Antiprotozoal Diseases and Aromatic Diamidine Derivatives Used in The Treatment of These Diseases

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ABSTRACT
Parasitic infections are quite common in developing countries and in immunocompromised individuals. Such infections are responsible for a considerable number of mortality, morbidity and economic distress. There is an urgent need for the development of novel antiparasitic compounds because existing drugs are resistant to the parasite species, are expensive and highly toxic, require long treatment regimens, and frequently exhibit significantly reduced activity. In this study, general information about antiprotozoal diseases was tried to be given and activity of aromatic diamidines against various microorganisms was investigated.

Keywords: Parasitic infections, Malaria, Sleep Disorder, Chagas disease, Leishmania, Aromatic diamidines

1. INTRODUCTION
Protozoal parasitic diseases have caused catastrophes in human history for centuries. At present, protozoal diseases continue to be a major health problem, especially in tropical and subtropical regions and backward countries [1]. These diseases include malaria, sleeping sickness, Chagas disease and visceral leishmaniasis. The antiprotozoal drugs currently being used in the clinic are expensive, they have high toxicity, the oral bioavailability is low, the requirement of long-term treatment cures and the decreasing effect against some parasite species due to the evolutionary stages and the presence of numerous microorganisms resistant to these drugs limits the use of these compounds in their treatment and new antiprotozoal compounds are urgently needed [2].

Malaria and Compounds Used in Treatment
Malaria (malaria) is the most important tropical parasitic disease in the world. Approximately 300-500 million people have this disease a year and 1-2 million people lose their lives for the reason of this disease [3]. There are four different types of Plasmodium parasites that cause malaria in humans. These parasites are Plasmodium falciparum, P. vivax, P. malaria and P. ovale. P. falciparum In South Africa and East Asia, P. vivax is more common in India, and these two parasites are responsible for more than 95% of the world's malaria cases. Malaria is spread through mosquitoes of anopheles, which have appropriate reproduction and living conditions in tropical regions. [4]. Malaria is an acute febrile illness. In immunocompromised individuals, the symptoms start about 10-15 days after they have bitten. The first symptoms are mild symptoms such as fever, headache, chills, nausea, vomiting, and may often be unrecognizable.
If treatment is not initiated within 24 hours after these symptoms, the disease can become serious and cause death due to serious anemia, especially in children, respiratory failure due to metabolic acidosis, and multiple organ dysfunctions in adults.

In the treatment of malaria since the 17th century, extracts obtained from shells of cinchona plants are used. This plant extract has been isolated in the middle of the 1820's and has been used for millions of malaria cases until daylight. Today, the most commonly used drugs in the treatment of malaria are the combination of chloroquine and sulfadoxine and pyrimethamine. Apart from some regions of Central America and Southeast Asia, P. falciparum has been reported to have resistance to chloroquine and to increase the rate of failure in treatment. The combination of sulfadoxine-pyrimethamine is used in the treatment of malaria cases caused by P. falciparum, which is resistant to chloroquine. In Southwest Asia and Northeast Africa good results are achieved in this combination, while activity in Southeast Asia and sub-Saharan Africa is low. Kinin and mfloquine still maintain their therapeutic activities today, except for some areas of the Thai coast [5]. Artemisinins are the newest class of compounds used in the treatment of malaria. Artemisinin is a natural compound isolated from Artemisia annua plant. This plant has been used by Chinese herbalists since 168 years ago in Milan [6]. While varying in different parts of the world, the development of resistance to all antimalarial drugs except artemisin derivative compounds is seen. Artemisinins are used in the treatment of patients with multiple resistance development. If arthritis develops resistance to the derivative compounds, it is likely to encounter untreatable malaria cases [7].

**Sleep Disorder and Compounds Used in Treatment**

Sleeping Disease in humans is a parasitic disease caused by Trypanosoma brucei rhodesiensense and Trypanosoma brucei gambiensense spread through bloodsucker flea sieve. T. b. gambiensense is more than 98% of sleeping cases. It is reported that approximately 300,000-500,000 people are infected with sleeping disease annually and 50,000 deaths occur [8]. During the past century, there have been many sleeping disease pandemics in Africa. The most recent epidemic occurred in 1970. In 2009, as a result of various attempts to control, the number of cases reported has fallen below 10,000 (9878) for the first time in the last 50 years, and this has continued in 2011 and 2012 as well. However, the estimated number of cases is 216 million, and the number of deaths from this disease worldwide is 445 thousand people. In the case of an epidemic, these figures show how the world is under threat. In the first stage of the disease trypanosomes multiply in subcutaneous, blood and lymphatic tissues. Hematologic and immunologic changes occur in this phase known as high fever episodes, headache, joint pain, and itching attacks that last for several days, and this phase is known as the hemolymphatic phase. In the second phase, parasites cross the blood-brain barrier and infect the central nervous system. In this phase, it is known as the neurological phase and more obvious symptoms of the disease are seen, such as behavior changes, confusion, sensory disorders and poor coordination. The most important symptom is the disorder in the sleep cycle that gives the disease its name; sleeping time is increasing, causing serious weight loss and eventually death [8, 9].

The drugs used in the treatment of the first stage of the disease are Suramin, which went into treatment in the 1920s and the pentamidine that started to be used in the 1940s. Suramin can cause side effects such as nausea, vomiting, shock, kidney damage, agranulocytosis, hemolytic anemia, jaundice and serious diarrhea with the use of i.v. pentamidin is preferred in the treatment of T. b. gambiensense infections in the first instance. It is used only in cases where Suramin is contraindicated in the treatment of T. b. rhodesiensense infections. Pentamidine is not suitable for advanced treatment of this disease. This is because the transition from blood-brain barrier to cerebral binding and tissue adhesion is reduced; the blood-brain barrier can be cleared by pulse pumps such as P-glycoprotein. For this reason, the effect is limited to the first stage and i.m. application is preferred [10]. Melarsoprol was started to be used in the second phase of sleeping disorder caused by T. b. rhodesiensense or T. b. gambiensense in 1949. Severe encephalopathy occurs in 5-10% of people using melarsoprol and approximately half of these patients lose their lives. Other important side effects include vomiting, abdominal colitis, peripheral neuropathy and thrombophlebitis. It is preferred that efionithine, synthesized in 1977, be used in the second stage of sleeping disorder caused by T. b. gambiensense. This drug, which is also ineffective for T. b. rhodesiensense infections, is not preferred due to its high cost and difficulty in application. In recent years, in addition to efionithine, niforoxim has also been used in the second-stage treatment of this disorder [8].
Leishmaniasis disease and Compounds Used in Treatment

Leishmaniasis is caused by Leishmania-type protozoal parasites and passes through Phlebotomus flies. Approximately 350 million people in 88 countries are at risk and it is estimated that 2 million new cases are seen annually [14]. Leishmaniasis infections also occur in rodents and other mammals. There are three main clinical variants of the disease-affecting Leishmaniasis; visceral leishmaniasis, cutaneous leishmaniasis and mucocutaneous leishmaniasis. Visceral leishmaniasis, also known as Kala scabies, is the disease-affecting L. donovani. This form of the disease is systemic. Patients have fever, scabies, cough, liver and spleen growth. Patients lose their lives due to diarrhea, superinfection or gastrointestinal bleeding within 20 months if the treatment is not performed. This disease can be seen in China, Latin America and Russia as well as being widespread in African and Mediterranean countries. Cutaneous and mucocutaneous leishmaniasis are characterized by localized lesions in one or several regions. These lesions are ulcerations that can slowly heal and cause pain and secondary bacterial infections can occur. Cutaneous leaching caused by L. topica is common in the Mediterranean coasts, the Middle East, southern Russia and India, as well as in children and adolescents. L. major is endemic in the African deserts, the Middle East and eastern Russia. L. aethiopica was in Kenya’s high regions and in Ethiopia. L. peruviana, L. braziliensis, L. panamensis are found in South and Central America, while L. mexicana is endemic in the southern and central parts of Texas [6].

Sodium stibogluconate and meglumine antimonate, which contain pentavalent antimony Sb (V), are the first preferred compounds in the treatment of leishmaniasis since 1940's. There is no significant difference between them in terms of efficacy and toxicity. I.v. or i.m. are administered by injection. Common side effects are nausea, hepatotoxicity and cardiotoxicity. Due to resistance development, these compounds are still being used in India, but still in use in the rest of the world. Amphotericin B is a compound used iv in the treatment of visceral leishmaniasis. It limits the use of toxic side effects. Toxic effects in the new clinical formulation obtained by preparing the lipid complex reduction has been achieved. However, the high cost creates a major problem for use in treating visceral leishmaniasis in developing countries. Furthermore, the lipid complex of both Amphotericin B and Amphotericin B is not considered suitable for the treatment of non-visceral leishmaniasis [14] Miltefosine is a compound that has been approved for use in the treatment of visceral leishmaniasis in India, and phase IV studies are in progress. The efficacy of cutaneous leishmaniasis is being investigated by phase III trials.

Some Antimicrobial Aromatic Diamidine Derivatives with Antiprotozoal Effect

Aromatic diamidine derivatives are used as promising compounds showing broad spectrum antimicrobial activity including protozoal diseases, in particular those caused by Trypanosoma and Plasmodium species. The mechanisms of action of aromatic diamidines are still unclear. The reason for this is probably due to their influence with more than one mechanism of action. The most commonly studied mechanism involves the interaction of aromatic diamidines with the DNA minor cavity. It is known that many active aromatic amidines and diamidine derivatives with different chemical structures link to the AT-rich DNA minuscule [2, 15]. These compounds, known to be molecules that interact reversibly with DNA, have been reported to exhibit their antimicrobial activity by inhibiting one or more DNA-dependent enzymes or by preventing direct transcription [16-21].

Aromatic diamidine derivatives; pentamidine, propamidine, stilbamidine, berenil (diminazene); also known as DNA-minor cavity binders or Topoisomerase I and / or II inhibitors, which can act against many bacterial species and amoebae, fungi and protozoa. Pentamidine is the most commonly used in clinical practice from aromatic diamides with wide activity.

Pentamidine

Pentamidine hypoglycemic agent was first synthesized in 1937 as an alternative to synthaline, and later found to be effective against African trypanosomes. It is widely used in the treatment of pneumonia (PCP) caused by P. carinii in patients with AIDS who can not tolerate the combination of antimony-resistant leishmaniasis and trimetoprim-sulfamethoxazole, in addition to its use in early stage sleep disorder treatment. [22]. PCP has become one of the leading causes of mortality and morbidity in AIDS patients. [23]. In a recent study, the combined effect of pentamidine with azoles against C. albicans strains isolated from the food borne of AIDS patients was examined and it was determined that azoles or pentamidine alone were ineffective at a concentration of 10 μg / ml pentamidine. The antifungal effect of itraconazole combined 10 μg / ml pentamidine against some fungi was examined and 72.7% fungicidal effect was observed. [24]. Table 1 summarizes the in vitro antimicrobial activities against various microorganisms of the pentamidine.

Table 1: Comparison of in vitro antimicrobial activities against certain microorganisms of pentamidine

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>IC₅₀</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trypanosoma brucei rhodesiens</td>
<td>2.8 nM</td>
</tr>
<tr>
<td>Trypanosoma cruzi</td>
<td>7.1 μM</td>
</tr>
<tr>
<td>Plasmodium falciparum</td>
<td>129 nM (W2), 51 nM (D6), 96 nM (K1)</td>
</tr>
<tr>
<td>Leishmania donovani</td>
<td>2.6 μM</td>
</tr>
<tr>
<td>Leishmania mexicana amazonensis</td>
<td>0.82 μM</td>
</tr>
<tr>
<td>Giardia lamblia</td>
<td>8.5 μM</td>
</tr>
<tr>
<td>Trichomonas vaginalis</td>
<td>Etki yok</td>
</tr>
<tr>
<td>Scedosporium prolificans</td>
<td>57 μg/ml</td>
</tr>
<tr>
<td>Candida albicans</td>
<td>0.78 μg/ml</td>
</tr>
<tr>
<td>Cryptococcus neoformans</td>
<td>3.12 μg/ml</td>
</tr>
<tr>
<td>Pneumocystis jiroveci</td>
<td>0.29 μg/ml</td>
</tr>
<tr>
<td>Aspergillus spp.</td>
<td>8 – 32 μg/ml</td>
</tr>
<tr>
<td>Fusarium spp.</td>
<td>2 – 4 μg/ml</td>
</tr>
</tbody>
</table>

One possible mechanism of antimicrobial action proposed for pentamidine is inhibition of DNA, RNA, phospholipid and protein synthesis and suppression of the development of Candida species in vivo / in vitro. [24]. The use of pentamidine is limited by the poor oral bioavailability and toxicity. Pentamidine has very serious side effects such as hypotension, abscess at the injection site, abnormal liver function, pancreatic complications, nephrotoxicity, leukopenia, thrombocytopenia, hypocalcemia, cardiotoxicity and hypoglycemia. [25, 26]. Pentamidine is administered in hospitals by following blood count, blood glucose level, liver and kidney functions due to toxicity. Recent studies have shown that pentamidine toxicity can be greatly reduced and drug absorption is enhanced by aerosol administration. [28].

Propamidine

Propamidine is clinically used for local treatment of Acanthamoeba keratitis, which is an important cause of vision loss in patients with severe ocular inflammation and contact lens use [27, 29]. The combined administration of polyhexamethyl biguanide and propamidine in patients is effective, well tolerated and nontoxic for the treatment of A. keratitis, but despite prolonged treatment with biguanides and / or diamidines such as propamidine.
and hexamidine, approximately 5% of patients treated with live parasites corneal and scleral inflammatory attacks have been reported. The amoebicidal activity of propamidine in vitro has shown that it may be an effective treatment option with high potency and low toxicity as effective as pentamidine against different strains of Acanthamoeba [30].

**Hexamidine**

The amoebicidal activity of propamidine alternative hexamidine was demonstrated against Acanthamoeba isolated in vivo and in vitro [31]. Pentamidine and its derivatives have been reported to inhibit replication of parasites in cell cultures and to act against Toxoplasma species by reducing the number of production of tixoisides. Thus, it has been suggested that these compounds may be useful in the treatment of toxoplasma [32].

**Berenil (Diiminazine)**

An aromatic diamidine developed in the mid-1950s as a cattle trypanoside, was found to have both in vivo and in vitro leishmanial and trypanocidal activity. It has also been observed that the dozza-dependent inhibition of the growth of L. donovani and L. tarentolea promastigotes. Treatment with beryllium in children with cutaneous leishmaniasis provides a rapid healing of the ulcer by applying topical medication in the treatment of this infection. Immediately after infection, there is an effect on L. major infected lesions. This compound has failed in 50-60% of drug-resistant T. brucei infection and the results have shown that recurrent infections are more severe than primary infections before treatment [33].

**Furamidine**

The prodrug of metamidoxime [34]. Many of the aromatic diamidine compounds exhibit very low oral bioavailability due to the cationic nature of furamidine and its derivatives and are therefore not effective by oral administration. As a result of in vivo experiments on rats, it has been reported that furamidine is much more active than pentamidine caused by Pneumocystis carinii and has lower toxicity, has no effect on toxicity orally, and has been shown to be effective at oral dose of 27 nM. In another study, it was determined that furamidin derivatives accumulate in the nucleus of the parasite and delay the maturation of the parasite. Thus, it was seen that the parasite population decreased its growth rate. [35]. Table 2 summarizes the in vitro antimicrobial activities of the furamidin against various microorganisms.

![Table 2: Comparison of in vitro antimicrobial activities against some microorganisms of furamidine](https://doi.org/10.24087/IAM.2018.2.11.640)

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>IC&lt;sub&gt;50&lt;/sub&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trypanosoma brucei</td>
<td>4.5 nM</td>
</tr>
<tr>
<td>rhodesiense</td>
<td></td>
</tr>
<tr>
<td>Trypanosoma cruzi</td>
<td>23.3 μM</td>
</tr>
<tr>
<td>Plasmodium falciparum</td>
<td>15.5 nM (K1)</td>
</tr>
<tr>
<td>Leishmania donovani</td>
<td>2.8μM</td>
</tr>
<tr>
<td>Giardia lamblia</td>
<td>0.2 μM</td>
</tr>
<tr>
<td>Trichomonas vaginalis</td>
<td>8.1-57.9 μM</td>
</tr>
<tr>
<td>Candida albicans</td>
<td>6.25 μg/ml</td>
</tr>
<tr>
<td>Cryptococcus neoformans</td>
<td>12.5 μg/ml</td>
</tr>
</tbody>
</table>

**Pafuramidine**

Furamidine's methoxy prodrug is the first oral trypanocidal drug to enter clinical trials and this compound has been extended to phase III clinical trials against pneumonias (PCP) caused by African Trypanosomiasis and Pneumocystis carinii [36]. However, while phase III clinical trials continued, prolonged phase I trials extended the duration of treatment from 10 days to 14 days, and renal toxicity occurred approximately 2 months after the end of the treatment and clinical trials of pafuramidine were discontinued. [37]. However, working on more effective derivatives like pafuramidin is still ongoing. Several prodrugs of azafuramidin and their derivatives show higher oral activity than furamidine. Because of the high activity of aromatic diamidines, the search for new prodrugs in this class of compounds continues to provide orally active compounds [39].

**CGP-40215A**

The dikatonic molecule is a meta-substituted diamidine derivative and is considered to be a pentamidine derivative due to its dichatonic character and long chain structure. Showed excellent antiparasitic activity against African trypanosomiasis with an IC50 of 4.5 nM in vitro and also remarkably remarkable in in vivo activity [40]. In vivo activity studies have been found to be highly effective after triple intraperitoneal dosing in trypanosomal mouse models. Eflornithine and CGP-40215A combination were effective in the rat model of central nervous system infection, but pharmacokinetik studies did not result in penetration into the blood-brain barrier and studies to develop new drugs were concluded. CGP-40215A contains a linear conjugated chain and interacts with DNA protonated in the physiological environment. It was found that both amidine groups
were positively charged under experimental conditions and the chain between the two amine groups was open to protonation with a pKa of 6.3. In studies of this molecule binding to DNA, it has been reported that binding affinity is related to electrostatic interactions and pH values and salt concentration depending on protonation [41, 42].

**DAPI**

This compound has been developed as an antiparasosomal agent for diminazene and stilbamidine derivatives but has been reported to have antibacterial, antifungal and antiviral activity as well as this effect. Nowadays, DAPI is used as a fluorescent dye that can bind to DNA at many binding sites. For this reason, many areas such as chromosome staining, DNA imaging and DNA structure lighting are used in molecular biology. Because of these areas of use, many minor cavitation binder compounds have been designed and synthesized [43].

**2. CONCLUSION**

Many drugs used for the current treatment for many parasitic infections present a significant toxicity to the host, with limited activity against various isolates of parasites and different stages of evolution and are expensive and do not meet the desired requirements to treat infected patients. Parasitic infections such as African sleeping sickness, malaria, Chagas disease affect millions of people worldwide; although incentives for the development of new drug treatments for most pharmaceutical industries are lacking. Parasitic diseases are an increasing concern for developed regions as the number of infected people increases due to increasing international travel and immune system-suppressing diseases. Among the many compounds tested to date, some aromatic diamidines represent an important group of drugs for antiparasitic treatment. New classes of orally active prodrugs of diamidines have also been developed and exhibit significant efficacy against a number of important infections, some of which offer fewer side effects than previous generation compounds. The claim that aromatic diamidines can be used against human malaria will provide an additional basis for ongoing drug discovery efforts. Given the potent antimicrobial activity of diamidines against many parasitic diseases, aromatic diamidines are an important class of drugs for the treatment of parasitic diseases.

**REFERENCES**


