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Ductus thoracicus Lymph in Mice. 2. Enzyme activities and protein content

J. Lindena and I. Trautschold

Department of Clinical Biochemistry, Medical School Hannover, Konstanty-Gutschow-Str. 8
3000 Hannover 61, FRG

Summary

Lymph was collected in anaesthetized mice by a newly developed cervical approach (see preceding paper). Short term drainage, which should not alter the colloid-osmotic forces, meets the physiological requirements for determination of protein content and enzyme activities in lymph and plasma. For cellular enzymes such as creatine kinase, lactate dehydrogenase, and aspartate aminotransferase, lymph plasma ratios higher than 1 are reported. Besides the well known lymphatic transport route for protein and albumin, the highly significant lymphatic transport of cellular enzymes to the actual intravasal enzyme activity is emphasized.

Introduction

Although methods for collection of thoracic duct lymph in mice have been in use for a long time (1) data on clinical-chemical analytes, to our knowledge, have not been presented. Besides the general and still ongoing interest in protein composition of different lymphatics, a special interest in the field of clinical enzymology on lymph has developed over the years. The virtually complete body lymph of the thoracic duct has been accepted as an important transport route of cellular enzymes from tissue to the actual intravasal enzyme content, since the direct interstitial-venous entry of enzyme molecules – which depends on the heterogeneity of the capillary barrier characteristic of the different organs – is more or less of secondary importance (for a review see: 2). With the aid of a recently developed approach to thoracic duct lymph in mice (1) this paper quantifies the significance of thoracic duct inflow into blood for several cellular enzymes, total protein and albumin.

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Animals and clinical chemical methods

The strain of mice, their conditions of housing, lymph and blood sampling have been described in detail in the previous paper (1). Enzyme activity, protein and albumin determination: The following enzymes were determined in lymph and plasma: lactate dehydrogenase LDH (EC 1.1.1.27), aspartate aminotransferase, ASAT (EC 2.6.1.1), alanine aminotransferase, ALAT (EC 2.6.1.2), creatine kinase, CK (EC 2.7.3.2), choline esterase, CHE (EC 3.1.1.8). Enzyme assays were carried out using UV or colorimetric tests on a microliter scale at 25 °C with optimized commercial tests (Boehringer, Mannheim) for LDH, ASAT, ALAT and CK according to the "Recommendations of the German Society for Clinical Chemistry" (3) and for CK with the revised method with the addition of EDTA (4). For CHE determination butyryl thiocholine was used as the substrate (Merck, Darmstadt). Protein was determined by the standard biuret method and albumin by the bromocresolgreen method (5). All measurements were in duplicate under quality control with plasma pool samples.

Table 1 Lymph flow (ml/h), enzyme activites (U/l), protein and albumin concentration (g/l) in lymph and plasma and the respective lymph plasma ratios (Ly/Pl). The exchange rate (d^{-1}) denominates to which extent the total intravasal enzyme activity, protein and albumin amount, respectively, is renewed by thoracic duct inflow each day (for calculation and underlying assumption see under results)

	Lymph	Plasma	Ly/Pl	Exchange rate
Lymph flow	0.396 ± 0.01	–	–	–
LDH	229.7 ± 33.2	130.7 ± 7.1	1.8 ± 0.25	18.6
ASAT	34.4 ± 3.3	18.2 ± 0.57	1.9 ± 0.18	20.0
ALAT	16.9 ± 0.73	19.4 ± 0.53	0.87 ± 0.04	9.2
CK	120.5 ± 22.1	37.9 ± 1.8	3.2 ± 0.58	33.6
CHE	539.7 ± 32.1	1.776 ± 35.9	0.30 ± 0.02	3.2
Prot	25.2 ± 1.0	53.2 ± 0.84	0.43 ± 0.04	5.0
Alb	9.9 ± 0.40	26.8 ± 1.3	0.35 ± 0.03	3.9

n = 14; mean ± standard error of the mean

Results

The directly measured parameters, lymph flow, enzyme activities, protein and albumin concentrations in lymph and plasma, together with the calculated values, i.e. lymph plasma ratio of these paramters (Ly/Pl) and the daily exchange rates between lymph and plasma are given in Table 1. The exchange rate per day is calculated from the extrapolated lymphatic transport of enzyme activities, protein and albumin amounts (on the basis of a flow of 0.396 ml/h) in relation to the actual enzyme activities, protein and albumin contents in the intravasal space of mice (mean body weight = 30 g; plasma space assumed as 30 ml/kg body weight). Lymph plasma ratios higher than 1 are obtained for the enzymes LDH, ASAT and CK. These enzymes also show remarkably high exchange rates.

Discussion

For determination of enzyme activity and protein in lymph and plasma chronically fistuled conscious mice cannot be used. After a delay phase, enzyme activities in lymph and plasma incrase due to enzyme release of surgically injured tissue and duration of the healing process. The short term collection (30–40 min) of lymph, however, did not alter enzyme activity in plasma as assured in preliminary experiments. The long term fistula technique additionally disturbs the colloid-osmotic equilibrium due to the permanent fluid and protein loss and thus alters the height and ratio of protein molecules in lymph and plasma. Therefore the lymph flow and protein content described here bear more resemblance to physiological conditions. Our data on enzyme activities in lymph can be interpreted only in relation to their activities in plasma. Higher activities of cellular enzymes in lymph than plasma such as CK, ASAT and LDH confirm our previous results on dogs, namely that cellular enzymes released from tissues are transported partly if not totally by the lymphatics (6, 7, 8, 9). CHE, in contrast, with a very low lymph plasma ratio is a so called plasma enzyme, which is synthetized in liver and can enter the intravasal space directly by traversing the sinusoidal space like albumin, having a similar lymph plasma ratio (10, 11). ALAT, a cellular enzyme, is certainly found in high activity in hematocytes, but other tissues also contribute to its activity in lymph, and therefore a higher lymph plasma ratio is found compared with CHE or Alb.

The calculated value for the exchange rate should give an estimate of the significance of lymphatic transport, especially for enzymes. Compared with dogs, where *Friedel* et al. (9) introduced this calculation, more than 10-fold higher values are found in mice, which is mainly due to the high lymph flow in mice in relation to body weight. We are unable to find comparative results on enzyme activities, protein and albumin in lymph of mice in the literature. For plasma this comparison is restricted to results from *Friedel* et al. (12, 13)

and *Metzenauer* and *Lutz* (14), in which only the latter used identical enzyme assays. Their value for CHE agrees quite well, whereas for ALAT and especially for ASAT they measured several fold higher activities. This is due to the fact that they used heart puncture as the technique for blood drawing which – in contrast to the catheter technique used by us – leads to the risk of aspirating minute amounts of disrupted muscle cells through the inserted needle and thus cause falsely elevated activities in plasma (13). This is why the intracellular activity of ASAT and ALAT is many fold higher (8×10^3 and 3×10^2 fold, respectively) than in plasma.

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Dr. med. vet. J. Lindena, and Prof. Dr. rer. nat. Dr. med. Ivar Trautschold, Department of Clinical Biochemistry, Medical School Hannover, Konstanty-Gutschow-Straße 8, 3000 Hannover 61, FRG