Pharmacological and Therapeutic Potential of Beauvericin: A Short Review

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Abstract

An entomopathogenic fungus, Beauveria spp. has been known to have numerous pharmacological and therapeutic implications, especially, in terms of human health making it a suitable for ethnopharmaceutical use. Beauvericin is a cyclic hexadepsipeptide mycotoxin, a novel bio-metabolite derived from this fungus, exhibiting a very potent anticancer, cytotoxic activities, antiplatelet aggregation and antimicrobial activities. The current review discusses the therapeutic potential of beauvericin including pharmacological and biological activities which will certainly draw the attention of scientific community to improve the production of beauvericin for its use in medical fields.

Keywords: Beauveria; Therapeutic; Anticancer; Cytotoxic; Antiplatelet

Introduction

Bioactive secondary metabolites produced by entomopathogenic fungi play a key role as virulence factors for fungi infecting arthropods [1,2]. In addition beauvericin can also be synthesized by several other fungal genera such as Paecilomyces, Polyporus, Isaria and Fusarium. Several low toxic compounds are produced by entomopathogenic fungi Beauveria bassiana like destruxins, bassianolide, Beauvericin, efrapeptins, tenellin, oosporein [3-5]. The biometabolite beauvericin is first isolated from liquid fermented culture of Beauveria bassiana, which is a common and commercial entomopathogenic mycoinsecticide [6,7]. The genus Beauveria is well known mycoinsecticide and exhibits antimicrobial, antitumor, antifungal and antiviral activities [8]. The entomopathogenic fungus, Beauveria bassiana belongs to Phylum Ascomycota. It has been found and isolated from a wide variety of insects of different orders [9-12]. It is most widely used fungal species which is commercially available [13]. This fungus is widely found on infected insects both in temperate and tropical areas throughout the world [14]. In addition to beauvericin, Beauveria also produces varieties of other pharmacologically active compounds such as enniatins, ketone, alphahydroxy isovaleric acid, hydrocarbons, fatty acids, and wax ester.

Beauvericin is a cyclic trimmer of a dipeptidol monomer synthesized from N-methyl phenylalanine and 2-hydroxyisovaleric acid which increases its potency for anti-cholesterol, and chemosensitizer activities, as well as repression of amyloid plaque formation in Alzheimer’s disease [15]. Recent studies have shown that other than the above biochemical and molecular processes, other molecular mechanism of beauvericin is cell apoptosis [16,17] and other molecular targets of beauvericin are antiangiogenic inhibition, inhibition of metastasis prostate and breast cancer [18]. Therefore, these fungal natural products represent promising leading anticancer potential. However, the cure of diseases such as cancer still remains elusive despite the availability of a variety of chemotherapeutics agents that exhibit sophisticated mechanisms of action [6]. The Figure 1 illustrates various interactions of beauvericin in biochemical processes, including metastasis, apoptosis, antiplatelet aggregation and antimicrobial activity.

Structure and Chemistry of Beauvericin

Beauvericin or cyclic hexadepsipeptide belongs to the family of enniatins with molecular formula C38H36N10O9, molecular weight 783.95, alkaline, needle like crystal, soluble in methanol, diethyl ether, chloroform, slightly soluble in water, melting point 95-97°C with a maximum absorption of 209 nm. The structure of beauvericin consists of three D-α-hydroxy-isovaleryl and N-methyl-L-phenylalanyl residues in alternating sequence as shown in Figure 2. Being a member of the enniatin family of antibiotics it has activity against Gram positive bacteria and mycobacteria, as well as against insects and brine shrimp. Its ion complexing capability allows beauvericin to transport alkaline earth metal and alkali metal ions across cell membranes [19,20]. Beauvericin has been shown to cause channel formation in patches of ventricular myocytes and synthetic membranes [21].

Figure 1: Illustration of the different interactions of beauvericin in biochemical processes, including metastasis, apoptosis, antiplatelet aggregation and antimicrobial activity.

Keywords: Beauveria; Therapeutic; Anticancer; Cytotoxic; Antiplatelet

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Mechanism of Extraction, Separation and Detection of Beauvericin

For the extraction of beauvericin, cereal and corn samples were blended by using an acetonitrile-water mixture. In 1999, homogenised the sample with methanol [22]. After blending, the extract was placed into the Liquid chromatography–mass spectrometry without any further clean up and resulting in an LOD of 8 µg/kg. Liquid-liquid-partitioning with dichloromethane is often combined with solid phase extraction (SPE) columns for cleaning processes. Separation and detection of beauvericin are performed by using a High-Performance Liquid Chromatography with Photodiode Array Detection (HPLC-DAD) system with a reversed phase C18 column and an acetonitrile-water mixture as mobile phase. The absorption spectrum is calculated at 192 nm and 209 nm. Besides this Nuclear Magnetic Resonance (NMR) Spectroscopy is also used for identification purposes.

Another technique used for separation and detection is high performance thin layer chromatography (HPTLC), using precoated silica gel 60 plates that are spotted with methanolic extracts. Mobile phases are toluene/acetone, chloroform/2-propanol and ethyl acetate/hexane. Detection is performed at 365 and 254 nm after colouring with iodine vapours with a detection limit in the mg/kg range. It is concluded that Liquid Chromatography-Mass Spectrometry is a powerful tool for the determination and identification of beauvericin. The major disadvantages of liquid chromatography, mass liquid chromatography and mass spectrometry techniques are the high costs involved and the professional experience that is required [23].

Biosynthesis of Beauvericin

Wang and Xu reviewed the biosynthesis of bioactive secondary metabolite beauvericin [8]. A multifunctional enzyme beauvericin synthetase with a molecular mass of 250 kDa helps in the synthesis of BEA [24]. The fermentation process for the production of BEA is shown in Figure 3. BEA synthetase, AdoMet, ATP and Mg2+ are the important constituent’s helps in the formation of cyclic ester of a trimer of (2R)-2-hydroxy-3-methylbutanoyl-N-methyl-L-phenylalanine. The nonribosomal peptide synthetase enzyme helps in the formation of cyclic ester of a trimer of (2R)-2-hydroxy-3-methylbutanoyl-N-methyl-L-phenylalanine [25]. The initial and important biosynthesis step involves transamination as L-phenylalanine and valine needs nitrogen and provided by any amino acids. The source of carbon can be pentose or hexose but the glucose was reported to be the most effective agent [26]. Beauvericin also requires several condensation, methylation and activation. S-Adenosyl methionine (AdoMet) acts as a source of methyl group for the L-phenylalanyl residues. Beauvericin biosynthesis is catalyzed by the beauvericin synthetase via a nonribosomal, thiol templated mechanism [7]. Beauvericin synthetase multienzyme activates the r-hydroxyxarboxylic acid (2r)-2-hydroxy-3-methylbutanoate (d-hydroxyisovalerate, d-hiv) and the amino acid phenylalanine (l-phe) as adenylates, captures the activated substrates with peptide bond and finally releases the free cyclic trimeric lactone or the linear hexadepsipeptide is cyclised to make beauvericin with optimum pH for beauvericin formation pH 7.2, and the optimum temperature is 25-27°C [27].

Sumalee states that, fungi are presently considered to be in a broad range of biological activities such as medicines and pesticides [28]. It is studied for the production of beauvericin, the fermentation conditions and techniques are widely accepted and fed-batch method is accepted as the most efficient method. B. bassiana, Fusarium spp., Paecilomyces fumoso-roseus and P. tenuipes are feasible and promising fungi for the production of beauvericin [28-32].

In fed batch fermentation, glucose is the main source to feed the culture and is successfully used in BEA production. It is concluded that carbon is the major source of glucose. Among peptone and NaNO3, peptone is the major source of nitrogen [33,34]. Being an intracellular product, only a small amount of BEA is exported into the medium [8,35]. Xu studied F. redolens Dzt2 mycelial culture along with macroporous polystyrene resin as sorbent increases the production of BEA from 194-265 mg/L. When the mycelial culture is optimized with glucose and resin, the yield is increased up to 400 mg/L. It is concluded that the quantity of BEA was low as compared to those of industrial and commercial products. Hence, the BEA can be extracted by using an organic and non-polar solution in the fermentation process to enhance production.

Biological Activities

Beauvericin displays a diverse array of biological activities including antibiotic, insecticidal, herbicidal, antiretroviral, cytotoxic, anti-haptotactic, anti-cholesterol and chemosensitizer, as well as repression of amyloid plaque formation in Alzheimer's disease [32]. According to Jow et al. [16] beauvericin increases antiproliferative activity against...
various human cancer cell lines by activating calcium sensitive cell apoptotic pathways. On the other hand, it also inhibits the directional cell motility of cancer cells at subcytotoxic concentrations as haptotaxis (outgrowth of cells) is essential for the formation of new blood vessels in tumors (angiogenesis), invasion of other tissues by cancer cells, and metastasis to the distant organs [18,36].

**Induction of Apoptosis and Cytotoxic and Antitumor Activity**

Apoptosis is synonyms to programmed cell death, which leads to series of distinct changes such as DNA fragmentation, alteration in cell morphology, such as blebbing, loss of cell attachment, cytoplasmic contraction and other biochemical changes including the activation of caspases through extrinsic and/or intrinsic mitochondrial pathways [6]. Zhan demonstrated the role of beauvericin in the induction of apoptosis which involves various changes in the cellular and molecular pathways [18]. This process involves the activation of proteins which are involved in programmed cell death, for example, the BCL2 family of proteins has both anti-apoptotic and pro-apoptotic members. Beauvericin has also been known to induce apoptosis in the human leukemia cell lines (CCRF-CEM) through nuclear fragmentation followed by the release of cytochrome C from mitochondrial membrane which leads to activation of caspase 3 and subsequent cellular changes in morphology [17]. Similarly the apoptotic effect of Beauvericin has also been investigated in human cell lines, HepG2 and MRC-5 to possess anti-convulsion and antihyphmic activities [37].

It is demonstrated in several laboratories that beauvericin induced apoptosis in different systems. It is reported that in CY-1 (monkey kidney) cells, beauvericin induces the stereotypical hallmarks of apoptosis, including the formation of DNA ladders, compaction of nuclear DNA and the subsequent appearance of apoptotic bodies [38]. Another study reported the apoptotic activity in neonatal human keratinocytes and human esophageal epithelial cells with beauvericin which has developed morphological changes in cell shrinkage and membrane blebbing [39]. Similar results are observed in a porcine kidney epithelial cell line when treated with beauvericin [40]. Nilanonta reported the cytotoxic effect of beauvericin on African green monkey kidney fibroblastin African Vero with IC\textsubscript{50} 10 µg/ml [30]. Additionally he also reported its effect on human breast cancer cells BC-1 and human epidermiod at 20 µg/ml. Nuclear fragmentation in the cell by the release of cytochrome C from mitochondria with the activation of caspase 3 and cellular changes in morphology with IC\textsubscript{50} 1-2 µg/ml leads to cytoytic effect on the cell lines of leukemia (CCRF-CEM) [16]. Calo studied the effect of mycotoxin BEA in human cell lines of myeloid origin which includes human monocyctic lymphoma cells U-937 with IC\textsubscript{50} 10 µg/ml and human promyelocytic leukemia HL-60 with IC\textsubscript{50} 12 µg/ml [41].

Lin demonstrated the induction of apoptosis by BEA in human non–small cell lung cancer-A549 [42] cell line which involves the BCL2 family, cytochrome C release and caspase 3 activation with IC\textsubscript{50} of 2.4-7.8 µg/ml, whereas Zhan studied the anticancerous activity against human non–small cell lung cancer [18] NCI-H460 cell line with IC\textsubscript{50} 1.1 µg/ml. Recently it was also demonstrated in human breast cancer MCF-7 cells, human CNS cancer (glioma) SF-268 cells and human pancreatic carcinoma MIA Pa Ca-2 cells with IC\textsubscript{50} from 1.3 to 1.8 µg/ml which involves searching for cell motility and angiogenesis inhibitors with potential anticancerous activity. Similar antiproliferative results were observed in human retinoblastoma Y79 cells with IC\textsubscript{50} 0.4-4 µg/ml [43].

In addition to its involvement in apoptotic pathways, beauvericin plays an important role in inhibition of cell cycle at certain check points. It is observed that beauvericin leads to the inhibition of P(III)Akt phosphorylation during T-cell activation. It is also observed that these cell lines show antiangiogenic activity in Human Umbilical Vein Endothelial Cells (HUVEC) cells at sublethal concentrations [18]. inhibition of angiogenesis is an effective cancer chemotherapy strategy, and thus may be a useful anticancer agent [44].

The first experimental study to demonstrate the anticancer properties of beauvericin is performed in mice that demonstrates that BEA leads to the inhibition of activated T cells via down regulation of the P(III)-Akt signaling pathway [45]. Furthermore it is showed that BEA plays an important role in the etiology of plant diseases. Preliminary studies reported that BEA is highly toxic to melon protoplasts as compared with fusaric acid and fumonisin B1 [46].

**Anti-Platelets Aggregation**

Platelets play an important role in regulation of tumor angiogenesis, its growth and metastasis. Earlier it is reported that cancer cells have remarkable property to activating platelets, helping their survival in the blood circulation during hematogenous metastasis by preventing tumor cell lysis by natural killer (NK) cells and cytotoxic T lymphocyte cells [47,48]. A variety of molecular mechanisms such as GP I b/IIIa, GP Ib-IX-V, P2Y receptors, and PAR receptors, have been proposed to describe tumor cell-induced platelet seggregation (TCIPA) [49,50].

**Antimicrobial Activity**

BEA showed a great antibacterial activity against many pathogenic Gram positive and Gram negative bacteria, which also includes human,
animal and plant pathogens mainly food crops. It was found that BEA targets the cell organelles such as ribosomes or cell nucleus and enzymes, as BEA is synthesized from amino acids. Unlike other antibiotics example as penicillin which blocks the peptidoglycan biosynthesis of Gram-positive bacteria whose cell wall is not the antibacterial mode of action of beaucerin, although these antibiotics and beaucerin are both from amino acids that are produced by fungi [8]. Bacterial strains which are inhibited by BEA are listed in Table 1. Till now investigation is going on, against the activity of drug resistant bacteria of BEA.

Viral infections lead to many fatal and epidemic diseases such as HIV, H1N1, SARS, AIV and HBV. Shin reported a new inhibitor of cyclic hexadepsipeptides i.e., BEA, which inhibits HIV type 1 integrase at IC_{50} of 1.9 μM [51-55]. Along with antibacterial and antiviral activity, BEA being a fungal product it lacks antifungal activity. BEA showed the antifungal activity only in combination with ketonazole at 0.5 mg/kg and miconazole at 0.5 mg/kg concentration against Candida parapsilosis which leads to high mortality rates especially in neonates [18,56]. Experimental research is still going on to study the effect of BEA in combination with other compounds as antifungal agent and as a result it can be utilized as therapeutic potential in many fields.

**Insecticidal Activity**

Hamill discovered firstly the insecticidal activity of BEA against a model organism Artina salina [7]. Similarly, various researchers investigated the insecticidal effect of BEA on Aedes aegypti, which is found to be more effective [57] and Calliphora erythrocephala, Lygus spp., Spodoptera frugiperda and Schizaphus graminus [58-60]. From the above listed functions the insecticidal activity of BEA is still under investigation and cannot be applied directly as commercial insecticidal agent.

**Conclusion**

BEA is a bioactive compound of a fungal product which shows different kinds of bioactivities (antimicrobial, anti-insecticidal, antitumor, and antiplatelet, etc.) at picogram level and with unique uncharacterized active mechanism [61-66]. BEA would be a great discovery in pharmacology, toxicology and medicines or pesticides which are inhibited by BEA are listed in Table 1. Till now investigation is going on, against the activity of drug resistant bacteria of BEA.

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<tr>
<th>Gram Positive Bacteria</th>
<th>Bacterial Strains</th>
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<tbody>
<tr>
<td>Bacillus spp.</td>
<td>Castelbury et al.</td>
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<tr>
<td>Bifidobacterium adolescentis</td>
<td>Castelbury et al.</td>
<td>[51]</td>
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<tr>
<td>Enterococcus faecium</td>
<td>Meca et al.</td>
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<td>Listeria monocytogenes</td>
<td>Meca et al.</td>
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<tr>
<td>Staphylococcus haemolyticus</td>
<td>Xu et al.</td>
<td>[54]</td>
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<tr>
<td>Agrobacterium tumefaciens</td>
<td>Xu et al.</td>
<td>[54]</td>
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<tr>
<td>Escherichia coli</td>
<td>Xu et al.</td>
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<tr>
<td>Pseudomonas lachrymans</td>
<td>Xu et al.</td>
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<tr>
<td>Shigella dysenteriae</td>
<td>Xu et al.</td>
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**Table 1:** Strains of bacteria inhibited by Beauvericin.

References


