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Secretory phospholipase A_2 mediates progression of acute liver injury in the absence of sufficient cyclooxygenase-2

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Abstract

Previous studies have shown that injury initiated by toxicants progresses even after most of the toxicant is eliminated from the body. One mechanism of progression of injury is the extracellular appearance of hydrolytic enzymes following leakage or upon cell lyses. Under normal conditions, after exposure to low to moderate doses of toxicants, secretory phospholipase A_2 ($sPLA_2$) and other hydrolytic enzymes are known to appear in the extracellular spaces in order to cleanup the post-necrotic debris in tissues. We tested the hypothesis that $sPLA_2$ contributes to progression of toxicant-initiated liver injury because of hydrolysis of membrane phospholipids of hepatocytes in the perinecrotic areas in the absence of sufficient cyclooxygenase-2 (COX-2). Male Sprague–Dawley rats were administered either a moderately hepatotoxic dose (MD, 2 ml CCl_4/kg, ip) or a highly hepatotoxic dose (HD, 3 ml CCl_4/kg, ip) of CCl_4. After MD, liver $sPLA_2$ and COX-2 were co-localized in the necrotic and perinecrotic areas and their activities in plasma and liver increased before decreasing in tandem with liver injury (ALT and histopathology) leading to 100% survival. In contrast, after the HD, high extracellular and hepatic $sPLA_2$ activities were accompanied by minimal COX-2 activity and localization in the liver throughout the time course. This led to progression of liver injury and 70% mortality. These data suggested a destructive role of $sPLA_2$ in the absence of sufficient COX-2. Time- and dose-dependent destruction of hepatocytes by $sPLA_2$ in isolated hepatocyte incubations confirmed the destructive ability of $sPLA_2$ when present extracellularly, suggesting its ability to spread injury *in vivo*. These findings suggest that $sPLA_2$, secreted for cleanup of necrotic debris upon initiation of hepatic necrosis, requires the co-presence of sufficiently induced COX-2 activity to prevent the run-away destructive action of $sPLA_2$ in the absence of the tissue protective mechanisms afforded by COX-2 induction.

Keywords: Arachidonic acid; Carbon tetrachloride; Cycloxygenase-2; Necrotic debris; Secretory phospholipase A_2; Prostaglandin E_2

Introduction

During the past several decades the mechanisms of initiation of drug/toxicant-induced tissue injury have been extensively investigated and a wealth of information is available (Williams, 1959, 1978; Holzbach, 1981; Lesca, 1981; Guengerich and Liebler, 1985; Parke and Sapota, 1996; Bessems and Vermeulen, 2001; Guengerich, 2005). It is known that once initiated, injury may progress even after the toxicant has been eliminated from the body regardless of the structure of the drug/toxicant or the mechanism of initiation of injury (Mehendale, 1995; Rao et al., 1997; Soni et al., 1998; Limaye et al., 2003; Mehendale and Limaye, 2005). In a pioneering study, Limaye et al. (2003) proposed and tested the concept that leakage of hydrolytic degradative enzymes (death proteins) along with other cellular contents, from the damaged or necrosed hepatocytes destroys the neighboring healthy or partially damaged hepatocytes upon activation in the calcium rich intercellular *milieu* (Fig. 1). The destructive effects of extracellular calpain in expanding liver

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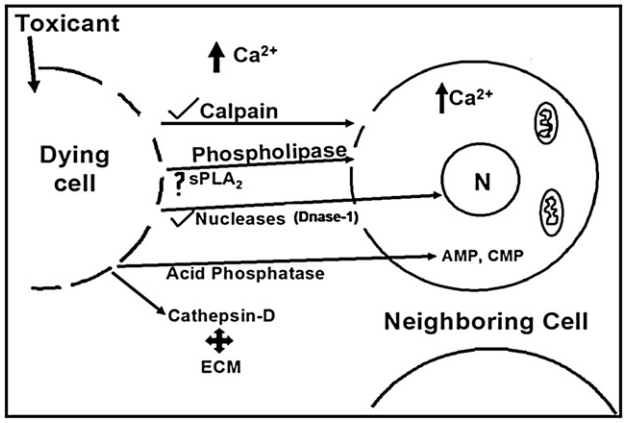

Fig. 1. Role of death proteins in progression of liver injury: mode of action. Adapted from Limaye et al., 2003 with modifications to reflect the current literature. Schematic representation of the lytic action of death proteins released from necrosed hepatocytes on the neighboring healthy or partially damaged hepatocytes. Death proteins, located in the cytosol as well as cell organelles such as lysosomes, or vesicles are tightly regulated by intracellular Ca^{2+}. These enzymes remain benign inside the cells until cellular integrity is perturbed by events such as a toxic episode or the vesicles are activated to release their contents into the intercellular space (e.g. $sPLA_2$). Upon toxicant exposure, cellular injury is initiated due to mechanism based cytotoxic events. The necrosed cells eventually release their contents into the Ca^{2+}-rich extracellular environment. Calcium dependent proteases, endonucleases, phospholipases, and other enzymes (death proteins) are activated in the Ca^{2+}-rich extracellular environment and hydrolyze their respective substrates in the plasma membrane of the neighboring hepatocytes. This causes plasma membrane blebbing and compromised plasma membrane permeability. Subsequently, other death proteins such as nucleases (such as Dnase-1) and acid phosphatases destroy their respective substrates, eventually leading to cell lysis. Consequently, more death proteins are released leading to the self-perpetuating sequel of progression of liver injury.

injury initiated by CCl_4 in rats and acetaminophen in mice have been demonstrated (Limaye et al., 2003; Mehendale and Limaye, 2005). Consistent with this proposal, Napirei et al. (2006) have reported that deoxyribonuclease-1 (Dnase-1) leaking out of necrosed hepatocytes causes progression of injury initiated by acetaminophen. While these pioneering studies have established the role of death proteins such as calpain and Dnase-1 (Fig. 1) in the progression of liver injury, the potential role of other death proteins has remained uninvestigated.

$sPLA_2$ is another member of death proteins that leaks out of the necrosed hepatocytes or is secreted in the intercellular space following drug/toxicant-initiated injury (Limaye et al., 2003; Ito et al., 2004; Mehendale and Limaye, 2005). $sPLA_2$ hydrolyzes the ester bond at the *sn*-2 position of glycerophospholipids in the presence of Ca^{2+} with a broad fatty acid and phospholipid specificity (Wolf et al., 1997; Winstead et al., 2000; Murakami and Kudo, 2004). $sPLA_2$ is expressed in hepatocytes and inflammatory cells like macrophages at basal levels and stored in cytosolic granules, or synthesized upon proinflammatory stimulation and then secreted in the intercellular space, its site of action (Akiba and Sato, 2004; Menschikowski et al., 2006). The physiological functions of $sPLA_2$ include release of arachidonic acid from dietary and membrane phospholipids (Murakami and Kudo, 2002). Serum and tissue concentrations of $sPLA_2$ correlate with disease severity in several immune-mediated inflammatory pathologies, such as rheumatoid arthritis, septic shock, psoriasis, Crohn's disease, respiratory distress syndrome, and asthma (Kramer et al., 1989; Seilhamer et al., 1989; Kudo et al., 1993; Murakami et al., 1997). Activation of $sPLA_2$ is associated with ischemic injury in rat kidney (Takasaki et al., 1998) and atherosclerotic lesions in $sPLA_2$ transgenic mice (Ivandic et al., 1999). Moreover, $sPLA_2$ has also been implicated in human liver diseases (Ito et al., 2004), neurodegenerative conditions (Cunningham et al., 2004), colitis (Woodruff et al., 2005), and myocardial ischemia/reperfusion injury (Ishikawa et al., 2005). Membrane rearrangement stimulated by proinflammatory cytokines, interleukin-1, tumor necrosis factor, and mitogens is accompanied by markedly induced expression

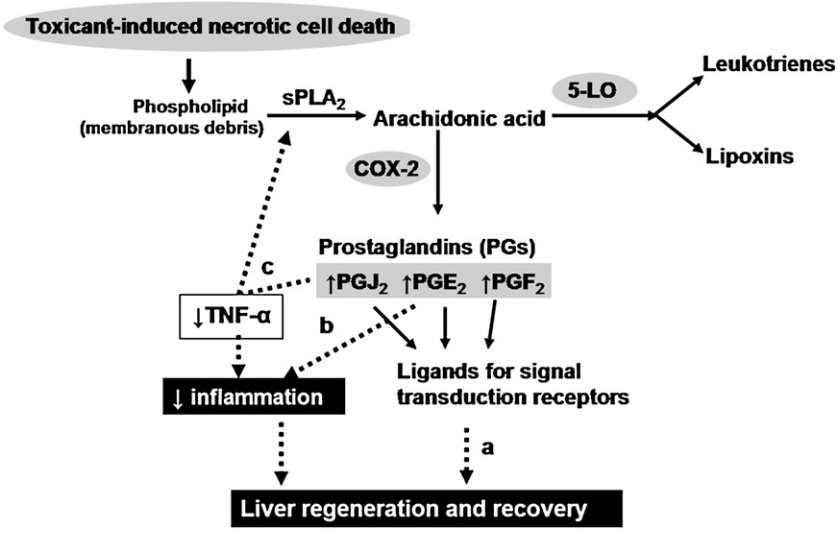

Fig. 2. Proposed mechanism of how adequate COX-2 induction curbs/mitigates $sPLA_2$-mediated progression of acute liver injury. Adequate induction and co-presence of COX-2 with $sPLA_2$ upregulates PG production that affords the following hepatoprotective effects: a) by acting as endogenous ligands for signal transduction pathways, thus enabling structural and functional restoration of the liver (pathway a); b) by upregulating anti-inflammatory cytokines (pathway b); and c) by regulating $sPLA_2$ transcription through the anti-inflammatory effects of PGE_2 and PGJ_2 (pathway c). Dotted lines exhibit the three ways in which PGs aid in tissue recovery.