A REVIEW ON BERBAMINE – A POTENTIAL ANTICANCER DRUG

Mohammed Rahmatullah¹*, Rownak Jahan², A.B.M. Anwarul Bashar¹, Abdullah Al-Nahain¹, Snehalal Majumder³, Md Tabibul Islam², Protiva Rani Das²

¹Department of Pharmacy, University of Development Alternative, Dhanmondi, Dhaka-1209, Bangladesh
²Department of Biotechnology & Genetic Engineering, University of Development Alternative, Dhanmondi, Dhaka-1209, Bangladesh
³Department of Microbiology and Serology, NH Health, Bangalore 560099, India

ABSTRACT
Berbamine belongs to the bisbenzylisoquinoline group of alkaloids and is present in a number of plant species belonging to the Berberis genus within the Berberidaceae family. The compound and various synthetic derivatives of the compound have shown remarkable success in in vitro trials to reduce cell viability and induce apoptosis in a number of cell lines obtained from a diverse variety of cancers. In this review, we explore the various studies conducted with berbamine and its derivatives and the possible molecular mechanisms behind their anticancer activities.

Key words: Berbamine, Berberis, Berberidaceae, anticancer.

INTRODUCTION
The family Berberidaceae comprises about 500 species worldwide. The two major compounds found in Berberidaceae family plants are berberine and berbamine. A number of important bioactivities have been reported for the plants, which include antimicrobial, antiemetic, antipyretic, antioxidant, antiinflammatory, anticancer, antiarrhythmic, sedative, anticholinergic, cholagogic, antileishmaniasis, and antimalaria.¹ Both berberine and berbamine have been recently gaining importance as possibly the next generation of anticancer drugs; among the two compounds, berberine has been well studied and found to be active against a number of cancers.²⁻⁴ In contrast, berbamine has been reported lesser number of times in the scientific literature regarding its anticancer potential, though existing
studies suggest that berbamine, like berberine, can also be of therapeutic value against different cancer types. This review shall explore the reported anticancer activities of berbamine through searches of reports published in databases like PubMed, SCOPUS, and Google Citations and examine the therapeutic potential of berbamine against cancer.

Berbamine belongs to the bisbenzylisoquinoline group of alkaloids like cepharanthine and isotetrandrine. A number of plants belonging to the Berberis genus and reported to contain berbamine are used in traditional Chinese medicine. [5] The structure of berberine, berbamine and some anticancer berbamine derivatives are shown in Figure 1.
The effect of berbamine has been studied in ChR-24 cells, which are derived from human KB carcinoma cells and are resistant to various anticancer drugs like vincristine, actinomycin D, daunomycin, and adriamycin. Berbamine partially overcame resistance to these anticancer drugs.\textsuperscript{[6]} In another study, berbamine was seen to reverse adriamycin and vincristine-induced drug resistance in MCF-7/ADR and KBv200 cell lines.\textsuperscript{[7]} The effect of berbamine has been examined in human erythroleukemic cell line K562 and its adriamycin-resistant counterpart K562/A02. Berbamine increased the chemosensitivity of adriamycin in K562/A02 cells in a dose-dependent manner. Treatment with berbamine decreased P-glycoprotein (P-gp) expression and down-regulated expression of MDR1 (multi-drug resistance1) and survivin mRNA in K562/A02 cells and also increased intracellular concentration of adriamycin in the cells.\textsuperscript{[8]} Since P-gp and survivin are inhibitors of apoptosis, and survivin transcription is associated with P-gp and MDR1 overexpression,\textsuperscript{[9]} it can be concluded that berbamine can act through promotion of apoptosis in K562/A02 cells by increasing sensitivity of K562/A02 cells to adriamycin and decreasing anti-apoptotic proteins like P-gp and survivin.

In K562/Adr (adriamycin-resistant) leukemia cells, berbamine reportedly inhibited cell growth in a dose-dependent manner, induced apoptosis, increased the protein expression of caspase-3, and reduced the mRNA and protein expression levels of MDR1 gene. Increase of protein expression of caspase-3 would induce apoptosis, and reducing the expression and protein levels of MDR1 gene would lead to reversal of drug-resistance.\textsuperscript{[10]}
The anticancer effects of berbamine have been shown in chronic myelogenous leukemia (CML). Gleevec (imatinib), a drug with the ability to block bcr/abl tyrosine kinase activity has shown remarkable success in treatment of this form of cancer; however, cells can develop resistance to this drug. Berbamine has been found to selectively induce cell death of both Gleevec-sensitive and -resistant Ph+ CML cells. The compound has also been found to display selective anti-proliferative activity of primary leukemia cells from CML patients. Berbamine has been found to down-regulate p210bcr/abl oncoprotein level, and induce apoptosis of bcr/abl+ cells through caspase-3-dependent pathway. [11] In a further study, it was shown that berbamine increased apoptosis of human Ph+ CML leukemia K562 cells, increased expression of caspase-3, and markedly inhibited phosphorylation of p210 bcr/abl protein. Berbamine also down-regulated level of chaperone Hsp90 protein in the cells. The berbamine-induced apoptosis of CML cells may involve a bcr/abl-Hsp90 mechanism. [12]

In imatinib-resistant bcr-abl-positive human chronic myelogenous leukemia K562 (K562-r) cells, berbamine inhibited proliferation of cells both in vivo as well as in vitro. Berbamine further induced apoptosis, and the apoptotic mechanism appeared to involve Bcl-2 family proteins, as well as MDR1 mRNA and P-gp protein. Berbamine also restored the chemosensitivity of the cells to imatinib. [13] Further studies with berbamine and these cells showed that berbamine was able to down-regulate bcr-abl and phospho-bcr-abl proteins by affecting bcr-abl mRNA expression and decrease expression of nuclear factor-κB (NF-κB), phospho-IκBα, IKKα, and survivin. Collectively, these factors induced apoptosis and stopped cell growth in K562-r cells. [14]

In a chronic myeloid leukemia cell line KU812, berbamine inhibited cell proliferation in a time- and dose-dependent manner, with IC₅₀ values for treatments of 24, 48, and 72 h at 5.83, 3.43, and 0.75 μg/ml, respectively. Berbamine induced cell cycle arrest at the G1 phase and also induced apoptosis. The compound up-regulated transcriptions of Smad3 and p21, and increased protein levels of both total Smad3 and phosphorylated Smad3. The protein levels of cyclin D1 and c-Myc were reduced. The levels of the anti-apoptotic proteins Bcl-2 and Bcl-xL were decreased, and the level of the pro-apoptotic protein Bax was increased. [15]

In chronic myeloid leukemia cells, berbamine may induce inhibition by acting through other signaling pathways. The compound has been shown to specifically bind to the ATP-binding pocket of calmodulin kinase (CAMK)IIγ, inhibit its phosphorylation, and trigger apoptosis.
The whole signaling mechanism may involve Stat-3 and β-catenin signaling networks. Berbamine has been shown to inhibit the in vivo tumorigenicity of liver cancer cells in NOD/SCID mice through inhibition of Ca\(^2+\)/calmodulin-dependent protein kinase II. As such, calcium/calmodulin-dependent protein kinases may also play an active role in the inhibitory effects of berbamine in various cancers. It may be noted that a berbamine derivative, O-4-ethoxy-butyl-berbamine (EBB) is a calmodulin antagonist and has been found to be a potent inhibitor against several forms of cancer (discussed in the next section).

In human multiple myeloma cell line KM3, berbamine inhibited the proliferation of these cells in a time- and dose-dependent manner. Antiproliferative effect was achieved through both arresting cell growth at the G1 phase as well as induction of apoptosis. Berbamine also caused increased expression of A20, down-regulation of IKKα, p-IκBα, and followed by inhibition of p65 nuclear localization. As a result, NF-κB downstream targets such as cyclinD1, Bcl-x(L), Bid and survivin were down-regulated. Some mechanisms through which berbamine exerts its anticancer effects have been shown in Figure 2.

![Figure 2. Mechanism of berbamine to inhibit cancer and reverse drug resistance (after 10,11,19,20)](image)

Berbamine also reportedly inhibited cell growth of the leukemia cell line, NB4, at different concentrations at different time points (IC\(_{50}\) value of 3.860 microg/ml at 48 hours). There was
induction of apoptosis and a drastic decrease in survivin mRNA levels. The protein expression of caspase-3 was increased; it has been hypothesized that the effects of berbamine on survivin and caspase-3 lead to the observed apoptotic effect.\textsuperscript{[19]}

Berbamine reportedly induced apoptosis in human hepatoma cell line SMMC7721 by inducing loss in mitochondrial transmembrane potential and caspase activation. The antiproliferative effect of berbamine was dose- and time-dependent. Berbamine arrested cell cycle at the G0/G1 phase and caused activations of caspases 3 and 9.\textsuperscript{[20]}

Berbamine has been shown to induce Fas-mediated apoptosis in human hepatocellular carcinoma HepG2 cells and inhibited its tumor growth in nude mice. Cell growth was decreased in a dose-dependent manner with an IC\textsubscript{50} value of 34.5 +/- 0.5 microM. Berbamine treatment increased the level of Fas and p53, caused depolarization of mitochondrial membrane and decrease of membrane potential, and activation of caspases 3, 8 and 9 in HepG2 cells. HepG2 human HCC xenograft mice treated with berbamine showed a significant reduction in tumor growth rates compared to saline-treated mice.\textsuperscript{[21]}

A therapeutic effect of berbamine has been seen in 405 patients with leukopenia. At the same time, berbamine has been shown to have leukogenic effects in rats and dogs injured by an anticancer agent.\textsuperscript{[5]} Amelioration of leukopenic complications in mice induced by cyclophosphamide (an anticancer agent) has been observed with berbamine. Administration of the compound for two weeks led to significant increases in neutrophil and leukocyte counts.\textsuperscript{[22]}

Topical application of berbamine to mouse skin at a dose of 2 mumol/mouse was found to suppress the tumor-promoting effect of 12-O-tetradecanoylphorbol-13-acetate (1 microgram) in mouse skin initiated with 7,12-dimethylbenz[a]anthracene (50 micrograms).\textsuperscript{[23]}

Berbamine has been shown to suppress the \textit{in vitro} and \textit{ex vivo} growth of human lung cancer A549 cell line. This cell line is from non-small cell lung cancer, and constitutes 80% of lung cancer cases associated with poor survival rates. The expression of the anti-apoptotic protein Bcl-2 was down-regulated by berbamine, while the level of the pro-apoptotic protein was increased by the compound. Berbamine also inhibited A549 cell migration. Berbamine also significantly enhanced the activity of anticancer drugs like trichostatin A and celecoxib.\textsuperscript{[24]} In another study, berbamine was found to be cytotoxic against lung cancer A549 cells.
Additionally, berbamine significantly reduced the growth of lung cancer in a dose-dependent manner in nude mice with prolonged survival time. [25]

Nanoparticles prepared from block copolymer of methoxy poly (ethylene glycol)-polycaprolactone (mPE-PCL) containing paclitaxel and berbamine has been shown to show dose- and time-dependent cytotoxicity against gastric cancer cell line BGC823 cells. The nanoparticles containing the two compounds demonstrated superior antitumor effect in vivo, when delivered intratumorally. [26]

Gemcitabine is used as a chemotherapeutic agent against pancreatic cancer; however, the drug suffers from the problem of quickly having drug-resistance. The effect of berbamine on the activity of gemcitabine has been evaluated in human pancreatic cancer cell lines Bxpc-3 and Panc-1. Berbamine was found to enhance gemcitabine-induced cell growth inhibition and apoptosis in the two cell lines. Berbamine, by itself, also showed a dose- and time-dependent inhibition of the pancreatic cancer cells. Treatment of berbamine and gemcitabine in combination resulted in down-regulation of anti-apoptotic proteins (Bcl-2, Bcl-xL) and up-regulation of pro-apoptotic proteins (Bax, Bid). Moreover, the drug combination activated the transforming growth factor-β (TGF-β)/Smad signaling pathway, as a result of a decrease in Smad7 and an increase in transforming growth factor-β receptor II (TβRII) expression. This resulted in up-regulation of cyclin-dependent kinase (cdk) inhibitors like p21, and down-regulation of c-Myc and cyclin D1, which would cause cell cycle arrest. [27]

ANTICANCER ACTIVITY REPORTS ON BERBAMINE DERIVATIVES

A berbamine derivative, O-4-ethoxy-butyl-berbamine (EBB), which is also a calmodulin antagonist, has been shown to exert a very strong inhibitory effect on human hepatoma cell line 7402 and mouse hepatoma cell line H22 in vitro, with IC₅₀ values of 3.312 microg/ml and 1.167 microg/ml, respectively. EBB also markedly enhanced the sensitivity of H22 cells to 5-flourouracil (5-FU). In vivo experiments showed that administration of EBB led to prolongation of lifespan in mice with ascites H22 to more than 3 months; 64% of the EBB-treated mice survived while 100% mortality was observed in non-EBB treated mice by the 18th day. Treatment of ascites H22 mice with a combination of EBB and 5-FU lead to 73% survival rate in mice versus only 27% in the 5-FU treated group. EBB increased the translation of tumor suppressor protein, p53, decreased the amount of calmodulin in hepatoma cells, and blocked hepatoma cell cycle proliferation at the G2/M phase. [28]
In multi-drug resistant MCF-7/ADR breast carcinoma cells, EBB was found to block the function of P-gp, and in combination with doxorubicin (Dox), arrested cell cycle progression at the G2/M phase, along with inducing apoptosis, and down regulating proteins cdc2/p34 and cyclin B1. \(^{29}\) The synergistic effects of EBB and pegylated liposomal doxorubicin (PLD) has been studied in hepatoma model, in where hepatoma was induced by inoculating H(22) cells into the right backs of mice. EBB (5 mg/kg) significantly augmented the antitumor activity of Dox or PLD and prolonged the survival time of mice. EBB significantly increased the liver levels of Dox and decreased toxicity of Dox and PLD. EBB also decreased the IC\(_{50}\) values of Dox and PLD from 0.050+/-.006 mg/L and 0.054+/-.004 mg/L to 0.012+/-.002 mg/L and 0.013+/-.002 mg/L, respectively (P<0.01). \(^{30}\) The mechanism of EBB anticancer activity was not determined in this study.

In human fibrosarcoma cells HT1080, EBB inhibited cell proliferation with an IC\(_{50}\) value of (8.2 +/- 1.2) microg/ml. EBB down-regulated the activities and mRNA levels of matrix metalloproteinases (MMP) 2 and 9, and up-regulated the mRNA levels of tissue inhibitor of metalloproteinases (TIMP) 1. The invasive ability of HT1080 cells was also inhibited by EBB. \(^{31}\) MMP2 and MMP9 are known to play an active role in angiogenesis, tumor growth and metastasis \(^{32,33}\), and as such, inhibition of these two MMPs can inhibit tumor development and invasion. Some mechanisms through which EBB exerts it anticancer effects have been shown in Figure 3.

![Figure 3. Mechanism of EBB as anti cancer agent (after 28,32-34)](image)

EBB was found to arrest human breast cancer cell line MCF-7 cells at the S phase with an IC\(_{50}\) value of (13.0 +/- 3.7) micromol/L. EBB caused depolymerization of the microtubule
and microfilament, impairment of the mitochondrion and swelling of endoplasmic reticulum, all of which may have lead to cell cycle arrest. [34] 

BBD (4-chlorobenzoyl berbamine) inhibited cell proliferation and induced apoptosis in Raji, L428, Namalwa and Jurkat lymphoma cells lines. Cell cycle was arrested at the G2/M phase through a signaling mechanism involving phosphoinositide 3-kinase (PI3/Akt) and NF-κB signaling pathways in a caspase-dependent manner. [35] 

In multiple myeloma cell lines (U266, RPMI 8226, MM1.R and MM1.S), BBD inhibited their growth after 24h treatment with IC_{50} values of 1.8, 2.3, 1.5 and 2.4 µg/ml, respectively. In U266 and RPMI 8226 cells, there was induction of apoptosis, including activation and cleavage of caspases 3, 8, 9 and PARP. Cell cycle was arrested at the G2/M phase in these two cell lines. Furthermore, BBD inhibited autocrine IL-6 production, and down-regulated membrane IL-6 receptor (IL-6R) expression. Crucial proteins downstream of the IL-6 signaling pathway, including AKT and STAT3, were inactivated in BBD-treated U266 cells. Forkhead transcription factor class 3a (FOXO3a), a nuclear transcription factor downstream from AKT, was up-regulated in the nuclei of BBD9-treated U266 cells. Bim, the target gene of FOXO3a, was up-regulated at both the protein and mRNA levels. The results suggest that BBD-induced apoptosis in various multiple myeloma cells can be through inhibition of the interleukin-6 (IL-6) pathway, leading to FOXO3a activation and upregulation of pro-apoptotic Bim. [36] 

BBD also demonstrated inhibitory effect against imatinib-resistant K562/IR cells both in vitro and in vivo. In cell cultures, the IC_{50} of BBD was 0.73 µg/ml, versus berbamine with IC_{50} value at 5.43 µg/ml. The expressions of p210 (Bcr-Abl), IKKα and nuclear NF-κB p65 (RelA) were all decreased following BBD treatment. BBD dose-dependently increased cell apoptosis and necrosis, along with dose-dependent increases in levels of cleaved caspase 3, caspase 9, poly ADP ribose polymerase (PARP), and microtubule-associated protein 1A/1B-light chain 3II (LC3II) expression. In tumor-bearing mouse model, BBD showed stronger effects than imatinib in reducing the tumor weight, promoting tumor regression, and increasing the body weight. [37] RelA has been found to be constitutively activated in human gastric carcinoma tissue, [38] and human prostate adenocarcinoma, [39] so BBD induced decrease in RelA expression can account for the observed inhibition by BBD. Increase in
LC3II expression suggests increase in cellular autophagy \cite{40}, which can also be another factor in the observed inhibition by BBD of K562/IR cells.

A series of berbamine glycosides were synthesized and tested for their anticancer efficacies \textit{in vitro} against a human leukemia cell line K562, a human lung adenocarcinoma cell line A549 and mouse lymphocytic leukemia cells L1210. Most of the glycosides demonstrated potent cytotoxic activities against the cell lines. Acetyl glycosyl berbamines were the most potent suggesting that these compounds have affinity to these cancer cells. \cite{41}

Thirteen synthetic berbamine derivatives were prepared and tested for their cytotoxic activities against human melanoma cells. Of these derivatives, one (BBMD3) exhibited over 6-fold activity than berbamine. BBMD3 inhibited Jak2 autophosphorylation kinase activity \textit{in vitro} with IC$_{50}$ value of 0.69 μM. The compound also inhibited autophosphorylation of Jak2 kinase at Tyr1007/1008 sites in the range of 15 μM in human melanoma cells at 4h after treatment. Following inhibition of Jak 2, constitutive activation of downstream signaling like Stat 3 was also blocked in the melanoma cells. The expressions of Stat 3 target proteins like Mcl-1 and Bcl-xL was also down-regulated along with induction of apoptosis. \cite{42}

BBMD3 has also been reported to decrease cell viability and induce apoptosis in G292, KHOS, and MG-63 human osteosarcoma cells. BBMD3 induced activation of caspase 3 and cleavage of PARP. The compound reportedly increased phosphorylation of c-Jun N-terminal kinase (JNK)/stress-activated protein kinase (SAPK), resulting in increased phosphorylation of c-Jun and total c-Fos (major components of transcriptional factor AP-1). Production of reactive oxygen species (ROS) was increased by BBMD3 in these cells. The compound increased the expression of the pro-apoptotic gene Bad, and decreased the expression of the positive cell cycle regulators cyclins D1 and D2. The results suggest that BBMD3 can trigger multiple cell inhibitory and apoptotic signaling pathways in osteosarcoma cells – stop cell cycle progression through decrease in cyclin D expression, and triggering apoptosis, possibly through increased ROS production leading to activation of caspase 3 and cleavage of PARP, as well as increasing expression of Bad. \cite{43} Regulation of ROS-mediated apoptosis and necrosis by caspase 3 has been reported previously. \cite{44} Activation of caspase 3 can lead to PARP cleavage. The normal function of PARP is routine repair of DNA damage induced by various stresses. However, cleavage of PARP by caspases into various fragments has been considered to be a hallmark of apoptosis \cite{45}, for these fragments, particularly 21 Kda and 55
Kda fragments generated from PARP-1 by caspases 3/7 can function as an inhibitor of PARP-1.\textsuperscript{[46]} The nuclear transcription factor AP-1 has also been implicated in apoptosis.\textsuperscript{[47]}

BBMD3 has been shown to inhibit cell viability and induce apoptosis of cancer stem-like cells (CSCs) in a time- and dose-dependent manner when the CSCs from four GBM patients (PBT003, PBT008, PBT022, and PBT030) were cultured. Induction of apoptosis was dependent on activation of caspase 3 and cleavage of PARP. MicroRNA-4284 (miR-4284) was shown to be over-expressed about 4-fold in the CSCs following BBMD3 treatment. The compound also increased phosphorylation of c-Jun N-terminal kinase (JNK)/stress-activated protein kinase (SAPK), resulting in increased phosphorylation of c-Jun and total c-Fos (major components of transcriptional factor AP-1).\textsuperscript{[48]}

**CONCLUSION**

Berbamine and more specially one of its synthetic derivatives BBMD3 have been shown to inhibit the proliferation and induce apoptosis in a variety of cancer cell types, thus opening up new avenues of anticancer drugs. Although the specific signaling mechanisms have not been fully elucidated yet, the low IC\textsubscript{50} values of BBMD3 against a number of cancer cell lines suggest that this compound merits further studies as to elucidate the molecular mechanisms behind its action. Both berbamine as well as its various synthetic derivatives can be considered as the next generation of anticancer drugs with activity against multiple cancer types. It is important to conduct studies on their short and long-term toxicities when administered. Furthermore, these studies open up new possibilities of other synthetic derivatives of berbamine as potentially more efficacious anticancer agents. A case in point is a number of berbamine derivatives, which selectively induces apoptosis of imatinib (IM)-resistant-Bcr/Abl-expressing leukemia cells from the K562 cell line and CML patients. In vitro studies have shown very high activities of these compounds with IC\textsubscript{50} values 0.36-0.55 microM.\textsuperscript{[49]}

**REFERENCES**


