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Schistosomiasis Chemotherapy

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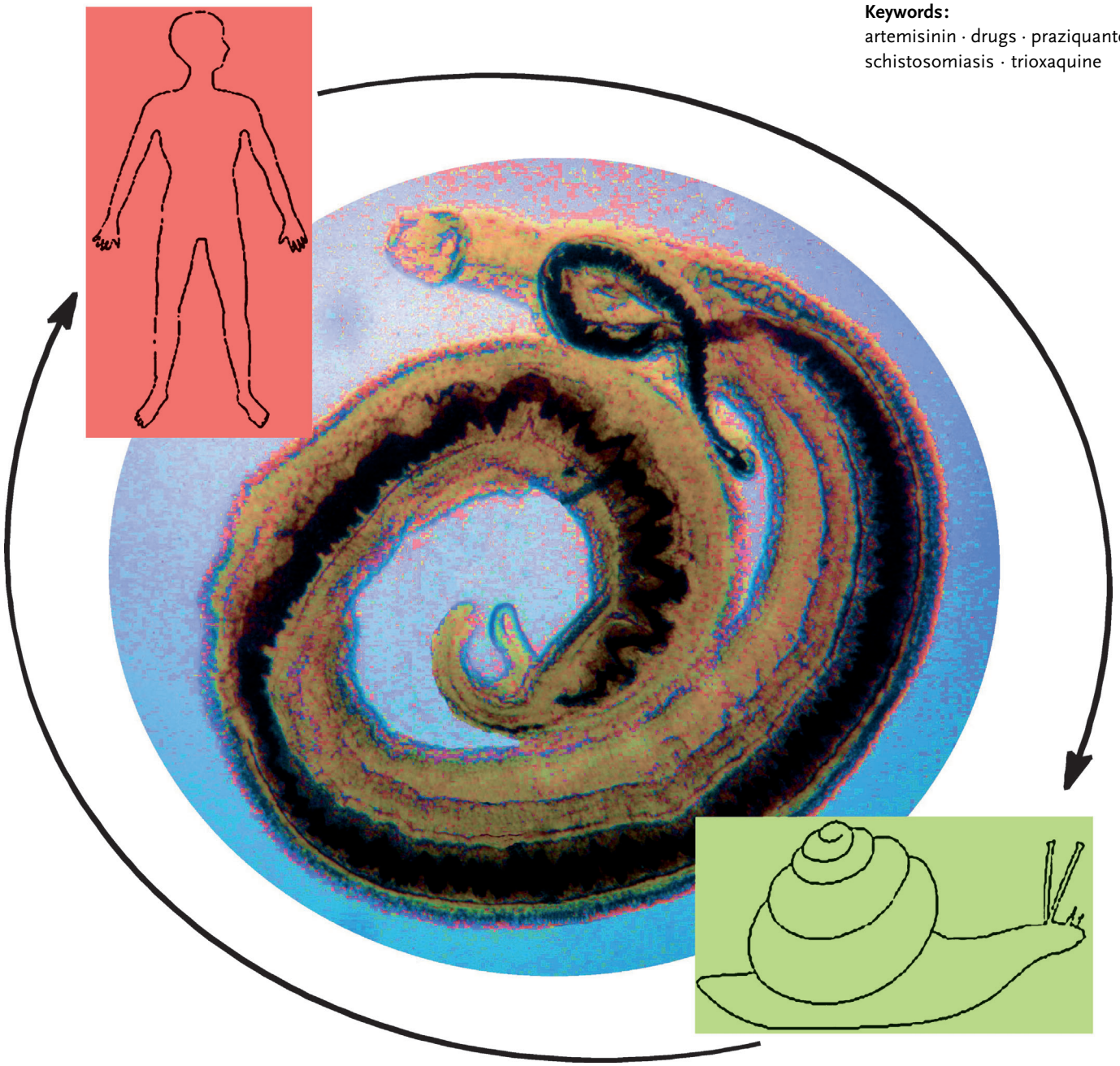

After malaria, schistosomiasis (or bilharzia) is the second most prevalent disease in Africa, and is occurring in over 70 countries in tropical and subtropical regions. It is estimated that 600 million people are at risk of infection, 200 million people are infected, and at least 200 000 deaths per year are associated with the disease. All schistosome species are transmitted through contact with fresh water that is infested with free-swimming forms of the parasite, which is known as cercariae and produced by snails. When located in the blood vessels of the host, larval and adult schistosomes digest red cells to acquire amino acids for growth and development. Vaccine candidates have been unsuccessful up to now. Against such devastating parasitic disease, the antischistosomal arsenal is currently limited to a single drug, praziquantel, which has been used for more than 35 years. Because the question of the reduction of the activity of praziquantel was raised recently, it is thus urgent to create new and safe antischistosomal drugs that should be combined with praziquantel to develop efficient bitherapies.

From the Contents

1. What is Schistosomiasis (Bilharziasis)?

In 1850, after his medical doctorate at the University of Tübingen, Theodor Maximillian Bilharz went to Egypt with a German scientific expedition, and became first chief of the Surgical Services at the Kasr El-Aïn Hospital and Medical School established by the French surgeon Antoine Clot in Cairo.[1] As early as in 1851, during the post-mortem examination of an Egyptian, Bilharz observed long white worms by naked eye in the blood of the portal vein. These worms were the a yet unknown trematode species *Distomum haematobium*, later renamed *Schistosomum haematobium*.[2] Bilharz performed autopsies and discovered peculiar changes in the mucous membranes of the bladder, intestines, ureters, and seminal glands as a result of schistosome infections. He described for the first time the eggs of *Schistosoma mansoni* in letters to his zoology teacher Carl T. von Siebold from May 1851 to January 1853.[1] It should be noted that *S. mansoni* was not mentioned until it was identified as a distinct species in 1907.

The terms schistosomiasis and bilharziasis were introduced into scientific nomenclature to depict a chronic disease caused by a trematode of the genus *Schistosoma* living in blood. There are five major species of schistosomes infecting man: *S. haematobium* confined in Africa, *S. mansoni* in Africa and South America, *S. intercalatum* in West Africa, and *S. japonicum* and *S. mekongi* located in Asia.

Schistosomiasis is known to have occurred in ancient times in Egypt and in China. Actually, it is the second most prevalent tropical disease after malaria in the developing world, occurring in over 70 countries in the tropics and subtropical regions.[3–5] The number of people treated for schistosomiasis rose from 12.4 million in 2006 to 33.5 million in 2010,[5b] in spite of treatment campaigns organized by the World Health Organization (WHO). It is estimated that 600 million people are at risk of infection, 200 million people are infected (85% in sub-Saharan Africa), and at least 200 000 deaths per year are associated with the severe consequences of infection, including fibrosis and calcification of the urinary tract, renal failure or bladder cancer (*S. haematobium*) and acute hepatitis, liver and intestine fibrosis, and portal hypertension (*S. mansoni*). Approximately 4% of people that are parasitized with *S. mansoni* but untreated develop severe hepatosplenic schistosomiasis,[6] and recent studies suggest that morbidity and mortality have been seriously underestimated.[7] In 2007, among schoolchildren of Southern Sudan, the prevalence of *S. haematobium* and *S. mansoni* infection was 73% and 70%, respectively.[8] Despite a national control program initiated in the 1950s,[9] schistosomiasis has re-emerged in the Peoples Republic of China in the early 2000s, and is regarded as the most serious parasitic disease in this country with 15 million cases.[10,11] Egypt is heavily affected with a prevalence higher than 60% in rural areas of Lower Egypt.[12]

Apart from places where schistosomiasis is or was recognized as an obvious health problem, as in Brazil,[13,14] China, Egypt, the Philippines,[15] and few African countries (Northern Senegal and Uganda), this disease is often not a priority for health authorities. The cost of control of the

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disease is too high with regard to the per capita health expenditure in sub-Saharan Africa.

In addition, the requirements of an increased population and development lead to large-scale water impoundments for electricity and irrigation, resulting in the increased transmission of parasitic diseases. Ecological changes and irrigation practices have introduced *Schistosoma haematobium* and *mansoni* and their respective snail vectors in many African areas,[16,17] but seem to have also changed the habitat for *Bulinus* snails, thus contributing to the decline of *S. haematobium* in Egypt.[4] Spatial epidemiology of schistosomiasis in Africa has been reviewed.[18] The implementation of major water resource schemes in Southern China may also have increased the transmission of *S. japonicum*, and the population movement from North-East Brazil has extended the transmission of *S. mansoni* by acclimatation of their respective host snails.[17]

With increasing people migrations, schistosomiasis is no longer a disease of distant lands, human cases exist now in American or European metropolitan areas. However, there is no accurate information about infection rates among return travelers and immigrants.[19] Fortunately, the proper intermediate-host snails cannot survive in temperate climates, blocking the life cycle of the parasite in these countries.

The multiplicity of potential hosts (pigs, cattle, cats, and rodents) for schistosome species complicates effective disease control. In many countries, including European countries, bovine and ovine livestocks are infested.[20] However, *S. japonicum* is the only schistosome with an important zoonotic transmission, with over 40 species of wild and domesticated animals suspected to serve as reservoir hosts. Chemotherapy of humans may be ineffective, if rapid re-infection occurs from animal hosts. Humans and bovines share *S. japonicum* transmission to a greater extent than with other mammalian species.[21,22]

2. Transmission of the Disease

Despite the early description of schistosomiasis in the 1850s, the way of transmission and the link to snails were not discovered until after the turn of the 20th century. All schistosome species penetrate humans during contact with water that is contaminated by cercariae, the larval, free-swimming form of the parasite produced by an intermediate-host snail.[19a] In theory, breaking of the schistosome life cycle should be easy: if human behaviour could be changed to stop the contamination of water with urine and feces that contain schistosome eggs, the transmission of the parasite would be stopped. The eggs that do not reach water are dessicated and play no part in transmission. The education of children to reduce water contamination, together with the provision of better sanitation and piped water are key parameters for a long-term control of the disease.

2.1. Cycle of Schistosome in the Molluscan Intermediate Host

Eggs that are present in the human excreta hatch on contact with water and release microscopic larvae called miracidia. In order to survive, the tiny larvae must find and penetrate a specific water snail. The main molluscans used by the human schistosomes are *Bulinus* (*S. haematobium* and *S. intercalatum*), *Biomphalaria* (*S. mansoni*), *Oncomelania* (*S. japonicum*), and *Neotricula* (*S. mekongi*). Once inside the host snail, the miracidium transforms into a sporocyst and multiplies. Daughter sporocysts migrate in the digestive glands of the mollusk, where they transform through an asexual reproduction cycle to produce thousands of new parasite larvae, cercariae, which leave the snail and enter the water (Figure 1). From the fourth week after the infestation of the snail with the miracidium, about 1500 cercariae are released every day. This asexual multiplication allows the parasite to increase dramatically in numbers, enhancing considerably the chances of re-infecting humans.[23]

For these reasons, there is a long history of attempts for control snails using molluscicides and reducing the favorable habitat of the host snail. However, repeated expensive treatment of the scattered aquatic species would be necessary to achieve these goals, and have consequences for the environment. During the 1960s, niclosamide (bayluscide) became the moluscicide of choice and was extensively used in some Africa countries.[4,24] However, it does not kill mollusks exclusively, and the toxicity of niclosamide to fish prevents its use as a viable long-term control strategy.[25]

Bernard Meunier was born in 1947 and received degrees from the universities of Montpellier and Paris-Orsay. After a post-doctoral stage at Oxford, he joined the "Laboratoire de Chimie de Coordination du CNRS" in 1979. His recent research interests include the mechanism of action of antimalarial and antischistosomal drugs. He is also developing specific copper chelators for potential anti-Alzheimer agents. He is a member of the French Academy of Sciences since 1999 and Foreign Member of the Polish Academy of Science since 2005. He is also the founder of the company Palumed (2000–2012). Currently, he is Emeritus Director of Research at the CNRS and Distinguished Professor at the Guangdong University of Technology (Guangzhou, China).

Anne Robert was born in 1959 and received degrees from the Chemical Engineering School of Toulouse and the University of Toulouse. She joined the research group of Bernard Meunier at the Laboratoire de Chimie de Coordination in 1985. She is currently Director of Research of the CNRS. Her major research interests are the chemical models of heme enzymes and the role of redox metals in biology. She especially studied the mechanism of action of peroxide-based antimalarial drugs, and the targeting of iron(II)–heme to develop drugs against parasitic diseases.

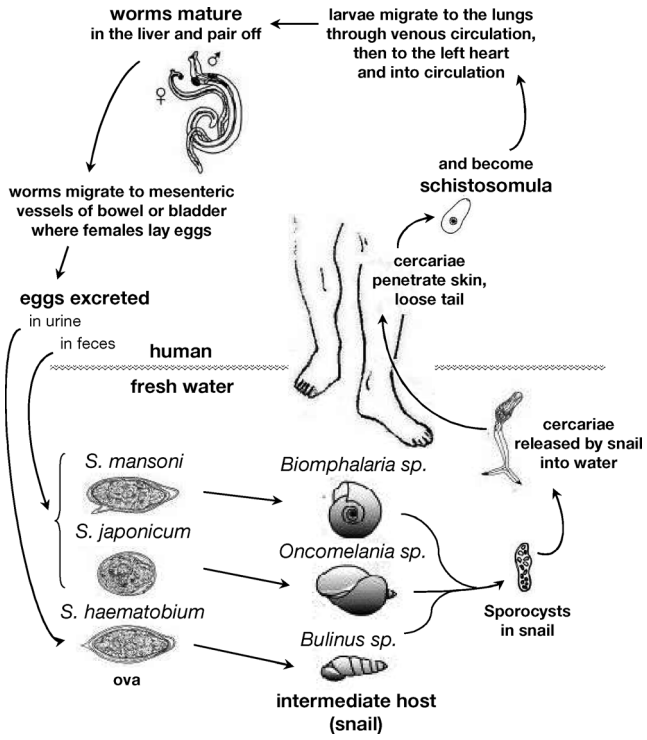


Figure 1. Life cycle of schistosomes.

2.2. Cycle of Schistosome in the Mammalian Host

People are infected with cercariae by contact with infested water during their normal daily activities, such as hygiene, recreation, fishing, or farming by irrigation. A minute or less of contact is sufficient for the larvae to pierce the horny layer of the host and penetrate the human skin.[26,27] During penetration, the cercariae shed their bifurcated tails, and the resulting schistosomula enter capillaries and lymphatic vessels. Within 12 hours of infection, people may complain about a tingling sensation or light rash, commonly referred to as "swimmer's itch", because of the irritation at the point of entrance.

Through the heart, schistosomula migrate to the lungs by the second or third day after penetration.[28] By the 15th day, they can be found accumulated in the liver, where they feed on portal blood and undergo rapid growth. These young larval and then adult schistosomes digest red cells. Their development takes three to four weeks after penetration. Then, mature worms pair off, with the male worm wrapped around the female. The male transports his mate to the mesenteric venous plexus for *S. mansoni*, *S. japonicum*, and *S. intercalatum* or to the vesical plexus for *S. haematobium*. The female is located under the digestive or vesical mucous membrane and lays 200 to 2000 eggs each day, starting from the 40th day after penetration. Eggs pass from the lumen of blood vessels into adjacent tissues and many are shed in the feces (*S. mansoni*, *S. japonicum*, and *S. intercalatum*) or urine *(S. haematobium)*.[23] The life span of the worm is five to ten years, and it is capable of evading the immune response of the host by a variety of different mechanisms.[29]

2.3. Health Consequences of Schistosomiasis

Acute schistosomiasis (Katayama fever), common in areas of high transmission rates, occurs four to six weeks after infection. Typical symptoms are an urticarial rash, enlarged liver and spleen, fever, myalgias, dry cough, and eosinophilia as a result of a systemic hypersensitivity reaction to migrating schistosomulae. These symptoms disappear as the infection becomes chronic. They are often nonspecific, and as a result the diagnosis is often missed, occasionally with disastrous results, such as schistosomal myelopathy with permanent neurological damage.

Light infections with schistosomiasis can be asymptomatic. Early signs of morbidity, common for *S. haematobium* and *S. mansoni*, which manifest in school-age children, are anaemia, impaired growth and development, poor cognition and school performance. The first obvious symptom of *S. haematobium* infection is the detection of blood in the urine (hematuria). Because *S. mansoni* eggs are excreted through the feces, intestinal symptoms, such as diarrhoea with or without blood, are found in heavy infections. However, none of these signs and symptoms are specific to schistosomiasis and a diagnosis from clinical manifestations is difficult. The late and life-threatening consequences of schistosomiasis include bladder cancer or serious kidney dysfunction, and severe complications with liver and spleen.

Each schistosome egg has a spine that tears the tissues it comes in contact with and creates a lesion. The eggs can also become embedded in the tissue. Each time this happens, the body tries to repair the damage, leading to the formation of a granuloma, which is an inflammatory response to eggs sequestered in the liver and other tissues.[29] Over time, the number of these granulomas can increase to the point where the organ calcifies and no longer functions as it should. In addition, small abscesses occur, and the occlusion of small vessels leads to necrosis and ulceration. Neurological symptoms caused by egg deposition in the brain and spinal cord constitute a severe presentation of the disease.[30,31] In addition, the adult *S. mansoni* worms are of relatively large size (0.3–0.4 mm in diameter and 8 mm long) in comparison to the size of the mesenteric veins (diameter 1–4 mm). Such obstacles in the vessels lead to severe disturbance of blood flow that predisposes to thrombotic complications.[32]

In addition, intestinal schistosomiasis was reported to impair the immune response of the host to other infectious agents.[33,34] Schistosomiasis and malaria share most of the same geographic areas, and co-infection is usual. Co-infection of mice with *S. mansoni* and *Plasmodium berghei* favors rapid *P. berghei* development and high parasitaemia, and delays plasmodium clearance during chloroquine treatment.[35]

3. Hemoglobin Digestion and Formation of Hemozoin

3.1. Globin as Nutriment

Schistosomes feed from the red blood cells of the host in order to acquire amino acids for growth, development, and

reproduction.[36,37] Red blood cells are ingested by schistosomes, and lysed within the esophagus of the parasite. The released hemoglobin flows into the cecum of the schistosome where it is degradated to amino acids that are necessary for the build up of parasite proteins.[38] Adult females, which are metabolically more active than males, ingest higher quantities of red blood cells.[36,39]

Hemoglobin is likely the only protein of the host that can supply schistosomes with amino acids.[40] In mice infected with *S. mansoni*, incorporation of tritiated L-leucine from labeled reticulocytes into schistosome tissues provides additional support regarding the nutritional significance of the amino acids of globin for *S. mansoni*.[36] In vitro, addition of globin to the medium increases growth and prolongs the survival of *Schistosoma mansoni*.[41]

3.2. Heme as Waste

Hemoglobin digestion also involves the release of heme, its prosthetic group. The ability of free iron(II)–heme to reduce molecular oxygen is the origin of its potential toxicity toward aerobic organisms as a result of the formation of reduced oxygen species (ROS), namely $O_2^{\bullet-}$, H_2O_2, and $HO^{\bullet}$, which may damage vital parasite biomolecules.

3.3. Detoxification of Heme

Hematophagous parasites that are unable to catabolize heme through the heme oxygenase pathway, such as *Plasmodium*[42] and *Rhodnius*,[43] detoxify free heme through its sequestration into a dark-brown crystalline pigment named hemozoin. This end product of hemoglobin digestion is also accumulated in the gut of schistosomes (Figure 2) and continuously regurgitated.[41,44] Interestingly, hemozoin synthesis in *S. mansoni* occurs not only in adult schistosomes, but also in the early larval stages of human infection.[45,46]

Figure 2. A schistosome pair. The female, completely filled with black hemozoin pigment, is located in a ventral groove of the male.

3.4. Structure of Hemozoin

Studies reported in 1972 indicated that the pigments formed during hemoglobin digestion by malarial and schistosomal parasites are "very similar if not identical".[47] After many controversies about the structure of malaria pigment, it is now considered that purified hemozoin of *Plasmodium* consists of ferriprotoporphyrin IX without any other components, such as proteins or fatty acids,[48] and is identical to synthetic crystalline β-hematin.[49] More recently, the heme-polymer pigment produced by *S. mansoni* was compared to β-hematin and to pigments produced by other blood-feeding organisms, such as *Plasmodium*, *Haemoproteus*, and *Rhodnius*.[47,50,51] Fourier-transform infrared spectroscopy data identify the same signature peaks of the iron–carboxylate bond characteristic in all four heme polymers. In β-hematin, heme molecules are linked to each other, forming dimers by means of reciprocal iron–carboxylate bonds with the propionates of the porphyrin rings, and the dimers form chains through hydrogen bonds (Figure 3).[52,53] The IR bands at 1660

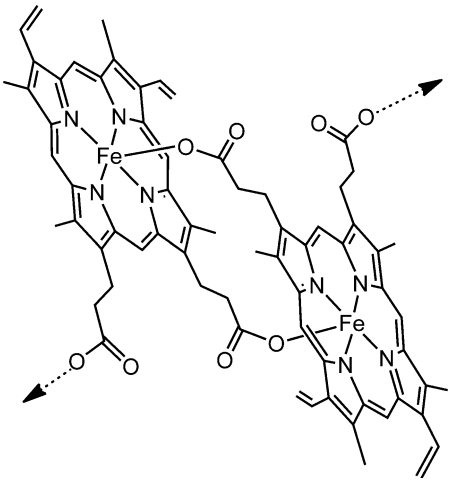


Figure 3. Structure of hemozoin.

and 1210 cm^{-1} are characteristic of iron–propionate bonds and were used to monitor the heme polymerization.[54] However, all four pigments could be distinguished by slightly different three-dimensional structures, indicating genus-specific formation and polymer extension.[50]

Aggregation of heme as dimers requires half of the heme propionate chains to be dissociated. This dissociation occurs at pH values near the pK_a of heme (4.8) in the food vacuole of *P. falciparum*. In *Schistosoma*, the surface of extracellular lipid droplets existing in the gut lumen seems to play a role in hemozoin crystallization.[55] Compounds able to interact with the mechanism of hemozoin formation in *Schistosoma*, *Plasmodium*, and *Rhodnius* could be used as new potential drugs against these diseases.

4. Anthelminthic Therapy

4.1. Vaccine

A strong argument for the development of a vaccine is the fact that schistosome infections are frequently undetected and significant pathology has time to develop before chemotherapy is administered. In addition, praziquantel, which is currently the only effective drug, is not completely effective in all patients and cannot prevent re-infection. Unlike other pathogens, schistosomes do not multiply in the mammalian host. The eggs they deposit in the tissues of the host are the principal cause of pathology, which develops roughly in proportion to the parasite burden. This means that sterile immunity induced by a schistosome vaccine is not essential. A significant reduction in worm burden would be useful, as would a vaccine-induced reduction in the fecundity of female worms or the viability of eggs.[56] Among continuous efforts to develop such vaccines, a vaccine based on *S. mansoni* cercariae giving rise to immature worms was elaborated in the 1960s. Unfortunately, the protection was limited to 7–30 days post-vaccination.[57]

Several antigens that were judged to be potential vaccine candidates induced less than 50% protection in the mouse model. Recombinant Sm-p80 protein provided levels of prophylactic efficacy of up to 58% in baboons.[58] The glutathione-S-transferase of *S. mansoni* was also considered.[59a] Recombinant Sh28GST (bilhvax, Eurogentec, Belgium) is currently considered as the best vaccine candidate for urinary schistosomiasis, because of its safety and immunogenicity. In phase I clinical trials, two injections of 100 μg of bilhvax were effective to initiate the production of antibodies specific of several Sh28GST isotypes in all the healthy volunteers that were treated. The vaccine has passed phase II trials, and recently entered phase III trials.[59b,c]

In fact, adult schistosomes are adapted to life in the human bloodstream, where they can survive for more than 30 years, so they must possess very efficient immune evasion strategies, indicating that the development of a schistosome vaccine for human use will not be easy.[60] In addition, large-scale production and conservation of a vaccine are expected to present obvious difficulties. Health education and prevention will remain decisive to reduce morbidity, and chemotherapy with simple and cheap drugs will be the only way to fight against schistosome-acquired infections.

Concerning antischistosomal chemotherapies, we will first briefly summarize drugs of historical value that were abandoned, mainly because of high levels of toxicity. The two compounds included in the WHO list of essential drugs, namely praziquantel and oxamniquine, will then be reviewed; as well as drugs that are not included in this list but which are clinically used under different circumstances. Then, recently developed agents that are in preclinical stages will be summarized. As far as possible, we favored the citation of original relevant reports in addition to key review articles.

4.2. Abandoned Drugs

4.2.1. Antimony Derivatives

At the beginning of the 20th century, reports indicated that Sudanese patients treated by intravenous injection of antimony tartrate (potassium bitartrate of antimony, so-called "tartar emetic", Figure 4) for leishmaniasis, experienced

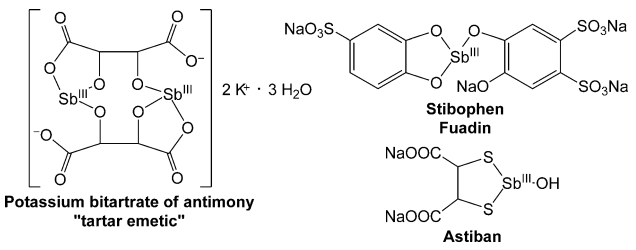


Figure 4. Structures of antischistosomal antimony derivatives.

interruption of their hematuria caused by schistosome co-infection. So, in 1918, the treatment of schistosomiasis with intravenous antimony tartrate begun, using multiple doses administered every day for a month or so to reach a total dosage of 15–30 g. In most cases, the disappearance of all symptoms without recrudescence was considered as a definitive cure.[61] Antimony tartrate is indeed active against *S. mansoni*, *S. haematobium* and, to a lesser extent, against *S. japonicum*. Used for years, antimony tartrate was then dismissed because of serious, and sometimes fatal, systemic reactions, including gastrointestinal, cardiovascular, hepatic, and dermatological disturbances. Accumulation of antimony in tissues as a result of its very slow excretion led to increasingly frequent toxic effects with the progress of therapy. In the search for better tolerated antimony drugs, it was found that only molecules containing trivalent antimony with oxygen ligands were maximally active (Stibophen, Figure 4),[62] while compounds with sulfur ligands were less effective, but better tolerated (Astiban, Figure 4). However, these drugs, given by intramuscular injection since the 1950s, caused severe side effects similar to those of antimony tartrate[63–65] and were discarded in the 1970s. In order to increase the benefit/risk ratio of antimonial derivatives, their formulation was attempted, for example through encapsulation in poly(*d*,*l*)-lactic acid[66] or in liposomes.[67] The development of pharmaceuticals with compositions based on a cyclodextrin and an antimony derivative for schistosomiasis and leishmaniasis treatment was reported.[68]

Mechanism of action: Glycolysis is the major pathway for energy production in adult *S. mansoni*, and is critically regulated by the thiol-containing phosphofructokinase (PFK). Antimonial drugs inhibit schistosome PFK in vivo,[69,70] resulting in the loss of attachment of the worms to the walls of blood vessels, and their migration to the liver after drug administration.[71] Disturbance of glycolysis also prevents the production of eggs by schistosome females.[72]

4.2.2. Other Abandoned Compounds

4.2.2.1. Emetine

The alkaloid emetine, isolated in 1817 from the roots of *Ipeca cuanha*, was first used to cure amoebic dysentery,[73,74] and its efficacy against *S. mansoni* was discovered when dysenteric patients that were co-infected with schistosomes were treated.[75] Efficacy was significant, but repeated injections over the course of a complete month were required.[76] Vomiting, cardiotoxicity, and neurotoxicity were drastic drawbacks at the therapeutic doses.[77–79] In search for less toxic derivatives, 2,3-dehydroemetine (Figure 5) was developed. However, the duration of the treatment and the poor therapeutic results, especially against *S. haematobium*, led to the renunciation of its clinical use.[64,80,81]

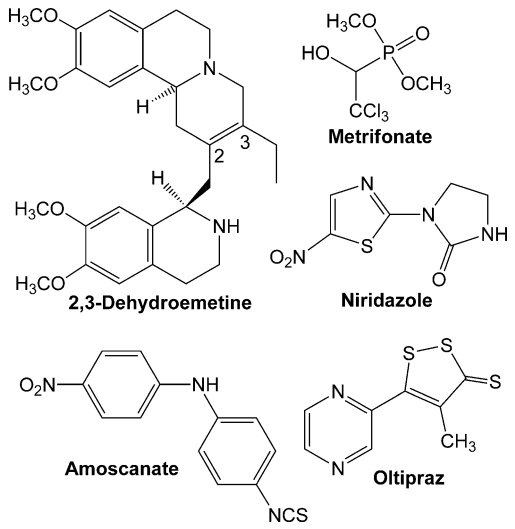


Figure 5. Structures of 2,3-dehydroemetine, metrifonate, amoscanate, niridazole, and oltipraz.

Mechanism of action: Emetine is a potent inhibitor of protein synthesis in eukaryotic cells, inhibiting the peptide-chain elongation by blocking the ribosome movement along mRNA.[82]

4.2.2.2. Metrifonate

Several organophosphorus compounds were evaluated as schistosomicides. Among them, metrifonate (2,2,2-trichloro-1-hydroxyethyl-dimethylphosphonate, Figure 5), previously used as insecticide under the trade names dipterex and dylox, was reported to have some activity against *S. japonicum* in mouse, dog, and man, and against *S. haematobium* in man. It was introduced for clinical use in 1955.[83,84] Metrifonate given to schoolboys infected with *S. haematobium* resulted in a significant reduction of the parasite load (50–60%), but without complete cure.[85] In addition, its activity against *S. mansoni* was only marginal.[84] Metrifonate is well tolerated and has a very low toxicity in warm-blooded animals. For this reason, this drug was recommended by the WHO for the treatment of urinary schistosomiasis (i.e., resulting from *S. haematobium*). However, the necessity of repeated doses tends to decrease compliance. The WHO stopped recommending metrifonate in 2000 because the drug did not appear to be as effective as the treatment of choice, praziquantel (see below). The therapeutic dose of metrifonate, consisting of three oral administrations of 7.5–10 mg kg^{-1} at 14-day intervals resulted in the reduction of egg production from 90–95%, and removal of symptoms in 44–93% of treated patients.[86]

Resistance to metrifonate was evidenced. Mollusks that were infected with *S. haematobium* and exposed to metrifonate produced schistosomules that were less sensitive to the drug in vitro.[87] A constant decrease of the response to metrifonate (from 79% in 1984 to 47% in 1987) was registered in Kenyan schoolchildren who were treated annually.[88] The mechanism of the loss of efficacy is unknown.

Mechanism of action: In humans, metrifonate is spontaneously transformed to 2,2-dichlorovinyl-dimethylphosphate (known as dichlorvos).[89] The good leaving-group properties of the dichlorovinyl moiety allows a transesterification with serine residue of the active site of acetylcholinesterase present in the tegument of the schistosome, resulting in a slow reversible inhibition of the enzyme. Accumulation of acetylcholine induces a reversible paralysis of the worm musculature, which results in its detachment from the wall of the blood vessels and migration to the liver (*S. mansoni* and *S. japonicum*) or to the lungs (*S. haematobium*).[90] The specific activity of metrifonate on *S. haematobium* is consistent with the fact that the specific activity of acetylcholinesterase is about four times higher in adult *S. haematobium* than in *S. mansoni*, and this difference is even bigger (20 times) in the tegument of the two species.[91] In addition, when the drug concentration decreases after treatment and the paralysis is reversed, *S. mansoni* that migrated to the liver is capable of returning to the mesenteric venous system, whereas *S. haematobium* remains trapped in the lungs and is unable to survive or, at least, to excrete eggs.[90] The mode of action of metrifonate has been reviewed in detail by Cioli et al.[64] The mechanism was questioned, because organophosphates do not specifically target cholinesterase enzymes, but inhibit also a large variety of esterases.[92]

Because of its long-lasting activity in the inhibition of acetylcholinesterase, metrifonate was evaluated in patients that were clinically diagnosed with Alzheimer's disease of mild to moderate severity. It significantly attenuated the deterioration in activities and the behavioral disturbances of the patients.[93]

4.2.2.3. Niridazole

This compound, which bears a nitrothiazole structure (Figure 5), was initially developed in the 1960s by Ciba–Geigy for use as an antibacterial agent.[94–96] In man, cure rates higher than 92% were observed in children or adults that were infected with *S. haematobium* and treated orally with 25 mg kg^{-1} d^{-1} for seven days.[97,98] The few patients who did not become parasite-free showed a significant reduction in egg output. For adults infected with *S. mansoni*, the cure rates observed with the standard schedule were ranging from 70 to

100%. Higher dosages of up to 35–40 $mg\,kg^{-1}\,d^{-1}$ were required in children infected with *S. mansoni*, to reach cure rates comparable with those observed in adults. These doses were possible because the tolerability of the drug was better in children than in adults.[97,99] However, at a therapeutic dosage, the drug had major side effects on the central nervous system in about 3% of adult patients (loss of consciousness, convulsions).[100] In patients with hepatosplenic *S. mansoni* schistosomiasis, the treatment had to be discontinued in almost 40% of cases because of neurological side effects.[101] Other side effects include alteration of the digestive and cardiac functions, but cardiac symptoms were considered less frequent than those occurring with antimony therapy, which was conventional during this time.[98] So, it was considered that niridazole could be employed on a large scale in children that suffer from *S. haematobium* or *S. mansoni* infections, that is, in those patients who are chiefly responsible for spreading the disease.[99] A long-term immunosuppressive activity was detected in man; carcinogenicity and embryotoxicity were documented in rodents.[64]

Mechanism of action: After a metabolic reduction of the nitro function of niridazole by adult *S. mansoni*, niridazole covalently binds to sulfhydryl groups of schistosome proteins and, to a lesser extent, to nucleic acids.[102] In addition, niridazole or its metabolites could suppress the immune hypersensitivity to schistosomal egg antigens in mice.[103] The P450-dependent oxidative metabolism in mammals (hydroxylation of the 2-imidazolidinone ring leading to 4-hydroxyniridazole) was considered to be responsible for different side effects of the drug, including mutagenic and carcinogenic effects.[64,104]

4.2.2.4. Amoscanate

Anthelmintic activity of amoscanate (Figure 5) was first reported in 1976. Upon administration of a single oral dose, this molecule was equally effective in various hosts (including primates) against *S. japonicum*, *S. haematobium*, and *S. mansoni* infections.[105] In humans, amoscanate was extensively tested in China, and cure rates higher than 92% were observed after a total dose of 7 $mg\,kg^{-1}$ administered over three consecutive days.[64] Because of its liver toxicity, amoscanate was supplanted by praziquantel.

Mechanism of action: The isothiocyanate group could acylate amine functions of schistosome plasma proteins.[105] However, this function might also be responsible for extensive binding of the drug to plasma proteins of the host (80%), suggesting that such acylation can not be the only factor responsible for the antischistosomal activity of amoscanate. In addition, derivatives in which the isothiocyanate function was replaced by a thionocarbamate or a methylpiperazine retained the antischistosomal activity.[64]

4.2.2.5. Oltipraz

The antischistosomal activity of a 1,2-dithiole-3-thione derivative named oltipraz (Figure 5) was also reported in the 1970s. This molecule was active against *S. haematobium*, *S. intercalatum*, and *S. mansoni*, but not against *S. japonicum*. Its bioavailability was increased by concomitant food absorption. Beside digestive side effects, the most serious drawback consisted of paresthesia and acute pain at the fingertips, a phototoxic reaction observed in up to 30% of treated patients exposed to sunlight. Photo-onycholysis was also observed in some cases. The toxicity led to the discontinuation of clinical trials.[64]

Mechanism of action: The worms that were recovered from infected mice treated with oltipraz exhibited reduced glutathione-reductase and glutathione-*S*-transferase activities.[106] With the depletion of its antioxidant defenses, the parasite might be more vulnerable to damage by oxidative stress induced by the host.[107] Conversely, levels of glutathione-*S*-transferase in the liver of treated mice increased in a dose-dependent manner.[108] The binding of a radiolabeled oltipraz metabolite to schistosome glutathione reductase was reported.[109,110] Beside antischistosomal activity, the regulation of glutathione-*S*-transferase activity by oltipraz may be responsible for the inhibition of carcinogenesis induced by different agents in different target organs.[111]

4.2.2.6. Myrazide, Benzodiazepines, Cyclosporin A, etc.

A preparation based on myrrh derivatives, myrazide, was marketed as schistosomicide since 2001. However, its efficacy, if existing, is very low.[112,113] A few other compounds were found to be active against one or several schistosome species, among them cyclosporin A, the benzodiazepine Ro 11-3128, or tubercidin (a purine analogue). Because of the lack of selectivity or intractable side effects at curative doses, further development of these drugs was abandoned. An acridanone–hydrazone derivative, Ro 15-5458 was proposed in the early 1990s as a treatment against *S. mansoni* schistosomiasis[114] and, more recently, against *S. haematobium*.[115] But, to the best of our knowledge, this drug was not developed further. Aromatic derivatives inspired from myracil A, lucanthone, hycanthone, and mirasan, will be reviewed in Section 4.3.1, which is devoted to oxamniquine (see below).

4.3. Clinically Used Compounds

4.3.1. Derivatives of Miracil A: Lucanthone, Hycanthone, Oxamniquine

The development of a whole family of compounds based on *p*-aminotoluene or *p*-aminobenzyl alcohol over a period of three decades resulted in the drug oxamniquine, which is still in the WHO List of Essential Medicines for antischistosomal use when praziquantel treatment fails.[116]

Miracil A, which contains a 9*H*-oxo-xanthene moiety (Figure 6), was isolated in 1938 after a systematic search for schistosomicides carried out by Bayer at Elberfeld (Germany). The thioxanthene analogue lucanthone (or miracil D, Figure 6), featuring the critical *p*-methylaniline moiety, exhibited some activity against *S. haematobium* and *S. mansoni* and was introduced into clinical practice. However, its efficacy was much lower than that of antimony-containing formulations and toxic side effects were observed, including

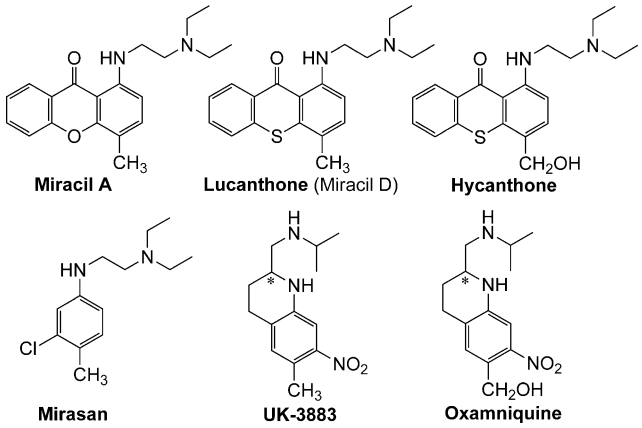


Figure 6. Structures of antischistosomals derived from or inspired by miracil A, including oxamniquine.

nausea, vomiting, and occasionally severe disturbance of the central nervous system and the cardiovascular system.[117]

Interestingly, lucanthone was active when given orally, but inactive in vitro and when given by injection. This observation suggests that lucanthone needs to be biotransformed by the host. In contrast to lucanthone, the derivative that results from hydroxylation of the aromatic methyl substituent, hycanthone (Figure 6), was active in vitro,[118] and was about five times more active when given intraperitoneally than orally, thus indicating that hycanthone was the biologically active metabolite of lucanthone.[119] Consequently, hycanthone replaced lucanthone in clinical practice for the treatment of both *S. mansoni* and *S. haematobium* patients, with oral doses of 3 mg kg^{-1} per day for four to five days, or a single intramuscular dose of 3 mg kg^{-1}. However, the acute liver toxicity (leading to death by hepatic necrosis), long-term mutagenic,[120] teratogenic,[121] and possibly carcinogenic activity[122] led to the discontinuation of the clinical use when the safer praziquantel became available. In addition, stable and transmissible resistance to hycanthone can be produced within a single generation in *S. mansoni* by exposing immature parasites in mice to the drug. Reproducible genomic rearrangements are induced during the acquisition of resistance and suggest that the resistant phenotype is induced rather than selected from preexisting forms.[123,124]

In an attempt to preserve the efficacy of hycanthone, a series of simple *N*-alkyl-*p*-methylaniline derivatives was synthesized. Mirasan (Figure 6) is active on mice but inactive on monkeys and humans. Development of UK-3883 in the 1960s by Pfizer and its oxidation product by *Aspergillus sclerotiorum* allowed the introduction of oxamniquine in 1969.[64] Regarding hycanthone/lucanthone, in vitro and in vivo activities are noticed for oxamniquine but not for UK-3883, which is a prodrug.[125] Side effects of oxamniquine are relatively mild compared with those of lucanthone, and oxamniquine is selective against *S. mansoni*. The therapeutic oral dose is 12–15 mg kg^{-1} (20 mg kg^{-1} for children weighting less than 25 kg). The systematic treatment of millions of people in a rural area in north-east Brazil, where severe schistosomiasis mansoni is endemic, was a potent method to cure the disease and drastically reduce transmission.[126] Unfortunately, the price of oxamniquine remains high and its continued commercial availability is uncertain.[127]

Beside its activity on adult worms, high doses of injected oxamniquine prevent the development of cercaria in mice.[128] Hycanthone-resistant schistosomes, which were invariably found to be resistant to oxamniquine, were isolated both from laboratory animals and humans.[124,129] This is a strong argument in favor of a common mechanism of action for both drugs.

Mechanism of action: First, it was proposed that hycanthone is an inhibitor of acetylcholinesterase from *S. mansoni*.[130] It is now considered that oxamniquine and hycanthone may inhibit the synthesis of nucleic acids in the parasite.[131,132] This inhibition is correlated with parasite death, and only transient in resistant schistosomes: when the drug pressure is removed (either in vitro or in vivo), the synthesis of nucleic acids takes place again.[132] Furthermore, tritiated hycanthone and oxamniquine bind irreversibly to the DNA of sensitive *S. mansoni*, but failed to do so in resistant schistosomes. In addition, the resistance is likely not a result of different membrane permeability or different uptake mechanisms between the two strains. This feature suggests that the mechanism of action of hycanthone is essentially based on the alkylation of schistosome DNA. It was proposed that is only in sensitive schistosomes oxamniquine enzymatically converted to an alkylating agent, an activated ester of the benzylalcohol function. The enzyme that activates oxamniquine is a schistosome sulfotransferase.[133,134]

Hycanthone and lucanthone intercalate between DNA base pairs, a mechanism responsible for their mutagenic ability. Oxamniquine, which lacks a 3-ring system, is not an intercalating agent. Beside alkylation of DNA, inhibition of several proteins, such as topoisomerase II, through direct binding, and inhibition of the base excision repair enzyme apurinic endonuclease (APE1) are likely responsible for anticancer properties of thioxanthenones.[135,136] Lucanthone prevents post-irradiation DNA repair in cancer cells by inhibiting human topoisomerase II. For this reason, lucanthone and an analogue were considered as adjuvants for anticancer radiotherapy.[135,137,138] It was recently reported that lucanthone inhibits autophagy by disrupting lysosomal function, and thus may enhance the pro-apoptotic effect of several anticancer agents.[139]

Noteworthy, oxamniquine is a chiral drug that is marketed as a racemate. We failed to find information on the activity/toxicity of oxamniquine enantiomers. Studies carried out with mouse liver fractions showed no enantioselective biotransformation of oxamniquine enantiomers,[140] while hydroxylation of UK-3883 to oxamniquine favored the dextrorotatory enantiomer of UK-3883.[141]

4.3.2. ***Praziquantel***

Since the 1980s, praziquantel (PZQ, Figure 7) is the drug of choice for the treatment of schistosomiasis, because it is orally effective against all five species of schistosomes with a single-day treatment. It is significantly more effective than oxamniquine in the treatment of an *S. mansoni* infection,[6] and also effective in patients infected with oxamniquine-

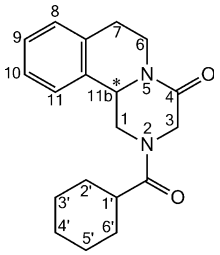


Figure 7. Structure of praziquantel.

resistant *S. mansoni*.[129] Praziquantel is very cheap (0.2 USD for a child) and well tolerated at therapeutic doses. The drug has also been extensively used in veterinary practice and was approved in 1980 for such use in the USA. The treatment of livestocks that serve as parasite reservoirs is required to interrupt disease transmission. Furthermore, it has a broad anthelmintic spectrum and is active against various cestodes.

4.3.2.1. *Discovery*

The activity of pyrazinoisoquinoline derivatives was first explored by Merck (Darmstadt) for the development of tranquilizers. Their anthelmintic activity was jointly discovered in 1972 by Merck and Bayer A.G. (Leverkusen), and readily appeared to compete favorably with former anthelmintic drugs.[142,143] Praziquantel was then developed, approved for human use by the Food and Drug Administration in 1982, and available on the international market in the 1980s. Merck is still providing praziquantel for Africa.[144]

In 1983, the Korean company Shin Poong patented a new synthetic method for PZQ, which was licensed to Eipico (Egypt) followed by other producers in many countries. This market competition, and the expiration of the initial patents from Merck and Bayer in the 1990s resulted in a drastic fall of praziquantel prices.[145] The recommended treatment consists of a single dose of 40 mg kg^{-1} (tablets of PZQ contain generally 600 mg of active ingredient). A syrup (600 mg/5 mL) is also available for children. The bioavailability of praziquantel drastically increases with concomitant administration of food.[146]

4.3.2.2. *Activity of Praziquantel*

Praziquantel or 2-(cyclohexylcarbonyl)-1,2,3,6,7,11b-hexahydro-4*H*-pyrazino[2,1a]isoquinolin-4-one is a colorless crystalline product that has a bitter taste and is stable and practically insoluble in water. The 4-oxo group and the 2-acyl functionionality are essential for the activity of the drug. Maximal activity is observed when a cyclohexylcarbonyl group is present at position 2, but benzoyl or *para*-aminobenzoyl residues also induce an activity.[147]

The drug bears an asymmetric center at position C11b. The marketed drug is a racemic mixture of 11b(*R*) and 11b(*S*), but only the *levo* enantiomer (−)(11b*R*)-PZQ has antischistosomal activity.[148] In man, a single dose of 20 mg kg^{-1} of (−)-PZQ displays the same activity as 40 mg kg^{-1} of the racemate, and both enantiomers of PZQ have similar toxicity.[149]

After oral administration of racemic PZQ in humans, the derivative that is hydroxylated at position 4′ of the cyclohexyl moiety, (−)-(11b*R*)-*trans*-4′-hydroxypraziquantel, was the major metabolite detected in serum. The inactive enantiomer (+)-11b*S*-PZQ was detected along with its 4′-hydroxylated derivative, thus suggesting an enantioselective first-pass liver metabolism of praziquantel.[150] (−)-(11b*R*)-*trans*-4′-Hydroxypraziquantel may substantially contribute to the antischistosomal efficacy of PZQ because of a similar activity and a longer in vivo half-life compared with that of the parent compound (−)-(11b*R*)-PZQ.[150,64]

4.3.2.3. *Toxicity/Safety of Praziquantel*

Contrary to many other antischistosomal drugs, praziquantel is not mutagenic in a number of different species, including bacteria, yeasts, insects, and mammals.[143] The acute toxicity in rats, mice, rabbits, and dogs is very low compared with that of other schistosomicides. Rats tolerate oral doses of up to 1000 mg kg^{-1} d^{-1} over a period of four weeks, and dogs up to 180 mg kg^{-1} d^{-1} over a period of 13 weeks without any organ damage. Praziquantel did not disturb reproduction in rats and did not have a teratogenic effect in rats, mice and rabbits.[143] In humans, PZQ induced no clinically relevant drug-related changes.[147,151]

4.3.2.4. *Mechanism of Action*

The efficacy of PZQ is dependent on the age of the infection, on the sex of the worms, and on their paired or unpaired status. During the earliest stages (from cercariae to the first few days after infection), the worms are susceptible, followed by progressive insensitivity down to a minimum at around three to four weeks weeks after infection (depending on the schistosome species). Schistosomes then gradually regain susceptibility until they are fully affected by the drug, around weeks 6–7 after infection. The ED_{50} of PZQ against immature *S. mansoni* worms in mice (4 weeks after infection) was at least 30 times higher than that observed for adult worms (6 or 7 weeks).[152,153] The striking drug insensitivity of immature worms is actually the most serious problem in the clinical use of PZQ.

Few alterations of the metabolism of *S. mansoni* and *S. japonicum* were reported after in vitro treatment at concentrations of praziquantel of 10^{-6} to 10^{-7} M. Glucose uptake, lactate excretion, concentration decrease of some enzymes (ATPase, alkaline phosphatase), or cholinesterase activity are modified in the presence of PZQ.[64] A transient decrease in glycogen content was observed in the parasites after treatment of the host mouse with subcurative doses of the drug.[147] These alterations are generally observed after long drug exposure (hours) and are generally thought as being "secondary" with respect to the modifications in Ca^{2+} content and tegumental morphology that occur at much shorter times (seconds).[64] No data support interactions of PZQ with nucleic acids or neurotransmitters.

Morphologic alterations: The direct exposure of adult schistosomes in vivo to praziquantel results in the immediate tetanic contraction of the musculature of the parasites, their detachment from the wall of mesenteric vessels, and their migration to the liver. Morphologic alterations of the tegument occur, including blebbing and vacuolization. In vitro, both effects occur 30 seconds after contact of the worms with PZQ, but the concentration of the drug required to produce tegumental vesiculation of *S. mansoni* was 10–60 times higher than the concentration producing increased motor activity or

muscular contraction. The minimum effective dose (MEC) of PZQ to induce contraction of *S. mansoni* was 16–32 nM (irrespective of the developmental stage that was tested), a concentration that resulted in no tegumental damage at any stage, even after 12–24 h. Immature developmental stages of *S. mansoni* were more resistant to vesiculation than mature worms (after day 35).[152] Early stages of schistosomula (days 0 to 7) were particularly resistant to tegumental damage. Adult worms (days 35 to 42) were the most susceptible, with extensive vesiculation and disintegration of the surface spines at a drug concentration of 0.3 μM. At this drug concentration, adult female worms displayed less vesiculation than male worms, and the vesicles usually appeared at around 2–4 h instead of 15 min after drug addition.[152]

In mice, the treatment of mature infections with subcurative doses of PZQ resulted in a prompt migration to the liver as a result of the drug-induced paralysis of the worm. But recovery from the paralytic effects of the drug can occur, with a resulting migration of the worms back to the mesenteric vessels. Thus, reduction of the worm burden in vivo was associated with the ability of the drug to produce tegumental damage rather than with the alteration of motor activity of the parasite.[152] However, after treatment with PZQ, the tegumental matrix, observed by transmission electron microscopy, was slightly modified. By contrast, significant swelling of the underlying muscle bundles occurred.[154] This feature implicates that the main PZQ target is the musculature, instead of the tegument, as previously reported.[155]

Immune Response of the Host: In vitro, exposure of male schistosomes to PZQ was accompanied by an increased exposure of the antigens of the parasites at the surface of their tegument.[156] Release of antigen components from or through the damaged parasite tegument may not only sensitize the immune response of the host, but possibly confer some degree of resistance to subsequent re-infection.[152] In T-cell-deprived mice infected with *S. mansoni*, PZQ (as well as hycanthone, oxamniquine, and antimonials) was found to be less effective than in immunologically intact animals, suggesting that the drug acts synergistically with immune effector mechanisms in killing adult schistosomes.[157] Conversely, the antischistosomal activity was restored when serum-containing antibodies were injected with PZQ. A glycoprotein of 200 kDa, located in the tubercules of parasites, would be directly implicated in a synergistic action between PZQ and the immune response of the host.[158]

Role of calcium: Although the molecular mode of action remains elusive, the two major effects, musculature contraction and tegument damages, are thought to be linked to praziquantel-dependent disruption of the Ca^{2+} homeostasis in worms.[159] There is some evidence of the interaction of PZQ with high-voltage-activated Ca^{2+} channels of schistosomes. It was proposed that PZQ may disrupt the interaction between the β and α_1 subunits of voltage-gated Ca^{2+} channels, thereby allowing more channels to be open, or allowing more current to flow through individual channels. An explanation for this unusual function may reside within the beta interaction domains (BID, site of interaction of the β and α_1 subunits).[160] Site-directed mutagenesis confirmed that the unusual structure of the β subunits of schistosomes is associated with susceptibility to PZQ.[161]

Other calcium-binding proteins were identified in schistosomes. A sarco/endoplasmic reticulum (Ca^{2+}–Mg^{2+})-ATPase (SERCA) of *S. mansoni* was found to be 140–250-fold less sensitive to selective inhibitors of mammalian SERCAs.[162] A calmodulin[163,164] and a calreticulin[165] from *S. mansoni* have also been cloned, expressed, and characterized.

Paramyosin and actin are also crucial proteins of the schistosomal tegument, with the actin/myosin interaction being regulated by Ca^{2+} ions. The surface spines of *S. mansoni* are composed of *para*-crystalline arrays of actin filaments. Actin is also present in areas that are recovering from damage, thus implying an important role of this structural protein in tegumental repair.[166] When adult *S. mansoni* worms were pre-exposed to the actin depolymerization agent cytochalasin D, they survived the exposure to 3 μM PZQ, which was lethal to schistosomes in control cultures.[167] Praziquantel was also proposed to directly bind to myosin light chain of schistosomes (SmMLC), to trigger its phosphorylation, and consequently to increase calcium mobilization.[168]

So, although treatment of *S. mansoni* with PZQ induced only moderate tegumental damage, this damage may be relevant. In addition, it was recently reported that in vitro treated worms exhibited a slightly enhanced production of nitric oxide (×1.5) along the tegument. The production of superoxide anion was two times higher than that in control worms, confirming the disturbance of the redox status.[154]

4.3.2.5. Resistance or Therapeutic Failures?

After thirty years of mass chemotherapy and long-term drug pressure, concerns are raised about the development of resistance to PZQ. It is possible to induce resistance of *S. mansoni* and *S. japonicum* to PZQ in mice under laboratory conditions. Cross resistance between praziquantel and oxamniquine did not occur, thus suggesting different mechanisms of action for these two drugs.[169]

Several therapeutic failures attributed to *S. mansoni* strains with a low sensitivity to praziquantel or possible drug resistance were identified.[169–172] But the isolates of uncured African patients had drug ED_{50} values 5–6 times higher than those of the drug-susceptible isolates. This is a low level of resistance, compared to that of hycanthone and oxamniquine resistance, which can rise 1000-fold,[127] but a small decrease in efficacy of PZQ can be clinically relevant. In addition, in areas with high rates of transmission, most patients harbor immature schistosome worms at the time of treatment (PZQ is ineffective against this stage). Low cure rates can be due to maturation of prepatent infections after treatment, or re-infections, and do not necessarily indicate the presence of drug-resistant schistosomes.[127,173] Failure of standard treatment with praziquantel (once at 40 mg kg^{-1}) was also reported in travelers returning from Africa to Spain with *S. haematobium* infections. But the initial dosage was clearly subtherapeutic, and these patients were cured after a treatment at 40 mg kg^{-1} d^{-1} for three consecutive days.[174]

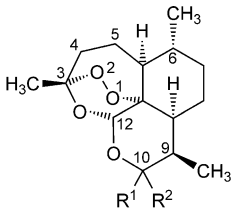
Recent studies suggested that no evidence of tolerance or resistance to praziquantel *in S. japonicum* was detected in China, at least in areas of low endemicity.[175]

As adult schistosomes living in blood vessels of the host must be able to take up nutriments and eliminate wastes, they are likely dependent on the use of multidrug transporters with broad substrate specificities. Consequently, multidrug-resistance (MDR) transporters may play a role in the resistance of the parasite to PZQ. *S. mansoni* strains express higher levels of multidrug-resistance-associated protein 1 (SmMRP1) in worms exposed to sublethal concentrations of praziquantel. A schistosomal homologue of the mammalian P-glycoprotein is also upregulated in PZQ-treated worms. Furthermore, these parasite proteins are expressed at significantly higher levels in juvenile worms, which are insensitive to PZQ.[176]

In conclusion, the occasional reports of individual failures of praziquantel treatment did not provide evidence for large-scale resistance of the parasite. However, laboratory studies leave little room for doubt that resistance to PZQ can occur, and this feature should encourage the search for new antischistosomal drugs.

4.4. Artemisinin Derivatives: Artemether and Artesunate; Complementarity to Praziquantel

Despite their high phylogenetic difference, the two hematophagous parasites *Plasmodium* and *Schistosoma* have a similar hemoglobin metabolism, leading to the formation of hemozoin. For this reason, antimalarial drugs that target heme were evaluated as potential antischistosomals. Among them, artemisinin (ART; Figure 8) and its



Artemisinin (ART): R^1, R^2 = O
Dihydroartemisinin (DHA): R^1 = H, R^2 = OH
Artemether (ARTM): R^1 = H, R^2 = β-OCH_3
Arteether (ARTE): R^1 = H, R^2 = α-OCH_2CH_3
Artesunate (ARTS): R^1 = H, R^2 = α-$OCO(CH_2)_2$–COOH

Figure 8. Structures of artemisinin and semisynthetic derivatives: dihydroartemisinin, artemether, arteether, and artesunate.

semisynthetic derivatives, especially artemether (ARTM), were recommended by the WHO as combination therapies against severe or resistant *P. falciparum* malaria. Artemisinin, extracted from the Chinese wormwood *Artemisia annua*, was also evaluated, first in China, against *S. japonicum* endemic infections, long before investigations on its mechanism of action.

4.4.1. Activity of Artemisinin Derivatives against Schistosomes

Mice infected with *S. japonicum* were treated with artemether at 200 mg kg^{-1} d^{-1} or 100 mg kg^{-1} d^{-1} for one to two days by oral or intramuscular route, respectively. When the treatment was carried out on day 7 after infection, the reduction rate for schistosomules was 74–92%. Adult schistosomes exhibited a lower sensitivity than schistosomula, with a reduction of the worm burden of only 47–70% when the treatment was carried out at day 35. The other developmental stages of schistosomes were less susceptible to ARTM treatment.[177] In mice infected with *S. mansoni* and orally treated with a dose of 1200 mg kg^{-1} (200 mg kg^{-1} d^{-1} for six days) on days 14 or 21 after infection, worm reduction rates of 83 and 98% were observed. When mice were treated on day 56 after infection, this high dose resulted in only 39% of worm reduction.[178] In mice, ARTM was reported to be inactive on adult *S. mansoni* at a dose of 800 mg kg^{-1}.[179] Thus, ARTM exhibited modest in vivo activity but was twofold more active against 2- to 3-week-old schistosomula than against adult parasites. ARTM exhibited also a significant activity against *S. haematobium* schistosomules aged less than 28 days in hamsters (300 mg kg^{-1}).[180] Artesunate (ARTS, Figure 8) was found less active than ARTM.[181] In vitro, toxic effects of ARTM on adult *S. mansoni* and *S. japonicum* were observed only at concentrations higher than 100 μg mL^{-1} or 40 μg mL^{-1}, respectively,[177,178] thus confirming that high doses of ART derivatives are required to kill schistosomes.

Field studies suggested some efficacy of repeated oral administration of ARTM against *S. japonicum*-[182] or *S. mansoni* infections.[183] In fact, artemisinin derivatives are currently not considered by the WHO for schistosomiasis treatment. However, many malaria patients treated with artemisinin-based combination therapy (ACTs) are indeed co-infected with schistosomes. Several reports indicate that ACT administered in accordance with the currently recommended malaria treatment schedule results in sharp reduction of *S. haematobium* loads,[184] reduction of *S. haematobium* eggs,[185] or decrease in *S. mansoni* morbidity.[186] Unfortunately, large scale studies failed to confirm these encouraging findings.[187,188]

So, the efficacy of ACTs against the two major schistosome species, *S. haematobium* and *S. mansoni*, is too moderate to consider them as a first-line antischistosomal treatment. In addition, using antimalarial drugs against schistosomiasis might select drug-resistant malaria parasites, and it is often pointed out that ACTs must be reserved for the control of malaria.[188,189] A recent report raised concerns about a possible decrease of the sensitivity of *S. japonicum* to ARTS after ten years of use in China.[190] However, this report was refuted because of flaws in the method.[191] In addition, the demand for ACT sharply increased since the late 1990s and artemisinin has been in short supply. But continuous-flow synthesis of artemisinin from artemisinic acid, its much less complex natural precursor extracted from the same plant in higher yield, should be capable of meeting the ever-growing demand for low-cost artemisinin.[192]

4.4.2. Combination Therapy Based on Artemisinin Derivatives and Praziquantel

Because ART derivatives exhibit their highest activity against the schistosomes in juvenile stages in contrast to PZQ, which shows its highest activity against adult worms, combined treatment with ARTM and PZQ would target all developmental stages of the parasite and hence may improve treatment outcome. In animals experimentally infected with *S. japonicum* or *S. mansoni*, the combination ARTM–PZQ induced a much higher reduction of worm burden than both drugs administered separately.[193] Unfortunately, combination of ARTS (12 $mg\,kg^{-1}$) and PZQ was not beneficial in further reducing *S. mansoni* egg counts compared with the treatment with PZQ alone in humans.[194]

4.4.3. Mechanism of Action of Artemisinin Derivatives

Heme and redox status: The peroxide bond included in a 1,2,4-trioxane cycle is the key structural feature responsible for the antimalarial activity of artemisinin derivatives. In mice infected with *Plasmodium*, reductive activation of the peroxide triggered by iron(II)–heme complexes leads to heme alkylation by the drug, resulting in the formation of covalent heme–drug adducts.[195,196] Similarly, heme–ARTM adducts were detected in extracts of adult *S. mansoni* worms treated with ARTM. A slight decrease in the hemozoin content of the gut was also observed (−25% with respect to untreated worms).[154] In addition, at a concentration where ARTM alone was inactive in vitro (5 $\mu g\,mL^{-1}$) against *S. japonicum*, addition of hemin in the culture medium resulted in some harmful effect on schistosomes, depending on the content of the culture.[197] In fact, the presence of hemin is not sufficient to trigger reductive activation of artemether. Instead, the iron must be present in oxidation state + II (the reduced form able to transfer an electron in the antibonding orbital of a peroxide). So, efficacy of peroxide drugs should depend on the availability of a reducing agent such as glutathione, able to reduce iron(III) to iron(II) (for memory: the oxidation state of iron in hemozoin is + III). Interestingly, the activity of glutathione *S*-transferases, and to a lesser extent also that of superoxide dismutase, were significantly lower in adult *S. japonicum* worms harvested from ARTM-treated mice compared with untreated animals.[198] In mice infected with *S. mansoni*, concomitant administration of *N*-acetylcysteine seems to enhance the therapeutic potential of ARTM.[199]

Other targets: Upon treatment of infected mice, ARTM caused sustained shrinkage followed by degeneration of the tegument, intestine, and genital gland of the worms that had migrated to the liver.[177,200] In vivo, 30 and 50% reduction in lengths of male and female *S. mansoni* worms, respectively, were observed 14 days after treatment, but after 56 days, worm dimensions had returned to control values. The effect on male testes and female ovaries were also reversible.[178] These observations suggest that ARTM-induced damages on the worm musculature or genital glands are not directly responsible for the death of the parasite. The most severe damage induced by ARTM was extensive tegumental erosion and peeling upon treatment of schistosomes[154,201,202] or other trematode parasites, such as *Chlonorchis sinensis*,[203] or *Fasciola hepatic*a.[204] However, tegumental damages remain moderate compared with those induced by other drugs, such as PA1259 (Figure 12) or mefloquine (Figure 9).[154] Ultrastructural damages were also observed in subtegumental musculature, parenchymal tissues, and gastrodermis in schistosomula. However, tegumental damages and other alterations were reversible in surviving larvae.[202]

Why young stages of schistosomes in the liver are more susceptible to artemisinin derivatives than adult stages is unclear. It was suggested that schistosomula do not possess an adequate antioxidant protective mechanism.[178] However, significant differences in the immunologic systems of schistosomula and adult worms may also be responsible for their difference of drug susceptibility.[205]

4.5. Mefloquine

In contrast to trioxane derivatives, which are heme-alkylating agents, antimalarial quinoline-based drugs are heme-stacking agents.[206] Several quinoline-containing drugs were evaluated against schistosomiasis, but only mefloquine (MFQ, Figure 9) was found active. Reduction of worm

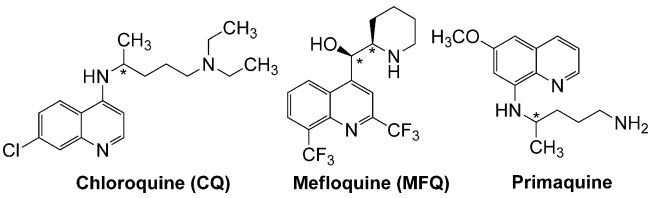


Figure 9. Structures of quinoline derivatives: chloroquine, mefloquine, and primaquine.

burdens as high as 87–95% were observed after a single oral dose of mefloquine on mice infected with either juvenile or adult *S. mansoni* or *S. japonicum*. In contrast to PZQ, high reductions of worm burdens were observed for young, developing stages.[207] As mefloquine at 5 $\mu g\,mL^{-1}$ kills cultured cercariae more efficiently than PZQ or ARTM, the drug is potentially active against all schistosome stages.[154]

Combination therapy with mefloquine–praziquantel was found more efficacious than each drug alone in mice harboring juvenile and adult *S. mansoni*.[208] In Ivorian schoolchildren infected with *S. haematobium*, the combination of MFQ and ARTS seems to act additively or even synergistically.[185] Millions of people are treated with MFQ and MFQ–ARTS combinations in areas where both malaria and schistosomiasis coexist. Hence, the potential benefit of antimalarial drug combinations containing mefloquine against schistosomiasis may be relevant.

Mechanism of action: In *Plasmodium spp.*, quinoline derivatives inhibit the formation of hemozoin. While MFQ is significantly active against schistosomes in the mouse model, other quinoline–methanol derivatives are only poorly active, or completely devoid of antischistosomal efficacy. Chloroquine, which is based on a 4-aminoquinoline motif, is also

inactive, as well as other antimalarials that have an extended aromatic moiety (acridine and benzoquinone derivatives), suggesting that aromatic π–π interaction between heme and the drug is not the only cause for the antischistosomal activity of mefloquine. A nonheme target has recently been proposed.[209]

Against malaria, mefloquine is used as a racemic mixture of the *erythro* enantiomers (11*S*,12*R*) + (11*R*,12*S*). The enantiomers of quinoline antimalarials have indeed the same antimalarial activity within the limits of experimental variability.[210a] Both *erythro* enantiomers of mefloquine showed also comparable antischistosomal properties when given to mice infected with *S. mansoni*.[207] In vivo, the antischistosomal activity of the *threo* enantiomers of MFQ was essentially the same as that of the *erythro* racemate. The activity of the *threo* racemate was significantly lower in vitro, suggesting that *threo* compounds undergo a metabolic asymmetric inversion in vivo to the *erythro* forms.[210b]

Administration of MFQ (400 mg kg^{-1}) to mice infected with adult *S. japonicum* resulted in extensive damage to the ultrastructure in tegument, subtegumental tissues, and vitelline cells.[211] Electron microscopy analysis of *S. mansoni* adult females treated with MFQ at 50 μg mL^{-1} showed a cavernous aspect of the worm surface with complete destruction of the external structures, and drastic alteration of vitelline cells. Noteworthy, a significant reduction of the hemozoin content in the worm gut (60%) was observed. Compared to PZQ, ARTM, and trioxaquine PA1259 (see below), MFQ was the most destructive drug, especially on vitelline cells.[154] The activity of MFQ on all the schistosome stages, the multiplicity of targets, and the various damages observed on treated worms suggest a complex mode of action, involving both a heme-dependent pathway and a heme-independent mechanism.

5. Future Treatments?

For an efficient treatment, it is considered that a reduction of the worm burden higher than 80–85% is necessary. Drugs (or drug combinations) recommended against soil-transmitted helminthiasis, lymphatic filariasis and onchocerciasis were evaluated against schistosomes. Albendazole, diethylcarbamazine, and ivermectin, or a combination of these drugs with PZQ did not add to the cure rate of PZQ in the treatment of *S. japonicum*, *S. mansoni*, and *S. haematobium*.[212] Antimalarials acting as folate antagonists (sulfadoxine, sulfamethoxypyrazine, pyrimethamine) are also inactive in the mouse model.[207] The screening of plants is still a research area for the development of new antischistosomal drugs.[213] Only the most promising new chemical entities will be reviewed below.

5.1. Drugs targeting Proteins of the parasite, Oxadiazoles

The schistosomes can survive for decades in a human host because of a unique set of antioxidant enzymes that continuously degrade the reactive oxygen species produced by the immune system of the host. Two principal components of this defense system were identified in *S. mansoni* as thioredoxin glutathione reductase (TGR) and peroxiredoxin (Prx). These enzymes present attractive targets for the development of antischistosomiasis drugs.[214] Among TGR inhibitors, oxadiazole-2 oxides were identified as new lead compounds for schistosomiasis chemotherapy (daily intraperitoneal injection at 10 mg kg^{-1} for five consecutive days). The most efficient oxadiazole derivative resulted in a decrease of worm burdens of 89% and 94% in mice infected with *S. mansoni* and treated 23 days or 37 days after infection, respectively. Interestingly, when treatment started one day after infection (skin-stage parasites), the worm burden was reduced by more than 99% compared with untreated mice. These protective effects exceed benchmark activity criteria set by the WHO for antischistosomal drug development.[215] The combined role of TGR inhibition by covalent modification of a cysteine or selenocysteine and the subsequent targeted release of NO are associated with worm killing by oxadiazoles.[215,216]

5.2. Trioxolanes

The efficacy of semisynthetic derivatives of artemisinin, in which the 1,2,4-trioxane moiety was replaced by a trioxolane (Figure 10), was in the same range as that of artemisinin. The

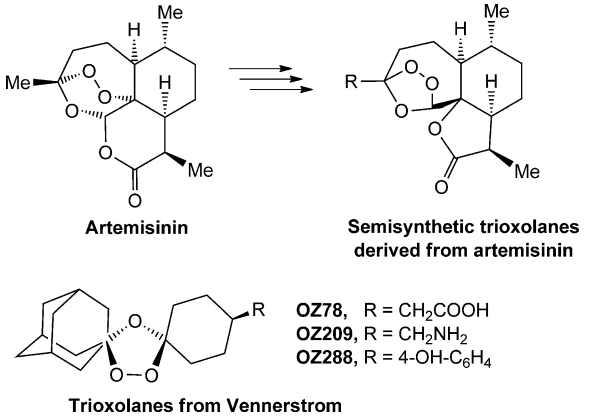


Figure 10. Structures of trioxolanes (Me = methyl, R = methyl, propyl, *iso*-butyl, or benzyl).

reduction of the worm burden was 30 to 60% in mice infected with *S. mansoni* and treated at a dose of 300 mg kg^{-1}. Nevertheless, the production of such compounds required several synthetic steps from artemisinin, which is not satisfactory in terms of cost of goods.[217] Vennerstrom and co-workers reported a series of new synthetic trioxolanes (OZs) essentially because of their antimalarial activities.[218] Antischistosomal activity of these compounds was also evaluated in mice infected with *S. japonicum*.[219] Among them, OZ78, OZ209, and OZ288 (Figure 10) exhibited high activities in mice with a 21-days-old *S. mansoni* infection, with a reduction of the worm burden of 79–96% following an administration of a single oral dose of 200 mg kg^{-1}. Against adult *S. mansoni* infection, OZ288, administered orally in a single dose of 400 mg kg^{-1}, was the only compound that exerted a significant

reduction in the worm burden (52–65 %). The OZ derivatives exhibited also high activities on both juvenile and adult stages of *S. mansoni* and adult *S. japonicum* in hamsters. The differences in activity of OZs against adult schistosomes between the hamster model and the mouse model might be explained by differences in the immunological responses or pharmacokinetic properties between the different animal species. In addition, the low toxicity, metabolic stability, and pharmacokinetic properties of the OZs underline the high potential of these drug candidates.[220]

5.3. Trioxaquantels

With regard to the complementarity of praziquantel and artemisinin derivatives, we designed new hybrid molecules, named trioxaquantels, which within a single drug combined the 1,2,4-trioxane moiety responsible for the activity of artemisinin, and the pyrazinoisoquinoline moiety of praziquantel (Figure 11). Their preliminary evaluation in mice

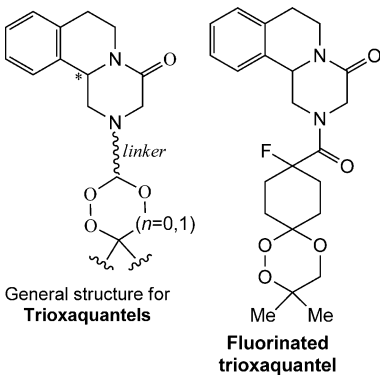


Figure 11. Structures of trioxaquantels.

infected with *S. mansoni* has recently been reported.[221] The fluorinated trioxaquantel depicted in Figure 11 exhibited the highest activity, with a reduction of the worm burden of 27 % after treatment of mice by oral route at a dose of 200 $mg\,kg^{-1}\,d^{-1}$ for five consecutive days. This activity, which is close to that of artemether (worm burden reduction of 41 % at 400 $mg\,kg^{-1}\,d^{-1}$ for five consecutive days) is only moderate. The low solubility of these drugs might be the reason for these rather low activities, as a result of poor oral absorption.

5.4. Trioxaquines PA1259 and PA1647; Synergy with Praziquantel

Trioxaquines (TXQ, Figure 12) are hybrid drugs that contain two antimalarial pharmacophores within a single molecule: a 1,2,4-trioxane and a 4-aminoquinoline.[222] Initially developed against malaria, they exhibit a dual mode of action: alkylation of heme with the trioxane entity and stacking of heme with the aminoquinoline moiety, thus leading to the inhibition of hemozoin formation in vitro.[223–225] As reported for artemisinin derivatives,[195,226,227] owing to their trioxane moiety, trioxaquines are indeed efficiently

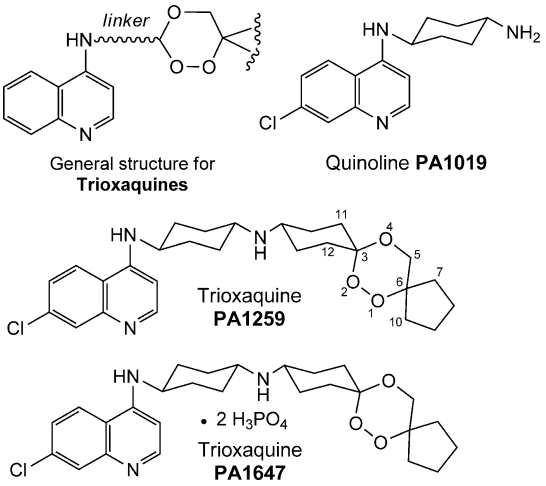


Figure 12. Structures of trioxaquines (Me = methyl).

activated by iron(II)–heme, leading to the formation of covalent heme–drug adducts, which were detected in the spleen of malaria-infected mice.[195,224]

5.4.1. In Vitro Activity

Several trioxaquines were found active on both larval and mature stages of cultured *S. mansoni*.[229] PA1259 exhibited a significant antischistosomal activity on all parasite stages. The efficacy of the trioxaquine PA1259 is similar to that of MFQ, and higher than that of PZQ on the free cercarial stage, whereas ARTM is inactive on cercariae. On mature parasites (49 days), the activities of PZQ, MFQ, and PA1259 are similar. On 21-day-old schistosomules, the activity of PA1259 is close to that of PZQ and significantly higher than that of MFQ and ARTM.[154]

5.4.2. In Vivo Activity

Mice infected with *S. mansoni* and treated at day 21 or day 49 post-infection were orally treated with four successive doses of 50 $mg\,kg^{-1}$ every three hours. The activity of PA1259 on the larval stage (53 % of worm burden reduction) was slightly higher than that of PZQ (41 % of worm burden reduction). For the adult stage, the worm burden reduction induced by PA1259 was half of that observed with PZQ (40 % compared to 86 %).[154] For comparison, ARTM was reported to be inactive at 800 $mg\,kg^{-1}$ in mice infected with *S. mansoni*.[179] Noteworthy, the efficacy of PA1259 in reducing the worm burden was similar on different parasite stages, suggesting a mode of action different to that of PZQ, which is stage-specific.

5.4.3. Synergy with Praziquantel

Mice infected with *S. mansoni* were treated at day 21 post-infection with a combination of PZQ and PA1647 (the diphosphate salt of PA1259, see Figure 12 for the structure). Treatment with 4 oral doses of 25 $mg\,kg^{-1}$ of each drug suggested a synergistic effect of the drug combination

(reduction of schistosomule burden was 73% with respect to untreated mice; for comparison, it was only 24% or 18% when PZQ or PA1647, respectively, were used as monotherapy). So, PA1259 and its phosphate salt PA1647 are actually among the most active drugs that have been tested to date against schistosomes, and the synergy of the combination of PZQ and PA1647 opens the route to an efficient bitherapy.[154]

Because trioxaquines are also developed for antimalarial activity, the possibility to select resistant *Plasmodium* strains when using trioxaquines against schistosomiasis may be a matter of concern. However, no trioxaquine-resistant *Plasmodium* strain could be selected after two years of continuous drug pressure (unpublished results by F. Benoit-Vical and co-workers).

5.4.4. Mechanism of Action of Trioxaquines

The drug PA1019 (Figure 12), which is the quinoline fragment of PA1259, is much less active on schistosomes than PA1259. This observation supports a synergistic effect of both quinoline and trioxane moieties of PA1259.[225] A general picture of damages induced by trioxaquine PA1259 in *S. mansoni* was recently reported and compared with that produced by three other reference drugs: PZQ, ARTM, and MFQ.[154]

5.4.4.1. Heme as Target

As a confirmation that heme is a relevant drug target in blood-feeding parasites, PA1259 alkylates heme in female adult *S. mansoni*, and heme–drug adducts were characterized in treated worms.[230] Upon in vitro treatment by PA1259, adult female *S. mansoni* readily regurgitated hemozoin (Hz), which is visible as a brown "cloud" in the right part of Figure 13a. A few minutes after treatment, the remaining Hz, which was black in control worms (Figure 13d,e), turned to light brown in treated worms (Figure 13b), suggesting the cleavage of the heme macrocycle. In treated schistosomes, products assigned to the oxidative cleavage of heme at the *meso* position were detected.[154] Some time after in vitro treatment, the brown pigment invaded the whole body of the worms (Figure 13c). These results were confirmed by transmission electron microscopy of ultrathin transverse sections of schistosomes: the hemozoin content in the gut of schistosomes treated with PA1259 was about 10% of that of untreated schistosomes. In addition, a part of the hemozoin pellets was found lining the external epithelium membrane, suggesting perforation of the gut epithelium upon treatment with PA1259.[154] Such modifications, observed with none of the reference drugs, namely PZQ, ARTM, and MFQ, suggest oxidative radical damages to the heme macrocycle and the gut wall.

5.4.4.2. Role of Nitric Oxide

The production of NO· (monitored by fluorescence in the presence of DAF2-DA) was measured in *S. mansoni* adult females treated with PA1259. Fluorescence was significantly enhanced with respect to control worms and mainly detected inside the gut and the vitelline cells. By comparison, treat-

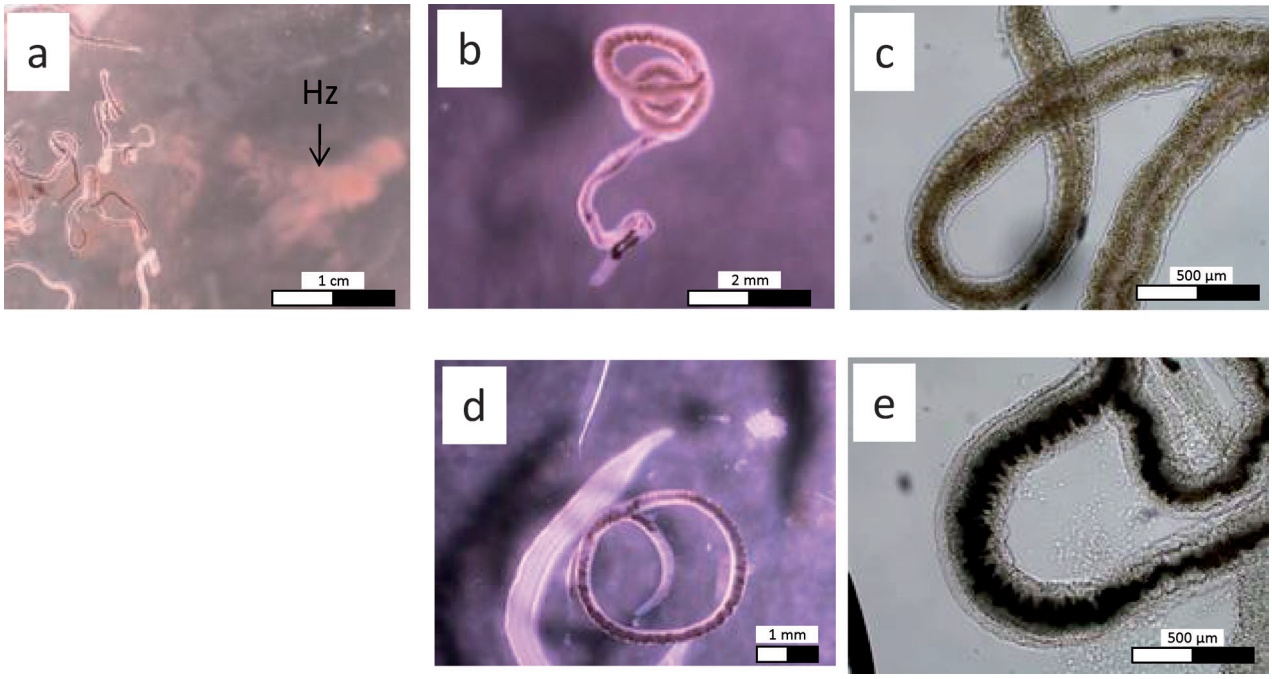


Figure 13. Photon microscopy images of *S. mansoni* females treated with PA1259 at 50 µg mL^{-1} (panels a, b, and c), in comparison to control worms (panels d and e). Upon treatment with PA1259, schistosomes regurgitate hemozoin (Hz) as a brown "cloud" visible in the right part of panel a. In panels d and e (untreated worms), black Hz is located in the gut, which is well defined. In panels d and c (treated worms), Hz turned to light brown and is present in the whole body of the worm, suggesting oxidative cleavage of the heme macrocycle and perforation of the gut epithelium induced by radical species.

ment with PZQ induced a smaller increase in NO· production near the tegument (Figure 14). The production of superoxide and hydrogen peroxide was not significantly enhanced in worms treated with PA1259. By contrast, in worms treated

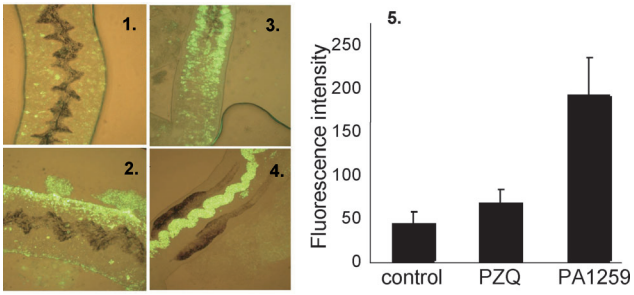


Figure 14. Dosage of nitric oxide (NO·) in adult *S. mansoni* females. The production of NO· (detected by DAF2-DA fluorescence) is low in control untreated worms (panel 1). In worms treated with PZQ, NO· is detected near the worm tegument (panel 2). In worms treated with PA1259, fluorescence resulting from NO· is mainly detected in vitelline cells (panel 3) and the gut (panel 4). Quantification of the total NO· fluorescence in PZQ- and PA1259-treated worms in comparison to controls is reported in panel 5 (fluorescence intensity 1.5 and 4.5 times higher in worms treated with PZQ and PA1259, respectively).

with PZQ, the production of $O_2^{\cdot -}$ was doubled in comparison to control worms. Again, these results indicated that these drugs have different targets and mechanisms of action, and are highly in favor of a bitherapy based on PZQ combined with a trioxaquine.

5.4.4.3. ***Other Targets than Heme***

Trioxaquine PA1259 and mefloquine (MFQ), which is a quinoline-based drug, are active on cercariae.[154] Because cercariae do not contain heme, activities of PA1259 (or MFQ) on that parasite stage suggest that heme is not the only target of these drugs. The quinoline part of PA1259 is likely to play a role in its activity against schistosomes, especially in the cercarial stage.

Musculature and tegument: *S. mansoni* strains treated with PA1259 and other different trioxaquines were convoluted without any exceptions, with a shape of a loose knot, suggesting an effect of the drug on the worm musculature.[229] TEM micrographs of worms treated with PA1259 exhibited severe swelling and disorganization of the subtegumental musculature. Extensive erosion or complete disappearance of the tegument also occurred upon treatment with trioxaquine. PA1259 and MFQ were the two drugs that induced the most severe damage to the tegument, which may lead to the exposure of the surface antigens of the worm, thus resulting in the attack of the parasite by the immune system of the host.[154]

Vitelline cells: The ultrastructure of vitelline cells of *S. mansoni* treated with PA1259 also exhibited extensive alterations. These damages were also induced in worms treated with MFQ, while most of the vitelline cells remained intact upon treatment with PZQ or ARTM. So, these alterations might be related to the quinoline part of the drug. Note that treatment with PA1259 induced the production of NO· in the vitelline cells (see above).[154] Despite some common effects of drugs that target heme, namely trioxane, quinoline, or hybrid molecules, such as trioxaquine, the morphological aspect and the intensity of effects vary according to the drug, suggesting some degree of specificity of the mode of action. The relative intensity of damages on cultured *S. mansoni*, as observed by electron microscopy (TEM and SEM), is reported in Table 1.

Table 1: Relative intensity of damages upon in vitro treatment of adult *S. mansoni* females with PA1259, compared with reference drugs praziquantel, mefloquine, and artemether.

	Treatment			
Target	PZQ	PA1259	MFQ	ARTM
Hemozoin	++	++++	++	+
Vitteline cells	+	++	+++	+
Tegument	+	+++	+++	++
Musculature	+++	+++	+++	–

Concerning the mechanism of action of trioxaquines against schistosomes, several questions remain to be addressed. Indeed, the fact that cercariae are heavily affected by treatment, the external tegumental alteration, and the effect on vitelline cells observed with PA1259 are not directly explained by the reaction of the drug with heme. But the sequence hemoglobin/heme/hemozoin is not the single source of iron in schistosomes, and several other iron-containing proteins may also play a role. Schistosomes have a high demand for iron and are dependent on iron from the host for early development within the mammalian host.[231–233] Several recent articles suggest that iron metabolism should be a valuable target for either chemotherapy or vaccine development against schistosomes.[59,234]

6. Conclusion

Schistosomiasis has some of the characteristics of an orphan disease, despite the large number of people that are contaminated in many tropical countries. After intensive research for the development of new chemotherapies in the middle of the last century, limited subsequent research efforts have reduced the therapeutic arsenal against this parasitic disease to one single drug, praziquantel. For this reason, it is urgent to develop new strategies, mainly based on "small molecules" (because the vaccine approach is facing a cliff) to control schistosomiasis in tropical countries.

The challenge in the treatment of schistosomiasis may be not to use "native" antimalarial drugs such as artemisinin derivatives, but to develop new drugs, considering the mechanism of action of antimalarial molecules with side-benefit against schistosomes, especially drugs targeting the heme digestion/polymerization metabolism. The co-occurrence of infections with multiple parasite species offers the opportunity for combined control. Under these conditions, it may be useful to develop antischistosomal peroxide-based

drugs that will also be active against malaria parasites, with the requirement that the drug should not easily induce the selection of drug-resistant strains of *Plasmodium*. This strategy might provide new molecules that are active on both parasites with limited side effects.

Acknowledgements

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