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 due to 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 46 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 canalicular 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
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 were not done. Autoantibody screening (ANA, AMA, SMA, ds DNA, 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 disease. 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 biochemistry in September 1991 showed complete normalisation 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 infiltrate and fibrosis. Dothiepin had showed severe degree of bile plugging which form intracanalicular precipitates in lobular zones 3 and 2 but sufficient to prevent canaliculic precipitates accumulating in zone 1. 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 solute which form intracanalicular precipitates in lobular zones 3 and 2 but sufficient to prevent canaliculic precipitates accumulating in zone 1.

In October 1997 he received fluoxetine and remained on it for 12 months. It did not affect his depression and therefore his local general practitioner switched him to paroxetine at a dose of 20 mg daily. Two months later he developed symptoms of cholestasis and presented with jaundice and pruritus. His LFTs showed bilirubin 260 µmol/l, ALP 544 U/l, AST 36 U/l, SBA 456 (normal <15), albumin 43 g/l, and INR 1.0. As in the first episode of cholestasis, autoantibody and viral screening were negative and abdominal ultrasound was normal. Paroxetine was stopped and his symptoms resolved within a couple of weeks. His LFTs in May 1999 were as follows: bilirubin 7 µmol/l; ALP 255 U/l; AST 35 U/l; SBA 15; albumin 43; and INR 1.0. He was recently seen in our clinic in May 2001 (fig 1C). His LFTs remain entirely normal and with respect to his liver, he is asymptomatic.

### 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 peroxide 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, Vectos, 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 SSRI, paroxetine and citalopram. Interestingly, before the onset of cholestasis both patients were treated for 12 months with another SSRI, fluoxetine. Unfortunately, that patient had also been simultaneously treated with several other drugs, including sulpiride, an agent which has been described as causing cholestasis. 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, 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.
HNF-1, downregulation occurring in response to bile acid lig-
mrp2 and ntcp expression in the rat occurs via RXR/RAR and
kind of biliary plugging seen in our patients. Regulation of
downregulation would protect the rodent liver against the
using endotoxin, oestradiol, and bile duct ligated rats.
early response to a variety of cholestatic insults in models
canalicular secretion of bilirubin, has been shown to be an
control and regulation of biliary transporter proteins. In the
models of cholestasis. This suggests species differences in the
cholestatic conditions, including those caused by sepsis,
and drugs, as well as in response to cytokines, and is associated with
nuclear factor κB stimulated upregulation of Mdr1b. Our
immunohistological observations provide no evidence in sup-
port 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 per-
ceived as more advanced in its hepatoprotective repertoire of
gene expression which confers protection against xenobiotics.
This study, performed on both proteins, 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, espe-
cially 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 canaliculus occurs via a sinusoidal mem-
brane, 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 main-
tained its characteristic and specifically canalicular distribu-
tion 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 elimina-
tion of the drug or its cholestatic metabolites from the hepa-
tocyte 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 redis-
tributed to the basolateral membrane of the hepatocyte in the
periphery of cirrhotic nodules in advanced primary biliary cir-
rhosis, 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 obstruc-
tion. Whether the redistribution occurs prior to insertion of
the transporter into the canalicular membrane or subse-
quently 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 precipi-
tated 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

![Image](http://gut.bmj.com/)

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.

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