

Clotrimazole as a Cancer Drug: A Short Review

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Abstract

Although clotrimazole was first used against fungal infections, a body of research was later developed indicating that this drug has anticancer properties as well. The mechanism of action is based on the inhibition of mitochondrial-bound glycolytic enzymes and calmodulin, which starves cancer cells of energy. Clotrimazole and its derivatives have been shown to decrease rates of cancer cell proliferation, induce G1 phase arrest, and promote pro-apoptotic factors, which lead to cell death.

Keywords: Clotrimazole; Calmodulin; Human melanoma; Hexokinase

Introduction

Clotrimazole, first synthesized by Karl Hienz Buchel (Bayer) in the late 1960s, was originally developed as an antifungal agent [1]. The first in a family ofazole derivatives, it was patented by Bayer in 1972 and introduced to German markets in 1973 under the brand name Canesten. This drug's unremarkable side effects and uncomplicated metabolic profile allowed it to enjoy worldwide acceptance for the treatment of mycotic outbreaks such as vaginal yeast infections and athlete's foot. A plethora of popular products (Desenex, Lotrimin, and Lamisil) followed and, because of its utility, clotrimazole was placed on the World Health Organization's list of essential medicines. Clotrimazole is a crystalline powder with a molecular formula of $C_{22}H_{17}ClN_2$ and an average molecular weight of 344.8 g/mol. It is a lipophilic molecule with a solubility of 0.49 mg/L in water. Figure 1 shows the 3-D representation of the clotrimazole molecule.

In addition to its use as an antifungal, there is promising research on using clotrimazole against other diseases (Table 1) such as sickle cell anemia, malaria, beriberi, tinea pedis, Chagas disease and cancer [2-7]. The mechanism of action of clotrimazole against fungi and the trypanosomes of Chagas disease is the inhibition of ergosterol synthesis which slows microbial growth. Clotrimazole also inhibits the Ca^{2+} -activated potassium (IK) channel and causes heme-induced membrane damage, which are responsible for its action against sickle cell disease and malaria, respectively.

In 1995, clotrimazole was shown to have an inhibitory effect on cancer cell proliferation and tumor growth when tested against human melanoma and glioblastoma cells *in vivo* [8,9]. Additional studies revealed clotrimazole to be a potent calmodulin inhibitor and that cells exposed to clotrimazole were found to have depleted calcium stores which were unable to be replenished by normal cell mechanisms [10,11]. Anti-cancer effects can also be traced to its targeting of the

glycolytic enzymes hexokinase, phosphofructokinase, and aldolase [12-14]. The inhibition of glycolysis was shown against breast cancer, MCF-7, MCF10A, MDA-MB-231, HeLa, B16-F10, and Lewis lung carcinoma cell lines [13,15-19]. Further research showed the cytotoxicity of derivative compounds when clotrimazole was used as a ligand in metal coordination complexes [20,21]. In each case, clotrimazole was a potent inhibitor of glycolysis, leading to cell-cycle arrest and apoptosis.

Mechanism of Action Against Cancer

Hexokinase (HK) is the enzyme that phosphorylates glucose in the first and the rate-limiting steps of glycolysis. HK has four isoforms HK_1 , HK_2 , HK_3 , and HK_4 (or glucokinase, GK). HK plays a crucial role in tumor growth and is the major enzyme expressed in various malignant tumors [22]. HK is bound to trans-membrane voltage-dependent anion channels (VDACs), which are located within the mitochondrial outer membrane [23]. HK will normally block the cytochrome c release from the mitochondrial outer membrane and protect the cells from apoptosis. Clotrimazole disrupts HK binding to mitochondria thus precipitating cell death [14]. Due to the fundamental role of HK in glycolytic flux in many cancers, this enzyme became an attractive target for cancer therapy. Chiara et al. has reported that clotrimazole specifically disrupts the HK-VDAC interaction in HeLa cells that causes the opening of the permeability transition pore complex, followed by release of apoptogenic proteins that cause indirect cell-death [17]. Palchaudhuri et al. treated B16-F10 cells with clotrimazole

| Disease | Mechanism of action |
|---|---|
| Sickle cell disease [2] | Inhibition of calcium-dependent potassium channel |
| Malaria [4] | Heme-induced membrane damage |
| Chagas disease [5], <i>Candida albicans</i> [7], Tinea pedis (Athlete's foot) [6] | Inhibition of ergosterol synthesis |
| Cancer [13] | Inhibition of calcium-dependent potassium channel, glycolytic enzymes, and G ₁ -phase arrest |

Table 1: Clotrimazole as a drug of interest against several diseases.

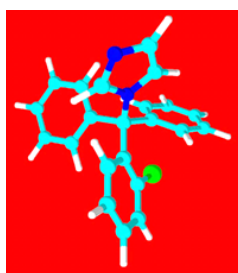


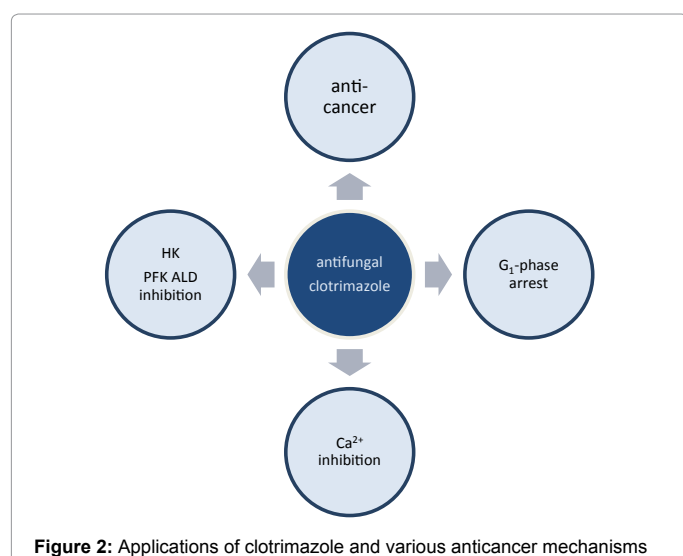
Figure 1: Structure of Clotrimazole

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to understand whether clotrimazole induces detachment of HK from the mitochondrial outer membrane and exhibits antiproliferative activity by inhibiting glycolysis and reducing the cellular ATP level [24]. This treatment showed a 71% reduction in mitochondrial-bound HK-consistent with previous observations and reveals the mechanism of inducing cell death. Other research showed that clotrimazole also inhibited the signaling kinase protein Akt, which enhances the effect of HK dissociation [25]. Clotrimazole most likely causes dissociation of HK, cytochrome c release, and apoptosis through an allosteric interaction [26].

However, HK is not the only cellular target affected by clotrimazole; it also affects the two other important glycolytic enzymes: phosphofructokinase (PFK) and aldolase (ALD) [14]. Treating B16-F10 cells with clotrimazole was found to cause detachment of these enzymes from the cytoskeleton [12,27]. Penso et al. treated Lewis lung carcinoma and colon adenocarcinoma (CT-26) cells with clotrimazole and found a dose-dependent detachment of the PFK and ALD from the cytoskeleton [18]. Since PFK is found to be overexpressed in aggressive tumors that have an increased glycolytic flux when compared with normal tissues, this enzyme is a promising target for antineoplastic agents [28]. In another study, clotrimazole inhibited breast cancer cell proliferation through apoptosis triggered by the displacement of key glycolytic enzymes [27]. Goncalves demonstrated that when clotrimazole was tested against tumoral and nontumoral intestinal epithelial (Caco-2) cell lines, clotrimazole inhibits oxidative phosphorylation and glycolysis. In addition to decreasing ATP production which slows cell growth and proliferation, detachment of PFK can cause significant changes in cytoskeleton conformation and cell shape, which eventually lead to apoptosis [19].

Another important feature of clotrimazole is its agonistic action on calmodulin. Interference with Ca²⁺ metabolism and blockage of the Ca²⁺-activated potassium (IK) channel by clotrimazole has been shown to reduce cancer cell proliferation [29-31]. Clotrimazole induces a release of Ca²⁺ stores and slows their replenishment, thus inhibiting translation and slowing tumor growth [32]. Furthermore, this disruption of ion transport is severe and induces apoptosis as demonstrated by Ito against acute lymphoblastic leukemia cells [9]. When clotrimazole was tested for 86Rb efflux inhibitions, it was found that clotrimazole blocks the IK channel with an average IC₅₀ value of 0.92 μM [24]. These results are remarkable in that the anticancer effects were observed without an accompanying cytotoxicity [8].

Since the discovery of the popular drug cisplatin, platinum complexes have become the most widely used metal-based drugs in anticancer research [33]. The nitrogen atom present in the imidazole ring of clotrimazole facilitates coordination with transition metal ions such as Pt, as well as Ru, Pd, Cu, Co, Zn and Ni. Ravera et al. synthesized Pt(II) complexes containing bis(clotrimazole) ligands which were shown to effectively inhibit the growth of MCF-7, SKBR-3, HT-29, and B16/BL6 cell lines [21]. In another study, a series of Ru-clotrimazole compounds were synthesized which were shown to be active against multiple cancer cell lines [34]. Finally, Soledad Betanzos-Lara et al. synthesized sixteen novel Cu^{II}, Co^{II}, Zn^{II}, and Ni^{II} coordination complexes of clotrimazole and observed cytotoxic activity against HeLa (cervix-uterine), PC3 (prostate), and HCT-15 (colon) cell lines [20]. The SAR studies in this research showed that Cu^{II} ligated clotrimazole complexes have superior IC₅₀ values compared with other metals. It is of interest that these metal complexes are inducing apoptosis superior to that of either the parent clotrimazole or cisplatin.

The triphenylmethyl pharmacophore present in clotrimazole is the crucial element in its anti-cancer activity. Small molecules containing the triphenylmethyl motif has demonstrated potent anticancer properties [24,35-38]. All of these triphenylmethyl analogues induce cell cycle arrest in melanoma and other cancer cell lines by inhibiting cellular growth in G₁- or M-phases [39]. Like S-trityl-L-cysteine, clotrimazole has no effect on tubulin polymerization which reduces the side effects observed compared with other classes of chemotherapeutic drugs [35]. Al-Qawasmeh et al. produced a series of 27 clotrimazole analogs without the imidazole moiety. This structure activity relationship study identified the large majority of these analogs as having similar G₀-G₁ phase arrest and antiproliferative effects as the parent molecule containing imidazole [39].

One limitation to clotrimazole's success is its poor water solubility that reduces bioavailability. Many attempts have been taken to improve clotrimazole's solubility through the use of microcapsules, thermo sensitive gels, liposomes, nanospheres or through a suspension with hydroxypropylmethyl cellulose [40-43]. Recently, inclusion of the poorly water-soluble clotrimazole with beta-cyclodextrins showed an enhanced solubility and a greater bioavailability in rats [44]. The future development of clotrimazole derivatives should center on retaining antiproliferative properties while increasing bioavailability-using information from structure relationship studies, biochemical, and cell-based assays.

Conclusions

Clotrimazole and its analogs have been shown to be clinically useful antitumor drugs, reducing the size and growth of neoplasms by interfering with plasma membrane ion transport, inducing G₁-phase cell cycle arrest, and inhibiting the metabolic enzymes HK, PFK, and ALD. A more complete understanding of the mechanism of action of clotrimazole in cancer cell death will accelerate research into beneficial applications of this drug against cancer.

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