Biochemistry & Physiology: Open Access

Hina K, Biochem Physio 2022 , 10:8

Research Article Open Acces

A Review: Molecular mechanism of CCl4 induced hepatotoxicity

Hina khalid[1*]

[1]*Department of Pharmacy, State University of New York at Albany, Pakistan*

ABSTRACT

The liver is vital to metabolic activities making it susceptible to liver injury. CCl4induced hepatotoxicity is widely studied in model organisms to assess liver damage as it directly mimics the damage in humans. CCl4 activation is ascribed to various cytochrome (CYP) 2E1, CYP2B1 or CYP2B2, and possibly CYP3A, resulting in the trichloromethyl radical, CCl3. This radical can bind to biological molecules (lipid, protein, nucleic acid) rendering the lipid metabolism non-functional and thus contribute to fatty degeneration. The formation of an adduct between DNA and CCl3 is considered as the initiator of hepatic carcinogenicity. CCl3 in presence of oxygen gave rise to far more reactive CCl3OO and initiate lipid peroxidation which ultimately leads to loss of membrane integrity.

At the molecular level, CCl4 mediate the release of several inflammatory cytokines (which may either be noxious or beneficial) among which TNF and NO appears to have a controversial role in CCl4 induced hepatotoxicity in that it can be either beneficial or detrimental. Such as among which TNF-α and NO accentuate the effect through cellular apoptosis and TGF-β contributes to fibro genesis. IL-6 and IL-10 are pivotal to a recovery process as it counteracts to minimize the anti-apoptotic activity and direct the cell towards regeneration. The intervention of any of the above-mentioned mechanistic aspects might aid in preventing more serious liver damage specifically employment of antioxidants, mutagenic agent, and maintenance of calcium sequestration. Moreover, CYP450 or the drugs preventing CCl4 induced cytotoxicity and proliferation would offer a protective response against hepatic cancer.

Introduction

Hepatotoxicity

Hepototoxicity refers to liver chemical mediated liver damage. The chemical which drives liver injury is termed hepatotoxicants. It includes various chemical such as agents in laboratories (CCL4, Paracetamol), industrial agents (lead, arsenic), natural toxins (aflatoxin, microcystins) , herbal medicine (Cascara saghrada, ephedra) . More than 900 drugs are reported to induce liver injury and is the most general reason for their withdrawal from the market[1]. CCl4 hepatotoxicity is governed by various factors lipid peroxidation, calcium sequesteration, oxidative stress, apoptosis or release of inflammatory response. It has been proposed that CCl4 elicit its effect either in a time dependent or dose dependent manner which if prolonged frequently results in persistent liver damage. Thus CCl4 toxicity ultimately causes fibrosis, cirrhosis and cancer.

Liver injury at random is divided into acute and chronic depending upon time span and persistence of injury. Acute injury is conquerable following withdrawal of toxic agent and associated with complete restoration of liver architecture and function. Chronic injury is partly reflected by continuous acute injury extended for prolonged duration and frequently results in end stage liver failure or hepatic cancer [2]. The CCl4 hepatotoxicity is widely studied in vivo and in vitro models thereby providing an important insight on various aspects of toxicity. The following review focuses on mechanistic aspects of toxicity together with molecular processes mediating the response.

CCl4 metabolism via cytochrome P450 (Biotransformation via CYP2EI)

CCL4 is widely used hepototoxicant model of liver injury. CCL4 is metabolized to trichloromethyl radical CCl3 via cytochrome (CYP) 2E1, CYP2B1 or CYP2B2, and possibly CYP3A. CCl3 further binds to oxygen resulting in trichloromethyl proxy radical CCl3OO. CCl3 and CCl3OO are highly reactive radicals that most often target protein, lipids or nucleic acid. CCl3 radical covalently binds to the cellular component hampered lipoprotein metabolism, leads to (fatty degeneration) steotosis. CCl3 and DNA interaction forms DNA adducts which may initiate carcinogenicity. CCl3OO attacks polyunsaturated lipid membrane initiating lipid peroxidation [3]. CCL4 toxication after several hours induces centrizonal necrosis of the hepatocyte, significantly elevating the level of Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) in the blood stream representing cellular damage [4].

CCL 4 metabolism is crucial for the induction of hepatotoxicity. The initial step in metabolism is CYP2EI mediated reductive dehalogenation of CCL4. The CYP2E1 inhibitors such as 2-diethylaminoethyl 2, 2-diphenyl valerate hydrochloride [SKF-525A], silymarin, allylisoproylacetamide employed in various studies prevented metabolism and CCL4 induced liver injury [5].

The CYP2E1 knockout mice (cyp2e1-/-) was intoxicated with a single dose of CCL4 (1ml/kg) to delineate the role of CYP2E1 in injury. The (cyp2e1-/-) mice after 24 hours showed no significant elevation in level of serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) relative to wild-type mice which showed 442-

***Corresponding author:** Khalid H, Department of pharmacy, State University of New York at Albany, Pakistan.Email : hinakhalid761@yahoo.com; Tel: 923058000000

Citation: Khalid H. (2022) A Review: Molecular mechanism of CCl4 induced hepatotoxicity. Biochem Physio.12:391

Received: 05-July-2021, Manuscript No. bcp-22-001- Pre Qc 22 ; **Editor assigned:** 03-Aug-2022, PreQC No, bcp-22-001 Pre Qc 22 (PQ); **Reviewed:** 17-Aug-2022, QC No. bcp-22-001 Pre Qc 22; **Revised:** 22-Aug-2022, Manuscript No. bcp-22-001 Pre Qc 22 (R); **Published:** 29-Aug-2022, **DOI:** 10.4172/2168-9652.1000391

and 125-fold increases in serum ALT and AST activities respectively, moreover significant centrilobular necrosis were apparent in wild type mice but was barely detectable in Cyp2e1-null mice demonstrating CYP2E1 as principle inducer of hepatotoxicity [5,6]

Lipid peroxidation

The CCl3OO and CCl3formed as a result of the metabolism of CCL4 are highly reactive entities which can covalently bind to biological macromolecule forming nucleic acid, protein and lipid adducts. Under aerobic condition, CCl3 is transformed to CCl3OOwhich is far more reactive than CCl3 and thus preferably (readily) abstracting hydrogen from polyunsaturated fatty acid initiating a series of reaction disrupting PUFA membrane. This whole process is termed as lipid peroxidation which results in loss of membrane integrity [3]. The process begins with removal of a hydrogen atom by free radical (hydroxyl (OH), alkyl (RO), peroxyl (ROO), andfrom PUFA to give a carbon-centered lipid radical (L) , followed by reaction with molecular oxygen producing lipid peroxyl radical (LOO) which in turn acquire hydrogen from adjacent fatty acid side chain generating lipid hydro peroxide (LOOH). The lipid hydro peroxide (LOOH) by exposure to metal ions form lipid alkoxyl radicals (LO•).Moreover, both LO• and LOO• may decompose into reactive aldehydes products such as malondialdehyde (MDA) and 4-hydroxynonenal (HNE) acrolein (ACR). This aldehyde readily modifies membrane protein, nucleic acid and is implicated in the pathophysiology of various disorders associated with oxidative stress and inflammation. Therefore, the resultant perturbation in membrane structure alters membrane permeability, ion movement and inactivation of metabolic processes [7, 8].

The HAS (high alcohol-sensitive) rats administered with CCL4 (1 ml/kg) resulted in the formation of 4-HNE and MDA protein adducts which was restricted to the mid - zone of hepatocyte by 6 hours of a dose , extended to zone 3 at 24 hours accompanied with zone 3 necrosis but was barely detectable by 36 to 72 h after dosing . Thus these aldehydes modify proteins in a time dependent manner and contribute to CCL4 injury. Furthermore, CCL4 treatment resulted in a dose- dependent elevation in DNA strand break and malondialdehyde deoxyguanosine (M1dG) adducts which was statistically evident at 1 and 4 mM. Additionally, CCL4 induced up regulation in the level of 8-oxodG accompanied by cytotoxicity at 4Mm after 2 hours of dosing. These effects implied the possibility of an increased risk of genotoxicity and carcinogenicity(10).

Disruption of calcium homeostasis

Calcium, homeostasis within the cell is achieved by balancing the entry of Ca2+ either from outside or from stored endoplasmic/sarcoplasmic reticulum (Cas2+) by continuous extrusion of Ca2+ through pumps of cell membrane or endoplasmic reticulum thereby maintaining low Ca2+ level in cytosol and high in of cell membrane or endoplasmic/sarcoplasmic reticulum (11). Halo alkane intoxication contributes to elevation of Ca2+ level by disruption of calcium pumps and by inhibiting sequestration of calcium out of the cytoplasm(12). CCL4 treatment resulted in reduced calcium transport across the plasma (37%) and mitochondrial membranes (59%), as well as the endoplasmic reticulum (46%) after 1 hour of treatment which might be associated with accumulation of calcium in parenchymal cell. Following 24 hours, altered pattern of calcium uptake was observed with mitochondrial membranes and plasma exhibited a continual drop (55%) and further decline (53%) respectively where as the endoplasmic reticular system displayed partial recovery (17%) in calcium uptake(13). It has been reported that a concentration of 0.172 mM carbon tetrachloride caused the release of calcium from the endoplasmic reticulum and mitochondria but was ineffective in up regulating cytosolic Ca2+ levels suggesting that calcium released might gain access to extracellular space in order to maintain a cytosolic pool of Ca2+ .Inhibition of CCL4 induced release of calcium by EGTA, a calcium chelatorindicated that LDH leakage and plasma membrane is associated with the influx of calcium into cells but EGTA failed to prevent cell death, suggesting some other factor might be involved in progression of injury (14). Moreover toxin mediated rise in calcium level activates a cytosolic and lysosome derivative enzyme most importantly calpain (Ca 2+ dependent cytosolic cysteine protease) and phospholipases. These are released by injured hepatocyte and attack plasma membrane of adjacent healthy hepatocyte ensuing cell death. Calpain interferes with cellular integrity by breakdown of various cytoskeleton and membrane protein including fodrin/spectrin, talin, filamin. Intervention with calpain inhibitor N-benzyloxycarbonyl-valine-phenylalanine methyl ester (CBZ), administered an hour later the administration of non-lethal dose of CCL4 (2 ml/kg) alleviated progression of liver injury with a concomitant decrease in calpain activity and afforded protection by 75% against lethality mediated by a lethal dose of CCL4 (3 ml/kg). Furthermore, the incubation of normal rat hepatocyte with calpain result in cell death which was subsequently prevented by administration of CBZ thus confirming the role of calpain in progression of injury.

CCL*4* induced apoptosis (mitochondrial dysfunction)

CCL4 is associated with increased ROS which mediate apoptosis in rat primary hepatocyte. Moreover , the decline in glutathione (GSH) along with an increase in malondialdehyde (MDA) level was observed following exposure to CCL4, the reliable evidence for oxidative stress. ROS drives onset of the Mitochondrial Permeability Transition or MPT which causes mitochondrial dysfunction mediated by members of the Bcl-2 family. The Bcl-2 family comprises of proapoptotic members Bax, Bcl-XS, Bak, Bad, Bik, Bim, Bid, Hrkand ant apoptotic members Bcl-2, Bcl-XL, A1, and Mcl-1. Following activation, Bax and Bak form pores on the outer mitochondrial membrane causing mitochondrial permeabilization thus releasing several apoptogenic factors including cytochrome c into cytosol. Cytochrome c binds to the adaptor apoptosis associated factor 1 (Apaf-1)and form apoptosome complex through recruitment of caspase-9 , which in turn induce caspase -3 activity leading to DNA fragmentation reflecting apoptosis and necrosis.Bcl-2 and Bcl-XL acts as an antagonist of Bax and Bak therefore confers resistance to apoptosis. Additionally significant downregulation of Bcl-XL , the only hepatocyte marker of Bcl-2 family along with concurrent up regulation of cytochrome c level were observed following 8 hr. of CCL4 administration.

Role of nitric oxide in CCL*4* injury

Nitric oxide and oxidative stress: The imbalance between ROS and antioxidants shifting towards the former results in a condition termed oxidative stress. Increase in ROS concentration reduces the bioavailability of NO. During oxidative state, tetrahydrobiopterin is reduced favoring uncoupling of NOS which results in production of superoxide. Excess superoxide can be produced enzymes including NAD (P) H oxidase, mitochondrial enzymes, xanthine oxidase, and myeloperoxidase by which reacts with NO and form toxic peroxynitrite (ONOO-) thereby reducing NO which is detrimental. In addition antioxidant enzyme SOD gave rise to hydrogen peroxide associated with increased oxidative stress. This is a common etiology

of vascular disease.

CCL4 induced cirrhosis were established to study the role of reduced O2-level in response to NO availability, cirrhosis result in increased oxygen content as evident from reduced hepatic cGMP . Inhibition of COX and XO and a reduced SOD activity causes decline in oxygen level suggesting these as a source of unregulated oxygen in cirrhosis. NOS inhibition showed no effect in oxygen content. However, in endothelial cell there is reciprocal regulation between NO and O therefore oxidative stress can be minimized by reducing oxygen thereby enhancing NO bioavailability. Endogenous NO is known to attenuate lipid peroxidation and oxidative stress in CCL4 induced chronic injury. Administration of NO inhibitors L-NAME or AG aggravated these effects. Administration of L-Arginine prevented a further increase in collagen content , bilirubin and alkaline phosphatase whereas shown no significant effect on lipid peroxidation, due to failure of L-Arginine to scavenge lipids (hydrophobic) free radical as NO is a short lived hydrophilic molecule.

iNOS in CCL4 induced injury

In the iNOS knockout mice after biweekly administration of CCL4 for 8 weeks there was decreased collagen production along with a less significant increase in aminotransferases indicating reduced necrosis but increased apoptosis of stellate cellsas compared to wild type mice. The mechanism underlying apoptosis are not completely understood. It was known that NO perform antiapoptotic activities via nitrosylation of caspases. Furthermore, MDA level was slightly increased in iNOS deficient mice ascribed to absence of peroxynitite radical which is formed as a result of interaction between iNOS derived NO and superoxide ion. Both decreased peroxynotrite radical and increased apoptosis participated in attenuation of fibrosis in iNOS deficient mice therefore demonstrating its protective effect. In acute liver injury model pretreated with iNOS inhibitor L-N6-(1-imino-ethyl) lysine (L-NIL) or (5-methylisothioureahemisulfate (SMT) and NO donor (Sodium nitroprusside) alleviated the effect of CCL4 induced liver injury as indicated from decreased aminotransferase and necrosis with the effect more pronounced in case of iNOS inhibitor which is attributable to reduce NO level than the NO donor which partially contributed its protective effect through scavenging (abstracting) lipid peroxy radicals LOO. Furthermore, iNOS inhibitor resulted in reduced expression of proinflammatory mediator and NF-κB activity whereas augmentation with NO donor merely decreased COX-2 expression where as the iNOS, NF-κB and TNF-α activity remain unaffected.

Nitric oxide in portal hypertension or CCL4 induced alteration in hepatic blood flow

Liver cirrhosis is characterized by portal hypertension caused by increased hepatic resistance to blood flow and hyper dynamic circulation. Increased resistance to blood flow is a consequence of architectural changes of parenchymal cell along with increased vascular tone in sinusoidal/post sinusoidal area. Dimininutiom in bioavailability of vasodilator NO and up regulation of vasoconstrictor (e-g cyclooxygenase-1 (COX-1)) in cirrhotic liver leads to intrahepatic resistance. Conversely, up regulation of NO contributes to hyper dynamic systemic/splanchnic circulation which is associated with a subsequent increase in blood flow and portal venous pressure reduced NO production is ascribed to the reduced eNOS activity and various studies reported reduced eNOS activity by 30-50% in response to CCL4 induced cirrhosis. The eNOS deficient mice injected with chronic CCL4 resulted in a decrease in the splanchnic circulation while an increase in hepatic resistance probably due to reduce NO in liver. IN addition the significance of iNOS in the splanchnic circulation cannot be excluded. Reported that the intervention of iNOS activity using1400W resulted in peripheral vasoconstriction in patients with cirrhosis and ascites suggesting iNOS might regulate vascular tone associated with hyper dynamic circulation. It had been proposed that endtoxemia, a complication in liver cirrhosis induces iNOS expression resulting in persistent production of NO and hyper dynamic circulation. Intriguingly, there exists a correlation between iNOS and eNOS activity in vasculature. The decrease in eNOS activity in liver sinusoids is associated with a concurrent up regulation of iNOS expression and enhanced NO concentration in the portal circulation mediated by endotoxemia in cirrhotic rat. Furthermore, iNOS up regulation in the splanchnic circulation mediate arterial vasodilation in portal hypertensive rats.

Liver fibrosis in CCL4 model

The prolonged chronic CCL4 treatment progresses to end stage liver fibrosis characterized by massive deposition of ECM which consists of collagens and other matrix components such as proteoglycans, fibronectins, and hyaluronic acid. Myofibroblast are central to the production of ECM scar in fibrosis thus replacing normal tissue with scar. The quiescent hepatic stellate cell is activated and transformed to myofibroblast that produce ECM and represents the major contributor of the myofibroblast population. The withdrawal of toxic agent (CCL4) causes spontaneous regression of fibrosis. During regression some of the myofibroblast underwent apoptosis, whereas others revert to inactive state similar to but distinct from, quiescent HSCs.These inactive HSCS is associated with a reduced expression of fibrogenic genes collagen-α1 (I), collagen-α1 [2], α-SMA, TGFβRI, and TIMP1 in the model of CCl4 and alcohol-induced liver fibrosis. It was also seen that members of HSP70 i-e Hspa1a and Hspa1b regulate the survival of HSCs in vitro and in vivo during recovery from liver fibrosis.

In addition to HSCs, adult hepatocyte also participated in accumulation of myofibroblast via EMT mediated by TGF-β1. The hepatocyte is found to express fibroblast-specific protein 1 (FSP1), a marker associated with mesenchymal cell morphology and motility suggesting their engagement in EMT and therefore fibrogenesis. The ECM remodeling during fibrosis is regulated by enzymatic synthesis and degradation of ECM. The enzyme crucial for degradation is matrix metalloproteinase (MMPs) whose activity is inhibited by Tissue Inhibitor of Metalloproteinases-1 TIMP-1. The liver-targeted TIMP-1 transgenic (TIMP-Tg) demonstrated marked reduction in spontaneous recovery following CCL4 induced fibrosis. It was facilitated by downregulation MMP activity and up regulation of HSC activation. Furthermore, TIMP-1 prevented the non-parenchymal apoptosis in vivo and HSC apoptosis in vitro along with suppression of caspase-3 activity thus significantly promoted liver fibrogenesis.

Molecular events: CCL4 mediated inflammatory response

Activation of Kuppfer cell: Activation of kuppfer cells by toxic substances (CCL4 or alcohol) causes the release of mediator including cytokine TNF-α, platelet derived growth factor (PDGF) and TGF-β as well as reactive oxygen species. Liver fibrosis model established by CCL4 or DMN showed up regulation of CD-68, a kuppfermarker and TNF-α production , which is colocalized adjacent to α-SMA positive hepatic stellate cell HSC hepatic lobules most predominantly in growing septa which showed that activated KC contributed to HSC

activation via TNF-α . Moreover, CD68 (+) cells underwent apoptosis as obvious from TUNEL signals and were found around the HSC. Thus KC contributes to hepatic apoptosis, inflammation and fibrogenesis.

Inactivation of kuppfer cell by GdCl3: GdCl3, a specific inhibitor of kuppfer cell in known to protect murine against hepatic injury induced by cholestasis, ethanol, endotoxemic and CCL4. GdCl3 induces apoptosis of kuppfer cell and reduction in the release of inflammatory cytokines. It exerts protective effect by destroying kuppfer cell there by reducing TGF- β and α SMA HSC expression and collagen production. Moreover it diminishes neutrophil infiltration and the potential of production of superoxide in turn reducing lipid peroxidation which is associated with necrosis, cholestasis and fibrosis .This can be used as a therapeutic modality to prevent fibrosis.

TNF-α

TNF-α is an inflammatory cytokine which mediates diverse physiological and pathological activities such as accentuate liver injury following exposure to toxins, drive repair process through liver proliferation regeneration, activation of NF-κb which exerts antiapoptotic effect. Above all it mediates apoptosis too via its receptor TNFR-1, TNF-α binds to its receptor TNF-R1 which passes conserved protein-protein interaction motif called the death domain of about 80 amino acids which is that interacts with the death domain of the adapter proteinTNF-R1-associated death domain protein (TRADD). TNF-R1 bound TRADDfacilitateTNF-R1 binding with Fas-associated death domain (FADD) mediating caspase-8 activation leading to apoptosis.

In TNFR-deficient mice, establishment of acute CCL4 injury revealed no difference in degree of necrosis and transaminase level where as neutrophil infiltration surrounding necrotic site as pronounced in TNFR.KO mice to some extent. It was further supported by the decrease in MPO myeloperoxidase activity and reduced expression of chemokine MIP, macrophage inflammatory protein and adhesion molecule I-CAM intercellular adhesion molecule. These responses were predominant in TNFR1 KO mice rather than TNFR 2 deficient. The chronic CCL4 exposure resulted in down regulation of TGF-β2 and Precollege α1 (III) gene expression indicating TNF-α is responsible for modulation of CCL4 induced inflammation and fibrosis mice.

NF-KAPPA (proinflammatory cytokine)

NF-κB a transcription factor plays a key role in regulating the inflammatory response. It is present in the cytosol in an inactive state being complexed with inhibitory protein IκBα.Following exposure to various stimuli such as oxidative stress, cytokines, LPS, NF-κB activation is achieved through phosphorylation IkBa at serines 32 and 36, which results in ubiquitination and eventual degradation of IkBa by proteasome. Activated NF-κB is transported to the nucleus where it binds to its target genes with sites regulating their expression.

Selective inactivation of NF-κB using decoy ODN, transferred via hem agglutinating virus of Japan (HVJ) to macrophages attenuated acute hepatic injury while reducing concentrations of IL-6, TNF-α, and IL-1and transaminase level. Furthermore, nuclear translocations of NF-κB were inhibited in macrophages. Moreover in liver fibrosis model, intoxicated for 8 weeks, transfection with NF-κB decoy resulted in suppressing fibrosis probably due to anti-inflammatory effect, concomitant with the reduced expression of transforming growth factor (TGF) -β, precollege type 1 α1, and α-smooth muscle actin (SMA) in macrophages.

NF-κB is known to confer protection against TNF-induced apoptosis .Both TNFR receptors participate in ant apoptotic activity. The initial event is formation of TNF/TNF-R1 complex which then interact with death domain of TRADD. Receptor-bound TRADD then recruit TRAF2 and into signaling complex thus mediating interaction ofTRAF2 and TNF-R1. Conversely, TRAF2can for homodimers or heterodimer TRAF1/TRAF2 which can directly bind to TNFR-2 . TNFR2 activate downstream signaling proteins including NF-κb , thus leading its activation. NF-B decoy ODN accelerated HSC apoptosis without affecting their morphological activation in a long-term CCL4 model. NF-κB also play a pivotal role during liver regeneration. The significant up regulation of HGF, NF-κB and AP-1 were observed in early stage in CCL4 intoxicated acute injury, TNF-α and IL-6and STAT-3 might trigger activation of these factors.

TGF-β

TGF-β is a multifunctional cytokine that regulates various processes including growth, differentiation, apoptosis and ECM deposition. TGF- β transmit its signals via binding its serine/threonine kinase receptor TGF-β receptor II and receptor I existing as homodynes in the plasma membrane. Ligand binding induces TGF-β receptor II to phosphorylate and heterodimerize with receptor l which subsequently phosphorylates Smad2 and Smad3 forming a heterodimer complex with Smad4. This complex moves into nucleus regulating expression of its target genes. It has been suggested that TGF-β signaling exerts its effect in activated hepatic stellate cell through diverse pathways modulated by various types of liver injury. CCL4 administration induced significant up regulation in Phospho-Smad3 but was absent in BDL-induced model. This differential expression of Smad is ascribed to differential regulation of the early growth response gene-1 (Egr-1) (up regulated in BDL induced model) which negatively regulate Smad3 expression. TGF- β mediate partial activation of the ERK pathway in BDL induced model, and p38 MAPK activity in CCL4 injury model implicating a crosstalk between p38 and Smad3 signaling. The possible mechanism underlying Erk MAPK activation initially involves auto phosphorylation of TβRII, TβRI on tyrosine residue following TGF- β activation. Activated receptors recruit Shc/Grb2 into a complex resulting in activation of the Erk MAPK pathway. Activated Erk down regulate PP2A (protein phosphatase 2A) which is known to dephosphorylate TβRI.

TGF-β utilize distinct signaling pathway in acute and chronic CCL4 model. During acute liver injury autocrine TGF-β signal induces up regulation of plasminogen activator inhibitor type 1 (PAI-1) and a2 (I) precollege (COL1A2). Moreover, phosphorylated Smad2 was significantly increased in activated HSCs followed by increase in Smad7 which is associated with gradual loss of the phosphorylated Smad2 thus resulting in transient and reversible ECM production. In contrast during chronic liver injury, TGF-β signal constantly phosphorylate Smad2 in the presence of low level of Smad7 in myofibroblast cells resulting in consistent ECM accumulation which leads to cirrhosis.

It has also been shown that TGF-βplays a critical role during regeneration it prevents hepatocyte proliferation by limiting hepatic DNA synthesis in hepatocytes once the liver has regenerated. In addition TGF-beta receptor (TBRI and TBRII) is transiently decreased in hepatocyte at an early stage and are restored in the later stages of regeneration thus preventing its proliferative activity which accounts for successful remodeling of hepatic structure.

Anti-inflammatory cytokine (IL-6, IL10)

Activated kuppfer cell releases both proinflammatory and anti-inflammatory cytokine in response to toxication. Among these IL-6 emerged as a hepatic-protective cytokine in various models (ccl4, ischemia, partial hepatectomy, alcoholic and nonalcoholic fatty liver. IL-10 is also known to regulate the inflammatory response mediate by CCL4 induced chronic injury and fibrosis model. IL-6 mediates its protective effect in hepatocyte through activation of STAT-3. IL-6 binds to its receptor gp80 and induces homodimerization of gp130 molecule. Dimerization subsequently causes receptor associated Janus kinases (JAKs) to auto phosphorylate and become activated. Activated JAKS then phosphorylate gp130 molecule which in turn phosphorylate STAT proteins. Activated STAT then dimerism and move to the nucleus where it activates its target gene including anti oxidative and anti-apoptotic gene thereby preventing hepatic damage. IL-6 plays a key role in both in liver injury and normal regeneration after injury. IL-6 −/− deficient mice injected with acute dose of CCL4 exhibited accentuated hepatocyte apoptosis and reduction in DNA synthesis and mitosis. Additionally it was associated with impaired liver regeneration and deficient in activation of (STAT3) and nuclear factor-kB (NF-kB) all of which was ameliorated following IL-6 treatment.

One of the study showed that IL-6 exerts its protective effect via downregulation of MMP-2 and implies that its low level is needed for recovery following injury. MMP-2 is known to potentiate liver injury and inflammation as its inhibition resulted in protection against TNF-α induced hepatitis and apoptosis. IL-10 knockout mice resulted in up regulation of tumor necrosis factor-alpha (TNF-a) and transforming growth factor-beta 1 (TGF-b1) level. It showed more pronounced neutrophil infiltration whereas marginal effect against hepatocyte necrosis.

References

1. Robin S, Sunil K, Rana A, Nidhi S Different models of hepatotoxicity and related liver diseases: a review. International Research J of pharm3.
2. Malhi H, Gores GJ. Cellular and molecular mechanisms of liver injury. Gastroenterology 2008;134(6):1641-54.
3. Weber LW, Boll M, Stampfl A. Hepatotoxicity and mechanism of action of haloalkanes: carbon tetrachloride as a toxicological model. Crit Rev Toxicol 2003;33(2):105-36.
4. Hosono-Fukao T, Hosono T, Seki T, Ariga T. Diallyl trisulfide protects rats from carbon tetrachloride-induced liver injury. J Nutr 2009;139(12):2252-6.
5. Manibusan MK, Odin M, Eastmond DA. Postulated carbon tetrachloride mode of action: a review. J Environ Sci Health C Environ Carcinog Ecotoxicol Rev 2007;25(3):185-209.
6. Wong FW-Y, Chan W-Y, Lee SS-T. Resistance to carbon tetrachloride-induced hepatotoxicity in mice which lack CYP2E1 expression. Toxicol Appl Pharmacol 1998;153(1):109-18.
7. Yadav U, Ramana KV. Regulation of NF-B-induced inflammatory signaling by lipid peroxidation-derived aldehydes. Oxid Med Cell Longev 2013;2013.
8. Fritz KS, Petersen DR. Exploring the biology of lipid peroxidation-derived protein carbonylation. Chem Res Toxicol 2011;24(9):1411-9.
9. Hartley DP, Kolaja KL, Reichard J, Petersen DR 4-Hydroxynonenal and malondialdehyde hepatic protein adducts in rats treated with carbon tetrachloride: immunochemical detection and lobular localization. Toxicol Appl Pharmacol 1999;161(1):23-33.
10. Beddowes EJ, Faux SP, Chipman JKChloroform, carbon tetrachloride and glutathione depletion induce secondary genotoxicity in liver cells via oxidative stress. Toxicology 2003;187(2):101-15.