COMMENTARY

Bufalin, a Traditional Oriental Medicine, Induces Apoptosis in Human Cancer Cells

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Abstract

Bufalin is a traditional oriental medicines which induces apoptosis in some lines of human tumor cells. It constitutes the major digoxin-like immunoreactive component of Chan Su, obtained from the skin and parotid venom glands of toads. Bufalin is cardioactive C-24 steroids that exhibits a variety of biological activities, such as cardiotonic, anaesthetic, blood pressure stimulatory, respiratory and antineoplastic effects. In terms of its anti-tumor activity, bufalin has been demonstrated to inhibit the growth of tumors, such as endometrial and ovarian cancers. This commentary introduces biologic and therapeutic effects of bufalin in treating some cancers. The compound is able to mediate inhibition of cell growth, cell cycle arrest, apoptosis, and expression of genes related to the malignant phenotype in human cancer cells.

Keywords: Traditional oriental medicines - bufalin - cell cycle - apoptosis - cancer

Asian Pacific J Cancer Prev, **13**, 399-402

Overview of bufalin

Bufalin is the major digoxin-like immunoreactive component of Chan Su, a traditional Chinese medicine obtained from the skin and parotid venom glands of the toad (Krenn and Kopp, 1998). Chan Su is the major component of such popular traditional Chinese medications as Liushenwan (Hong et al., 1992), Shexiangbaoxinwan (Song et al., 2000), Lu-Shen-Wan and Kyusin (Hong et al., 1992). These traditional Chinese medications have long been widely applied in China, Japan, Korea, and other Asian countries, and are currently used as alternative medicines (Morishita et al., 1992). The chemical structure of bufalin is shown in Fig. 1. Bufalin and other bufadienolides are cardioactive C-24 steroids that exhibit a variety of biological activities, such as cardiotonic, anesthetic, blood pressure stimulation, respiration and antineoplastic activities (Krenn and Kopp, 1998). In terms of its anti-tumor activities, bufalin has been demonstrated to induce growth inhibition, cell cycle arrest and apoptosis of tumor cells. Bufalin can be safely used for long periods without severe side effects. At high dosages, however, cardioactive steroids cause cardiac arrhythmia, breathlessness, seizure and coma (Panesar, 1992). The structural similarity between bufadienolides and digoxin accounts for the toxic effects.

Preclinical studies

Gynecologic cancers

Bufalin inhibited cell proliferation by the G0/G1-arrest of the cell cycle and by inducing the apoptosis of endometrial cancer and ovarian cancer cells in vitro. In contrast, bufalin has little effect on normal human endometrial epithelial cells, suggesting that the effects of bufalin might be cell-type specific and could be weaker on the normal endometrium. Western blot analysis showed the down-regulation of the expression of cyclin A, bcl-2 and bcl-xL, and the simultaneous up-regulation of p21WAF1 and activated caspase-9 expression in endometrial cancer and ovarian cancer cells (Takai et al., 2008).

Gastric cancer

Bufalin inhibited the proliferation of gastric cancer MGC803 cells in a dose-dependent and time-dependent manner. At a low concentration (20 nmol/l), bufalin induced M-phase cell cycle arrest, whereas at a high concentration (80 nmol/l) it induced apoptosis in MGC803 cells. Bufalin increased the Bax/Bcl-2 ratio and activated caspase-3 during the apoptotic process of MGC803 cells. It should be noted that bufalin transiently activated the phosphatidylinositol 3-kinase (PI3K)/Akt signaling pathway and then inhibited it completely, and upregulated the Casitas B-lineage lymphoma (Cbl) family of ubiquitin ligases, upstream modulators of PI3K. A combination of bufalin and LY294002, a PI3K-specific inhibitor, enhanced apoptosis, but PD98059, an extracellular-regulated protein kinase-specific inhibitor, had no significant effect on bufalin-induced apoptosis. These results suggested that the PI3K/Akt pathway might play a key role in bufalin-induced apoptosis in gastric cancer MGC803 cells (Li et al., 2009).

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Prostate cancer

The antiproliferative and apoptotic mechanisms of bufalin on prostate cancer cells were investigated. Proliferation of LNCaP, DU145, and PC3 cells was measured by 3-(4,5-dimethylthiazol-2-yle)-2,5-diphenyltetrazolium bromide assay and the doubling time (tD) was calculated. Bufalin caused changes in the tD of three prostate cancer cell lines, which were more significant than that of human mesangial cells. In addition, bufadienolides induced prostate cancer cell apoptosis more significantly than that in breast epithelial cell lines. After treatment, the caspase-3 activity and protein expression of caspase-3, -8, and -9 were elevated. The expression of other apoptotic modulators, including mitochondrial Bax and cytosolic cytochrome c, were also increased. However, expression of p53 was only enhanced in LNCaP cells. Downregulation of p53 by antisense TP53 restored the cell viability suppressed by bufalienolides. Furthermore, the increased expression of Fas was more significant in DU145 and PC3 cells with mutant p53 than in LNCaP cells. Transfection of Fas small interfering RNA restored cell viability in the bufadienolide-treated cells. These results suggest that bufalin suppress cell proliferation and cause apoptosis in prostate cancer cells via a sequence of apoptotic modulators, including Bax, cytochrome c, and caspases. The upstream mediators might be p53 and Fas in androgen-dependent LNCaP cells and Fas in androgen-independent DU145 and PC3 cells (Yu et al., 2008).

Bufalin inhibited proliferation of cancer cells at doses of 0.1, 1, or 10 microM after 2-4 days of culture. Cytotoxicity of bufalin on the DU145 and LNCaP cells was dose-dependent. Bufalin increased Ca2+(i) and apoptosis in cancer cells after a 24-hr culture as well as caspase 3 activities in DU145 and PC3 cells and caspase 9 activities in LNCaP cells. Bufalin may inhibit the proliferation of prostate cancer cell lines associated with sustained elevation of the Ca2+(i) and that of apoptosis (Yeh et al., 2003).

Hepatocellular carcinoma

BEL-7402 cells of human hepatocellular carcinoma were inoculated to form subcutaneous tumors, and were implanted into the liver to establish orthotopic transplantation tumor models of human hepatocellular carcinoma in nude mice. Bufalin has significant anti-tumor activities in the orthotopic transplantation tumor model of human hepatocellular carcinoma in nude mice with no marked toxicity and was able to induce apoptosis of transplanted tumor cells. This apoptosis may be mediated mainly via up-regulating the expression of apoptosis-regulated gene bax, which may be involved in its anti-tumor mechanism of bufalin (Han et al., 2007).

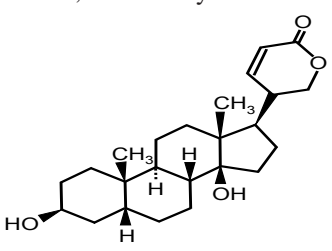

Figure 1. The Chemical Structure of Bufalin

Table 1. Clinical and Pathologic Characteristics of the 356 CRC Cases in this Study

Characteristics	<40	≥40	p value
Gender	106	250	0.332
Female	56 (52.8)	146 (58.4)	
Male	50 (47.2)	104 (41.6)	
Tumour histologic type	106	250	
Adenocarcinoma	97 (91.5)	241 (96.4)	0.054
Non-adenocarcinoma	9 (8.5)	9 (3.6)	
Colon tumour	68	155	-
Adenocarcinoma	60 (88.2)	150 (96.8)	
Carcinoid	0	0	
GIST	0	0	
Leiomyosarcoma	1 (1.5)	0	
Lymphoma	7 (10.3)	5 (3.2)	
Rectum tumour	38	95	-
Adenocarcinoma	37 (97.4)	91 (95.8)	
Carcinoid	0	1 (1.1)	
GIST	0	1 (1.1)	
Leiomyosarcoma	1 (0.8)	2 (2.1)	
Lymphoma	0	0	
Tumour histologic grade	59	146	<0.001
Grade 1 & 2	29 (49.1)	113 (77.4)	
Grade 3 & mucin	30 (50.9)	33 (22.6)	
UICC stage	40	106	0.457
Stage I	6 (15.0)	27 (25.5)	
Stage II	9 (22.5)	27 (25.5)	
Stage III	20 (50.0)	43 (40.6)	
Stage IV	5 (12.5)	9 (8.5)	
Locations	106	250	0.700
Colon	68 (64.2)	155 (62.0)	
Rectum	38 (35.9)	95 (38.0)	
Colon	68	155	0.087*
Descending colon	17 (25.0)	65 (41.8)	
Ascending colon	23 (33.8)	50 (32.6)	
Transverse colon	5 (7.4)	3 (1.8)	
Colon, unspecified	23 (33.8)	37 (23.8)	

* 'Colon, unspecified' excluded from correlation analysis; UICC, Union for International Cancer Control

Leukemia

Bufalin was found to be a potent inducer of differentiation in human leukemia cells by examination of various differentiation markers (as assessed by the morphology, histochemistry, and the abilities to phagocytose latex particles, to reduce nitro-blue tetrazolium and to develop Fc receptors). Bufalin, at a concentration as low as 10 nM, also produced a strong differentiation-inducing activity in three other human leukemia-derived cell lines (human promyelocytic HL60, monoblastic U937 and myeloblastic ML1). Treatment of K562 cells with other cardiotonic steroids, such as cinobufagin, ouabain and digitoxigenin, at the concentration of 10 nM for four days resulted in weak or no effect on the cells. These findings suggest that bufalin might have potentiality as a new agent in the differentiation therapy for human myelogenous leukemia (Zhang et al., 1991; Masuda et al., 1995; Watabe et al., 1998; Kawazoe et al., 1999).

In contrast to gynecologic cancer results, bufalin has been reported to induce cell cycle arrest in the G2/M phase of leukaemic cells (Numazawa et al., 1994; Jing et

al., 1994a). Interestingly, Jing et al. (1994b) demonstrated that apoptosis was not induced by bufalin in normal mononuclear and polymorphonuclear cells, suggesting that the effects of bufalin may be cell-type specific.

Lung cancer

Apoptotic cell death was induced in human non–small cell lung cancer A549 cells by treatment with bufalin. Bufalin suppressed the A549 cell proliferation in time- and dose-dependent manners and induced cell cycle arrest at G1 phase. Bufalin affected the protein expressions of Bcl-2/Bax, cytochrome c, caspase-3, PARP, p53, p21WAF1, cyclinD1, and COX-2 in A549 cells. Bufalin-induced decrease of the protein levels of receptor expressions and/or phosphorylation of VEGFR1, VEGFR2, EGFR and/or c-Met was confirmed using immunoblotting. Bufalin inhibited the protein expressions and phosphorylation of Akt, NF-jB, p44/42 MAPK (ERK1/2) and p38 MAPK in A549 cells (Jiang et al., 2010).

Breast cancer

Bufalin greatly sensitized ER-alpha-positive MCF-7 and ER-alpha-negative MDA-MB-231 human breast cancer cells to TRAIL-induced apoptosis. Enhanced apoptotic effects by TRAIL/bufalin combo were associated with augmentation of caspases activation. Further mechanistic investigation demonstrated that bufalin was able to significantly decrease Mcl-1 and Bcl-XL expression levels, and inhibit the transcription factor STAT3. The important consequence of downregulation Mcl-1 in the enhancement action by combining bufalin with TRAIL was confirmed by either knockdown or overexpression of Mcl-1 approach (Dong et al., 2011).

Colon cancer

The effects of bufalin were evaluated and characterized in HT-29 and Caco-2 human colon cancer cells. Contrary to its well-documented apoptosis-promoting activity in other cancer cells, bufalin induces caspase-independent cell death in colon cancer cells, as indicated by the absence of significant early apoptosis as well as PARP and caspase-3 cleavage. Instead, bufalin activated an autophagy that was linked to the generation of reactive oxygen species (ROS) and c-Jun NH2-terminal kinase (JNK). JNK activation increased expression of ATG5 and Beclin-1. ROS antioxidants (N-acetylcysteine and vitamin C), the JNK-specific inhibitor SP600125, and JNK2 siRNA attenuated bufalin-induced autophagy (Xie et al., 2011).

Osteosarcoma

Bufalin is highly effective in suppressing the growth of human osteosarcoma U-2 OS cells. Bufalin inhibited the cell migration and invasion of U-2 OS cells in vitro. Moreover, bufalin reduced MMP-2 and MMP-9 enzyme activities of U-2 OS cells. Bufalin also suppressed the protein level of MMP-2 and reduced the levels of mitogen-activated protein kinases (MAPKs) such as JNK1/2 and ERK1/2 signals in U-2 OS cells (Chueh et al., 2011).

Bladder cancer

Treatment of human bladder carcinoma T24 cells with bufalin had a strong growth inhibitory effect. The prominent arrest of cancer cells in the G2/M phase of the cell cycle is likely to account for this effect. Bufalin also stimulates apoptosis in human bladder cancer cells. Apoptosis induction of T24 cells by bufalin showed correlation with proteolytic activation of caspase-3, -8 and -9, and concomitant degradation of PARP, and collapse of the mitochondria membrane potential. Furthermore, these events are accompanied by induction of Bax/Bcl-2 (or Bcl-xL) ratio and down-regulation of inhibitor of apoptosis protein family members (Hong et al., 2012).

Clinical trials

Meng et al. conducted a pilot study, using a phase 1 trial design, of huachansu in patients with advanced cancer. Huachansu was administered intravenously for 14 days followed by 7 days off (1 cycle). Fifteen patients (hepatocellular cancer, n=11; nonsmall cell lung cancer, n=2; pancreatic cancer, n=2) were enrolled in the trial. Six (40%) had stable disease (median duration, 6.0 months; range, 3.5-11.1 months). One of these patients (with hepatocellular cancer) had 20% regression (duration, 11 months). Plasma bufalin concentration reached maximal levels at the end of the 2-hour infusion and was proportional to the amount of drug being administered (0.81-3.38 ng/mL) (Meng et al., 2009).

In conclusion, many questions are currently still unanswered with respect to the molecular mechanisms underlying how traditional oriental medicines inducedifferentiation, cell cycle arrest and apoptosis. Certainly, further work will be required to improve the understanding on why transformed cells are more susceptible to the effects of bufalin than normal cells. Also, combinations of bufalin with differentiation-inducing agents, with cytotoxic agents and even with gene therapy may represent novel therapeutic strategies and new hope on the horizon in the treatment of cancer.

Acknowledgements

This study was supported by a grant (project code FK344 to NT) from the Japan Society of Gynecologic Oncology, a Grant-in-Aid (No. 21592139 to NT) for Scientific Research from the Ministry of Education, Culture, Sports, Science, and Technology, Japan, and a Research Fund at the Discretion of the President, Oita University.

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