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NEW DRUGS AGAINST INTESTINAL PARASITOSES

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Introduction

This article will consider the drug therapy of three major groups of parasitic intestinal infections: protozoa, nematodes and cestodes. The intestinal flukes (trematodes) have a limited geographic distribution, in Asia primarily, and disease attributed to them is relatively rare. The far more common and important liver, lung and blood flukes are not, strictly speaking, intestinal helminths, although their eggs may be present in faeces. Schistosomiasis has already been considered by the World Health Organization and will only be mentioned in this paper.

The major intestinal protozoan infections are caused by Entamoeba histolytica and Giardia intestinalis. Intestinal nematode infections of public health importance are caused by Enterobius vermicularis, Ascaris lumbricoides, Necator americanus, Ancylostoma duodenale, Trichuris trichiura and Strongyloides stercoralis. Intestinal cestodiasis caused by Taenia solium, Taenia saginata and Hymenolepis nana are much less prevalent than nematodiasis. Nevertheless, human cysticercosis caused by Taenia solium is a serious public health problem. More that 2.5 billion of the world population are infected by one or more of these parasites and, according to Stoll, the reinfection rates are high. Out of seven infections, probably six could be considered as reinfections. A considerable amount of research, pursued all over the world, has produced in the last ten years a few very good therapeutic agents. Our review will be concentrated on these new drugs which can be given over a short period of time, as a single dose if possible, and reliably eliminate multiple intestinal protozoan and helminthic infections.

Treatment of intestinal protozoan infections

The 5-nitroimidazole derivatives are the most advanced drugs for the treatment of amoebic trophozoites in the intestinal wall and other body tissues, as well as giardiasis (Rossignol et al., 1984). Metronidazole, the first introduced in therapeutics, is 2-methyl-5-nitro-1H-imidazole-1-ethanol. Tinidazole is 1-(2-(ethylsulfonyl) ethyl)-2-methyl-5-nitro-1H-imidazole. Ornidazole is α -(chloromethyl)-2-methyl-5-nitro-1H-imidazole-1-ethanol. The last derivative developed is secnidazole, which is 1-(2-hydroxypropyl)-2-methyl-5-nitroimidazole. Metronidazole, ornidazole and secnidazole appear as light yellow microcristalline powders that are soluble in polar solvents and water; tinidazole is colourless and soluble in benzene. Nitroimidazoles are quickly absorbed with a peak level approximately two hours after a single oral dose of two grammes. Metronidazole has a short half-life of 5.4 hours, tinidazole, ornidazole and secnidazole have much longer half-lives of 13, 14.4 and 17 hours respectively. Blood assays have been developed using both bioassays and high pressure liquid chromatography techniques.

Metronidazole and secnidazole are metabolized in the liver by oxidation of the lateral chain. The parent drugs are recovered from urine, but not from faeces, among metabolites most of which are not identified. Tinidazole remains unchanged in the blood for a long period of time and is recovered from the urine 50 per cent unchanged and 50 per cent as metabolites, which are not identified for most cases. Ornidazole is excreted through urine (63 per cent) and faeces (22 per cent), and 96 per cent of the total oral dose is metabolized, half being conjugated and the metabolic pathway has been identified.

The mode of action of nitroimidazoles has been studied with Trichomonas vaginalis and Clostridium wellchii. In vitro, metronidazole at 200 µg/ml destroys the nucleus, axostyle, and undulating membrane within 60-70 minutes; death and lysis are observed at 60 and 75 minutes, respectively. When added to a culture of Trichomonas vaginalis in Bushby's medium, metronidazole abolishes H_2 evolution in 15-20 minutes and CO_2 evolution in 60 minutes, resulting in parasite death. This suggests that nitroimidazoles are active on the hydrogenase component of the phosphoroclastic reaction which appears to be ferredoxin. While there is no evidence that it could be found in Trichomonas spp., this enzyme is formed only in anaerobic organisms. Thus, we would expect to find it in all organisms that evolve hydrogen, including protozoa. Moreover, metronidazole has a redox potential of -0.56 V which is much more electronegative than the potential of ferredoxin (-0.46 V), and therefore acts as a better acceptor of electrons generated by the phosphoroclastic system. The site of this electron capture was demonstrated by Edwards. He showed that hydrogen does not accumulate in the organisms (Trichomonas vaginalis and Clostridium wellchii) after contact with metronidazole, suggesting that the imidazole ring is not reduced during the experiment. On the contrary, it could be clearly established that the nitro group is reduced during the same process by accepting electrons from reduced ferredoxin; this reduction is irreversible. In general, nitroimidazoles inhibit not only the hydrogenase system but also other electron transfer mechanisms. This mechanism of action could explain failures observed during the therapy with metronidazole. These are not attributed to the development of metronidazole-resistant strains, since the isolated strains are always sensitive in vitro to the drug. Several organisms commonly present in the vagina absorb and inactivate significant amounts of metronidazole which acts by reducing the nitro group. This phenomenon, which is greater under anaerobic than aerobic conditions, is more pronounced for some organisms, such as Escherichia coli or Klebsiella aerogenes. This inactivation is not unique to metronidazole but, may reflect a general property of 2-nitro and 5-nitroimidazole derivatives. The activity of this family of drugs seems to be related to the redox potential of each derivative when compared to the ferredoxin potential. This is also clearly related to the presence and the position of the nitro-group, which is reduced during the drug effect. The corresponding reduced compound has no efficacy against the original sensitive organisms. In contrast, the non-nitro compounds have low efficacy. Efficacy increases beginning with the 4-nitro derivatives, which are poorly effective, to the 2-nitro, and finally the 5-nitro, which are the best. This difference in activity is probably due to the degree of ionization of this function when substituted between the two N atoms.

In mice, the acute oral LD_{50} is 4,350mg/kg for metronidazole, 3,600mg/kg for tinadazole, 2,400mg/kg for secnidazole and 1,420mg/kg for ornidazole. In subacute toxicity studies, doses as high as 50mg/kg for metronidazole, 800mg/kg for tinidazole and 400mg/kg for secnidazole were administered orally to rats, for four to 13 weeks. No severe toxic effects were recorded. In dogs, 100mg/kg of metronidazole, 450mg/kg of tinidazole and 100mg/kg of secnidazole, administered daily during 4 to 13 weeks, never produced a marked toxicity, Ornidazole and secnidazole, administered to the same species at doses of 250 and 200mg/kg respectively, for three to 14 weeks, showed neuro-toxicity as early as week three of the studies. This toxicity was reversible after termination of the drug administration. Ornidazole was also given by the i.v. route for four weeks; only a slight sedative effect was observed, with ataxia and salivation lasting 15-30 minutes after high-dose injection (150mg/kg/day). Induration at the site of infection was observed in all groups from week three for low and medium doses and from week two for high doses. No other sign of intoxication was recorded. No teratogenic effects were observed with any of the four derivatives in mice, rats and rabbits when administered during pregnancy. Metronidazole and tinidazole were also administered to rats, before mating: no influence on male fertility and

general reproductive performance was observed for either drug. Nitroimidazoles are generally considered mutagenic chemicals. Mutagenicity was observed with Klebsiella pneumoniae and Salmonella typhimurium TA.100. Metronidazole was reported mutagenic in urine metabolites from humans. Ornidazole was also revealed to be mutagenic in Salmonella typhimurium, but negative results have been observed in other tests, such as micronucleus in mice and chromosome aberrations. Long-term carcinogenicity studies were conducted with the four compounds in rats and mice. Lung adenomas in mice, hepatocellular carcinomas, and mammary fibroadenomas in rats were considered to be related to the administration of the test compounds. Nevertheless, these drugs have been widely used throughout the world, in the last 20 years, and no reports of increased incidences of cancer attributable to them have been made. The carcinogenic risk, related to these products, may be negligible.

In the treatment of acute amoebic dysentery, the four drugs are highly effective, giving cure rates over 90 per cent. Activity against cysts, is quite weak, probably due to the quick metabolization of the drugs. Metronidazole is administered at a dose of 10mg/kg twice daily for eight to 10 days. Tinidazole is administered in a single daily dose of 20mg/kg, repeated for two consecutive days. Ornidazole is prescribed at a dose of 7.5mg/kg, twice daily for five to 10 days. Secnidazole is administered at a dose of 12mg/kg, twice daily for three days. This drug seems to have a much higher cysticidal effect than the other three drugs. This is probably due to a lower rate of metabolization of the drug, after oral administration. A single two gram dose of secnidazole has been reported to be highly cysticidal. Clinical investigators reported that 10 per cent failure was observed with ornidazole administered at a dose of two gram daily for five to seven days, and 30 per cent failure, when the treatment was administered for less than five days. Metronidazole showed 30-40 per cent failures in treating patients with Entamoeba histolytica minuta. It seems that, at least for metronidazole, tinidazole and ornidazole, a minimum of five consecutive days of treatment should be prescribed in order to obtain a very high cure rate in amoebic dysentery and a reasonable cysticidal effect. In addition, it is appropriate also to prescribe a luminal amoebicide, such as the dichloroacetamide derivatives, for maximum effectiveness.

All four nitroimidazole derivatives are highly effective in treating intestinal giardiasis caused by Giardia intestinalis. Metronidazole is prescribed at a dose of eight mg/kg daily for five consecutive days; single doses of 30mg/kg of tinidazole are also highly effective. Ornidazole and secnidazole have not been extensively evaluated in giardiasis, but there is little doubt that a single 30mg/kg dose is also very effective.

Nitroimidazoles are considered safe drugs and only minor side effects in about 15-30 per cent of the patients have been observed. The most common adverse reactions are dizziness, muscle and joint pains, epigastric pains, nausea and vomiting. A metallic, sharp unpleasant taste has also been reported. Alcohol is contraindicated during treatment because nitroimidazoles interfere with the metabolism of alcohol, with the exception of ornidazole. Because moderate leukopenia was occasionally observed, these drugs are contraindicated in patients with evidence of blood dyscrasia. Some central nervous system toxicity being observed in animals, nitroimidazoles are probably be contraindicated in patients with active organic central nervous system diseases.

Treatment of intestinal helminthic infections

Several anthelminthics have been developed in the last 20 years, for the treatment of nematode, cestode and trematode infections. In fact, few of them are widely used throughout the world, all being broad-spectrum drugs.

Two benzimidazoles, mebendazole which is methyl(5-benzoyl-1H-benzimidazol-2-yl) carbamate and albendazole which is methyl-5-propylthio-1H-benzimidazol-2-yl carbamate, are currently available in more than 100 countries. They are presented as white to yellowish powders, very slightly soluble in water and tasteless. Mebendazole is poorly absorbed from the gastro-intestinal tract and, following an oral administration, 90 per cent of the drug is excreted unchanged in the faeces within 24 hours. Mebendazole and hydroxymebendazole, its first blood metabolite could be assayed by liquid chromatography with electrochemical

detection. If very low mebendazole levels could be assayed in the blood, approximately 80 to 100ng/ml, following a 800mg oral dose, hydroxymebendazole could be detected at a much higher level, approximately 10 times that of the parent drug (Oosterhuis et al., 1984). Albendazole is also very poorly absorbed from the intestine. The lack of radiolabelled human studies does not allow any quantification of the amount absorbed. Nevertheless, if albendazole itself could not be detected by high pressure liquid chromatography in the blood, its first metabolite albendazole-sulfoxide could be easily identified. Following a 400mg oral dose, approximately 250ng/ml of albendazole sulfoxide are detected and the half-life of this metabolite could be calculated as 8.30 hours. The little fraction absorbed is excreted through urine, metabolized as six main metabolites, all identified (Rossignol et al., 1984). The mode of action of these two drugs is through inhibition of glucose uptake by the helminths, resulting in a depletion of glycogen and adenosine triphosphate, which are necessary for parasite survival, thus leading to the slow death of the worms.

In rats and mice, the oral LD_{50} is higher than 1280mg/kg for mebendazole and 1320mg/kg for albendazole. In subacute toxicity studies, in rats, doses as high as 140mg/kg for mebendazole and 168mg/kg for albendazole produced toxicities, such as irreversible testicular degeneration. The same studies carried out in dogs showed toxicity at 48mg/kg daily dose for albendazole. Leucopenia was seen in one animal. Life-time carcinogenicity studies, carried out in rats and mice, with both drugs, did not produce any evidence of carcinogenicity. Nevertheless, these two drugs are embryotoxic and teratogenic in rats, causing abnormalities of the fetuses, mainly skeletal deformities. After several years during which these two anthelminthics have been used extensively, no reports of human teratogenicity have been recorded, suggesting that, at the doses prescribed, the drugs may be safe in pregnancy. Nevertheless, their use during pregnancy is contraindicated.

Mebendazole is highly effective in treating nematode infections. The same dose level is prescribed in adults and children. A single 100mg dose is 100 per cent effective in enterobiasis. One hundred mg, twice a day, for three consecutive days, is 100 per cent effective in ascariasis, 92 per cent effective in hookworm infection (Necator americanus and Ancylostoma duodenale) and 85 per cent effective in trichuriasis (Keystone et al., 1979). Two hundred mg, twice daily, for three consecutive days, may have some effect in large tapeworm infections.

Albendazole, administered as a single 400mg dose in adults and children, is 100 per cent effective in enterobiasis and ascariasis, 91 per cent effective in hookworm infection (Necator americanus and Ancylostoma duodenale), and 80 per cent effective in trichuriasis. Four hundred mg per day, for three consecutive days, is 80 per cent effective in strongyloidiasis. In fact, 100 per cent cure rate could be observed at a dose of 800mg daily for three days, repeated twice at two weeks interval. Some effects were observed in large tapeworm infections, following 400mg per day, for three consecutive days.

Mebendazole and albendazole are ovicidal against Ascaris lumbricoides, Necator americanus, Ancylostoma duodenale and Trichuris trichiura. In addition, a larvicidal effect against the 14-day old larvae of Necator americanus, during their migrating stage, was observed following a single 400mg dose of albendazole in experimentally infected volunteers.

Benzimidazoles are generally extremely well tolerated causing minimal clinical side effects in less than five per cent of the patients. Epigastric pains, nausea, light diarrhoea and headache are the most often reported side effects. Haematological and biochemical abnormalities are not observed when these drugs are administered as a single dose or repeated for three days.

A few clinical studies were recently reported in the literature, where mebendazole was used as a single 500mg or even 1000mg dose. Cure rates are generally low, but the egg reduction in ascariasis, ancylostomiasis and trichuriasis is quite high (90 per cent).

Another related imidazole, levamisole/tetramisole, is currently used for the treatment of ascariasis and to some extent, ancylostomiasis. Because of its narrow spectrum and its use in immunodeficiency diseases, this drug is not a drug of choice for the treatment of nematodes.

Pyrantel-Oxantel, two cyclic amidines, are combined to produce a broad-spectrum anthelminthic. Pyrantel is (E)-1,4,5,6-tetrahydro-1-methyl-2-(2-(2-thienyl) ethenyl) pyrimidine 4,4'-methylenebis (3-hydroxy-2-naphthalenecarboxylate), insoluble in water and very slightly absorbed from the intestine. Oxantel is (E)-(2-(1,4,5,6-tetrahydro-1-methyl-2-pyrimidinyl) ethenyl) phenol 4,4'-methylenebis (3-hydroxy-2-naphthalenacarbobylate), practically insoluble in water and not absorbed from the intestine. Their mode of action is by inhibiting neuromuscular transmission, producing paralysis of the worms. The oral LD_{50} in rats and mice is above 2000mg/kg. Both drugs are not teratogenic and/or embryotoxic in several animal species. A daily dose of 10-15mg/kg for two to three days of the combination is 100 per cent effective in enterobiasis and ascariasis and above 90 per cent effective for both species of hookworms and trichuriasis. Tolerance is generally good and four to 20 per cent of the patients experience clinical side effects such as abdominal cramps, nausea, vomiting, headache and dizziness.

Praziquantel is 2-(cyclohexylcarbonyl)-1,2,3,6,7,11 b-hexahydro-4H-pyrazino(2,1-a) isoquinolin-4-one, an isoquinolinepyrazine derivative. It is a colourless crystalline powder with a bitter taste, insoluble in water. After oral administration, the drug is quickly absorbed from the intestine, with a peak concentration at two hours. It is excreted through urine after being metabolized in the liver. Drug excretion is complete within 24 hours. The acute oral LD_{50}s in rats and mice is above 2000mg/kg. The drug is not teratogenic in various animal species, and not carcinogenic during life-time carcinogenicity studies in rats and mice. Praziquantel has been primarily developed for the treatment of schistosomiasis caused by Schistosoma mansoni, Schistosoma haematobium, Schistosoma intercalatum and Schistosoma japonicum. It is a major breakthrough, being the only drug highly effective against the four species of schistosomes, as a single dose or on a single day of treatment (Davis et al., 1979). It is also very effective in treating liver and lung flukes.

The interest of praziquantel in our review is limited to its use in the treatment of intestinal cestodiasis. As a 25mg/kg single oral dose, it is highly effective (80 per cent) against Hymenolepis nana and probably Hymenolepis diminuta, while single five to 10mg/kg doses are almost 100 per cent effective against large tapeworms, such as Taenia saginata, Taenia solium and Diphyllobothrium latum (Baranski et al., 1980; Bylund et al., 1977). The drug is also effective against brain and cutaneous cysticercosis (Botero et al., 1982).

Conclusions

In general, very few chemotherapeutic agents are available for the treatment of parasitic infections. This is obviously true for the treatment of protozoan and helminthic infections which affect large populations in the Third world. In order to contribute to a decrease in the prevalence of such infections, new antiparasitic agents should have a broad spectrum of action, a good safety profile and should be effective in a single dose.

In fact, the available drugs do not completely meet these requirements. Amoebic dysentery and giardiasis may easily be treated by nitroimidazoles. Tinidazole and secnidazole, which have long half-lives allowing a single daily administration, seem the most convenient agents. Secnidazole, as a single two gramme dose, should be further investigated to confirm the high effectiveness observed in preliminary reports and because it also shows better cysticidal activity in amoebiasis. Nematode infections may easily be treated by benzimidazoles, albendazole used in a single dose, or perhaps mebendazole if high single doses (500 to 1000mg) can produce acceptable cure rates besides a high egg reduction. These drugs are not fully effective in a single dose. In addition, nitroimidazoles are suspected to be carcinogenic while benzimidazoles may be teratogenic.

The ideal drug, which has to be considered as a model, is praziquantel. This highly effective anthelminthic against numerous cestodes and trematodes is prescribed in a single dose or on a single day. It is very well tolerated and does not have any carcinogenic or teratogenic potential. Such a drug is still desperately needed for the treatment of protozoan or nematode infections.

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