The use of zeolites as slow release anthelmintic carriers

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Abstract

This work examines the ability of commerical zeolite Y to act as a slow release agent for a number of anthelmintic drugs. Administration to rats, dosed with *Nippostrongylus brasiliensis*, of pyrantel and/or fenbendazole and pigs, dosed with *Ascaris* and *Oesophagostomum*, of dichlorvos (DDVP) loaded onto zeolite Y was more sucessful in killing adult worms than administration of the pure drug alone. The zeolite Y was used as supplied for initial studies and then later dealuminated for further studies. The drug loadings were monitored by thermal analysis and the loaded zeolites were used in several field trials. The results indicate that zeolite Y is a suitable vehicle for the slow release of some anthelmintics. The slow release of drug from the zeolite matrix improved its efficacy.

Introduction

Little is known about the influence of zeolites on intestinal physiology. Pond (1984) has shown that following zeolite feeding there is a reduction in the uptake of ammonia via the intestinal wall, and this may lead to improved food utilization.

Mumpton & Fishman (1977) summarize information concerning the properties of zeolites and the results of their use as supplements in the diets of farm animals. Several claims were made that diarrhoea was reduced in pigs and calves and that the droppings of chickens had a reduced water content. These reports may have implications for diarrhoeas of helminth origin since loss of water and hence dehydration, when combined with other pathological effects, is one of the major causes of death in young animals. Claims were also reported of improved weight gain, digestibility and utilization of feed in animals that received zeolite supplements; these also have implications for animals infected with parasites. A general review of the use of zeolites in agriculture is given by Mumpton (1978).

Wells & McHugh (1983) showed that in rats fed with clinoptilolite at the rate 100 g kg^{-1} of conventional diet for a short period there was a posteriad displacement of the nematode, *Nippostrongylus brasiliensis* in the small intestine. It was suggested that this might be a beneficial response to the feeding of zeolite, with an earlier start to the expulsion of the worm population. This was due to zeolite characteristics influencing directly or indirectly the earlier commencement of enzyme activity restoration and the reduction of physical damage caused by the worms in the gastrointestinal tract.

It is possible that zeolites provide protection for drugs that are easily decomposed due to humidity. The vinyl phosphate anthelmintic dichlorvos (2,2-dichlorovinyl dimethyl phosphate) is one of the most widely used, due to its biocidal activity, volatility and rapid detoxification. Dichlorvos reponds well as a plasticizer with various resins, thus making possible a variety of slow release formulations. It is effective against *Ascaris suum*, *Oesophagostomum* spp. and *Trichuris suis* and in large doses it has been found to be effective against *Hyostrogylus rubidus* (Jacobs, 1968). Pyrantel (trans-1-methyl-2-[2-(2-thienyl)vinyl]-1,4,5,6 tetrahydropyrimidine) is a broad base anthelminitic, effective against most adult and immature gastro-intestinal, pulmonary and ocular nematodes. At therapeutic doses, pyrantel shows no toxicity. It is fast acting, and most worms are expelled within 24 h. Fenbendazole is a benzimidazole-based wide range anthelminitic, but its mode of action is slow and repeated normal administrations are required. Prichard *et al.* (1976) speculated on how the efficiency

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of the benzimidazole-based anthelmintics could be improved by the use of a slow release mechanism.

The present paper investigates the effects of short term administration of a number of anthelmintic loaded zeolite preparations on worm numbers and distribution in pigs receiving a low infection of *Ascaris* and *Oesophagostomum* and rats receiving a low infection of *Nippostrongylus brasiliensis*. Dyer *et al*. (1986) demonstrated that the known beneficial effects of certain drugs, including dichlorvos, fenbendazole and pyrantel, could be enhanced by incorporating them into a zeolite matrix from which they are released slowly, thus prolonging drug delivery in the therapeutic range. Shaker *et al*. (1992) showed how zeolites could be used to release tetramisole loaded on zeolite Y. Most drug molecules are quite large, but dichlorvos is quite a small molecule and could diffuse into the framework of zeolite Y, and this was the main reason for choosing zeolite Y. Dealuminating zeolite Y increased the loading of hydrophobic drugs.

Materials and methods

The zeolite Y was supplied in the sodium form by Laporte Industries, Widnes, UK. Dichlorvos was supplied by SDS Biotechnology, and its purity was determined at 98% + using gas chromatography. The operating conditions were as follows: a AI 92 gas chromatograph was fitted with an FID detector and a 5% SE30 on WDMCS 60/85 column. The injection and detector temperatures were 210°C and 200°C respectively, while the column was at 95°C. The sample volume was 4 μl. Pyrantel pamoate and fenbendazole were supplied by Farbwerke Hoechst A.G. All thermal analysis was carried out on a Mettler TG50 system conected to a TA 3000 processor. All samples were heated at 10°C min^{-1} in a flowing nitrogen atmosphere (20 ml min^{-1}).

Drug loading

A batch system was used to load the anthelmintics onto the zeolite in which a weighed amount of calcined zeolite (550°C for 16 h) was placed in a round-bottomed flask and refluxed with a 1% drug solution in a suitable solvent. The system was stirred continuously for approximately 50 h, the solvent was then filtered off to leave a zeolite/drug matrix which was then dried at 60°C for 3 h in a vacuum oven. The amount of drug contained within the zeolite and on the surface was determined by thermogravimetry. The chemical composition of zeolite Y used in these experiments was determined using X-ray fluorescence spectroscopy (table 1). The crystal size was determined to be around 2 μm, using scanning electron microscopy.

Anthelmintic treatment of pigs

Three groups of eight pigs aged 8 weeks and of mixed sex were housed at the University of Liverpool Department of Animal Husbandry. All pigs were infected with between 2000 and 4000 *Ascaris* and *Oesophagostomum*, then left for 2 weeks to allow for a period of worm establishment and then dosed with the drug/zeolite Y matrix. One group of pigs acted as a control, a second group was treated with a low dose 3 mg kg^{-1} dichlorvos loaded zeolite and a third group with a high dose of 14 mg kg^{-1} dichlorvos loaded zeolite. To dose the pigs, an aliquot of the dichlorvos/sodium Y matrix was accurately weighed according to pig weight, and then slurried with sunflower oil which was then dosed via gavage. After a period of 3 days, the pigs were killed and the stomach, small intestine and large intestine removed for examination of parasites.

Table 1. Chemical composition of zeolite Y.

Chemical	Weight (%)
Aluminium oxide	21.9
Silicon oxide	62.6
Sodium oxide	12.7
Potassium oxide	0.3
Iron oxide	0.57
Other *	1.93

*Various metal oxides such as titanium, manganese and chromium.

Anthelmintic treatment of rats

One hundred 12-week-old Wistar rats from a closed colony maintained at the University of Salford were assigned to treatment groups (ten rats per group) as shown in tables 2 and 3 The rats were housed individually and given water and pelleted rat cake (Labsure CRM®) *ad libitum* prior to being placed in their treatment groups. Rats were infected with 500 third stage larvae of *Nippostrongylus brasiliensis* in 0.2 ml of dechlorinated water by subcutaneous infection. The animals were dosed via gavage at day 6 and autopsied at day eight post-infection. To assess the worm burden and its distribution, the small intestine was divided into eight sections and the worms counted. Total counts from each section were then grouped as indicated in the tables.

Due to previously poor loading results obtained when fenbendazole was loaded onto zeolite Y, the zeolite used was dealuminated to enhance the loading of the drug as previously described by Kerr (1968), in which ethylenediaminetetraacetic acid (H_4EDTA) was added directly to a slurry of zeolite in water. The mixture was stirred for 6 h, then filtered and calcined in air at 550°C.

Data analysis

Worm counts for anthelmintic treatment of rats were transformed using a sine transformation: $\sin^{-1}\sqrt{(x/n)}$, where x = count per region per rat, n = total count per rat and calculated in the radian mode, thus the proportion of worms present in each region (eight regions in total) was calculated for individual rats. The transformed worm

Table 2. Treatment of rats with pyrantel pamoate.

Rat group	Treatment
1	Control
2	20 mg kg^{-1} pure drug
3	10 mg kg^{-1} pure drug
4	20 mg kg^{-1} drug loaded zeolite
5	10 mg kg^{-1} drug loaded zeolite

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Table 3. Treatment of rats with fenbedazole.

Rat group	Treatment
1	Control
2	5 mg kg^{-1} pure drug
3	2.5 mg kg^{-1} pure drug
4	5 mg kg^{-1} drug loaded zeolite
5	2.5 mg kg^{-1} drug loaded zeolite

counts were subjected to a factorial analysis with further investigation of statistically significant effects being performed using Student *t*-tests. NS indicates that there was no significant difference at $P = 0.05$.

Results and Discussion

Drug loading

Dichlorvos is a relatively small molecule and therefore had no problems entering the zeolite Y windows. Drug loadings of between 39 and 64 mg g^{-1} were recorded in the treatment of pigs. In rats, fenbendazole did not initially give a favourable loading onto zeolite Y, but following partial dealumination a much better loading occurred both internally and on the zeolite surface, as quantified by thermal analysis. Pyrantel pamoate has a large pamoic acid molecule of 9.1 Å in length and 8.44 Å in diameter compared to zeolite Y with windows of 7.4 Å. A large amount of surface loading would therefore be expected to occur and the results confirmed this although internal loading was also achieved at a reasonable level.

Anthelmintic treatment of pigs

At first glance, it seems as if the results are inconclusive, but if one pig from each group is ignored because of vomiting soon after dosing, the results for *Ascaris* are very promising (table 4), especially when the dose administered (14 mg kg^{-1}) is half of the therapeutic value. If the dose was doubled then it is likely that all the worms would have been removed. In the case of *Oesophagostomum* (table 5) the results are disappointing as the zeolite has not held its drug through to the large intestine. However by using a 2-dimensional zeolite pore system rather than a 3-dimensional one as used in the present study, it is likely that the diffusion of the drug would be slowed down. Zeolite L has the same dimension windows as zeolite Y but has a 2-dimensional pore system, therefore zeolite L may prove to be more effective at delivering drugs into the large intestine.

Table 4. Mean worm numbers of *Ascaris* in pigs treated with dichlorvos.

Pig group		1	2
	Mean	27.4	0.43
3	3.0	0.05	NS
2	0.43	0.025	–

1, Control; 2, high dose dichlorvos (14 mg kg^{-1}); 3, low dose dichlorvos (3 mg kg^{-1}); F = 0.24; P = NS. One pig from each group was ignored due to vomiting after dosing.

Table 5. Mean worm numbers of *Oesophagostomum* in pigs treated with dichlorvos.

Pig group		1	2
	Mean	104.43	46.71
3	82.43	NS	NS
2	46.71	NS	–

1, Control; 2, high dose dichlorvos (14 mg kg^{-1}); 3, low dose dichlorvos (3 mg kg^{-1}); F = 0.22; P = NS. One pig from each group was ignored due to vomiting after dosing.

Anthelmintic treatment of rats

All the experimental groups treated with pyrantel pamoate (table 6) had significantly less *N. brasiliensis* than those in the control groups. Rats receiving a full dose (20 mg kg^{-1}) of pure drug compared with a full dose of drug loaded zeolite showed no significant differences although worm counts were lower in the zeolite supplemented group. This was also true in rats receiving a half dose (10 mg kg^{-1}) of pure drug compared with a half dose of drug loaded zeolite.

With fenbendazole, rats receiving a full dose (5 mg kg^{-1}) of pure drug compared with a full dose of drug loaded zeolite showed a reduction in worm numbers in the zeolite supplemented group (table 7) although the reduction was not significant. However, worm numbers were higher in rats receiving a half dose (2.5 mg kg^{-1}) of pure fenbendazole compared with those receiving a half dose of drug loaded zeolite, and the difference in worm number between these groups was significant.

Table 6. Mean worm numbers of *Nippostrongylus brasiliensis* in rats treated with pyrantel pamoate.

Rat group		1	2	3	4
	Mean	2226.5	6.1	3.4	16.8
5	7.0	<0.001	NS	NS	NS
4	16.8	<0.001	NS	NS	–
3	3.4	<0.001	NS	–	–
2	6.1	<0.001	–	–	–

1, Control; 2, full dose; 3, full dose/zeolite; 4, half dose; 5, half dose/zeolite; F = 23.99; $P < 0.001$.

Table 7. Mean worm numbers of *Nippostrongylus brasiliensis* in rats treated with fenbendazole/dealuminated zeolite Y.

Rat group		1	2	3	4
	Mean	133.6	17.8	0.1	26.7
5	6.2	<0.001	0.25	NS	0.05
4	26.7	<0.001	0.50	0.01	–
3	0.1	<0.001	0.10	–	–
2	17.8	<0.001	–	–	–

1, Control; 2, full dose; 3, full dose/zeolite; 4, half dose; 5, half dose/zeolite; F = 73.67; $P < 0.001$.

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Table 8. Transformed worm numbers of *Nippostrongylus brasiliensis* in regions 1–8 of the small intestine of rats treated with pyrantel pamoate. (See table 6 for details of rat groups.)

Region 1 (F = 1.649; *P* = NS)

Rat group		1	2	3	4
	Mean	0.205	0.219	0	0.195
5	0.117	0.50	0.50	0.50	0.50
4	0.195	NS	NS	0.50	–
3	0	0.10	0.05	–	–
2	0.219	NS	–	–	–

Regions 2, 3 and 4 (F = 53.91; *P* < 0.001)

	Mean	1.498	0.503	0.579	0.868
5	0.475	0.005	NS	NS	0.25
4	0.868	0.05	0.50	0.50	–
3	0.579	0.005	NS	–	–
2	0.503	0.005	–	–	–

Regions 5 and 6 (F = 2.16; *P* = NS)

	Mean	0.320	0.120	0.103	0.476
5	0.276	NS	0.25	NS	0.25
4	0.476	0.25	0.05	0.005	–
3	0.103	0.25	NS	–	–
2	0.120	0.25	–	–	–

Regions 7 and 8 (F = 1.25; *P* = NS)

	Mean	0.189	0.179	0	0.236
5	0.142	NS	NS	0.25	0.50
4	0.236	0.25	0.25	0.10	–
3	0	NS	NS	–	–
2	0.179	NS	–	–	–

Table 9. Transformed worm numbers of *Nippostrongylus brasiliensis* in regions 1–8 of the small intestine of rats treated with fenbendazole/dealuminated Y. (See table 7 for details of rat groups.)

Region 1 (F = 9.00; *P* = 0.001)

Rat group		1	2	3	4
	Mean	0.329	0.326	0	0.435
5	0.234	NS	NS	0.01	0.025
4	0.435	NS	NS	0.001	–
3	0	0.001	0.001	–	–
2	0.326	NS	–	–	–

Regions 2, 3 and 4 (F = 27.57; *P* = 0.001)

	Mean	1.152	0.918	0	0.786
5	0.539	0.001	0.005	0.001	0.05
4	0.786	0.01	NS	0.001	–
3	0	0.001	0.001	–	–
2	0.918	NS	–	–	–

Regions 5 and 6 (F = 2.33; *P* = NS)

	Mean	0.210	0.386	0.1	0.368
5	0.237	NS	NS	NS	NS
4	0.368	NS	NS	0.05	–
3	0.1	NS	0.025	–	–
2	0.386	NS	–	–	–

Regions 7 and 8 (F = 4.5; *P* = 0.01)

	Mean	0.0956	0.110	0	0.268
5	0.103	NS	NS	NS	NS
4	0.268	0.025	0.025	0.001	–
3	0	NS	NS	–	–
2	0.110	0.05	–	–	–

Transformed worm counts of N. brasiliensis *in rats*

With pyrantel pamoate, in region 1 of the small intestine there were significantly more worms in the full dose pure drug group when compared with the equivalent zeolite supplemented group (table 8). In regions 2, 3 and 4 combined, all experimental groups had a significantly lower proportion of worms present than the control group. There were no relevant comparisons of significance in regions 5 and 6 combined. This was also the case in regions 7 and 8 combined.

With fenbendazole, in region 1 of the small intestine, a significant difference was observed between the full dose pure drug group and the loaded zeolite group, there being a greater proportion of worms in the former than the latter (table 9). In regions 2, 3 and 4 combined there was also the tendency for the zeolite groups to have a smaller proportion of worms than the pure drug and control groups. This was also the case for regions 5 and 6 where those animals receiving full dose pure drug had a significantly greater proportion of worms than the equivalent zeolite supplemented group. In regions 7 and 8 there was a significant comparison between those rats receiving pure drugs at full and half dose and the control group, with more worms being present in the pure drug groups than the controls (table 9).

Carrier substances have been found to act as slow release agents giving an improved performance by the therapeutic agent involved (Dyer *et al.*, 1986). The present results support the idea that pyrantel pamoate loaded onto zeolite Y is more effective at expelling *N. brasiliensis* in rats than the same amount of pure drug substance. This was also true of fenbendazole when loaded onto dealuminated zeolite Y. Worm populations were significantly reduced by drug loaded zeolite in the upper regions of the intestine, as worms shifted to the posterior regions. Wells & McHugh (1983) also showed a similar posterior shifting in worm populations in rats fed quantities of zeolites in the diet. Thus, the posterior shifting and early removal of worms from the intestine observed in drug loaded zeolite treated rats was due to the presence of zeolite initiating a hostile environment, enhancing the effect of the drug and releasing it slowly in those regions normally favoured by the worms.

Drug stability

It was also found that all drugs used in the trials remained stable and active within the zeolite matrix at different temperatures for over 4 months and over 6 months for dichlorvos. This has obvious commercial advantages helping to protect the drug and improve the shelf life of unstable anthelmintics such as dichlorovos which is normally bound by expensive plastic beads to prevent excessive release and breakdown.

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