Shiga toxin (Stx) type 2-induced increase in O-linked N-acetyl glucosamine protein modification: a new therapeutic target?

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Shiga toxin (Stx)-producing Escherichia coli (STEC) causes bloody diarrhea, which may progress to the potentially fatal hemolytic uremic syndrome (HUS). Development of HUS after STEC infection is dependent on Stx, and is particularly linked to Stx type 2a, Stx2a (Melton-Celsa, 2014; Scheutz, 2014). In this issue of EMBO Molecular Medicine, Lee et al report that O-linked N-acetyl glucosamine protein modification (O-GlcNAcylation) is increased in host cells after Stx exposure and the subsequent endoplasmic reticulum (ER) stress response. The elevated O-GlcNAcylation resulted in increased inflammatory and apoptotic processes. Inhibition of O-GlcNAcylation with OSI-1 protected cells from the Stx2a-induced damage. In mice intoxicated with Stx2a, OSI-1 treatment reduced kidney damage and increased mouse survival.

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See also: K-S Lee et al (January 2022)

Shiga toxin (Stx)-producing E. coli are food- and water-borne causes of hemorrhagic colitis and hemolytic uremic syndrome (HUS). Production of Stx2a is particularly linked to HUS development (Melton-Celsa, 2014; Scheutz, 2014). The Stxs consist of a catalytically active A subunit and a pentamer of B subunits that bind to target cell receptors (globotriaosylceramide,Gb3). Treatment for STEC infection is limited to supportive therapies because antibiotic regimens can increase the chance of developing HUS (Tarr et al, 2018).

Potential interventions for STEC infection in development consist largely of antitoxin antibodies or vaccines, and receptor-blocking analogs (Mühlen & Dersch, 2020). An anticomplement therapy that is successful in noninfectious HUS, Eculizumab, has not consistently demonstrated similar benefits for STEC-related HUS (Mühlen & Dersch, 2020). However, Lee et al describe a novel strategy for interrupting Stx2a-based damage and death in multiple cell types and organoids, and demonstrated protection of mice injected with Stx2a by an inhibitor of O-linked N-acetyl glucosamine protein modification O-GlcNAcylation, OSI-1 (Lee et al, 2021). Protein modification by the addition of O-GlcNAc affects many cellular processes, including the cell cycle and stress responses (Martinez et al, 2017; Estevez et al, 2020). The addition of O-GlcNAc to proteins is facilitated by O-GlcNAc transferase (OGT), which can be inhibited by OSI-1 (Alteen et al, 2021).

The decision to test whether OSI-1 could protect from Stx2a-mediated damage came from the authors’ original finding that THP-1 cells treated with Stx2a had increased levels of O-GlcNAcylation associated with protein in cell lysates (Lee et al, 2021). This result revealed yet another cellular process altered by Stx intoxication. The Stxs, which travel through the cell in a retrograde manner from the endosome to the Golgi to the endoplasmic reticulum (ER), are known to induce both ER and ribotoxic stress responses in addition to halting protein synthesis in susceptible cells [see Fig 1 and (Lee et al, 2016)]. The increase in O-GlcNAcylation observed by Lee et al may well be a reaction by the cell to ER stress induced by Stx2a; as such, O-GlcNAcylation has been shown to decrease injury due to ER stress [see review (Martinez et al, 2017)]. Importantly, the authors showed that a catalytically inactive version of Stx2a that cannot target the ribosome was unable to increase O-GlcNAcylation (Lee et al, 2021). This study demonstrated that in addition to an ER stress response, that an overall increase in O-GlcNAc levels occurred, and that both pro-inflammatory (p65) and pro-apoptotic (Akt and Bad) signaling proteins were O-GlcNAcylated, their phosphorylation status altered, and function increased. These pro-inflammatory and apoptotic cellular changes could be inhibited by pretreatment of the THP-1 cells with OSI-1 or an inhibitory RNA directed toward OGT.

The authors found a similar O-GlcNAcylation response in endothelial cells, which are the target cell type in HUS. They used primary human renal proximal tubular endothelial cells (HRPTEpi) for these studies and demonstrated that the inflammatory cytokine and chemokine response to Stx2a were reduced by OSI-1 treatment, thereby showing that the response observed in THP-1 cells was conserved in cells highly sensitive to Stx2a. The authors then moved to three-dimensional (3D) human-mini-kidney spheroids and induced pluripotent stem cells (iPSC)-derived renal organoids and demonstrated downregulation of pro-inflammatory and apoptotic signals, as well as kidney injury marker Kim-1 induced by Stx2a when these models were treated with OSI-1.

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With a plethora of data supporting the hypothesis that inhibition of O-GlcNAcylation is protective to cells and organoids *in vitro*, the authors used a mouse toxin injection model to test the potential protective efficacy of OSMI-1 *in vivo*. Mice were injected daily with OSMI-1 starting from the day before intoxication. Mice given OSMI-1 were significantly protected from weight loss, elevated kidney injury markers, and death as compared to animals given the vehicle control.

The results from this paper demonstrate that inhibition of O-GlcNAcylation could be a possible target for treatment to mitigate the effects of Stx. It will be exciting to observe whether similar findings can be demonstrated in an STEC infection model. Although OGT mutations may be lethal (Estevez *et al.*, 2020), and OSMI-1 may have some toxicity (Alteen *et al.*, 2021), the limited time course of an STEC infection likely provides an acceptable framework to test inhibitors of the cellular O-GlcNAcylation process in additional *in vivo* models.

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