Aldosterone glucuronidation inhibition as a potential mechanism for arterial dysfunction associated with chronic celecoxib and diclofenac use in patients with rheumatoid arthritis

M.A. Crilly[1], A.A. Mangoni[2], K.M. Knights[3]

[1]Institute of Applied Health Sciences, and [2]Division of Applied Medicine, Section of Translational Medical Sciences, University of Aberdeen Medical School, Aberdeen, Scotland; [3]Department of Clinical Pharmacology, School of Medicine, Faculty of Health Sciences, Flinders University, Adelaide, South Australia.

Abstract

Objective

Adverse cardiovascular (CV) effects of non-steroidal anti-inflammatory drugs (NSAIDs) are largely independent of their cyclooxygenase (COX) enzyme selectivity, but could be a consequence of aldosterone 18ß-glucuronidation inhibition (AGI), which varies between NSAIDS. This study assesses the chronic effects of celecoxib (selective COX-2 inhibitor) versus diclofenac (non-selective NSAID) therapy on arterial dysfunction in patients with rheumatoid arthritis (RA).

Methods

AGI was assessed in vitro using human kidney cortical microsomes. Arterial function was measured clinically as the extent (augmentation index, AIX%) and timing (reflected wave transit time, RWTT, msec) of arterial wave reflection using radial applanation pulse wave analysis (SphygmoCor PWA device) in 39 RA patients without overt CV disease aged 40-65. A higher AIX% (and lower RWTT) indicates arterial dysfunction. Clinical assessment on a single occasion included a fasting blood sample, patient questionnaire and medical record review. Multivariable analysis was used to adjust for sex, mean blood pressure, arthritis duration, cumulative ESR-years and current DMARD therapy.

Results

The inhibition constant (K_i) for celecoxib was lower than that of diclofenac (K_i, 3.5 vs. 8.4 μM). Chronic celecoxib use was associated with a higher AIX% (34.8 vs. 32.3) and lower RWTT (130.1 vs. 132.7 msec) compared with diclofenac. Adjusted mean differences were AIX% 4.7 (95%CI 0.6 to 8.9; p=0.03) and RWTT -3.6 (95%CI -10.0 to 2.7; p=0.26).

Conclusion

Celecoxib has a greater potency for AGI than diclofenac and its use is associated with a significantly higher AIX%. Our findings support AGI as a plausible mechanism for the CV toxicity of NSAIDs.

Key words

rheumatoid arthritis, non-steroidal anti-inflammatory drugs, aldosterone, arterial dysfunction, pulse wave analysis

Michael A. Crilly, MD, MPH, MRCGP, MFPHM
Arduino A. Mangoni, PhD, FRCP
Kathleen M. Knights, PhD

Please address correspondence to: Michael A. Crilly, MD,
Senior Lecturer in Clinical Epidemiology, Institute of Applied Health Sciences, University of Aberdeen Medical School Polwarth Building at Foresterhill, Aberdeen, Scotland AB25 2ZD.
E-mail: mike.crilly@abdn.ac.uk

Received on January 14, 2013; accepted in revised form on February 25, 2013.

Funding: the in vitro aldosterone inhibition study was funded by a Grant-in-Aid (G12A6511) from the National Heart Foundation of Australia.
The use of human renal tissue was approved by the Southern Adelaide Clinical Human Research Ethics Committee (study reference: 059.056).
The RAAIX study was funded by NHS Rheumatology Endowments and was approved by Grampian Research Ethics Committee (study reference: 04/S0801/67).

Competing interests: none declared.

Introduction

The relative cardiovascular (CV) safety of non-steroidal anti-inflammatory drugs (NSAIDS), a class of drugs commonly prescribed to patients with arthritis, is the subject of considerable attention (1, 2). NSAIDs with varying degrees of selectivity for cyclo-oxygenase (COX-1, COX-2) increase the risk of adverse CV events such as myocardial infarction (1, 2). Since COX selectivity does not fully account for the CV toxicity of NSAIDs other mechanisms must play a role (3-7). One mechanism through which NSAIDS may increase CV risk is through increased plasma concentrations of aldosterone (3).

Patients with rheumatoid arthritis (RA) are at a higher risk of CV death than the general population (8) and also have higher levels of arterial dysfunction (assessed using pulse wave analysis, PWA) compared to healthy controls (9-11). 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 (12, 13). In each cardiac cycle the outgoing systolic pressure wave generated by the left ventricle is also reflected back towards the heart, where it returns to augment the central aortic pressure (12, 13). The speed of travel of both outgoing and reflected waves are greater in patients with stiffer arteries, which increases the extent of augmentation (higher augmentation index, AIX%) and reduces the reflected wave transit time (RWTT, msec). A higher AIX% has been shown to be independently associated with surrogate markers of cardiovascular disease in RA patients (9, 14). Moreover, it is a significant predictor of cardiovascular events and all-cause mortality in other patient groups (15).

Recent *in vitro* research has demonstrated that several non-selective NSAIDS exert significant inhibitory effects on aldosterone metabolism, with a consequent potential increase in serum aldosterone concentrations (16). Aldosterone has generally adverse effects on the CV system with elevated serum concentrations being associated with endothelial dysfunction, arterial stiffening, increased arterial wall reflection, myocardial fibrosis and an increased risk of CV death (17-20). Aldosterone is metabolised by 18ß-glucuronidation in the kidney (~80%) and the liver (~20%) by the enzyme UDP-glucuronosyltransferase 2B7 (UGT2B7). Non-selective NSAIDS have been shown *in vitro* to inhibit aldosterone 18ß-glucuronidation with a rank order of diclofenac >naproxen >indomethacin >ibuprofen (16). We have recently reported an association between the inhibition constant (K_i) of several non-selective NSAIDs and arterial dysfunction in chronic RA users of these drugs (21). These observations suggest that AGI might represent a novel mechanism of toxicity of non-selective NSAIDS.

Although celecoxib is widely used in the management of arthritis, inhibition of aldosterone glucuronidation and the level of arterial dysfunction associated with celecoxib administration have not previously been reported (21). The 'Rheumatoid Arthritis Augmentation Index' (RAAIX) study was originally undertaken to assess the relationship between cumulative inflammatory burden and arterial dysfunction among patients with RA (22, 23). Diclofenac was taken by one third of patients and was the most commonly used NSAID in the RAAIX study (22).

The aim of this study was to determine *in vitro* the extent of inhibition of aldosterone 18β-glucuronidation by celecoxib and to assess the level of arterial dysfunction associated with celecoxib (compared with diclofenac) using data from the previous RAAIX clinical study (22).

Methods

Inhibition of aldosterone 18β-glucuronidation was studied using five concentrations of celecoxib (1–25μM), three concentrations of aldosterone (150–600μM) and the same human kidney cortical microsomes (HKCM) and techniques as described previously (16). Briefly, aldosterone 18β-glucuronide was quantified by reference to an external aldosterone standard curve (50–1000μM) following chromatographic separation using a Waters Nova-Pak

C18 column (150×3.9 mm, 4 μm; Milford, MA, USA) at a mobile phase flow rate of 1 ml min^{-1}. The mobile phase comprised component A (95% water, 5% acetonitrile, 0.002% v/v acetic acid) and component B (100% acetonitrile). The gradient conditions were 95%A: 5%B for 1 min then 35%A: 65%B over 8 min. Analytes were monitored at 241nm and the retention times for aldosterone18β-glucuronide and aldosterone were 4.68 and 6.36 min, respectively. Aldosterone standard curves were linear (r^2>0.99). Coefficients of variation were 2.1% for inter-day variability and <6% at 100μM and <2% at 1000μM aldosterone for overall within-day assay reproducibility.

The identity of aldosterone 18β-glucuronide in incubation samples was confirmed by liquid chromatography-tandem mass spectrometry (LC-MS/MS) as reported previously (16). Using a Micromass Quattro micro tandem quadropole mass spectrometer (Waters Associates, Manchester, UK) electrospray mass spectrometry was performed in the negative ion mode for the duration of the LC run, with scan duration 1 s, mass range 80–1000 m/z, capillary voltage -3.5 kV, cone voltage 25 V, source temperature 80°C, desolvation temperature 350°C and desolvation gas flow 410 l h^{-1}. For the peak eluting at 4.68 min the MS analysis in the negative ion mode gave a molecular ion ($[M-H]^-$) with *m/z* of 535 (c.f. known molecular mass of aldosterone 18β-glucuronide, 536.5 g mol^{-1}) and a fragment ion $[M-GA-H]^-$ *m/z* 359.0 corresponding to the loss of the glucuronic acid moiety.

Inhibition of aldosterone 18β-glucuronidation was studied using five concentrations of celecoxib (1–25μM), three concentrations of aldosterone (150–600μM) and the same human kidney cortical microsomes (HKCM) and techniques as described previously (16). The inhibition constants (K_i) for diclofenac (and four other non-selective NSAIDS) using these same methods have previously been reported (16). The inhibition constant (K_i) for celecoxib was determined by fitting untransformed data to the equations for competitive, non-competitive and mixed inhibition using a non-linear least-squares modelling program (EnzFitter, version 2.0.18.0; Biosoft, Cambridge, UK) (16).

In the original RAAIX study we recruited 114 patients aged between 40–65 years with a diagnosis of rheumatoid arthritis (RA) of more than 6 months duration. This was done by reviewing the medical records of a consecutive series of ambulatory patients attending hospital rheumatology clinics in Aberdeen. We excluded patients with overt arterial disease (angina, prior myocardial infarction, transient ischaemic attack, stroke, arterial revascularisation, intermittent claudication, and peripheral arterial disease), atrial fibrillation, heart failure and valvular heart disease. Exclusions were based on an initial screening patient-questionnaire, resting 12-lead ECG (reported by a cardiologist for pathological Q-waves, conduction defects, minor Q-waves associated with ST-segment/T-wave anomalies), and medical record review. We have described our methods in detail elsewhere (22, 23).

Patients attended for assessment on a single occasion in the morning (after fasting overnight and abstaining from smoking, alcohol and caffeine) by a single research nurse. No participants had a history of recent infection, antibiotic treatment or immunisation within the previous two weeks. Standardised assessment included brachial blood pressure (BP) measurement, pulse wave analysis (PWA), fasting venous blood sample for erythrocyte sedimentation rate (ESR), rheumatoid factor (RF), and fasting lipid profile. Current medication use (including assessment of NSAID use) was reviewed by the research nurse. The nurse was otherwise blind to the patients' previous medical records, which were not made available at assessment. A self-completed patient questionnaire included smoking habit, the disease-specific Stanford Health Assessment Questionnaire (HAQ) and the generic EuroQoL questionnaire (24, 25).

Patients rested supine in a quiet side-room for at least 10 mins before undergoing three BP/PWA measurements according to current guidelines (26). BP was measured at the right brachial artery using a validated automatic oscillometric BP machine (Omron HEM757 IntelliSense BP monitor; Omron Healthcare, Illinois, USA) (27). 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. High levels of both between-observer and within-observer repeatability for PWA have previously been demonstrated for our research nurse; with between-observer Bland-Altman 95% limits of agreement (LoA, mean difference ±2SD) of 1.0±3.9 and within-observer LoA of 1.5±7.0 for AIX standardised to a heart rate of 75 beats per minute (28). The 'SphygmoCor' PWA device employs a validated 'generalised transfer function' to derive the central aortic pulse waveform from the peripheral waveform (29). All three PWA recordings were required to have an in-built SphygmoCor quality index score of at least 95% (based on average pulse height, pulse height variation and diastolic variation). A single rheumatologist undertook a detailed retrospective review of the medical records blind to all PWA results.

Statistical analysis

The RAAIX study adhered to the principles of the Declaration of Helsinki. The *in vitro* aldosterone inhibition study was funded by a Grant-in-Aid (G12A6511) from the National Heart Foundation of Australia. The use of human renal tissue was approved by the Southern Adelaide Clinical Human Research Ethics Committee (study reference: 059.056). The RAAIX study was funded by NHS Rheumatology Endowments and was approved by Grampian Research Ethics Committee (study reference: 04/S0801/67). All patients provided informed written consent. The funders played no role in the analysis or reporting of this study.

Normally distributed continuous variables are summarised as means (standard deviation, SD) and skewed continuous variables are summarised as medians (inter-quartile range, IQR). All *p*-values are 2-sided. Analysis is based on the mean of the three BP/PWA measurements. Since AIX% varies with heart rate in an individual it was

standardised to 75 beats-per-minute (30). No formal power calculation was undertaken for this comparison of arterial dysfunction among 39 chronic users of celecoxib/diclofenac. In order to ensure that there were 10 subjects for each covariate included in the regression analysis the original RAAIX study recruited 114 RA patients against an intended sample size of 110 patients.

ESR is routinely measured in Aberdeen at almost all out-patient visits including at annual RA-assessment. The availability of an annual ESR for individual patients for each year since the onset of arthritis was a median of 100% (IQR 69% to 100%). The availability of an annual ESR did not differ by age, gender, rheumatoid factor positivity, or Stanford disability index (data not shown). The 'cumulative inflammatory burden' for each individual patient, since the onset of arthritis, was estimated using the area-under-the-curve approach (AUC in ESR-years). The 'trapezium rule' was used to derive the AUC, based on the highest ESR recorded in the medical records during each year of follow-up, with linear interpolation when data for a given year was missing (31). For example, an annual ESR of 20, 10 and 5 mm/hour over 3 years would equate to ~ 35 ESR-years.

Adjusted analysis of mean differences in arterial function were undertaken using multiple linear regression (MLR) with IBM SPSS Statistics (version 19, IBM Corporation Software Group, Somers, NY). Celecoxib/diclofenac therapy was directly entered into the multivariable model. Other variables for inclusion in the multivariable model were selected from the 17 patient characteristics listed in Table II (smoking status included as 'ever smoked'; blood pressure as 'mean arterial pressure'; drug therapy as 'current DMARD' and 'current cardiovascular drug' use) using the SPSS algorithm for 'forward' variable selection (a variable selection technique) with augmentation index (AIX%) as the dependant variable (probability-of-F-for-entry ≤0.10; probability-of-F-for-removal ≥0.20). The independent variables selected were: cumulative ESR-years, mean arterial blood pressure, sex, arthritis duration and current DMARD therapy. The same five variables were also used to adjust mean differences in the reflected wave transit time (msec). We confirmed that the assumptions of linearity, normal distribution and equal variance for MLR were met. There was no evidence of multicollinearity in the regression models produced. 'Goodness to fit' was assessed using the adjusted R square.

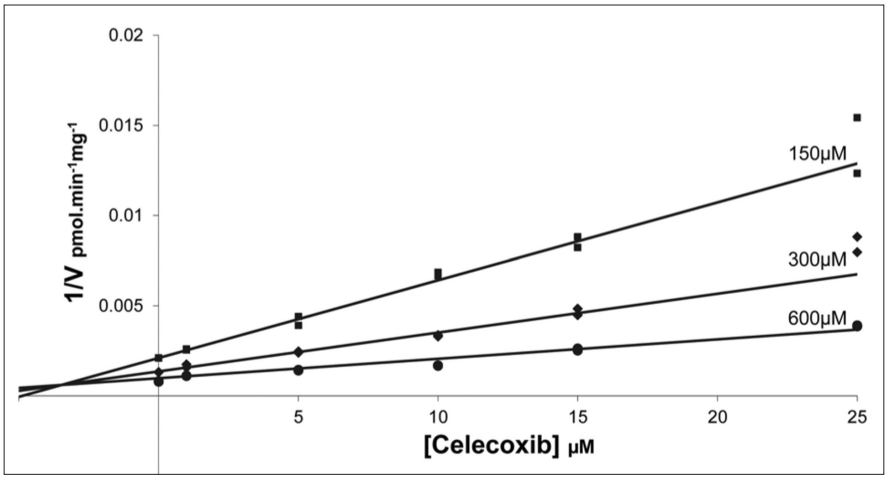

Fig. 1. Dixon plot for the inhibition of aldosterone 18β-glucuronidation by celecoxib at three concentrations of aldosterone.

Results

The effect of celecoxib on aldosterone 18β-glucuronidation catalysed by HKCM was best modelled using the equation for competitive inhibition (Figure 1). The derived K_i value was 3.5 µM (standard error, SE, of parameter fit ± 0.18 µM). The comparative data for diclofenac is shown in Table I. It should be noted that the K_i may be over-estimated by approximately an order of magnitude because of the additive inhibitory effect of unsaturated long-chain fatty acids that are released from microsomal membranes during the course of an incubation (32). 'True' K_i values may be obtained experimentally when bovine serum albumin (BSA), which sequesters the released unsaturated fatty acids, is added to microsomal incubations. In this study the extensive protein binding of celecoxib (>97%) precluded the addition of BSA to the incubations (33).

We identified 31 chronic users (>3 months) of diclofenac and 8 chronic users of celecoxib from the original RAAIX study of 114 patients with RA. No patients were taking more than one NSAID concurrently. All patients had previously received DMARD therapy and no patients were currently taking aspirin. The rheumatological and cardiovascular features of these 39 patients (87% female; mean age 53.9, SD 6.8 years) are shown in Table II. The duration of arthritis and Stanford HAQ disability score were similar for both groups, although patients taking diclofenac had moderately higher study-assessment ESR and cumulative ESR-years. The combined previous/current use of methotrexate was similarly high in both groups (diclofenac 65%, celecoxib 75%). The higher use of CV drugs in the diclofenac group was mainly attributable to a higher prevalence of treated hypertension (23% *vs*. 0%) and brachial BP was lower in the diclofenac group (126/83 *vs*. 137/85 mmHg). Resting heart rate was also higher in the diclofenac group compared to celecoxib (71 *vs*. 64 beats per minute).

The level of arterial dysfunction was higher in the celecoxib group (AIX% 34.8 *vs*. 32.3 and RWTT 130.2 *vs*. 132.7 msec) compared to the diclofenac group (Table II). On crude analysis the mean difference in AIX% was 2.4 (95%CI -3.3 to 8.2, p=0.40) and on adjusted analysis the difference in AIX% was 4.7 (95%CI 0.6 to 8.9, p=0.03). The mean difference in RWTT on crude analysis was -2.6 msec (95%CI -9.0 to 3.9, p=0.42) and -3.6 (95%CI -10.0 to 2.7, p=0.26) on adjusted analysis (Table III). Excluding the seven patients on drug

treatment for hypertension (all taking diclofenac) produced comparable adjusted mean differences to those reported in Table III (AIX% 5.0, 95%CI 0.3 to 9.7, p=0.04; RWTT -4.4 msec, 95%CI -11.8 to 3.0, p=0.23). Our findings also remained robust when additional variables known to be associated with arterial stiffness (heart rate and height) were directly added to these multivariable models.

Resting heart rate was 6.5 beats-per-minute higher in the diclofenac group. Although this was already accounted for by the standardisation of AIX% to heart rate (30), forcing heart rate into the final multivariable model produced comparable results (AIX% 5.3, 95%CI 0.9 to 9.8, p=0.02; RWTT -3.4 msec, 95%CI –10.4 to 3.7, p=0.34) to those reported in Table III.

A higher proportion of women were taking diclofenac and patients in the diclofenac group were 7.8 cms shorter than those taking celecoxib (Table II). The inclusion of sex in the model already accounted for much of the influence of height on arterial wave reflection, although forcing height in the final multivariable model produced rather larger adjusted mean differences (AIX% 6.0, 95%CI 1.5 to 10.4, p=0.01; RWTT -4.9 msec, 95%CI -12.1 to 2.4, p=0.18) compared to those reported in Table III.

Table I. Inhibition of aldosterone 18β-glucuronidation by celecoxib and diclofenac using human kidney cortical microsomes.

Drug	Dose (mg)	Cmax (μM)[$]	Unbound fraction	Unbound Conc. (μM)	K_i (μM)
Celecoxib	200[*]	1.9	0.026	0.05	3.5
Diclofenac	50[**]	6.7	0.005	0.03	8.4

[*]DAVIES NM, MCLACHLAN AJ, DAY RO, WILLIAMS KM: Clinical pharmacokinetics and pharmaco-dynamics of celecoxib. *Clin Pharmacokinet* 2000; 38: 225-42.
[**]WILLIS JV, KENDALL MJ, FLINN RM, THORNHILL DP, WELLING PG: The pharmacokinetics of diclofenac sodium following intravenous and oral administration. *Eur J Clin Pharmacol* 1979; 16: 405-10.
[$]Plasma Cmax values: celecoxib 0.72 μg/mL; diclofenac 2 μg/mL

Table II. Characteristics of patients with rheumatoid arthritis who are chronic users of diclofenac or celecoxib (n=39).

Figures are numbers (%) unless otherwise indicated	Diclofenac (n=31)			Celecoxib (n=8)		
Mean age, years (SD)	53.7	6.5		55.0	8.4	
Female	28	90%		6	75%	
University education	8	26%		2	25%	
Mean arterial blood pressure, mmHg	98.9	12.2		104.0	12.3	
Mean brachial systolic pressure, mmHg (SD)	125.8	17.6		136.8	17.1	
Mean brachial diastolic pressure, mmHg (SD)	82.6	9.9		85.1	9.7	
Mean ratio total/HDL-cholesterol (SD)	3.7	1.0		3.1	0.6	
Mean height, cms (SD)	162.7	5.5		170.5	9.2	
Mean waist-hip ratio (SD)	0.85	0.08		0.80	0.08	
Never smoked	14	45%		3	38%	
Ex-smoker	11	36%		4	50%	
Current smoker	6	19%		1	13%	
Current cardiovascular drug therapy	8	26%		1	13%	
Bendroflumethiazide	4	13%		1	13%	
Calcium channel blocker	3	10%		0	0%	
Atenolol	1	3%		0	0%	
Angiotensin converting enzyme inhibitor	1	3%		0	0%	
Family history premature coronary heart disease	10	32%		2	25%	
Median Stanford HAQ disability (IQR)	1.4	0.8	2	1.3	0.8	1.8
Median EuroQoL score (IQR)	0.66	0.52	0.69	0.60	0.25	0.73
Median arthritis duration, years (IQR)	9	3	15	10	5	15
Median ESR at assessment, mm/hour (IQR)	22	8	36	18	9	25
Median cumulative ESR-years (IQR)	221	99	526	206	144	320
Rheumatoid factor 'positive' (>30 IU)	26	84%		6	75%	
Current rheumatoid drug therapy[*]	28	90%		7	88%	
Methotrexate	13	42%		4	50%	
Sulphasalazine	10	32%		2	25%	
Leflunomide	2	7%		1	13%	
Cytokine modulator	3	10%		0	0%	
Azathioprine	1	3%		0	0%	
Prednisolone	3	10%		0	0%	
Mean augmentation index, AIX% (SD)	32.3	7.1		34.8	7.2	
Mean reflected wave transit time, msec (SD)	132.7	7.3		130.1	9.3	

[*]No patients were currently prescribed other DMARDs such as gold, penicillamine or cyclosporine.
Erythrocyte sedimentation rate (ESR), Health Assessment Questionnaire (HAQ), standard deviation (SD), inter-quartile range (IQR).

Discussion

Our *in vitro* study found that in comparison to diclofenac, celecoxib is a potent non-substrate inhibitor of aldosterone 18β-glucuronidation catalysed by human kidney cortical microsomes. In addition, the *in vivo* chronic use of celecoxib was associated with a higher level of arterial dysfunction compared to diclofenac, although only the adjusted difference in augmentation index (AIX%) reached statistical significance.

Relatively few studies have assessed arterial dysfunction in RA patients (9-11, 35, 36). Only two previous studies have assessed the influence of NSAID-use on arterial dysfunction (35, 36). In men aged >50 years in the UK attending for community-based ultrasound abdominal aortic aneurysm screening, NSAID-use (predominantly diclofenac, ibuprofen and indomethacin) was associated with a reduced aortic wall distensibility (35). In a second study two weeks therapy with indomethacin was associated with a 1.2 point increase in AIX% among 12 patients with RA (36). The adjusted difference of almost 5 points in AIX% observed between celecoxib and diclofenac is likely to be of clinical as well as statistical significance. Pooled data from a recent systematic review of prospective cohort studies indicates that a 10 point increase in AIX% is associated with a 32% increase in the relative risk of CV

events and a 39% increase in all-cause mortality (15). In a separate study, 3 months atorvastatin therapy in patients with RA was associated with a 4 point reduction in AIX% (11).

A strength of the RAAIX study is that a single research nurse undertook high quality PWA in a controlled environment among a consecutive series of patients with RA recruited from routine clinical practice. The characteristics of our participants are similar to RA patients receiving ambulatory care elsewhere in the UK (37). We measured and adjusted for several important CV and rheumatological factors in our analysis, including factors known to be independently associated with arterial dysfunction. Although age is strongly related to arterial dysfunction, the restricted age range of participants (40–65 years) meant that age was not selected as a variable for inclusion in the regression model. Our multivariable analysis for AIX% explained 55% of the variability in AIX% between patients taking diclofenac and celecoxib. In this study brachial BP was higher in the celecoxib group and a recent meta-analysis found that selective NSAIDS may induce a greater rise in brachial BP compared with other NSAIDs (38). Although this cannot be the explanation for the higher AIX% associated with celecoxib use, since BP was adjusted for in the analysis.

A limitation of the observational cross-sectional design of the RAAIX study is that we cannot infer a temporal relationship, nor exclude residual confounding as an explanation for our findings. The relatively small number of RA patients limited the number of rheumatological/CV factors that could be included in the multivariable analysis and there is a risk that we may have over-fitted the model to the data. The study was not formally powered to detect differences in AIX (and RWTT) in relation to the chronic use of celecoxib and diclofenac. Based on the data from this study an estimated 250 participants, half taking celecoxib and half taking diclofenac, would be required to confirm an unadjusted difference of 2.5 points in AIX or RWTT with a SD of 7.0 (*p*-value of 0.05 and 80% power).

Table III. Chronic use of celecoxib and diclofenac: mean differences in arterial dysfunction (n=39).

	Mean difference	(95% CI)	*p*-value	Multivariable model summary R	Adj R Sq	*p* (ANOVA)
Augmentation index, AIX%						
Unadjusted	2.4	(-3.3; 8.2)	0.40			
Adjusted*	4.7	(0.6; 8.9)	0.03	0.79	0.55	0.00001
Reflected wave transit time, msec						
Unadjusted	-2.6	(-9.0; 3.9)	0.42			
Adjusted*	-3.6	(-10.0; 2.7)	0.26	0.54	0.15	0.09

*Adjusted using multiple linear regression for: cumulative ESR-years, mean arterial blood pressure, sex, arthritis duration, and current disease-modifying anti-rheumatic drug (DMARD) therapy. Adjusted R Square (Adj R Sq); Analysis of variance (ANOVA).

The metabolism of aldosterone *in vitro* is not influenced by posture, diurnal variation or electrolytes (sodium and potassium). Thus the *in vitro* assessment of aldosterone inhibition may not translate into the less controlled human *in vivo* environment. Aldosterone concentrations were not measured in study participants and so we cannot substantiate directly that NSAID-related arterial dysfunction is mediated through the inhibition of aldosterone metabolism. However, as indicated if a 10-fold over-estimation of K_i is assumed the ratios of the peak plasma concentration to K_i would suggest potential inhibition of aldosterone glucuronidation by celecoxib and diclofenac at therapeutic plasma concentrations with standard dosing (32).

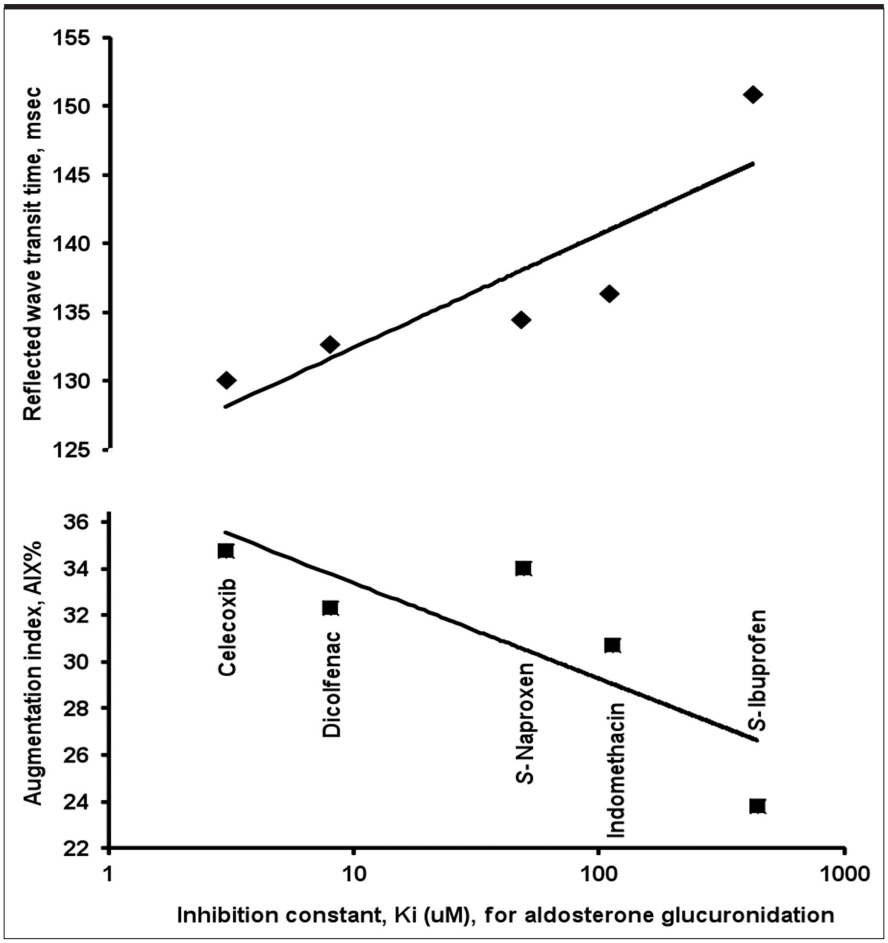

Fig. 2. Central arterial function with NSAID use and inhibition of aldosterone 18β-glucuronidation.

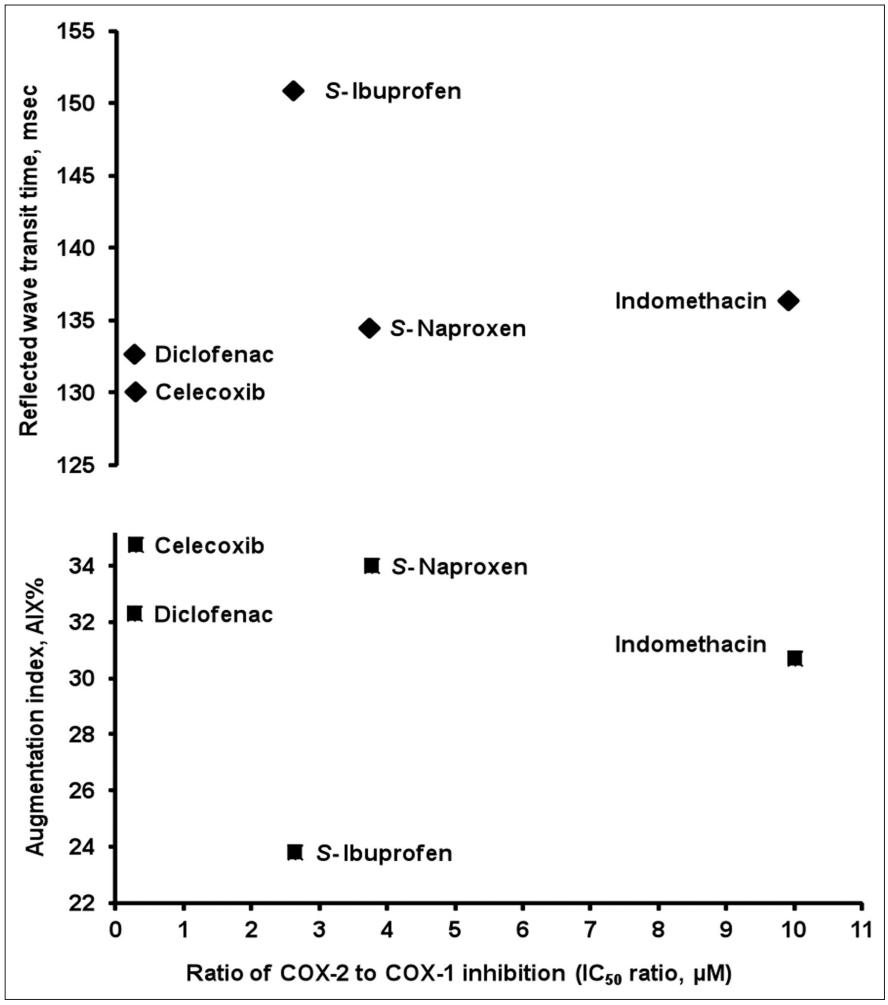

Fig. 3. Central arterial function with NSAID use and ratio of COX-2 to COX-1 inhibition.

Based on cross-sectional correlation, aldosterone 18β-glucuronidation inhibition appears to be a more plausible explanation for arterial dysfunction associated with NSAID use than COX-2/COX-1 selectivity. The *in vitro* inhibition constant (K_i 3.5 μM) for celecoxib was obtained using the same methods in the same laboratory as for other non-selective NSAIDS (16). The inhibition of aldosterone glucuronidation by diclofenac (K_i 8.4 μM) and other non-selective NSAIDs (*S*-naproxen 48.7 μM, indomethacin 113 μM; *S*-ibuprofen 441 μM) have previously been published (16). Figure 2 shows the relationship between the inhibition (K_i) of aldosterone glucuronidation *in vitro* and the level of central arterial dysfunction (AIX% and RWTT) associated with these NSAIDS in the RAAIX study; the total number of patients taking naproxen, ibuprofen and indomethacin for more than three months were 16, 7 and 6 respectively (22). In Figure 2 increasing potency of NSAID-related aldosterone glucuronidation inhibition (AGI) is associated with a higher level of arterial dysfunction (higher AIX% and lower RWTT). Celecoxib appears to be a potent inhibitor of aldosterone glucuronidation and is also associated with the highest level of arterial dysfunction among the NSAIDS previously studies. Ibuprofen is a weak inhibitor of aldosterone glucuronidation and is associated with the lowest level of arterial dysfunction. Both AIX% and RWTT were stongly correlated with K_i, with a Pearson correlation co-efficient (r) for AIX% of –0.97 (95%CI -0.61 to -1.0, p=0.007) and +0.99 (95%CI +0.85 to +1.0, p=0.001) for RWTT.

Based on the 'William Harvey Human Modified Whole Blood Assay' (WHMA), other investigators in a single laboratory have previously published the COX-2 and COX-1 inhibition constants (cyclo-oxygenase IC_{50} values) for a wide range of NSAIDS (34). The COX-2/COX-1 ratios reported for diclofenac, celecoxib, ibuprofen naproxen, indomethacin being 0.27, 0.28, 2.63, 3.76 and 10.00 respectively (Table I of the original publication) (34). Figure 3 shows the absence of any important relationship between *in vitro* COX-2/COX-1 ratios and the level of arterial dysfunction associated with the chronic use of these five NSAIDS. The Pearson correlation coefficient (r) for AIX% was -0.16 (95%CI -0.91 to +0.84, p=0.80) and +0.15 (95%CI -0.84 to +0.91, p=0.81) for RWTT.

Our results are preliminary and require confirmation in larger studies that include additional markers of arterial dysfunction. But our finding that the degree of AGI is greater for celecoxib than for diclofenac, and that chronic celecoxib use also appears to be associated with a higher level of arterial dysfunction adds support to the possibility that aldosterone may be the link between the use of NSAIDS and adverse CV outcomes.

Acknowledgements

We are grateful to all our previous collaborators involved with the original RAAIX study: Hazel Clark, David Crosbie, Vinod Kumar, Alan G. Macdonald, John Meecham, Neil Scott, and David J Williams.

References

1. KEARNEY PM, BAIGENT C, GODWIN J, HALLS H, EMBERSON JR, PATRONO C: Do selective cyclo-oxygenase-2 inhibitors and traditional non-steroidal anti-inflammatory drugs increase the risk of atherothrombosis? Meta-analysis of randomised trials. *BMJ* 2006; 332; 1302-8.
2. MCGETTIGAN P, HENRY D: Cardiovascular risk and inhibition of cyclooxygenase: a systematic review of the observational studies of selective and nonselective inhibitors of cyclooxygenase 2. *JAMA* 2006; 296; 1633-44.
3. KNIGHTS KM, MANGONI AA, MINERS JO: Non-selective non-steroidal anti-inflammatory drugs and cardiovascular events: is aldosterone the silent partner in crime? *Br J Clin Pharmacol* 2006; 61; 738-40.
4. KNIGHTS KM, MANGONI AA, MINERS JO: Defining the COX inhibitor selectivity of NSAIDs: implications for understanding

toxicity. *Expert Rev Clin Pharmacol* 2010; 3; 769-76.
5. MANGONI AA, CRILLY MA, KNIGHTS KM: Cardiovascular toxicity of nonsteroidal anti-inflammatory drugs: moving beyond cyclooxygenase selectivity. *Expert Rev Clin Pharmacol* 2011; 4; 299-302.
6. WARNER TD, MITCHELL JA: COX-2 selectivity alone does not define the cardiovascular risks associated with non-steroidal anti-inflammatory drugs. *Lancet* 2008; 19; 371; 270-3.
7. KIRKBY NS, LUNDBERG MH, HARRINGTON LS *et al*.: Cyclooxygenase-1, not cyclooxygenase-2, is responsible for physiological production of prostacyclin in the cardiovascular system. *Proc Natl Acad Sci USA* 2012; 109; 17597-602.
8. AVINA-ZUBIETA JA, CHOI HK, SADATSAFAVI M, ETMINAN M, ESDAILE JM, LACAILLE D: Risk of cardiovascular mortality in patients with rheumatoid arthritis: a meta-analysis of observational studies. *Arthritis Rheum* 2008; 59; 1690-7.
9. AVALOS I, CHUNG CP, OESER A *et al*.: Increased augmentation index in rheumatoid arthritis and its relationship to coronary artery atherosclerosis. *J Rheumatol* 2007; 34; 2388-94.
10. KLOCKE R, COCKCROFT JR, TAYLOR GJ, HALL IR, BLAKE DR: Arterial stiffness and central blood pressure, as determined by pulse wave analysis, in rheumatoid arthritis. *Ann Rheum Dis* 2003; 62; 414-8.
11. VAN DOORNUM S., MCCOLL G, WICKS IP: Atorvastatin reduces arterial stiffness in patients with rheumatoid arthritis. *Ann Rheum Dis* 2004; 63; 1571-5.
12. MACKENZIE IS, WILKINSON IB, COCKCROFT JR: Assessment of arterial stiffness in clinical practice. *QJM* 2002; 95; 67-74.
13. SMULYAN H, SIDDIQUI DS, CARLSON RJ, LONDON GM, SAFAR ME: Clinical utility of aortic pulses and pressures calculated from applanated radial-artery pulses. *Hypertension* 2003; 42; 150-5.
14. PIERINGER H, SCHUMACHER S, STUBY U, BIESENBACH G: Augmentation index and large-artery remodeling in patients with longstanding rheumatoid arthritis compared with healthy controls. *Semin Arthritis Rheum* 2009; 39: 163-9
15. VLACHOPOULOS C, AZNAOURIDIS K, O'ROURKE MF, SAFAR ME, BAOU K, STEFANADIS C: Prediction of cardiovascular events and all-cause mortality with central haemodynamics: a systematic review and meta-analysis. *Eur Heart J* 2010; 31; 1865-71.
16. KNIGHTS KM, WINNER LK, ELLIOT DJ, BOWALGAHA K, MINERS JO: Aldosterone glucuronidation by human liver and kidney microsomes and recombinant UDP-glucuronosyltransferases: inhibition by NSAIDs. *Br J Clin Pharmacol* 2009; 68; 402-12.
17. MAHMUD A, FEELY J: Arterial stiffness and the renin-angiotensin-aldosterone system. *J Renin Angiotensin Aldosterone Syst* 2004; 5; 102-8.
18. STRUTHERS AD: Aldosterone blockade in cardiovascular disease. *Heart* 2004; 90; 1229-34.
19. TOMASCHITZ A, PILZ S, RITZ E, MEINITZER A, BOEHM BO, MARZ W: Plasma aldosterone levels are associated with increased cardiovascular mortality: the Ludwigshafen Risk and Cardiovascular Health (LURIC) study. *Eur Heart J* 2010; 31; 1237-47.
20. BERNINI G, GALETTA F, FRANZONI F *et al*.: Arterial stiffness, intima-media thickness and carotid artery fibrosis in patients with primary aldosteronism. *J Hypertens* 2008; 26; 2399-405.
21. CRILLY MA, MANGONI AA: Non-steroidal anti-inflammatory drug (NSAID) related inhibition of aldosterone glucuronidation and arterial dysfunction in patients with rheumatoid arthritis: a cross-sectional clinical study. *BMJ Open* 2011; 1: e000076.
22. CRILLY MA, KUMAR V, CLARK HJ, SCOTT NW, MACDONALD AG, WILLIAMS DJ: Arterial stiffness and cumulative inflammatory burden in rheumatoid arthritis: a dose-response relationship independent of established cardiovascular risk factors. *Rheumatology* (Oxford) 2009; 48; 1606-12.
23. CRILLY MA, CLARK HJ, KUMAR V, SCOTT NW, MACDONALD AG, WILLIAMS DJ: Relationship between arterial stiffness and Stanford Health Assessment Questionnaire disability in rheumatoid arthritis patients without overt arterial disease. *J Rheumatol* 2010; 37; 946-52.
24. BRUCE B, FRIES JF: The Stanford Health Assessment Questionnaire: dimensions and practical applications. *Health Qual Life Outcomes* 2003; 1: 20.
25. RABIN R, DE CHARRO F: EQ-5D: A measure of health status from the EuroQol Group. *Ann Med* 2001; 33; 337-43.
26. VAN BORTEL LM, DUPREZ D, STARMANSKOOL MJ *et al*.: Clinical applications of arterial stiffness, Task Force III: recommendations for user procedures. *Am J Hypertens* 2002; 15; 445-52.
27. EL ASSAAD MA, TOPOUCHIAN JA, ASMAR RG: 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; 127-33.
28. CRILLY M, COCH C, BRUCE M, CLARK H, WILLIAMS D: Indices of cardiovascular function derived from peripheral pulse wave analysis using radial applanation tonometry: a measurement repeatability study. *Vasc Med* 2007; 12; 189-97.
29. PAUCA AL, O'ROURKE MF, KON ND: Prospective evaluation of a method for estimating ascending aortic pressure from the radial artery pressure waveform. *Hypertension* 2001; 38; 932-7.
30. WILKINSON IB, MACCALLUM H, FLINT L, COCKCROFT JR, NEWBY DE, WEBB DJ: The influence of heart rate on augmentation index and central arterial pressure in humans. *J Physiol* 2000; 525; 263-70.
31. MATTHEWS JN, ALTMAN DG, CAMPBELL MJ, ROYSTON P: Analysis of serial measurements in medical research. *BMJ* 1990; 300; 230-5.
32. ROWLAND A, GAGANIS P, ELLIOT DJ, MACKENZIE PI, KNIGHTS KM, MINERS JO: Binding of inhibitory fatty acids is responsible for the enhancement of UDP-glucuronosyltransferase 2B7 activity by albumin: implications for in vitro-in vivo extrapolation. *J Pharmacol Exp Ther* 2007; 321; 137-47.
33. DAVIES NM, MCLACHLAN AJ, DAY RO, WILLIAMS KM: Clinical pharmacokinetics and pharmacodynamics of celecoxib: a selective cyclo-oxygenase-2 inhibitor. *Clin Pharmacokinet* 2000; 38; 225-42.
34. WARNER TD, GIULIANO F, VOJNOVIC I, BUKASA A, MITCHELL JA, VANE JR: Nonsteroid drug selectivities for cyclo-oxygenase-1 rather than cyclo-oxygenase-2 are associated with human gastrointestinal toxicity: a full *in vitro* analysis. *Proc Natl Acad Sci USA* 1999; 96; 7563-8.
35. CLARIDGE M, HOBBS S, QUICK C, DAY N, BRADBURY A, WILMINK T: Nonsteroidal antiinflammatory drugs are associated with increased aortic stiffness. *Vasc Health Risk Manag* 2005; 1; 149-53.
36. WONG M, JIANG BY, MCNEILL K, FARISH S, KIRKHAM B, CHOWIENCZYK P: Effects of selective and non-selective cyclo-oxygenase inhibition on endothelial function in patients with rheumatoid arthritis. *Scand J Rheumatol* 2007; 36; 265-9.
37. PANOULAS VF, DOUGLAS KM, MILIONIS HJ *et al*.: Prevalence and associations of hypertension and its control in patients with rheumatoid arthritis. *Rheumatology* (Oxford) 2007; 46; 1477-82.
38. CHAN CC, REID CM, AW TJ, LIEW D, HAAS SJ, KRUM H: Do COX-2 inhibitors raise blood pressure more than nonselective NSAIDs and placebo? An updated meta-analysis. *J Hypertens* 2009; 27; 2332-41.