MINI-REVIEW

Anti-tumor Activity and Apoptosis-regulation Mechanisms of Bufalin in Various Cancers: New Hope for Cancer Patients

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Abstract

The induction of apoptosis in target cells is a key mechanism for most anti-tumor therapies. Bufalin is a cardiotonic steroid that has the potential to induce differentiation and apoptosis of tumor cells. Research on bufalin has so far mainly involved leukemia, prostate cancer, gastric cancer and liver cancer, and has been confined to in vitro studies. The bufadienolides bufalin and cinobufagin have been shown to induce apoptosis in a wide spectrum of cancer cell. The present article reviews the anticancer effects of bufalin. It induces apoptosis of lung cancer cells via the PI3K/Akt pathway and also suppressed the proliferation of human non-small cell lung cancer A549 cell line in a time and dose dependent manner. Bufalin, bufotalin and gamabufotalin, key bufadienolides, significantly sensitize human breast cancer cells with differing ER-alpha status to apoptosis induction by the TNF-related apoptosis-inducing ligand (TRAIL). In addition, bufadienolides induce prostate cancer cell apoptosis more significantly than that in breast epithelial cell lines. Similar effects have been observed with hepatocellular carcinoma (HCC) but the detailed molecular mechanisms of inducing apoptosis in this case are still unclear. Bufalin exerts profound effects on leukemia therapy in vitro. Results of multiple studies indicate that bufalin has marked anti-tumor activities through its ability to induce apoptosis. Large-scale randomized, double-blind, placebo or positive drug parallel controlled studies are now required to confirm the efficacy and apoptosis-inducing potential of bufalin in various cancers in the cliniucal setting.

Keywords: Chemotherapy - PI3K/Akt pathway - Bufalin - anti-apoptotic proteins - Bcl-2 - caspase-3

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Background

It's been 50 years since chemotherapy was introduced into the clinical practice of cancer treatment. Chemotherapy has been successful in the treatment of some forms of cancer; however, it is not the case for the majority of epithelial tumors of the breast, colon, lung, and ovary. Initially, the development of chemotherapeutic agents was based on the original strategy, to interfere with DNA replication and to stop proliferation of cells. Many observations have indicated that cell death plays a significant role during physiological processes of multicellular organisms, particularly during embryogenesis and metamorphosis (Gluecksmann et al., 1951; Lockshin et al., 2001). The molecular mechanisms of apoptosis and its function in normal physiology are crucial in understanding the effect of chemotherapy and the mechanisms of chemoresistance.

Recent observations suggest that the induction of apoptosis in target cells is a key mechanism for most anti-tumor therapies, including chemotherapy, γ-radiation, immunotherapy, and cytokines (Kaufmann et al., 2000). Therefore, defects in apoptosis may cause resistance. The

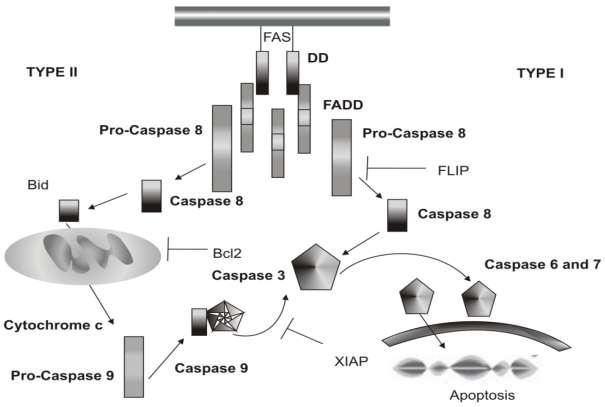

Figure 1. Apoptotic Cascade

realization that apoptosis is a key factor that contributes to the anti-tumor activity of chemotherapeutic drugs has allowed us to understand how drug resistance may arise and to look for new approaches for the treatment of cancer. Apoptosis is characterized by morphological changes including cell shrinkage, membrane blebbing, chromatin condensation, and nuclear fragmentation (Wyllie et al., 1980; Kerr et al., 1994). All these changes are the result of

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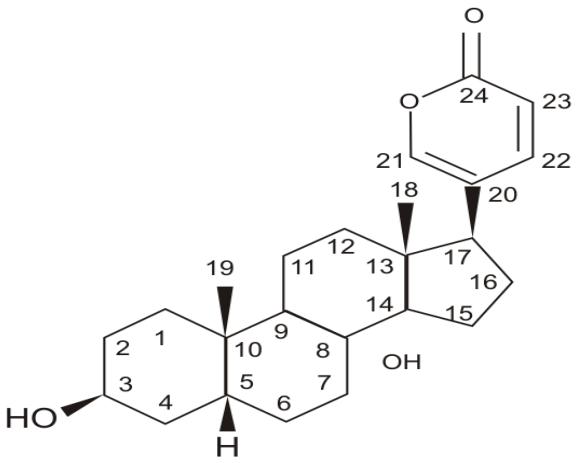

Figure 2. Chemical Structure of Bufalin

the activation of a cascade of intracellular factors known as caspases (Figure 1). Each step in the apoptotic cascade is delicately controlled by intracellular factors that can block the apoptotic pathway either at the "initiator" or "effector" level. There are three groups of proteins that are known to inhibit apoptosis (Figure 1), first is the bcl2 family of proteins (Cory et al., 2002; Willis et al., 2003), second is the FADD-like interleukin-1 converting enzyme (FLICE)-inhibitory proteins (FLIP) (Krueger et al., 2001; Thome et al., 2001); and third is the inhibitors of apoptosis proteins (IAP) (Hashimoto et al., 1997; Kawazoe et al., 1999; Chen et al., 2009). Bcl-2 family plays a crucial role in the control of apoptosis and can be classified into two functionally distinct groups: antiapoptotic proteins and pro-apoptotic proteins. Bcl-2, an antiapoptotic protein, is known to regulate apoptotic pathways and protect against cell death. Bax, a pro-apoptotic protein is expressed abundantly and selectively during apoptosis and promotes cell death. Increasing the ratio of bcl-2 to bax has commonly been used to determine the induction of apoptosis in several tissues (Krueger et al., 2001; Thome et al., 2001).

It is well established that anti cancer agents induce apoptosis and interruption of apoptosis inducing regimens may reduce treatment sensitivity. However, anti cancer agents induce apoptosis in normal tissues as well as in tumors. Bufalin is a cardiotonic steroid isolated from the Chinese toad venom Chansu, a galenical preparation of the dried white venom of Chinese Bufo gargarizans (Asiatic toad) (Hong et al., 1992; Panesar et al., 1994), the molecular formula of which is C24H3404 with a relative molecular weight 386.5 g/mol (Figure 2). Bufalin has the capacity of inducing differentiation and apoptosis of tumor cells. Bufalin suppress cell proliferation and cause apoptosis in hu¬man prostate cancer cells, via an action of sustained election of the [Ca^{2+}], and apoptotic modulators including Bax, cytochrome c, and caspases (Yeh et al., 2003; Yu et al., 2008). Bufalin effectively inhibits Leukaemia (Zhang et al., 1991; Watabe et al., 1997; Hashimoto et al., 1997; Kawazoe et al., 1999; Chen et al., 2009), ovarian cancer cells and endometrial cancer cells (Takai et al., 2008), gastric cancer (Li et al., 2009), pancreatic cancer and lung cancer (Meng et al., 2009), and hepatocellar carcinoma (Han et al., 2007, Qi et al., 2007).

Research on Bufalin mainly involves tumor spectra of leukemia, prostate cancer, gastric cancer and liver cancer, and is confined to in vitro studies (Masuda et al., 1995; Watabe et al., 1996; Hashimoto et al., 1997; Watabe et al., 1997; Yamada et al., 1998; Wu et al., 2000; Chen et al., 2001; Yeh et al., 2003; Tian et al., 2006; Zhu et al., 2006; Han et al., 2007; Chen et al., 2009). In this review article we have reviewed the anticancer properties of Bufalin from the perspective of an emerging treatment option for cancer patients.

Bufalin Induced Cell Apoptosis In Lung Cancer

The anti-tumor effects of Bufalin have not been demonstrated in lung cancer. Bufalin could induce apoptosis of lung cancer cells via the regulation of PI3K/Akt pathway. Zhitu et al. (2012) have used A549 human lung adenocarcinoma epithelial cell line as the experimental model to evaluate the effects of Bufalin in lung cancer chemotherapy. A549 cells were treated with Bufalin, wherein the proliferation was detected by MTT assay and apoptosis was detected by flow cytometry analysis and Giemsa staining. The results showed that Bufalin inhibits the proliferation of A549 cells and induces apoptosis of A549 cells in a dose and time dependent manner. Moreover, western blot analysis revealed that Bufalin synergized with Akt inhibitor to induce apoptosis of A549 cells and this was associated with the upregulation of Bax expression, the downregulation of Bcl-2 and livin expression, and the activation of Caspase-3 (Zhitu et al., 2012). Livin is a newly discovered member of inhibitor of apoptosis protein family (Kasof et al., 2001). The BIR domain of livin has a new type of zinc finger structure which could bind Caspase-3 to inhibit its activity, thereby inhibiting apoptosis (Sanna et al., 2002). A recent study showed that inhibiting livin could induce the apoptosis of human bladder cancer cells via a mechanism involving caspase-3 (Liu et al., 2010). The activated receptors, VEGFR, EGFR, and c-Met play crucial role in the proliferation of lung cancer cells (Morelli et al., 2006; Kim et al., 2008; Puri et al., 2008; Naumov et al., 2009; Colon et al., 2011). Another study by Jiang et al. (2011), demonstrated that Bufalin suppresses the proliferation of human NSCLC A549 cell line in time- and dose dependent manner. The results suggest that Bufalin inhibits the human lung cancer cell proliferation via VEGFR1/VEGFR2/EGFR/c- Met–Akt/p44/42/p38-NF-kB signaling pathways.

Bufalin Induced Cell Apoptosis In Breast Cancer

In spite of best practices to improve awareness leading to better screening and detection rates, as well as treatment, breast cancer remains the world's second most cancer-related death in women.

The death receptor ligand TNF-related apoptosis-inducing ligand (TRAIL) has preferential toxicity to malignant cells and it's been considered a promising candidate for cancer therapy (Dong et al., 2011). However

number of resistance mechanisms alters its efficacy. Bufalin, bufotalin and gamabufotalin, key members of bufadienolides have significantly sensitized human breast cancer cells with different status of ER-alpha to apoptosis induction of TRAIL, as evidenced by enhanced Annexin V/FITC positive cells (apoptotic cells), cytoplasmic histone-associated DNA fragments, membrane permeability transition (MPT), caspases activation and PARP cleavage (Dong et al., 2011). Further mechanistic investigation demonstrated that Bufalin was able to significantly decrease Mcl-1 expression and modestly decrease Bcl-XL expression level. Down-regulations of these anti-apoptotic proteins were well correlated with inhibition of transcription factor STAT3 activation. Previous study results confirmed that suppression of Akt and the NF-kB/Bcl-2 pathway by anticancer agents inhibited growth and induced apoptosis and cell cycle arrest in A549 cells and breast cancer MDAMB- 231 cells (Wang et al., 2009; Yu et al., 2009).

Bufalin Induced Cell Apoptosis In Prostrate Cancer

Prostate cancer has its highest incidence in the USA and is becoming a major concern in Asian countries. Bufalin and cinobufagin at the concentration of 1-10 μM inhibit proliferation and induce cell apoptosis of androgen-dependent (LNCaP) and independent (DU145 and PC3) prostate cancer cell lines (Yu et al., 2008). In addition, bufadienolides induces 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. The sustained elevation of the intracellular calcium concentration of anticancer studies might not completely reflect the in vivo expressions of whole body systems in response to the anticancer therapy of cardiotonic steroids (Yu et al., 2008).

Bufalin Induced Cell Apoptosis in Hepatocellular Carcinoma

Bufadienolides Bufalin and cinobufagin have been shown to induce a wide spectrum of cancer cell apoptosis. However, the detailed molecular mechanisms of inducing apoptosis in hepatocellular carcinoma (HCC) are still unclear. Qi et al. (2011), found that Bufalin and cinobufagin induce marked changes in apoptotic morphology and significantly increased the proportion of apoptotic cells. This apoptotic induction was associated with an increase in Fas, Bax and Bid expression, a decrease in Bcl-2 expression, disruption of the mitochondrial membrane potential, release of cytochrome c, activation of caspase-3, -8, -9 and -10, and the cleavage of poly (ADP-ribose) polymerase (PARP), which indicates that Bufalin and cinobufagin induces apoptosis through both Fas and mitochondria-mediated pathways (Qi et al., 2011). 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 (Yin et al., 2011). 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).

Bufalin Induced Cell Apoptosis in Leukemia

Bufalin inhibits APL cell proliferation in a time and dose dependent manner and induced NB4 cell apoptosis accompanied by Survivin downregulation and activation of Caspase-3. MEK/ERK signaling pathway is negatively regulated in Bufalin induced apoptosis in NB4 human leukemia cell line. Bufalin enhances ATRA induced differentiation in NB4 cell line and primary culture in APL cells (Yamada et al., 1998). Bufalin in combination with VP16, alltrans retinoic acid, lα, 25-dihydroxyvitamin D3, rTNF-α or γ-interferon may be very useful in the differentiation of human leukemia (Zhang et al., 1992). When human leukemia HL-60 cells were treated with 10−7 M Bufalin, the amounts of both topoisomerase (topo) IIα and IIβ and the activity of topo II decreased markedly and were almost undetectable 18 h after the start of treatment. The level of topo II mRNA started to decrease immediately after the start of treatment with Bufalin, with a subsequent decrease in the amount of topo IIα protein. These changes preceded the fragmentation of DNA, a typical feature of apoptosis (Hashimoto et al., 1997).

Bufalin Induced Cell Apoptosis in Gastrc Cancer

Gastric cancer is one of the most common causes of death from cancer in China (Ferlay et al., 2010). Bufalin induces MGc-803 cell death characterized by apoptotic phenotypes DNA content changes and chromosome DNA fragmentation Bufalin showed a remarkable cell cycle specificity in inducing apoptosis in G1 phase The induction of apoptosis would be a very important mechanism of Bufalin in treatment of gastric cancer (Chen et al., 2000).

Bufalin induces apoptosis by altering the expression of apoptosis-related genes c-myc and bcl-2 (Masuda et al., 1995). The activation of mitogen-activated protein kinase (MAPK) may be involved in Bufalin induced apoptosis in U93T cells (Watabe et al., 1997). Over-expression of Bcl-2 inhibits the Bufalin-induced MAPK activation and the subsequent AP-I activation and cell apoptosis in U937 cells. Using the inhibitor of the biosynthesis of AP-1 and the dominant negative c-Jun reduces the activation of AP-I and the induction of apoptosis after Bufalin treatment. Bufalin induces apoptosis via the activation of AP-I through MAPK cascade including JNK in human leukemia U937 cells (Watabe et al., 1997). In addition, Bufalin induces HL-60 cell apoptosis via the activation of the transcription factor NF-kB and AP-I (Chen et al., 2009).

Bufalin reveals the profound effects on leukemia therapy in vitro. The mechanisms of Bufalin induced

apoptosis have been deeply understood. This review of literature shows that Bufalin has marked anti-tumor activities and is able to induce apoptosis. However, large scale randomized, double-blind, placebo or positive drug parallel controlled studies are required to study the efficacy or apoptosis inducing potential of Bufalin in various cancers. Bufalin shows enough promise to emerge as a potent, novel and effective anti cancer therapy for cancer patients across the globe.

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