Novel Mechanisms of Toxic Bile Salt-Induced Hepatocellular Apoptosis

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Cholestatic liver injury results from the accumulation of toxic bile salts within the liver. The aim of the present study was to understand the mechanism of bile salts-induced hepatocellular apoptosis in bile duct-ligated (BDL) rats, using Western blot and immunohistochemical analysis. The mechanisms of hepatoprotective effects of *Salvia miltiorrhiza* (SM) was also studied in this model. Apoptotic cell death was increased five-fold after three days of BDL, decreased over two weeks and remained constant thereafter. Total cellular Bax protein was increased three days after BDL and decreased over time thereafter. We observed the translocation of Bax to mitochondria and subsequent release of cytochrome *c*. Nuclear p53 increased three days after BDL, but most p53 was expressed in the cytoplasm after one week. The expression of c-Myc was inhibited by three days, but increased at later stages following BDL. Bcl-2 was increased over time in BDL rats. Fas expression was not changed and activation of caspase 8 did not occur. Fas immunoreactivity was exclusively observed in the cytoplasm of hepatocytes, indicating that Fas expressed in rat hepatocytes is predominantly a soluble form. Thus our data suggest that nuclear p53 regulates the expression of Bax in BDL rats. The translocation of Bax to mitochondria supports the hypothesis that this may lead to release of cytochrome *c* and transduce the signal for apoptotic death of hepatocytes by toxic bile salts. The lack of changes in Fas suggests that Fas may not be playing a role in apoptosis in this model.

Salvia miltiorrhiza BUNGE, a traditional chinese herbal medicine, has been commonly used to treat chronic hepatitis and liver fibrosis. However, the machanism of action is not yet fully understood. The treatment of extract of SM to BDL rat reduced the number of TUNEL positive cells to the control level after 10 days of BDL. The expression of Bax was reduced with time by the treatment of SM. The treatment of SM accelerated the cytoplasmi sequesteration of p53. The expression of Bcl-2 was increased, hence the Bax to Bcl-2 ratio was decreased significantly. These results suggest that SM may used as hepatoprotective agent in extrahepatic cholestasis model. (This research was supported by a grant (PF002105-01) from Plant Diversity Research Center of 21st Frontier Research Program funded by MOST and Spella Co.)