# ABSTRACT

Ischemia reperfusion (IR) injury is a common phenomenon associated with the surgical treatment of hepatocellular carcinoma (HCC) and metastatic cancer of the liver. Rapamycin, an effective immunosuppressant and inhibitor of growth of cancer cells, is often employed in the management of HCC patients who undergo liver resection or liver transplantation. Rapamycin has also been shown to induce the synthesis of the antioxidant enzymes heme oxygenase 1 (HO-1) and peroxiredoxin 1 (Prx-1). However, rapamycin also inhibits bile flow. Oltipraz, is a very effective inducer of antioxidant enzymes and inhibits cancer cell growth, is currently undergoing clinical trials as a chemopreventive agent for the treatment of HCC. Strategies employing pre-treatment with rapamycin and oltipraz have the potential to reduce IR injury. However, their abilities to induce antioxidant enzymes and potential detrimental effects on both normal and transformed liver cells are not well understood. Therefore, the aim of this study was to characterize the abilities of rapamycin and oltipraz to induce the expression of HO-1 and Prx-1 in normal and transformed liver cells. Another aim of this study was to determine the effects of rapamycin and oltipraz on the expression of bile acid transporters in normal and transformed liver.

To achieve these goals, a rat model of segmental hepatic ischemia and reperfusion, isolated cultured rat hepatocytes and transformed rat liver (H4IIE) cells were employed. Quantitative PCR (qPCR) was conducted for measurement of the expression of mRNA encoding antioxidant enzymes and bile acid transporters. Probe-based strategies, β-actin as reference RNA, and the ΔΔCT method were employed.

Rapamycin increased HO-1 and Prx-1 mRNA expression in rat liver *in vivo* and in cultured rat hepatocytes. On the other hand, rapamycin inhibited expression of mRNA encoding the sinusoidal bile acid transporters Ntcp, Oatp1 and Oatp2 mRNA. But rapamycin induced the canalicular transporter Mrp2 and Bsep mRNA expression in cultured rat hepatocytes. However, in transformed rat liver (H4IIE) cells rapamycin inhibited HO-1 and Prx-1 expression whereas oltipraz induced HO-1 and Prx-1 mRNA expression. Expression of three bile acid transporters, Bsep, Bcrp and Oatp9, were not detected in H4IIE cells. Moreover, the relative expression of mRNA encoding some bile acid transporters in H4IIE cells were significantly different

compared to that in normal rat hepatocytes. Rapamycin induced the sinusoidal transporters Ntcp and Oatp1 mRNA expression but inhibited Oatp2 mRNA expression in H4IIE cells. In contrast, oltipraz inhibited sinusoidal transporters Ntcp, Oatp1 and Oatp2 mRNA in H4IIE cells. Rapamycin and oltipraz both induced Mrp2 mRNA expression in H4IIE cells.

It is concluded that pharmacological pre-treatment with rapamycin may not be so effective in reducing IR injury since the induction by rapamycin of antioxidant enzyme expression in normal liver cells is modest, while in transformed cells expression of antioxidant enzymes is inhibited. The inhibition of bile flow associated with pre-treatment with rapamycin is likely due to inhibition of the expression of sinusoidal BA transporters in normal liver cells. In the clinical treatment of HCC patients with rapamycin, attention needs to be paid to the blood concentration of the drug as different concentrations can have markedly different effects on expression of antioxidant enzymes and BA transporters. The ability of transformed liver cells to transport bile acids is likely impaired compared to that of normal liver cells, and the actions of both rapamycin and oltipraz on the expression of bile acid transporters are mixed. Further studies are warranted to determine conditions under which pre-treatment with oltipraz can be effective to reduce IR injury with minimal effects on bile flow.