Mechanism of N-(4-hydroxyphenyl)retinamide-induced apoptosis

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Background The synthetic retinoid N-(4-hydroxyphenyl)retinamide (4HPR) induces apoptosis in a variety of human cancer cells, and inhibits carcinogenesis of breast, bladder, lung, ovarian, and prostate cancer in animal model. It has been suggested that nuclear retinoid receptors, reactive oxygen species (ROS), ceramide, and some other unknown signals mediate 4HPR-induced apoptosis. To determine the mechanism of 4HPR-induced apoptosis, we investigated the possible role of ceramide, an important second messenger in the signaling of apoptosis, and galectin-3, an inhibitor of apoptosis.

Methods We treated cervical carcinoma (C33A), lung adenocarcinoma (H522), and head and neck squamous cell carcinoma (22B) cell lines with 4HPR and different inhibitors of ceramide-mediated signaling pathway and measured the levels of ceramide with mass spectrometry (MS). In addition, galectin-3 transfected breast carcinoma cell line (BT549) was treated with 4HPR and analyzed for apoptosis, ROS generation, and expression of apoptosis-related proteins.

Results After 1-3 days of treatment, 5 μM 4HPR inhibited the growth of C33A, H522, and 22B cell lines by 70-80%. Fumonisin B1 (FB1), an inhibitor of ceramide synthase, sphingosine-1-phosphate, which inhibits activation of caspase-3 by ceramide, and 12-O-tetradecanoylphorbol-13-acetate, an activator of protein kinase-C which counteracts ceramide-mediated apoptosis did not decrease the cytotoxicity of 4HPR in any of the three cell lines. However, antioxidant butylated hydroxyanisole (BHA) inhibited the effect of 4HPR on these cell lines. MS demonstrated that 4HPR significantly increased the levels of several ceramide species compared to controls in all three cells. BHA did not inhibit the increase in the ceramide levels after 4HPR treatment, while FB1 almost completely blocked the 4HPR-induced increase in the ceramide levels. In galectin-3 study, both vector and mutant galectin-3 transfected BT549 cell lines underwent significant apoptosis after treatment with 4HPR (5 μM). 4HPR stimulated ROS generation, which was followed by bcl-2 down regulation, release of cytochrome c, increased caspase-3 activity, and PARP cleavage in vector and mutant cell lines. However, all these effects of 4HPR including ROS production as well as the induction of apoptosis were significantly inhibited in galectin-3 overexpressing cell line.

Conclusion These two studies suggest that increased ROS generation is an important early event of 4HPR-induced apoptosis in various carcinoma cell lines, while ceramide does not play a critical role in lung, head and neck, and cervical carcinoma cell lines.