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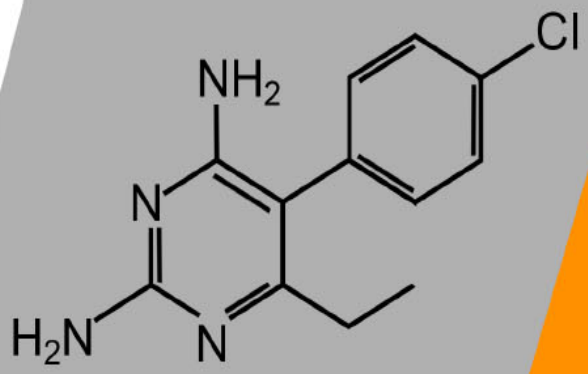
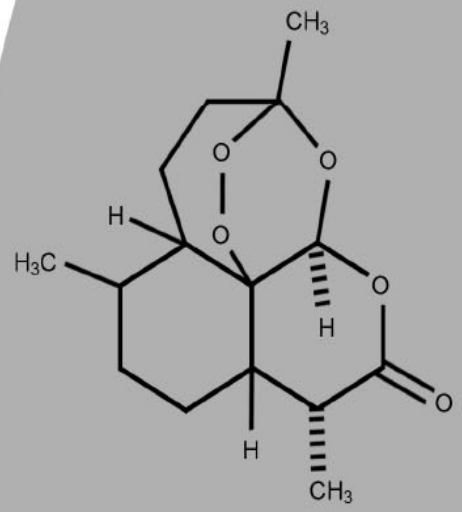
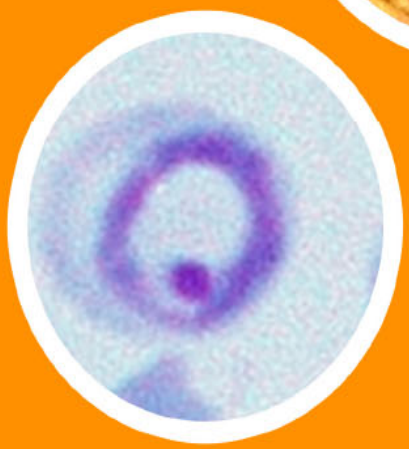
ANTIPROTOZOAL DRUG DISCOVERY:
A CHALLENGE THAT REMAINS







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Antiprotozoal Drug Discovery: A Challenge That Remains

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FOREWORD

All living creatures have to adjust to the cycle of life. All species have a beginning and have an end and during this period, they experience a breakdown of the homeostasis, call disease. Disease in man is associated to pain, anguish and suffering of the sick individual. More than a million years have been expended to search the causes of diseases, their effects in the body, and how to return patients to health. So many treatment procedures have emerged through human history, from natural products and magic healers to synthetic drugs design and physicians. As you can see, the application of curative measures have not frontiers nor limits.

Probably plants have been the principal source of remedies against disease. Actually, the use of plants, is not very practiced in occidental official medicine, instead, drugs are synthetic compounds that were primarily isolated from plants, fungus, algae, or bacteria. Nowadays, pharmacological industries, make big investments to develop new compounds for chronic and degenerative diseases, but it seems that investment on research to develop new drugs for diseases of the poor, is not one of their goals.

This book approaches the different facets of some of the most important diseases produced by protozoan parasites, under the analysis of epidemiological facts, economical and social impact, prevention and control problems. An analysis of the studies on new drugs for protozoan parasitic diseases, their hopes and achievements, is offered. An overview of the use of medical plants to treat specifically Malaria and Chagas disease, is also presented, as well as the experimental results obtained with the application of various metals in the treatment of malaria, leishmaniasis, toxoplasmosis and trichomoniasis.

This book will be of interest to parasitologists, pharmacologists and chemists and those involved or interested in the isolation and synthesis of active compounds against parasites.

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PREFACE

Protozoan parasites are a worldwide public health problem that challenge the immune system of millions of humans and non-humans animals, resulting in devastating social and economic consequences. Until now, chemotherapy is central to the control of most of the infections caused by these parasites in both medical and veterinary practice. Chemotherapy control, has been mainly compromised by the development of parasite drug resistance, drugs severe side-effects and toxicity, low to medium efficacy, high cost drugs, and the introduction of counterfeit drugs that are a threat to public health with many overwhelming effects for patients; consequently, the search for new antiprotozoal drugs is an urgent need.

The development of new drugs against these parasites, has been largely neglected because they mainly affect people living in extreme poverty with limited or non-marketing possibilities; for these reasons, in some endemic countries, herbal medicine has become an important and sustainable source of control.

This eBook is an effort of researches who work in the field of antiparasitic experimental chemotherapy and medical parasitology and it summarizes the progresses made in the field of chemotherapy and drug research against protozoan diseases. Synthetic and natural treatments to control Chagas disease, malaria, toxoplasmosis and other parasitic diseases, are discussed in order to open the possibility for applying these scientific progress in the field of antiprotozoal chemotherapy.

The editor and the authors would like to thank Bentham Science Publishers for providing the opportunity to bring this knowledge to the scientific community and to the general public.

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CHAPTER 1

Protozoal Diseases: Importance and Prophylaxis

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Abstract: This chapter focuses on the effect that five parasitic diseases, produced by five protozoa, have on human health, namely: malaria, toxoplasmosis, Chagas disease, leishmaniasis and trichomoniasis. Our approach is the following: An analysis of the epidemiological components of these diseases in the context of the parasite's life cycle; an analysis of the modifications in the natural history of these diseases introduced by human activities such as: migration, iatrogenesis and increased human mobility due to technological advances in the transportation of merchandise and persons; an analysis of how immune suppression produced by other living agents and chemotherapy affects these diseases, and their dispersion by tissue grafts; an analysis of the impact that these diseases have on the society, communities and family economies of countries affected by them, as well as what is being done to prevent them. In conclusion we find that research on new drugs is absolutely necessary to alleviate suffering, but given that these diseases are inextricably linked to poverty, we have to think of poverty as a disease, and as such we have an obligation to prevent it and eradicate it, as in the many other diseases and ills that befall mankind.

Keywords: Chagas disease, Chemotherapy, Leishmaniasis, Malaria, New drugs, Parasitic diseases, Poverty, Prohylaxis, Protozoa, Toxoplasmosis, Trichomoniasis.

INTRODUCTION

The simplest forms of life among the eukaryote are the protozoa, just formed by one cell. The first protozoa probably evolved from the fusion of two bacteria 1.45 to 2.5 billion years ago [1]. These primogenital protozoa, diversified, organized,

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and evolved into multicellular creatures from the trilobites to man. When studding protozoan parasites of man, I suggest not to lose the perception that we, humans, descend from those primogenital protozoa. If we think of humans in this fashion, it is not difficult to recognize that when the cells that conform us face a pathogen protozoa they initiate a relation or confrontation eukaryote to eukaryote and if the balance favors the host the infection is eradicated, but if it favors the parasite, the infection is established [2]. However, in this interaction both may come to an understanding, *i.e.* if the parasite doesn't behave as a parasite then the host will tolerate it. This mutual acceptance is possibly the most common.

Of all the protozoa in nature a minuscule number have learned to use humans as substrate, and of all these, only five are discussed in this book because they are of special significance to human health. The five protozoa referred to in this chapter are: *Plasmodium spp*., *Toxoplasma gondii*, *Trypanosoma cruzi*, *Leishmania* spp. and *Trichomonas vaginalis*. We will systematically discuss each one of these parasites in the following chapters. In this first chapter we will have a general discussion about all five regarding issues which we find of particular relevance to better understand their importance. The reader will find that in some sections the five parasites are addressed together, while in others we will deal with one parasite or disease specifically.

ECONOMIC IMPORTANCE

When we talk about the economic importance of a disease, we normally talk about the burden that the disease creates at a local, national or global level. Most measurements take into account the premature mortality, morbidity, impairment and disability caused by the disease in study. The study of the global burden of diseases has developed a tool to quantify and compare health between populations, using a joint measure of both mortality and disability called disability-adjusted life year or DALY that measures the loss expressed in years of life due to death or disability caused by a disease [3, 4]. There are however other values that can also give us an idea of the burden produced by a disease.

A general rule created by Sachs and Malaney states that where malaria prospers more, human societies will prosper less. There is an unmistakable correlation

between malaria and poverty. A comparison between the gross domestic product (GDP) of malarial and non malarial countries of similar development showed a GDP of US$1,526 for the malarial countries while in the non malarial countries it was US$8,268. This represents a five-fold difference between the two, which suggests that malaria exerts a considerable pressure on the economic development of populations. In a study of the rate of economic growth over a period of 25 years (1965-1990), countries with large populations living in areas of high malarial transmission were compared with countries of similar socioeconomic status but with a lower rate of malaria transmission. The results showed that the former countries had an annual per capita growth of 0.4% while for the latter it was 2.3%. [5].

In a study examining the burden of neglected tropical diseases within the framework of the global burden of disease, malaria was listed as the 9th cause of human death at global level, with 39 million DALYs, and held 1st place in the mortality burden among the tropical diseases of the world in the year 2002 [3].

An analysis of malarial deaths in the sub-Saharan region of Africa, made in 2010, showed a mortality between 655,000 and 1.24 million, with an estimated 82.69 million years lost by disability and death (DALYs), and an associated economic cost of 5.8% of the total GDP of the African Sub-Saharan region. Very important improvements have been achieved in the control of malaria in this region, with the use of residual insecticides impregnated in bed-nets and sprayed in houses, in conjunction with the artemisinin-based combination therapy. However, *Anopheles* resistance to the four classes of insecticides used has been documented, including pyrethroids, the only insecticides used to impregnate bed-nets; the resistance of *P. falciparum* to artemisinin has also been documented [6].

Among human infections caused by parasites, toxoplasmosis is one of the more prevalent, affecting 30 to 50% of the human population. Despite the fact that the infection is long-lived, the vast majority of people remain asymptomatic. The lowest prevalence of toxoplasmosis, of about 1% occurs in some countries in the Far East, and the highest rates, above 90% are found in European and South-American countries. A study of ocular toxoplasmosis in 1042 patients in Southern Brazil showed a prevalence 30 times higher than in any other part of the world

CHAPTER 2

Drugs for Parasitic Diseases - Strategies to Solve a Global Problem

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Abstract: For many years, one of the driving forces for the development of drugs has been, and still is, the pharmaceutical industry, but the need for therapeutic agents regarding unprofitable diseases makes necessary a change in this situation. Parasitic diseases are a good example since the quantity and safety of many drugs currently in use are inadequate, and the development of new agents is insufficient. In the paper herein, we discuss the main methodologies used for the development and discovery of antiparasitics, the new trends and prospects, as well as the first results achieved by supranational programs and consortia created for such purposes.

Keywords: New approaches, New drugs, Parasitic diseases.

INTRODUCTION

Parasitic diseases are a major socioeconomic challenge either affecting humans or animals. They do not only pose a huge health risk, but also limit the proper development of the affected communities, which, in some cases, represent a large part of the population. Apart from the obvious health risks, these infections have a direct impact on the economic development of the population. Humans affected by certain chronic parasitoses typically develop comorbidities which, either directly or indirectly, affect their quality of life, reduce their productive capacity and limit their potential for economic progress. Moreover, parasitic diseases also affect animals that are in direct or indirect contact with humans. The existence

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of infected animals, which are, in some cases, a reservoir allowing these diseases to spread, is also a severe economic damage and results in the loss of animals because they are sacrificed in order to prevent the disease from spreading. Perhaps this is the reason why the parasitic diseases of animals have received comparatively more attention than those of humans from pharmaceutical companies [1].

Part of the lack of interest in human parasitic diseases has been a logical consequence of the roles adopted by different actors in the discovery and development of drugs. Remarkably, the pharmaceutical industry has usually been in charge of said developments, and these companies are profit-driven commercial enterprises. The benefits provided by the commercialization of these drugs do not normally compensate for the large investments needed to develop antiparasitic agents, especially because these diseases are restricted to developing countries with little funding for public health. According to Doctors without borders/Médecins Sans Frontières (MSF), the treatment cost for visceral leishmaniasis is US$450 per patient in their program in India, but this price can climb to US$2,500 per patient, which is too expensive for most government Ministries of Health [2]. This is one of the reasons why parasitic diseases have historically been considered “orphan diseases”. The scarce attention received depends exclusively on the academy and/or special programs of either international organizations or nonprofit foundations. Even the resources provided by foundations and nonprofit entities are modest compared to those allocated to the study of other diseases, such as HIV. These huge differences derive from the lack of visibility of parasitic diseases, even when they incapacitate millions of people annually. The World Health Organization (WHO) includes some parasitoses, such as Chagas disease, human African trypanosomiasis (HAT, or sleeping sickness) and leishmaniasis, within the Neglected Tropical Diseases (NTDs). According to the WHO, NTDs do not only affect millions of people across 149 countries, deriving in high costs for developing economies, but also have a high mortality rate if they are not treated properly [2].

In a publication of 2013, Jaggi reported that in the last 20 years the Food and Drug Administration (FDA) has approved 1,200 new drugs and only 3 of them are antiparasitics. Furthermore, not only are there few new antiparasitic drugs, but,

considering the time needed for such drugs to reach the market, there is no prospect either for new alternatives to appear in the near future [3]. This lack of new antiparasitic agents means that drugs developed between 1930 and 1970 are still in use. The constant use of these drugs since then has led to resistance phenomena, thereby reducing their effectiveness. However, the international interest in parasitic diseases is growing because the global landscape of infections is changing. There are two main factors driving this change:

- *The increase in the area of influence of parasitic diseases:* The recrudescence of situations generating social, economic, ethnic, religious and/or political tension turns the migrant issue into a problem difficult to solve. There are thousands of people displaced from their usual areas of residence, leaving behind their past in search of better places to live. In these mass movements, other less obvious "immigrants" come along, which should not be neglected. On the other hand, many people in developed countries travel around the globe, both for professional reasons and out of a desire to visit new places, exposing themselves to health risks which are unusual in their places of residence. In a few words, globalization is also a driving force for the spread of parasitic diseases. The truth is that the emergence of new areas affected by parasites (outside their endemic regions) does not only have an impact on developed countries. In some cases, they are the "exporters" of parasitosis. In Argentina, there have been cases of infections with *Diphyllobothrium latum,* a tapeworm of salmonids found in Canada and Japan, which has apparently been introduced during the planting of trouts. Moreover, in the City of Buenos Aires, an increase of parasitosis has also been verified in recent years, previously rare in said area, along with an increasing incidence of infections with *Taenia saginata* and *Taenia solium*, including the development of cases of neurocysticercosis [4].

While this "importation" of pathologies is often limited to affected individuals, if the parasite finds the appropriate proliferation medium, such as poor hygiene, or the appropriate vectors are present, it can quickly become a mass phenomenon. Regardless of the obvious concern for global health, the increase of people affected by parasites makes this issue more interesting for the pharmaceutical industry. As for the availability of vectors, there is a change that is related to the second factor:

CHAPTER 3

Crude Extracts and Isolated Compounds with Trypanocidal Activity

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Abstract: Chagas' disease, or American trypanosomiasis, is a potentially mortal disease caused by the protozoan *Trypanosoma cruzi*. It is included in the list of neglected diseases or poverty-linked diseases. An estimate of 5.7 million people in the endemic areas are infected, resulting in 7000 yearly deaths. The disease is distributed in Central and South America, Mexico, and the southern United States. Due to rural-urban migration, the parasite is transmitted by blood transfusion, by vertical transmission from mother to child, or by organ transplantation; on the other side, some cases have been detected in non-endemic zones both in America and in Europe. Chagas' disease is among the 17 neglected diseases; it is a complex zoonosis, involving interaction of vectors species with wild, peridomestic, and domestic mammals, showing several clinical pictures and transmission modes. It poses the highest economic burden among parasitic diseases in Latin America due to its long chronicity. The clinical manifestations and epidemiologic traits of the disease vary from region to region. The current medication against *T. cruzi*, nifurtimox and benznidazole, has adverse effects. Aiming to make new treatment alternatives known, the activity of plant-isolated compounds from Central America, South America, and Mexico is herein discussed, along with their action against different stages of *Trypanosoma cruzi*.

Keywords: Chagas disease, Crude extracts, Medicinal plants, *Trypanosoma cruzi*.

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INTRODUCTION

Chagas' disease, or American trypanosomiasis, is a potentially mortal disease caused by the protozoan parasite *Trypanosoma cruzi* and transmitted by hematophagous hemipterans of the Reduviidae family. It is included in the list of neglected diseases or poverty-linked diseases. An estimate of 5.7 million people are infected in the endemic areas, which includes the 21 countries in Latin America, resulting in 7000 yearly deaths and over 70 million are at risk for becoming infected. The disease is distributed in Central and South America, Mexico, and the southern United States [1 - 5]. Rural-to-urban migration in Latin America during the 1970s and 1980s changed the epidemiologic patterns of the disease, turning it into an urban infection where the parasite can be transmitted without a vector [3]. Due to an increase in population movements, the number of diagnosed cases increased even in non-endemic countries both in America and in Europe. A 2011 study reported between 68,000 and 122,000 cases in European countries [6]; this increase poses additional risks for parasite transmission by blood transfusion, vertical transmission from mother to child, or organ transplantation [7 - 9]. Currently regarded as a public health issue in several countries of the Americas, the World Health Organization/Pan American Health Organization (WHO/PAHO) place Chagas' disease among the 17 priority neglected diseases [10 - 12].

The disease was discovered in 1909 by Carlos Chagas, in Brazil. The acute form of Chagas' disease was first described in Berenice, a 2-year-old girl who died at age 82 from other causes. Although infected, she did not acquire the disease. Chagas also described the morphology and the life cycle of the parasite in vertebrate hosts and in vectors insects, and made observations on the course of infection in laboratory animals and in cultures. This clinical entity is a complex zoonosis, with recurrent infections involving the interaction of vectors species with wild, peridomestic, and domestic mammals, showing several clinical pictures and transmission modes. It poses the highest economic burden among parasitic diseases in Latin America due to its long chronicity. Since 1909, Carlos Chagas refers to the rural housing conditions as posing risk or promoting vector propagation, and these conditions are still prevalent nowadays [13]. The clinical manifestations and epidemiologic traits of the disease vary from region to region.

Biology of Chagas' Disease

Trypanosoma cruzi is a flagellate protozoan of the phylum Sarcomastigophora, order Kinetoplastida, and family Trypanosomatidae. The parasite goes through three main developmental stages: trypomastigote (bloodborne when it is in the bloodstream of its mammal host, and metacyclic in the feces of vectors), epimastigote in the intestine of the triatomine and in culture media, and amastigote when located inside the cells of the mammal host. The trypomastigote stage shows a 10-20 μm elongated body, vesicular nucleus, slightly granular cytoplasm, and a terminal kinetoplast with diffuse chromatin from which the membrane emerges. Epimastigote is fusiform and can be up to 30-μm long; kinetoplast has a chromatin organized in a compact manner at the anterior portion, near the nucleus; the parasite has a flagellum and an undulating membrane. Amastigote does not exhibit an external flagellum, but nucleus and kinetoplast are visible; the cell, 3-5 μm in diameter, is a multiplication or fission form inside host cells [14 - 17]. Both vectors arthropods and humans play a role in *T. cruzi* life cycle, along with a large number of reservoir mammals, mainly possums, armadillos, squirrels, field mice, rats, donkeys, pigs, dogs, and cats [18].

Vectors insects carry the infective forms (metacyclic trypomastigotes) of *T. cruzi* in feces, which are deposited on the skin during or after feeding; after entering the host through skin or mucosa, the parasite is able to invade a wide variety of cells where it transforms into the amastigote stage, a replicative intracellular form. Eventually, amastigotes will give rise to trypomastigote forms, which disseminate to other cells and can be ingested by the vector. When a triatomine ingests blood from an infected mammal, the parasite enters the bug; epimastigotes develop in the intestine lumen of the insect, multiplying by binary fission, and about 15-30 days later the metacyclic trypomastigote stage, the infective form, is produced and expelled with the feces of triatomines. When a parasite-infected triatomine feeds again on a healthy mammal, its stomach fills to repletion and discharges feces containing metacyclic trypomastigotes (Fig. **1**).

Trypanosoma cruzi has a wide array of surface molecules that allow it to make contact with mammal cells and complete the infection process. Membrane molecules start a host-parasite signaling process; among these, lipid rafts in

CHAPTER 4

Malaria, Generalities and New Treatments

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Abstract: Malaria is still a grave public health problem in tropical areas of the world. The greater genetic diversity of *Plasmodium vivax* at geographic sites with less control over infection reveals the importance of genetic studies of these parasites. *Plasmodium vivax* is the main cause of human malaria in Asia and Latin America but is absent from most of central Africa due to the near fixation of a mutation that inhibits the expression of its receptor, the Duffy antigen, on human erythrocytes. The search for vaccine development remains an issue of global concern.

Keywords: Dufy antigen, Malaria, New Treatments, *Plasmodium vivax*, Quinine, Vaccines.

MALARIA GENERALITIES

Malaria is an acute or chronic tropical disease caused by protozoans of the genus *Plasmodium*. This protozoan occurs in red blood cells. The hosts of *Plasmodium* are humans and other mammals as well as reptiles and birds. Malaria in humans is caused by five species of *Plasmodium*: *Plasmodium falciparum*, occurring mainly

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in countries with a tropical climate, in particular in Sub-Saharan Africa (south and center of the continent); *Plasmodium vivax*, having the broadest range of occurrence (from the tropical to the moderate zone); *Plasmodium malariae*, occurring sporadically in countries with a subtropical and tropical climate; *Plasmodium ovale*, occurring in the west of Africa, the Philippines, east of Indonesia and in Papua New Guinea; and *Plasmodium knowlesi*, occurring in south-east Asia, Singapore, Malaysia and Thailand. Among the five species, the most dangerous is *Plasmodium falciparum* .

In the malarian transmission approximately 30–40 species of mosquitoes of the genus *Anopheles* fulfill the significant role of vectors. The mosquitoes responsible for spreading the disease breed in various bodies of water; they like puddles and also rice fields. The number of mosquitoes depends on a large extent on the rainfall intensity, temperature and humidity. The most suitable conditions for mosquito development and malaria transmission are the air temperature of 17–33 ºC and the mean relative humidity above 60%. Most blood- sucking mosquitoes are crepuscular feeders [1].

Plasmodium Life Cycle

The Life Cycle is almost the same for all the species that infect humans (Fig. **1**).

In humans, the parasites grow and multiply first in the liver cells and then in the blood red cells. In the blood, successive broods of parasites grow inside the red cells and destroy them, releasing daughter parasites ("merozoites") that continue the cycle by invading other red cells [2 - 5].

The blood stage parasites are those that cause the symptoms of malaria. When certain forms of blood stage parasites ("gametocytes") are picked up by a female *Anopheles* mosquito during a blood meal, they start another, different cycle of growth and multiplication in the mosquito [2 - 5].

After 10-18 days, the parasites are found (as "sporozoites") in the mosquito's salivary glands. When the *Anopheles* mosquito takes a blood meal on another human, the sporozoites are injected with the mosquito's saliva and start another human infection when they parasitize the liver cells [6 - 9].

Thus the mosquito carries the disease from one human to another (acting as a

"vector"). Differently from the human host, the mosquito vector does not suffer from the presence of the parasites [6 - 9].

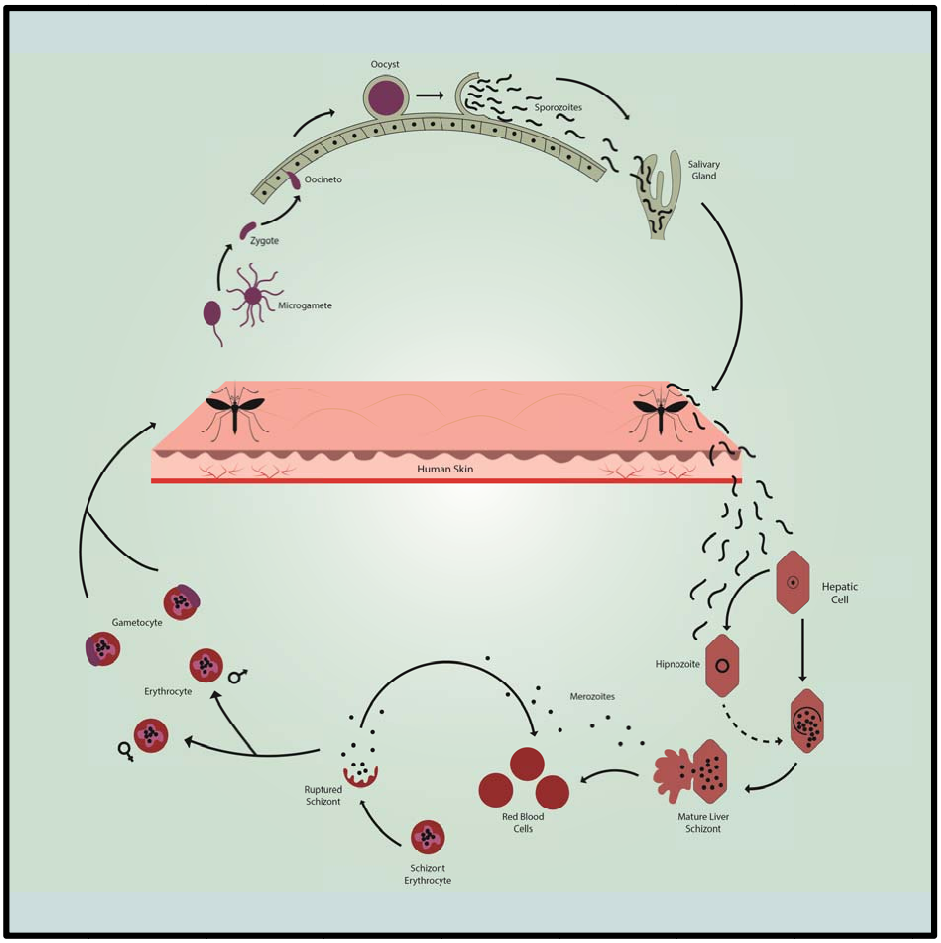

Fig. (1). Life cycle of the malaria parasite. (Modified by Alcántara-Quintana L, of the one of a series 'Malaria: Parasite Biology Tutorial', www.wellcome.ac.uk) [2 - 5].

NEW TREATMENTS

Quinine was the first natural antimalarial agent, this compound is an alkaloyd extracted from the bark of the cinchona tree (*Cinchona calisaya*) [10]. The first records from 1600's mention that the bark was used to make infusions and it was referred to as the "Jesuits' bark", "cardinal's bark", or 'sacred bark'; in 1820 it was first isolated by Pierre Joseph Pelletier and Joseph Caventou remaining as the

CHAPTER 5

Update on Advances in Plant-based Antimalarial Drug Discovery

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Abstract: Malaria remains one of the major infectious diseases with high morbidity and mortality in developing and under-developed countries of the world. Although readily curable, the development of resistance by the malaria parasite especially *Plasmodium falciparum*, to existing antimalarials necessitates the need for continuous search for new antimalarial drugs. Several approaches have been adopted in this search including those based on natural products, particularly medicinal plants and herbs. As exemplified in the discovery of quinine and artemisinin, there has been intensified effort in the search of safe and efficacious plant-based antimalarial compounds. This search has yielded moderate success. The current chapter highlights the most promising plant-based drug discovery success with particular emphasis on studies that have reached some advanced clinical stages. With many plant-based compounds exerting antimalarial activities through multiple mechanisms, it is expected that further studies will complement current research efforts towards successful antimalarial drug discovery and development.

Keywords: Artemisinin, Chloroquine, Drug discovery, Herbal medicine, Malaria, Natural products, Plant-derived drugs, *Plasmodium falciparum*, Quinine, Reversed pharmacology.

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INTRODUCTION

Malaria has remained a major health challenge in several countries of the world and according to the World Health Organization (WHO) 2014 malaria statistics, 97 countries and territories of the world have ongoing malaria transmission with 3.3 billion people at risk, an estimated 198 million cases and 584000 deaths in 2013, 85% of who are children under 5 years old [1]. Pregnant women are among the group hardest hit by malaria prevalence, and made worse by limited drug choices. *Plasmodium falciparum*, the most virulent malaria parasite, has developed resistance to most of the available antimalarial drugs [2]. The current standard of treatment as recommended by WHO is artemisinin (ART)-based combination therapy (ACT) comprising artemisinin (or its derivative) in combination with another antimalarial usually an arylamino alcohol or 4-aminoquinoline [3]. Increasing reports of malaria resistance to ACTs, occasioned by delayed cure of parasitaemia and fever symptoms in patients in Southeast Asia and Africa, is a current threat which can only be offset, in the long term, by the discovery of new antimalarial drugs [4, 5].

There is a general consensus on what an ideal antimalarial drug should be. Apart from effectiveness against malaria and demonstration of superiority over existing drugs, an acceptable antimalarial drug should exert actions *via* novel mechanisms that will safeguard against parasite resistance. More important, however, is the economic factor. Malaria is prevalent in developing and under-developed countries of the world. The resource limitation implies that effective drugs should be affordable in order to reach the target population and enhance treatment compliance. Several approaches have been adopted in antimalarial drug discovery and development. These include optimization of therapy through existing drugs. This generally involves the combination of unrelated antimalarial drugs to explore multiple mechanisms of action against the parasite as seen in various marketed antimalarial formulations [6]. Another approach is the development of analogs of existing antimalarial drugs with the purpose of improving efficacy and safety. This approach has been successful with, for example, the development of several aminoquinolines based on quinine [7]. And yet another approach is the search for bioactives and lead compounds from natural products.

The historical relevance of plant-derived compounds in antimalarial chemotherapy suggests that, despite the limited success in recent times, successful antimalarial drug discoveries are possible from botanicals. Natural products present an endless source of potential new pharmacophores upon which synthetic analogues and modifications can be based. Several novel compounds are yet undiscovered from plants many of which, like quinine and ART, are not only therapeutically active on their own, but provide new leads into synthetic antimalarial drug discovery. The high perception of safety of natural products by health consumers, often founded on centuries of traditional use, has made natural products more appealing and acceptable as potential sources of therapeutic agents. While this perception seems to favor popular trust in herbal products, it should be noted that phytochemicals are not always free of toxicity and safety data are required before regulatory approval.

In most malaria-endemic communities, there is anecdotal evidence of antimalarial activity for a wide range of ethnobotanical remedies. This provides the basis for initial antimalarial screenings and bioassay-guided isolation and purification in drug discovery [8]. Additionally, there is some commitment to research and preservation of traditionally useful ethnobotanical resources in malaria-endemic countries [8] especially in Africa. Apart from global climate change, which poses a threat to expand malaria towards the areas of high elevation in Africa and South Asia, as well as the resultant climate change due to rapid urbanization and other human activities has been recognized as responsible for increasing the number of valuable traditional plants going into extinction [9]. These factors highlight the urgency and favorable considerations in antimalarial drug discovery focusing on natural products to provide leads.

In this chapter, an update on plant-based antimalarial drug discovery efforts is provided with emphasis on those lead compounds and products that have reached some stages of assessment in humans since after the discovery of quinine. A brief description of the discovery of the prototypical natural product antimalarial drugs quinine and ART is included to provide historical perspective of the relationship between malaria drug discovery and phytochemicals.

CHAPTER 6

Metals as an Option for Protozoa Treatment

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Abstract: Protozoa infections are a worldwide health problem that preferentially affects populations in underdeveloped countries. *Plasmodium, Leishmania, Trypanosoma, Toxoplasma, Neospora, Entamoeba* and *Trichomonas* are some of the most important genuses of protozoan parasites that infect and invade cells, tissues and organs. These parasites proliferate, migrate and mutate to evade the chemotherapeutic effects of drugs that were previously effective for their treatment. The use of metals and metals complexes to control and treat these parasites, seems a very prominent alternative. Palladium, platinum, ruthenium, antimonium, gold, iron and vanadium are some of the metals that have been evaluated as antiprotozoal treatments with favorable results. A review of the experimental effects of metal complexes on these parasites follows, along with commentary.

Keywords: Antimonium, Gold, *Leishmania*, Metal treatments, *Plasmodium*, Platinum, Palladium, Ruthenium, *Toxoplasma gondii*, *Trypanosoma*, Vanadium.

INTRODUCTION

There are 38 transition metals included in the periodic table. These metals contents ductility and malleability characteristics, frequently display numerous

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regular oxidation states, have electrons in more than one group and are capable of conduct electricity and heat. There is a positive charge in the center of these metals that let them bind to other biomolecules of negative charge; by using these main features of the transition metals, the development of new metal-based compounds with favorable therapeutic uses, has been possible. With the discovery of cisplatin in 1965, a new field of research emerged to investigate the therapeutic features of metals [1].

The advantage in using metal based compounds instead of carbon compounds when designing new therapeutic drugs, is the ability of metals to coordinate ligands in a three dimension shape, in order to define molecular targets [2].

Treatment of protozoan experimental infections with metal compounds, are now being recognized. Protozoan parasites infect over a half billion people worldwide and continue to be one of the most significant public health problems causing a global impact. Some important human diseases caused by these pathogens include malaria, leishmaniasis, Chagas disease, toxoplasmosis, amebiasis and trichomoniasis. Most of the current drugs for these illnesses have been used for decades and have many limitations, such as the emergence of drug resistance, severe side-effects and low-to-medium drug efficacy; these drugs have been largely neglected as models for drug development because they are mainly used in countries with limited resources and as a consequence, with scarce marketing possibilities. Currently, there is a demand to develop new drug-based antiprotozoan therapies [3].

According to a report from the WHO, in 2013, malaria caused 584,000 deaths, with African children being the most affected population. Trypanosomiasis (sleeping sickness) has decreased in frequency, and new cases are located in Africa. From 100 to 200 new cases were reported in Chad and South Sudan in 2013. For Chagas disease, approximately 6 to 7 million people are estimated to be infected worldwide, mostly in Latin America; almost 30% of the infected people develop to a chronic phase with cardiac complications, and about 10% of these chronic patients may present digestive or neurological symptoms [4]. In addition, because the affected populations are usually located in underdeveloped countries, new treatments are not a priority for large pharmaceutical laboratories.

Leishmaniasis is distributed worldwide and is an endemic disease in more than 90 countries across almost every continent. For the treatment of leishmaniasis in humans, a selection of therapeutic compounds is offered, but the drugs are toxic and present adverse effects [5].

Worldwide, around 30% of people is infected with *Toxoplasma gondii*. Severe disease and major complications like encephalitis, myocarditis and pneumonitis are described in immunocompromised patients as a consequence of a primary infection or reactivation of a chronic one; also the fetus can be severely damage during gestation with long-term disabling sequelae, stillbirths or fetal death [6]. *Neospora caninum*, the etiologic agent of neosporosis, is an overwhelming apicomplexan parasite with worldwide distribution that causes reproductive loss in cattle and neuromuscular damage in dogs [7]. Human amebiasis caused by *E. histolytica*, is responsible for almost 50,000,000 diarrheal infections and 100,000 deaths per year. This infection is remarkably common in children from developing nations [8]. A very important sexually transmitted disease is caused by *Trichomonas vaginalis*. Trichomoniasis is a very important public health problem because it produces reproductive damage and enables the acquirement and the transmission of HIV [9].

The research of bioactive metal complexes is an opportunity to find new and less expensive treatments for these diseases [10]. This review identifies the history of these parasite infections and recollects the chemotherapy and different metals already in use along with those proposed as possible treatments for the aforementioned protozoa [11].

Table 1. Experimental use of metals against some protozoan parasites.

Parasite	***Metal/compound***	***Reference***
Leishmania	Copper	[12]
	Nickel	[13]
	Zink	[14]
	Iridium	[15]
	Palladium	[16]
	Osmium	[17]
	Vanadium	[18]

SUBJECT INDEX

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