International Journal of Pharmaceutics, 91 (1993) 105–109

IJP 02986

Research Papers

Comparative effects of anionic, natural bile acid surfactants and mixed micelles on the intestinal absorption of the anthelmintic albendazole

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(Received 9 March 1992)
(Modified version received 17 July 1992)
(Accepted 29 July 1992)

Key words: Albendazole absorption; Intestinal perfusion; Surfactant; Sodium taurocholate; Sodium lauryl sulfate; Mixed micelles

Summary

The intestinal absorption of albendazole using surfactants (sodium taurocholate, sodium lauryl sulfate and mixed micelles) was studied. All experiments were performed on rat small intestine following intestinal perfusion. Solubilization of albendazole with sodium taurocholate improves the absorption constant of albendazole when compared to sodium lauryl sulfate induced solubilization (2.80 and 1.51 h^{-1}, respectively). When mixed micelles (sodium taurocholate-oleic acid) were used the values for the absorption constant obtained (ranging from 1.056 to 1.135 h^{-1}) showed a prevailing effect of oleic acid.

Introduction

Albendazole (ABZ) is a wide-spectrum anthelmintic often used against gastrointestinal parasites. Its action is related to the inhibition of the development in different larval stages of parasites (Marriner and Bogan, 1980). ABZ (methyl[5-(propylthio)-H-benzimidazol-2-yl]carbamate) (Theodorides et al., 1976; Van den Bossche, 1985), belongs to the benzimidazole group and, as such, it is nearly insoluble or only slightly soluble in water (Theodorides et al., 1976). Since the efficacy of ABZ in the control of hydatidosis was first demonstrated, this use has become generalized. Its anthelmintic action is due to inhibition of the fumarate-reductase activity in the parasite. Its mode of action is the same as all benzimidazole derivatives, causing degenerative changes in the intestinal and tegumental cells on the helminths, via an interaction effect with the cytoplasmic microtubules (Lacey, 1988, 1990). Its limited absorption is probably related to the poor water solubility of this drug.

The amphiphilic properties of surfactants allow for stable dispersions of lipophilic substances to be formed, as well as for increase in the permeability of biological membranes (Kakemio et al., 1970). These factors contribute to increase

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the gastrointestinal absorption of drugs. Surfactants can increase the dissolution rate of poorly water-soluble drugs by micellar solubilization of the compound (Poelma et al., 1990).

The aim of this work was to improve the intestinal absorption of ABZ by increasing its solubility by the addition of the surfactants, sodium lauryl sulfate (SLS) (anionic surfactant); sodium taurocholate (STC) (natural bile acid surfactant); and mixed STC-Oleic acid (OA) micelles.

Materials and Methods

Animals

Male Wistar rats, ranging between 250 and 300 g, were used throughout this study. The animals had been fasted for 18–20 h before surgery. Five animals per condition surfactant were used.

Reagents

The sample of ABZ, supplied by Smith Kline and Beecham, SAE (Madrid, Spain).

Taurocholic acid sodium salt was used as representative of the naturally occurring bile surfactants, since it has been shown to be the major bile salt in the rat (Saunders, 1975). It was purchased from Sigma Chemical Co. (Madrid, Spain), with a purity of 98%. The concentration of taurocholate in the perfusion fluid was 50 mM.

In other experiments, SLS (Scharlau; Barcelona, Spain) was used as the most genuine representative of the anionic sulfate amphiphiles (Garrigues et al., 1992). The concentrations of SLS in the perfusion fluid were 38 and 50 mM.

Mixed micelles were composed of 0.1–1% w/v of a fusogenic lipid, solubilized by addition of a surfactant, usually to 2% w/v of a bile salt (Van Hoogdalen et al., 1989). In all experiments 0.1 and 0.5% OA were used, being solubilized by addition of STC at 38 mM (2% w/v).

All other chemicals were of analytical-grade purity (Scharlau; Barcelona, Spain).

Absorption technique

The intestinal absorption of ABZ was studied using the different surfactants (STC and SLS) and mixed micelles (STC-OA) in a phosphate buffer adjusted to a value of pH 6.7.

The animal was anaesthetized with urethane (Merck, 1.3 g per kg i.p.). The general perfusion method was described elsewhere (Del Estal et al., 1991). In situ rat gut preparation was performed by means of the technique of Doluisio et al. (1969) for the absorption test, as modified by Foradada et al. (1974) and Plá-Delfina et al. (1987), and 5 ml of the solution were perfused.

TABLE 1

Percent average albendazole concentration (±SD) relative to initial (0.0025 mg ml^{-1}) level remaining in the intestinal lumen after intestinal perfusion with sodium taurocholate (38 mM) and mixed micelles (sodium taurocholate, 38 mM and oleic acid, 0.1 and 0.5%), apparent first-order rate constant fitting each data set (K_a, h^{-1}), intercept slope (A_0) and correlation coefficients found (n = 5)

Time (min)	Percent remaining albendazole in lumen			
	Surfactant:	STC (38 mM) Mean ± SD	STC (38 mM) + OA (0.1%) Mean ± SD	STC (38 mM) + OA (0.5%) Mean ± SD
5		59.88 ± 2.23	73.26 ± 6.76	72.60 ± 6.65
10		46.71 ± 4.79	68.09 ± 7.39	66.11 ± 7.64
15		36.19 ± 3.93	59.10 ± 5.91	60.68 ± 6.64
20		30.06 ± 2.63	55.22 ± 5.91	56.39 ± 6.86
25		23.46 ± 1.49	49.50 ± 5.20	51.01 ± 6.00
30		18.30 ± 1.40	46.13 ± 3.03	46.39 ± 7.33
K_a (h^{-1})		2.810 ± 0.116 [a]	1.135 ± 0.055	1.056 ± 0.119
A_0		74.66 ± 7.50	80.38 ± 6.70	79.20 ± 6.59
r		0.999	0.996	0.999

[a] $P < 0.05$.

The initial concentration of ABZ used was 0.0025 mg/ml in the solution of surfactant. The sampling intervals were 5 min over a total time of 30 min.

Drug analysis

The concentrations of ABZ were determined by using reversed-phase LC, with slight modifications on previous studies (Bogan and Marriner, 1979; Prieto et al., 1988). The stationary phase was column Nova-Pak ODSC-18, 4 μm (Waters) and the mobile phase was acetonitrile-triethylamine-water (30 : 1.5 : 68.5 v/v; pH 3 using phosphoric acid), and before utilization it is outgassed in an ultrasonic sound bath and filtered with a vacuum pump using Millipore filters (0.45 μm mesh). The flow rate was set to 1 ml min^{-1}. A variable wavelength detector set at 292 nm was used.

The absorption rate constant, K_a was calculated by linear regression from

$$\ln C = \ln C_0 - K_a t$$

where C and C_0 are the concentration at time t and the initial concentration, respectively.

Statistical analysis

All results were statistically analyzed by means of Student's t-test. Levels less than 0.05 were considered not significant.

Results

The absorption rate constants of ABZ found in the presence of STC and mixed micelles are listed in Table 1. In a preliminary study, the optimal concentrations to be used were determined, and the experiment was carried out using 5% Tween 80 (polyoxyethylene sorbitan monooleate), which is equivalent to 38 mM STC on a molar basis (Del Estal et al., 1991). The initial concentration of ABZ and 0.0025 mg ml^{-1}.

Table 2 shows the percent average ABZ concentrations remaining in the intestinal lumen after intestinal perfusion with 5% Tween 80, 38 and 50 mM SLS as well as the absorption rate constants of ABZ found in the presence of these surfactants.

Discussion

The kinetics of ABZ absorption in the stomach follows a passive diffusion process (Prieto et al., 1991), and apparently the same kinetics occurs when ABZ is perfused with surfactants (Tween 80) in the duodenal tract (Del Estal et al., 1991). Improved absorption of ABZ could be achieved, using the same conditions of drug initial concentration, and varying the perfused surfactants. The study of the influence of different surfactants to improve the apparent absorption

TABLE 2

Percent average albendazole concentration (±SD) relative to initial (0.0025 mg ml^{-1}) level remaining in the intestinal lumen after intestinal perfusion with Tween 80 (5%) and sodium lauryl sulfate (38 and 50 mM), apparent first-order rate constants fitting each data set (K_a, h^{-1}), intercept slope (A_0) and correlation coefficients found ($n = 5$)

Time (min)	Percent remaining albendazole in lumen		
Surfactant:	Tween 80 (5%) Mean ± SD	SLS (38 mM) Mean ± SD	SLS (50 mM) Mean ± SD
5	77.98 ± 9.60	82.36 ± 2.95	82.06 ± 1.84
10	69.62 ± 10.5	71.23 ± 4.01	70.80 ± 0.75
15	64.52 ± 8.9	62.54 ± 4.41	61.64 ± 3.64
20	56.57 ± 9.9	54.20 ± 1.37	53.82 ± 1.06
25	51.05 ± 8.5	48.76 ± 4.36	50.67 ± 1.16
30	45.59 ± 8.5	44.75 ± 3.71	42.72 ± 2.09
K_a (h^{-1})	1.300 ± 0.230	1.490 ± 0.260	1.511 ± 0.113
A_0	87.52 ± 0.23	91.57 ± 5.79	91.47 ± 1.96
r	0.998	0.996	0.995

constant of ABZ, shows that STC increases ABZ absorption significantly in the duodenal tract of the rat.

STC is bile salt, and results in disaggregation in the mucosa of the intestinal membrane, besides micellar solubilization, and advances the drug-membrane interaction (Poelma et al., 1990). Higher concentrations of STC have been used for a similar purpose by others (Kakemi et al., 1970).

The results show that the addition of ABZ to STC significantly increases the apparent absorption rate constant (K_a) of ABZ with respect to the other surfactants used.

Concerning mixed micelles, several mechanisms could explain their action in the absorption increase; Murakami et al. (1985) suggested a decrease in the barrier function of the mucous layer containing mixed micelles; a transcellular mechanism may also be involved, due to the incorporation of solubilized fusogenic lipid into the epithelial membrane, resulting in an increase in membrane fluidity.

On the other hand, there are contradictory studies on the influence of mixed micelles on the increase in drug absorption; most workers have used mixed micelles with poorly soluble drugs, improving their absorption (Van Hoogdalem, 1989; Poelma et al., 1990) but others have observed no improving effects (Hikal et al., 1976; Muranishi et al., 1979). The exact role of mixed micelles in drug absorption is not well known. In this respect, Poelma et al. (1990) suggest that mixed micelles produce an increase in the disolution rate and/or facilitate drug transport to absorbent membrane, however, they conclude that little is known about the exact role of the pre-epithelial diffusion layer in the absorption process of drugs and the interaction of bile salts with this absorption barrier.

The results on the effects mixed micelles on ABZ absorption have been obtained after perfusion of an initial ABZ concentration of 0.0025 mg/ml in a solution with mixed micelles of 38 mM STC and 0.1 and 0.5% OA, no significant differences in the effect on the absorption rate of ABZ with either OA concentration being achieved. The K_a values ranged between $1.135 \pm 0.055\ h^{-1}$ with 0.1% OA and $1.056 \pm 0.119\ h^{-1}$ with 0.5% AO (Table 1). These data are slightly lower in magnitude than those obtained using 5% Tween 80, although there are no significant differences. Comparing these results with those obtained using STC one can observe that they are significantly smaller in magnitude, and the conclusion can be reached that in these mixed micelles the main effect is that due to OA, and for an increase in micellar solubility, with the reduction of consequent drug absorption.

SLS was used to compare its effect in the absorption of ABZ with Tween 80 and STC. Values of $1.490 \pm 0.260\ h^{-1}$ for 38 mM SLS and $1.511 \pm 0.113\ h^{-1}$ for 50 mM SLS were determined. The increase in SLS concentration from 38 to 50 mM does not increase significantly the K_a of ABZ.

On the other hand, SLS shows a non-significant tendency towards an increase in the ABZ absorption rate constant when compared to Tween 80 (Table 2).

The lesser extent of micellization of SLS with respect to Tween 80 (nonionic surfactant), increases the ABZ absorption constant as has been shown recently by Garrigues et al. (1992). This could be related to the slightly higher absorption constant obtained for SLS. This lower degree of micellization could be due not only to the ionic nature of SLS (anionic), but also to the SLS micelles themselves which would be less lipophilic and would include less drug.

In summary, the highest intestinal absorption rate of ABZ was obtained using STC, probably because the dissociation of ABZ micelles is faster in STC, thus enhancing the absorption rate. Moreover, STC would be less potent at micellization than SLS and Tween.

When mixed micelles are used the main effect on absorption is due to OA, through increasing micellar solubilization and hence decreasing the absorption rate.

The results obtained with SLS are intermediate between those obtained with Tween 80 and STC and are justified since this compound is less potent at micellization than Tween 80 and more powerful than STC.

Greater bioavailability of ABZ with STC can be expected, as well as a decreasein the therapeu-

tic dose and therefore a possible reduction of secondary effects (Gil-Grande et al., 1987). Further studies will be directed to studies of the pharmacokinetics and bioavailability of ABZ using these surfactants in order to determine when their use is appropriate.

Acknowledgements

The authors are grateful to the Department of Clinical Research of SmithKline & Beecham Pharmaceutics (Madrid) for cooperation, and Dr J.M. Plá-Delfina and his team for advice. This study was partially supported by CICYT (PTR 91-0025) and Junta de Castilla y León (Ref 11/04/91).

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