Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Elsevier hereby grants permission to make all its COVID-19-related research that is available on the COVID-19 resource centre - including this research content - immediately available in PubMed Central and other publicly funded repositories, such as the WHO COVID database with rights for unrestricted research re-use and analyses in any form or by any means with acknowledgement of the original source. These permissions are granted for free by Elsevier for as long as the COVID-19 resource centre remains active.
Azithromycin and glucosamine may amplify the type 1 interferon response to RNA viruses in a complementary fashion

James J. DiNicolantonio a, Jorge Barroso-Aranda b, Mark F. McCarty c,∗

a Mid America Heart Institute, Kansas City, MO, United States
b Clínica Libre de Adicciones, Tijuana, B.C, Mexico
c Catalytic Longevity Foundation, United States

A R T I C L E   I N F O

Keywords:
Type 1 interferon
MDA5
MAVS
IRF-3
Azithromycin
Glucosamine

A B S T R A C T

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...

* Corresponding author.
E-mail address: markfmccarty@gmail.com (M.F. McCarty).

https://doi.org/10.1016/j.imlet.2020.09.008
Received 21 August 2020; Received in revised form 9 September 2020; Accepted 22 September 2020
Available online 28 September 2020
0165-2478/© 2020 European Federation of Immunological Societies. Published by Elsevier B.V. All rights reserved.
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 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 alkalization 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-inflammatoryary 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 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-GlcNaclylation 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 diabetic or pre-diabetic should take these findings into consideration if early during the course of a viral infection—phycoerythrin, may have potential in this regard [9,37].

Funding

No research grants funded this work.

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.

References

[1] V. Giele, S.L. Johnston, M.R. Edwards, Azithromycin induces anti-viral responses in bronchial epithelial cells, Eur. Respir. J. 36 (September (3)) (2010) 664–654.[2] M. Menzel, H. Akbarshahi, L. Bjermgren, L. Uller, Azithromycin induces anti-viral effects in cultured bronchial epithelial cells from COPD patients, Sci. Rep. 28 (June (6)) (2016) 28698.[3] M. Menzel, H. Akbarshahi, E. Tufvesson, C. Persson, L. Bjermgren, L. Uller, Azithromycin augments rhinovirus-induced IFNα via cytosolic MDA5 in experimental models of asthma exacerbation, Oncotarget 8 (May (19)) (2017) 31601–31611.[4] C. Li, S. Zh, Y.Q. Deng, et al., Azithromycin protects against Zika virus infection by upregulating virus-induced type I and III interferon responses, Antimicrob. Agents Chemother. 63 (September (12)) (2019).[5] J.D. Porter, J. Watson, L.R. Roberts, et al., Identification of novel macrolides with antibacterial, anti-inflammatory and type 1 and III IFN-agonizing activity in airway epithelium, J. Antimicrob. Chemother. 71 (October (10)) (2016) 2767–2781.[6] A. Schogler, B.S. Kopf, M.R. Edwards, et al., Novel antiviral properties of azithromycin in cysic fibrosis airway epithelial cells, Eur. Respir. J. 45 (February (2)) (2015) 428–439.[7] B. Huang, J. Li, X. Zhang, Q. Zhao, M. Lu, Y. Lv, RIG-1 and MDA-5 signaling pathways contribute to IFNβ production and viral replication in porcine circovirus type 2-infected PK-15 cells in vitro, Vet. Microbiol. 211 (November (2017)) 36–42.[8] N. Song, Q. Qi, R. Cao, et al., MAVS O-GlcNacylation is essential for host antiviral immunity against lethal RNA viruses, Cell Rep. 28 (August (9)) (2019) 2386–2396.[9] M.F. McCarthy, J.J. DiNicolaontonio, Nutraceuticals have potential for boosting the type 1 interferon response to RNA viruses including influenza and coronavirus, Prog. Cardiovasc. Dis. 63 (May (3)) (2020) 383–385.[10] A. Knob, H. Kai, H. Harada, H. Niiyama, H. Ikeda, Oral administration of glucosamine improves vascular endothelial function by modulating intracellular redox state, Int. Heart J. 58 (December (6)) (2017) 926–932.[11] S.A. Yuzwa, M.S. Macauley, J.E. Heinonen, et al., A potent mechanism-based O-GlcNAc inhibitor that blocks phosphorylation of tau in vivo, Nat. Chem. Biol. 4 (August (8)) (2008) 483–490.[12] J.-C. Lagier, M. Million, P. Gautret, et al., Outcomes of 3,737 COVID-19 patients treated with hydroxychloroquine/azithromycin and other regimens in Marseilles, France: a retrospective analysis, Travel Med. Infect. Dis. 36 (2020) 101791.[13] M. Scholz, R. Derwand, V. Zelenko, COVID-19 outpatients - Early risk-stratified treatment with zinc plus low-dose hydroxychloroquine and azithromycin: a retrospective case series study, Preprints (2020), https://doi.org/10.20944/preprints202007.0252v1.[14] J.M. Scherrmann, Intracellular ABCB1 as a possible mechanism to explain the synergistic effect of hydroxychloroquine/azithromycin combination in COVID-19 therapy, AAPS J. 22 (June (4)) (2020) 86.[15] M.J. Vincent, E. Bergersen, S. Perrein, et al., Chloroquine is a potent inhibitor of SARS coronavirus infection and spread, Virol. J. 22 (August (2)) (2005) 69.[16] I. Sentikar, M.A. Pacini, L. Revel, Antiarthritis effects of glucosamine sulfate in animal models, Arzneimittelforshung 41 (May (5)) (1991) 542–545.[17] S. Yomogida, Y. Kojima, Y. Tsutsumi-Ishii, J. Hua, K. Sakamoto, Nagaoka I. Glucosamine, A naturally occurring amino monosaccharide, suppresses dextran sulfate sodium-induced colitis in rats, Int. J. Mol. Med. 22 (September (3)) (2008) 381–387.[18] K.H. Chuang, Y.C. Peng, H.Y. Chien, M.L. Lu, Wu Y.L.A. Du HI, Treatment with zinc plus low-dose hydroxychloroquine and azithromycin: a retrospective case series study, Preprints (2020), https://doi.org/10.20944/preprints202007.0252v1.[19] S.L. Navarro, E. White, E.O. Kantor, et al., Randomized trial of glucosamine and chondroitin supplementation on inflammation and oxidative stress biomarkers and plasma proteomcs profiles in healthy humans, PLoS One 10 (2) (2015) e0117534.[20] J. Gao, X. Huang, H. Gu, L. Lou, Z. Xu, Predictive criteria of severe cases in COVID-19 patients of early stage: a retrospective observational study, J. Clin. Lab. Anal. 6 (September (2)) (2020) e23662.[21] L.A. Potempa, I.M. Rajab, P.C. Hart, J. Bordon, R. Fernandez-Botran, Insights into the use of C-Reactive protein as a diagnostic index of disease severity in COVID-19 infections, Am. J. Trop. Med. Hyg. 103 (August (2)) (2020) 561–563.[22] D. Yao, L. Xu, O. Xu, et al., O-linked fN-Acetylgalactosamine modification of A20 enhances the inhibition of NF-κB (Nuclear Factor-κB) activation and elicits vascular protection after acute endolamelar arterial injury, Arterioscl. Thromb. Vasc. Biol. 38 (June (12)) (2019) 1309–1319.[23] S.C. Lin, J.Y. Chung, B. Lamote, et al., Molecular basis for the unique deubiquitinating activity of the NF-kappaB inhibitor A20, J. Biol. Mol. Chem. 376 (February (2)) (2020) 526–540.[24] L. Rossetti, M. Hawkins, W. Chen, J. Gindi, N. Bazzilli, In vivo glucocorticoid infusion induces insulin resistance in normoglycemic but not in hyperglycemic conscious rats, J. Clin. Invest. 96 (July (1)) (1995) 132–140.[25] A.D. Baron, J.S. Zhu, J.S.H. Zhu, H. Weldon, L. Miauna, W.T. Garvey, Glucocorticoid induces insulin resistance in vivo by affecting GLUT 4 translocation in skeletal
muscle. Implications for glucose toxicity, J. Clin. Invest. 96 (December (6)) (1995) 2792–2801.

[26] S.B. Peterson, G.W. Hart, New insights: a role for O-GlcNAcylation in diabetic complications, Crit. Rev. Biochem. Mol. Biol. 51 (May (3)) (2016) 150–161.

[27] N.R. Dostrovsky, T.E. Towheed, R.W. Hudson, T.P. Anastassiades, The effect of glucosamine on glucose metabolism in humans: a systematic review of the literature, Osteoarthr. Cartil. 19 (April (4)) (2011) 375–386.

[28] G. Pocobelli, A.R. Kristal, R.E. Patterson, et al., Total mortality risk in relation to use of less-common dietary supplements, Am. J. Clin. Nutr. 91 (June (6)) (2010) 1791–1800.

[29] G.A. Bell, E.D. Kantor, J.W. Lampe, D.D. Shen, E. White, Use of glucosamine and chondroitin in relation to mortality, Eur. J. Epidemiol. 27 (August (8)) (2012) 593–603.

[30] T.M. Brasky, J.W. Lampe, C.G. Slattery, E. White, Use of glucosamine and chondroitin and lung cancer risk in the VITamins and Lifestyle (VITAL) cohort, Cancer Causes Control 22 (September (9)) (2011) 1333–1342.

[31] E.D. Kantor, J.W. Lampe, U. Peters, D.D. Shen, T.L. Vaughan, E. White, Use of glucosamine and chondroitin supplements and risk of colorectal cancer, Cancer Causes Control 24 (June (6)) (2013) 1137–1146.

[32] K. Sacre, L.A. Criswell, J.M. McCune, Hydroxychloroquine is associated with impaired interferon-alpha and tumor necrosis factor-alpha production by plasmacytoid dendritic cells in systemic lupus erythematosus, Arthritis Res. Ther. 14 (June (3)) (2012) R155.

[33] A. Gardet, A. Pellerin, C.A. McCarl, et al., Effect of in vivo hydroxychloroquine and ex vivo Anti-BDCA2 mAb treatment on pDC IFN-$\alpha$ production from patients affected with cutaneous lupus erythematosus, Front. Immunol. 10 (2019) 275.

[34] K. Sakata, S. Nakayamada, Y. Miyazaki, et al., Up-regulation of TLR7-Mediated IFN-$\alpha$ production by plasmacytoid dendritic cells in patients with systemic lupus erythematosus, Front. Immunol. 9 (2018) 1957.

[35] L. Ronnblom, The type I interferon system in the etiopathogenesis of autoimmune diseases, Ups. J. Med. Sci. 116 (November (4)) (2011) 227–237.

[36] T. Celhar, R. MagalhÃ£es, A.M. Fairhurst, TLR7 and TLR9 in SLE: when sensing self goes wrong, Immunol. Rev. 53 (September (1–3)) (2012) 58–77.

[37] E.E. To, R. Vlahos, R. Luong, et al., Endosomal NOX2 oxidase exacerbates virus pathogenicity and is a target for antiviral therapy, Nat. Commun. 8 (July (11)) (2017) 69.