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Short Communication

The intestinal absorption of Luxabendazole in rats

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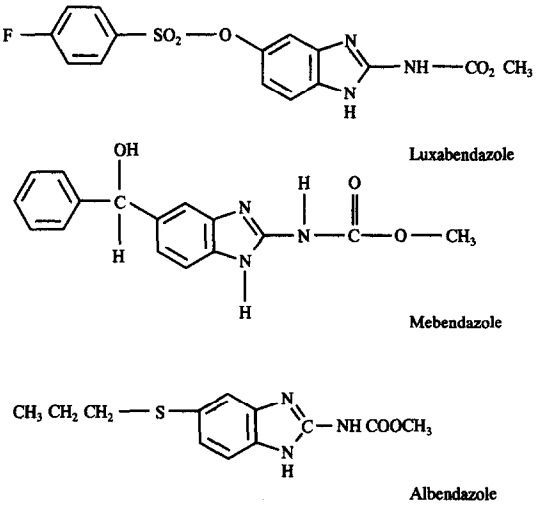
Introduction

Luxabendazole, methyl 5-(4-fluorophenyl-sulphoniloxy)-benzimidazole-2-carbamate, (LBZ) is a new compound developed by Hoechst A.G. laboratories (Frankfurt/Main, Germany), which has been shown to exhibit high efficacy against Trematodes (*Fasciola*, *Dicrocoelium*), Cestodes (*Moniezia*) and Nematodes (*Haemonchus*, *Ostertagia*, *Trichostrongylus*, *Trichinella*) [1].

This drug has been successfully tested against both larval and adult forms of *Fasciola hepatica* in sheep. The efficacy of LBZ at single dose of 10 mg kg^{-1} body weight against immature and mature liver flukes of *Fasciola hepatica* was 95.2 and 95.9%, respectively [2].

The main differences in benzimidazole efficacy have been attributed to their pharmacokinetics in the host [3]. Plasma levels of these compounds are related to their absorption rate and water solubility. Thus the less-soluble benzimidazoles remain in host plasma for longer periods of time than their more soluble counterparts. Assuming the presence of an equilibrium between plasma and gastrointestinal tract, the period of time during gastrointestinal parasites are exposed to the drug is extended [4]. In addition the absorption rate of these compounds can be of therapeutic interest against tissue dwelling parasites and liver flukes, i.e. *Fasciola* and *Dicrocoelium* spp.



Scheme 1
Chemical structure of benzimidazole compounds.

On the other hand, the absorption rate constant (*Ka*) in a simple diffusion process is closely related to the chemical structure of the compounds ready to be absorbed by the gastric and/or intestinal mucose Plá Delfina *et al.* [5] have established this relationship between the *in vitro* octanol/water partition coefficients and the first order absorption rate constants for a series of related drugs. These authors have proposed a logarithmic equation that related both parameters and is useful to predict the *Ka* value from the partition coefficient for a homologous series of compounds [6].

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Therefore, due to the relevance of benzimidazole intestinal transport on their efficacy *in vivo*, we have studied the absorption of Luxabendazole in rats. Furthermore, the kinetic constants of two well known structural analogues, albendazole (ABZ) and mebendazole (MBZ) belonging to the less soluble benzimidazoles were also determined in order to compare these constants with their octanol/water partition coefficients (Scheme 1).

Materials and Methods

Experimental animals

Male Wistar rats ranging between 250 and 300 g in weight were used throughout the study. The animals were fasted for 18–20 h before surgery.

Reagents

LBZ was kindly supplied by Hoechst (Frankfurt, Germany). The sample of ABZ, was supplied by SmithKline Beecham, SAE (Madrid, Spain). HPLC grade acetonitrile and dimethylsulphoxide were supplied by Farmitalia Carlo Erba (Milan, Italy). N,N′, Dimethylformamide for UV spectroscopy, was supplied by Fluka Chemika (Buchs, Switzerland).

Absorption technique

An *in situ* rat gut preparation was made according to the technique described by Doluisio *et al.* [7] and modified by Plá Delfina *et al.* [8]. Briefly, the small intestine of animals anaesthetized with urethane (1.3 g kg^{-1} i.p.), was exposed, and an input and output cannulae was placed 30 cm downward from the pylorus in a duodeno-jejunal segment. Bile ducts were tied off in order to interrupt the enterohepatic circulation. After flushing with saline (in order to wash the internal surface of the gut), 5 ml of the test solution using DMSO as vehicle, at 37°C, was introduced into the cannulated segment. Due to their poor solubility, the drugs were dissolved in 1% DMSO [9]. At fixed time intervals after dosing, the segment was completely emptied. Samples of 0.1 ml were removed for drug assay and the solution reintroduced into the gut.

The sampling intervals were of 5 min each for a total of 30 min. The initial concentrations of LBZ and ABZ used were: 10, 100, 200, 300 and 500 mg ml^{-1}.

Drug analysis

The HPLC system comprised a Shimadzu LC 6 A solvent delivery pump, a Shimadzu C12-6A detector and Reodyne 7125 with 20-μl loop injector. Analyses were performed on a reversed-phase Nucleosil C-18 column (5 mm particle size, 20 cm × 2 mm i.d.) purchased from Teknokroma (Barcelona, Spain), for LBZ, and Nova-Pack C-18 column (4 mm particle size, 15 cm × 3.9 mm i.d.) purchased from Waters (Barcelona, Spain) for ABZ. A guard column (2 cm × 2 mm i.d.) packed with Perisorb R.P. 18 (30–40 mm pellicular), supplied by Upchurch Scientific (Oak Harbor, WA, USA), was used when biological samples were injected.

Preparation of samples. Samples were centrifuged for 15 min at 10,000*g* and a portion of 0.5 ml of the supernatant was passed through a C-18 Sep-Pack cartridge previously conditioned with 5 ml each of methanol and water. Then 2 ml of water and 1 ml of N,N′, dimethylformamide were run through the cartridge to wash out the drug.

HPLC analysis of drug. The concentrations of LBZ and ABZ in the samples were determined by reversed-phase HPLC [10]. The mobile phase consisted of acetonitrile–0.05 M phosphate buffer (pH 7.0) (40:60, v/v). The flow rate was 0.5 ml min^{-1} and the detection wavelength was 290 nm.

The octanol/water partition coefficient was determined by the method described by Irwin *et al.* [11].

Statistical analysis

All results were statistically analysed by analysis of variance (ANOVA-MANOVA, CSS Statistical). *P* values of less than 0.05 were not considered significant.

Results

Perfusion of different initial doses of both LBZ and ABZ (10–500 mg ml^{-1}) changed the corresponding drug concentration remaining unabsorbed at sampling times (5–30 min). These data were fitted to the following equation:

$$\ln (DG) = \ln (FD) - Ka\, t$$

where *D* is the amount of drug introduced into

the intestinal segment, G is the volume in the gut, F is the bioavailability, Ka the absorption rate constant and t the time post dose.

Table 1 shows that the Ka value for LBZ ranged between 0.752 and 1.065 h^{-1}, whilst the F factor ranged between 0.944 and 0.989. Similarly, Table 2 shows the values obtained for ABZ were Ka (0.833–1.058 h^{-1}) and F (0.947–1.007).

The representation of absorption rate values against their initial concentrations shows a good linearity for LBZ ($r > 0.994$). The absorption of LBZ from the intestinal lumen followed first order kinetics (Fig. 1) and the slope value of the representation of the absorption rate values initial concentration produced a diffusion constant value for this process of 0.990 h^{-1}.

Similarly, Fig. 1 shows the individual values of the apparent absorption rate constant of ABZ vs each real concentration perfused, being the diffusion constant of this drug estimated to be 0.893 h^{-1} ($r > 0.987$).

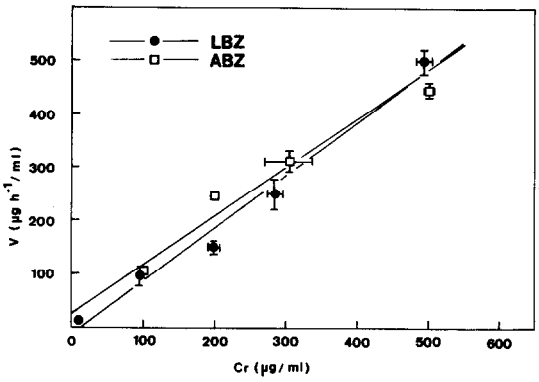

Figure 1
Relationship between the rate absorption (v) and real concentrations of LBZ (●) and ABZ (□), in perfused rat small intestine. Points represent the average ±SD of five determinations.

Data analysis shows that there are no significant differences in the apparent absorption rate constant with respect to the initial concentration perfused.

The representation of these values indicates that the intestinal absorption of LBZ may be

Table 1
Remaining concentrations of LBZ in the intestinal lumen at each sampling time (±SD, average of five animals). First-order absorption rate constants, bioavailability factor ($F1$ and correlation coefficients are also shown

	Remaining concentration of Luxabendazol in intestinal lumen (μg ml^{-1}) Initial concentration of Luxabendazol (μg ml^{-1})				
Time (min)	10	100	200	300	500
5	8.85 ± 0.31	89.6 ± 1.60	182.3 ± 12.4	273.3 ± 3.8	455.9 ± 10.3
10	7.89 ± 0.43	85.8 ± 4.7	171.8 ± 10.8	252.9 ± 2.0	418.1 ± 11.4
15	7.06 ± 0.26	74.1 ± 2.6	158.6 ± 8.8	244.5 ± 9.7	397.6 ± 21.5
20	6.69 ± 0.10	68.2 ± 2.2	149.8 ± 7.7	222.1 ± 4.7	383.2 ± 29.7
25	6.13 ± 0.30	65.5 ± 3.2	145.7 ± 8.3	201.4 ± 1.2	351.5 ± 42.6
30	5.63 ± 0.14	59.8 ± 0.9	133.1 ± 5.83	186.8 ± 5.8	296.9 ± 9.1
Ka (h^{-1})	1.065 ± 0.081	0.999 ± 0.090	0.752 ± 0.044	0.913 ± 0.071	1.043 ± 0.126
F	0.944 ± 0.042	0.961 ± 0.036	0.982 ± 0.046	0.986 ± 0.025	0.989 ± 0.024
r	0.988 ± 0.003	0.995 ± 0.059	0.997 ± 0.015	0.995 ± 0.001	0.991 ± 0.005

Table 2
Remaining concentrations of ABZ in the intestinal lumen at each sampling time (±SD, average of five animals). First-order absorption rate constants, bioavailability factor ($F1$ and correlation coefficients are also shown

	Remaining concentration of Albendazole in intestinal lumen (μg ml^{-1}) Initial concentration of Albendazol (μg ml^{-1})				
Time (min)	10	100	200	300	500
5	9.25 ± 0.01	89.9 ± 0.6	180.4 ± 0.30	284.3 ± 5.02	445.8 ± 3.2
10	8.52 ± 0.30	80.1 ± 0.8	165.5 ± 0.12	261.2 ± 3.03	413.2 ± 4.6
15	7.83 ± 0.34	74.3 ± 3.2	147.4 ± 1.61	241.3 ± 12.3	387.8 ± 4.2
20	7.21 ± 0.31	71.1 ± 4.1	130.6 ± 0.04	218.6 ± 10.5	362.3 ± 3.3
25	6.81 ± 0.42	68.1 ± 5.7	124.1 ± 1.21	198.7 ± 15.9	337.8 ± 3.5
30	5.92 ± 0.22	55.6 ± 0.2	188.3 ± 0.97	185.5 ± 14.9	316.6 ± 3.6
Ka (h^{-1})	1.058 ± 0.079	1.052 ± 0.080	1.288 ± 0.040	1.049 ± 0.100	0.833 ± 0.043
F	1.002 ± 0.001	0.973 ± 0.009	1.007 ± 0.022	1.031 ± 0.008	0.947 ± 0.007
r	0.997 ± 0.007	0.995 ± 0.040	0.998 ± 0.021	0.998 ± 0.014	0.998 ± 0.001

due to a kinetic mechanism of simple diffusion and therefore no energy-dependent saturable kinetic is involved.

Discussion

Plasma levels of orally administered benzimidazolic compounds are demonstrative of their antiparasitic activity in animals, hence the determination of the absorption process and their kinetic constants is of importance to discover how these compounds reach the blood. The absorption process of LBZ has been studied *in vitro* in rat gut, and the absorption rate constant has been estimated to be 0.990 h^{-1}, which compares closely with that calculated for ABZ, $Ka = 0.893\ h^{-1}$ (a well-known benzimidazolic compound). This similar behaviour can be explained due to their close structural similarity and their low solubility in water, manifested in their high octanol/water partition coefficient, chemical parameter closely linked to drug diffusibility through biological membranes (Table 3). However, the *Ka* previously reported for mebendazole (MBZ), another less soluble benzimidazolic compound, was significantly larger using the same tissue sample [12]. When the absorption rate constants of these three compounds are compared with their octanol/water partition coefficients (Table 3) show a direct relationship between both parameters, being the *Ka*/*Kr* ratio *ca.* 2 for the benzimidazoles used in present study and MBZ.

There is scarce information about intestinal absorption of benzimidazolic compounds in other organisms to compare with our results. However, del Estal *et al.* using different ionic and nonionic surfactants like sodium dodecyl sulphate and Tween 80 [13] and bile salts like sodium taurocholate [14] found an improvement of the absorption rate of ABZ reflected in a significant increase of its *Ka* in a rat *in vitro* model.

It is worth noting the relationship found between the absorption constants of LBZ and ABZ and their octanol/water partition coefficients when compared with that calculated for the less soluble benzimidazole MBZ [12]. Plá Delfina *et al.* [5, 6] have previously reported the existence of parabolic patterns between the octanol/water partition coefficient and the diffusibility through biological membranes for a large series of chemically analogous structures. Although it is very early to establish a similar plot to that obtained by these authors, it may be advisable to complete it with all the benzimidazolic structures available at this time.

Table 3
Values of the octanol/water partition coefficients (*Kr*), absorption rate constants (*Ka*), and relationship between both parameters (*Kr*/*Ka* ratio)

Drug	*Kr*	*Ka*	*Kr*/*Ka*
Luxabendazole	1.9	0.990	1.92
Albendazole	1.8	0.893	2.02
Mebendazole*	2.7	1.270	2.12

* Del Estal [12].

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