Appl Microbiol Biotechnol (2001) 57:614–619
DOI 10.1007/s00230100720

MINI-REVIEW

J. C. Harris · S. Plummer · D. Lloyd

Antigiardial drugs

Received: 23 February 2001 / Received revision: 14 May 2001 / Accepted: 18 May 2001 / Published online: 21 September 2001

Abstract *Giardia intestinalis* is a world-wide cause of intestinal infection. Treatment of this debilitating disease is usually accomplished using one of several drugs. Metronidazole is the treatment of choice, but benzimidazoles are now being used more frequently. Other treatments include quinacrine, paromomycin and furazolidone. Even though these drugs are all used to treat the same disease, their modes of action differ in all cases. However, resistance is increasing and new alternatives are being sought. New wave antigiardials all appear to have their roots in natural herbal remedies. This mini-review looks at the current treatments available, their efficacy, side effects and different modes of action and addresses a possible way forward using natural products.

Introduction

Giardia intestinalis, a microaerophilic, flagellated, parasitic protozoan of man was first observed by Antony van Leeuwenhoek in 1681. The protozoan is a world-wide cause of intestinal infection that results in severe and explosive diarrhoea. Two million cases of symptomatic giardiasis were reported in Asia, Africa, and Latin America, with over 500,000 new cases being reported annually (WHO 1995). In most cases the infection is self-limiting; however, infants, the elderly and those that are immunocompromised are more susceptible and the disease can result in morbidity (Ortega and Adam 1997) and has even resulted in mortality (Shukry et al. 1986).

Several drugs are used to treat this disease, usually one of a family of nitroimidazoles, benzimidazoles, quinacrine, paromomycin or furazolidone. Recently there has been an increase in the number of resistant cases of giardiasis, so the quest is on for new effective alternative treatments.

D. Lloyd
Microbiology Group, School of Biosciences, University of Wales, Cardiff, PO Box 915, Cardiff CF10 3TL, UK

J.C. Harris (✉) · S. Plummer
Cultech Biospeciality Products, York Chambers, York St, Swansea SA1 3NJ, UK
e-mail: janine@jharris2000.fsnet.co.uk

Metronidazole

The use of nitroimidazoles as antimicrobials began with the discovery of azomycin, 2-nitroimidazole (Maeda et al. 1953), and its synthetic 5-nitroimidazole derivative, metronidazole (Fig. 1), commercially known as Flagyl (Cosar and Julou 1959). Both compounds were shown have trichomonacidal activity (Horie 1956; Durel et al. 1959), and it was later shown that metronidazole was also antigiardial (Mandoul et al. 1961; Schneider 1961).

It is an effective antigiardial both in vitro (Boreham et al. 1986; Paget et al. 1989) and in vivo (Webster 1990), and is routinely used for the treatment of giardiasis. However, it has been shown that this drug has no effect on the viability of cysts (Paget et al. 1989).

Metronidazole as such is inactive, and only forms active metabolites when it is reduced by PFOR (Lloyd and Pedersen 1985; Moreno et al. 1984). Selective toxicity is achieved because the drug is only reduced in an anaerobic microenvironment and it is prevented by oxygen (Lloyd and Pedersen 1985). Therefore, action is restricted to anaerobic protozoa and obligate anaerobic bacteria. *Trichomonas vaginalis* and *Entamoeba histolyica* reduce metronidazole to give acetamide and *N*-(2-hydroxyethyl) oxamic acid as end-products, which are not active in mammalian cells (Thompson et al. 1993). Reduction creates a concentration gradient allowing accumulation of metronidazole within the cell (Pratt and Fekety 1986). As metronidazole has a lower redox potential (–460 mV) than ferredoxin (–320 mV), it acts as an electron sink, drawing electrons away from ferredoxin to become reduced active species. The active species are thought to be either nitro or nitroso radical anions or intermediates, such as hydroxylamines.

Two modes of action are proposed: either DNA damage, strand breakage and crosslinking (Muller 1983;

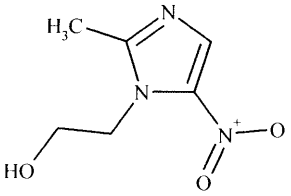

Fig. 1 Metronidazole, 2-methyl-5-nitroimidazole-1-ethanol

Edwards 1986) which may inhibit DNA segregation, or modification of the genes involved in the completion of mitosis. This is shown by the ability of metronidazole to arrest the cell cycle in the G2+M stage (Hoyne et al. 1989). Oxygen consumption in *Giardia muris* and *T. vaginalis* is inhibited because metronidazole acts as an alternate electron acceptor (Paget et al. 1989).

Side effects have been noted in 7.1% of cases (Davidson 1990). These include an unpleasant metallic taste, which can result in poor compliance, gastrointestinal disturbance, such as vomiting, nausea, diarrhoea and abdominal cramps, pancreatitis, vertigo, headaches, a disulfarum-like reaction, CNS toxicity, transient leukaemia, dizziness, drowsiness, lassitude, paraethesias, urticaria and pruritis (Davidson 1990; Thompson et al. 1993). It has also been shown to be mutagenic in the bacterium *Salmonella* (Legator et al. 1975; Rosenkranz and Speck 1975; Lindmark and Muller 1976), and induces tumours in rodents (Legator et al. 1975; Rustia and Shubik 1979).

Failure of treatment has been assigned to poor intestinal adsorption (Kane et al. 1961). It was shown in *T. vaginalis* that metronidazole resistance is correlated with a decrease in PFOR, resulting in a deficient oxygen scavenging mechanism (Lloyd and Pederson 1985; Yarlett et al. 1986). However, this was shown not always to be the case in *Giardia*, as even though the concentrations of PFOR were 17 times lower in a resistant strain, the oxygen scavenging ability was not significantly different in the resistant and sensitive strains (Ellis et al. 1993). In some resistant strains there was an increase in the activity of NADPH oxidase. The $NADP^+$/NADPH couple (E_h=–320 mV) competes with metronidazole for electrons. Drug uptake changes have been observed in some resistant strains and could be part of a blockade; alternatively an active efflux mechanism may operate (Upcroft 1994). In some laboratory-induced, drug-resistant strains the resistance correlates with diminished thiol-dependent peroxidase and reductase levels. However, it was concluded that this must be a secondary line of defence, because if it was a fundamental characteristic the same would be seen in resistant strains from patients.

Other nitroimidazoles

Several other nitroimidazoles have been synthesised: benzoyl metronidazole, secnidazole, nimorazole, tinidazole and ornidazole (Fig. 2).

Benzoyl metronidazole, the tasteless form of metronidazole, is often used in paediatric cases of giardiasis as it does not have the bitter taste of metronidazole and comes as a suspension (Homeida et al. 1986). Tinidazole, known commercially as Fasigyn, has been used in several in vitro studies and has proved 100% successful in a single oral dosage of 2 g (Jokipii and Jokipii 1979; Webster 1990). Secnidazole has also shown to be effective in a single oral dose 50 mg kg^{-1} (Cimerman et al. 1990). It has mild side effects of nausea, anorexia and abdominal pain (Katz et al. 1989). Ornidazole, known commercially as Tiberal, was shown to be 100% effective in a trial in Malaysia, with no apparent side effects (Iyngkaran et al. 1978), and nimorazole was >90% effective in a 5- to 7-day regime. Only mild gastrointestinal side effects were seen (Levi et al. 1977).

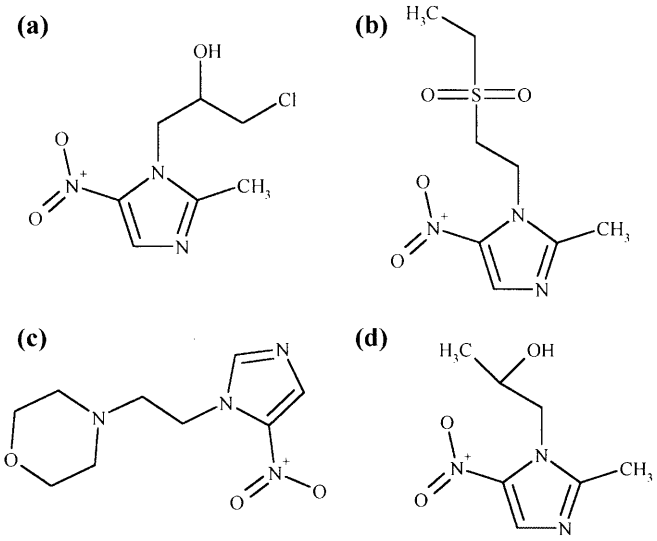

Fig. 2a–d The nitroimidazole family: **a** ornidazole, **b** tinidazole, **c** nimorazole and **d** secnidazole

Benzimidazoles

Mebendazole, the first benzimidazole used against *G. intestinalis*, was reported to be ineffective (De Souza et al. 1973), killing only 37% of the parasites, but showed the possibility of antiprotozoal activity in the homologous drug series (Fig. 3) (Hutchinson et al. 1975). Since these studies were conducted, there have been several positive reports on the use of mebendazole and albendazole in giardiasis (Meyer and Randulescu 1979; Zhong et al. 1986; Al-Waili et al. 1988; Al-Waili 1990) and trichomoniasis (Al-Waili 1987).

Selective toxicity is achieved because the drug is adsorbed minimally from the host intestine. There is no genotoxicity (Botero 1986) and no tumour induction subsequent to high-dose regimes (Thoedorides and Daly 1989).

It has been suggested that benzimidazoles act by interacting with the colchicine site in tubulin in the microtubules, resulting in the disruption of assembly and disassembly (Lacey 1988; Meloni et al. 1990; Magne et al.

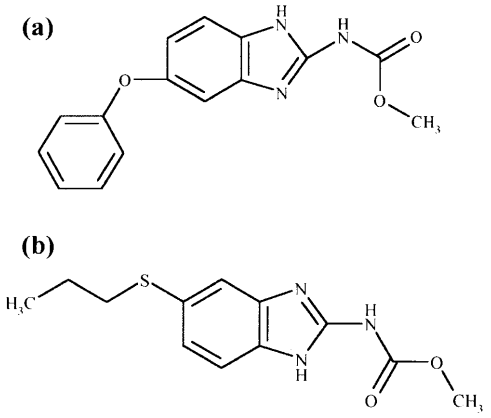

Fig. 3a, b Commonly used benzimidazoles: **a** mebendazole and **b** albendazole

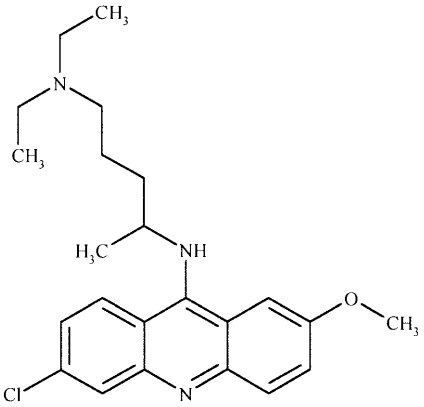

Fig. 4 Quinacrine [*N*-4-(6-chloro-2-methoxy-9-acridinyl)-*N*1,*N*1-diethyl-1,4-pentanediamine]

1991). Other researchers have proposed that it affects bioenergetics (McCracken and Stillwell 1991). However, mutation in the tubulin gene reveals the primary site of action. The benzimidazoles do not effect the flagellar tubulin, which has a different subunit structure (Clark and Holberton 1988).

Quinacrine

Quinacrine (Fig. 4) was shown to be effective against *Giardia* as early as 1937 (Brumpt 1937; Galli-Valerio 1937) and was recommended for the treatment of giardiasis as early as 1941 (Hartman and Kyser 1941). Also known as Atabrine and Mepacrine, it is an acridine yellow dye developed as an anti-malarial and anti-cestode.

Quinacrine is effective in vitro and in vivo, and has proven itself clinically. It has an advantage over nitroimidazoles in that it also kills cysts, as shown by decreased excystation from both in vitro derived (Gillin and Diamond 1981) and patient-derived cysts (Namgung et al. 1985).

The precise mechanism of action is unknown, but there are several alternative proposals. Firstly, it is thought that it interferes with the flavin components of enzymes such as NADH oxidase, thereby decreasing oxygen consumption (Paget et al. 1989). Secondly, it is a known cholinesterase inhibitor. Finally, it is thought that by binding to DNA it inhibits nucleic acid synthesis (Thompson et al. 1993). Quinacrine is taken up by the organisms, but it does not accumulate within the nucleus, suggesting that DNA binding is not the primary site of action. After overnight exposure, the plasma membranes appear fragile, suggesting that this is a more likely target (Upcroft et al. 1996).

Selective toxicity is established through preferential absorption by the organism. Side effects have been observed, including gastrointestinal disturbances such as vomiting, nausea, a bitter taste which results in poor compliance, blood dycrasias, ocular toxicity, sweating, vertigo, retinal pigmentation, headache, dizziness, fever, pruritis, skin and urine discoloration, corneal oedema, myalgias, toxic psychosis, insomnia, haemolysis in glucose-6-phosphate dehydrogenase-deficient patients, disulfuram-like reaction, seizures and central nervous system stimulation (Davidson 1990). It also crosses the placenta, so is contra-indicated in pregnancy.

Resistant cells are shown to exclude the drug (Upcroft 1994). Quinacrine resistance is rapidly induced in furazolidone-resistant lines, suggesting that the exclusion may be aided by this resistance mechanism.

Furazolidone

Furazolidone, commercially known as Furoxone (Fig. 5), is a synthetic nitrofuran. It was first reported as an antigiardial in 1960 (Webster 1960) and has been shown to be effective both in vitro (Craft et al. 1981) and in vivo (Craft et al. 1981; Crouch et al. 1986). It is selective for the protozoa because it is only minimally absorbed from the intestine, and like metronidazole, is only activated by the parasite (Pratt and Fekety 1986). The precise mechanism of action is unknown, but it is thought it could act as an electron acceptor, as is the case for metronidazole (Crouch et al. 1986). It inhibits bacterial enzymes and forms toxic radical anions in trypanosomes (Davidson 1990) As nitrofurans have a more positive redox potential than nitroimidazoles, they may be reduced by NAD(P)H oxidases as well as by PFOR (Moreno et al. 1984). This reduction to an active species may occur whenever a reduced flavin is present. The active species are thought to act by damaging DNA. In one study, furazolidone was shown to act on the S and G2+M stage of the cell cycle, indicating inhibition DNA synthesis and of completion of the cell cycle (Hoyne et al. 1989).

Side effects are seen in 10% of patients, and include gastrointestinal disturbance, nausea, vomiting, diarrhoea, malaise, pruritis, urticaria, hypersensitivity, haemolysis in glucose-6-phosphate dehydrogenase-deficient patients. As it is a monoamine oxidase inhibitor, it may cause hypertensive crisis on exposure to tyramine-con-

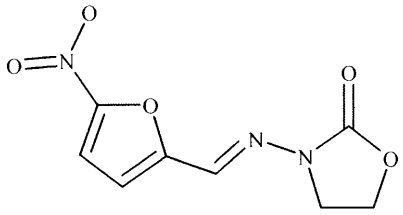

Fig. 5 Furazolidone

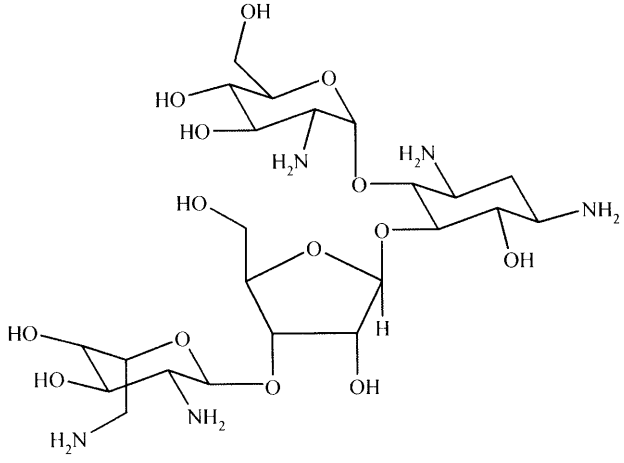

Fig. 6 Paromomycin

taining foods. It is carcinogenic in rodents (McCalla 1979) and mutagenic in bacteria.

Furazolidone-resistant cells have similar oxygen affinity and enzyme activities to metronidazole-sensitive cells. It therefore appears that nitrofurans and nitroimidazoles have completely different modes of action. This correlates with the thiol-dependent reductase and peroxidase activities (Smith et al. 1988).

Paromomycin

Paromomycin, or humatin, is a broad-spectrum aminoglycoside antibiotic (Fig. 6). The giardial rRNA sequence was predicted to be susceptible to it (Edlind 1989).

Clinical efficacy in a 10-day trial has been confirmed (Carter et al. 1962; Kreutner et al. 1981) as well as in vitro activity (Gillin and Diamond 1981; Boreham et al. 1986; Edlind 1989). It is selectively toxic as it is minimally absorbed from the gastrointestinal tract of the host: its action depends on the unique giardial rRNA structure. The mechanism of action depends on the size and sequence of the small subunit (16S-like) rRNA. Binding to the rRNA inhibits protein synthesis.

Side effects are observed: they include gastrointestinal intolerance resulting in ulceration. Renal intolerance is seen if given parenterally (Davidson 1990). No teratogenicity is observed, so it is the drug of choice during pregnancy.

Natural remedies – the way forward?

Due to the occurrence of unpleasant side effects and increasing resistance to the synthetic pharmaceuticals recommended for the treatment of giardiasis, there has been increasing interest in the quest for natural alternatives. Researchers are looking at plants that have been used for gastrointestinal disturbance by users of alternative therapies for generations.

Whilst investigating the historical use of garlic, Bolton et al. (1982) noted that it was often used for gastrointestinal complaints. At the time of their study, it had already been established that garlic has antibacterial, antifungal and antiviral properties. This prompted an investigation into its possible use as an antiprotozoal against *E. histolyica* (Mirelman et al. 1987; Ankri et al 1997). Inhibitory activity was noted with crude extract at 25 μg ml^{-1} and lethal dosage was established as ≈50 μg ml^{-1}. Encouraged by these results, a clinical trail was carried out on patients suffering from giardiasis (Soffar and Mokhtar 1991). Garlic was established as an antigiardial, resolving the symptoms in all patients within 24 h and completely removing any indication of giardiasis from the stool within 72 h, at a dosage of 1 mg ml^{-1} twice daily. No in vitro calculations were possible as the workers could not culture the protozoa in vitro. Recent work has established that a freeze-dried garlic powder is an effective antigiardial in vitro (Harris et al. 2000).

Under certain conditions allicin, a principle component of garlic, shown to be antibacterial, degrades to diallyl trisulphide. This chemical is more stable than the extremely volatile allicin and is easily synthesised. In China it is commercially available as a preparation called Dasuansu and has been prescribed for *E. histolyica* and *T. vaginalis* infections (Lang and Zhang 1981). The antigiardial activity of this garlic component was assessed by Lun et al. (1994). It gave an IC_{50} of 14 μg ml^{-1} and was shown to affect cell morphology.

In a new quest for medicinal botanicals several other plant extracts noted for their gastrointestinal effects have been screened for antigiardial activity. Mexico has a high number of catalogued medicinal plants, 14 of which are antidiarrhetics and antiparasitics. Three species tested, *Justicia spicigera* (Acanthus), *Lipia beriandieri* (Oregano) and *Psidium guajava* (Guava) proved more potent than the control tinidazole (Ponce-Macotela et al. 1994). Further work with the Maya communities of Southern Mexico tested the six most important botanicals in the treatment of gastrointestinal disturbance against the protozoa *E. histolyica* and *Giardia lamblia*. *Cuphea pinetorum* (Loosestrife) was antiprotozoal in both cases, and *Rubus corifolius* (Rose) and *Helianthemum glomeratum* (Rock rose) were antigiardial. Methanol extracts were fractionated on a cellulose column to yield six fractions. Only one fraction was shown to maintain activity. This fraction was shown to contain kaempferol and quercetin. Standards of these were tested for activity and it was established that kaempferol was responsible for the activity. Quercetin was also antigiardial but not antiamoebic (Calzada et al. 1998).

The Luo of East Africa are also alternative therapists. Work with their gastrointestinal botanicals established seven plants species that were lethal antigiardials in 500 ppm and 15 species that were lethal at 1,000 ppm (Johns et al. 1995).

Pippali rasayana, an Ayervedic herbal preparation, originally mentioned in the Veda, the ancient scribes of India, was assayed for its antigiardial activity (Agrawal et al. 1994). It was shown to have activity in vivo but not in vitro. It was therefore tested to see if it was immunomodulatory. There was no marked change in haemagglutination titre or in the plaque forming cell assay, but there was an increase in phagocytic activity and macrophage migration index. In clinical trials, at a dose of 1 g three times daily for 15 days it was 92% effective (Agrawal et al. 1997).

Even though new effective alternative therapies have been identified, and trials have been carried out in vitro, metronidazole remains the treatment of choice for giardiasis. However, if trends in resistance continue to increase, the exploration and utilisation of alternatives will have to be pursued with more alacrity.

References

Agrawal AK, Singh M, Gupta N, Saxena K, Puri A, Verma AK, Saxena RP, Dubey CB, Saxena KC (1994) Management of giardiasis by an immunomodulatory herbal drug *Pippali rasayana*. J Ethnopharmacol 44:143–146

Agrawal AK, Tripathi DM, Sahai R, Gupta N, Saxena RP, Puri A, Singh M, Misra RN, Dubey CB, Saxena KC (1997) Management of giardiasis by *Pippali rasayana*: a clinical study. J Ethnopharmacol 56:233–236

Al-Waili NS (1987) Mebendazole in *Trichomonas hominis*. Clin Exp Pharmacol Physiol 14:679–680

Al-Waili NS (1990) Mebendazole in giardial infections: inappropriate doses. Trans R Soc Trop Med Hyg 84:753–754

Al-Waili NS, Al-Waili BH, Saloom KY (1988) Therapeutic use of mebendazole in giardial infections. Trans R Soc Trop Med Hyg 82:438

Ankri S, Miron T, Rabinkov A, Wilchek M, Mirelman D (1997) Allicin from garlic strongly inhibits cysteine proteinases and cytopathic effects of *Entamoeba histolyica*. Antimicrob Agents Chemother. 41:2286–2288

Bolton S, Null G, Troetel WM (1982) The medicinal uses of garlic - fact and fiction. Am Pharm NS 22:40–43

Boreham PFL, Phillips RE, Shepherd RW (1986) The activity of drugs against *Giardia intestinalis* in neonatal mice. J Antimicrob Chemother 18:393–398

Botero D (1986) Nematode infections of man: intestinal infections. In: Campbell WC, Rew RS (eds) Chemotherapy of parasitic diseases. Plenum Press, New York, pp 267–276

Brumpt L (1937) Traitment experimental de la lamblaise. CR Séances Soc Biol Fil 124:1040–1042

Calzada F, Alanis AD, Meckis M, Contreras A, Cedillo-Rivera R (1998) *In vitro* susceptibility of *Entamoeba histolyica* and *Giardia lamblia* to some medicinal plants used by the people of southern Mexico. Phyto Res 12:70–72

Carter CH, Bayles A, Thompson PE (1962) Effects of paromomycin sulphate in man against *Entamoeba histolyica* and other intestinal protozoa. Am J Trop Med Hyg 11:448–451

Cimerman B, Katz N, Zingano RG, Rocha RS (1990) Treatment of intestinal giardiasis with a single dose of secnidazole. Bull Soc Fr Parasitol 8:423

Clark JT, Holberton DV (1988) Triton labile antigens in flagella isolated from *Giardia lamblia*. Parasitol Res 74:415–423

Cosar C, Julou L (1959) Activité de 1'(hydroxy-2' éthyl)-1 méthyl-2 nitro-5 imidazole (8823 R. P.) vis-à-vis des infections expérimentales a *Trichomonas vaginalis*. Ann Inst Pasteur 96:238–241

Craft JC, Murphy T, Nelson JD (1981) Furazolidone and quinacrine. Am J Dis Child 135:164–166

Crouch AA, Snow WK, Thong YH (1986) Effect of twenty-three chemotherapeutic agents on the adherence and growth of *Giardia lamblia in vitro*. Trans R Soc Trop Med Hyg 80:893–896

Davidson RA (1990) Treatment of giardiasis: the North American perspective. In: Meyer EA (ed) Human parasitic diseases, vol III. *Giardiasis*. Elsevier, Amsterdam, pp 325–334

De Souza DW, De Souza MS, Neves J (1973) Therapeutic action of mebendazole (R17,635) in multiple parasitized patients. Preliminary results. Rev Inst Med Trop Sao Paulo 15:30–33

Durel P, Roiron V, Sibloulet A, Borel LJ (1959) Essai d'un antitrichomonas dérivé de l'imidazole (8823 R. P.). C R Soc Fr Gynecol 29:36–45

Edlind TD (1989) Susceptibility of *Giardia lamblia* to aminoglycoside protein synthesis inhibitors: correlation with rRNA structure. Antimicrob Agents Chemother 33:484–488

Edwards DI (1986) Reduction of nitroimidazoles *in vitro* and DNA damage. Biochem Pharmacol 35:53–58

Ellis JE, Wingfield JM, Cole D, Boreham PFL, Lloyd D (1993) Oxygen affinities of metronidazole resistant and sensitive stocks of *Giardia intestinalis*. Int J Parasitol 23:35–39

Friedman GD (1980) Cancer after metronidazole. N Engl J Med 302:519

Galli-Valerio B (1937) La lambliase et son traitment par l'atebrine. Schweiz Med Wochenschr 67:1181–1182

Gillin FD, Diamond LS (1981) Inhibition of clonal growth of *Giardia lamblia* and *Entamoeba histolyica* by metronidazole, quinacrine and other antimicrobial agents. J Antimicrob Chemother 8:305–316

Harris JC, Plummer S, Turner MP, Lloyd D (2000) The microaerophilic flagellate *Giardia intestinalis*: *Allium sativum* (garlic) is an effective antigiardial. Microbiology 146:3119–3127

Hartman HR, Kyser FA (1941) Giardiasis and its treatment. J Am Med Assoc 116:2835–2839

Homeida MA, Daneshmena TK, Ali HM, Kaye CM (1986) Metronidazole metabolism following oral benzoylmetronidazole suspension in children with giardiasis. J Antimicrob Chemother 18:213–219

Horie H (1956) Anti-trichomonas effect of azomycin. J Antibiot Ser A 9:168

Hoyne GF, Boreham PFL, Parsons PG, Ward C, Biggs B (1989) The effect of drugs on the cell cycle of *Giardia intestinalis*. Parasitology 99:333–339

Hutchinson JGP, Johnston NM, Plevey MVP, Thongkhiew CA (1975) Clinical trial of mebendazole: a broad spectrum antihelminthic. Br Med J 2: 309–310

Iyngkaran N, Lee EL, Robinson MJ (1978) Single dose treatment with tiberal of *Giardia lamblia* infection in children. Scand J Infect Dis 10:243–246

Johns T, Faubert GM, Kokwaro JO, Mahunnah RCA, Kimanani EK (1995) Antigiardial activity of gastrointestinal remedies of the Luo of East Africa. J Ethnopharmacol 46:17–23

Jokipii L, Jokipii AMM (1979) Single dose metronidazole and tinidazole as therapy for giardiasis: success rates, side effects and drug adsorption and elimination. J Infect Dis 140:984–988

Kane PD, McFadzean JA, Squires S (1961) Adsorption and excretion of metronidazole. II. Studies on primary failures. Br J Vener Dis 37:276–277

Katz N, Cimerman B, Zingano AG, Zingano RG, Rocha RS (1989) A clinical trial using secnidazole suspension in children infected with *Giardia lamblia*. In: Katz N, Willis AT (eds) Secnidazole: a new approach to 5-nitroimidazole therapy. Excerpta Medica, Amsterdam, pp 35–40

Kreutner AK, Del-Bene VE, Amstey MS (1981) Giardiasis in pregnancy. Am J Obstet Gynecol 140:895–901

Lacey E (1988) The role of the cytoskeletal protein tubulin in the mode of action and mechanism of drug resistance to benzimidazoles. Int J Parasitol 18:855–936

Lang YJ, Zhang KY (1981) Studies on the effective components of garlic (*Allium sativum* L.). Chin Herbal Med (Zhong Cao Yao) 4:4–6

Legator MS, Connor TH, Stoekel M (1975) Detection of mutagenic activity of metronidazole and niridazole in body fluids of humans and mice. Science 188:1118–1119

Levi GC, De Avila CA, Neto VA (1977) Efficacy of various drugs for the treatment of giardiasis: a comparative study. Am J Trop Med Hyg 26:564–565

Lindmark DG, Muller M (1976) Antitrichomonacidal action, mutagenicity and reduction of metronidazole and other nitroimidazoles. Antimicrob Agents Chemother 10:476

Lloyd D, Pedersen JZ (1985) Metronidazole radical-anion generation *in vivo* in *Trichomonas vaginalis* oxygen quenching is enhanced in the drug resistant strain. J Gen Microbiol 131:87–92

Lun ZR, Burri C, Menzinger M, Kaminsky R (1994) Antiparasitic activity of diallyl trisulphide (dasuansu) on human and animal pathogenic protozoa (*Trypanosoma* sp. *Entamoeba histolyica* and *Giardia lamblia*) *in vitro*. Ann Soc Belg Med Trop 74:51–59

Maeda K, Osato T, Umezawa H (1953) A new antibiotic azomycin. J Antibiot A 6:82

Magne D, Favennec L, Chochillon C, Gorenflot A, Meillet D, Kapel N, Raichvarg D, Savel J, Gobert JG (1991) Role of cytoskeleton and surface lectins in *Giardia duodenalis* attachment to Caco2 cells. Parasitol Res 77:659–662

Mandoul R, Dargelos R, Millan J (1961) Destruction des protozoaires intestinaux de la souris par un dérivé nitré de l'imidazole. Bull Soc Pathol Exot 54:12–16

McCalla DR (1979) Nitrofurans. In: Hahn FE (ed). Antibiotics, vol 1. Springer, New York Berlin Heidelberg, pp 176–213

McCracken RO, Stillwell WH (1991) A possible mode of action of benzimidazole antihelminthics. Int J Parasitol 21:99–104

Meloni BP, Thompson RCA, Reynoldson JA, Seville P (1990) Albendazole: a more effective antigiardial agent *in vitro* than metronidazole or tinidazole. Trans Soc Trop Med Hyg 84:375–379

Meyer EA, Randulescu S (1979) *Giardia* and giardiasis. Adv Parasitol 17:1–48

Mirelman D, Monheit D, Varon S (1987) Inhibition of growth of *Entamoeba histolyica* by allicin, the active principle of garlic extract (*Allium sativum*). J Infect Dis 156:243–244

Moreno SNJ, Mason RP, Docampo R (1984) Distinct reduction of nitrofurans and metronidazole to free radical metabolites by *Tritrichomonas foetus* hydrogenosomal and cytosolic enzymes. J Biol Chem 259:8252–8259

Muller M (1983) Mode of action of metronidazole on anaerobic bacteria and protozoa. Surgery 93:165–171

Namgung R, Ryu J-S, Kee KT, Soh CT (1985) The effect of metronidazole and quinacrine on the morphology and excystation of *Giardia lamblia*. Yonsei Rep Trop Med 16:28ó44

Ortega YR, Adam RD (1997) *Giardia*: overview and update. Clin Infect Dis 25:545–550

Paget TA, Jarroll EL, Manning P, Lindmark DG, Lloyd D (1989) Respiration in the cyst and trophozoite forms of *Giardia muris*. J Gen Microbiol 135:145–154

Ponce-Macotela M, Navarro-Alegria I, Martinez-Gordillo MN, Alvarez-Chacun R (1994) Efecto antigiardi·sico *in vitro* de 14 extractos de plantas. Rev Invest Clin 46:343–347

Pratt WB, Fekety R (1986) The antimicrobial drugs. Oxford University Press, New York

Roe FJC (1985) Safety of nitroimidazoles. Scand J Infect Dis Suppl 46:72–81

Rosenkranz HS, Speck WT (1975) Mutagenicity of metronidazole: activation by mammalian liver microsomes. Biochem Biophys Res Commun 66:520–525

Rustia M, Shubik P (1979) Experimental induction of hepatomas, mammary tumours and other tumours with metronidazole and non-inbred Sas:MRC(WI)BR rats. J Natl Cancer Inst 63:863

Schneider J (1961) Traitment de la giardiase (lambliase) par le métronidazole. Bull Soc Pathol Exot 54:84–93

Shukry S, Zaki AM, Dupont HL, Shoukry I, El Tagi M, Hamed S (1986) Detection of enteropathogens in fatal and potentially fatal diarrhea in Cairo, Egypt. Clin Microbiol 24:959–962

Soffar SA, Mokhtar GM (1991) Evaluation of the antiparasitic effect of aqueous garlic (*Allium sativum*) extract in hymenolepiasis *Nana* and giardiasis. J Egypt Soc Parasitol 21:497–502

Smith NC, Bryant C, Boreham PFL (1988) Possible roles for pyruvate ferredoxin oxidoreductase and thiol dependant peroxidase and reductase activities in resistance to nitroheterocyclic drugs in *Giardia intestinalis*. Int J Parasitol 18:991–997

Thompson RCA, Reynoldson JA, Mendis AHW (1993) *Giardia* and giardiasis. Adv Parasitol 32:71–160

Theodorides VJ, Daly IH (1989) Human safety studies with albendazole. Proc World Assess Adv Vet Parasitol 13S

Upcroft P (1994) Multiple drug resistance in the pathogenic protozoa. Acta Trop 56:195–212

Upcroft JA, Campbell RW, Upcroft P (1996) Quinacrine resistance in *Giardia duodenalis*. Parasitology 112:309–313

Voogd CE (1981) On the mutagenicity of nitroimidazoles. Mutat Res 86:243–277

Webster LT (1960) Drugs used in protozoal infections. In: Gilman AG, Rall TW, Nies AS, Taylor P (eds) Goodman and Gillmans pharmalogical basis of therapeutics, 8th edn. Pergamon Press, New York, pp 978–1007

World Health Organization (1995) An overview of selected curable sexually transmitted diseases. In: Global program on AIDS. World Health Organization, Geneva, Switzerland

Yarlett N, Yarlett NC, Lloyd D (1986) Metronidazole resistant clinical isolates of *Trichomonas vaginalis* have lowered O_2 affinities. Mol Biochem Parasitol 19:111–116

Zhong H, Cao W, Rossignol JF, Feng M, Gans SB, Tan W (1986) Albendazole in nematode, cestode, trematode and protozoan (*Giardia*) infections. Chin Med J Eng Edn 99:912–915