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Lymphatic Bioavailability of Diazepam and Desmethyldiazepam in the Rat

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Summary

The lymphatic bioavailability (F_L) of diazepam (DZ) and its major metabolite desmethyldiazepam (DDZ) was studied. DZ was administered in intravenous and intraduodenal boluses, and in intravenous infusion in three groups of rats with different total lipid (TL) content in the central lymph. The effect of a) different lipophilicity of DZ and DDZ, b) lymphatic TL content, and c) route of DZ administration on F_L was determined. It was found that a) F_L values of DZ exceeded the F_L values of DDZ and b) F_L values of DZ increased with increasing TL content in the lymph (an opposite relation was found in DDZ), and c) the highest F_L value of DZ + DDZ sum after intravenous bolus administration was attained contrary to the lowest one after intraduodenal bolus administration.

Key words

Diazepam – Desmethyldiazepam – Lymphatic bioavailability

Introduction

The factors affecting lymphatic bioavailability (F_L) were studied in our previous papers using model drugs in the form of parent molecules (Lamka *et al.* 1990, Lamka *et al.* 1991). Some of these drugs are easily biotransformed and in the sum of parent molecules and metabolites distributed in the organism and excreted. Thus, metabolites are also distributed into the lymphatic system. The F_L values of parent molecules in comparison to their metabolites can be very different due to the unequal physico-chemical characteristics of these molecules. To verify this assumption, we followed the F_L values of diazepam (DZ) and its major metabolite desmethyldiazepam (DDZ) after DZ administration in rats. We were interested in a) the F_L values of DZ and DDZ molecules, b) the effect of lymphatic TL content on F_L of DZ and DDZ, c) the effect of the route of administration of DZ on the DZ and DDZ lymphatic bioavailability.

Materials and Methods

Animals

The experimental procedure was described in detail in a previous paper (Lamka *et al.* 1989). Briefly, three groups of rats were prepared in which different total lipid (TL) contents in their central lymph were achieved by means of different diets (unfed group – the lowest TL content, fed group – the mean TL content, oil-fed group – the highest TL content). The rats were anaesthetized before the experiment (Pentobarbital inj., Spofa). The carotid artery and thoracic duct in the neck were cannulated and the blood and lymphatic samples were collected at regular intervals.

Routes of DZ administration

DZ was administered in bolus intravenously (i.v.) and, following laparotomy, intraduodenally (i.d.) in a dose 5 $mg.kg^{-1}$, or in infusion (blood steady-state concentrations 0.4–0.5 $mg.l^{-1}$) (details see Lamka *et*

al. 1990). The concentrations of DZ and its metabolite DDZ were determined in the blood and lymphatic samples.

Determination of DZ and DDZ

DZ and DDZ in the blood and lymph were determined simultaneously by gas-liquid chromatography (GLC). GLC was performed using a Packard Model 428 gas chromatograph with an ECD-detector and a 3 % OV-17 on GasChromQ 80-100 column (glass, 2 mm I.D. x 1 m). The working temperature of the column was 245 °C, the flow rate of carrier gas nitrogen was 20 $ml.min^{-1}$. DZ and DDZ were extracted from the biological material into a double amount of benzene containing griseofulvin as the internal standard. The calibration curve of the method was linear in the concentration range 0.04–5.00 $mg.l^{-1}$ for both substances, the determination RSD was 4 %. The detection limits of the method with 50 μl samples were 0.02 and 0.04 $mg.l^{-1}$ for DZ and DDZ, respectively.

Determination of polarity of model drugs

The polarities of DZ and DDZ were compared on the basis of different behaviour of these drugs in HPLC analysis on octylsilane and octadecylsilane columns. The more polar substances exhibited lower retention times than substances with lower polarity in reversed-phase HPLC analysis on the above mentioned columns, so the lipophilicity of substances can be compared on the basis of their retention times.

A relation of DZ and DDZ retention times on a RP-HPLC analysis was determined on SP8000 Liquid Chromatograph (Spectra-Physics, USA) equipped with Spectroflow Monitor SF770 (Schoeffel, USA) UV-detector with a set wavelength at 254 nm. The analyses were performed on the octylsilane column RP-8 10 μm (stainless-steel column 3.3x250 mm, Spectra-Physics, USA) and on the octadecylsilane column Separon SGX C18 7 μm (glass column 3.3x150 mm, Tessek, CZ). A methanol-water (50:50) mixture, flow rate 0.5 $ml.min^{-1}$ was used as the mobile phase. The standard solutions of DZ, DDZ and DZ+DDZ in mobile phase in concentration of 10 $mg.l^{-1}$ and 10+10 $mg.l^{-1}$ respectively, were applied into the columns in 10 μl samples. The retention times of DZ and DDZ were determined under the above described conditions and then compared. The measurements were done in triplicates.

Mathematical evaluation of the results

Using Biofit software, the areas under the blood (AUC_B) and lymphatic (AUC_L) concentration curves were calculated for time intervals 0–120 min and 0–115 min, respectively, in cases of bolus administration. The time interval for infusion administration of DZ used for the calculation of AUC_B and AUC_L was 0–85 min. The F_L values of DZ and DDZ were calculated as the AUC_L/AUC_B ratio (Lamka *et al.* 1990). The statistical significance of differences in the mean values of the experimental parameters was evaluated by the unpaired t-test, the significance level was $P<0.05$.

Results

Determination of the lymphatic TL content.

The significantly different TL values were found in lymphatic samples depending on the experimental group investigated: unfed group 5.35 ± 1.13 $g.l^{-1}$, fed group 15.63 ± 3.65 $g.l^{-1}$, and oil-fed group 44.00 ± 5.44 $g.l^{-1}$.

Determination of DZ and DDZ polarity.

DDZ exhibited shorter retention times than DZ in both types of reversed-phase chromatographic columns (see Table 1). These results confirmed that DDZ had a higher polarity (lower lipophilicity).

Lymphatic availability of DZ and DDZ.

All the routes of DZ administration used led to the rapid formation of DDZ, the blood and lymphatic concentrations of DDZ exceeded the detection limit value of this drug (except in the case of i.d. administration at the time interval 85–115 min) (see Figs 1–3). The concentration curves of DDZ resembled the course of blood concentration curves of parent DZ with a slight time delay. The resulting F_L values are presented in Table 2.

Effect of DZ and DDZ lipophilicity.

All differences between the availabilities of parent drug DZ and its metabolite DDZ were statistically significant (with the exception of intravenous infusion in the unfed group), and the availability of DDZ was mostly lower than that of DZ.

Effect of the lymphatic quality.

The F_L values of DZ increased with increasing TL content in contrast to DDZ, the availability of which decreased with increasing TL content (with the exception of i.v. administration) (see Table 2).

Effect of the routes of administration.

Depending on the different routes of administration, F_L values of both DZ and DDZ were significantly different in the most cases. The highest F_L values for DZ and DDZ sum were found after i.v. administration, the lowest ones were obtained after i.d. administration.

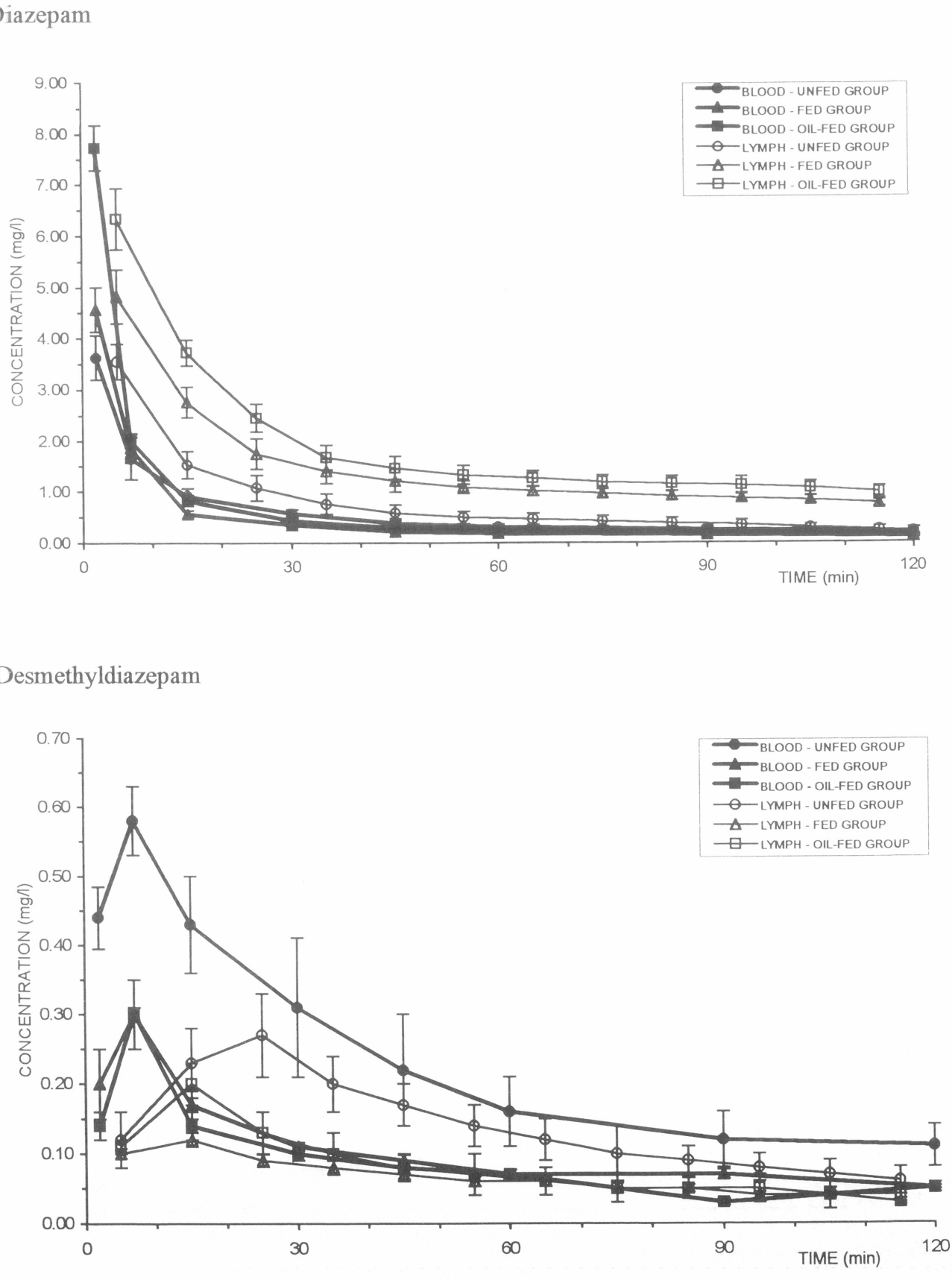


Fig. 1
Diazepam and desmethyldiazepam blood and lymphatic concentrations – intravenous bolus administration

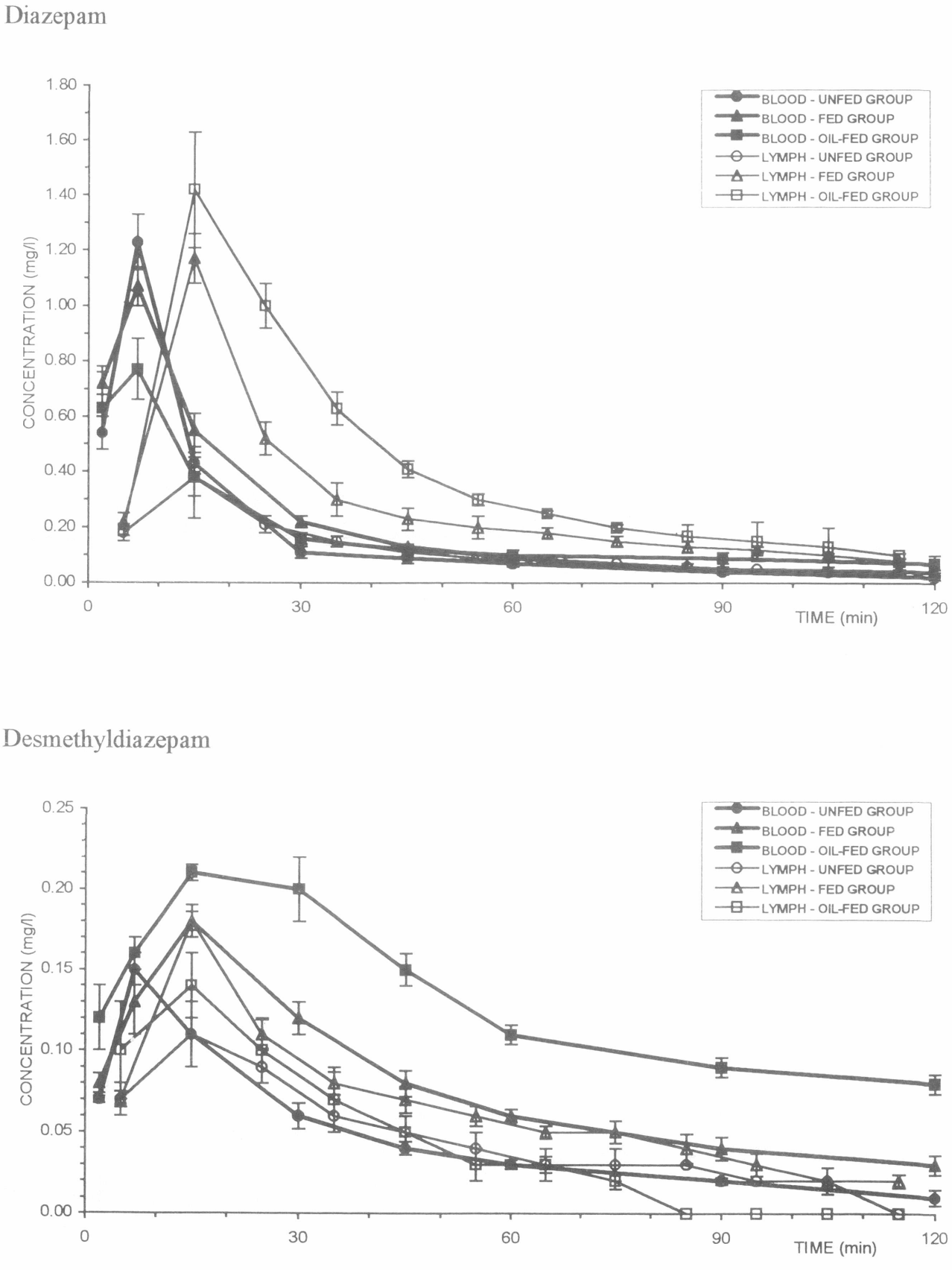
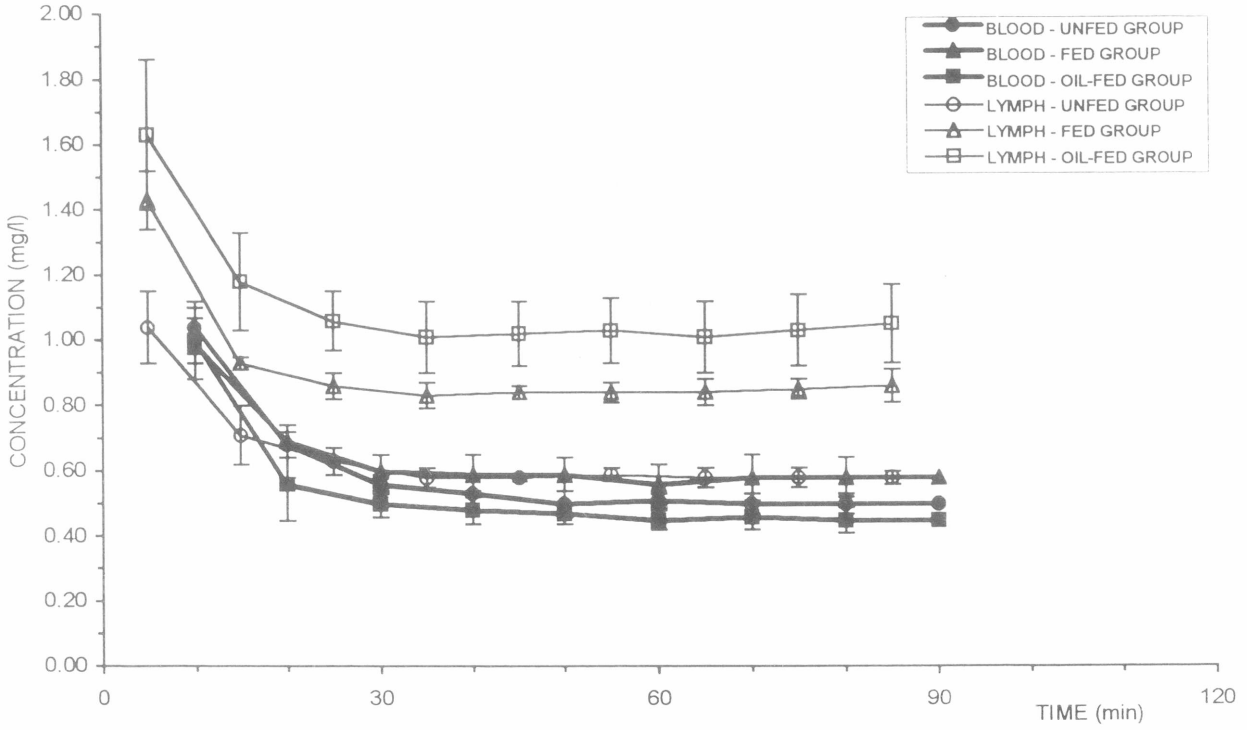


Fig. 2
Diazepam and desmethyldiazepam blood and lymphatic concentrations – intraduodenal bolus administration

Diazepam



Desmethyldiazepam

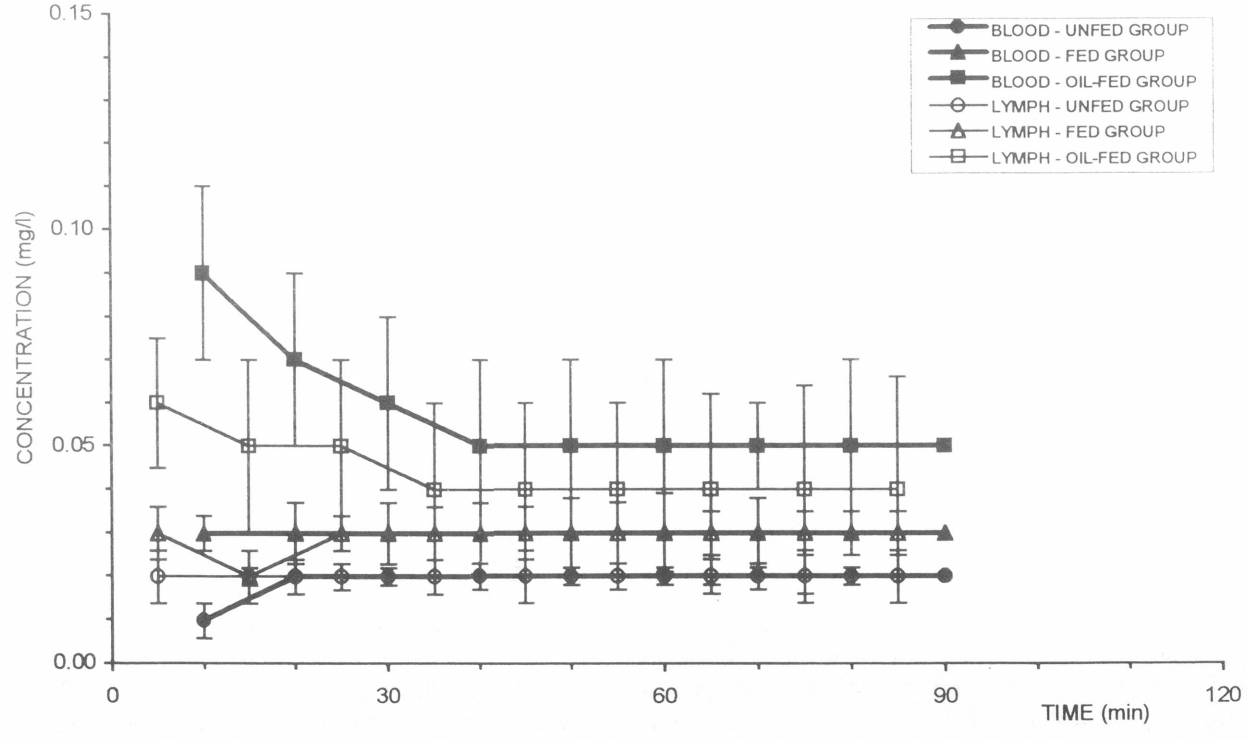


Fig. 3
Diazepam and desmethyldiazepam blood and lymphatic concentrations – intravenous infusion administration

Table 1
Retention times of diazepam (DZ) and desmethyldiazepam (DDZ)

	Retention time (min)			
Column type	DZ (n=3)	DDZ (n=3)	DZ+DDZ (n=3)	
Octylsilane	15.48 ±0.01	13.77 ±0.01	15.50 ±0.01	13.77 ±0.01
Octadecylsilane	5.45 ±0.02	4.42 ±0.02	5.45 ±0.01	4.41 ±0.01

Data are mean ± S.D., n = number of assays

Table 2
Lymphatic bioavailability (F_L) of diazepam (DZ) and desmethyldiazepam (DDZ) in the rat

Route of DZ administration	Experimental group	Number of animals	F_L DZ	DDZ	Mean of DZ + DDZ
Intravenous (Bolus)	Unfed	8	1.51±0.19	0.69±0.09	
	Fed	6	3.22±0.27	0.66±0.07	
	Oil-fed	7	2.95±0.15	0.84±0.16	
Mean			*2.56±0.20*	*0.73±0.11*	*1.64±0.15*
Intravenous (Infusion)	Unfed	6	1.13±0.05	1.25±0.21	
	Fed	5	1.47±0.06	0.96±0.30	
	Oil-fed	6	2.22±0.40	0.80±0.20	
Mean			*1.61±0.17*	*1.00±0.24*	*1.30±0.20*
Intraduodenal (Bolus)	Unfed	6	0.75±0.11	0.98±0.19	
	Fed	6	1.36±0.05	0.80±0.07	
	Oil-fed	6	2.19±0.18	0.36±0.03	
Mean			*1.43±0.11*	*0.71±0.10*	*1.07±0.10*
Mean			1.87±0.16	0.81±0.15	

Data are Mean ± S.D.

Discussion

The lymphatic bioavailability of some model drugs and the effect of their different lipophilicity on the F_L values was reported in our previous papers comparing F_L values of structurally very different molecules (diazepam, inulin, o-I-hippurate, o- and p-I-benzoates) (Lamka *et al.* 1991). Using DZ and its metabolite DDZ in this experiment enabled us to evaluate the effect of lipophilicity by comparing the lymphatic bioavailabilities of molecules with a very similar structure.

The polarity of DZ expressed as a partition coefficient in the octanol-water system was reported to range from 2.66 to 2.86 (Hansch and Leo 1979), only one reference mentioned the polarity of DDZ to be 2.94 (Golovenko 1976). Unfortunately, no paper refers to simultaneously determined polarity values for both DZ and DDZ. It was thus impossible to make any conclusions on DZ and DDZ polarity relations based

on published sources. This is the reason why we carried out HPLC determinations of DZ and DDZ polarities simultaneously. Considering their chromatographic behaviour, we concluded that the polarity (of DDZ is higher the lipophilicity is lower) as compared to DZ.

The effect of DZ and DDZ lipophilicity on their distribution into the lymph was most marked in comparison with mean F_L values of both drugs, where F_L values of DZ exceeded F_L values of DDZ when administered by whatever route. This finding is a result of the lower DDZ lipophilicity and consequently of its lower ability to become distributed in the rat central lymph in contrast to DZ.

The F_L values of DZ increased with increasing TL content in the lymph, the opposite relation was evident in DDZ. This is the general conclusion of the effect of different lymphatic quality on the F_L parameter studied. This characteristic reflects a state, that the more lipophilic is the medium for distribution of both lipophilic and hydrophilic molecules, the more lipophilic molecules are attracted and the more hydrophilic molecules are rejected.

The highest F_L value of the sum of model drugs DZ+DDZ was reached on i.v. administration (bolus), contrary to the lowest F_L values on i.d. administration. This result corresponds with the rate of DZ administration into the rat organism. In the case of high rate of administration, the drug was distributed within whole organism with a high and fast saturation of all fluids and tissues by the parent molecules of drug. These molecules also reached the lymphatic system to be later slowly eliminated with the lymph (lipids in chylomicrons). The remaining high concentration of DZ was established there by this process. At the same time, DDZ, a metabolite with a lower but still high enough lipophilicity for absorption into the chylomicrons, was quickly formed. Thus, the distribution of DDZ (the concentration courses) was similar to that of parent DZ molecules. This relation is the most evident on i.v. (bolus) administration, less evident on infusion or intestinal bolus administration. From the point of view of the lipophilic agent targeting into the lymphatic system, i.v. administration (bolus) provides the highest availability in the mentioned system.

It can be assumed that three factors mentioned above affecting the distribution of DZ and DDZ (different lipophilicity of DZ and DDZ, lymphatic TL content, route of DZ administration) are not the only ones involved in the overall pharmacokinetics. The effect of different binding of DZ and DDZ into the blood components (plasmatic proteins, erythrocytes) and the effect of lipids as binding competitors also have to be taken into consideration.

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Reprint Requests

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