Antidepressant induced cholestasis: hepatocellular redistribution of multidrug resistant protein (MRP2)

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Background: We report two cases of antidepressant induced cholestasis. Case reports: We describe the first reported case of acute cholestasis due to citalopram (selective serotonin reuptake inhibitor) occurring in a patient who also experienced obstetric cholestasis in association with each of three pregnancies; in a second patient cholestasis developed due to dothiepin (tricyclic antidepressant), and six years later due to paroxetine. In both cases liver biopsies showed features of a “pure” cholestasis with total resolution within 1–6 months after withdrawal of the causative drug. Immunostaining for the canalicular transporter, multidrug resistant protein 2 (MRP2), responsible for biliary secretion of several organic anions including bilirubin glucuronides, showed sustained expression in both biopsies as well as relocation with appearance of strong staining of the basolateral membrane of the hepatocyte. This finding has also not been reported previously. Conclusions: We postulate that intracellular redistribution of MRP2 may reflect an adaptive compensatory mechanism which helps in the elimination of the drug or its cholestatic metabolites from the hepatocyte back to the sinusoidal space and subsequent excretion in urine. Changes seen in these two patients differ from findings previously reported in rats where downregulation of mrp2 occurs in response to experimentally induced cholestasis. We speculate that the rat is more advanced than humans in its ability to downregulate canalicular transporter expression as protection against progressive intrahepatic cholestasis.

Antidepressive drugs may occasionally cause impairment of liver function. Both tricyclic antidepressant and monoamine oxidase inhibitors have been reported to induce prolonged or even fatal jaundice. Selective serotonin reuptake inhibitors (SSRI), such as paroxetine, have been shown to induce severe acute or chronic hepatitis. The mechanisms by which antidepressive drugs induce cholestatic changes are not known. Recent cloning of proteins involved in the secretion of bile and its constituents has helped to provide a better understanding of the processes involved in the pathogenesis of some cholestatic disorders. However, data on expression of these transporters in drug induced cholestasis do not exist.

In this paper, we describe two cases of antidepressant induced cholestasis. The first patient developed acute cholestasis during treatment with citalopram, an SSRI, which is the first report of such a cholestatic reaction. The second patient developed features of cholestasis after dothiepin (tricyclic antidepressant) and six years later he presented with similar symptoms associated with treatment with paroxetine. Neither the occurrence of two episodes of cholestasis following two different antidepressants in one patient nor acute cholestasis caused by paroxetine have been reported previously. We also looked (for the first time in drug induced cholestasis) at the hepatocellular distribution of multidrug resistant protein 2 (MRP2), one of the key canalicular proteins responsible for the transport of several organic anions, including bilirubin glucuronides, from the hepatocyte to bile.

PATIENTS
Patient No 1
A 30 year old female presented initially with symptoms of obstetric cholestasis (OC) which developed in two successive pregnancies and responded well to treatment with ursodeoxycholic acid. In October 1999 she was found to have clinical symptoms of depression and was given fluoxetine. As there had been no significant improvement in her depression over a 12 month period she was changed to citalopram. The initial dose of 10 mg daily was increased to 20 mg after four weeks. She presented one month later with symptoms of jaundice and pruritus. Citalopram was her only treatment at the onset of cholestasis. Her liver function tests (LFT) at presentation were as follows: bilirubin 75 μmol/l (normal <22 μmol/l); alkaline phosphatase (ALP) 637 U/l (normal 120–360 U/l); aspartate transaminase (AST) 33 U/l (normal 5–35 U/l); albumin 43 g/l (normal 35–50 g/l); and international normalised ratio (INR) 0.9 (normal 0.8–1.2). Autoantibody screening (ANA, AMA, SMA, ds DNA, ssDNA) was negative as was viral screening for hepatitis A, B, and C. Abdominal ultrasound was normal. A liver biopsy showed severe canicular cholestasis, mainly centrilobular in distribution. There was no evidence of inflammation, bile duct loss, or any underlying chronic liver damage. Her symptoms resolved within a couple of months after citalopram was withdrawn. In June 2000 her LFTs showed complete normalisation and were as follows: bilirubin 6 μmol/l; ALP 277 U/l; AST 13 U/l; serum bile acids (SBA) 15; albumin 46 g/l; and INR 1.0 (fig 1A).

Patient No 2
A 63 year old male with depression presented in May 1991 with a three week history of malaise, pruritus, and subsequent jaundice. Prior to the onset of cholestasis he had been treated with dothiepin at a dose of 75 mg daily for three months. Dothiepin was his only treatment at that time. His

Abbreviations: MRP2, multidrug resistant protein 2; SSRI, selective serotonin reuptake inhibitor; LFTs, liver function tests; ALP, alkaline phosphatase; AST, aspartate transaminase; INR, international normalised ratio; SBA, serum bile acids; TBS, Tris buffered saline; OC, obstetric cholestasis.
INR 1.0. He was recently seen in our clinic in May 2001 (fig 1). The striking zonal distribution of canalicular plugging supported the theory that in these cholestatic drug reactions biliary bile acids are insufficient for solubilisation of biliary solutes which form intracanalicular precipitates in lobular zones 3 and 2 but sufficient to prevent canalicular precipitates accumulating in zone 1.

Figure 1 Values for alkaline phosphatase (ALP; normal 120–360 U/l) and bilirubin (BL; normal <22 μmol/l) in both patients during their episodes of cholestasis. Pred., prednisolone.

LFTs at presentation were as follows: bilirubin 161 μmol/l; ALP 600 U/l; AST 40 U/l; albumin 42 g/l; and INR 1.0. SBA was not done. Autoantibody screening (ANA, AMA, SMA, dsDNA, ssDNA) was negative as was viral screening for hepatitis A and B. Abdominal ultrasound was normal. Liver biopsy showed severe pure cholestasis without inflammation, bile duct loss, or features of chronic liver damage. Dothiepin had been stopped at presentation and his symptoms persisted for a couple of weeks. His pruritus was treated with cholestyramine and rifampicin with no effect. In June 1991 he was placed on a short course of oral prednisolone and his symptoms resolved within the next four weeks. His pruritus was treated with cholestyramine and rifampicin with no effect. In June 1991 he was placed on a short course of oral prednisolone and his symptoms resolved within the next four weeks. His liver biopsy in September 1991 showed complete normalisation with the tricyclic antidepressant dothiepin. His liver biopsy in September 1991 showed complete normalisation with the tricyclic antidepressant dothiepin. His liver biopsy in September 1991 showed complete normalisation with the tricyclic antidepressant dothiepin.

**IMMUNOHISTOCHEMISTRY**

**Methods**

Formalin fixed paraffin embedded liver sections were subjected to antigen retrieval by microwave treatment for 30 minutes. Before the primary antibody was applied, sections were pretreated with 3% hydrogen peroxidase in methanol to block endogenous peroxidase activity and with 10% normal horse serum. Tissues were then exposed to monoclonal anti-MRP2 antibody (Alexis, UK) diluted 1:20 in Tris buffered saline (TBS) for one hour at room temperature. After triple washing in TBS, biotinylated antimouse secondary antibody (1:150) was applied for one hour, followed by detection using the avidin-biotin-immunoperoxidase method (ABC Kit, Vector, UK) for 30 minutes. To obtain a brown reaction product, the slides were exposed to 3,3 diaminobenzidine (Vector UK Ltd) and finally a counterstain with Mayer’s haematoxylin (Vector UK Ltd) was applied.

**Results**

Histologically normal liver tissue obtained from donor liver used for transplantation was used as a normal control. This showed diffuse canalicular immunostaining for MRP2 with no obvious zonal variation in staining intensity (fig 2A). Immunostaining was also present in the epithelium of bile ducts and ductules. This was mainly luminal with focal basolateral staining being evident.

Immunostaining for MRP2 in both patients was very similar. Diffuse canalicular immunoreactivity was present, with stronger staining seen in dilated canaliculi in the perivenular regions (fig 2B). In addition, membranous staining of hepatocytes was present in periportal and perivenular regions (fig 2C, D), a feature not seen in normal liver. Biliary epithelial staining was present although in patient No 2 staining appeared to be weaker than in normal liver.

**DISCUSSION**

Both patients described here manifested clinical, biochemical, and histological features of cholestasis associated with antidepressive drugs. The second episode of cholestasis in patient No 2 and symptoms of cholestasis in patient No 1 occurred after exposure to two different SSRIs, paroxetine and citalopram. Interestingly, before the onset of cholestasis both patients were treated for 12 months with another SSRI, fluoxetine. Without any side effects but also without significant improvement of their depression. Symptoms of cholestasis in both cases started suddenly approximately two months after the introduction or incremental dose increase of either paroxetine or citalopram. Features of cholestasis have not yet been described in association with the above mentioned drugs. To the best of our knowledge there is only one case report of SSRI induced cholestasis published in the literature. In their letter, Cosme et al described a 49 year old man in whom symptoms of cholestasis occurred five months after introduction of fluoxetine. Unfortunately, that patient had also been simultaneously treated with several other drugs, including sulpiride, an agent which has been described as causing cholestasis.

The first episode of cholestasis in patient No 2 was associated with the tricyclic antidepressant dothiepin. His liver biopsy showed features of a pure cholestasis, without impairment of liver architecture. This is in contrast with previously described cases of tricyclic antidepressant induced liver damage where cholestasis was associated with progressive inflammatory infiltration, fibrosis, and bile duct loss.

The striking zonal distribution of canalicular plugging supports the theory that in these cholestatic drug reactions biliary bile acids are insufficient for solubilisation of biliary solutes which form intracanalicular precipitates in lobular zones 3 and 2 but sufficient to prevent canalicular precipitates accumulating in zone 1.
cholestatic conditions, including those caused by sepsis, drugs, and haemolysis, but not classically seen in animal models of cholestasis. This suggests species differences in the control and regulation of biliary transporter proteins. In the rat, downregulation of mrp2, the transporter responsible for canalicular secretion of bilirubin, has been shown to be an early response to a variety of cholestatic insults in models using endotoxin, oestradiol, and bile duct ligated rats. This downregulation would protect the rodent liver against the kind of biliary plugging seen in our patients. Regulation of mrp2 and ntcp expression in the rat occurs via RXR/RAR and HNF-1, downregulation occurring in response to bile acid ligands as well as in response to cytokines, and is associated with nuclear factor κB stimulated upregulation of Mdr1b. Our immunohistochemical observations provide no evidence in support of MRP2 downregulation in our severely cholestatic patients. The high serum bile acid level of >400 is of an order we see in patients with complete deficiency of expression of BSEP and suggests that biliary plugging has resulted from maintained levels of bilirubin and other organic anion secretion despite poor biliary bile acid output. In turn, it suggests that human MRP2 expression is not as susceptible to downregulation as it is in the rat, which may therefore be perceived as more advanced in its hepatoprotective repertoire of gene expression which confers protection against xenobiotics. Recent immunostaining studies performed on both patients showed significant redistribution of one of the key canalicular transporters, multidrug resistant protein 2 (MRP2). MRP2 is a recently cloned protein responsible for the canalicular transport of several organic anions, including bilirubin. We have tested a wide range of antibodies against hepatocellular transporters but it was only MRP2 antibody which worked in paraffin embedded sections. Despite the fact that in both biopsies analysed cholestasis was induced by two different agents, MRP2 stainings showed striking similarities, especially in their strong basolateral membrane reaction, not seen in normal liver. Redistribution of MRP2 into the basolateral membrane has been observed in vitro in HepG2 cells. Under physiological conditions, transport of MRP2 from the Golgi apparatus to the canalculus occurs via a sinusoidal membrane, as demonstrated by Boyer and Soroka. One may assume that membranous staining observed by us may be explained by cholestasis induced disturbance of the polarity of hepatocytes. We have recently shown however that in another cholestatic condition, primary biliary cirrhosis, MRP2 maintained its characteristic and specifically canalicular distribution without any detectable staining of the basolateral membrane of the hepatocyte which would be indicative of lost hepatocellular polarity. It seems therefore more likely that basolateral membrane expression of MRP2 reflects an adaptive compensatory mechanism which helps in elimination of the drug or its cholestatic metabolites from the hepatocyte back to the sinusoidal space, and then the subsequent excretion of the drug (metabolite) in urine. Our finding that MDR3, a phospholipid flippase, is uncharacteristically redistributed to the basolateral membrane of the hepatocyte in the periphery of cirrhotic nodules in advanced primary biliary cirrhosis, in association with upregulation of MRP3 which is able to excrete bile acids into the blood, was interpreted by us as an adaptive response of the cholestatic hepatocyte facilitating extrusion of bile acids and “biliary” phospholipid into plasma. We can postulate that the parallel uncharacteristic basolateral expression of MRP2 in our patients is a response of the hepatocyte to an extreme degree of canalicular obstruction. Whether the redistribution occurs prior to insertion of the transporter into the canalicular membrane or subsequently by lateral diffusion from the canalicular domain via tight junctions rendered incompetent as a consequence of the drug toxicity per se, or secondary to plugging, remains to be determined.

It is likely that antidepressant induced cholestasis may occur in predisposed individuals. The observation that patient No 1 developed OC in addition to citalopram jaundice and patient No 2 developed cholestatic jaundice in response to two drugs of different classes strongly supports the hypothesis that they represent genetically predisposed individuals who are susceptible to cholestatic jaundice which can be precipitated by a variety of stimuli. The predisposed liver thus responds with a limited repertoire to diverse insults. We were not able to identify predisposing factors in patient No 2. Patient No 1 however presented a prior history of OC. In fact, she became pregnant soon after recovery from her citalopram induced cholestasis and again developed features of OC. OC

Figure 2. Immunohistochemical staining reaction for multidrug resistant protein 2 (MRP2). (A) Normal liver; (B, C) biopsy from patient No 2; (D) biopsy from patient No 2. In normal liver, MRP2 expression was present in canaliculi and bile duct epithelium. In the liver biopsy from patient No 2 (B, C) there was stronger staining of MRP2 in dilated canaliculi in acinar zones 2–3. Focal basolateral membranous immunoreactivity was present in perivenular hepatocytes (C). Basolateral membranous immunoreactivity was also present in periportal hepatocytes (D, arrow).
can be associated with malfunction of other canalicular transporters, or a mutation causing disturbed trafficking of MDR3 to the canalicular membrane, as recently shown by us and others. Whether a molecular defect(s) leading to OC predisposes to SSRI induced cholestasis remains to be elucidated.

References


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