The molecular mechanism of 20(S)-Protopanaxdiol, a metabolite of ginseng, induced hepatocellular carcinoma HepG2 cell apoptosis and new ginsenosides from the root of panax ginseng C. A. Meyer
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The Molecular Mechanism of 20(S)-Protopanaxdiol, A Metabolite of Ginseng, Induced Hepatocellular Carcinoma HepG2 Cell Apoptosis and New Ginsenosides from the Root of *Panax ginseng* C. A. Meyer

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ABSTRACT

Ginseng, clinically used for thousands of years in China, is now one of the most popular phytomedicines worldwide. Ginsenosides are widely accepted as pharmacologically active components of ginseng from which more than 80 protopanaxadiol (PPD) and protopanaxatriol (PPT) type ginsenosides have been identified and characterized. Upon oral consumption, PPD and PPT type ginsenosides were partly transformed into their respective end metabolites, PPD and PPT. It has been reported that PPD has cytotoxicity on various cancer cell lines, but the molecular mechanism of the action is largely undefined. In this study, we showed the chemical diversity and complexity of ginsenosides in the root of *P. ginseng* and the molecular mechanism of PPD-induced HepG2 cell apoptosis.

Chapter 3 demonstrates the phytochemical studies on the n-butanol fraction of ethanol extracts of root of *Panax ginseng* which led to the isolation of 41 compounds. Six new PPT-type ginsenosides (ginsenosides Re1~6, 1-6), six new PPD-type ginsenosides (ginsenosides Ra4~9, 7-12) and a new phenolic glycoside [4-(1-E-propenyl)-2-methoxyphenyl 1-O-β-D-apiofuranosyl (1→6)-β-D-glucopyranoside, 13] were determined together with twenty eight known compounds including ten PPT-type ginsenosides [ginsenosides Re (14), Rf (15), Rg1 (16), Rg2 (17), 20-gluco-ginsenoside Rf (18), koryoginsenoside R1 (19), notoginsenoside R1 (20), R2 (21), N (22), and yesanchinoside D (23)], fourteen PPD-type ginsenosides [ginsenoside Ra1 (24), Ra2 (25), Rb1 (26), Rb2 (27), Rb3 (28), Rc (29), Rd (30), Rs1 (31), Rs2 (32), malonyl-ginsenoside Rb1 (33), gypenoside XVII (34), pseudoginsenoside RC1 (35), quinquenoside R1 (36), and vinaginsenoside R16 (37)], an oleanane-type triterpenoid [ginsenoside Ro (38)], two phenolic glycosides [4-allyl-2-methoxyphenyl 6-O-β-D-apiosyl(1 → 6)-β-D-glucoside 39], twelve other known compounds (40-51).
(39) and eugenol 4-O-rhamnosyl (1 → 2) glucoside (40)] and a purin glycoside [adenosine (41)]. Their structures were elucidated by using spectroscopic methods including 1D and 2D-NMR, high resolution MS and chemical transformation. The unusual α-D-glucopyranosyl-(1→3)-β-D-glucopyranosyl side chain (as found in compounds 1 and 2) and the eugenol derivatives (compounds 13, 39 and 40) were reported in the genus Panax for the first time.

Studies in Chapter 4 showed that PPD inhibited the cell growth and induced apoptosis in human hepatocarcinoma HepG2 cells. PPD induced the intrinsic as well as the extrinsic apoptotic pathways. PPD treated cells showed a massive cytoplasmic vacuolization and a dramatic change of Endoplasmic reticulum (ER) morphology. The occurrence of ER stress was supported by the observations that PPD treatment affected the ER stress-associated genes and proteins expression. PPD induced the phosphorylation of PERK and eIF2α, the splicing of XBP1 mRNA, and the cleavage of AFT6, suggested that PPD activated the three previously described arms of unfolded protein response (UPR). These observations suggest that PPD-induced apoptosis involves an ER stress response. Knockdown of one of the three UPR limbs by specific siRNA did not affect the PPD-induced apoptosis but transfection of cells with CHOP siRNA significantly reduced the PPD-mediated apoptosis. Western blotting analysis showed that PPD-stimulated downregulation of Bcl-2 protein, increase of DR5 protein, activation of caspase-8 and cleavage of PARP were significantly inhibited in CHOP knockdown cells. Taken together, these observations support the hypothesis that PPD induces HepG2 cell apoptosis through ER stress pathway which may provide a new target pathway for cancer chemotherapy using ginsenosides.
In conclusion, this study provides a new insight to the role of ER stress in PPD-induced HepG2 cell apoptosis and the exploitation of these findings may facilitate the establishment of a new molecular target of PPD and PPD-type ginsenosides and lead to the development of new PPD analogs for cancer chemotherapy. As for phytochemical study of ginseng, 13 new compounds have been isolated and characterized. The existence of these new components illustrates the chemical diversity and complexity of saponins in P. ginseng.
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