

# openheart Supplemental N-acetylcysteine and other measures that boost intracellular glutathione can downregulate interleukin-1 $\beta$ signalling: a potential strategy for preventing cardiovascular events?

James J DiNicolantonio,<sup>1</sup> James H O'Keefe,<sup>1</sup> Mark F McCarty<sup>2</sup>

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## INTERLEUKIN-1 — LIKELY MEDIATOR OF CRP-ASSOCIATED CARDIOVASCULAR RISK

A proinflammatory milieu associated with elevations of C-reactive protein (CRP) and homocysteine has been linked epidemiologically to increased risk for myocardial infarction (MI). Nonetheless, there is good reason to suspect that neither CRP nor homocysteine are mediators of this risk, but rather serve as markers for increased activity of another agent or agents that are true mediators.<sup>1-4</sup> The search for such a mediator has cast suspicion on interleukin (IL)-1 $\beta$ , which, in conjunction with IL-6, boosts hepatic expression of CRP.<sup>5,6</sup> While the effects of IL-1 $\beta$  on hepatic acute phase protein expression are variable, IL-1 $\beta$  also promotes hepatic synthesis of serum amyloid A, another acute phase reactant linked to increased cardiovascular (CV) risk.<sup>5,7</sup> Furthermore, there is some reason to suspect that IL-1 $\beta$  may act on the liver to boost homocysteine levels.<sup>8</sup> Rodent and cell culture studies indicate that IL-1 $\beta$  can promote atherogenesis by via effects on endothelial, smooth muscle and foam cells.<sup>9-16</sup> Conversely, apolipoprotein E knockout mice in which IL-1 $\beta$  has likewise been knocked out, or that are treated with a monoclonal antibody targeting IL-1 $\beta$ , are less prone to atherogenesis.<sup>17-19</sup> The impact of canakinumab, a monoclonal antibody targeting IL-1 $\beta$ , is now being studied for secondary prevention of MI in patients with elevated CRP in the Canakinumab Anti-inflammatory Thrombosis Outcomes Study (CANTOS) multicenter trial.<sup>20</sup> This trial should provide important insight regarding the suspected role of IL-1 $\beta$  in coronary events. A pilot study

has already confirmed that canakinumab can markedly lower elevated CRP; its impact on homocysteine has not yet been evaluated.<sup>21</sup> The ability of potent statin therapy to decrease risk for coronary events in patients with elevated CRP but low-normal low density lipoprotein (LDL) cholesterol might reflect a suppressive impact of statins on IL-1 $\beta$  activation via inflammasomes.<sup>22-25</sup> While canakinumab may prove to be an important therapeutic asset for patients with established coronary disease, the availability of IL-1 $\beta$ -antagonist measures that are less expensive and more practical for use in primary prevention of atherosclerotic disorders would be welcome.

## ROLE OF NEUTRAL SPHINGOMYELINASE IN SUSTAINED IL-1 SIGNALLING

Recent studies, employing rat primary hepatocytes as well as a human embryonic kidney cell line transfected with the IL-1 $\beta$  receptor, have defined a feed-forward mechanism that controls the intensity of IL-1 $\beta$  signalling. IL-1 receptor associated kinase-1 (IRAK-1) is an obligate mediator of this signalling, a key component of the complex formed with the activated IL-1 $\beta$  receptor, and its level is a determinant of the intensity of IL-1 $\beta$  signalling.<sup>26</sup> When IRAK-1 is phosphorylated by IRAK-4, another member of this complex, its kinase activity is enabled; IRAK-1-mediated phosphorylations then lead to transmission of the IL-1 $\beta$  signal, with downstream activation of transforming growth factor beta-activated kinase 1 (TAK1), c-Jun N-terminal kinases (JNK) and nuclear



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<sup>1</sup>Saint Luke's Mid America Heart Institute, Kansas, USA

<sup>2</sup>Catalytic Longevity, Encinitas, California, USA

## Correspondence to

Dr James J DiNicolantonio, Saint Luke's Mid America Heart Institute, Kansas, MO 64111, USA; jjdinicol@gmail.com

factor kappa-light-chain-enhancer of activated B cells (NF- $\kappa$ B).<sup>27–29</sup> However, IRAK-1 also autophosphorylates certain serine/threonine residues in its C-terminal region, and some of these phosphorylations enable polyubiquitination and subsequent proteasomal degradation of the protein.<sup>26</sup> For this reason, the strength of the IL-1 $\beta$  signal tends to fade over time.

One downstream effect of IL-1 $\beta$  signalling is activation of neutral sphingomyelinase 2 (NSMase-2), leading to generation of ceramide.<sup>30</sup> Ceramide, in turn, binds and activates protein phosphatase 2A (PP2A), which dephosphorylates IRAK-1 in such a manner as to protect it from ubiquitination and proteasomal degradation.<sup>31–32</sup> Hence, this arm of the IL-1 $\beta$  signalling network functions to sustain the intensity of the IL-1 $\beta$  signal.

However, NSMase-2 is subject to redox regulation, as it is inhibited by free glutathione in physiological millimolar concentrations.<sup>33–34</sup> This inhibition blunts the generation of ceramide induced by IL-1 $\beta$  activity, leading to accelerated degradation of IRAK-1 and downregulation of the IL-1 $\beta$  signal.<sup>32–35</sup> It follows that measures which boost cellular levels of glutathione can suppress, though not eliminate, IL-1 $\beta$  signalling. (Such signalling would continue with diminished intensity, as IRAK-1 levels would be decreased but not eliminated.)

### BOOSTING CELLULAR GLUTATHIONE LEVELS DOWNREGULATES IL-1 SIGNALLING

Glutathione, present in low millimolar concentrations in most cells, constitutes the chief water-soluble intracellular oxidant scavenger, protecting the membranes, DNA and proteins of oxidatively stressed tissues. (Ascorbate is likewise of importance in this regard.) It also opposes the proinflammatory/proapoptotic signalling effects of hydrogen peroxide, both by enabling the degradation of this compound via glutathione peroxidase and by working with glutaredoxin to promote the restoration of sulfenic acids to sulfhydryl form.<sup>36–40</sup> As we have seen, it can also oppose IL-1 $\beta$ -induced inflammation via its inhibitory impact on NSMase-2. A further role of glutathione is to aid excretion of relatively hydrophobic compounds via conjugation reactions catalysed by glutathione-S-transferases.<sup>41</sup>

Intracellular cysteine availability is rate limiting for glutathione synthesis, such that increased cysteine levels boost this synthesis.<sup>42</sup> N-acetylcysteine (NAC) is a venerable nutraceutical which acts as a well-tolerated delivery vehicle for cysteine when administered orally. Both rodent and clinical studies show that NAC supplementation can boost tissue glutathione levels; the clinical doses employed have usually been in the range of 600–1800 mg daily.<sup>43–44</sup> NAC supplementation may have particular utility in the elderly, as a decline in tissue glutathione levels is a characteristic feature of ageing; nonetheless, these levels can be boosted by NAC supplementation or a diet richer in cysteine.<sup>45–48</sup> *In vitro*, as expected, NAC treatment has been shown to blunt the intensity of IL-1 $\beta$

signalling in hepatocytes from ageing rats by promoting degradation of IRAK-1.<sup>49</sup>

If clinical administration of NAC can indeed antagonise IL-1 $\beta$  signalling, and if CRP serves as a marker for hepatic IL-1 $\beta$  activity, one would expect supplemental NAC to decrease elevated CRP levels. Indeed, several studies evaluating intravenous or oral NAC administration in various proinflammatory conditions have demonstrated precisely this.<sup>50–53</sup>

The other key determinant of the rate of glutathione synthesis is the expression of gamma-glutamylcysteine synthetase, a phase 2 enzyme whose transcription is stimulated by activation of the nuclear factor (erythroid-derived 2)-like 2 (nrf2) transcription factor.<sup>54–55</sup> A wide range of nutraceuticals and drugs, characterised as ‘phase 2 inducers’, are known to activate nrf2, leading to induction of a range of antioxidant enzymes, as well as gamma-glutamylcysteine synthetase. Perhaps, the most clinically developed of these is the natural cofactor lipoic acid (transported most effectively as its natural R-isomer<sup>56</sup>), which has documented clinical utility in diabetic neuropathy when administered at 1200–1800 mg daily—a clinical dose range similar to that of NAC.<sup>45–57–5</sup> In ageing rats, oral administration of R-lipoic acid has been shown to boost myocardial glutathione levels, while decreasing those of ceramide<sup>60</sup>—an effect likely attributable to inhibition of NSMase-2. Lipoic acid has likewise been shown to diminish ceramide levels and the activities of NSMase-2 and PP2A in endothelial cells *in vitro*.<sup>61</sup>

Since free, rather than oxidised, glutathione inhibits NSMase-2; it follows that the rate of intracellular generation of hydrogen peroxide—which promotes the oxidative conversion of glutathione to diglutathione—can also modulate NSMase-2 activity. Hence, rapid intracellular production of superoxide, which yields hydrogen peroxide after its dismutation, tends to elevate NSMase-2 activity and the intensity of IL-1 $\beta$  signalling.<sup>62–65</sup> Hence, practical measures which quell superoxide generation by NADPH oxidase complexes, mitochondria or uncoupled nitric oxide synthase may have potential for downregulating IL-1 $\beta$  signalling in certain clinical circumstances.

### OXIDATIVE STRESS AND INFLAMMASOME ACTIVATION

Consideration should also be given to the possibility that NAC and other antioxidant strategies might decrease production of IL-1 $\beta$  by limiting inflammasome activation. Inflammasomes featuring the protein NLRP3 play an obligate role in generation of IL-1 $\beta$ ; they activate caspase-1, which cleaves the pro-IL-1 $\beta$  protein to yield mature IL-1 $\beta$ .<sup>66</sup> Oxidative stress can activate NLR Family Pyrin Domain Containing 3 (NLRP3)-dependent inflammasomes, apparently by inducing increased expression of the thioredoxin-interacting protein (TXNIP), which binds to NLRP3; oxidants also increase the availability of free TXNIP by inhibiting its binding to thioredoxin.<sup>67–69</sup> A number of studies show that oxidants generated by NADPH oxidase complexes can promote activation of

NLRP3-dependent inflammasomes.<sup>70–73</sup> The fact that statins can downregulate NADPH oxidase activation by suppressing isoprenylation of Rac1 may well be pertinent to the ability of these drugs to decrease inflammasome activity and decrease IL-1 $\beta$  production.<sup>74–76</sup> Free intracellular bilirubin generated by haeme oxygenase-1 induction functions physiologically as an inhibitor of NADPH oxidase complexes, and the structurally-related compound phycocyanobilin, a prominent chromophore in edible cyanobacteria such as spirulina, appears to mimic this activity; this natural compound may thus have potential for suppressing inflammasome activation and IL-1 $\beta$  production.<sup>77–81</sup> In a large number of cell culture studies, NAC has been reported to inhibit inflammasome activation and IL-1 $\beta$  production.<sup>82–88</sup> However, these studies do not clarify whether increased glutathione mediates this effect or whether the direct scavenging activity of NAC does; in the latter case, these effects might be of limited clinical relevance. Further research to clarify this point could be worthwhile. Many cell signalling effects of oxidative stress reflect hydrogen peroxide-mediated oxidation of protein sulphhydryl groups to sulfenic acid; glutathione functions to reverse this modification.<sup>38–40</sup> Glutathione also promotes elimination of hydrogen peroxide via glutathione peroxidase.<sup>37</sup>

## SUMMING UP

In the event that the ongoing CANTOS study confirms that IL-1 $\beta$  is an important mediator of CV disease, measures in addition to canakinumab for downregulating the signalling and production of IL-1 $\beta$  should be evaluated. The impact of high-dose rosuvastatin on elevated CRP suggests that it may be suppressing IL-1 $\beta$  production, and there is reason to suspect that NAC, lipoic acid and phycocyanobilin may have practical potential as nutraceutical measures for controlling IL-1 $\beta$  activity.

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