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HEPARAN SULFATE PROMOTES RECOVERY FROM ACUTE LIVER INJURY: INHIBITION OF PROGRESSIVE CELL DEATH OR ENHANCED REGENERATION?

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Heparan sulfate proteoglycans (HSPG), syndecans and glycosyl-phosphoinositide linked glypicans are present in all adherent cells. Heparan sulfate (HS), attached to these proteins, is a strongly anionic linear polysaccharide, which allows the cell surface to bind a variety of effectors and matrix components. Syndecan ectodomains can be proteolytically cleaved releasing HS-peptides that exert a variety of biological effects. HSPGs have roles in morphogenesis, tissue repair, host defense, energy metabolism, and act as co-receptors, potentiating the action of low concentrations of ligands. Shed ectodomains play a critical role in wound repair by binding cytokines, growth factors, protecting proteases from their inhibitors and coordinating the repair process.

Among the four-member syndecan gene family, syndecan-1 (Sdc1) is mainly expressed in epithelia including hepatocytes. Prior work by Park et al. suggested that ectodomain-shedding protects against dysregulated inflammation (1). Syndecan-1 shedding facilitates the removal of CXC chemokines leading to the resolution of neutrophilic infiltration. Sdc1 $-/-$ mice developed normally but were more susceptible to LPS exposure, due to increased levels of chemokines and neutrophils. Delayed inhibition of metalloproteinases in WT mice caused a similar phenotype as Sdc1 $-/-$ mice. Delayed administration of HS, the linear polysaccharide ligand binding moiety of syndecan-1, protected the knockout mice from cell death (1).

Nam et al in this issue of *Hepatology* extend their work on the role of syndecan-1 to acute liver injury and repair using the acetaminophen (APAP) mice model, which closely resembles the human disease (2). APAP hepatotoxicity can be broadly divided into three phases (3, 4). During initiation or APAP metabolism phase, APAP is metabolized to its reactive metabolite NAPQI, which is quenched by GSH leading to GSH depletion and covalent protein binding, forming APAP adducts. This leads to the progression phase, which can be divided into two categories- first, intracellular events which include mitochondrial dysfunction, mitochondria ROS mediated by self-sustaining MAPK signaling via JNK (5). After a few hours during which antioxidants, GSH repletion, and JNK inhibitors are still effective in inhibiting cell death, a point of no return is reached when sufficient oxidative stress causes MPT, ATP depletion, and release of mitochondrial proteins, which induce DNA

damage. The hallmark of injury at this stage is necrosis, which peaks in the mouse model at 12–24 hours. This necrosis occurs mainly in zone 3, but there is a penumbra, i.e. an area surrounding the necrosis where hepatocytes appear to be reversibly stressed and where proliferation occurs. Though some controversy exists, most studies indicate that apoptosis does not occur, presumably because GSH depletion, ROS, and ATP depletion incapacitate caspases. Following necrosis, the extracellular part of progression phase starts where DAMPs are released from dying cells. DAMPs are implicated in propagation of injury, triggering inflammation and in initiating repair (3, 4). The third and final stage is recovery phase where initially the hepatocytes surrounding necrotic zones divide and regenerate the damaged liver. Later hepatocyte proliferation spreads well beyond the penumbra (6).

Nam et al now demonstrate that global syndecan-1 deletion unmasks enhanced liver injury from APAP resulting in apparent delayed apoptosis usually not seen in wild type (WT) mice. The early phase of APAP toxicity up to 12 hours was not affected either *in vivo* or *in vitro* (though very high APAP concentration (50mM) was used). After 12 hours, injury began to resolve in the WT but worsened in the knockout mice, suggesting syndecan-1 normally halts the progression of liver injury and/or is required to facilitate repair. Surprisingly, caspase 3 was activated in Sdc1 $-/-$ beginning at 12 hours. This was seen in the penumbra, the area surrounding the necrosis, the area where liver cell proliferation is seen in WT mice (6). The Sdc1 $-/-$ exhibited suppressed AKT activation and increased GSK3 β activation, importance of which was confirmed by inhibition experiments. Caspase 3 and GSK3 β inhibitors arrested the progression of injury whereas AKT inhibition worsened it. Interestingly, in WT mice given a lethal dose of APAP, which is known to impair regeneration (6), the delayed administration of caspase 3 and GSK3 β inhibitors protected against progressive injury. The authors interpret this as GSK3 β mediated apoptosis. Whereas, the authors do acknowledge the alternative, that GSK3 β inactivation may promote liver repair (via β -catenin activation) and the delayed apoptosis may be a default pathway due to failure to regenerate, these studies need further detailed analysis to determine the effect of GSK3 β inhibition on various downstream targets.

Among the derivatives of HS the authors tested heparin, which is similar to heparan but has less glucuronic acid in the disaccharide chain than iduronic acid and is highly sulfated. Heparin was also protective in the APAP model. Although not addressed here, the authors previously showed that the protective effect of HS could be disassociated from its anticoagulant property. Further analysis of heparin's protective role is needed in light of the recent report that coagulation pathways may stimulate liver regeneration after APAP overdose and thus could be actually beneficial (7). HS can either modulate several growth factors and cytokines, and such interactions may be involved in its protective effect in acute liver injury. For e.g. Heparin activates hepatocyte growth factor (HGF), one of the primary mitogens for hepatocytes (8), which may in turn drive liver repair following APAP overdose.

The authors tested the therapeutic potential of syndecan-1 ectodomain, which contains HS as well as HS alone. Pretreatment of Sdc1 $-/-$ mice protected, but even more remarkably, when the ectodomains or HS was administered 3 or 10 hours after APAP, beyond when NAC is effective. This approach also was beneficial in WT mice given a lethal dose of APAP. Structural analysis revealed that 2-O-sulfated domains in syndecan-1 HS were critical for

inhibiting progression of liver injury. Although this approach did not seem to affect liver injury in the ConA model, it did show efficacy in the furosemide-induced hepatic necrosis, suggesting potential value beyond APAP. It is important to note that protection was seen in WT mice at the lethal dose, which impairs regeneration, highlighting the potential therapeutic value of HS in the treatment of human APAP toxicity.

A major missed opportunity in this study is to test the role of HS and Syndecan-1 in liver regeneration after APAP. Although the authors briefly investigated mechanisms of liver repair, lack of an extended time course complicates data interpretation. Previous studies have shown that the final recovery phase starts around 24 hours after APAP exposure in mice where cells in necrotic penumbra enter cell cycle by expressing Cyclin D1 (6). They undergo S-phase DNA synthesis around 48 hours and undergo mitosis around 72 hours after APAP exposure. The proliferation ceases by 96 hours and regenerative process is completed by 120 hours. The duration and extent of the liver regeneration is based on the dose and initial injury. High doses of APAP and/or higher initial liver injury as observed in *Sdc* $-/-$ mice results in delayed liver regeneration. The role of proteoglycans in liver regeneration has long been suspected. HS is required for proper functioning of various growth factors involved in hepatocyte proliferation and Syndecan-1 overexpression resulted in increased hepatocyte proliferation (9). Taken together with the pro-mitogenic effects of Heparin it is very likely that Syndecan-1 and other HS proteoglycans are involved in stimulation of liver regeneration after APAP overdose, which needs further investigations.

In summary, these novel data demonstrate that HS proteoglycans and specifically Syndecan-1 is critically involved in APAP-induced liver injury and liver repair via multiple mechanisms including preventing progression of liver injury and initiation of liver repair. These studies have highlighted HS proteoglycans as yet another promising therapeutic targets in treatment of APAP-induced ALF.

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