Choleretic Effect of *Curcuma longa* and Verapamil in Mice

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**Abstract:** Choleretic drugs are used in some conditions e.g., fat malabsorption. Verapamil is a calcium channel blocker, which inhibits p-glycoprotein (P-gp) and may affect bile accumulation. *Curcuma longa* is traditionally used for treatment of malabsorption and as food additive. In this study, the effect of verapamil and *Curcuma longa* was compared on bile accumulation in gall bladder of mice. This simple animal model is approved and used for evaluation and screening pharmacological effect of agents on bile production or secretion. In this model, the mice are off-feed for 24 h. The mice were divided in 3 groups. Saline was administrated in group 1, an aqueous suspension of *Curcuma longa* and verapamil was orally administrated in groups 2 and 3 respectively. One hour after drug administration, the mice were euthanized and gall bladder was immediately removed. The weight of bile was measured in each mouse and compared between groups. The mean of bile weight was 4.67±0.615, 14.33±0.792 and 14.17±1.641 mg in groups 1 to 3 respectively. Verapamil as well as *Curcuma longa* significantly increased the accumulation of bile in gall bladder in comparison to saline. Thus verapamil and *Curcuma longa* may similar action on bile secretion via p-glycoprotein.

**Key words:** *Curcuma longa*, choleretic effect, mice, verapamil

**INTRODUCTION**

Bile production and secretion is changed by several factors including nutrition, diseases and drugs. Choleretic drugs are used in some conditions such as fat malabsorption. The Choleretic drugs stimulate production or secretion the bile. Ultimately, drugs and their metabolites which present in bile pass into the intestinal tract during the digestive process. P-Glycoprotein (P-gp) also is involved in the excretion of endogenous compounds (Groen et al., 1995; Eytan and Kuchel, 1999; Leung and Bendayan, 1999; Johnston et al., 2000; Hardman and Limbird (2001) and this carrier has a significant impact on systemic and tissue/cellular bioavailability of drugs (Fromm, 2000; Liu and Hu, 2000; Hendriks and Vaalburg, 2002).

The inhibition of canalicular glycoprotein transporters has been suggested as the cause of familial intrahepatic cholestasis. Mutations in multidrug resistance 3-glycoprotein (MDR3) gene induce progressive familial intrahepatic cholestasis type 3 (PFIC3) (Belinsky et al., 2005; Elamiri et al., 2003). PFIC2 patients secrete less than 1% of bile salts compared with normal infants (Wang et al., 2001). Phenotypically, mutation in mdr2 in mice resembles the disruption of MDR3 in human. Secondly, mutation in the bile salt exporting pump (BSEP)/sister of P-glycoprotein (spgp) gene causes progressive familial intrahepatic cholestasis type 2 in human. However, in spgp knock-out mice only a mild persistent cholestasis occurs (Elamiri et al., 2003; Lam et al., 2005).

Multidrug resistance especially in cancers relates to P-gp function (Orlowskis and Garrigos, 1999; Srinivas et al., 2006). Also, P-gp inhibition or induction can affect pharmacokinetics of drugs and may cause drug interaction (Verschraagen et al., 1999; Yu, 1999). Class HI multidrug resistance (MDR) P-glycoproteins (P-gp), mdr2 in mice and MDR3 in man; mediate the translocation of phosphatidylcholine across the canalicular membrane of the hepatocyte. Mice with a disrupted mdr2 gene completely lack biliary phospholipid excretion and develop progressive liver disease, characterized histologically by portal inflammation, proliferation of the bile duct epithelium, and fibrosis. This disease phenotype is very similar to a subtype of progressive familial intrahepatic cholestasis. It was demonstrate that mutations in the human MDR3 gene lead to progressive familial intrahepatic cholestasis with high
serum γ-GT. The histopathological picture in these patients is very similar to that in the corresponding mdr2(-/-) mouse, in which mdr2 P-gp deficiency induces complete absence of phospholipid in bile (De Vree, 1998).

Curcuma longa L., which belongs to the Zingiberaceae family, is a perennial herb that measures up to 1 m high with a short stem, distributed throughout tropical and subtropical regions of the world, being widely cultivated in Asiatic countries, mainly in India and China. Its rhizomes, a powder, called turmeric, it has been in continuous use for its flavoring, as a spice in both vegetarian and non-vegetarian food preparations and it also has digestive properties. Current traditional Indian medicine claims the use of its powder against biliary disorders, anorexia, coryza, cough, diabetic wounds, hepatic disorder, rheumatism and sinusitis (Ammon and Wahl, 1991; Araújo and Leon, 2001; Chainani-Wu, 2003; Zeng et al., 2007).

The extract from C. longa specifically inhibits gastric acid secretion by blocking H2 histamine receptors in a competitive manner. C. longa has specifically antidepressant effects in vivo. The activity of C. longa in antidepressive may mediated in part through MAO A inhibition in mouse brain (Yu et al., 2002; Kim et al., 2005). The main constituents of the extract were identified as curcumin, demethoxycurcumin, bisdemethoxycurcumin, and ar-turmerone. Both curcuminoids and sesquiterpenoids in turmeric exhibit hypoglycemic effects (Kuroda et al., 2005; Nishiyama et al., 2005).

Verapamil, a calcium channel blocker, is specific inhibitor of the P-glycoprotein (mdr1) in the hepatocyte canalicular membrane and other tissue. This drug is used as antihypertensive and antiarrhythmic agent (Mickisch et al., 1991; Barth et al., 2006).

Thus in this study, the effect of verapamil and Curcuma longa was compared on bile accumulation in gall bladder of mice for evaluation their potency and possible mechanism.

MATERIALS AND METHODS

This study was conducted in Department of pharmacology and toxicology Shahid Chamran University, Ahvaz, Iran during 2008-2009.

Mice were purchased from lab animals' research center, Jundishapour university of Ahvaz. Mice (NMRI strain) with 20±3 grams weight were divided 3 groups (6 mice in each group). The mice were kept without feed but water ad libitum in 24 hours prior study.

In day of study, in group 1, saline was administrated (as control group). Suspension of Curcuma longa was orally administrated in 20 mg/10 g body weight of mice in group 2. This suspension was prepared by 600 mg powder of Curcuma longa and 10 mL distilled water. The volume of suspension for each mouse was nearly 0.5 mL. Suspension of verapamil was orally administrated in 0.24 mg/10 g body weight of mice in group 3. One hour later, the mice were euthanized by diethyl ether and immediately the gall bladder was carefully removed. The weight of gall bladder (with bile) for each mouse was measured with analytical scale. Then, the bile is extracted from gall bladder and again it was weighted. The difference of weight of full and empty gall bladder was concerned as amount of bile.

The mean of amount of bile in groups was compared by SPSS program and ANOVA test. The p-values less than 0.05 were concerned as significant.

RESULTS AND DISCUSSION

The mean of bile in gall bladder was 4.67±0.615 mg in group 1 as control. This amount was 14.33±0.792 mg in group 2 that received Curcuma longa. The mean of bile in gall bladder was 14.17±1.641 mg in group 3 that received verapamil.

The bile accumulation in gall bladder was significantly increased by Curcuma longa and verapamil in comparison to saline (p = 0.001).

Final effect of verapamil and Curcuma longa was similar on the bile accumulation probably by same mechanism on P-glycoprotein. P-glycoprotein (Mdr1) is capable of transporting bile acids (Lam et al., 2005). Verapamil was studied for its effects on secretive function of liver in rats. In the animals with low initial level of bile secretion, infusion of verapamil resulted in increase of the bile flow. In the animals with high initial level of the liver secretive function, verapamil decreased the bile flow. The changes of bile flow and biliary secretion of bile acids and lipids in two groups of animals suggest that verapamil could be influenced in regulation of bile secretion depending on its initial level (Tukaev et al., 2002).

The similar effects of Verapamil and Cyclosporin A, on bile acid transport and metabolism, can be explained by mdr1 mediated disturbances of cellular ATP transport rather than by inhibition of individual bile acid transporters (Barth et al., 2006).

The effects of verapamil on the dopamine-induced pancreatic exocrine secretion were investigated in the isolated and blood-perfused canine pancreas. Dose-related increases in the volume of pancreatic secretion induced by dopamine (1-10 micrograms) were reduced by infusion of 50 and 100 μg/min of verapamil (Iwatsuki et al., 1986).

The effects of various P-glycoprotein transporters is evaluate on bile formation and the canalicular transport of taurocholic acid in an attempt to understand the combined role of these transporters in the pathogenesis of familial intrahepatic cholestasis. Total bile acid and cholic acid secretion rate were decreased in the mdr2 knock-out mice.
However, bile flow and the secretion of muricholic acids were increased (Elamiri et al., 2003).

The pathogenesis of manganese-bilirubin (Mn-BR) induced cholestasis has only been studied in rats and is associated with alteration in the hepatic homeostasis of cholesterol and phospholipids. Multidrug resistance-2 (mdr2) transporter, which mediates excretion of these lipids, is suggested to be involved in this phenomenon. Akoume et al. (2004) examined the role of mdr2 in its pathogenesis, using mice with disrupted mdr2 gene (mdr2 (-/-)). Results showed that Mn-BR combination decreased bile flow in mice. These data indicate that Mn-BR induced cholestasis is reproducible in mice (Akoume et al., 2004).

The knockout of spgp gene in mice results in intrahepatic cholestasis, but with significantly less severity than PFIC2 in humans. Notably, although the secretion of cholic acid in mutant mice is greatly reduced (6% of wild-type), total bile salt output in mutant mice is about 30% of wild-type. Also, secretion of an unexpectedly large amount of tetra-hydroxylated bile acids (not detected in wild-type) is observed. These results suggest that hydroxylation and an alternative canalicular transport mechanism for bile acids compensate for the absence of Spgp function and protect the mutant mice from severe cholestatic damage. In addition, the spggp-/- mice display a significant increase in the secretion of cholesterol and phospholipids into the bile. This latter observation in spggp-/- mice suggests that intrahepatic, rather than intracanalicular, bile salts are the major driving force for the biliary lipid secretion. The spggp-/- mice thus provide a unique model for gaining new insights into therapeutic intervention for intrahepatic cholestasis and understanding mechanisms associated with lipid homeostasis (Wang et al., 2001).

Former studies have shown that curcumin, which can be extracted from different Curcuma species, is able to stimulate bile flow in rats, whereas bisdemethoxycurcumin, which is mainly found in rhizomes of Curcuma longa, is believed to inhibit bile flow. The influence of both curcuminoids on bile flow, bile acid concentration and excretion over a time period of 180 min in the bile fistula model in rats. Furthermore, it was tested the ability of both curcuminoids to reduce cyclosporin-induced cholestasis. The choleretic effect of bisdemethoxycurcumin lasted longer than that of curcumin. However, only bisdemethoxycurcumin statistically significantly attenuated cyclosporin-induced reduction of bile acid excretion (Deters et al., 1999).

In conclusion, from present study it is concluded that Verapamil has choleretic effect similar to Curcuma longa in mice as experimental model. The important finding of this study is the choleretic effect of Curcuma longa may be carried by p-glycoprotein (P-gp). But more study is needed for finding the exact its mechanism and this effect of Curcuma longa can be evaluated in human.

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