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RESISTANCE OF INTESTINAL PARASITOSEs TO ANTIPARASITIC DRUGS

by

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Introduction

"Drug resistance has accompanied the development of chemotherapy like a faithful shadow and the history of chemotherapy is also a history of drug resistance". This quotation, taken from the classical review of drug resistance by Schintzer and Grunberg (1957), is of course true for the antibiotics.

Even in the early days when penicillin was developed for therapeutic use, it was found that certain bacteria were not killed by this B-lactam. Since those days the problem of bacterial resistance to antibiotics has reached huge proportions. As soon as a new antibiotic was used (overused) on a large scale, it was rapidly followed by the appearance of a high proportion of resistant organisms up to a point at which the value of the antibiotic as a therapeutic agent became doubtful (for reviews see Gale *et al.*, 1981, Levy, 1982).

The quotation of Schnitzer and Grunberg (1957) is nowhere more true than in the poultry industry which depends upon drugs for the control of coccidiosis. The prophylactic medication in which drugs are included in the ration of the broiler chicken from 1-day-old until slaughter, has inevitably resulted in widespread drug resistance (Chapman, 1982). Indeed, the continuous exposure to a drug is one of the experimental methods to develop resistance *in vivo*. Continuous exposure is also the reason that mutation to resistance to a systemic fungicide occurs when it is applied to the soil and taken up by the plant during an extended period of time, resulting in a high and continuous selection pressure (Dekker, 1977). It is interesting to note that a high potential for development of fungicide resistance does not always imply that problems will arise in practice. Indeed the resistant mutants show quite often a reduction in fitness and pathogenicity (Fuchs *et al.*, 1977; Dekker, 1982). The study of fitness and pathogenicity of a resistant strain should be included in studies with protozoa and helminths too.

In this short overview the potential of gastrointestinal protozoa and helminths to develop resistance to different groups of antiparasitic agents will be discussed.

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Concepts

According to WHO, resistance to drugs can be defined as the ability of the parasites to survive and/or multiply despite the administration and absorption of a drug in doses equal to or higher than those usually recommended but within the limits of tolerance of the host (taken from Peters, 1984). Kelly and Hall (1979) defined resistance as a significant increase in the ability of individuals within a strain to tolerate doses of the compound, which would prove lethal to the majority of individuals in a normal population of the same species.

A decrease in sensitivity may be caused by nongenetic or genetic changes in the target cell. If a change in sensitivity does not involve a change in chromosomal or extrachromosomal DNA it is thought to be the result of phenotypic adaptation. This may take place in culture after long exposure to the drug under conditions favourable for the cell or organism. The phenotypic resistance is not stable and is usually lost upon transfer to a drug-free medium. Of course this instability should not be confused with regression of resistance, which may be due to reduced fitness of genetic mutants (Georgopoulos, 1982). Involvement of extrachromosomal genes in resistance to drugs is common in bacteria and has been described in yeast and fungi. However, most genetic studies on resistance to fungicides have revealed the involvement of chromosomal genes. Decrease in sensitivity due to genetic changes in the invasive organism is of course a serious problem. The question is: "Is resistance to the common drugs used for the treatment of intestinal protozoa an existing problem?"

Protozoa

The most commonly used tissue amoebicides belong to the chemical class of nitroimidazoles. Metronidazole was the first used clinically in protozoan diseases. First, it was used for Trichomonas vaginalis infections and, later, its use was expanded to the treatment of amoebiasis and giardiasis. Most of the information available is on the use of metronidazole against the cosmopolitan common parasite of the vagina, T. vaginalis. Growth of T. vaginalis in the presence of sub-lethal concentrations of metronidazole has caused the development of some resistance (i.e. somewhat higher MIC-values are needed) to the drug (Coombs, 1976). However, in this study it was not examined whether the change in sensitivity was the result of phenotypic adaptation or due to genetic changes. Induced resistance to metronidazole administered to hamsters infected intravaginally with Trichomonas foetus and treated with sub-curative levels of metronidazole (50 mg/kg; 12 doses over a 3-week period) was demonstrated in vitro and in vivo (Actor et al., 1969). A strain of T. foetus recovered from a bull and maintained in hamsters showed cross resistance to demetridazole, metronidazole and aminitroazole (McLoughlin, 1967). In 1965 Robinson and Mirchandani reported that two patients with T. vaginalis showed relative resistance to metronidazole. Three T. vaginalis isolates were obtained in the USA from three women with trichomoniasis refractory to routine treatment with metronidazole (Müller et al., 1980). Cure was obtained with increased dosage of metronidazole. In experimental mouse infections, all three strains showed about 10 to 20 times lower susceptibility to this nitroimidazole derivative than did a number of control strains. In 1981 Waitkins and Thomas reported on a metronidazole resistant T. vaginalis strain isolated from a patient. However, it is not clear whether this treatment failure was due to a natural resistance of this strain or had been developed during previous exposure to other drugs. The patient had been treated on five occasions with the polyene macrolide, nystatine, known to form complexes with sterols, thus causing changes in the membranes that may lead to decreased permeability of the cell membrane.

Thus Trichomonas strains with lowered metronidazole susceptibility exist. However, as compared with the number of patients treated only a limited number of strains with decreased susceptibility has been found. In a letter to The Lancet, Benazet and Guillaume (1972) wrote: "The production of resistant strains of trichomonads to metronidazole is indeed possible in the laboratory under conditions far removed from those in which the organisms exist in man, but the strains thus produced can be considered as curiosities of no practical relevance". This was written after metronidazole had been used on a very large scale for 12 years. Now, thirteen years later strains resistant to this nitroimidazole are still rare. A survey of our own data bank and of MEDLARS indicates that resistance of Entamoeba and Giardia to metronidazole can, at the moment, be considered a minor problem.

In his review on amoebiasis, Harries (1982) mentioned cases of resistance to metronidazole. It should not be surprising because tinidazole and metronidazole are chemically very close and have the same mode of action. In the paper of Datta et al. (1974), to which Harries (1982) referred, 7 patients out of 20 with amebic liver abscess were not cured after treatment with metronidazole (400 mg three times a day for 10 days). In 4 of these patients *E. histolytica* was demonstrated in the hepatic aspirate after the end of therapy. Although the authors take drug resistance as a possibility for treatment failure, they found this speculative since data regarding blood levels and sensitivity tests were absent (Datta et al., 1974). Nair and Nagarajan (1983) mention in their review on nitroimidazoles, a Russian study indicating that the sensitivity of two strains of *E. histolytica* decreased in 3 years. Smith et al. (1982) isolated *Giardia lamblia*, from a patient with chronic symptomatic giardiasis despite seven separate courses of either quinacrine or metronidazole. This strain was *in vitro* not more drug resistant than three other isolates. However, monocytes-macrophages from this patient exhibited reduced killing for *G. lamblia* compared with normal subjects.

Thus the resistance of the patient's infection might be due to reduced cellular cytotoxicity for *G. lamblia* and not to resistance of the *Giardia* strain to metronidazole or quinacrine.

Helminths

The number of reports on resistance against anthelmintics in nematodes of veterinary importance is quite impressive. Most information is summarized by Kelly and Hall (1979). Benzimidazoles have been breakthroughs in the control of helminthiasis and in the development of systemic fungicides for plant disease control. Some of them also became valuable tools in the study of the structure and role of the microtubular system. These facts were at the origin of a number of studies to search for the mechanisms of resistance. The breakthrough in the development of systemic fungicides for plant disease control was certainly the introduction of benzimidazole carbamate, benomyl (for review see Dekker, 1985). Benomyl is converted to carbendazim (MBC) which is the main toxic principle. Benomyl and its conversion product carbendazim have been shown to interfere with mitosis in fungi this results from interference with microtubule assembly (Davidse, 1982).

The protein, tubulin, is the building block of microtubules. Binding of carbendazim to this tubulin is at the origin of its interference with the assembly of the microtubules. Binding studies with carbendazim in cell-free extracts of fungi, plants and mammalian brain have led to a better understanding of the biochemical basis of the selective toxicity of this benzimidazole carbamate (Davidse, 1982). In extracts of carbendazim-resistant *Aspergillus nidulans* and *Penicillium expansum* no or only a low binding activity appeared to be present. The mutation responsible for the behaviour of the *Aspergillus nidulans* strains occurred in the structural gene for β -tubulin, one of the monomers of the tubulin molecule (Van Tuyl, 1977; Sheir-Neiss, 1978). The mutations leading to resistance to carbendazim in *A. nidulans* and the plant pathogenic, *P. expansum*, which involve changes in affinity of tubulin to carbendazim, do not impair the normal functioning of the microtubular system, as the growth rate or sporulation of the strains does not change (Davidse, 1982).

So far no research has been published on whether a decreased affinity of tubulins from tiabendazole resistant strains is also involved in the mechanism of resistance. However, it was found that tiabendazole and oxfendazole had virtually no effect on brain tubulin assembly whereas they were both good inhibitors of *Ascaridia galli* tubulin polymerization (Dawson et al., 1984). The affinity of mammalian brain tubulin for carbendazim is also low (Davidse, 1982). As with carbendazim in fungi the differential binding found with tiabendazole might explain its selectivity as an anthelmintic. Although so far the literature did not reveal evidence for the involvement of a decreased affinity of tubulin from benzimidazole-resistant strains of *Haemonchus*, *Ostertagia*, *Trichostrongylus* spp. in sheep and goats or from equine strongyles, this decreased affinity might be a good working-hypothesis.

A number of other mechanisms of resistance have been postulated. For example, Coles (1977) suggested a reduced uptake of tiabendazole as a mechanism of tiabendazole resistance in the eggs of *Haemonchus contortus* but Sangster and Prichard (1984) did not find a difference in tiabendazole uptake by eggs of resistant and susceptible *Trichostrongylus*

colubriformis. Furthermore, the final concentrations of tiabendazole in resistant worms were significantly greater than in the susceptible strain. Sangster and Prichard (1984) also proved that inhibition of glucose uptake is not a mechanism of action of tiabendazole nor is it associated with mechanisms of drug resistance.

Fumarate reductase activity in a tiabendazole-resistant strain of H. contortus was found to be significantly lower than that of a susceptible strain (Bryant & Bennett, 1983). However, the activity of this important enzyme system in a mebendazole resistant strain did not differ from that in the susceptible strain even though this H. contortus was cross-resistant to tiabendazole. Furthermore, the same authors found that one H. contortus strain resistant to tiabendazole and one to cambendazole possess fumarate reductase activities indistinguishable from the corresponding susceptible strains. Therefore, benzimidazole resistance cannot be generally correlated with diminished fumarate reductase activity.

Although other mechanisms of resistance might operate, the working hypothesis suggesting that differences in susceptibility might be based on a different affinity of tubulins to benzimidazole carbamates is worthwhile to explore.

Cases of resistance have been found in nematodes in sheep, goats and horses. This resistance against anthelmintics has been reported mainly from countries where anthelmintics have been used at a high frequency such as Australia, South Africa and New Zealand (Boersema, 1985). In other words, resistance against anthelmintics is mainly found when a relative high and continuous selection pressure is present. Most of the modern anthelmintics used in the treatment of gastro-intestinal worms in man are used in single doses (mass control programmes) or during a short period of time. Therefore, resistance of e.g. Ascaris, hookworm, Trichuris, Taenia, Hymenolepis against the normally used anthelmintics should be rare.

In their review on resistance of animal helminths to anthelmintics Kelly and Hall (1979) report that bephenium hydroxynaphthoate is less effective against Necator americanus than against Ancylostoma duodenale in man. They also refer to a study of Comney and Haddock who found 5 cases of N. americanus which were not responsive to repeat doses of bephenium. Two of these cases were also treated with tiabendazole without effect. According to Kelly and Hall these results suggest a possible resistance to bephenium and a possible cross-resistance against both A. duodenale and N. americanus (Janssens, 1985). Furthermore, as reviewed by Janssens (1985), the results of the treatment with bephenium hydroxynaphthoate of 2141 patients infected with hookworm indicate that the cure rates vary from 16 to 98%. More should be known on the pharmacokinetics before one can suggest that treatment failures are due to resistance to tiabendazole and/or bephenium hydroxynaphthoate.

A further survey of our data bank and that of MEDLARS did not reveal strains of gastrointestinal helminths in man that showed resistance against benzimidazole carbamate (e.g. flubendazole and mebendazole) levamisole, pyrantel, niclosamide or praziquantel. Based on the literature it can be concluded that resistance of gastrointestinal parasites in man against the common antiparasitic drugs seems to be a nonexistent problem.

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