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Azithromycin and glucosamine may amplify the type 1 interferon response to RNA viruses in a complementary fashion

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ABSTRACT

Previous research demonstrates that, in clinically relevant concentrations, azithromycin can boost the ability of RNA viruses to induce type 1 interferon by amplifying the expression and virally-mediated activation of MDA5. O-GlcNAcylation of MAVS, a down-stream target of MDA5, renders it more effective for type 1 interferon induction. High-dose glucosamine administration up-regulates O-GlcNAcylation by increasing the cellular pool of UDP-*N*-acetylglucosamine. Hence, it is proposed that joint administration of azithromycin and high-dose glucosamine, early in the course of RNA virus infections, may interact in a complementary fashion to aid their control by enhancing type 1 interferon induction

In clinically meaningful concentrations (2–10 μM), azithromycin has been reported to amplify type 1 interferon response to RNA viruses (rhinoviruses, Zika virus) and poly(I:C) in human bronchial epithelial (HBE) and in human colorectal adenocarcinoma-derived HT-29 cells [1–6]. For unclear reasons, this response is more sensitive in cells obtained from patients with asthma or COPD than healthy subjects [3].

Two cytosolic receptors for double-stranded RNA, melanoma differentiation-associated protein 5 (MDA5) and retinoic acid-inducible gene 1(RIG-1), initiate a signal, transmitted through mitochondrial antiviral-signaling protein (MAVS) and the transcription factor interferon regulatory factor 3 (IRF3), that up-regulates at the transcriptional level the expression of type 1 interferons [7]. The up-regulatory effect of azithromycin on RNA virus stimulated type 1 interferon production is not affected by siRNA RIG-1 knock-down in bronchial epithelial cells, but is suppressed by MDA5 knock down [3]. Furthermore, whereas 2 μM azithromycin does not influence the mRNA expression of RIG-1 in HBE cells from asthmatics, it markedly boosts the mRNA expression of MDA5 [3]. Hence, a reasonable interpretation of these findings is that, in clinically feasible concentrations, azithromycin boosts the type 1 interferon response to RNA viruses by up-regulating MDA5 expression. However, it should be noted that, in HT-29 cells, azithromycin also up-regulated RIG-1 mRNA [4].

MAVS oligomerizes in response to activated MDA5 or RIG-1, promoting subsequent activation of IRF3. Duan and colleagues have shown that RNA virus infection prompts an O-GlcNAcylation of MAVS that renders it more susceptible to a K63-linked ubiquitination that enables it to activate IRF3 [8]. Moreover, this response can be enhanced by boosting the cellular pool of UDP-*N*-acetylglucosamine, the donor substrate for O-GlcNAcylation. One way to achieve this is to expose cells to exogenous glucosamine. These researchers found the feeding mice ample amounts of glucosamine (2.5 % of diet) could markedly enhance their survival when challenged with influenza virus 3 days after initiation of glucosamine feeding ($p = 0.0015$); mortality was cut roughly in half [8]. This protection was lost in mice in which either MAVS or O-GlcNAc transferase (the mediator of protein O-GlcNAcylation) had been knocked out. Dietary glucosamine was also shown to protect mice from mortality induced by infections with vesicular stomatitis virus and coxsackievirus. The dietary dose of glucosamine employed in these studies has been analogized to an intake of about 10 g daily in humans [9].

These considerations suggest that azithromycin and high-dose glucosamine may interact in a complementary fashion to boost the type 1 interferon response to RNA viruses by up-regulating activity of both MDA5 and MAVS. Clinically, glucosamine has been administered at up to 3 g daily without clear adverse consequences [10]. A dose of 3 g, 2 or 3 times daily, conceivably could replicate a measure of the marked protection documented in RNA virus-exposed mice. It can be anticipated that glucosamine supplementation will gradually but progressively

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enhance cellular pools of UDP-GlcNAc – which is why glucosamine was administered for 3 days prior to viral challenge in the study by Duan's group [8].

As a possibility for the future, it may be noted that Thiamet-G, a specific and high potent inhibitor of the O-GlcNAcase that removes N-acetylglucosamine from proteins (Ki = 92 nM), could be expected to boost MAVS O-GlcNAcylation more rapidly and at far lower dose than supplemental glucosamine; this agent it not yet clinically available [11].

A further implication is that the hydroxychloroquine/azithromycin regimen evaluated by Raoult and Zelenko in COVID-19 (Zelenko adds zinc) might perhaps be even more effective if complemented with high-dose glucosamine [12,13]. Measures which up-regulate type 1 interferon response logically should provide the greatest benefit if employed early during the course of a viral infection – consistent with the recommendations of Raoult and Zelenko. Within the context of this regimen, it has also been proposed that azithromycin should potentiate the alkalinization of endosome and Golgi bodies achieved with hydroxychloroquine therapy [14]. This effect is suspected to lessen the ability of SARS-CoV-2 to gain access to the cytoplasm of cells via endosomal uptake, and also to impair the glycosylation of ACE2 in golgi bodies, making this membrane protein a less effective receptor for the SARS-CoV-2 spike protein [14,15].

Rodent studies, and also some clinical results, indicate that glucosamine has anti-inflammatory properties; [16–18] conceivably, these might be of some utility in the later-stage cytokine storm phase of COVID-19. In particular, supplementation with glucosamine/chondroitin has been shown to lower C-reactive protein levels (CRP) in healthy overweight adults; elevated CRP is a marker for poor prognosis in COVID-19 [19]. [19–21] Recent research indicates that O-GlycNAcylation of the anti-inflammatory deubiquitinase A20 enhances its ability to inhibit TRAF6-dependent pathways of NF-kappaB activation, which often mediate induction of pro-inflammatory cytokines [22,23].

Intravenous administration of glucosamine in rodents has been shown to compromise insulin sensitivity in rodents, and excessive O-GlcNAcylation of proteins is thought to mediate some of the complications of diabetes [24–26]. Studies evaluating the impact of oral glucosamine on insulin sensitivity in humans have yielded mixed results, with several noting an increase of insulin resistance in subjects who were mildly insulin resistant at baseline [27]. Hence, people who are diabetic or pre-diabetic should take these findings into consideration if they contemplate using high-dose glucosamine on a chronic basis for protection from viral infections. On a positive note, long-term use of glucosamine has been associated with decreased total mortality and decreased risk for colon and lung cancers in epidemiological studies – perhaps a reflection of glucosamine's anti-inflammatory potential [28–31].

It should be noted that hydroxychloroquine's impact on endosomes may lessen the efficacy of the viral RNA-induced, TLR7-mediated endosomal pathway of type 1 interferon induction. [32,33] (Indeed, enhanced TLR7-mediated signaling triggered by endosomal uptake autoantibody/RNA complexes in plasmacytoid dendritic cells may be a key driver of systemic lupus erythematosus, and hydroxychloroquine's down-regulatory impact on this may help to rationalize its efficacy in this disorder [34–36].) For which reason, azithromycin's (and glucosamine's) up-regulatory impact on interferon induction by cytosolic double-stranded RNA receptors may provide compensation for a flaw in hydroxychloroquine's anti-viral activity – a fact which may well be pertinent to the complementarity of these two drugs in COVID-19 therapy. Of related interest is evidence that the TLR7-mediated pathway of type 1 interferon induction can be up-regulated by inhibition of endosomal NADPH oxidase; spirulina, and its key chromophore phycocyanobilin, may have potential in this regard [9,37].

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Declaration of Competing Interest

MFM is co-inventor and co-owner of a US patent on nutraceutical uses of phycocyanobilin oligopeptides derived from spirulina. JJD is Director of Scientific Affairs at AIDP.

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