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

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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 (Kᵢ) for celecoxib was lower than that of diclofenac (Kᵢ, 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
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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 18β-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 18β-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 (150x3.9 mm, 4 μm; Millford, 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 241 nm and the retention times for aldosterone18β-glucuronide and aldosterone were 4.68 and 6.36 min, respectively. Aldosterone standard curves were linear (r²>0.99). Coefficients of variation were 2.1% for inter-day variability and <6% at 100 μM and <2% at 1000 μM 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 quadrupole 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 0.7 kV, cone voltage 25 V, source temperature 150°C, and sheath gas flow 5 L min⁻¹. The identity of aldosterone 18β-glucuronide 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.

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 Kᵢ 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 Kᵢ 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’ Kᵢ 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 into 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.

**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,

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

<table>
<thead>
<tr>
<th>Drug</th>
<th>Dose (mg)</th>
<th>Cmax (μM)</th>
<th>Unbound fraction</th>
<th>Unbound Conc. (μM)</th>
<th>( K_i ) (μM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Celecoxib</td>
<td>200*</td>
<td>1.9</td>
<td>0.026</td>
<td>0.05</td>
<td>3.5</td>
</tr>
<tr>
<td>Diclofenac</td>
<td>50**</td>
<td>6.7</td>
<td>0.005</td>
<td>0.03</td>
<td>8.4</td>
</tr>
</tbody>
</table>


Plasma Cmax values: celecoxib 0.72 μg/mL; diclofenac 2 μg/mL.

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

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Celecoxib (n=31)</th>
<th>Diclofenac (n=8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean age, years (SD)</td>
<td>53.7</td>
<td>55.0</td>
</tr>
<tr>
<td>Female</td>
<td>28 90%</td>
<td>6 75%</td>
</tr>
<tr>
<td>University education</td>
<td>8 26%</td>
<td>2 25%</td>
</tr>
<tr>
<td>Mean arterial blood pressure, mmHg</td>
<td>98.9</td>
<td>104.0</td>
</tr>
<tr>
<td>Mean brachial systolic pressure, mmHg (SD)</td>
<td>125.8</td>
<td>136.8</td>
</tr>
<tr>
<td>Mean brachial diastolic pressure, mmHg (SD)</td>
<td>82.6</td>
<td>85.1</td>
</tr>
<tr>
<td>Mean ratio total/HDL-cholesterol (SD)</td>
<td>3.7</td>
<td>3.1</td>
</tr>
<tr>
<td>Mean height, cms (SD)</td>
<td>162.7</td>
<td>170.5</td>
</tr>
<tr>
<td>Mean waist-hip ratio (SD)</td>
<td>0.85</td>
<td>0.80</td>
</tr>
<tr>
<td>Never smoked</td>
<td>14 45%</td>
<td>3 38%</td>
</tr>
<tr>
<td>Ex-smoker</td>
<td>11 36%</td>
<td>4 50%</td>
</tr>
<tr>
<td>Current smoker</td>
<td>6 19%</td>
<td>1 13%</td>
</tr>
<tr>
<td>Current cardiovascular drug therapy</td>
<td>8 26%</td>
<td>1 13%</td>
</tr>
<tr>
<td>Bendroflumethiazide</td>
<td>4 13%</td>
<td>1 13%</td>
</tr>
<tr>
<td>Calcium channel blocker</td>
<td>3 10%</td>
<td>0 0%</td>
</tr>
<tr>
<td>Atenolol</td>
<td>1 3%</td>
<td>0 0%</td>
</tr>
<tr>
<td>Angiotensin converting enzyme inhibitor</td>
<td>1 3%</td>
<td>0 0%</td>
</tr>
<tr>
<td>Family history premature coronary heart disease</td>
<td>10 32%</td>
<td>2 25%</td>
</tr>
<tr>
<td>Median Stanford HAQ disability (IQR)</td>
<td>1.4</td>
<td>0.8</td>
</tr>
<tr>
<td>Median EuroQol score (IQR)</td>
<td>0.66</td>
<td>0.69</td>
</tr>
<tr>
<td>Median arthritis duration, years (IQR)</td>
<td>9</td>
<td>15</td>
</tr>
<tr>
<td>Median ESR at assessment, mm/hour (IQR)</td>
<td>22</td>
<td>18</td>
</tr>
<tr>
<td>Median cumulative ESR-years (IQR)</td>
<td>221</td>
<td>256</td>
</tr>
<tr>
<td>Rheumatoid factor ‘positive’ (&gt;30 IU)</td>
<td>26 84%</td>
<td>6 75%</td>
</tr>
<tr>
<td>Current rheumatoid drug therapy*</td>
<td>28 90%</td>
<td>7 88%</td>
</tr>
<tr>
<td>Methotrexate</td>
<td>13 42%</td>
<td>4 50%</td>
</tr>
<tr>
<td>Sulphasalazine</td>
<td>10 32%</td>
<td>2 25%</td>
</tr>
<tr>
<td>Leflunomide</td>
<td>2 7%</td>
<td>1 13%</td>
</tr>
<tr>
<td>Cytokine modulator</td>
<td>3 10%</td>
<td>0 0%</td>
</tr>
<tr>
<td>Azathioprine</td>
<td>1 3%</td>
<td>0 0%</td>
</tr>
<tr>
<td>Prednisolone</td>
<td>3 10%</td>
<td>0 0%</td>
</tr>
<tr>
<td>Mean augmentation index, AIX% (SD)</td>
<td>32.3</td>
<td>34.8</td>
</tr>
<tr>
<td>Mean reflected wave transit time, msec (SD)</td>
<td>132.7</td>
<td>130.1</td>
</tr>
</tbody>
</table>

*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).

NSAID-use (predominantly diclofenac, ibuprofen and indomethacin) was associated with a reduced aortic wall stiffness (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
Inhibition of aldosterone glucuronidation by celecoxib and diclofenac / M.A. Crilly et al.

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).

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

^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 Ki is assumed the ratios of the peak plasma concentration to Ki 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.](image-url)

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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 strongly 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 1 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.

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