

PEER REVIEW HISTORY

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ARTICLE DETAILS

TITLE (PROVISIONAL)	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
AUTHORS	Crilly, Michael; Mangoni, Arduino

VERSION 1 - REVIEW

REVIEWER	Nicola Goodson Senior Lecturer in Rheumatology University of Liverpool UK
REVIEW RETURNED	28-Feb-2011

THE STUDY	<p>The title of this study suggests that the study exploring associations between NSAID related inhibition of aldosterone glucuronidation and arterial dysfunction in RA. This is an interesting and valid approach to try and explore potential mechanisms for explaining increased CVD associated with RA.</p> <p>However, the small numbers of patients on different NSAIDs did not have these measurements of AGI performed and I think as a "proof of concept study" that this is a weakness of the current methodology.</p> <p>I would also be concerned about the lack of information on dose of NSAID used as this is likely to be an important confounding variable as this would influence AGI.</p> <p>Would prior NSAID use have influenced AGI and arterial dysfunction? Attempts to adjust for time varying inflammation have been used in the multivariate analysis. However, it is possible that other NSAID exposures during this time may have influenced arterial stiffness.</p> <p>In addition more accurate assessment of inflammatory disease burden would strengthen this work. The idea that cumulative annual ESR reflects disease activity overtime is likely to be an underestimation. One way round this might be to use propensity scores to adjust for disease variables that are associated with use of different nsaids. The analysis could then be weighted for these. This would overcome some of the problems with use of multivariate linear regression to adjust for so many potential confounders in the small groups of patients in the different NSAID groups.</p> <p>There is some over interpretation of the results presented in the key messages. These hint at the importance on AGI in the associations between diclofenac and ibuprofen and pulse wave analysis. However the results demonstrate that augmentation index was higher in naproxen users (who would be expected to have a higher AGI) than in diclofenac users (lowest AGI). This raises suspicion that</p>
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	correlation between AGI and PWA may not be as clear as suggested
RESULTS & CONCLUSIONS	I would have concern that in this very small study of 60 patients there has been some overinterpretation of the results. Also as AGI by different NSAIDs is likely to be heterogeneous in individuals the AGI should have been measured in all participants rather than refer to previous assessments by NSAID in another patient group.

REVIEWER	<i>Dr Gene-Siew Ngian</i> Rheumatologist University of Melbourne No competing interests to declare
REVIEW RETURNED	16-Mar-2011

THE STUDY	As this is an analysis of a previously performed study, power calculations were not carried out to determine the number of patients needed in each group to demonstrate a significant difference. As a result, there are small numbers of patients in each group. The number of patients meeting ACR criteria was reported in the STROBE checklist but not in the manuscript.
RESULTS & CONCLUSIONS	Whilst the authors found that patients taking ibuprofen, the ns-NSAID with the lowest AGI had significantly lower arterial than patients taking diclofenac, the ns-NSAID with the highest AGI, there was no significant difference in arterial stiffness between patients taking diclofenac and those taking ns-NSAIDs with intermediate AGI. Therefore, a dose-response relationship was not conclusively demonstrated. The authors make no mention of the fact that the mean AIX of the seven patients taking ibuprofen was very low (and the RWT very high), indeed much lower than in patients not taking NSAIDs. In several parts of the manuscript, the authors do not distinguish between significant and non-significant differences between groups.
GENERAL COMMENTS	The authors did not report either dose or frequency of NSAID use. Although they stated that the small numbers in each group would preclude analysis of differences in arterial stiffness based on high/low dose therapy, it would be useful to have some information about quantity of use.

VERSION 1 – AUTHOR RESPONSE

REVIEWER #1

R1.1 "Proof of concept study"

We have removed 'proof of concept' from the title and the manuscript. We did not measure the level of 'inhibition of aldosterone glucuronidation' (AGI) as this would entail studying kinetic parameters from hepatic and renal specimens in our participants. Instead we used AGI data for NSAIDs from a published in vitro study using human liver and kidney microsomes (also see R1.8). The use of human microsomes provides a strong justification for testing the in vitro data in our cohort.

The title has been changed from "a proof of concept study" to "a cross-sectional clinical study".

R1.2 Lack of information on NSAID dose/frequency (also raised R2.6)

The numbers involved in this study are too small to permit us to adequately assess the influence of individual NSAIDs doses on arterial dysfunction. For example, in relation to diclofenac (which was the most commonly used NSAID; n=31): the majority of patients (n=18) were prescribed 150mg daily; 7 patients were prescribed a lower dose (one patient 75 mg; 6 patients 100 mg daily); a further 3 patients took diclofenac 'as required'; only 3 patients took a higher dose (2 patients 200 mg; one patient 225 mg daily).

In the 'discussion' we already mention as a study limitation that:

"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 with each NSAID with arterial dysfunction."

We agree with the Reviewer that the potential effects of different doses should be considered in larger studies.

R1.3 Would prior NSAID use influence AGI and arterial dysfunction?

From the hospital medical records we extracted data concerning previous NSAID-use. But we did not extract details of which particular NSAID(s) had been used in the past. Consequently we are not able to assess the influence of previous NSAID-use on arterial dysfunction. Among patients who had not taken an NSAID within the previous 3-months the vast majority (88%) had previously taken an NSAID. Only 3% of patients (based on their hospital records) had never taken an NSAID. However, given the kinetics of the inhibitory effects of ns-NSAIDs on AGI in vitro, it is very unlikely that significant residual effects on AGI would be present for more than 1-2 weeks after discontinuation.

In the results section we already report that:

"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)".

R1.4 Assessment of inflammatory disease burden

We think that our measurement of the cumulative 'inflammatory disease burden' using ESR-years is one of the particular strengths of our study. Most previous research in this area has relied upon a single ESR measurement. We are not aware of a "more accurate assessment of inflammatory disease burden [that] would strengthen this work" based on the review the previous medical records.

Since patients are much more likely to attend rheumatology clinics (and attend more frequently) when their disease is active, we suspect that 'ESR-years' are more likely to 'over-estimate' (rather than 'under-estimate') cumulative disease activity.

Additional text added to 'methods - statistical analysis':

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).

Additional text added to discussion:

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]

R1.5 Use propensity scores to adjust for disease variables

The use of propensity scores to adjust for independent variables in observational studies is an alternative to the conventional approach of multiple linear regression (MLR) that we have employed in this study. The propensity score approach has not been shown to be superior to more established approaches such as MLR. Whilst propensity scores have a theoretical advantage in permitting the inclusion of a greater number of covariates, their practical limitation is that the approach relies upon utilising two regression models (a 'propensity model' and a 'treatment effect model'), rather than just a single model. Consequently the propensity score approach introduces additional variance into the adjusted estimate of the treatment 'effect size'. This may be the explanation as to why propensity score methods have not been shown to be superior to conventional multivariable analysis.

Stürmer T, et al. A review of the application of propensity score methods yielded increasing use, advantages in specific settings, but not substantially different estimates compared with conventional multivariable methods. *J Clin Epidemiol.* 2006;59(5):437-47.

Shah BR, et al. Propensity score methods gave similar results to traditional regression modelling in observational studies: a systematic review. *J Clin Epidemiol.* 2005;58(6):550-9.

We have previously used propensity scores in a previous cohort study [McCowan C, et al. Cohort study examining tamoxifen adherence and its relationship to mortality in women with breast cancer. *Br J Cancer.* 2008; 99 (11): 1763-8.], but on balance our preference for the analysis of this study remains based on the more established technique of MLR.

R1.6 Small number of patients on different NSAIDS

The analysis 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. The sample size of the original study was based on the statistical 'rule of thumb' of having 10 subjects for each independent variable included in multiple linear regression (MLR). A sample size of 110 was our intention to ensure that there were 10 subjects for each of the 11 factors we anticipated including in the original regression analysis.[reference #13]

The important study limitation of the 'small number of patients on different NSAIDS' is already highlighted several times in the manuscript:

Abstract: "The study findings are limited by the small number of patients involved and require further replication in a much larger study." Article Summary: "The small number of patients taking each NSAID means that the confidence intervals around our findings are wide." Discussion: "Our assessment of RA patients included a relatively small number of patients taking each NSAID and only 7 patients were chronic users of ibuprofen."

Additional text added to 'discussion':

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.

R1.7 Over interpretation of the results presented in the key messages

We are conscious of the dangers of over-interpreting our results based on only 60 patients and have attempted be appropriately cautious in our report. We have re-drafted the 'Article Summary - Key

messages' and 'Abstract-results'.

Also see R1.6, R1.8 and R2.3 .

We have re-drafted the 'Article Summary - Key messages', so that this now reads:

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 fully fit the anticipated pattern. Our findings support the concept that AGI may play a role in the cardiovascular toxicity of some ns-NSAIDS commonly used in routine clinical practice.

We have also re-drafted the 'Abstract-results' ["Indomethacin and naproxen demonstrated an intermediate level of arterial dysfunction, although both were more similar to diclofenac than they were to ibuprofen."], so that this now reads:

Indomethacin demonstrated an intermediate level of arterial dysfunction. In relation to arterial dysfunction both indomethacin and naproxen were more similar to diclofenac than ibuprofen

R1.8 Aldosterone glucuronidation inhibition (AGI) should have been measured in all participants
The citation in the manuscript to a previous study reporting on NSAID-related AGI refers to in vitro research using human liver and kidney microsomes [Knights K et al. Brit J Clin Pharmacol 2009;68:402-412]. It does not refer to "previous assessments by NSAID in another patient group". The technique applied to assessing NSAID-related AGI in vitro is not currently applicable to individual patients as it would require kidney and liver biopsies from each participant. Based on the findings reported in this manuscript we are currently submitting a grant application to study the influence of NSAID-related AGI on arterial function in an adequately powered clinical study.

In the 'discussion' we already mention as a study limitation that
"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."

Additional text added to 'discussion' as a 'study limitation':

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.

REVIEWER #2

R2.1 Analysis of previous study data with small number of patients taking each NSAID
See response to R1.6 above (which relates to the same issue).

R2.2 Number patients meeting ACR criteria (reported in STROBE checklist, but not in manuscript)
We have now reported the number (%) of patients meeting American College of Rheumatology (ACR) criteria in the main text (under 'methods'). We have also added this data as an additional row to both Tables 1 and 2. Under 'statistical analysis we have also added "RA-criteria" as another of the 'additional variables' that did "not alter the adjusted values for AIX and RWT reported, nor improve the goodness to fit of the final regression model."

Additional text added to 'methods':

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.

R2.3 NSAID-AGI relationship with arterial dysfunction not conclusively demonstrated
Also see response to R1.7 above

Only the differences in arterial dysfunction between diclofenac and ibuprofen reached statistical significance at the 5% level (p-value). Indomethacin (intermediate AGI) was associated with an intermediate level of arterial dysfunction. Naproxen was associated with an intermediate level of arterial dysfunction based on the reflected wave transit time (RWT), but was associated with a higher augmentation index (AIX%) than would be anticipated from its in vitro AGI. We would argue, however, that a dose-response relationship study, even in a much larger population, does not necessarily entail the existence of statistically significant differences between all the different drugs/doses investigated.

We have modified the 'Abstract' to indicate that [only] "Indomethacin demonstrated an intermediate level of arterial dysfunction." [rather than both indomethacin AND naproxen].

'Discussion' (1st paragraph) has been re-written:

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.

We have also re-drafted the 'Article Summary - Key messages', so that this now reads:

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 fully fit the anticipated pattern. Our findings support the concept that AGI may play a role in the cardiovascular toxicity of some ns-NSAIDs commonly used in routine clinical practice.

R2.4 Mention AIX% in ibuprofen-patients much lower than in patients not taking NSAIDs

Additional text added to 'Results' under 'Differences in arterial function':

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 patients not currently taking a NSAID (unadjusted mean difference in AIX% 7.1, 95%CI -1.4 to 25.6).

R2.5 Distinguish between 'significant' and 'non-significant' differences

We have intentionally attempted to avoid an overreliance on 'hypothesis testing/p-values' and we have reported 95%CI's around all of the differences we observed in this study. We find the arguments concerning the overreliance of clinical research on 'hypothesis testing/p-values' that have been made by several statisticians compelling. For example, see 'Statistics with confidence, 2nd Ed (2000), BMJ Books. Chapter 3: Confidence intervals rather than p-values; by Douglas Altman and Martin Gardiner.'

R2.6 Dose/frequency NSAID-use not reported

See response to R1.2 above - which relates to the same issue.

Michael A. Crilly
Arduino A. Mangoni

VERSION 2 - REVIEW

REVIEWER	<i>Dr Gene-Siew Ngian</i>
REVIEW RETURNED	17-Apr-2011

GENERAL COMMENTS	Reviewer completed checklist only. No further comments
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REVIEWER	<i>Nicola Goodson</i>
REVIEW RETURNED	18-Apr-2011

THE STUDY	I still have concerns over the small sample size of patients studied, particularly in the multivariate analysis comparing arterial dysfunction with different NSAID exposures. This was conducted in only 60 patients (31 reporting diclofenac, 16 reporting Naproxen, 6 reporting indomethacin and 7 reporting ibuprofen use) and was repeated using Augmentation index and reflected wave transit as outcomes.
RESULTS & CONCLUSIONS	<p>Whilst it is possible that the nsNSAID associated AGI may be associated with augmentation index and reflected wave transit I think that the very small study numbers and within patient variability make it difficult to conclude this.</p> <p>The comment that Coxib users had similar vascular dysfunction to diclofenac users is interesting as Coxibs and oxicams do not contain a carboxylic acid functional group and should not be able to inhibit glucuronidation. Therefore the finding that these patients using coxibs have a similar level of vascular dysfunction to users of high glucuronidation inhibitors is interesting and suggests that this may be due to other factors rather than nsnsaid related aldosterone inhibition. This certainly warrants discussion.</p>
GENERAL COMMENTS	I feel that with the small sample size and even smaller numbers of patients exposed to the individual NSAID drugs that it is very difficult to place any meaningful interpretation on the findings. The authors have toned down their key points and discussion. However, it is difficult to be confident that any association exists between the nsNSAID associated AGI and vascular dysfunction. The finding that Coxib users have a similar if not worse vascular dysfunction to diclofenac (the drug with highest AGI) also complicates this association as these drugs do not inhibit AG. These findings suggest that the vascular dysfunction may not be associated with nsNSAID associated AGI.

VERSION 2 – AUTHOR RESPONSE

REVIEWER #1

[A] Similar arterial dysfunction observed among diclofenac and coxib users

It is suggested that selective COX-2 inhibitors (coxibs) are unlikely to inhibit aldosterone glucuronidation as they do not contain a carboxylic acid functional group. Selective COX-2 inhibitor users had a similarly high augmentation index (AIX%) compared to diclofenac (the ns-NSAID with

highest level of aldosterone glucuronidation inhibition, AGI). This suggests that the relatively high AIX% observed with selective COX-2 inhibitors may be due to factors other than NSAID-related AGI.

This is an interesting point and we agree that AGI may not be the explanation for the relatively high AIX% associated with selective COX-2 inhibitor use in our study.

A recent review of randomised clinical trials suggests that selective COX-2 inhibitors may have a greater propensity to elevate brachial blood pressure than ns-NSAIDs.[Chann, 2009]. In our study treatment with selective COX-2 inhibitors was associated with higher brachial blood pressure compared to diclofenac (brachial blood pressure 135/86 vs. 125/82 mmHg; pulse pressure 47 vs. 33 mmHg). As a higher brachial blood pressure (systolic, diastolic, mean and pulse) is also associated with higher AIX%, [Tarumi, 2010] one plausible explanation is that the relatively high AIX% observed among selective COX-2 inhibitor users may be primarily due to a higher brachial blood pressure, rather than the AGI phenomenon.

Additional text added to Results (final paragraph):

"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. "

Additional text added to Discussion:

"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]

Additional References added:

[34] Chan CC, Reid CM, Aw TJ, Liew D, Haas SJ, Krum H. Do COX-2 inhibitors raise blood pressure more than non-selective NSAIDs and placebo? An updated meta-analysis. *J Hypertens*. 2009;27(12):2332-41.

[35] Tarumi T, Sugawara J, Tanaka H. Association between ankle blood pressure and central arterial wave reflection. *J Hum Hypertens*. Published Online First: 21 Oct. 2010. doi:10.1038/jhh.2010.100

[B] Concerns over the small number of patients on different NSAIDS

We accept that the small number of patients on different NSAIDS is an important limitation. This is mentioned several times in the manuscript.

Abstract:

"The study findings are limited by the small number of patients involved and require further replication in a much larger study."

Article Summary:

“The small number of patients taking each NSAID means that the confidence intervals around our findings are wide. “

Discussion:

“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.”

Michael A. Crilly
Arduino A. Mangoni