Distribution of Subcutaneously Administered Inulin between Blood and Peripheral Lymph in the Rabbit

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Abstract—In experiments on rabbit peripheral lymph, the contribution of the blood and lymphatic system to the whole-body distribution of inulin after subcutaneous administration has been investigated and the effects of hyaluronidase and of thermal stimulus at the administration site examined. Inulin concentrations in lymph exceeded plasma concentrations by more than 100-fold. At the end of the experiment (90 min) the amount of drug in the total lymph collected was about one-seventh the amount found in urine. The blood system, as a result of higher circulation at the administration site distributes inulin from the subcutis more rapidly than does lymph. Hyaluronidase did not influence inulin concentrations in blood and lymph but thermal stimulus significantly decreased both concentration and total distribution. The decrease resulted from a developed oedema and vasoconstriction in the skin and subcutis of the cannulated extremity.

Lymph and lymphatics participate in the biodistribution of substances naturally occurring in the body as well as xenobiotics. This distribution is influenced by factors such as the size of the drug molecule (Grotte 1956; Olszewski & Engeset 1978), the pathological state of the organism or tissue (DeMarco & Levine 1969; Deak & Csaky 1984), or the physicochemical properties of xenobiotics and the composition of lymph (Lamka et al 1989).

The property of the lymphatics in their ability to accept large drug molecules is utilized in indirect lymphography. Preclinical testing of the distribution of the lymphographic carrier is either by scintigraphy (Henze et al 1982) or, as reported by the present authors (Lamka et al 1986), by direct determination of lymphatic and blood concentrations of drugs.

In the study of the distribution of drugs between blood and lymph, inulin has been used as a model drug. The present paper aims at investigating the behaviour of inulin in peripheral (hind limb) rabbit lymph after its subcutaneous (s.c.) administration to intact anaesthetized animals, and to evaluate the effects of hyaluronidase and thermal damage.

Materials and Methods

Animals
Female Chinchilla rabbits, 2·9–4·0 kg, were fed a standard diet and had free access to water. They were divided into three groups. In the first group inulin was administered alone (Inulin), in the second, inulin was administered with hyaluronidase (Inulin + Hya), and in the third inulin was administered to animals prepared according to Jonsson et al (1979) (Inulin + Inf).

The rabbits were anaesthetized by i.v. pentobarbitone (Pentobarbital SPOFA) at an initial dose of 30–35 mg kg^{-1} and subsequent doses as needed.

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Cannulation of vessels
One of the common carotid arteries of the anaesthetized animal was cannulated and heparin in 0·9% NaCl (saline) (150 int. units kg^{-1}) administered. In the proximal part of the thigh the femoral artery and vein were exposed, the primary femoral lymphatics (Courtice 1959) ligated, and the main lymphatic cannulated; the cannula was held in place with Histoacryl blue (B. Braun, Melsungen AG).

Withdrawal of samples
Lymph was collected at 10 min intervals. The flow was maintained by passive movement of the limb, i.e. at 3 min intervals the limb was flexed and extended five times with slight manual massage of the popliteal nodes. Blood was withdrawn at 2, 7, 15, 30, 45, 60 and 90 min, centrifuged and the inulin concentration determined in the plasma.

At the end of the experiment (at 90 min), urine was quantitatively withdrawn from the urinary bladder.

The flow of lymph was determined from the volumes of collected lymph at the individual withdrawal intervals.

Model drug administration
Inulin (inulin purissimum, Laevosan-Gesellschaft Linz/Donau) was administered (s.c. 50 mg kg^{-1}) into the dorsum of the paw. The total volume of solution (0·5 mL kg^{-1}) was divided into two identical doses administered in close proximity. Hyaluronidase was administered in a dose of 0·3 mg kg^{-1} as a mixed solution with inulin.

Determination of inulin and total proteins
Inulin in plasma, lymph and urine was determined biochemically (Homolka 1971); the original method was adapted to a sample volume of 0·1 mL. Total proteins of lymph and plasma were determined using the Bio-La-Total Proteins Set (Lachema Brno).

Mathematical evaluation of results
Areas under the concentration curves of plasma (AUC$_P$) and lymph (AUC$_L$) of the individual animals were determined at intervals during the experiment (Reinsch 1967). The values

obtained were averaged and analysed for statistical significance of differences (Student's t-test). Comparison was always between the Inulin group and the Inulin + Hya and Inulin + Inf groups, respectively.

Results

Distribution of inulin from the subcutis of the rabbit hind limb is mediated both by the blood and lymphatic system (Figs 1, 2). Lymphatic concentrations always exceeded blood concentrations, the differences being greater in the Inulin and Inulin + Hya groups than in the Inulin + Inf. group.

AUC values correspond to the different inulin concentrations in plasma and lymph (Table 1). AUC_L is larger than AUC_P by about 100 times (Inulin); the ratio does not change
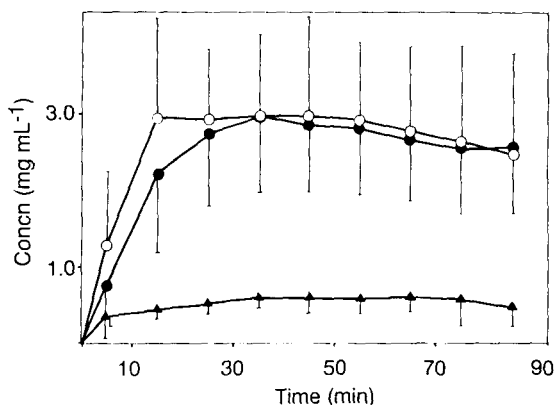

FIG. 1. Inulin concentrations in lymph of experimental groups. Symbols: group administered inulin alone O——O, a combination of inulin + hyaluronidase ●——●, and inulin alone + thermal stimulus ▲——▲.
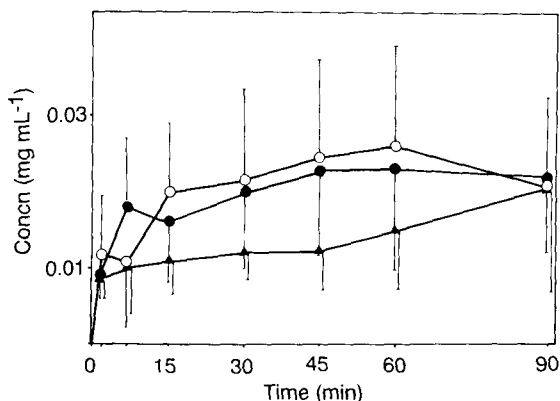

FIG. 2. Inulin concentrations in plasma of experimental groups. For further explanation see text to Fig. 1.

Table 1. Areas under the concentration curves (AUC) of experimental groups.

Experimental group	n	Biological fluid	AUC (mg mL^{-1} min)
Inulin	13	Lymph	226·40 ± 74·82
		Plasma	1·97 ± 1·30
Inulin + Hya.	10	Lymph	201·11 ± 66·12
		Plasma	1·83 ± 0·88
Inulin + Inf.	6	Lymph	44·62 ± 13·38***
		Plasma	1·19 ± 0·51

n—Number of animals. ***—$P < 0.001$.

Table 2. Total protein concentrations in plasma and lymph and lymphatic flow of experimental groups.

Experimental group	n	Concentration (g L^{-1}) Plasma	Lymph	Lymphatic flow (mL h^{-1} kg^{-1})
Inulin	5	52·12 ± 4·80	22·09 ± 4·25	0·458 ± 0·112
Inulin + Hya.	5	50·08 ± 4·12	23·64 ± 6·23	0·667 ± 0·253
Inulin + Inf.	5	53·55 ± 2·50	42·70 ± 3·15***	0·586 ± 0·256

n—Number of animals. ***—$P < 0.001$.

when enzyme is added (Inulin + Hya). A statistically significant decrease in the AUC_L value was found in the group Inulin + Inf; the decrease in AUC_P was not significant.

In the individual experimental groups, the flow of lymph and the concentrations of total proteins in lymph were also determined and compared (Table 2). There was no statistically significant difference in flow between the groups; a significant shift from the Inulin group in the concentration of total proteins occurred only in the Inulin + Inf animals.

When the contribution of the blood and lymphatic system to the body distribution of inulin was assessed (Table 3), it was found that over the time of the study about 7 times more inulin was taken up by blood than by lymph. In the Inulin + Inf animals, absorption was diminished with the decrease being more marked for the absorption by lymph. Hyaluronidase had no effect on the parameters under study.

Discussion

One of the factors influencing the two-way transport of drugs through the blood-lymph barrier is the size of the drug molecule (Grotte 1956; Lamka et al 1986). Inulin, with dimensions of 5·9 × 1·6 nm (Eigner et al 1988), lies between low- and high-molecular weight drugs. It is therefore used to study transport processes through physiological barriers.

Biochemical determination of inulin (which requires a minimum volume of 0·1 mL of biological material) was used.

Table 3. Inulin excreted in urine and lymph in experimental groups.

Experimental group	n	Urine mg	% dose	Lymph mg	% dose
Inulin	5	35·30 ± 14·65	20·95 ± 5·11	5·27 ± 0·46	3·08 ± 0·61
Inulin + Hya.	5	28·93 ± 11·02	21·01 ± 9·67	4·92 ± 1·39	3·98 ± 0·91
Inulin + Inf.	5	11·87 ± 8·88*	8·55 ± 5·63	1·23 ± 0·42***	0·87 ± 0·31***

n—Number of animals. *—$P < 0.05$. ***—$P < 0.001$.

In plasma and urine this volume is easily obtainable, but for lymph it was necessary to measure the flow under experimental conditions. It was found that with regular movement and massage of the hind limb the flow of lymph is continuous, 0·15–0·30 mL/10 min, the yield being dependent on the capacity of the cannulated vessel. The required volume of the sample can be obtained over a time interval short enough to enable a detailed description of the lymphatic concentration curve to be made. Hyaluronidase and thermal stimulus of the limb slightly increased the lymph flow, but these shifts result from variable oscillations of the measured values only. Thus it was assumed that, after local administration, the enzyme did not influence the flow of the lymph. On the other hand, the thermal stimulus produced changes in the limb, which affected lymphatic flow (Jonsson et al 1979), particularly in the initial phase. In our experiments the flow was measured as late as 30 min after the stimulus and later, when the flow conditions approached the values of intact animals.

Qualitative parameters of lymph are also dependent on experimental conditions. A significant increase in total protein content in the lymph from the thermally treated limb resulted from the inflammation in the skin and subcutis caused by a release of tissue mediators of the histamine type and prostaglandins, and their effect on the blood vascular wall (Jonsson et al 1979). Our present finding thus demonstrates the efficacy of this type of stimulus. No similar effect with hyaluronidase was observed.

The starting point of distribution experiments was administration of inulin alone to intact animals. High inulin concentrations found in lymph exceeded those in plasma by two orders of magnitude. However, it was simultaneously found that in urine at 90 min a substantially larger amount of inulin was present compared with that in collected lymph; the preferential route of inulin biodistribution from the site of administration is via the blood.

The explanation must be sought in the size of the inulin molecule and in the flow conditions of the subcutis. The molecule is large enough to hinder unlimited transfer into the blood capillaries. At the same time a rapid absorption of the vehicle (saline) from the subcutis takes place (rapid disappearance of blisters developed due to administration), and inulin is "filtered" through the subcutis. This results in a high concentration of inulin at the administration site. The molecule then passes into the permeable lymphatics. Also blood flow in the subcutis is much greater than lymphatic flow and although there is a limited escape of inulin directly into the blood, it carries away a larger amount of inulin from the site of its administration. The low inulin concentrations in plasma at the same time result from the high capability of the kidneys to excrete it (Smith 1956).

An accelerating effect of hyaluronidase on the absorption of drugs (Wenke et al 1984) was not demonstrated in our conditions. Nevertheless, there was a change in the absorption in animals with a thermally stimulated limb which resulted in an increase in the permeability of blood vessels. There is a nociceptive response and the activity of the sympathetic nerve is increased and hence blood pressure is raised (Hamar et al 1979). After initial vasodilatation, vasoconstriction occurs with a decrease in the permeability of the vessels. The oedema developed in the initial phase causes pressure on the constricted vessels, further limiting the flow (Jonsson et al 1979). Hence there is a decrease in inulin absorption from the treated area compared with untreated animals, absorption being more limited from the lymphatic system.

The concentrations of inulin in plasma and lymph are substantially different. The ratio AUC_L/AUC_P is greater than that for dextran of molecular weight 40 000 (Lamka et al 1986). The binding of a suitable radionuclide such as ^{99m}Tc could test this difference in concentrations from the standpoint of usability in indirect lymphography. The advantages of inulin compared with the dextrans would be a shorter period of radiation load of the organism and a lower risk of allergic reactions.

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