ***cross-sectional studies***

Non-steroidal anti-inflammatory drug (NSAID) related inhibition of aldosterone glucuronidation and arterial dysfunction in patients with rheumatoid arthritis: a cross-sectional clinical study.

Michael A Crilly (26 January 2011)

	Item No	Recommendation
Title and abstract	1	(*a*) Indicate study's design in title: **"cross-sectional clinical study"**
		(*b*) Abstract: **Structured abstract provided**
Introduction		
Background/rationale	2	Scientific background: **NSAID-related inhibition of aldosterone metabolism is described**
Objectives	3	State specific objectives: **"Aim of this exploratory analysis was to assess the association between ns-NSAID-related aldosterone glucuronidation inhibition (AGI) and established markers of arterial dysfunction using data from a previous study of patients with RA**."
Methods		
Study design	4	Key elements of study design presented early: **"Our methods have been described in detail elsewhere." [ref #13. Crilly MA, Kumar V, Clark HJ, et al .Arterial stiffness and cumulative inflammatory burden in rheumatoid arthritis: a dose-response relationship independent of established cardiovascular risk factors. Rheumatology. 2009;48:1606-1612.] [ref # 14. Crilly MA, Clark HJ, Kumar V, et al. Relationship between arterial stiffness and Stanford Health Assessment Questionnaire disability in rheumatoid arthritis patients without overt arterial disease. J Rheumatol. 2010;37:946-952.]** **"In the original study we recruited patients with a consultant rheumatologist diagnosis of rheumatoid arthritis (RA) by reviewing the medical records of a consecutive series of patients attending hospital-based rheumatology clinics in the city of Aberdeen. We identified patients aged between 40-65 years with RA for more than 6 months duration."**
Setting	5	Setting, locations, and relevant dates: **"In the original study we recruited patients ... by reviewing the medical records of a consecutive series of patients attending hospital-based rheumatology clinics in the city of Aberdeen."** **"Patients attended the Clinical Pharmacology Department at Aberdeen Royal Infirmary (ARI) on a single occasion and underwent assessment by a single clinical research nurse (April-December 2006)."**
Participants	6	(*a*) Eligibility criteria, sources and methods of selection of participants: **"We excluded patients with overt arterial disease (angina, prior myocardial infarction, transient ischaemic attack, stroke, arterial revascularisation, intermittent claudication, peripheral arterial disease), atrial fibrillation, heart failure and valvular heart disease. The exclusion of patients with arterial disease was based upon an initial screening patient-questionnaire, resting 12-lead ECG (independently reported by a cardiologist to identify pathological Q-waves, conduction defects, minor Q-waves associated with ST-segment/T-wave anomalies), and a detailed medical record review by a rheumatologist. No participants had a history of recent infection, antibiotic treatment or immunisation within the previous two weeks."**

Variables	7	Define outcomes, exposures, and potential confounders: **"Standardised assessment included blood pressure (BP) measurement, pulse wave analysis (PWA), fasting venous blood sample (including erythrocyte sedimentation rate [ESR], rheumatoid factor [RF], and lipid profile). A self-completed patient questionnaire included smoking habit and the Stanford Health Assessment Questionnaire (HAQ). Current medication use was comprehensively reviewed by the research nurse and included use of over-the-counter (without the need for a prescription) NSAIDS. A detailed retrospective review of the medical records using a previously piloted study form, was undertaken by a single rheumatologist blinded to all PWA results and included date of arthritis onset, previous blood test results (erythrocyte sedimentation rate, rheumatoid factor), joint surgery and co-morbidity (including treated hypertension)."** Diagnostic criteria: **"Consultant rheumatologist diagnosis of rheumatoid arthritis (RA)"** **"Our methods have been described in detail elsewhere.[ref #13. Rheumatology. 2009;48:1606-1612.]" -- we have previously reported on the proportion of patients with a consultant rheumatologist diagnosis of rheumatoid arthritis (RA) who meet "American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis.[Arnett FC, et al. Arthritis Rheum 1988 Mar;31(3):315-24].** **[Text from Rheumatology. 2009;48:1606-1612] "Whilst all of our study participants had a clinical diagnosis of RA made by a rheumatologist, only 56% met ACR (4/7) criteria for RA. This may be attributable to a typographical error in our questionnaire (which asked about morning stiffness for 6 months rather than 6 weeks). Whilst 39% of patients reported more than one hour of morning stiffness for more than 6 months in the past, only 18% of patients reported such stiffness over the previous week. Some clinical heterogeneity may exist in our study population of patients with a clinical rheumatological diagnosis of RA, although the inclusion of 'ACR criteria' as a variable in the fully adjusted analysis made no difference to our results. ACR-criteria also 'accumulates' over time. For example, in our study the median duration of arthritis was almost 10 years: some 66% (36/55) of patients with arthritis duration greater than 10 years met ACR-criteria, compared to 48% (28/59) with a shorter duration."**
Data sources/ measurement	8*	Sources of data and details of methods of assessment (measurement): **"Analysis is based on the mean of the three PWA measurements. The principle measures of arterial dysfunction are augmentation index (AIX%) and reflected wave transit time (RWT, msec). Since AIX% varies with heart rate in an individual it was standardised to 75 beats-per-minute."** **"Aldosterone 18ß-glucuronidation inhibition constants, Ki , derived from in vitro studies of human kidney cortical microsomes (HKCM), have been published for 4 of the ns-NSAIDS taken by patients in the RAAIX study (diclofenac 8 µM, naproxen 49 µM, indomethacin 113 µM, ibuprofen 441 µM; a lower Ki indicates greater inhibition). [ref #6: Knights KM, Winner LK, Elliot DJ, et al. Glucuronidation by human liver and kidney microsomes and recombinant UDP-glucuronosyltransferases: inhibition by NSAIDs. Br J Clin Pharmacol 2009;68:402-412.] Nabumetone is a close structural analogue of naproxen and the two were combined together in the analysis."**
Bias	9	Efforts to address potential sources of bias: **"BP was measured at the right brachial artery using an validated automatic oscillometric BP machine (Omron HEM757 IntelliSense BP monitor; Omron Healthcare, Illinois, USA)." [El Assaad MA, et al. Evaluation of two devices for self-measurement of blood pressure according to the international protocol: the Omron M5-I and the Omron 705IT. Blood Press Monit 2003;8:127-133.]**

		"**A detailed retrospective review of the medical records, using a previously piloted study form, was undertaken by a single rheumatologist blinded to all PWA results".** **"The nurse remained blind to the patients' previous medical records (which were not made available at assessment) and only reviewed current medication and questionnaire responses (to ensure that all questionnaires items were fully completed), after PWA assessment had been completed".**
Study size	10	Study size: **"Our methods have been described in detail elsewhere.[ref #13. Rheumatology. 2009;48:1606-1612.]"** **The original study recruited 114 patients.** **[Text from Rheumatology. 2009;48:1606-1612] "A sample size of 110 patients with RA was our intention, in order to ensure that there were 10 subjects for each CV risk factor included in the multi-variable analysis. We included 11 cardiovascular risk factors in our analysis, based on those contributing 90% of the 'population attributable risk' to myocardial infarction in the recent INTERHEART study."**
Quantitative variables	11	Quantitative variables handled: **AIX% and RWT were Normally distributed and summarised as mean (SD).** **"Analysis is based on the mean of the three PWA measurements"** Groupings chosen: **Not applicable**
Statistical methods	12	(*a*) Statistical methods (including control for confounding): **Multiple linear regression (MLR) was used to adjust mean differences in AIX% (and RWT) for variables known to be associated with AIX%, namely: age, sex, mean arterial blood pressure, ever smoked, Stanford HAQ disability score and cumulative ESR-years.**
		(*b*) Subgroups and interactions: **Not applicable**
		(*c*) Missing data: **We had negligible levels of missing data due to the implementation of a rigorous study design and the diligence of our research nurse (and rheumatologist reviewing the medical records). Except for the calculation of 'ESR-years' (as a measure of cumulative inflammatory burden) the level of missing data was minimal.** **"Cumulative ESR-years were derived from the highest single annual ESR recorded in the medical record during each year of follow-up and calculated using the 'trapezium rule' with linear interpolation when data for a given year was missing."** **"Our methods have been described in detail elsewhere. [ref #13. Rheumatology 2009;48:1606-1612.]"** **[Text from Rheumatology. 2009;48:1606-1612] "Medical records were available for 112 patients (complete medical records could not be obtained for two patients) who contributed a total of 1,040 person-years of rheumatoid disease. An annual ESR was available for 77% (797/1,040) of these person-years. Missing ESR data was imputed as previously described. For individual patients the average availability of an annual ESR, for each year since the onset of arthritis, was a median of 93% (IQR 67% to 100%). The availability of an annual ESR did not differ by age, gender, rheumatoid factor, ACR-RA criteria or Stanford disability index (data not shown)."** **Of the 114 patients recruited, 112 had complete data and were included in the fully adjusted regression analysis/model [see Table 3 from ref #13; Rheumatology. 2009;48:1606-1612]**

		(*d*) Analytical methods taking account of sampling strategy: **Not applicable**
		(e) Sensitivity analyses: **"The inclusion of additional variables (study ESR, duration of arthritis, fasting cholesterol, smoking pack-years, treated hypertension and current DMARD use) did not alter the adjusted values for AIX and RWT reported, nor improve the goodness to fit of the final regression model."**
Results		
Participants	13*	(a) Report numbers of individuals at each stage of study: **"The original study recruited 114 patients. We excluded 9 patients from the analysis who were not currently taking NSAIDS, but had done so within the previous 3 months; and excluded 2 users of infrequently prescribed NSAIDS (ketoprofen and tiaprofenic acid)."** **"Our methods have been described in detail elsewhere. [ref #13. Rheumatology 2009;48:1606-1612.]" – we had negligible levels of missing data due to the implementation of a very careful study design and the diligence of our research nurse (and rheumatologist reviewing the medical records). Of the 114 patients recruited 112 had complete data and were included in the fully adjusted regression analysis/model [see Table 3 from ref #13; Rheumatology. 2009;48:1606-1612]** **We have no data available concerning the number of RA patients excluded at the rheumatology clinic level because they were already known to have arterial disease.** **We also have no data concerning the number of eligible RA patients attending rheumatology clinic who declined to be contacted by our research nurse. All of the RA patients who did agree to being contacted by the research nurse subsequently attended for assessment (N=114) and have been included in this analysis as described.** **No patients were excluded because of technical failure to undertake PWA. Despite initial (pre-study) concerns that it might not be feasible to undertake PWA in some RA patients, due to diseased wrists, this proved not to be the case. The research nurse successfully undertook PWA on all participants.**
		(b) Reasons for non-participation: **Not known/recorded**
		(c) Flow diagram: **None/ not applicable**
Descriptive data	14*	(a) Characteristics of study participants: **Shown in detail in Table 1**
		(b) Indicate number of participants with missing data: **Of the 114 patients recruited, 112 had complete data and were included in the fully adjusted regression analysis/model [see Table 3 from ref #13; Rheumatology. 2009;48:1606-1612]**
Outcome data	15*	Report summary measures: **"Analysis is based on the mean of the three PWA measurements. The principle measures of arterial dysfunction are augmentation index (AIX%) and reflected wave transit time (RWT, msec). Since AIX% varies with heart rate in an individual it was standardised to 75 beats-per-minute."**
Main results	16	(*a*) Unadjusted estimates and confounder-adjusted estimates (95%CI): **Table 3 reports unadjusted and adjusted mean differences in AIX% and RWT (with their related 95%CI's)** Make clear which confounders were adjusted for and why they were included **"Multiple linear regression (MLR) was used to adjust mean differences in AIX% (and RWT) for variables known to be associated with AIX%, namely: age, sex, mean arterial blood pressure, ever smoked, Stanford HAQ disability score and cumulative ESR-years. Analysis was undertaken using SPSS v 17.We confirmed that the assumptions of**

		linearity, normal distribution and equal variance for MLR were met. 'Goodness to fit' was assessed using the adjusted R^2. The inclusion of additional variables (study ESR, duration of arthritis, fasting cholesterol, smoking pack-years, treated hypertension and current DMARD use) did not alter the adjusted values for AIX and RWT reported, nor improve the goodness to fit of the final regression model."
		(*b*) Category boundaries (continuous variables categorized): **Not applicable**
		(*c*) Estimates of absolute risk: **Not applicable**
Other analyses	17	Subgroup analysis: **None** Sensitivity analyses: **see STROBE #12e (above)**
Discussion		
Key results	18	Summarise key results with reference to study objectives: **"In summary we found that the current use of an ns-NSAID with a higher level of aldosterone glucuronidation inhibition (AGI), in patients with RA, appears to be associated with a higher level arterial dysfunction. Arterial dysfunction was highest in patients taking diclofenac and lowest in those taking ibuprofen. These difference in arterial function were not attributable to other important CV and rheumatological features that are known to influence arterial function, since the observed differences remained after adjusting for such features. Consequently differences in AIX and RWT appear to be genuinely related to the use of different ns-NSAIDs. Patients using ns-NSAIDS with intermediate levels of AGI, naproxen and indomethacin, demonstrated intermediate levels of arterial dysfunction."**
Limitations	19	Limitations of the study, sources of potential bias or imprecision: **"The main limitation of our study is its cross-sectional nature which means that we can only assess association rather than direct causation. Since we did not measure serum aldosterone and ns-NSAID levels in these patients with RA we are unable to directly confirm the previous in vitro findings. Our assessment of RA patients included a relatively small number of patients taking each NSAID and only 7 patients were chronic users of ibuprofen. The number of patients taking individual NSAIDS were too small to permit a comparison of association of low/high-dose NSAIDS with arterial dysfunction. The relatively small size of our study restricted the number of potential confounding factors (rheumatological and CV) that could be included in the multivariate analysis without running the risk of over-fitting the data. …. As with all observational studies we cannot excluded the possibility of residual confounding as an explanation for our findings."**
Interpretation	20	Cautious overall interpretation of results: **"Our results are preliminary and require confirmation in larger studies looking at different ns-NSAIDS, serum aldosterone concentrations and surrogate markers of arterial dysfunction. Several important questions remain to be addressed, including whether high/low ns-NSAID dosage is related to arterial dysfunction."**
Generalisability	21	Generalisability (external validity) of the study results: **"The characteristics of our participants are similar to RA patients receiving outpatient care elsewhere in the UK. [ref #32. Panoulas VF, Douglas KM, Milionis HJ et al. Prevalence and associations of hypertension and its control in patients with rheumatoid arthritis. Rheumatology 2007;46:1477-1482]"**
Other information		
Funding	22	Source of funding and role of the funders: **"The original study was supported by charitable funding from NHS Grampian Rheumatology Endowments. The funders played no role in the analysis or reporting of this study."**

*Give information separately for exposed and unexposed groups.

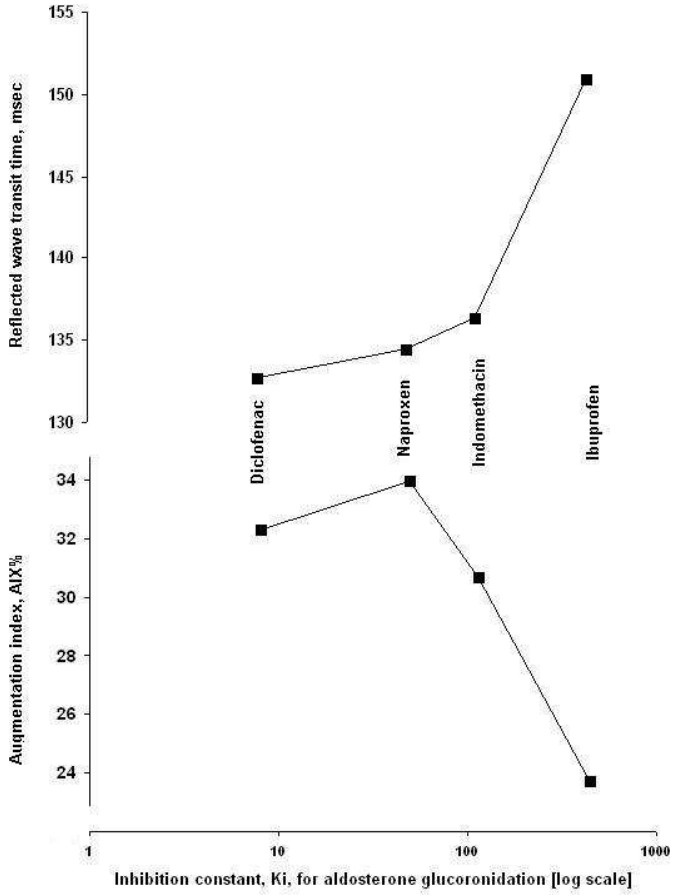

Figure 1. Use of NSAIDS and central arterial function in patients with rheumatoid arthritis
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Non-steroidal anti-inflammatory drug (NSAID) related inhibition of aldosterone glucuronidation and arterial dysfunction in patients with rheumatoid arthritis: a cross-sectional clinical study

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TITLE PAGE

Non-steroidal anti-inflammatory drug (NSAID) related inhibition of aldosterone glucuronidation and arterial dysfunction in patients with rheumatoid arthritis: a cross-sectional clinical study

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KEY WORDS:
Arterial dysfunction; Pulse wave analysis; Non-steroidal anti-inflammatory drugs; Rheumatoid arthritis; Aldosterone.

WORD COUNT = 3,253
WORD COUNT: ABSTRACT = 292

NUMBER OF TABLES = 3
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ABSTRACT

Objective:
Patients with rheumatoid arthritis (RA) are at increased risk of cardiovascular (CV) disease and are also commonly prescribed non-selective non-steroidal anti-inflammatory drugs (ns-NSAIDS). New *in vitro* evidence suggests that this increased CV risk may be mediated through aldosterone glucuronidation inhibition (AGI), which differs between NSAIDS (diclofenac > naproxen > indomethacin > ibuprofen). Our aim was to explore the association between ns-NSAID-related AGI and arterial dysfunction.

Methods:
The extent (augmentation index, AIX%) and timing (reflected wave transit time, RWT, msec) of aortic wave reflection (measured using radial applanation pulse wave analysis, PWA, SphygmoCor device) were assessed on a single occasion in 114 consecutive RA patients without overt CV disease aged 40-65 years. A 'higher AIX%' and 'lower RWT' indicate arterial dysfunction. Assessment included fasting blood sample, patient questionnaire and medical record review. Multivariate analysis was used to adjust for age, sex, mean blood pressure, smoking, cumulative erythrocyte sedimentation rate (ESR-years) and Stanford disability score.

Results:
We identified 60 patients taking ns-NSAIDS and 25 non-users. Using a ns-NSAID with the highest AGI was associated with a higher AIX% (and lower RWT) versus treatment with a ns-NSAID with the lowest AGI (diclofenac AIX% 32.3, RWT 132.7 msec; versus ibuprofen AIX% 23.8, RWT 150.9 msec): adjusted mean differences AIX% 6.5 (95%CI 1.0 to 11.9; p=0.02); RWT –14.2 milliseconds (95%CI –22.2 to –6.3; p=0.001). Indomethacin demonstrated an intermediate level of arterial dysfunction. In relation to arterial dysfunction both indomethacin and naproxen were more similar to diclofenac than ibuprofen

Conclusions:
ns-NSAID-related AGI is associated with arterial dysfunction in patients with RA These findings provide a potentially novel insight into the CV toxicity of commonly used ns-NSAIDS. The study findings are limited by the small number of patients involved and require further replication in a much larger study.

KEY WORDS: Arterial dysfunction; Pulse wave analysis; Non-steroidal anti-inflammatory drugs; Rheumatoid arthritis; Aldosterone.

ABSTRACT WORD COUNT = 292

ARTICLE SUMMARY

Article focus

Aldosterone glucuronidation inhibition (AGI) potentiates the adverse cardiovascular effects of aldosterone. Recently published *in vivo* research suggests that such inhibition differs between non-selective non-steroidal anti-inflammatory drugs (ns-NSAIDS); with a ranked order of diclofenac > naproxen > indomethacin > ibuprofen. No previous studies have assessed the relationship between ns-NSAID-related AGI and arterial dysfunction in chronic users. This study assessed arterial dysfunction using pulse wave analysis (PWA).

Key messages

In patients with rheumatoid arthritis (RA) we found that chronic use (> 3 months) of diclofenac (high AGI) was associated with greater arterial dysfunction compared to ibuprofen (lower AGI). This association was independent of other cardiovascular and rheumatological factors. Indomethacin (intermediate AGI) was associated with an intermediate level of arterial dysfunction, although naproxen (intermediate AGI) did not fit the anticipated pattern. Our findings support the concept that AGI may play a role in the CV toxicity of some ns-NSAIDS commonly used in routine clinical practice.

Strengths and limitations of this study

A single research nurse assessed RA patients who were recruited from a consecutive series attending hospital rheumatology clinic. We adjusted for several important cardiovascular and rheumatological factors known to be independently associated with arterial function and our multivariate analysis explained a high proportion of the variability in arterial dysfunction among chronic ns-NSAID users. The observational cross-sectional design of our study means that we cannot assess causation, nor exclude residual confounding as an explanation for our findings. The small number of patients taking each NSAID meant that the confidence intervals are wide.

INTRODUCTION

Non-steroidal anti-inflammatory (NSAID) are among the most commonly prescribed drugs in clinical practice. Their relative safety has attracted considerable interest, particularly in relation to their association with adverse cardiovascular (CV) events.[1;2] Most of this interest has focused on the role of selective cyclo-oxygenase-2 (COX-2) inhibitors,[3] but non-selective NSAIDS (ns-NSAIDS) also have the potential to increase the risk of adverse CV events. For example, the use of the ns-NSAID diclofenac has been shown to increase the risk of adverse CV events.[2] ns-NSAID use is associated with adverse CV effects including reduction of renal perfusion, electrolyte disturbances (sodium and water retention) and increase in blood pressure. The detrimental effects in renal function are thought to be secondary to a reduced synthesis of vasodilatory prostaglandins such as PGE2. However, ns-NSAIDS have been shown to exert adverse renal effects disproportionate to the level of inhibition of prostaglandin synthesis.[4] This suggests that there might be other yet unknown mechanisms responsible for the potential increase in CV risk associated with ns-NSAID use.[5]

Aldosterone metabolism

Very recent *in vitro* evidence suggests that ns-NSAIDS enhance the action of aldosterone through the inhibition of aldosterone metabolism.[6] Aldosterone is metabolised by 18ß-glucuronidation in both the liver and kidneys in a reaction that is catalyzed by the enzyme UDP-glucuronosyltransferase-2B7. Several ns-NSAIDS have been shown *in vitro* (human kidney cortical microsomes) to inhibit aldosterone 18ß-glucuronidation and individual NSAIDS vary in their ability to inhibit aldosterone glucuronidation.[6] Diclofenac, for example, is a strong inhibitor of glucuronidation, whereas ibuprofen is a weaker inhibitor.[6]

Aldosterone is a mineralocorticoid which plays an important role in the renin-angiotensin-aldosterone system and has generally deleterious effects on the CV system. Higher aldosterone levels are associated with endothelial dysfunction, arterial stiffening, increased arterial wall reflection, myocardial fibrosis and an increase in the risk of CV death.[7-10] Drugs that block the action of aldosterone (such as spironolactone) have been shown to reduce the risk of CV death in patients with heart failure and following myocardial infarction.[11] Aldosterone receptors predominate in the aorta and spironolactone has been shown to improve arterial function assessed using the technique of pulse wave analysis (PWA).[7] Consequently the increased risk of adverse CV events in patients taking diclofenac may also be related to the enhanced effects of aldosterone as a consequence of ns-NSAID-related aldosterone glucuronidation inhibition (AGI).

RAAIX study

Patients with rheumatoid arthritis (RA) are known to be at a higher risk of CV death.[12] The RAAIX (RA augmentation index) study was undertaken to assess the relationship between the cumulative inflammatory burden and arterial dysfunction in patients with rheumatoid arthritis (RA).[13;14] RAAIX involved a detailed assessment of both CV and rheumatological features; including the assessment of the use of NSAIDS. Other researchers have subsequently reported the relative level of aldosterone 18ß-glucuronidation inhibition (AGI) for several of the ns-NSAIDS taken by patients in the RAAIX study.[6] In the RAAIX study arterial dysfunction was assessed non-invasively using radial applanation tonometry and pulse wave analysis (PWA).[13;14] PWA is based on the phenomenon of 'arterial wave reflection' which is influenced by pulse wave velocity, endothelial dysfunction, peripheral arterial resistance and left ventricular ejection.[15;16] In each cardiac cycle the outgoing systolic pulse wave is also reflected back towards the heart, predominantly at the level of arterial bifurcations,[17] and returns to the heart during

systole where it augments the central aortic pressure.[15;16] The speed of travel of both outgoing and reflected waves are greater in patients with stiffer arteries; which increases the extent of augmentation (higher AIX%) and reduces the reflected wave transit time (lower RWT).

Study aim

The aim of this exploratory analysis was to assess the association between ns-NSAID-related aldosterone glucuronidation inhibition (AGI) and established markers of arterial dysfunction using data from a previous study of patients with RA.

METHODS

In the original study we recruited patients with a consultant rheumatologist diagnosis of rheumatoid arthritis (RA) by reviewing the medical records of a consecutive series of patients attending hospital-based rheumatology clinics in the city of Aberdeen. We identified patients aged between 40-65 years with RA for more than 6 months duration. Whilst all of our study participants had a clinical diagnosis of RA made by a rheumatologist, only 56% (64/114) met 'American College of Rheumatology' criteria (ACR 4/7) for RA. We excluded patients with overt arterial disease (angina, prior myocardial infarction, transient ischaemic attack, stroke, arterial revascularisation, intermittent claudication, peripheral arterial disease), atrial fibrillation, heart failure and valvular heart disease. The exclusion of patients with arterial disease was based upon an initial screening patient-questionnaire, resting 12-lead ECG (independently reported by a cardiologist to identify pathological Q-waves, conduction defects, minor Q-waves associated with ST-segment/T-wave anomalies), and a detailed medical record review by a rheumatologist. No participants had a history of recent infection, antibiotic treatment or immunisation within the previous two weeks.

Clinical assessment

Patients attended the Clinical Pharmacology Department at Aberdeen Royal Infirmary (ARI) on a single occasion and underwent assessment by a single clinical research nurse (April-December 2006). Assessment took place in the morning after participants had fasted overnight and abstained from smoking, alcohol and caffeine. Standardised assessment included blood pressure (BP) measurement, pulse wave analysis (PWA), fasting venous blood sample (including erythrocyte sedimentation rate [ESR], rheumatoid factor [RF], and lipid profile). A self-completed patient questionnaire included smoking habit and the Stanford Health Assessment Questionnaire (HAQ).[18] Current medication use was comprehensively reviewed by the research nurse and included use of over-the-counter (without the need for a prescription) NSAIDS. A detailed retrospective review of the medical records using a previously piloted study form, was undertaken by a single rheumatologist blinded to all PWA results and included date of arthritis onset, previous blood test results (erythrocyte sedimentation rate, rheumatoid factor), joint surgery and co-morbidity (including treated hypertension). Our methods have been described in detail elsewhere.[13;14] The nurse remained blind to the patients' previous medical records (which were not made available at assessment) and only reviewed current medication and questionnaire responses (to ensure that all questionnaires items were fully completed), after PWA assessment had been completed.

Pulse wave analysis (PWA)

Patients rested supine in a quiet side-room for at least 10 minutes before undergoing three BP/PWA measurements according to current guidelines.[20] BP was measured at the right brachial artery using an validated automatic oscillometric BP machine (Omron HEM757 IntelliSense BP monitor; Omron Healthcare, Illinois, USA).[21] Pulse wave analysis (PWA)

was undertaken using the SphygmoCor device (AtCor Medical, Sydney, Australia) with a hand-held tonometer (Millar, Texas, USA) 'applanated' at the right radial artery. The 'SphygmoCor' PWA device employs a validated 'generalised transfer function' to derive the central aortic pulse waveform from the peripheral waveform.[22] All three PWA recordings were required to have a in-built SphygmoCor quality index score at least 95% (based on average pulse height, pulse height variation and diastolic variation). We have previously demonstrated her high levels of within-observer and between-observer repeatability.[19] The research nurse remained blind to the patients' previous medical history until PWA was completed.

Aldosterone glucuronidation inhibition (AGI)

Aldosterone 18ß-glucuronidation inhibition constants, Ki , derived from *in vitro* studies of human kidney cortical microsomes (HKCM), have been published for 4 of the ns-NSAIDS taken by patients in the RAAIX study (diclofenac 8 µM, naproxen 49 µM, indomethacin 113 µM, ibuprofen 441 µM; a lower Ki indicates greater inhibition).[6] Nabumetone is a close structural analogue of naproxen and the two were combined together in the analysis.

Statistical analysis

Analysis is based on the mean of the three PWA measurements. The principle measures of arterial dysfunction are augmentation index (AIX%) and reflected wave transit time (RWT, msec). Since AIX% varies with heart rate in an individual it was standardised to 75 beats-per-minute.[23] The UK version of the Stanford-HAQ was scored using standard methods without any imputation required for missing data.[18] Cumulative ESR-years were derived from the highest single annual ESR recorded in the medical record during each year of follow-up and calculated using the 'trapezium rule' with linear interpolation when data for a given year was missing.[24] ESR-years reflects both the duration and level of inflammatory burden (e.g. 5 years of arthritis and annual ESR's of 30, 20, 10, 10, 20 mm/hour would equate to approximately 90 ESR-years). ESR is routinely measured for almost all RA-patients attending rheumatology out-patient clinic in Aberdeen, where the policy is to review all RA-patients at least annually. Complete medical records were available for 112 patients who contributed a total of 1,040 person-years of rheumatoid disease. An annual ESR was available for 77% (797/1,040) of these person-years and the availability of an annual ESR for each year since the onset of arthritis for individual patients was a median of 93% (IQR 67%–100%). The availability of an annual ESR did not differ by age, gender, rheumatoid factor positivity, RA-criteria (ACR 4/7), or Stanford HAQ disability index (data not shown).

Multiple linear regression (MLR) was used to adjust mean differences in AIX% (and RWT) for variables known to be associated with AIX%, namely: age, sex, mean arterial blood pressure, ever smoked, Stanford HAQ disability score and cumulative ESR-years. Analysis was undertaken using SPSS v 17.We confirmed that the assumptions of linearity, normal distribution and equal variance for MLR were met. 'Goodness to fit' was assessed using the adjusted R^2. The inclusion of additional variables (study ESR, duration of arthritis, RA-criteria, fasting cholesterol, smoking pack-years, treated hypertension and current DMARD use) did not alter the adjusted values for AIX and RWT reported, nor improve the goodness to fit of the final regression model.

The study adhered to the principles of the Declaration of Helsinki and was approved by Grampian Research Ethics Committee (study reference: 04/S0801/67). All participants provided informed written consent. The study was funded from a charitable source (NHS Grampian Rheumatology Endowments).

RESULTS

The original study recruited 114 patients. We excluded 9 patients from the analysis who were not currently taking NSAIDS, but had done so within the previous 3 months; and excluded 2 users of infrequently prescribed NSAIDS (ketoprofen and tiaprofenic acid). The characteristics of the remaining 103 patients (82% female) are shown in **Table 1**.

Table 1. Characteristics of patients with rheumatoid arthritis

Figures are numbers (%) unless otherwise indicated

	Current NSAID use					
	Yes (n=78)			No (n=25)		
Cardiovascular features						
Female	64	82%		20	80%	
Mean age, years (SD)	53.4	6.9		53.9	5.7	
Mean heart rate, beats per minute (SD)	69.7	10.6		64.5	8.6	
Mean systolic BP, mmHg (SD)	126.2	16.9		124.7	12.1	
Mean diastolic BP, mmHg (SD)	82.9	10.0		81.3	7.6	
Mean pulse pressure, mmHg (SD)	35.6	9.1		36.9	7.4	
Mean arterial BP, mmHg (SD)	99.5	12.2		97.8	8.8	
Mean fasting cholesterol, mmol/L (SD)	5.3	1.2		5.6	1.1	
Mean fasting glucose, mmol/L (SD)	5	0.8		5	0.5	
Mean waist-hip ratio (SD)	0.85	0.08		0.85	0.08	
Ever smoked for 12 months or more	44	56%		17	68%	
Treated hypertension	12	15%		7	28%	
Bendroflumethiazide	10	13%		5	20%	
Atenolol	6	8%		5	20%	
Calcium channel blocker	3	4%		1	4%	
Angiotensin converting enzyme inhibitor	2	3%		3	12%	
Rheumatological features						
Mean age onset arthritis, years (SD)	41.8	10.4		41.4	12.1	
Median duration arthritis, years (IQR)	9	4	15	8	4	16
Median Stanford HAQ disability (IQR)	1.4	0.6	1.9	0.6	0.3	1.3
Median study ESR, mm/h (IQR)	19	8	30	10	6	18
Median cumulative ESR-years (IQR)	206	99	468	93	58	297
Rheumatoid factor positive (>=30 IU/ml)	66	85%		18	72%	
Rheumatoid arthritis criteria (ACR 4/7)	47	60%		10	40%	
Previous joint surgery	17	22%		4	16%	
Current DMARD therapy	70	90%		24	96%	
Current prednisolone therapy	8	10%		3	12%	
Current NSAID therapy (>3 months)	78	100%		0	0%	
Diclofenac	31	40%		--	--	
Naproxen/ Nabumetone	16	21%		--	--	
Celecoxib/ Etoricoxib	11	14%		--	--	
Ibuprofen	7	9%		--	--	
Meloxicam	7	9%		--	--	
Indomethacin	6	8%		--	--	
Other (ketoprofen, tiaprofemic acid)	2	3%				
Current cytokine therapy	4	5%		1	4%	
Proton pump inhibitor	27	35%		4	16%	
Misoprostol	6	8%		0	0%	
Central arterial function						
Mean augmentation index, AIX% (SD)	31.9	7.9		30.9	8.3	
Mean reflected wave transit time, msec (SD)	135.7	11.4		133.1	12.4	

Blood pressure (BP), disease modifying anti-rheumatic drug (DMARD), erythrocyte sedimentation rate (ESR), American College of Rheumatology (ACR), Health Assessment Questionnaire (HAQ), non-steroidal anti-inflammatory drug (NSAID), standard deviation (SD), inter-quartile range (IQR)

Table 2. Patient characteristics and use of non-selective non-steroidal anti-inflammatory drugs

Figures are numbers (%) unless otherwise indicated

	Diclofenac (n=31)			Naproxen (n=16)			Indomethacin (n=6)			Ibuprofen (n=7)		
Aldosterone 18ß-glucuronidation inhibition constant (Ki), µM	**Ki = 8**			**Ki = 49**			**Ki = 113**			**Ki = 441**		
Cardiovascular features												
Female	28	90%		12	75%		5	83%		5	71%	
Mean age, years (SD)	54	6.5		55	5.6		57	5.9		50	9.5	
Mean heart rate, beats per minute (SD)	70.7	11.9		73.0	10.5		68.9	4.6		70.0	10.9	
Mean systolic BP, mmHg (SD)	126	17.6		125	20.3		118	14.0		123	13.4	
Mean diastolic BP, mmHg (SD)	83	9.9		82	12.1		75	7.4		84	7.9	
Mean pulse pressure, mmHg (SD)	35.3	10.5		35.3	9.0		34.4	6.1		31.1	8.1	
Mean arterial BP, mmHg (SD)	99	12.2		99	15.3		92	9.8		100	10.3	
Mean fasting cholesterol, mmol/L (SD)	5.4	1.3		5.4	1.5		5.8	0.9		5.1	1.0	
Mean fasting glucose, mmol/L (SD)	4.9	1.0		5.1	0.6		5.0	0.4		4.9	0.5	
Mean waist-hip ratio (SD)	0.85	0.08		0.86	0.09		0.89	0.08		0.88	0.09	
Ever smoked for 12 months or more	17	55%		9	60%		4	67%		3	43%	
Treated hypertension	7	23%		3	19%		2	33%		0	0%	
Rheumatological features												
Mean age onset arthritis, years (SD)	43	11.2		42	8.4		39	7.1		37	11.9	
Median duration arthritis, years (IQR)	9	3	15	10	2	17	19	11	22	11	7	20
Median Stanford HAQ (IQR)	1.4	0.8	2.0	1.6	1.2	1.9	1.5	0.9	1.9	0.6	0.0	1.9
Median ESR-years (IQR)	221	99	526	186	77	435	706	140	825	315	81	829
Rheumatoid factor positive (>=30 IU/ml)	26	84%		13	81%		6	100%		7	100%	
Rheumatoid arthritis criteria (ACR 4/7)	14	45%		11	69%		4	67%		5	71%	
Previous joint surgery	9	29%		4	25%		1	20%		2	29%	
Current DMARD therapy	28	90%		15	94%		5	83%		6	86%	
Central arterial function												
Mean augmentation index, AIX% (SD)	32.3	7.1		34.0	7.3		30.7	3.6		23.8	14.1	
Mean reflected wave transit time, msec (SD)	132.7	7.3		134.5	12.6		136.4	8.7		150.9	18.0	

Blood pressure (BP), disease modifying anti-rheumatic drug (DMARD), erythrocyte sedimentation rate (ESR),
American College of Rheumatology (ACR), Health Assessment Questionnaire (HAQ), standard deviation (SD), inter-quartile range (IQR)

Table 3. Differences in central arterial function associated with the use of non-selective non-steroidal anti-inflammatory drugs

	Unadjusted mean difference				Adjusted mean difference				Summary of model		
		95% CI		P		95% CI		P	R	Adj. R^2	P (ANOVA)
Augmentation index (AIX%)											
Diclofenac	'Reference group'										
Naproxen/ Nabumetone	**1.7**	-2.8	6.1	0.46	**1.7**	-2.0	5.4	0.35	0.70	0.40	0.0003
Indomethacin	**-1.6**	-7.7	4.5	0.59	**-1.0**	-7.8	5.8	0.77	0.65	0.28	0.02
Ibuprofen	**-8.6**	-15.9	-1.2	0.02	**-6.5**	-11.9	-1.0	0.02	0.86	0.67	0.000001
Reflected wave transit (RWT), msec											
Diclofenac	'Reference group'										
Naproxen/ Nabumetone	**1.9**	-3.9	7.7	0.52	**1.3**	-4.5	7.0	0.66	0.54	0.16	0.05
Indomethacin	**3.8**	-3.0	10.6	0.26	**3.5**	-4.8	11.7	0.40	0.56	0.14	0.13
Ibuprofen	**18.2**	9.8	26.6	0.0001	**14.2**	6.3	22.2	0.001	0.82	0.59	0.00001

Adjusted using multiple linear regression (MLR) for age, sex, mean arterial blood pressure, ever smoked, Stanford HAQ score and cumulative ESR-years

No patients were taking aspirin or more than one NSAID concurrently. All NSAID-users had been taking their current NSAID for more than 3 months. Diclofenac was the most commonly used NSAID and was taken by almost a third of patients. One quarter of patients had not taken an NSAID within the previous 3 months (although 88% of these patients had been prescribed an NSAID in the past). All patients, both NSAID-users and non-users, had previously received DMARD therapy.

Patient characteristics

NSAID-users and non-users were similar for age, sex, waist-hip ratio, fasting lipids, glucose, arthritis duration, age arthritis onset and previous joint surgery **(Table 1).** Based on the Scottish 'ASSIGN' score,[25] NSAID-users had a slightly lower 10 year predicted risk of a CV event compared to non-users (median 6% versus 8%; Mann-Whitney U test, p=0.38).[25] NSAID-users had a higher brachial blood pressure at assessment, lower prevalence of treated hypertension and a lower exposure to tobacco. The level of current inflammation (study ESR), cumulative inflammation (ESR-years) and arthritis related disability (Stanford HAQ) were higher in current NSAID-users. The use of proton pump inhibitors and misoprostol was also higher among current NSAID-users. The current use of prednisolone and DMARD was similar for both groups; the overall use of cytokines was relatively low. The rheumatological and CV features of ns-NSAID-users (with NSAIDS ordered by their level of aldosterone glucuronidation inhibition) are shown in **Table 2**

Differences in arterial function

Increasing levels of ns-NSAID-related AGI were associated with a higher AIX% and a lower RWT (**Figure 1**). Mean differences in arterial function between patients taking diclofenac and the other three ns-NSAIDS (naproxen, indomethacin, ibuprofen) are shown in **Table 3**. Diclofenac was selected as the 'reference group' as it has the highest level of *in vitro* aldosterone glucuronidation inhibition (AGI) and was also the most commonly used ns-NSAID. Compared to the unadjusted comparisons, the adjustment for other CV and rheumatological factors reduced the differences between ns-NSAIDS, but the observed trend of a higher degree of arterial dysfunction (higher AIX% and lower RWT) being associated with increasing levels of ns-NSAID-related AGI persisted. The exception to this pattern was the slightly higher AIX% for naproxen compared to diclofenac. The differences between ibuprofen and diclofenac reached statistical significance (AIX% 6.5, 95%CI 1.0 to 11.9, p=0.02; and RWT –14.2 msecs 95%CI –22.2 to –6.3, p=0.001); although only 7 patients were chronic users of ibuprofen. The differences between diclofenac and the two other nsNSAIDS (indomethacin and naproxen) were not statistically significantly different.

Patients with no NSAID use over the previous 3 months (n=25) had a mean AIX% of 30.9 (SD 8.3) and RWT 133.1 (SD 12.5) msec. On adjusted comparison with current NSAID-users (all NSAIDS listed in **Table 1** combined) AIX% was –1.0 (95%CI –3.9 to 1.9; p=0.49) lower and RTW 4.3 msec (95%CI –0.6 to 9.2; p=0.09) higher among non-current users. The use indomethacin was associated with a similar level of dysfunction (AIX%) compared to patients not currently taking a NSAID. Interestingly the use of ibuprofen was associated with a lower level of AIX% compared to non-current users (unadjusted mean difference in AIX% 7.1, 95%CI -1.4 to 25.6) .

Eleven patients were currently taking a selective COX-2 inhibitor (8 celecoxib; 3 etoricoxib) and had a mean AIX% of 33.6 (6.4) and RWT 132.8 (SD 8.8) msec. On adjusted comparison with diclofenac, AIX% was non-significantly higher for patients taking a selective COX-2 inhibitor (2.9, 95%CI –1.4 to 6.8; p=0.19), whereas mean RWT was similar (adjusted difference –0.7 msec, 95%CI –6.7 to 5.4; p=0.80). Brachial blood

pressure (135/86 vs. 125/82 mmHg) and pulse pressure (47 vs. 33 mmHg) were higher for patients taking a selective COX-2 inhibitor compared to diclofenac.

DISCUSSION

In summary we found that the current use of an ns-NSAID with a higher level of aldosterone glucuronidation inhibition (*in vitro* AGI), in patients with RA, appears to be associated with a higher level arterial dysfunction. Arterial dysfunction was highest in patients taking diclofenac (high AGI) and lowest in those taking ibuprofen (low AGI); difference in arterial function were not attributable to other important CV and rheumatological features that are known to influence arterial function, Patients using indomethacin (intermediate AGI) had an intermediate levels of arterial dysfunction, but we were not able to demonstrate an entirely consistent relationship since naproxen had a higher AIX% than would be anticipated from its relative level of in vitro AGI.

Comparison with existing literature

A small number of previous studies have assessed arterial dysfunction in patients with RA using pulse wave analysis (PWA).[13;26-29] The difference observed in AIX% in this analysis (of 6.5) between diclofenac and ibuprofen is of a statistically and clinically relevant magnitude. It compares, for example, with a 4.2 point reduction in AIX% associated with 3 months atorvastatin therapy in patients with RA.[28] At coronary angiography a 10-point higher AIX% at baseline is associated with a 27% increased risk of CV events over 4 years follow-up; a 10 millisecond higher RWT is associated with a 15% reduction in CV events.[30] Published research concerning NSAID-related arterial dysfunction is currently limited. Only one previous study has directly assessed the influence of NSAID-use on AIX%. In 12 patients with RA 14-days of therapy with indomethacin (75 mg BD) was associated with a 1.2 (95%CI -2.1 to 4.5) increase in AIX%.[29] In a study of men aged >50 years undergoing community-based screening for abdominal aortic aneurysm in the UK, the use of an NSAID (75% took either ibuprofen, diclofenac or indomethacin) was significantly associated with reduced aortic wall distensibility assessed using M-mode ultrasound.[31]

Study strengths and limitations

The strengths of our study are that a single research nurse undertook high quality PWA in a controlled environment among individuals with RA recruited from a consecutive series of patients attending rheumatology clinic. Our measurement of the 'cumulative inflammatory burden' is a particular strength of our study. ESR area under the curve (cumulative ESR-years) reflects both the level and duration of inflammatory burden. Only one previous study investigating the relationship between arterial dysfunction and inflammation has attempted to assess inflammatory burden', but this was restricted to only the most recent 5 years of rheumatology clinic follow-up.[13] We measured and adjusted for several important CV and rheumatological factors in our analysis, including factors known to be independently associated with arterial function. PWA has previously been shown to be predictive of CV events in patients after coronary angiography.[30] Our multivariate model explained a high proportion of the variability (60%-70%) in arterial dysfunction between patients taking diclofenac and ibuprofen. The characteristics of our participants are similar to RA patients receiving outpatient care elsewhere in the UK.[32] The study prevalence of treated hypertension is lower than that reported elsewhere and probably relates to the exclusion of patients with overt arterial disease.[33]

The main limitation of our study is its cross-sectional nature which means that we can only assess association rather than direct causation. Since we did not measure serum aldosterone and ns-NSAID levels in these patients with RA, we are unable to directly

confirm the previous *in vitro* findings. Whilst *in vitro* research is particularly useful for the investigation of biological mechanisms, such laboratory based findings may not translate exactly to the less controlled situation of patients with rheumatoid arthritis. The analysis reported is based on data from an existing study, rather than from a study specifically designed to assess the influence of different NSAIDS on arterial dysfunction. Our assessment of RA patients included a relatively small number of patients taking each NSAID and only 7 patients were chronic users of ibuprofen. The number of patients taking individual NSAIDS were too small to permit a comparison of the association of low/high-dose NSAIDS with arterial dysfunction. The relatively small size of our study restricted the number of potential confounding factors (rheumatological and CV) that could be included in the multivariate analysis without running the risk of over-fitting the data. Although the inclusion of additional variables in the multivariate model, such as treated hypertension, did not improve the goodness to fit, nor substantially alter the adjusted values reported for AIX and RWT. As with all observational studies we cannot excluded the possibility of residual confounding as an explanation for our findings.

Study implications

The finding that ns-NSAID-related AGI appears to be associated with arterial dysfunction in patients with RA provides a potentially novel insight into the CV toxicity of commonly used ns-NSAIDS. Our results are preliminary and require confirmation in larger studies looking at different ns-NSAIDS, serum aldosterone concentrations and surrogate markers of arterial dysfunction. Several important questions remain to be addressed, including whether high/low ns-NSAID dosage is related to arterial dysfunction and if switching ns-NSAIDS (from high-AGI to low-AGI) improves arterial function. If the adverse CV events associated with ns-NSAID-use are due to AGI, then switching to an alternative 'lower-AGI ns-NSAID' may be an appropriate option for patients heavily dependant on NSAIDS for symptomatic relief.

Selective COX-2 inhibitors lack a carboxylic acid functional group and would not be anticipated to inhibit aldosterone glucuronidation to the same extent as diclofenac. It is therefore interesting that chronic users of selective COX-2 inhibitors and diclofenac had similarly high AIX% values. If AGI is not the explanation for the relatively high level of arterial dysfunction associated with selective COX-2 inhibitor use, then alternative mechanisms must be involved. A recent meta-analysis suggests that selective COX-2 inhibitors may induce a greater rise in brachial blood pressure compared with ns-NSAIDs.[34] This might account for the relatively high AIX% values associated with selective COX-2 inhibitor use in our study, since higher brachial pressures (diastolic, systolic, mean and pulse) also correlate with a higher AIX%.[35]

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Competing interests

We have no competing interests to declare.

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Author contributions
MAC - original study conception and design; analysis and interpretation of the data; initial drafting and re-drafting of the article; final approval of the version to be published.
AAM - original hypothesis; interpretation of data; revising the article critically for important intellectual content; re-drafting of the article; final approval of the version to be published.

Data-sharing
Consent for data-sharing was not obtained from study participants at the time of recruitment, but the presented data are held in an anonymised dataset. Access to the dataset is available from the corresponding author (at mike.crilly@abdn.ac.uk) in SPSS format for clinical academic researchers interested in undertaking a formally agreed collaborative research project(s). Although the risk of individual patient identification is low any research involving the release of the dataset to other clinical academics would require approval by Grampian Research Ethics Committee.

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FIGURE LEGEND

Figure 1. Use of NSAIDS and central arterial function in patients with rheumatoid arthritis

STROBE Statement—Checklist of items that should be included in reports of ***cross-sectional studies***

Non-steroidal anti-inflammatory drug (NSAID) related inhibition of aldosterone glucuronidation and arterial dysfunction in patients with rheumatoid arthritis: a cross-sectional clinical study.

Michael A Crilly (26 January 2011)

	Item No	Recommendation
Title and abstract	1	(*a*) Indicate study's design in title: **"cross-sectional clinical study"**
		(*b*) Abstract: **Structured abstract provided**
Introduction		
Background/rationale	2	Scientific background: **NSAID-related inhibition of aldosterone metabolism is described**
Objectives	3	State specific objectives: **"Aim of this exploratory analysis was to assess the association between ns-NSAID-related aldosterone glucuronidation inhibition (AGI) and established markers of arterial dysfunction using data from a previous study of patients with RA**."
Methods		
Study design	4	Key elements of study design presented early: **"Our methods have been described in detail elsewhere." [ref #13. Crilly MA, Kumar V, Clark HJ, et al .Arterial stiffness and cumulative inflammatory burden in rheumatoid arthritis: a dose-response relationship independent of established cardiovascular risk factors. Rheumatology. 2009;48:1606-1612.] [ref # 14. Crilly MA, Clark HJ, Kumar V, et al. Relationship between arterial stiffness and Stanford Health Assessment Questionnaire disability in rheumatoid arthritis patients without overt arterial disease. J Rheumatol. 2010;37:946-952.]** **"In the original study we recruited patients with a consultant rheumatologist diagnosis of rheumatoid arthritis (RA) by reviewing the medical records of a consecutive series of patients attending hospital-based rheumatology clinics in the city of Aberdeen. We identified patients aged between 40-65 years with RA for more than 6 months duration."**
Setting	5	Setting, locations, and relevant dates: **"In the original study we recruited patients ... by reviewing the medical records of a consecutive series of patients attending hospital-based rheumatology clinics in the city of Aberdeen."** **"Patients attended the Clinical Pharmacology Department at Aberdeen Royal Infirmary (ARI) on a single occasion and underwent assessment by a single clinical research nurse (April-December 2006)."**
Participants	6	(*a*) Eligibility criteria, sources and methods of selection of participants: **"We excluded patients with overt arterial disease (angina, prior myocardial infarction, transient ischaemic attack, stroke, arterial revascularisation, intermittent claudication, peripheral arterial disease), atrial fibrillation, heart failure and valvular heart disease. The exclusion of patients with arterial disease was based upon an initial screening patient-questionnaire, resting 12-lead ECG (independently reported by a cardiologist to identify pathological Q-waves, conduction defects, minor Q-waves associated with ST-segment/T-wave anomalies), and a detailed medical record review by a rheumatologist. No participants had a history of recent infection, antibiotic treatment or immunisation within the previous two weeks."**

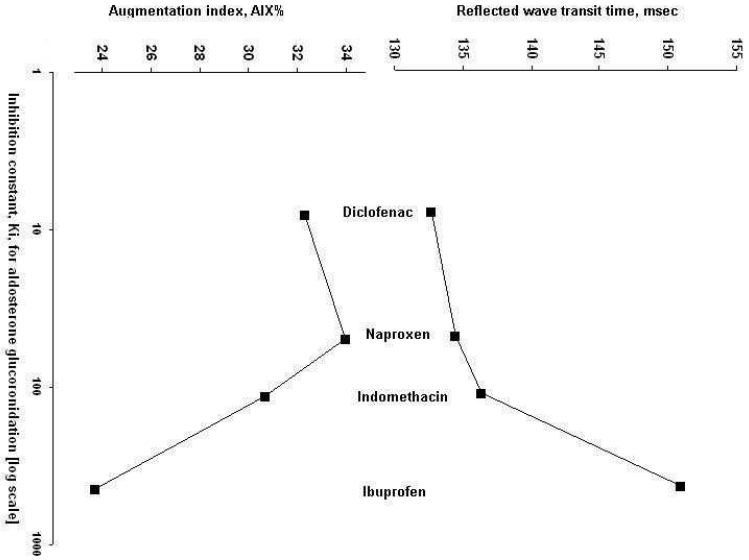
Variables	7	Define outcomes, exposures, and potential confounders: **"Standardised assessment included blood pressure (BP) measurement, pulse wave analysis (PWA), fasting venous blood sample (including erythrocyte sedimentation rate [ESR], rheumatoid factor [RF], and lipid profile). A self-completed patient questionnaire included smoking habit and the Stanford Health Assessment Questionnaire (HAQ). Current medication use was comprehensively reviewed by the research nurse and included use of over-the-counter (without the need for a prescription) NSAIDS. A detailed retrospective review of the medical records using a previously piloted study form, was undertaken by a single rheumatologist blinded to all PWA results and included date of arthritis onset, previous blood test results (erythrocyte sedimentation rate, rheumatoid factor), joint surgery and co-morbidity (including treated hypertension)."** Diagnostic criteria: **"Consultant rheumatologist diagnosis of rheumatoid arthritis (RA)"** **"Our methods have been described in detail elsewhere.[ref #13. Rheumatology. 2009;48:1606-1612.]" -- we have previously reported on the proportion of patients with a consultant rheumatologist diagnosis of rheumatoid arthritis (RA) who meet "American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis.[Arnett FC, et al. Arthritis Rheum 1988 Mar;31(3):315-24].** **[Text from Rheumatology. 2009;48:1606-1612] "Whilst all of our study participants had a clinical diagnosis of RA made by a rheumatologist, only 56% met ACR (4/7) criteria for RA. This may be attributable to a typographical error in our questionnaire (which asked about morning stiffness for 6 months rather than 6 weeks). Whilst 39% of patients reported more than one hour of morning stiffness for more than 6 months in the past, only 18% of patients reported such stiffness over the previous week. Some clinical heterogeneity may exist in our study population of patients with a clinical rheumatological diagnosis of RA, although the inclusion of 'ACR criteria' as a variable in the fully adjusted analysis made no difference to our results. ACR-criteria also 'accumulates' over time. For example, in our study the median duration of arthritis was almost 10 years: some 66% (36/55) of patients with arthritis duration greater than 10 years met ACR-criteria, compared to 48% (28/59) with a shorter duration."**
Data sources/ measurement	8*	Sources of data and details of methods of assessment (measurement): **"Analysis is based on the mean of the three PWA measurements. The principle measures of arterial dysfunction are augmentation index (AIX%) and reflected wave transit time (RWT, msec). Since AIX% varies with heart rate in an individual it was standardised to 75 beats-per-minute."** **"Aldosterone 18ß-glucuronidation inhibition constants, Ki , derived from in vitro studies of human kidney cortical microsomes (HKCM), have been published for 4 of the ns-NSAIDS taken by patients in the RAAIX study (diclofenac 8 µM, naproxen 49 µM, indomethacin 113 µM, ibuprofen 441 µM; a lower Ki indicates greater inhibition). [ref #6: Knights KM, Winner LK, Elliot DJ, et al. Glucuronidation by human liver and kidney microsomes and recombinant UDP-glucuronosyltransferases: inhibition by NSAIDs. Br J Clin Pharmacol 2009;68:402-412.] Nabumetone is a close structural analogue of naproxen and the two were combined together in the analysis."**
Bias	9	Efforts to address potential sources of bias: **"BP was measured at the right brachial artery using an validated automatic oscillometric BP machine (Omron HEM757 IntelliSense BP monitor; Omron Healthcare, Illinois, USA)." [El Assaad MA, et al. Evaluation of two devices for self-measurement of blood pressure according to the international protocol: the Omron M5-I and the Omron 705IT. Blood Press Monit 2003;8:127-133.]**

		"**A detailed retrospective review of the medical records, using a previously piloted study form, was undertaken by a single rheumatologist blinded to all PWA results".** **"The nurse remained blind to the patients' previous medical records (which were not made available at assessment) and only reviewed current medication and questionnaire responses (to ensure that all questionnaires items were fully completed), after PWA assessment had been completed".**
Study size	10	Study size: **"Our methods have been described in detail elsewhere.[ref #13. Rheumatology. 2009;48:1606-1612.]"** **The original study recruited 114 patients.** **[Text from Rheumatology. 2009;48:1606-1612] "A sample size of 110 patients with RA was our intention, in order to ensure that there were 10 subjects for each CV risk factor included in the multi-variable analysis. We included 11 cardiovascular risk factors in our analysis, based on those contributing 90% of the 'population attributable risk' to myocardial infarction in the recent INTERHEART study."**
Quantitative variables	11	Quantitative variables handled: **AIX% and RWT were Normally distributed and summarised as mean (SD).** **"Analysis is based on the mean of the three PWA measurements"** Groupings chosen: **Not applicable**
Statistical methods	12	(*a*) Statistical methods (including control for confounding): **Multiple linear regression (MLR) was used to adjust mean differences in AIX% (and RWT) for variables known to be associated with AIX%, namely: age, sex, mean arterial blood pressure, ever smoked, Stanford HAQ disability score and cumulative ESR-years.**
		(*b*) Subgroups and interactions: **Not applicable**
		(*c*) Missing data: **We had negligible levels of missing data due to the implementation of a rigorous study design and the diligence of our research nurse (and rheumatologist reviewing the medical records). Except for the calculation of 'ESR-years' (as a measure of cumulative inflammatory burden) the level of missing data was minimal.** **"Cumulative ESR-years were derived from the highest single annual ESR recorded in the medical record during each year of follow-up and calculated using the 'trapezium rule' with linear interpolation when data for a given year was missing."** **"Our methods have been described in detail elsewhere. [ref #13. Rheumatology 2009;48:1606-1612.]"** **[Text from Rheumatology. 2009;48:1606-1612] "Medical records were available for 112 patients (complete medical records could not be obtained for two patients) who contributed a total of 1,040 person-years of rheumatoid disease. An annual ESR was available for 77% (797/1,040) of these person-years. Missing ESR data was imputed as previously described. For individual patients the average availability of an annual ESR, for each year since the onset of arthritis, was a median of 93% (IQR 67% to 100%). The availability of an annual ESR did not differ by age, gender, rheumatoid factor, ACR-RA criteria or Stanford disability index (data not shown)."** **Of the 114 patients recruited, 112 had complete data and were included in the fully adjusted regression analysis/model [see Table 3 from ref #13; Rheumatology. 2009;48:1606-1612]**

		(*d*) Analytical methods taking account of sampling strategy: **Not applicable**
		(e) Sensitivity analyses: **"The inclusion of additional variables (study ESR, duration of arthritis, fasting cholesterol, smoking pack-years, treated hypertension and current DMARD use) did not alter the adjusted values for AIX and RWT reported, nor improve the goodness to fit of the final regression model."**
Results		
Participants	13*	(a) Report numbers of individuals at each stage of study: **"The original study recruited 114 patients. We excluded 9 patients from the analysis who were not currently taking NSAIDS, but had done so within the previous 3 months; and excluded 2 users of infrequently prescribed NSAIDS (ketoprofen and tiaprofenic acid)."** **"Our methods have been described in detail elsewhere. [ref #13. Rheumatology 2009;48:1606-1612.]" – we had negligible levels of missing data due to the implementation of a very careful study design and the diligence of our research nurse (and rheumatologist reviewing the medical records). Of the 114 patients recruited 112 had complete data and were included in the fully adjusted regression analysis/model [see Table 3 from ref #13; Rheumatology. 2009;48:1606-1612]** **We have no data available concerning the number of RA patients excluded at the rheumatology clinic level because they were already known to have arterial disease.** **We also have no data concerning the number of eligible RA patients attending rheumatology clinic who declined to be contacted by our research nurse. All of the RA patients who did agree to being contacted by the research nurse subsequently attended for assessment (N=114) and have been included in this analysis as described.** **No patients were excluded because of technical failure to undertake PWA. Despite initial (pre-study) concerns that it might not be feasible to undertake PWA in some RA patients, due to diseased wrists, this proved not to be the case. The research nurse successfully undertook PWA on all participants.**
		(b) Reasons for non-participation: **Not known/recorded**
		(c) Flow diagram: **None/ not applicable**
Descriptive data	14*	(a) Characteristics of study participants: **Shown in detail in Table 1**
		(b) Indicate number of participants with missing data: **Of the 114 patients recruited, 112 had complete data and were included in the fully adjusted regression analysis/model [see Table 3 from ref #13; Rheumatology. 2009;48:1606-1612]**
Outcome data	15*	Report summary measures: **"Analysis is based on the mean of the three PWA measurements. The principle measures of arterial dysfunction are augmentation index (AIX%) and reflected wave transit time (RWT, msec). Since AIX% varies with heart rate in an individual it was standardised to 75 beats-per-minute."**
Main results	16	(*a*) Unadjusted estimates and confounder-adjusted estimates (95%CI): **Table 3 reports unadjusted and adjusted mean differences in AIX% and RWT (with their related 95%CI's)** Make clear which confounders were adjusted for and why they were included **"Multiple linear regression (MLR) was used to adjust mean differences in AIX% (and RWT) for variables known to be associated with AIX%, namely: age, sex, mean arterial blood pressure, ever smoked, Stanford HAQ disability score and cumulative ESR-years. Analysis was undertaken using SPSS v 17.We confirmed that the assumptions of**

		linearity, normal distribution and equal variance for MLR were met. 'Goodness to fit' was assessed using the adjusted R^2. The inclusion of additional variables (study ESR, duration of arthritis, fasting cholesterol, smoking pack-years, treated hypertension and current DMARD use) did not alter the adjusted values for AIX and RWT reported, nor improve the goodness to fit of the final regression model."
		(*b*) Category boundaries (continuous variables categorized): **Not applicable**
		(*c*) Estimates of absolute risk: **Not applicable**
Other analyses	17	Subgroup analysis: **None** Sensitivity analyses: **see STROBE #12e (above)**
Discussion		
Key results	18	Summarise key results with reference to study objectives: **"In summary we found that the current use of an ns-NSAID with a higher level of aldosterone glucuronidation inhibition (AGI), in patients with RA, appears to be associated with a higher level arterial dysfunction. Arterial dysfunction was highest in patients taking diclofenac and lowest in those taking ibuprofen. These difference in arterial function were not attributable to other important CV and rheumatological features that are known to influence arterial function, since the observed differences remained after adjusting for such features. Consequently differences in AIX and RWT appear to be genuinely related to the use of different ns-NSAIDs. Patients using ns-NSAIDS with intermediate levels of AGI, naproxen and indomethacin, demonstrated intermediate levels of arterial dysfunction."**
Limitations	19	Limitations of the study, sources of potential bias or imprecision: **"The main limitation of our study is its cross-sectional nature which means that we can only assess association rather than direct causation. Since we did not measure serum aldosterone and ns-NSAID levels in these patients with RA we are unable to directly confirm the previous in vitro findings. Our assessment of RA patients included a relatively small number of patients taking each NSAID and only 7 patients were chronic users of ibuprofen. The number of patients taking individual NSAIDS were too small to permit a comparison of association of low/high-dose NSAIDS with arterial dysfunction. The relatively small size of our study restricted the number of potential confounding factors (rheumatological and CV) that could be included in the multivariate analysis without running the risk of over-fitting the data. …. As with all observational studies we cannot excluded the possibility of residual confounding as an explanation for our findings."**
Interpretation	20	Cautious overall interpretation of results: **"Our results are preliminary and require confirmation in larger studies looking at different ns-NSAIDS, serum aldosterone concentrations and surrogate markers of arterial dysfunction. Several important questions remain to be addressed, including whether high/low ns-NSAID dosage is related to arterial dysfunction."**
Generalisability	21	Generalisability (external validity) of the study results: **"The characteristics of our participants are similar to RA patients receiving outpatient care elsewhere in the UK. [ref #32. Panoulas VF, Douglas KM, Milionis HJ et al. Prevalence and associations of hypertension and its control in patients with rheumatoid arthritis. Rheumatology 2007;46:1477-1482]"**
Other information		
Funding	22	Source of funding and role of the funders: **"The original study was supported by charitable funding from NHS Grampian Rheumatology Endowments. The funders played no role in the analysis or reporting of this study."**

*Give information separately for exposed and unexposed groups.



Use of NSAIDS and central arterial function in patients with rheumatoid arthritis
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