Nippostrongylus brasiliensis infection in the rat: effect of iron and protein deficiency and dexamethasone on the efficacy of benzimidazole anthelmintics

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SUMMARY Malnutrition, anaemia, and gut parasites are commonly interrelated. Using the *Nippostrongylus brasiliensis*-rat model, the effect of iron and protein deficiency on the efficacy of benzimidazole anthelmintics was studied. It was demonstrated that the anthelmintics mebendazole and fenbendazole were significantly less effective in eradicating parasites when animals were deficient in iron and protein. This decreased efficacy of anthelmintics in iron and protein deficiency could not be overcome by intraperitoneal administration of the drug. Since nutritional deficiencies may act via impairment of the immune response, anthelmintic efficacy was determined in adequately nourished rats treated with the immunosuppressive drug dexamethasone. A similar decrease in efficacy of mebendazole was shown when these animals were treated with dexamethasone. Thus it is possible that lowered anthelmintic efficacy in iron and protein deficient animals is mediated by immune deficiency. These findings may be relevant to anthelmintic programmes in malnourished communities.

There is increasing evidence that nutritional deficiencies are associated with impairment of immune defence mechanisms in the host, and contribute to persistence or recurrence of infection (see review by Scrimshaw, 1975).

It has recently been shown that iron and protein deficiency potentiate helminth infections in an animal model (Bolin *et al.*, 1977). Furthermore, it is recognised that certain parasites can induce deficiencies in infected hosts (reviewed by Symons, 1969). For example, the human hookworm *Necator americanus* can be associated with hypoproteinaemia and iron deficiency anaemia.

The effect on anthelmintic efficiency of nutrient imbalances induced by diet or parasites has not been investigated, although there are reports in the literature of reduced drug efficacy in severe helminth infections. For example, mebendazole is much less effective in heavy *Trichuris trichiura* infections in man (Peña Chavarria *et al.*, 1973; Wolfe and Wershing, 1974). As severe trichuriasis is associated with anaemia and diarrhoea, this therapeutic failure

with anthelmintics may be related to nutrient deficiencies.

The present experiment was designed to evaluate this hypothesis using the rat-*Nippostrongylus brasiliensis* model. In this system, experimental infection is produced by subcutaneous inoculation of infective larvae. These migrate to the lungs, up the trachea, and down the oesophagus to the small intestine where, six days after infection, they develop into mature worms. A complex multiphasic immune response is then mounted by the host which initiates expulsion of adult worms four days later (Kelly and Dineen, 1972; Kelly *et al.*, 1973; Ogilvie and Jones, 1973; Kelly and Dineen, 1976). The animal then becomes resistant to re-infection, with less than 2% of a secondary challenge developing into mature worms (Africa, 1931; Love *et al.*, 1974).

The mode of action of benzimidazole anthelmintics is not well understood. Recent work has suggested that they may have a selective biochemical action by inhibiting the fumarate reductase system in nematodes (Prichard, 1970). This pathway, exclusive to the parasite, is important in energy production from carbohydrate metabolism. It is not clear whether the drug is effective by absorption through

the parasite cuticle or whether serum levels in the host are important in worm eradication.

Methods

PARASITE/ANIMALS

Methods for culture and inoculation of *N. brasiliensis*, estimation of worm burdens, and details of rat strains used have been described (Kelly and Dineen, 1972; Love *et al.*, 1974).

PREPARATION OF NUTRIENT DEFICIENT ANIMALS

A balanced synthetic diet was prepared for nutrient sufficient rats (Bolin *et al.*, 1971). An iron and protein deficient diet was prepared by withdrawing iron from the diet and reducing the protein content from 30% to 10%. The deficient animals were fed distilled water and housed in brass cages.

ANTHELMINTICS

Mebendazole¹ (methyl-5(6)-benzoyl 1-2 benzimidazole carbamate) and fenbendazole² (methyl 5-(phenylthio)-2-benzimidazole carbamate) were obtained commercially. Recommended dose rates for these drugs are 12.5 and 5 mg/kg, respectively.

STATISTICS

Analysis of variance was carried out on all data after transformation to $\log_{10}(x + 1)$.

Experimental design and results

EXPERIMENT 1

Effect of iron and protein deficiency on anthelmintic efficacy

Six groups of Wistar strain rats were weaned onto synthetic diets at 4 weeks of age (Table 1). Groups 1 and 2 were fed a sufficient diet and groups 3 to 6 were fed an iron and protein deficient diet. After four weeks all animals were injected subcutaneously with 1000 infective larvae of *N. brasiliensis*. At the same time blood was collected for haemoglobin estimation, and deficient animals had a mean haemoglobin of 8.2 ± 0.2 g/dl and a mean body weight of 89 ± 4.1 g, compared with a mean haemoglobin of 15.4 ± 0.3 g/dl and a mean weight of 213 ± 6.7 g for sufficient animals.

Six days after inoculation four groups were treated with anthelmintics (mebendazole or fenbendazole) and two groups were left untreated. Thus one

sufficient group and one deficient group were untreated and one sufficient group and one deficient group were treated with mebendazole at a dose rate of 25 mg/kg. Of the remaining two groups one was treated with fenbendazole (10 mg/kg) and one was treated with mebendazole at 50 times the recommended dose (625 mg/kg). All animals were killed four days after treatment—that is, 10 days after infection—and total worm counts performed (Table 1).

Table 1 *Anthelmintic efficacy of mebendazole and fenbendazole in iron and protein deficient rats infected with Nippostrongylus brasiliensis.*

Group and dietary iron and protein status	Treatment	Dose rate (mg/kg)	Total worm count (mean value and SE)		P value
			Day 6	Day 10	
Sufficient					
1	Nil	—	358 $\pm$ 43	Gp 2 v gp 3	< 0.01
2	Mebendazole	25	1 $\pm$ 0	Gp 2 v gp 5	< 0.01
Deficient					
3	Mebendazole	25	69 $\pm$ 21		
4	Nil	—	374 $\pm$ 33		
5	Fenbendazole	10	187 $\pm$ 32		
6	Mebendazole	625	0.75 $\pm$ 0		

Mebendazole at a dose rate of 25 mg/kg administered by gastric intubation was very effective in eradicating parasites from animals on a sufficient diet, group 2 having a mean worm count of only 1, representing an anthelmintic efficacy of greater than 99%. In contrast, when the same dose of mebendazole was administered to iron and protein deficient animals a significant worm burden remained, the mean count for group 3 being 69 ± 21 , an efficacy of approximately 80%. Fenbendazole was even less effective with a mean worm burden of 187 ± 32 , an efficacy of 50% (group 5). The difference in anthelmintic efficacy between deficient animals treated with either mebendazole (group 3) or fenbendazole (group 5) and sufficient animals (group 2) is significant at the 1% level.

When iron and protein deficient rats were treated with mebendazole at 50 times the normal dose rate—that is, 625 mg/kg, group 6—the parasites were effectively cleared, the mean remaining worm burden being less than 1. In this group, however, all animals suffered from severe diarrhoea.

The mean worm burden in untreated animals from both sufficient (group 1) and deficient (group 4) groups was 358 ± 43 and 374 ± 33 respectively and this difference was not significant.

¹Telmin (Ethnor Pty. Ltd., 1-5 Khartoum Rd., Nth. Ryde, NSW, Australia).

²Panacur (Hoechst (Aust.) Ltd., 606 St. Kilda Rd., Melbourne, Vic., Australia).

EXPERIMENT 2

Effect of route of administration on anthelmintic efficacy in iron and protein deficiency

Although mebendazole and fenbendazole are insoluble some absorption from the gastrointestinal tract does occur (Prichard and Kelly, 1978). Despite the fact that the serum levels that are achieved are low these drugs are effective in eradicating parasites from sites outside the gastrointestinal tract such as the respiratory tract (Kelly *et al.*, 1975).

Thus it was possible that malabsorption was responsible for the decreased anthelmintic efficacy in iron and protein deficient rats, as adult worms, normally confined to the upper jejunum, are located throughout the entire small intestine in deficient rats (Bolin *et al.*, 1977).

This experiment was designed to evaluate the above hypothesis. Six groups of five animals were weaned onto a synthetic diet, with three groups on a sufficient diet (groups 1-3) and three groups on a deficient diet (groups 4-6). After four weeks all rats were inoculated with 1000 infective larvae of *N. brasiliensis* and haemoglobin levels performed. In addition, group 6 was pretreated with 5 mg iron dextran³ intramuscularly two days before inoculation with larvae. The mean haemoglobin levels were 15.3 ± 0.3 g/dl, 12.1 ± 0.3 g/dl, and 8.0 ± 0.1 g/dl for sufficient, replenished, and deficient groups respectively. Rats on a sufficient diet weighed 195 ± 13.8 g compared with 151 ± 5.7 g for rats on a deficient diet.

Six days later all groups were treated with mebendazole at a dose rate of 12.5 mg/kg by either the intraperitoneal or intragastric route, except for group 1, which was left untreated and served as an infection control. All animals were killed four days after treatment and total worm counts performed. The results summarised in Table 2 show that intragastric mebendazole was significantly less effective in deficient animals (group 4, mean worm count 37 ± 14) compared with sufficient animals (group 2, mean worm count 0). Furthermore, intraperitoneal administration did not improve the efficacy of mebendazole in iron and protein deficiency, the mean worm count for group 5 being 33 ± 7 . On the other hand, intraperitoneal mebendazole cleared all parasites in iron and protein sufficient animals, the mean worm count for group 3 being 0.

In group 6, which was iron replenished, there was a marked improvement in drug effectiveness, the mean worm count being only 7 ± 3 compared with worm counts of 37 and 33 in non-repleted iron deficient animals (groups 4 and 5 respectively). There

³Imferon (Fisons Pty. Ltd., North Sydney, NSW, Australia).

Table 2 Anthelmintic efficacy of mebendazole in iron and protein deficient rats infected with *Nippostrongylus brasiliensis*: intraperitoneal and intragastric routes of administration compared.

Group and dietary iron and protein status	Anthelmintic* treatment	Route of administration	Total worm count (mean value and SE)	P value
Sufficient				
1	Nil	—	366 ± 33	Gp 2 v gp 4 < 0.01
2	Mebendazole	Intragastric	0	Gp 6 v gp 2 NS†
3	Mebendazole	Intraperitoneal	0	Gp 4 v gp 5 NS
Deficient				
4	Mebendazole	Intragastric	37 ± 14	
5	Mebendazole	Intraperitoneal	33 ± 7	
6†	Mebendazole	Intragastric	7 ± 3	

*Mebendazole was given at a dose rate of 12.5 mg/kg.

†This group iron replenished two days before inoculation with *N. brasiliensis*.

‡NS: not significant.

is no significant difference between anthelmintic efficacy in the iron replenished animals (group 6) and rats on a sufficient diet (group 2).

EXPERIMENT 3

Effect of dexamethasone on anthelmintic efficacy

Nutritionally induced iron and protein deficiency has been shown to affect both worm expulsion and anthelmintic efficacy. As delayed worm clearance is a result of impairment of the immune process governing expulsion (Bolin *et al.*, 1977), it may be that immune deficiency also mediates decreased anthelmintic efficacy. This experiment was designed to evaluate the effect of steroid-induced immune deficiency on the chemotherapeutic activity of mebendazole.

Twenty adult Wistar rats fed a nutritionally sufficient commercial diet⁴ were divided into four equal groups. All were injected subcutaneously with 1000 infective larvae of *N. brasiliensis*. Animals in group 1 received no further treatment and acted as infection controls. On the day of inoculation, intramuscular injections of dexamethasone⁵ were started in groups 2 and 3 at a total dose rate of 0.4 mg/animal daily (Ogilvie, 1965). Six days after infection groups 3 and 4 were treated with mebendazole at a dose rate of 12.5 mg/kg, and total worm counts were performed on all animals four days later.

⁴Allied Feeds (42 Walker St., Rhodes, NSW, Australia).

⁵Decadron (Merck, Sharp & Dohme (Aust. Pty. Ltd.), Granville, NSW, Australia).

Table 3 Effect of dexamethasone on anthelmintic efficacy of mebendazole in rats infected with *Nippostrongylus brasiliensis*.

Group	Steroid*	Anthelmintic†	Total worm count (mean value and SE)	P value
		Day 6	Day 10	
1	Nil	Nil	397 ± 20.9	Gp 3 v gp 4 < 0.05
2	Dexamethasone	Nil	442 ± 32.7	
3	Dexamethasone	Mebendazole	229 ± 32.0	
4	Nil	Mebendazole	29 ± 9.1	

*Dexamethasone was given at a dose rate of 0.4 mg/animal daily.

†Mebendazole was given at a dose rate of 12.5 mg/kg.

In the non-steroid treated rats, mebendazole was effective in clearing more than 90% of parasites (group 4, mean worm count 29 ± 9.1), when compared with the infection control group (group 1, mean worm count 397 ± 20.9). In contrast, in steroid treated rats (group 3), mebendazole was only 50% effective in eradicating parasites, with a mean worm burden of 229 ± 32 . This difference is significant at the 5% level. Worm counts in steroid-treated rats not given mebendazole (group 2, 442 ± 32.7 worms) were not significantly different from the infection controls (group 1).

Discussion

These studies indicate that in the *N. brasiliensis*-rat model, iron and protein deficiency reduces the efficacy of the benzimidazole anthelmintics mebendazole and fenbendazole. This decreased efficacy can be overcome by the use of very large doses of mebendazole or by pretreating the rats with intramuscular iron. In addition, dexamethasone treatment reduces the efficacy of mebendazole in nutritionally sufficient rats.

The finding of decreased anthelmintic efficacy in iron and protein deficiency has not been demonstrated before. Mebendazole is a potent anthelmintic which is effective in man against trichuriasis (Miller *et al.*, 1974), enterobiasis (Brugmans *et al.*, 1971), ascariasis (Hutchison *et al.*, 1975), and hookworm (Peña Chavarria *et al.*, 1973). Furthermore, mebendazole is considered to be a notable advance in the treatment of trichuriasis as the first effective therapy without major side-effects (Archer, 1974). Thus, it is of interest that it is less effective in curing patients with severe trichuriasis (Peña Chavarria *et al.*, 1973; Wolfe and Wershing, 1974). As severe infections are associated with chronic diarrhoea and anaemia,

failures in treatment may be related to nutrient deficiencies.

It is possible that decreased anthelmintic efficacy in iron and protein deficiency is mediated by a defect in immune response. This concept is supported by the demonstration of reduced efficacy of mebendazole in dexamethasone-induced immunodeficient rats. Steroids, however, have a wide range of biological effects and a direct relationship between reduced anthelmintic efficacy and immune suppression remains to be demonstrated.

Hass (1973) investigated the effect of steroids on anthelmintic efficacy in this model. He postulated that in steroid-treated rats *N. brasiliensis* should be more susceptible to anthelmintics, but concluded that his results did not support this hypothesis. However, analysis of his figures shows that steroids, in fact, reduce the efficacy of several benzimidazole anthelmintics. For example, in his study, thiabendazole reduced parasite populations in steroid-treated rats by only 47%, compared with 99% in control rats.

In the *N. brasiliensis*-rat model dietary induced iron and protein deficiency not only reduced anthelmintic efficacy, but also delayed worm expulsion (Bolin *et al.*, 1977) and decreased acquired resistance to reinfection (Duncombe *et al.*, unpublished).

There is evidence that an association exists between helminthiasis, malnutrition, and immunodeficiency states in man. Jose and Welch (1970) in a study on 2250 Aboriginal children found marked increases in total parasite load and number of species present in growth retarded compared with normal children. Cruz *et al.* (1966) and Rivera *et al.* (1970) have reported hyperinfection with *Strongyloides stercoralis* in a variety of immunodeficiency states, including steroid administration.

It therefore seems likely that dietary and/or parasite induced nutritional deficiency may play a role in potentiating gut parasites in man. Field studies using iron and protein supplementation are necessary to determine the validity of this concept.

We would like to thank Miss Glenda Walsh, Mr. Christopher Porter, and Mr. Ian Weston for their excellent technical assistance. This study was supported by a grant from the National Health and Medical Research Council.

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