EXPERIMENTAL METHODS FOR CLINICAL PRACTICE

Study of Drug Transport between the Blood and Lymph in the Predominant Direction

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Our method for evaluating the time course and intensity of antibiotics and other drugs transport in the predominant direction between the blood and lymph in humans promotes a more objective evaluation of drug circulation mechanisms, which is essential for determining the time of their repeated administration and route of administration. Calculation of the lymph/blood difference coefficient, based on parallel repeated measurements of the drug concentration in the lymph and blood, and of the lymph/blood coefficient provides complete data on the direction and time course of drug transport between the lymph and blood in the predominant direction.

Key Words: *blood; lymph; lymph/blood coefficient; drug transport direction*

Evaluation of the time course of hyperintense transport of a drug and lymph components between the blood and lymph in the predominant direction is sometimes essential in clinical practice and in experimental studies, during antibiotic or other drug therapy of infections [5,7,13,15], oncological diseases [8,10,14], in lymphosorption and other manipulations with the lymph system, in order to improve the therapy efficiency.

New important data on the lymph system functioning were obtained during recent years. It was found that water transport in the lymph nodes and through the lymph vessel membranes is realized by means of aquaporine, a specialized protein discovered in 2003 [6,11]. Thirteen aquaporines were found in different organs. The lymph system functions with participation of transforming growth factor (TGF-β) with its TβR-I receptor, vascular endothelium growth factor (VEGF-C) [12], TNF-α [9], granulocyte colony stimulating factor (G-CSF) [7], *etc.* However these data are still insufficient to solve the problem.

We have developed a new method, based on the patented method for evaluating the time course of radioactive agent transport intensity in the predominant direction between the blood and nonmineralized organ [4], which is based on repeated calculations of the percent incorporation of the label in the blood and nonmineralized organ during different periods after injection of the isotope to the rat. The calculation of the blood/nonmineralized organ percent radioactivity (PRA) is calculated by the formula:

$$\text{PRA} = \frac{\%\ \text{incorporation of radioisotope in nonmineralized organ}}{\%\ \text{incorporation of radioisotope in blood}}.$$

The intensity of transport in the predominant direction is calculated using the PRA difference

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coefficient (CD_{PRA}) by the formula: $CD_{PRA1}=PRA_2-PRA_1$, $CD_{PRA2}=PRA_3-PRA_2$, *etc.* The radioisotope transport from the blood to the organ predominates if CD_{PRA} is above zero (when the next PRA value is more than the previous one). When the next PRA value is less than the previous one, transport in the opposite direction predominates. At CD_{PRA} below zero (when the next PRA value is lower than the previous one) the radioisotope transport from the organ to the blood predominates. When the next PRA value is higher than the previous one, transport in the opposite direction predominates. The transport intensity is the greatest when the difference between two nearest CD_{PRA} is the greatest. The transport intensity is calculated by comparing the two nearest CD_{PRA} with the same sign ("+" or "-") by deducing the lesser CD_{PRA} from the greater one. If two CD_{PRA} have different signs, they are summed up.

However the prototype method is used to evaluate the time course of transport between the rat blood and nonmineralized organ, but not the transport of an antibiotic or another drug between two biological liquids (blood and lymph). The biochemistry, cellular composition, lymph and blood capillary permeability, and morphology of the lymph and blood are different in health and disease. Moreover, the lymph differs from the blood by the characteristics of its formation and by function.

Study of the time course and intensity of antibiotic and other drugs transport in the predominant direction between the blood and lymph in humans provides data for more objective evaluation of the mechanisms of drug circulation, which is essential for choosing the time of repeated drug administration and evaluation of the routes of administration. For this, the lymph/blood coefficient (CLB) should be calculated after the drug injection by the formula:

$$CLB=\frac{\text{drug content in lymph}}{\text{drug content in serum}},$$

then calculate the transport intensity in the predominant direction using the CLB difference (D_{CLB}): $D_{CLB1}=CLB_2-CLB_1$, $D_{CLB2}=CLB_3-CLB_2$, *etc.* At D_{CLB} above zero (when the next CLB value is more than the previous one) transport from blood to lymph predominates. When the next CLB value is less than the previous one, transport in the opposite direction predominates. At D_{CLB} below zero (when the next CLB value is lower than the previous one) transport from lymph to blood predominates. If the next CLB value is higher than the previous one, transport in the opposite direction predominates. The drug transport intensity is greater if the difference between the two nearest D_{CLB} values is greater. In order to compare the transport intensities, the values of the two nearest D_{CLB} with the same sign ("+" or "-") should be compared by deducing the lesser D_{CLB} from the greater one. For comparing the two D_{CLB} values with different signs, their values should be summed up.

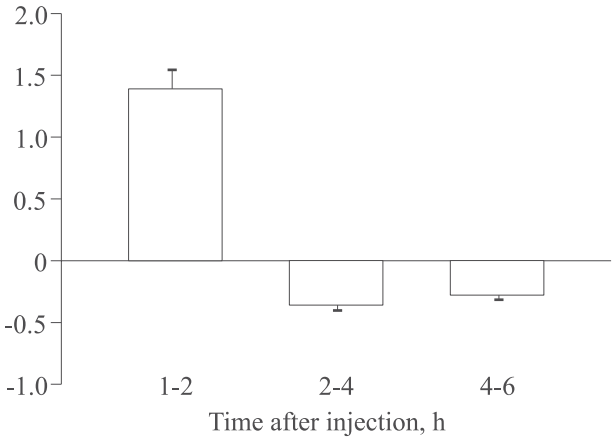

Fig. 1. Lymph/serum difference coefficient during 6 h after a single intramuscular injection of 300 mg tetraolean to 10 patients with acute peritonitis.

The time course of antibiotic (tetraolean, ampicillin, kanamycin sulfate) transport in the predominant direction between blood and lymph was evaluated in 37 patients with acute peritonitis, hospitalized at Hospital Surgery Clinic of Moscow Stomatological Institute. Tetraolean consists from tetracycline (tetracycline group; $^1/_3$) and oleandomycin (macrolide antibiotic; $^2/_3$), ampicillin is a penicillin, and kanamycin sulfate is an aminoglycoside [3]. Antibiotic concentrations in the lymph were evaluated by the agar diffusion test with the following

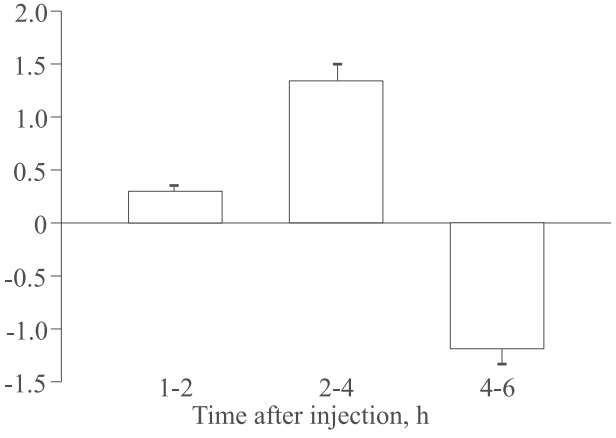

Fig. 2. Lymph/serum difference coefficient during 6 h after a single intramuscular injection of 500 mg ampicillin to 15 patients with acute peritonitis.

TABLE 1. Changes in the Mean Concentration of Tetraolean (μg/ml) in the Lymph (Thoracic Lymph Duct), Blood Serum (*M*±*m*), and CLB and D_{CLB} in 10 Patients with Acute Peritonitis

Liquid	Hour after injection			
	1	2	4	6
Lymph	1.78±0.76	8.29±2.12	5.17±0.70	4.20±0.74
Serum	1.72±0.81	3.44±1.11	2.52±0.57	2.40±1.12
CLB	1.03	2.41	2.05	1.77
D_{CLB}	+1.39	-0.36	-0.28	

TABLE 2. Changes in the Mean Concentration of Ampicillin (μg/ml) in the Lymph (Thoracic Lymph Duct), Blood Serum (*M*±*m*), and CLB and D_{CLB} in 15 Patients with Acute Peritonitis

Liquid	Hour after ampicillin injection			
	1	2	4	6
Lymph	8.33±2.91	7.92±2.42	6.90±2.47	3.80±1.55
Serum	9.70±0.96	6.84±0.80	2.75±0.70	2.750±0.789
CLB	0.86	1.16	2.50	1.38
D_{CLB}	+0.30	+1.34	-1.19	

test bacteria: *Bacillus subtilis* ATCC-6633 for analysis of kanamycin, *Bacillus mycoides* HB_2 for analysis of ampicillin, and *Bacillus subtilis* L_2 variant for analysis of tetraolean.

The CLB rapidly increased in 10 patients injected with 300 mg tetraolean, reaching the maximum 2 h after injection due to predominant release of the antibiotic from blood to lymph (Table 1, Fig. 1). Starting from hour 2 the CLB slowly reduced till hour 6 because of predominating transport of the antibiotic from lymph to blood. Between hours 1 and 2 after the antibiotic injection D_{CLB} increased because of its predominant transport from blood to lymph. During the next period (between hours 2 and 6 after injection) it decreased because of predominant transport of tetraolean from lymph to blood.

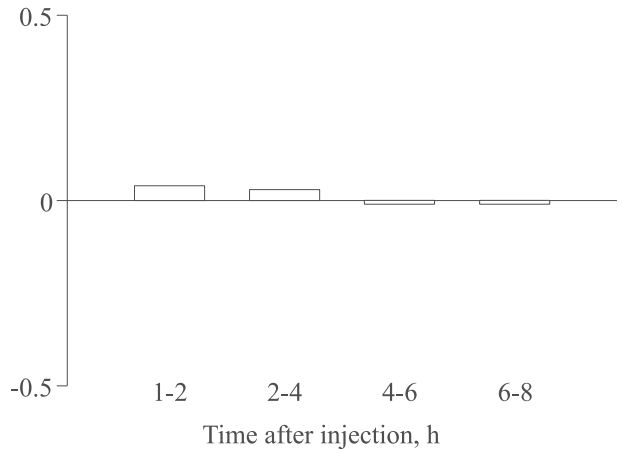

Fig. 3. Lymph/serum difference coefficient during 8 h after a single intramuscular injection of 500 mg kanamycin sulfate to 12 patients with acute peritonitis.

Injection of 500 mg of ampicillin to 15 patients with acute peritonitis was followed by a later elevation of CLB (by hour 4 postinjection) and a more rapid reduction of the coefficient by hour 6 in comparison with the time course of tetraolean due to predominant transport of ampicillin during phase I (by hour 4) from blood to lymph, while during phase II (from hour 4 to hour 6) transport in the opposite direction predominated (Table 2). Elevation of D_{CLB} between hours 1 and 2 postinjection and its great increase between hours 2 and 4 with a rapid drop by hours 4-6 postinjection were observed (Fig. 2).

One hour after injection of 500 mg kanamycin sulfate to 12 patients with acute peritonitis its levels in the serum and lymph in the thoracic lymph duct were measured throughout 8 hours (Table 3, Fig. 3).

Time course of kanamycin sulfate was quite different in comparison with tetraolean and ampicillin. The concentrations of kanamycin in the lymph and serum were highly stable from hour 1 till hour 6 after injection. No statistically significant differences in the lymph (p>0.1) and serum (p>0.5) concentrations were detected. A reduction of the antibiotic concentrations in the lymph and serum from hour 6 towards hour 8 in fact did not tell on the CLB and D_{CLB}, which virtually did not change between hours 1 and 8.

TABLE 3. Changes in the Mean Concentration of Kanamycin Sulfate (μg/ml) in the Lymph (Thoracic Lymph Duct), Blood Serum (*M*±*m*), and CLB and D_{CLB} in 12 Patients with Acute Peritonitis

Liquid	Hour after kanamycin injection				
	1	2	4	6	8
Lymph	11.9±1.7	12.7±1.3	12.3±1.4	11.3±1.2	6.1±0.3
Serum	12.6±0.7	12.9±0.8	12.1±1.0	11.5±0.7	6.3±0.2
CLB	0.94	0.98	1.01	0.98	0.97
D_{CLB}		+0.04	+0.03	-0.01	-0.01

Comparing the lymph and blood, we should take into consideration the probable difference in the time course of the antibiotic and other drugs transport in the predominant direction between the lymph of different regions and blood, as it is known that the biochemical parameters and cellular composition of the lymph in different organs and even in different portions of the same vessel are often different [1,2]. Therefore, measurements of the lymph concentration in a certain site of the lymph system will be more accurate.

We conclude that calculation of the D_{CLB} on the base of measurements of the drug concentration in the lymph and blood and of CLB calculation provide ample data on the direction and time course of the drug transport in the predominant direction between the lymph and blood.

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