OFF-TARGET INTERACTIONS AS NOVEL EXPLANATION OF NSAID CV RISK.

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Introduction: Non-steroidal anti-inflammatory drugs (NSAIDs) are a clinically useful and widely used group of compounds, which are associated with an increased cardiovascular (CV) risk which is not well explained by existing theories. The extent of the CV risk differs amongst NSAIDs, they can be ranked as follows in terms of their CV safety profiles (most favourable to least favourable): Naproxen > ibuprofen > diclofenac = coxib's > rofecoxib. Recent evidence used *in silico* approaches to predict NSAID interactions with off-target nuclear receptors and a reporter assay then confirmed *in vitro* that celecoxib and diclofenac, but not naproxen, are antagonists of the Thyroid hormone receptor beta (Zloh, Perez-Diaz, Tang, Patel & Mackenzie, 2016). Due to the fact that many drugs exhibit poly-pharmacology the present work builds on the off-target theory of NSAID CV risk with a comprehensive investigation of NSAID off-target interactions to explain NSAID CV risk and why it differs from NSAID to NSAID.

Methods: VirtualToxLab and PharmMapper were used to predict the off-target interactions of a range of NSAIDs with off-target nuclear receptors and other proteins (Vedani, Dobler, Hu & Smiesko, 2015; Wang et al, 2017). A luminescent reporter assay was used to confirm an NSAID interaction with aldosterone nuclear receptor (ANR). 2 way analysis of variance (ANOVA) was used to determine the significance of differences observed between NSAIDs compared to diclofenac. A fluorescent assay was used to investigate an NSAID angiotensin converting enzyme 1 (ACE1) interaction. Non-linear regression analysis within GraphPad Prism5 was used to determine Michaelis-Menten Km and Vmax of ACE1 in the presence and absence of diclofenac and naproxen. Plotted data are expressed as background normalised values. Michaelis-Menten Km and Vmax analysis was conducted using data that had not been normalised.

Results: Diclofenac, celecoxib, naproxen, rofecoxib, aspirin and valdecoxib modestly induce the ANR. Diclofenac induces the ANR more than these other NSAIDs. The difference in expression between diclofenac and other NSAIDs was significant only compared to some NSAIDs and at some concentrations. Compared to native ACE1 diclofenac reduced Vmax and increased Km and naproxen increased Vmax and reduced Km, as did trandolapril, an inhibitor of ACE1.

Discussion: ANR and ACE1 play key roles in positively regulating blood pressure. An NSAID-ANR interaction would provide novel explanation for NSAID CV risk. An increased level of ANR induction by diclofenac compared to other NSAIDs would help to explain the less favourable CV safety profile of diclofenac compared to other NSAIDs. Additionally, an increase in the velocity of ACE1 by diclofenac and a decrease in the velocity by naproxen would provide new explanation for the CV safety profiles of naproxen and diclofenac.

References:

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