Treatment Effect of Rifampicin on Cholestasis

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Citation

Abstract
Rifampicin, a semisynthetic antibiotic, has been discovered serendipitously to have beneficial effect on cholestasis. The mechanism, as recently demonstrated, is its induction of pregnane X receptor to regulate the genes involved in bile acids biosynthesis, detoxification and transportation. Thus, toxic bile acids like lithocholic acid are hydrolysed by increased CYP3A4 into a less toxic form. Activation of efflux pumps also facilitates excretion of bile acids from hepatic cells. Furthermore, rifampicin inhibits CYP7A1, a rate-limiting enzyme in the conversion of cholesterol into bile acids, and so reducing bile acids synthesis. However, rifampicin also causes hepatotoxicity in some cases. This limits its use in the treatment of cholestasis. The prospective approaches to overcome the problems could be to find modification of rifampicin chemical structure or find other inducers.

INTRODUCTION
Cholestasis is a very common pathophysiologic state due to impaired secretion of bile. It can be caused by obstruction within the liver (intrahepatic) or outside the liver (extrahepatic). At present, there are no effective long-term treatments for the condition.

Rifampicin, a semisynthetic antibiotic derived from the rifamycin, has been used effectively in the treatment of cholestasis since discovered serendipitously. But hepatotoxicity has been a problem in some cases. Recently, great progress has been made to understand the mechanisms for the effects of rifampicin in cholestasis. Thus, the relevant knowledge in the field is reviewed in order to help the development of better drugs for the treatment of cholestasis.

CHARACTERISATION OF CHOLESTASIS AND EFFECTS OF BILE ACIDS
Cholestasis is characterized by accumulation of serum and hepatic bile acids, the main constituents of the primary biliary fluid, plus nutritional imbalance due to malabsorption of lipids and fat-soluble vitamins. In a rat cholestasis model made by bile duct-ligation, the serum concentration of total bile acids was about 30 - 50 folds higher than normal. Bile acids have detergent effects that can be extremely toxic if their levels become much higher than normal. The consequences are irreversible liver damages including elevated serum levels of liver enzymes, severe itching (pruritus), lethargy, jaundice and, ultimately, liver failure.

The majority of bile acids are cholic acid, chenodeoxycholic acid, deoxycholic acid and lithocholic acids. Cholic acid and chenocholic acid are primary bile acids synthesized in liver from cholesterol while deoxycholic acid and lithocholic acid are secondary bile acid formed in the intestine by the bacterial 7α-dehydroxylation of cholic acid and chenocholic acid and absorbed into blood for enterohepatic circulation. Their chemical structures are shown in Fig1. Cholic acid is trihydroxy bile acid, chenodeoxy cholic acid and deoxycholic acid are dihydroxy and lithocholic acid is monohydroxy.

From in vitro study with rat microsomes, all bile acids have non-specific inhibitory effects on both CYP enzymes such as CYP2A1, CYP2C11, CYP3A2 and non-CYP enzymes such as steroid 17 beta-dehydrogenase and P450-reductase. However, the inhibitory potential of bile acids was inversely related to their extent of hydroxylation and thus, the lithocholic acid is the most toxic. Indeed, administration of lithocholic acid to rodents caused cholestasis.

TREATMENT EFFECT OF RIFAMPICIN ON CHOLESTASIS
Rifampicin has been used to successfully treat pruritus, a prominent feature of cholestasis. The pathophysiologic mechanism of pruritus is still not well understood. It could be related with high serum and tissue bile salt concentrations because biliary drainage reduced...
pruritus in both extrahepatic and intrahepatic cholestasis.\(^{(10,11)}\)

In the treatment of pruritus, the first choice is the anion exchange resin cholestyramine.\(^{(16)}\) It can bind to bile acids in gastrointestinal tract to increase fecal excretion of bile acids. However, the use of cholestyramine has poor adherence with therapies, probably due to gastroenterological side effects especially constipation, and is not overly effective. Rifampicin is often used when patients are intolerant to cholestyramine.\(^{(17)}\) Another choice is the opiate antagonist drugs naloxone and naltrexone that may cause opiate withdrawal-like symptoms.

The effectiveness of rifampicin has been demonstrated by several studies. In a clinical study, six patients with primary biliary cirrhosis were treated with a daily dose of 600 mg rifampicin for 2 weeks. Five of them experienced a pronounced decrease of their pruritus.\(^{(12)}\) Rifampicin has been used to treat pruritus of 33 children with chronic cholestatic liver diseases with the median dose 5 mg/kg/day and duration 36 weeks.\(^{(18)}\) Five patients (15%) had complete relief of pruritus, and 12 (36%) a partial response.

Recent studies have shown that the mechanism of the effect of rifampicin is mediated by a nuclear receptor, pregnane X receptor (PXR), an integral component of the body defense mechanism against chemical insult.\(^{(11,13)}\) PXR activates many genes to detoxify xenobiotics by increasing their metabolism.\(^{(13a)}\) The genes so activated by PXR benefit cholestasis.\(^{(17)}\) The encoded enzymes and transporters involved in bile acids biosynthesis, detoxification and transportation are listed in Table 1.

![image:2]

**MECHANISM: ROLE OF CYP3A4 IN THE RIFAMPICIN TREATMENT**

It is well established that CYP3A4 is activated by environmental inducers mediated by PXR to protect the body from harmful substrates.\(^{(16,19,20,21,22)}\) Rifampicin can also activate PXR, which in turn, increases expression of CYP3A4 to accelerate metabolism of toxins and drugs. In cholestasis, activation of CYP3A4 produces bile acids that are less toxic.\(^{(23,24,25)}\)

CYP3A4 is the most abundant P450 enzyme in the liver. It is responsible for the oxidative metabolism of a wide variety of substrates including steroid catabolism and metabolism of foreign compounds, with the majority of pharmaceutical compounds being substrates for CYP3A4.\(^{(26,27)}\) The reactions catalysed by CYP 3A4 include N-oxidation, C-oxidation, N-dealkylation, O-dealkylation, nitro-reduction, dehydration and C-hydroxylation.\(^{(26,27)}\)

It has been demonstrated that CYP is involved in the 6 alpha-hydroxylation of both taurochenodeoxycholic acid and lithocholic acids. The resulting bile acids are less toxic.\(^{(28,29)}\) The relative cytotoxicity of each bile acid is attributable to its hydrophobicity, and both ring hydroxylations and conjugation reduce hydrophobicity and effectively detoxify bile acids as well as rendering them accessible to excretory transporters and so facilitate its excretion in the feces or urine.\(^{(30)}\) Thus, rifampicin induction of CYP3A4 mediated by PXR is an important step in the detoxification of bile acids accumulated in cholestasis.

The mechanism of how rifampicin activates CYP3A4 mediated by PXR has been elucidated. Firstly, PXR binds to response elements of the CYP3A4 gene and confers transactivation. PXR binds to CYP3A promoters together with 9-cis retinoic acid receptor (RXR) as a heterodimer to ER6 (everted repeat with a 6 bp spacer) elements.\(^{(29,30)}\) Secondly, rifampicin has been identified as a PXR activator by binding to PXR and modulates PXR binding to PXR elements of CYP3A4 promoter.\(^{(31,32)}\)

**MECHANISM: ROLE OF CYP7A1 IN THE RIFAMPICIN TREATMENT**

PXR could inhibit cholesterol 7α-hydroxylase (CYP7A1) and inhibit bile acids biosynthesis.\(^{(33,34)}\) CYP7A1 is a hepatic enzyme in the neutral pathway for the conversion of cholesterol into bile acids.\(^{(33)}\) It is the first and rate-limiting step and thus plays key roles in cholesterol/bile acid homeostasis.\(^{(33)}\) The enzyme is feedback down-regulated at the transcriptional level by bile acids.

In human primary hepatocyte cell culture, rifampicin reduced CYP7A1 mRNA expression as determined by quantitative real-time PCR.\(^{(33)}\) In another study performed in HepG2 cell culture, treatment of rifampicin also resulted in repression of CYP7A1 expression.\(^{(33)}\) This effect was reversed by the addition of a PXR small interfering RNA to block PXR function.

The mechanism was further demonstrated when rifampicin was found to enhance PXR interaction with HNF4alpha and therefore reduce the PGC-1alpha (peroxisome proliferator-activated receptor gamma coactivator) interaction with HNF4alpha that strongly induces CYP7A1 expression.
Chromatin immunoprecipitation assay showed that PXR, HNF4alpha and PGC-1alpha bound to CYP7A1 chromatin, and rifampicin dissociated PGC-1alpha from chromatin. Consequently, cholesterol conversion to bile acids was reduced.

MECHANISM: ROLE OF BILE ACIDS TRANSPORTERS IN THE RIFAMPICIN TREATMENT

Rifampicin also acts via PXR on bile acids transporters that pump bile acids out from or into hepatocytes. These transporters include the multidrug resistance-associated protein 2,3 (MRP2, MRP3) and Na+-independent organic anion-transporting polypeptides 2 (OATP2).

MRP2 is an ATP-binding cassette transporter. It transports a wide range of conjugated and unconjugated organic anions including bile acids, bilirubin and drugs into bile. MRP2 mRNA is regulated by nuclear receptors PXR, FXR (farnesoid X-receptor) and CAR (constitutive androstane receptor).

It has been shown that MRP2 level is regulated by rifampicin in hepatocytes. As low as 1 µM of rifampicin resulted in significant increase of MRP2 mRNA levels paralleled with increased CYP3A4 mRNA. The consequence of rifampicin-induced MRP2 expression is the increased excretion of bile acids from hepatocyte into bile.

MRP3 ia a basolateral efflux transporter that transports bile acids. The expression of MRP3 in rat and human liver is low under normal condition but is induced in cholestasis or in the absence of MRP2 or bile salt export pump.

In HuH7 cells, treatment of rifampicin can induce MRP3 mRNA levels 4 folds. A similar result was also obtained from HepG2 cells although the induction fold is only 2 fold. Thus, induction of MRP3 could play important role in cholestasis treatment by rifampicin.

OATP2, a membrane transporter mediating sodium-independent hepatocellular uptake of bile salts, is also involved. Concomitant PXR-dependent up-regulation of OATP2 and CYP3A4 represents an important constitutive response in the hepatic detoxification of cholestatic bile salts.

PROBLEMS IN RIFAMPICIN TREATMENT

However, rifampicin treatment has been also reported to cause hepatotoxicity. This was originally described in 1974 when rifampicin was used together with other drugs for 11 patients with tuberculosis, with 10 of them having hepatitis. Bachs reported two cases of hepatitis caused by using rifampicin only in 1992.

In a recent investigation of 41 patients treated with rifampicin, 50% patients had increases in their liver function tests (bilirubin, alanine transaminase, or alkaline phosphatase). Among them, three cases were reported to have hepatitis. Two of them had impaired hepatic synthetic function detected by increased prothrombin time and decreased serum albumin.

PROSPECTIVE APPROACHES TO IMPROVE THE TREATMENT

There are probably two ways to find a better treatment for cholestasis. The first is to modify the chemical structure of rifampicin to increase its ability to reduce bile acids accumulation in cholestasis and decrease its hepatotoxicity. The structure complex of rifampicin has provided many opportunities to generate pharmacologically useful analogs.

Numerous derivatives have been synthesized from natural products for the better treatment of tuberculosis through the modification at the C-3 position of the naphthoquinone chromophore, such as rifabutin and rifapentine. The new derivatives have different properties. Rifabutin has low ability to induce PXR and been used in the treatment of HIV to reduce drug-drug interactions between anti-tuberculosis and HIV proteinase inhibitors. Rifapentine has longer half life and reduced dosing interval compared to rifampicin. Thus, the potential to modify rifampicin for better derivatives in the treatment of cholestasis is great.

Another alternative is to use other PXR inducers that have the ability to induce PXR but have lower hepatotoxicity. The range of PXR inducers are very broad as listed in Table 2. Indeed, some of them have been studied such as phenobarbital, St John’s wort. However, their effectiveness is not as good as rifampicin.

CONCLUSIONS AND DISCUSSION

The use of rifampicin in the treatment of cholestasis has been demonstrated to be mediated by PXR. Activation of PXR co-coordinately regulates genes involved in bile acids biosynthesis, detoxification and transportation. Thus, it reduces bile acids biosynthesis by inhibition of CYP7A1, increases detoxification by induction of CYP3A4, increases...
uptake of bile acids from blood by induction of OATP2 and increases pumping out of bile acids from hepatocyte by induction of MRP2 and MRP3.

These effects could co-ordinated with the CYP changes in cholestasis. There are three types of CYP alterations in bile duct-ligated rats. In general, CYPs were impaired by bile acids due to their detergent effects. However, sex-specific CYPs were subjected to regulation of increased serum estradiol concentration in the model with male-specific 2C11 disproportionally decreased and female-specific 2C12 up-regulated. In addition, CYPs in the bile acids synthesis pathway are regulated by bile acids. For example, it was shown that lithocholic acid itself could activate CYP3A4 mediated by FXR and farnesoid X receptor (FXR), another nuclear receptor. Cholesterol 7α-hydroxylase is inhibited by bile acids by FXR. Thus, rifampicin could exit synergistic effects together with protective effects activated by increased bile acids in cholestasis.

The major problem for the use of rifampicin in the treatment of cholestasis is its inherent hepatotoxicity. This could be overcome by synthesizing rifampicin derivatives from modification of its chemical structure. Another option is to find another suitable inducers among the wide range of chemicals that activate PXR.

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References
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