International Journal of Oriental Medicine 2000 1(2), 1-5 **Review**

Induction of apoptosis and inhibition of cancer cell growth by Oriental medicine

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Recently, considerable attention has been focused on identifying naturally occurring chemopreventive substances capable of inhibiting, retarding, or reversing the multi-stage carcinogenesis. In this article, the studies, which reported oriental medicine-induced apoptosis and its mechanism, will be reviewed.

Among various oriental medicines, two components, arsenic trioxide and bufalin, were massively studied being focused on apoptosis.

Arsenic trioxide (As_2O_3), a major ingredient of arsenic compounds in traditional Chinese medicine, have been recorded to have therapeutic effects on the treatment of psoriasis, syphilis, rheumatosis and a number of malignant tumours. The As_2O_3 had been known a very effective treatment for acute promyelocytic leukemia (APL). A study of cellular and molecular mechanisms of this treatment by using NB4 cells as a model suggested that induction of apoptosis could be one of the mechanisms of the therapeutic effect of As_2O_3. This apoptosis induction occurred independently of the retinoid pathway and was mediated, at least partly, through the modulation of bcl-2, as well as PML-RAR alpha and or PML proteins (Chen *et al.*, 1996). As_2O_3 was also demonstrated to inhibit the growth and survival of oesohageal carcinoma cell line EC8712 by apoptosis (Shen et al. 1999). In addition, a study reported that As_2O_3 induced apoptosis of HCE16/3 cell (HPV 16 DNA-immortalized human cervical epithelial cells) and selectively inhibited expression of viral early gene in HCE16/3 cell. According to the study, induction of apoptosis of HCE16/3 cells by As_2O_3 treatment might be associated with down-regulation of viral oncogene expression (Zheng *et al.*, 1999). Another study showed that As_2O_3 inhibited growth and induced apoptosis in six human malignant cell lines, MGC-803, HIC, MCF-7, HeLa, BEL-7402 and A549 cells, at varying degrees, in a time dose-dependent manner. The sensitivity of these cells to As_2O_3 was shown to be correlated with the increase in intracellular Ca^{2+}, possibly indicating that a critical intracellular Ca^{2+} signal transduction pathway could be involved in As_2O_3-mediated cell-death and its selectivity (Zhang *et al.*, 1999).

Bufalin is an active principle of the Chinese medicine chan'su. Since its selective inhibitory effects on the growth of various human cancer cell was reported, numerous studies have been followed. A study

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demonstrated that the human leukemia cell line HL-60, treated with bufalin, exhibited the growth-inhibitory effect associated with the induction of apoptosis. The result suggested the usefulness of bufalin for differentiationapoptosis-inducing therapy for cancer (Jing *et al.*, 1994). In another study, a low concentration of bufalin was shown to induce apoptosis of a broad range of human leukemia cell lines, such as HL60, ML1, but not in mouse leukemia M1 cells. Bufalin-induced apoptosis in HL60 cells was inhibited by $ZnCl_2$, an inhibitor of endonuclease, but not by cycloheximide, an inhibitor of protein synthesis. The levels of expression of the c-myc and bcl-2 genes in HL60 cells decreased with time after treatment with bufalin. These results suggested that bufalin induced apoptosis specifically in human leukemia cells by altering the expression of these genes involved in apoptosis (Masuda *et al.*, 1995). Bufalin also caused apoptosis in human leukemia U937 cells by anomalous activation of mitogen-activated protein kinase (MAPK) via the signaling pathway of Ras, Raf-1, and MAPK kinase-1 (Watabe *et al.*, 1996). The apoptosis induced by bufalin in U937 cells was significantly inhibited by overexpression of the Bcl-2 protein. No significant difference of the activation of MAPK kinase-1 that was induced by bufalin in wild-type or Bcl-2-overexpressed U937 cells suggested that Bcl-2 act downstream of MAPK kinase-1 (Watabe *et al.*, 1997). According to a recent study, apoptosis was strongly induced by bufalin in cells expressing sense RNA for Taim1. However cells expressing antisense RNA for Taim1 were more resistant than the control bufalin-treated cells. Moreover, sense transformants had elevated activities of Rac1, PAK and c-Jun NH2-terminal kinase (JUK). Accordingly Tam1 might play a critical role in bufalin-induced apoptosis through these activation (Kawazoe, Watabe *et al.*, 1999). In another recent study, bufalin specifically inhibited the Na+, K(+)-ATPase, which act upstream of the bcl-2 protein, of human tumor cells, for example, monocytic leukemia THP-1 cells, humanlymphoblastic leukemia MOLT-3 cells, and human colon adenocarcinoma COLO320DM cells but not normal human leukocytes and not murine leukemia P388D1 and M1 cells and induced apoptosis in human tumor cells selectively via this inhibition (Kawazoe, Aiuchi *et al.*, 1999).

A variety of the other oriental medicine was also investigated focusing on their apoptosis-inducing capability.

Chinonin, a natural antioxidant extracted from a Chinese medicine, was demonstrated to have preventive effects against apoptotic and necrotic cell death of cardiomyocytes in hypoxia-reoxygenation process and its protective mechanisms were related to the antioxidant properties of scavenging nitric oxide and oxygen free radicals, and the modulating effects on the expression levels of bcl-2 and p53 proteins (Shen *et al.*, 2000).

Indirubin, the active ingredient of Danggui Longhui Wan, a mixture of plants that is used in traditional Chinese medicine to treat chronic diseases, was identified as potent inhibitors of cyclin-dependent kinases (CDKs). Indirubin-3'-monoxime inhibits the proliferation of a large range of cells, mainly through arresting the cells in the G_2/M phase of the cell cycle (Hoessel et *al.*, 1999).

Extract of Tripterygium wilfordii Hook. f (TWHf) has immunosuppressive activity and has been used as anti-inflammatory agent in traditional Chinese medicine for centuries. In a study triptolide, the major active component in the extract, which inhibited antigen or mitogen-induced T cell

proliferation, induced T cell apoptosis through activating caspases and growth arrest (Yang *et al.*, 1998).

Elemene, isolated from the Chinese medicinal herb Rhizoma zedoariae, and allicin, a natural organosulfide from garlic, were shown to exhibit antitumor activity in human and murine tumor cells *in vitro* and *in vivo*, and the inhibitory effect of elemene and allicin on proliferation of HL-60 cells was associated with cell cycle arrest from S to G_2M phase transition and with induction of apoptosis (Zheng *et al.*, 1997).

Several Kampo medicines, e.g. Orengedoku -to and San'o-shashin-to, were found to inhibit dexamethasone-induced apoptosis in murine thymocytes. Some of these medicines contain Coptidis rhizoma (CR) as the major constituent. In a study the CR extract showed the most potent inhibitory activity on thymocyte apoptosis of more than 200 species of herbal extracts. The benzodioxolo-benzoquinolizine alkaloid, berberine, and five berberine-type alkaloids were isolated from CR extract and they had an inhibitory effect (Miura *et al.*, 1997).

Coriolus versicolor polysaccharide peptide (CVP) and the bis-benzylisoquinoline alkaloids, tetrandrine (TET) and berbamine (BER), the active ingredients isolated from Chinese medicinal herbs, were known to possess antitumor activities, concentration-dependently inhibited the proliferation of human leukemic HL-60 cells. However, according to a study TET and BER, but not CVP, inhibited the proliferation of HL-60 cells via induction of apoptosis (Dong *et al.*, 1997).

A wide array of phenolic substances, particularly those present in dietary and medicinal plants, have been reported to possess substantial anticarcinogenic and antimutagenic activities. The majority of these naturally occurring phenolics retain antioxidative and anti-inflammatory properties which appear to contribute to their chemopreventive or chemoprotective activity. Recently, it was suggested that these compounds had ability to suppress proliferation of human cancer cells via induction of apoptosis (Surh, 1999).

Shikonin isolated from Lithospermum erythrorhizon, a traditional oriental medicinal herb, also was shown to induce apoptosis in HL60 human premyelocytic leukemia cell line (Yoon Y *et al.*, 1999).

The water fraction of Panax ginseng, one of traditional oriental medicine herbs can exert a potent effect on the recovery of the hair follicles. A study reported that the action might depend on its combined effects on proliferation and apoptosis of the cells in the hair follicle (Kim *et al.*, 1998).

Syzygium Aromaticum (L.) Merr. Et Perry flower bud (SAFB) has been success- fully used for the asthma and many allergic disorders. Recently we studied whether SAFB induced apoptosis of mast cells. In our study, SAFB induced apoptosis of mast cells, and mitochondrial events was also demonstrated in this type of apoptosis.

In conclusion, recent studies on the apoptosis inducing mechanism by various oriental medicine will provide more information of their pharmacological action and scientific basis of clinical application.

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