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**A Ginseng Saponin Metabolite-Induced Apoptosis in HepG2 Cells Involves a Mitochondria-Mediated Pathway and its Downstream Caspase-8 Activation and Bid Cleavage**

Seon-Hee Oh, Bang-Wool Lee , Hu-Quan Yin, Hyun-Mi Kim, and Byung-Hoon Lee  
*College of Pharmacy, Wonkwang University*

20-O-( $\beta$ -D-Glucopyranosyl)-20(S)-protopanaxadiol (IH901), an intestinal bacterial metabolite of ginseng saponins formed from ginsenosides Rb1, Rb2 and Rc, is suggested to be a potential chemopreventive agent. Here we show that IH901 induces apoptosis in human hepatoblastoma HepG2 cells. IH901 led to an early activation of procaspase-3 (6 h posttreatment), and the activation of caspase-8 became evident only later (18 h posttreatment). Caspase activation was a necessary requirement for apoptosis because caspase inhibitors significantly inhibited cell death by IH901. Treatment of HepG2 cells with IH901 also induced the cleavage of cytosolic factors such as Bid and Bax and translocation of truncated Bid to mitochondria. A time-dependent release of cytochrome c from mitochondria was observed, which was accompanied by activation of caspase-9. zVAD-fmk and zIETD-fmk abrogated Bid processing and translocation, cytochrome c release and caspase-3 activation. The activation of caspase-8 was inhibited not only by zIETD-fmk but also by zVAD-fmk, which is known to inhibit caspase-1, -3, -4 and -7. The results, together with the kinetic change of caspase activation, indicate that activation of caspase-8 occurred downstream of caspase-3 and -9. These results suggest that the activation of caspase-8 after early caspase-3 activation might act as an amplification loop necessary for successful apoptosis. Levels of neither Fas mRNA nor protein were changed by IH901. Preincubation of HepG2 cells with antagonistic anti-Fas antibody showed little protective effect, if any, on IH901-induced cell death, indicating the possibility that the Fas/FasL system is not involved in IH901-induced apoptosis in HepG2 cells. Primary hepatocytes isolated from normal SD rats were not affected by IH901 (0-60  $\mu$ M). The very low toxicity in normal hepatocytes and high activity in hepatoblastoma cells suggest that IH901 is a promising cancer chemopreventive agent.

**Keyword** : ginseng saponin metabolite, HepG2, apoptosis, caspase, bax/bid, cytochrome c