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Pharmacokinetics of ortho-I-hippurate in the Blood and Central Lymph of the Rat

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Summary

The authors studied ortho-I-hippurate kinetics in the blood and central lymph in two groups of intact rats and three groups of animals with induced pathological states (cirrhosis, uraemia, malabsorption). A differentiated lipid concentration in the central lymph was induced in intact animals by depriving them of food (the unfed group) or allowing them food (the fed group) before the experiment. All the hippurate kinetic parameters, including lymphatic bioavailability (F_L), in the fed group were very close to those in the unfed group, which was also used as the control for the groups with induced pathological states. Cirrhosis, uraemia and malabsorption altered the blood and lymphatic kinetic parameters in many cases, but the changes mostly followed a parallel course so that F_L was maintained (except in the uraemia group, in which it fell).

Key words
Central lymph – ortho-I-hippurate – Pharmacokinetics – Bioavailability

Introduction

One of the factors which can markedly modify the pharmacokinetics of drugs is a pathological state of the organism. This has been discussed by many authors (e.g. Janků *et al.* 1986). Disorders of liver, kidney, cardiac and thyroid function have mainly been presented; pathological states influencing interstitial fluid formation and the lymphatic circulation have been described far less. Studies in small laboratory animals include (for example) those by Kotani *et al.* (1967) in rabbits with cirrhosis of the liver, by Bloom *et al.* (1978) in rats with biliary obstruction and by Jonsson *et al.* (1979) in rabbit limbs inflamed by scalding. All the above and similar studies, however, utilized the pathological state to study its effect on the quantitative and qualitative characteristics of the relevant type of lymph.

Description of direct pharmacokinetics in the lymphatic system in pathological states are even rarer. Deak and Csaky (1984) studied the effect of cirrhosis on the transport of model substances across the plasma-lymph barrier, Roberts *et al.* (1979) described the penetration of antibiotics into the peripheral lymph of an inflammation-damaged limb (by a bacterial noxa) and De Marco and Levine (1969) demonstrated an increase in the lymph-absorbable portion of para-aminosalicylic acid after obstruction of the superior mesenteric vein. These are the only studies known to us and it seems safe to say that the above problem is not an object of experimental attention.

Our laboratory, which studies pharmacokinetics in the lymphatic system, has included the effect of pathological states in its investigation of factors able to influence pharmacokinetics. In a previous study we demonstrated that pathological states (cirrhosis, uraemia, malabsorption) had a marked effect on the flow and composition of the lymph (Lamka *et al.* 1986) and the same experimental states were used in the present study to investigate the kinetics of the model drug ortho-I-benzoate. The influence of the lipid content in the lymph was also studied.

Material and Methods

Animals. The experiments were carried out with Wistar rats weighing 240–280 g. Except for the unfed group, which was deprived of food 18 h beforehand, the animals were allowed food *ad libitum* up to the start of the experiment; all the animals were allowed water ad libitum.

The rats were divided into five groups. The first (unfed) group acted as the control. The second (intact animals with unrestricted access to food) was termed the fed group. The groups with pathological states were termed cirrhotic (chronic administration of CCl_4), uraemic (a single dose of uranyl nitrate) and malabsorptive (a single dose of methotrexate). The methods have already been described in details (Lamka *et al.* 1986).

Cannulation, sampling. The experiments were performed under pentobarbitone general anaesthesia (Pentobarbital inj. Spofa, i.p., 35 mg/kg). Blood samples were taken from one of the carotid arteries and lymphatic samples from the thoracic duct in the region of the neck (details see in Lamka *et al.* 1986). Blood samples were collected at intervals of 2, 7, 15, 30, 45, 60 and 120 min, lymphatic samples at 10-min intervals.

Model drug administration. The drug was ortho-^{125}I-hippurate sodium (Nuclear Research Center, Řež; volume activity 20 MBq/ml, chemical concentration 10 mg/ml, radiochemical purity over 97 %) that was injected in a dose of 1 mg/kg into the v. saphena as a bolus.

Mathematical evaluation. A pharmacokinetic analysis was carried out with a Biofit programme (Trilobyte Inc. Prague 1990) on a personal computer. It was based on the two-compartment model of the behaviour of substances in the organism as demonstrated in preliminary experiments. The basic kinetic parameters, computed from the blood and lymphatic concentration curves, were used to compute the derived parameters – the half-time of distribution ($T_{1/2(\alpha)}$) and elimination phase ($T_{1/2(\beta)}$), the time when the maximum concentration was attained in the lymph (T_{max}), total clearance (Cl_{tot}), distribution volume ($V_{d(area)}$) and the areas under the lymphatic (AUC_L) and blood (AUC_B) concentration curves. Lymphatic bioavailability was computed as the AUC_L/AUC_B ratio.

The numerical values are given as means ± the standard deviation. The means were computed from the experimental and mathematically derived data for the individual animals. Statistical significance of the differences was determined by Student's t-test, comparing the blood and lymphatic values in the experimental groups with those in the control group.

Results

The blood kinetic parameters of hippurate in control group (Tab. 1) confirm experimentally its properties already demonstrated (Lázníček and Květina 1984) and particularly that it is a substance with short half-times, so that the AUC value is correspondingly low. The $V_{d(area)}$ value indicates that it also penetrates intracellularly (its accumulation in the plasma on the basis of low transport binding is unlikely – Lázníček *et al.* 1987). The lymphatic half-times are very similar to those for the blood; the T_{max} values are low and AUC_L is mildly higher than AUC_B. The concentration curves are very similar to each other (Fig. 1).

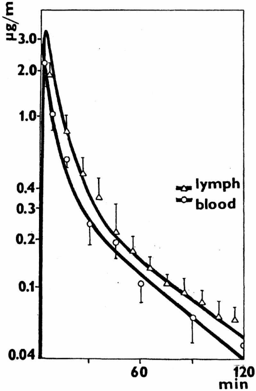

Fig. 1.
Ortho-I-hippurate concentrations in the blood and central lymph of the rat.

The chronic administration of CCl_4 (the cirrhotic group) was manifested in blood parameters by a decrease in $T_{1/2(\beta)}$ and the AUC and by an increase in Cl_{tot}. The lymphatic parameters did not differ markedly (except T_{max}) from the control values and F_L attained a mean value of 1.51 ± 0.31.

The administration of uranyl nitrate (the uraemic group) caused massive damage to the renal tubules. This impairment of the eliminative capacity of the organism was clearly manifested by lengthening of $T_{1/2(\beta)}$), leading to an increase in both AUC and a decrease in Cl_{tot} and $V_{d(area)}$. Interesting among the lymphatic parameters is the relatively shorter $T_{1/2(\beta)}$ as compared to the respective blood value what results consequently in a low F_L value.

Table 1
Kinetic parameters of ortho-I-hippurate in blood and lymph

Experimental group	n	Biological fluid	$T_{1/2(\alpha)}$ (min)	$T_{1/2(\beta)}$ (min)	Cl_{tot} (ml/min/kg)	$V_{d(area)}$ (ml/kg)	T_{max} (min)	AUC (μg/ml/min)	F_L
Controls (unfed)	6	Blood	3.96 ± 0.42	35.48 ± 5.94	24.18 ± 2.53	1237.8 ± 131.1	–	41.70 ± 4.09	1.29 ± 0.15
		Lymph	5.75 ± 0.91	38.04 ± 10.90	–		1.52 ± 0.28	53.88 ± 9.33	
Cirrhotic	6	Blood	3.77 ± 0.85	24.06* ± 8.31	35.80** ± 9.61	1434.8 ± 325.0	–	24.15*** ± 5.14	1.51 ± 0.31
		Lymph	5.45 ± 1.42	50.95 ± 12.13	–		3.82*** ± 0.93	36.16* ± 10.35	
Uraemic	6	Blood	7.20** ± 1.31	370.70*** ± 140.10	1.41*** ± 0.41	689.2*** ± 89.1	–	601.41*** ± 71.93	0.95* ± 0.19
		Lymph	3.98 ± 1.77	280.08*** ± 92.30	–		1.96 ± 0.41	545.50*** ± 116.10	
Malabsorptive	7	Blood	4.08 ± 0.59	78.40** ± 23.70	13.17** ± 6.09	1067.8 ± 412.8	–	89.97* ± 36.40	1.28 ± 0.21
		Lymph	6.36 ± 2.43	90.40** ± 31.80	–		1.83 ± 0.26	114.90* ± 49.20	
Fed	6	Blood	3.78 ± 0.40	30.13 ± 6.12	22.50 ± 1.89	1178.6 ± 165.3	–	44.43 ± 5.85	1.35 ± 0.17
		Lymph	8.15 ± 1.12	43.30 ± 9.13	–		1.70 ± 0.30	60.27 ± 8.12	

* – $p<0.05$

** – $p<0.01$

*** – $p<0.001$

n – Number of animals

$T_{1/2(\alpha)}$ – *distribution half-time*

$T_{1/2(\beta)}$ – *Elimination half-time*

Cl_{tot} – *Total blood clearance*

T_{max} – *time of attainment of maximum lymphatic concentration*

AUC – Area under concentration curve

F_L – *Lymphatic bioavailability*

$V_{d(area)}$ – *Distribution volume*

The administration of methotrexate induced a state of malabsorption. Lengthening of the blood $T_{1/2(\beta)}$ led to an increase in AUC_B and a decrease in Cl_{tot}. The lymphatic parameters showed a similar shift; F_L was the same as in the control group.

Kinetic parameters (including F_L) in the fed group were practically identical to those in the control (unfed) group.

Discussion

Hippurate was chosen for our experiments as a model drug with a small molecule (length 1.24 nm) (Motais 1977). Because of their small size, it is easy for hippurate molecules to be transferred to the interstitium and from there into the lymph, where their concentration is very quickly stabilized at values similar to those in the blood as regards both the time course and the absolute values. The lymphatic concentration curve remains throughout at mildly higher concentration values; F_L attained a value of 1.29. An explanation of this F_L can be found in the very rapid decrease of the blood concentration. Despite the brief interval for the lymph formation, mildly higher values of lymphatic concentrations were demonstrated with some delay.

After the induction of cirrhosis, changes in the distribution of hippurate into the lymph, expressed as the F_L value, were minimal, but course of the concentration curves altered. The blood kinetic parameters were indicative of interference with the blood circulation (acceleration) – a finding also described in patients with cirrhosis ascites (Pacovský *et al.* 1986). It is also possible, however, that reduced transport binding of hippurate had an effect in this pathological state, with a resultant impact on excretion as described by Lázníček *et al.* (1987). Lengthening of the lymphatic $T_{1/2(\beta)}$ is determined by the appearance of intestinal oedema with the development of ascites after the disturbance of flow relationships in the liver (Witte *et al.* 1981). The lymphatic concentrations were stabilized with a more pronounced delay than in the control group (see also the longer T_{max}). The resultant F_L was higher than in intact animals, although the difference was not statistically significant.

Minimal Cl_{tot} values are typical in acute uraemia. Owing to a marked decrease in renal hippurate excretion the drug is retained in the organism (lengthening of $T_{1/2(\beta)}$). The relatively lower AUC_L in relation to AUC_B in a comparison with intact rats is stabilized through the effect of the large amount of fluid which collects in the renal capsula and the surrounding serous membranes. The fluid here is under pressure and the superfluous interstitium fluid is drained away in large quantities *via* the renal lymphatic vessels into the thoracic duct. Hippurate, which is transported passively in the fluid, reaches the central lymphatic vessel in concentrations corresponding to its blood concentrations. Because of the very slow decrease in the blood concentrations, the situation typical of the control group does not develop in uraemic rats, in which F_L is consequently lower.

In malabsorption, some of the blood kinetic parameters likewise differ from the control values. The changes in Cl_{tot} and both $T_{1/2(\beta)}$ are indicative of lowered excretory performance – described in the literature after the administration of methotrexate (Fox 1979). A comparison of the blood and the lymphatic $T_{1/2(\beta)}$ with the corresponding values in the control group shows that mean values of the lymphatic half-time are relatively lengthened. This finding evidently corresponds to the formation of oedema in the intestinal mucosa described in an earlier paper (Lamka *et al.* 1986). Nevertheless, the mean F_L is at the control level.

The total lipid content of the central lymph can be modified by food deprivation or free access to food and the lipids can then markedly influence the distribution of lipophilic substances in the lymph (Lamka *et al.* 1989). As anticipated, no such effects were demonstrated in the case of hydrophilic substances of the type of hippurate.

In general, it can be claimed that, despite the marked changes brought about in hippurate kinetics by induced pathological states, lymphatic bioavailability was maintained (except in the uraemic group) at the same level as in healthy animals.

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