ELSEVIER

International Journal of Pharmaceutics 104 (1994) 175–179

international journal of pharmaceutics

Notes

Improved peroral bioavailability of mebendazole in rabbits by administration of various *N*-alkoxycarbonyl derivatives of mebendazole

Lise Sylvest Nielsen [1]

The Royal Danish School of Pharmacy, Department of Pharmaceutics, 2 Universitetsparken, DK-2100 Copenhagen, Denmark

(Received 14 July 1993; Accepted 15 September 1993)

Abstract

The absorption of mebendazole, a very poorly soluble benzimidazole carbamate, used in the treatment of hydatid disease, and various *N*-alkoxycarbonyl prodrugs, was investigated after peroral administration to rabbits. The compounds were administered in the form of aqueous suspensions at pH 5.0. All prodrugs were rapidly hydrolyzed to mebendazole after absorption. The bioavailability was improved for all prodrugs. The highest bioavailability was obtained with the *N*-methoxycarbonyl derivative which showed a 13-fold improvement in bioavailability compared to mebendazole. The improved bioavailability of the prodrugs was ascribed to the increased water solubilities and appropriate lipophilicities. The *N*-alkoxycarbonylation of the benzimidazole moiety in mebendazole and in other widely used benzimidazole carbamates may be a promising prodrug approach to enhance poor absorption which follows peroral administration of this type of poorly soluble drug.

Key words: Mebendazole; Bioavailability; *N*-Alkoxycarbonyl derivative; Prodrug

Alvolar and cystic echinococcosis (hydatide disease), which is caused by infection with the larval stage of the cestodes *Echinococcus multilocularis* and *E. granulosus*, is one of the most lethal of all human helminth infections (Eckert, 1986). The only curative treatment is surgery, however, it is often ineffective due to extensive secondary hydatid disease (Schantz et al., 1982). Benzimidazole carbamates such as mebendazole have been used for more than a decade in inoperable cases. Long-term peroral treatment has in most cases proved to suppress the disease (Davis et al., 1989; Wilson et al., 1992). The clinical response in patients treated with, e.g., mebendazole, is variable, and is related to its low and variable bioavailability, which is most likely due to a combination of a very low solubility and extensive first pass metabolism of the drug (Dawson and Watson, 1985; Dawson et al., 1985).

In an attempt to overcome or diminish this delivery problem by the prodrug approach, several *N*-alkoxycarbonyl derivatives of thiabendazole and mebendazole with increased water-solu-

[1] Present address: Pharmaceutical Development, A/S DUMEX (Dumex Ltd), 11 Dalslandsgade, DK-2300, Copenhagen S, Denmark.

SSDI 0378-5173(93)E0280-4

bilities and appropriate lipofilicities have been syntized and evaluated in our laboratory (Nielsen et al., 1992, 1994). It was found that the derivatives were rapidly cleaved in biological media, the *N*-alkoxycarbonyl derivatives being up to 16 times more soluble than the parent compound. Thus, the major objective of the present study was to identify mebendazole prodrugs showing increased absorption characteristics compared to the parent drug. Therefore, in order to establish the most promising derivative, the relative bioavailability of mebendazole was determined after peroral administration of mebendazole and its derivatives (II–IV, shown in Scheme 1), to rabbits. The derivatives II and IV consisted of two isomers in the proportion 1 : 1.

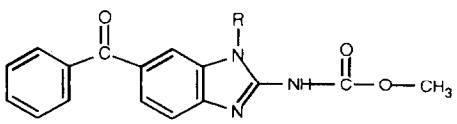
Mebendazole was obtained from Sigma Chemical Co., St. Louis. The *N*-alkoxycarbonyl derivatives (II–IV) were prepared as previously described (Nielsen et al., 1994).

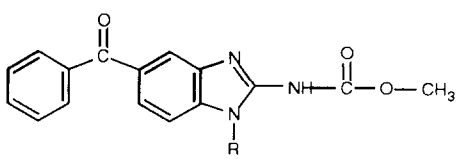
A radioimmunoassay (RIA) (Michiels et al., 1982) was employed to assess the level of mebendazole in the plasma samples obtained from the bioavailability studies. The analyses were kindly performed by Janssen Pharmaceutica, Belgium. The applied RIA method was compared to an HPLC method (unpublished method, but mentioned in Woestenborghs and Heykants, 1990) by comparing the mebendazole average values found by RIA with the level of mebendazole in pooled samples obtained by HPLC. The regression equation for the correlation graph was $y = 1.09x^{1.021}$ ($r = 0.986$; $n = 46$). The correlation with the HPLC method indicates that the applied RIA method has a good accuracy. The limit of detection of mebendazole in the rabbit plasma was about 2 ng ml^{-1} for the RIA method and about 5 ng ml^{-1} for the HPLC method.

The hydrolysis of the derivatives (II–IV) was studied in 20% rabbit gut homogenate diluted with 0.05 M phosphate buffer of pH 7.4 at 37°C. The gut homogenate, comprising segments from jejunum, ileum and colon, was prepared as previously described (Møss et al., 1990). The analytical procedure is described in a previous work (Nielsen et al., 1994).

Four male albino rabbits weighing 2.0–2.5 kg were included in the bioavailability study. The rabbits were fasted for 20 h prior to drug administration, but allowed free access to water.

Mebendazole and the three derivatives (II–IV) were given to each rabbit using an open cross-over design with at least 7 days between each drug administration. Rabbits 1 and 2 received the compounds in the following order: I, III, II, IV and I. Rabbits 3 and 4 received the compounds in the following order: I, IV, III, II and I. Suspensions (10 ml), containing mebendazole at a dose of 30





I R = H

IIa R = $-C(=O)-OCH_3$

IIIa R = $-C(=O)-OCH_2CH_3$

IVa R = $-C(=O)-OCH_2CH_2CH_3$

IIb R = $-C(=O)-OCH_3$

IVb R = $-C(=O)-OCH_2CH_2CH_3$

Scheme 1.

mg kg^{-1} or equimolar amounts of compound (II–IV), were given perorally through a stomach tube, followed by 10 ml of water.

The suspensions were prepared by dispensing the compounds in a 0.02 M acetate buffer of pH 5.0. The suspensions were placed in an ultrasonic water bath for 15 min and then rotated on a mechanical spindle for 18–20 h.

Approx. 2–5% of the substances were lost during the administration due to adsorption of undissolved substance in the auxiliary equipment.

Blood samples (1 ml) were collected from a marginal ear vein in heparinized test tubes prior to and 20, 40 min, 1, 2, 3, 4, 6, 8 and in most cases also 24 and 48 h after drug administration. Plasma samples obtained after centrifugation for 10 min at 6000 rpm were stored at −20°C until analysis.

The areas under the plasma concentration-time profiles (AUC) were calculated using the trapezoidal rule, with addition of the residual area from the last sampling point to infinity, calculated as the last measured concentration divided by the slope (k_e) of the terminal log-linear phase. In those instances where k_e could not be estimated, the mean k_e value in the group was used to estimate the residual area.

The relative bioavailability (F_{rel}) of the prodrugs after peroral administration was calculated using AUC values for mebendazole obtained at the end of the experimental series B, since blood-sampling in series A was only performed from 0 to 8 h after administration.

Fig. 1 shows the mebendazole average plasma concentration-time curves obtained after administration of mebendazole and its derivatives II–IV. The extent of absorption, judged by the AUC values (Table 1), varies both within and between the rabbits. This might be explained by the fact that the absorption of mebendazole from the gastro-intestinal tract is probably dissolution-rate limited (Dawson et al., 1985) and due to the variable rate of stomach emptying time in the fasted rabbit as reported by Chiou et al. (1969).

The relative bioavailability shows a marked improvement in the bioavailability of all prodrugs compared to the parent drug (Table 1). The most promising prodrug, the *N*-methoxycarbonyl derivative (II), showed a 13-fold improvement in bioavailability. The relative bioavailability was calculated using the higher AUC values for mebendazole obtained at the end of experimental series B compared to that at the beginning of experimental series A (Table 1), thereby giving lower F_{rel} values. The dose given to the rabbits was adjusted by weight, and the difference between the average $AUC_{0-8\ h}$ values obtained in

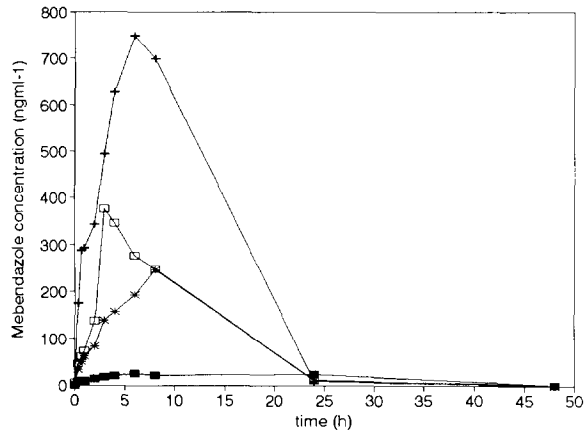


Fig. 1. Mean mebendazole plasma concentrations of mebendazole in four rabbits following peroral administration of mebendazole (■) and the *N*-alkoxycarbonyl derivatives, II (+), III (*) and IV (□) in an amount corresponding to 30 mg mebendazole per kg body weight.

Table 1
Average AUC values ($n = 4$) and relative bioavailabilities (F_{rel}) of mebendazole after oral administration of mebendazole (I) and various *N*-alkoxycarbonyl derivatives of mebendazole (II–IV)

Compound	$AUC_{0-8\ h}$ [a] (ng ml^{-1} h)	$AUC_{0-\infty}$ [b] (ng ml^{-1} h)	±SD	F_{rel} [c]
I,A	87		23	
I,B [d]	139		23	
I,B [d]		751	73	1
II		9564	3357	12.7
III		2970	1809	4.0
IV		4036	4351	5.4

[a] Student's *t*-test was performed on the $AUC_{0-\infty}$ values, and the significance level obtained between the groups was 0.032.
[b] The residual area was in most cases 0–30%, but in a few it exceeded 30% of the $AUC_{0-\infty}$, therefore the values are predicted with some uncertainty.
[c] Calculated from the $AUC_{0-\infty}$ values for mebendazole obtained at the end of experimental series B.
[d] $n = 3$.

the two periods (significance level = 0.032) may partly be ascribed to a change in the metabolic pattern of mebendazole and/or a change in the distribution pattern of mebendazole due to the rabbits being fatter at the end of the experiment. The rabbits gained typically 600–700 g in weight during the experimental period.

The half-lives ($t_{1/2}$ = 2–8 h) determined from the slope of the terminal log-linear phase of the plasma profile showed great variation both between the rabbits and within the drug compounds. The half-lives were much longer than that ($t_{1/2}$ = 0.93 h) reported by Dawson et al. (1985) after intravenous administration of a tracer dose to man, but were in good agreement with the values obtained in man after peroral administration of large doses of mebendazole (Münst et al., 1980; Witassek et al., 1981; Braithwaite et al., 1982). Consequently, the half-lives determined in this study might not be true elimination half-lives, but absorption/distribution half-lives.

The hydrolysis experiments in rabbit gut homogenate showed that the *N*-alkoxycarbonyl derivatives (II–IV) were enzymatically cleaved in the homogenate (Table 2) and, therefore, after peroral administration of the derivatives, absorption of both mebendazole and the derivatives is expected to take place. After peroral administration of the derivatives (II–IV), only mebendazole could be detected in the plasma samples. This is in good agreement with the previous findings in vitro showing that the derivatives were rapidly cleaved by rabbit plasma and liver enzymes (Nielsen et al., 1994).

Table 2
Half-lives ($t_{1/2}$) for the degradation of the *N*-alkoxycarbonyl derivatives (II–IV) in 0.02 M phosphate buffer solution (pH 7.4) and in gut homogenate [a] at 37°C

Compound	$t_{1/2}$ (h)	
	Buffer pH 7.4	20% rabbit gut homogenate
II	139	30
III	347	60
IVa	517	110
IVb	462	90

[a] The homogenates were derived from a mixture of the jejunum, ileum and colon.

In conclusion, this study shows that it is possible to improve the poor bioavailability of mebendazole by peroral administration of *N*-alkoxycarbonyl derivatives, with increased water solubilities and appropriate lipophilicities, to rabbits. However, studies on the effect of particle size of the compounds on the bioavailability are required since the compounds were given as suspensions.

1. Acknowledgements

I am very grateful to Dr Robert Woestenborghs and co-workers of Janssen Pharmaceutica, Beerse, Belgium, for carrying out the RIA analysis of my samples and to Dr Keld Hermansen, Department of Biological Sciences, The Royal Danish School of Pharmacy, for the assistance given with the animal experiment. Dr Lona Christrup, The Royal Danish School of Pharmacy, Department of Pharmaceutics, and Dr Claus Selch Larsen, Lundbeck A/S, are acknowledged for valuable discussions concerning handling of the experimental data.

2. References

Braithwaite, P.A., Roberts, M.S., Allan, R.J. and Watson, T.R., Clinical pharmacokinetics of high dose mebendazole in patients treated for cystic hydatid disease. *Eur. J. Clin. Pharmacol.*, 22 (1982) 161–169.

Chiou, W.L., Riegelman S. and Amberg, J.R., Complications in using rabbits for the study of oral drug absorption. *Chem. Pharm. Bull.*, 17 (1969) 2170–2173.

Davis, A., Dixon, H. and Pawlowski, Z.S., Multicentre clinical trials of benzimidazole carbamates in human cystic echinococcosis (phase 2). *Bull. WHO*, 67 (1989) 503–508.

Dawson, M. and Watson, T.R., The effect of dose form on the bioavailability of mebendazole in man. *Br. J. Clin. Pharmacol.*, 19 (1985) 87–90.

Dawson, M., Braithwaite, P.A., Roberts, M.S. and Watson, T.R., The pharmacokinetics and bioavailability of a tracer dose of [3H]-mebendazole in man. *Br. J. Clin. Pharmacol.*, 19 (1985) 79–86.

Eckert, J., Prospects for treatment of the metacestode stage of Echinococcus. In Thompson, R.C.A. (Ed.), *The Biology of Echinococcus and Hydatide Disease*, George Allen & Unwin, London, 1986, pp. 250–290.

Michiels, M., Hendriks, R., Heykants, J.A. and Van den Bossche, H., The pharmacokinetics of mebendazole and flubendazole in animals and man. *Arch. Int. Pharmacodyn. Ther.*, 256 (1982) 180–191.

Münst, G., Karlaganis, G. and Bircher, J., Plasma concentrations of mebendazole during treatment of echinococcosis. *Eur. J. Clin. Pharmacol.*, 17 (1980) 375–378.

Møss, J., Buur, A. and Bundgaard, H., Prodrugs of peptides: 8. In vitro study of intestinal metabolism and penetration of thyrotropin-releasing hormone (TRH) and its prodrugs. *Int. J. Pharm.*, 66 (1990) 183–191.

Nielsen, L.S., Bundgaard, H. and Falch, E., Prodrugs of thiabendazole with increased water-solubility. *Acta Pharm. Nord.*, 4 (1992) 43–49.

Nielsen, L.S., Sløk, F. and Bundgaard, H., *N*-alkoxycarbonyl prodrugs of mebendazole with increased water-solubility. *Int. J. Pharm.*, 102 (1994) 231–239.

Schantz, P.M., Van den Bossche, H. and Eckert, J., Chemotherapy for larval echinococcosis in animals and humans: report of a workshop. *Z. Parasitenkd.*, 67 (1982) 5–26.

Wilson, J.F., Rausch, R.L., McMahon, B.J. and Schantz, P.M., Parasiticidal effect of chemotherapy in alveolar hydatid disease: review of experience with mebendazole and albendazole in Alaskan eskimos. *Clin. Infect. Dis.*, 15 (1992) 234–249.

Witassek, F., Burkhardt, B., Eckert, J. and Bircher, J., Chemotherapy of alveolar echinococcosis. Comparison of plasma mebendazole concentrations in animals and man. *Eur. J. Clin. Pharmacol.*, 20 (1981), 427–433.

Woestenborghs, R. and Heykants, J., Assay methods for imidazole and triazole-like antifungals and for some antihelminthics. In Reid, E. and Wilson, I.D. (Eds), *Methodological Surveys in Biochemistry and Analysis*. Vol. 20, Royal Society of Chemistry, Cambridge, 1990, pp. 153–156.