Coordinated defence and the liver

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ABSTRACT – The liver is strategically placed to protect the body against a vast array of potentially harmful compounds. The steps involved include phase I metabolism which makes molecules more reactive and phase II reactions which generally enhance solubility in bile or urine. Recent discoveries have shown how regulation of these reactions is also closely allied to expression of membrane transporters which excrete the products of biotransformation into bile and prevent their reabsorptoion via the intestine. The coordinated activity of these various functions is orchestrated by orphan nuclear receptors which, in response to an encounter with a potential toxin, are able to induce expression of the genes involved in its biotransformation and excretion. Lithocholic acid (LCA) is routinely produced in our intestine by bacterial deconjugation of chenodeoxycholic acid a major bile acid in humans. In human liver the presence of LCA is sensed by the pregnane X receptor (PXR) which has the potential to switch on all the genes required for safe metabolism and elimination of LCA from the body. These include cytochrome P450 3A which hydroxylates LCA to more soluble forms and sulfotransferase (SULT2A1) which by sulphation of LCA makes it more readily soluble in bile and enhances its faecal excretion. Similarly, PXR exposure to LCA produces up-regulated expression of the membrane transporters MDR1 and MRP2 which excrete metabolites of LCA. Evidence is accumulating in support of the hypothesis that deficiencies in these defence mechanisms underlie susceptibility to primary sclerosing cholangitis and ulcerative colitis.

KEY WORDS: cytochrome P450 3A, lithocholic acid, MDR1 and MRP2, orphan nuclear receptors, pregnane X receptor, sulphation, susceptibility to primary sclerosing cholangitis, ulcerative colitis

The liver metabolises and excretes a host of endogenous molecules and stands at the portal of entry for all kinds of ingested xenobiotics, whether food or drug. The fate of these compounds depends largely on the efficiency of their absorption from the gut and uptake and biotransformation by the liver. The mechanisms involved include metabolic transformation and active transport which usually work in combination to enhance elimination from the body whether in urine or faeces. In this the liver is closely allied with the intestine in maintaining the functionality of bile as a safe route for elimination of any potentially toxic compound that lacks sufficient solubility in water to permit its efficient excretion via the kidneys. The detergent properties of bile facilitate efficient excretion of compounds which are poorly soluble in water.

Coordinated biliary defence mechanisms

Bile acids provide the required detergency and are essential for biliary cholesterol secretion and absorption of fat and fat soluble vitamins. The synthesis of bile acids by the liver is closely regulated to compensate for faecal loss, thus maintaining a consistent pool of bile acids in the entero-hepatic circulation which re-circulate from liver to intestine six to ten times a day. The primary bile acids in man are cholic and chenodeoxycholic acid. They are metabilised by enteric bacteria to the secondary bile acids deoxycholic and lithocholic respectively. Lithocholic acid (LCA) derives its name from *lithos* (Greek for stone) to reflect its poor solubility. It naturally partitions into lipid membranes where it exerts many potentially harmful effects. Only the highest primates, chimpanzee and humans, are able to live with bile containing LCA and the chenodeoxycholate from which it is derived. For this reason, all the preclinical work on cholesterol gallstone dissolution by chenodeoxycholic acid had to be conducted in chimpanzees. Animals which do not possess these metabolic defensive mechanisms are unable to survive feeding of chenodeoxycholic acid or LCA in their diet. When male New Zealand white rabbits were fed regular laboratory chow containing ursodeoxycholic, chenodeoxycholic, or LCA at a concentration of 0.5% (w/w) it resulted in a 14-day mortality of 6/12 for LCA, 2/8 for chenodeoxycholic acid and 0/6 for ursodeoxycholic acid.[1]

Much of the liver's capacity to defend the body against potentially harmful substances resides in the combination of metabolic transformation with active transport of material into faeces. These protective mechanisms are effective against a wide spectrum of both endogenous and exogenous xenobiotics with toxic potential. The chemical reactions

are generally grouped as phase I and phase II reactions, the former largely converting inert materials into more reactive molecules which, in phase II, can be conjugated with solubilising ligands such as sulphate, glucuronide and glutathione. Thus the human liver possesses two methods for protection against lithocholate toxicity. Cytochrome P450 3A is the most highly expressed of microsomal cytochromes in man and renders LCA more soluble by hydroxylation to non-toxic bile acids such as hyodeoxycholic acid or murideoxycholic acid. Alternatively sulfation by dehydroepiandrosterone (DHEA) sulfotransferase (SULT2A1) converts LCA to a less toxic and more water-soluble form. Sulphation of LCA greatly reduces its reabsorption from the gut and so promotes its excretion in faeces, minimising its entero-hepatic circulation (Fig 1).

CYP3A is largely responsible for phase I conversion not only of bile acids but also of steroid hormones. Many potentially cholestatic compounds have their toxicity attenuated in this way, and as a result can be harmlessly excreted via bile and/or urine. Female sex hormones are generally cholestatic and in susceptible individuals may induce cholestatic jaundice associated with pregnancy or oral contraceptive use. The most cholestatic metabolite of oestrogens is generally recognised to be 17β-estradiol glucuronide . The liver's mechanism for preventing this toxicity is identical to that which de-toxifies LCA by 3-O-sulphonation. This reaction is performed by dehydroepiandrosterone sulphotransferase (SULT2A1) with 3'-phosphoadenosine phosphosulphate synthetase 2 (PAPSS2) as the sulphate donor. It is now recognised that it is the ability of human and chimpanzee liver to sulphate LCA which largely enables these species to tolerate having chenodeoxycholic acid and its derivative LCA as biliary constituents.

Further evidence of a coordinated defence is demonstrated by the fact that the tri-partite mechanisms provided by phase I and phase II biotransformation followed by elimination via active transport exists as a barrier at the intestinal level as well as within the liver. The highly effective combination is provided by CYP3A, SULT2A1, MRP2 and MDR1 at both intestinal and hepatic level. An excellent example of the efficacy of this system was provided by Dilger *et al* (2005)[2] who administered a single dose of the synthetic corticosteroid budesonide to healthy human volunteers and charted its concentration in peripheral blood. Remarkably, following a 5-day course of rifampicin which is a known inducer of CYP3A, virtually no budesonide could be detected in the systemic circulation following an oral dose. This excellent example of first-pass extraction illustrates the strategic positioning of the gut and liver in intercepting ingested xenobiotics and the amazing efficiency of defence provided by coordinated induction of biotransformation enzymes and elimination transporters. How is such coordination achieved?

Chemical ligands entering the liver encounter a series of ligands of diverse specificities known as orphan receptors. The best known are those whose ligands are products of the endocrine system. The ligand–receptor complex typically binds to another factor to form a heterodimer which in combination with co-factors is able to exert an inducer or suppressor effect on

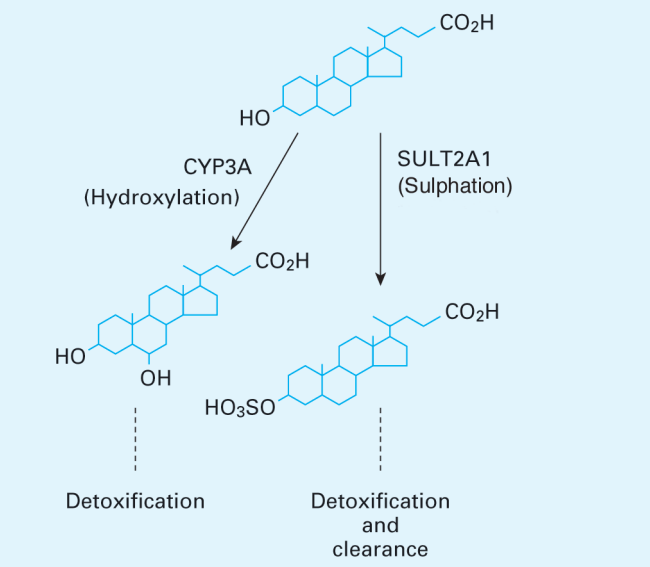

Fig 1. Human liver possesses two methods for protection against lithocholate toxicity. Cytochrome P450 3A renders LCA more soluble by hydroxylation to non-toxic bile acids such as hyodeoxycholic acid or murideoxycholic acid. Sulfation by SULT2A1 greatly reduces LCA reabsorption from the gut and so promotes its excretion in faeces.

target genes. Thus the Farsenoid X receptor (FXR) binds the primary bile acids cholic and chenodeoxycholic acid and forms a heterodimer with the retinoic acid receptor RXR. The FXR:RXR heterodimer is then able to regulate many of the genes involved in bile acid synthesis including the key rate-limiting enzyme cholesterol 7α-hydroxylase and thus provide a sensor and effector mechanism within the entero-hepatic circulation of bile acids which precisely matches *de novo* bile acid synthesis with faecal loss.

The pregnane X receptor (PXR) is unique among human nuclear receptors because of its promiscuous capacity for interacting with a widely diverse group of ligands. LCA is a preferred ligand of PXR in contrast to the other bile acids which act as FXR ligands.[3] Rifampicin is a powerful inducer of PXR and its downstream gene transcription products include CYP3A, SULT2A1, PAPSS2, MRP2 and MDR1 which in combination provide a highly effective and efficient system to protect us from accumulation of LCA within the body (Fig 2). Sonoda *et al* (2002)[4] have shown that LCA in micromolar amounts is a powerful inducer of SULT2A1 only in the presence of the RXR:PXR heterodimer. It can thus be seen that PXR has a critical and central role in orchestrating the intestine and liver's response to LCA and many other hazardous chemicals.

Defective mechanisms and disease susceptibility

It may be that defective functioning of these defence mechanisms in humans would produce susceptibility to disease in the liver and intestine. One strong contender for such a liver disease is primary sclerosing cholangitis (PSC). In PSC the intrahepatic and extrahepatic bile ducts become chronically inflamed with

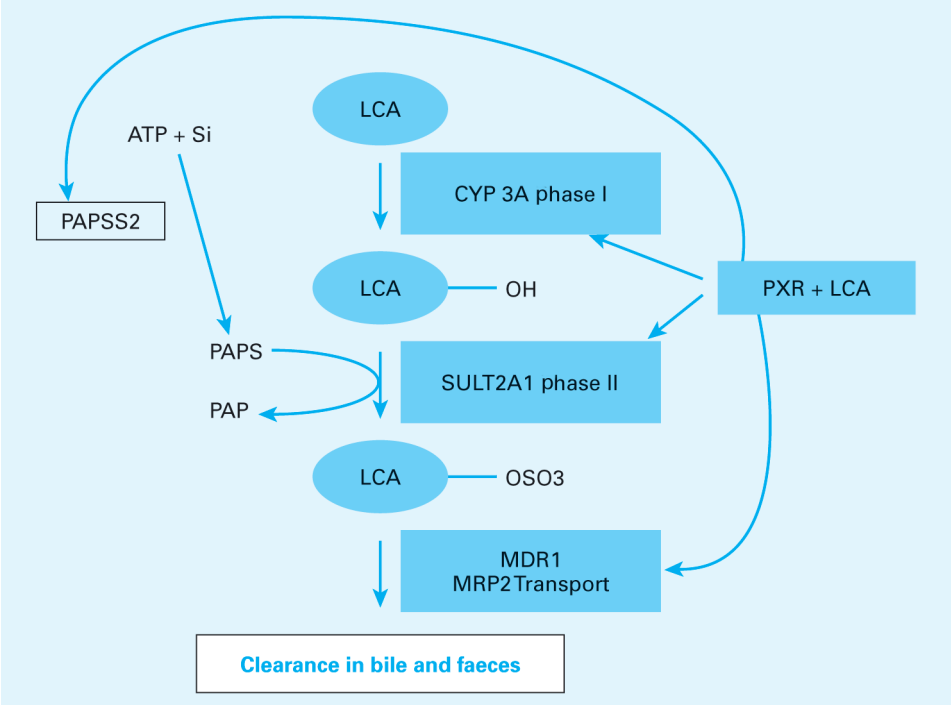

Fig 2. Lithocholic acid (LCA), along with a wide range of other ligands, is bound by the orphan nuclear receptor PXR which then moves into the nucleus where it binds with RXR, its heterodimer. This complex then sits on the PXR response element of the various genes whose coordinately expressed products work in close combination to attenuate the potential toxicity of the LCA via phase I hydroxylation (CYP3A), phase II conjugation (SULT2A1 and PAPSS2) and active transporter function (MDR1 and MRP2).

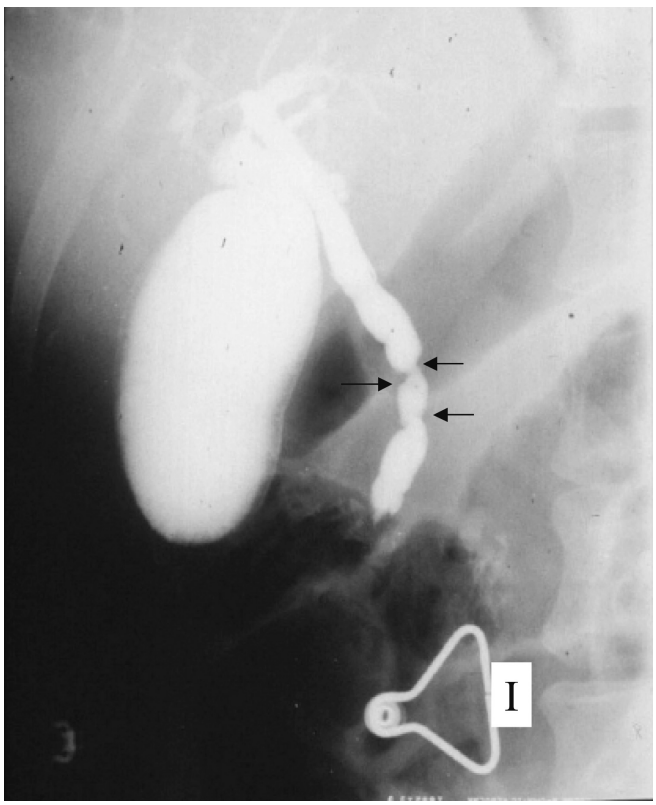

Fig 3. Strictures of the common bile duct arrowed are typical of primary sclerosing cholangitis (PSC). PSC is associated with ulcerative colitis in most cases. An ileostomy appliance (I) is apparent as evidence of a total colectomy.

resultant fibrosis which produces stricturing and may culminate in a classically beaded appearance of the ducts. PSC is associated with ulcerative colitis (UC) in some 70% of cases. Typically ulcerative colitis begins at the ano-rectal junction and spreads to a variable degree from the left to right colon and sometimes as far as the ileo-caecal junction. In contrast the colitis associated with PSC begins in the caecum and right colon and spreads distally. This would be entirely compatible with colonic injury in association with reabsorption of toxic bile acids with extension distally along the colon as the injury diminishes the absorptive capacity proximally. The tendency for the distal colon to be spared until the later stages of the disease accounts for the clinical observation that patients diagnosed with extensive colitis associated with PSC seldom give a history of troublesome diarrhoea. The risk of carcinoma is increased both in the colon with UC and in the bile ducts with PSC. However, in idiopathic UC the risk is highest for carcinoma of the distal colon and rectum compared to the caecum and proximal colon in patients with PSC. In follow-up of patients transplanted for PSC, our Liver Unit's experience has been that the risk of recurrent PSC in the transplanted liver is much reduced in those who have undergone colectomy compared with those whose colon is intact.[5] These clinical observations strongly support the hypothesis that the distribution of inflammation, fibrosis and neoplasia in patients affected by the combination of UC and PSC is related to intestinal function and to the flux of the entero-hepatic circulation (Fig 3). Ursodeoxycholic (Urso) acid is widely used in hepatology for its hepatoprotective effects. Urso normally constitutes about 1% of bile acids in human bile but on sustained oral feeding this rises to 50%. Urso is much less metabolised to LCA than the chenodeoxycholic acid it replaces and is conjugated with N-acetyl aminoglucosamine, thus sparing sulphate

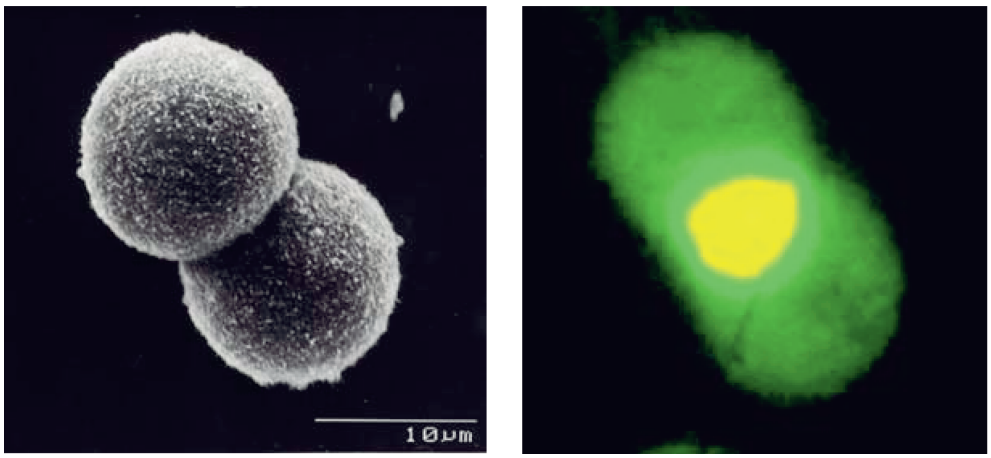

Fig 4. Two hepatocytes are shown in which the bile canaliculus has become a closed space into which both cells secrete bile as normal. In this photograph accumulation of the fluorescent bile acid cholyl lysyl fluorescein, synthesised by Dr Charles O Mills in our laboratory, can be seen as a measure of bile formation.

reserves. Patients treated with Urso for primary biliary cirrhosis have a significant reduction in their colon cancer risk, supporting the hypothesis that Urso replaces constituents of the naturally occurring human bile acid pool which may be promoting colonic cancer.

The liver's ability to sulphate lithocholic acid is reduced in explanted liver removed at the time of transplantation for end-stage liver disease caused by primary biliary cirrhosis and a group of other liver diseases including PSC.[6] When later it became clear that lithocholic acid sulphotransferase activity resided in dehydroepiandrosterone sulphtransferase (DHEAST) (still later isoform SULT2A1) we demonstrated reduced activity in end-stage liver tissue from primary biliary cirrhosis, primary sclerosing cholangitis and other selected liver diseases.[7] These studies were not able to distinguish diminished enzyme activity that was primary and potentially causal from diminished function that arose as a consequence of the disease within the liver. We therefore performed a series of *in vitro* experiments utilising the hepatocyte couplet model (Fig 4) in the laboratories of the late Professor Roger Coleman. The model enabled us to observe bile formation as it accumulated within a sealed canalicular vacuole between coupled hepatocytes. We were able to demonstrate that toxicity of LCA caused disruption of the peri-canalicular microfilament network, blebbing of hepatocyte membrane and redistribution of bile canalicular transporters out of the canalicular membrane. Protection against these toxic effects of LCA was provided by both co-administration of ursodeoxycholic acid with LCA,[8] and supplementation of sulphate supply via the addition of S-adenosyl methionine(SAMe). Co-administration of SAMe and dehydroepiandrosterone (DHEA) abrogated the hepatoprotective effect of SAMe thus demonstrating competition between LCA and DHEA for DHEAST activity and providing direct evidence that sulphation of LCA was a key aspect of SAMe's hepatoprotective action.[9]

Recent observations have demonstrated impaired functioning of MDR1 and SULT2A1 in the colon in patients with ulcerative colitis. Langmann *et al* (2004) showed an eleven-fold reduction in the expression of SULT2A1 in the colon of patients with ulcerative colitis.[10] Ho *et al* (2006) observed a highly significant association between the common MDR1 haplotypes and UC but not Crohn's disease (CD), which was critically dependent on a single nucleotide polymorphism (SNP).[11] The association with UC was strongest with the phenotype of extensive disease, providing compelling evidence to support the contribution of genetically determined impairment of MDR1 expression which determines risk to UC. Given that both MDR1 and SULT2A1 are coordinately regulated by PXR, these data strongly support our hypothesis for a potential association of a defect in PXR function in PSC. Susceptibilty to inflammatory bowel disease has been shown to be associated with genetic variation in the PXR encoding gene.[12] In a study of the PXR gene, Karlsen *et al*[13] did not find that susceptibility to PSC was associated with any of the polymorphisms which they chose to study but found that certain genotypes exerted a significant effect on diminished survival in PSC patients. The impaired functioning of PXR coordinated biotransformation and transport mechanisms shared by liver and intestine could also be anticipated to impact upon the susceptibility to recurrent PSC in the transplanted liver which pertains in those whose colon is intact but not in those who have undergone total colectomy (Fig 5).[5]

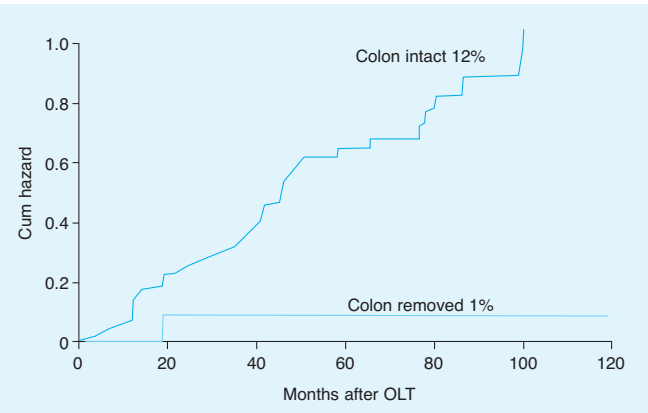

Fig 5. The recurrence rate of primary sclerosing cholangitis (PSC) is shown in patients who received their liver transplant for PSC in Birmingham. It appears that the risk of recurrent disease is associated with an intact colon and that total colectomy with ileostomy protects against this. OLT = orthotopic liver transplantation. Reproduced from *The Lancet* with permission from Elsevier.[5]

Conclusion

Clear evidence exists that closely coordinated actions of both hepatic and intestinal biotransformation mechanisms protect the body from injury by both endogenous and exogenous chemicals. The promiscuous nuclear binding receptor PXR binds a wide variety of known toxicants including the potentially lethal secondary bile acid LCA. PXR's ability to promote expression of the genes which code for the CYP3A4, SULT2A1, PAPSS2, MRP2 and MDR1 makes it of vital importance in protecting the liver and gut from such toxic agents. Evidence is now accumulating that deficiencies in this system of coordinated defence is associated with inflammatory bowel and hepatobiliary disease.

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