Potential Hexokinase II Inhibition by Benzimidazole Anthelmintics: Albendazole, Mebendazole and Fenbendazole

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Authors’ contributions

This work was carried out in collaboration between both authors. Author HL wrote the first draft and managed the literature searches. Author SKK wrote and managed the manuscript. Both authors read and approved the final manuscript.

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ABSTRACT

The nitrogen heterocycle benzimidazoles have been known to be anthelmintic drugs. Beyond that role, the benzimidazoles exhibit other pharmacological potential as anti-inflammatory, antiulcer, anti-hypertensive and anticancer agents. A growing body of evidence supports the anticancer efficiency via treatment with benzimidazoles. The target proteins for pharmacological effect appear to be cell microtubules. However, it has been reported that the benzimidazoles could inhibit hexokinase II, which is a critical factor in the glycolysis pathway in humans. Here, we discuss on the most common benzimidazoles such as albendazole, mebendazole and fenbendazole and focus on the potential anticancer activities of the target enzyme hexokinase II. This review would give better insight in development of target-specific benzimidazole derivatives as potential anticancer therapeutics.

Keywords: Benzimidazole; albendazole; mebendazole; fenbendazole; hexokinase.

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1. INTRODUCTION

A nitrogen heterocycle benzimidazole was first synthesized by Hoebrecker [1], and after 80 years the therapeutic activity as an anthelmintic drug was recognized [2]. Today, benzimidazoles are the most important anthelmintics serving as effective drugs for intestinal and tissue-dwelling parasites [3]. Benzimidazoles are bicyclic aromatic compounds with an imidazole ring fused with benzene [4]. Although benzimidazoles are primarily utilized as anthelmintic drugs, they have also presented strong therapeutic application against pathogenic bacteria and fungi [5]. Benzimidazole derivatives are also associated with a variety of types of pharmacological activities such anti-inflammatory [6], antiulcer [7], anti-hypertensive [8] and even possibly anticancer [9].

The most popular benzimidazole anthelmintics such as albendazole, mebendazole, and fenbendazole have been used throughout the world since their introduction in the 1960s, showing efficacy against parasites in livestock due to their high activity and low toxicity [10-11]. Researchers revealed some benzimidazoles appear to lead to inhibition of microtubule polymerization as antimitotic drugs and result in critical transition of the cell cycle leading to cell death [12-13]. Additionally, it has been reported that a benzimidazole derivative may inhibit hexokinase II, a key glycolytic enzyme in cancer cell metabolisms [14].

The benzimidazoles have revolutionized the drug discovery process by showing their diverse range of biological activities. The power of potential of benzimidazoles has drawn interest in developing potent benzimidazole derivatives containing broad spectrum pharmacological activities. In this review, we focus on the antitumor therapeutic effects of the following benzimidazole anthelmintics: albendazole, mebendazole, and fenbendazole by selectively surveying literatures from 1984 to 2019 (Fig 1). In addition, we will review the target enzyme hexokinase II for anticancer effect by the above mentioned benzimidazoles.

2. ALBENDAZOLE

Albendazole is notably low in toxicity and considerably safer for treatment due to its benzimidazole carbamates. In 1975, albendazole was invented by Robert J. Gyurik and Vassilios J. Theodorides for treatment of parasites in sheep [15]. This drug became registered for use in humans in 1982 [16]. Albendazole (5-(propylthio-1H-benzimidazol-2-yl) carbamic acid methyl ester is insoluble in water and most organic solvents. It is poorly absorbed in the GI tract, and after absorption, albendazole is rapidly converted by the liver to its primary metabolite, albendazole sulfoxide, which is

![Fig. 1. Benzimidazoles. 2D structures of molecules: Albendazole (a), Mebendazole (b) and Fenbendazole (c)]
Albendazole can treat neurocysticercosis, which is an infection by the pork tapeworm in the muscles, brain, and eyes. This condition has the potential to cause seizures, brain swelling, and vision problems [18]. This drug is commonly coupled with surgery to target cystic hydatid disease, an infection caused by dog tapeworm in the liver, lung, and lining of the abdomen. Human hydatid cyst (cystic echinococcosis) is a zoonotic disease that is recognized as a major public health issue on a global scale. Human hydatid cysts is the result of ingestion of *Echinococcus granulosus* eggs, and as the worms enter the larval stage, hydatid cysts form in the liver, lungs, and other organs in the human body. This life-threatening disease is typically treated with hydatid cyst open surgery, but there is a high-risk recurrence. Albendazole has been used alone or coupled with surgery to treat the condition of hydatid cysts. Efficacy is still not well documented, and hydatid cyst treatment with albendazole is still undergoing further research in case studies and clinical trials in humans and animals [19]. According to Fattahi Masoom et al. (2017), albendazole may have benefits for patients with inoperable cystic echinococcosis with multiple cysts [20]. There were 164 patients with echinococcosis referred to Ghaem hospital between 2001 and 2013 who were diagnosed with alveolar echinococcosis. Patients who underwent higher phases had notably increased chances of better response. Albendazole has shown promising results in pharmaceutical application of treating human hydatid cysts in neurocysticercosis.

3. MEBENDAZOLE

Mebendazole (2-benzimidazolecarboxylic acid methyl ester), another class of benzimidazoles that came into use in 1971, was developed by Janssen Pharmaceutica in Belgium [21]. Mebendazole is on the World Health Organization's List of Essential Medicines and is available as a generic medication. Compared to albendazole, which was moderately toxic to humans, Mebendazole was the first benzimidazole carbamate to be used on humans [22]. Mebendazole halts glucose uptake and can also be prescribed to treat parasitic pinworm infections including threadworm, tapeworms, roundworms, and other nematode infections in humans and livestock [23]. Mebendazole has typically been used in low dosages for local gut helminthic infections, but requires increasingly high amounts in months to years for chronic echinococcus infections. Current research indicates that mebendazole has potential antitumor properties [24]. This drug shows early clinical efficacy in glioblastoma and medulloblastoma. In experiments performed by Bai et al. (2015), researchers explored three different mebendazole polymorphs (A, B, and C) that can penetrate the brain [25]. Results observed by polymorph C had the highest efficacy by therapeutically reaching the mouse brain tissue and tumor with fewer side effects when coupled with elacridar than cases observed with other therapeutics [25]. Customized mebendazole formulated tablets were observed for the polymorph content by IR spectroscopy and for efficacy and tolerability. Polymorph A presented lower levels in the plasma and brain, and B and C showed greater toxicity and increased survival. Elacridar is a drug that acts as a bioenhancer that inhibits multiple drug resistant tumors. Therefore, mebendazole, which is enhanced by elacridar, has high efficacy and is a better choice of brain cancer therapy [25].

4. FENBENDAZOLE

Fenbendazole (methyl N-(6-phenylsulfanyl-1H-benzimidazol-2-yl) carbamate) is a broad-spectrum benzimidazole anthelmintic that contains anti-proliferative activity and is typically used in veterinary medicine to target parasitic pinworms. Fenbendazole is also known to have low degree of toxicity and high degree of safety in animals [26-30]. Fenbendazole is used to target gastrointestinal parasites including: giardia, roundworms, hookworms, whipworms, the tapeworm genus *Taenia* - excluding dipylidium caninum, which is a common dog tapeworm -, pinworms, aelurostrongylus, paragonimiasis, strongyles, and strongyloides. Fenbendazoles can be administered to experimental animals such as sheep, cattle, horses, dogs, cats, and rabbits [31]. This broad-spectrum anthelmintic disrupts microtubules and proteasomes, reduces glucose uptake from glucose transporter isof orm 4 (GLUT4), expresses glycolytic enzymes such as hexokinase II, starving cancer cells [14]. When
insulin initiates the glucose uptake in cells, GLUT4 translocates from the intracellular vesicles directly to the plasma membrane where glucose is taken up. The linear movement of GLUT4 towards the plasma membrane takes place along microtubules. When microtubules are disrupted with fenbendazole, GLUT4 is disrupted simultaneously, thus reducing insulin-stimulated glucose absorption [32]. Fenbendazole can also reactivate the p53 gene that functions to suppress tumors and inhibit some cancers [33]. Accumulation of ubiquitylated derivatives including p53 appears to result in apoptosis in the mitochondrial pathway.

Recent clinical studies explore future therapies that can lead to anticancer effects. A research experiment demonstrated the inhibition of proteosome function and induction of apoptosis in human lung cancer cells by fenbendazole [34]. Fluorogenic substrates were used to show that fenbendazole results in the decrease of proteasomal activity in the cells. Non-small cell lung carcinoma (NSCLC) were treated with fenbendazole and accumulated green fluorescent protein with an increased half-life. Apoptosis regulatory proteins are typically degraded by ubiquitin-proteosome pathway and accumulate within cells treated by fenbendazole. NSCLC cells with fenbendazole lead to endoplasmic reticulum stress, reactive oxygen species production, decreased mitochondrial membrane potential, and cytochrome c release [34]. Fenbendazole leads to cancer cell death, which may lead to new anticancer agents with high efficacy and specificity for tumor cells by inhibition of hexokinase in glycolysis.

5. HEXOKINASE

Hexokinases play a key role in cellular glucose metabolism. Hexokinase is the first step in glycolysis and uses an ATP molecule in the initial process, influencing the direction of glucose flux within the cell. This is the first step of all major pathways of glucose usage including glycolysis, pentose phosphate pathway, and glycogenesis. Glycolysis and gluconeogenesis are critical for energy metabolism and storage. Hexokinases transfer a phosphate from ATP to glucose, which yields glucose-6-phosphate. This results in a downstream effect of the concentration gradient and allows facilitation of cellular glucose influx. This reaction must be shielded from water in order to keep ATP molecules from being cleaved off by water. As a result, hexokinase performs an induced fit, which encloses glucose and ATP post binding. The open structure of hexokinase is shown in Fig 2a. Once glucose is bound, hexokinase undergoes a conformation change as shown in Fig 2b. Due to the possibility of substantial conformational change, some benzimidazoles may have an opportunity to bind the active site of hexokinases. For example, Zeng et al. (2015) revealed the complex structure between human hexokinase II and a potential inhibitor 2,6-disubstituted glucosamine, indicating that the active site of hexokinase is potentially a good binding target for finding inhibitors [35].

There are four isoforms of hexokinase: hexokinases I, II, III, and IV. Hexokinases I, II, and III are inhibited noncompetitively by glucose 6-phosphate and competitively by ATP. Hexokinase I catalyzes the first step in glycolysis. Hexokinase inhibits glycolysis by 2-deoxy-D-glucose (2-DG), a glucose analog, which is the rate-limiting step of glycolysis via hexokinase. 2-DG assists in the development of anticancer strategies that include radio, chemosensitization, and oxidative stress. Hexokinase phosphorylates this analog to 2-DG-phosphate (2-DG-P) and metabolism is disrupted by phosphoglucone isomerase [36]. This results in 2-DG-P accumulation and ATP depletion in cells. Furthermore, 2-DG-P increases the activity of autophagy, enhances reactive oxygen species production, and activates AMP-activated protein kinase-9.

Hexokinase II takes part in glycolysis and in the transports into and out of the mitochondrial intermembrane space through the association with a voltage-dependent anion channels; thus, the depletion of hexokinase II may inhibit the glycolytic pathway [37]. There are several compounds synthesized in order to inhibit hexokinase II. The compound 2,6-disubstituted glucosamine was found by a high throughput screen, and the compound showed a competitive inhibition with a micro-range inhibition equilibrium constant (Ki = 2.9 μM) [35]; the complex structure is shown in Fig 2c. As another example, 3-bromopyruvic acid showed its amazing ability to inhibit hexokinase II and became an antitumor agent with high efficacy [38]. In addition, researchers synthesized a compound called as lonidamine (LND), which serves as a hexokinase inhibitor, antitumor agent, mitochondrial pyruvate carrier, and plasma membrane monocarboxylate transporters inhibitor [39]. More specifically, lonidamine disrupts energy metabolism of cancer cells, specifically inhibiting aerobic glycolytic activity affecting the mitochondrial complex II.
and hence, LND might impair energy-requiring processes [39]. Fenbendazole also impairs enzymatic activity of hexokinase II, eventually leading to the activation of apoptotic signals [14].

6. CONCLUSION

Benzimidazoles that have historically been used in veterinary medicine may be beneficial in treating cancer in humans. Traditionally, they have been prescribed as antimicrobial and antiparasitic agents, but clinical studies show these agents offer anticancer effects with low toxicity and are highly effective by starving cells of glucose through apoptosis. By interrupting the energy metabolism of parasites by binding to tubulin, microtubule polymerization can be disrupted causing nutrient uptake inhibition and prevention of the major pathways of glucose by hexokinases. Glycolysis is prevented by hexokinase and therefore inhibits glucose pathways. Benzimidazoles such as albendazole, mebendazole, and fenbendazole have been shown to inhibit the progression of difficult-to-treat cancer types. By inhibiting glucose from being upregulated by the enzyme hexokinases and the binding site of the protein, the cells cannot complete the glycolysis pathway and die. Thus, researchers across the world need to design new benzimidazole derivatives that could specifically target hexokinase II in an attempt to treat cancerous cells with low toxicity and no adverse effects.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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