Distribution and Elimination of Milk Angiogenin in Mouse Immunocompetent Organs and Brain

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Dynamics of angiogenin content in the serum, thymus, bone marrow, and brain of mice was studied after intravenous injection. The maximum angiogenin retention was detected in the thymus, while high rate of its elimination in the brain.

Key Words: *angiogenin; pharmacokinetics; lymphoid organs; brain*

Angiogenin (ANG), a polyfunctional protein, is expressed by all mammalian cells and is present in the blood and (in higher concentrations) in milk [2]. It can be hypothesized that ANG participates in the mechanisms of passive immunity transfer from the mother to the progeny. ANG is a potent inductor of blood vessel growth in tissues and participates in other processes essential for the maintenance of homeostasis in animals. The immunomodulating effect of ANG was described [4,7,9], which prompted using it as the basis for the creation of new effective immunocorrective drugs. However, there are in fact no data on the pharmacokinetics of ANG in systems determining the immune status of animals [8].

We studied the pharmacokinetics of ANG isolated from cow milk in mouse serum, thymus, bone marrow, and brain.

MATERIALS AND METHODS

The study was carried out on male BALB/c mice (18-20 g). The animals were kept in a vivarium on standard fodder and drinking ration. ANG was isolated from fresh cow milk by chromatography [5]. Experimental mice were injected (into the caudal vein) with ANG in saline (580 ng/g body weight). Controls were intravenously injected with the same volume of saline.

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The animals were decapitated, the blood was collected, the thymus, bone marrow, and brain were removed. The organs were cleansed from adipose tissue, washed in saline, weighed, and homogenized on cold in a glass homogenizer in 10 ml 0.2 M Tris-HCl buffer at pH 7.0. The homogenate was mixed on a magnetic mixer for 15 min centrifuged for 15 min at 10,000 rpm; the supernatant was collected. In order to separate the serum, blood was incubated for 30 min at 37°C and cooled at 4°C. The serum was centrifuged at 10,000 rpm. The content of ANG in tissue extracts and serum was evaluated by enzyme immunoassay (EIA) [6].

Based on the dynamics of ANG changes in the serum and tissue extracts after injection of exogenous ANG, the main pharmacokinetic parameters were calculated using a standard one-compartment pharmacokinetic model characterizing the "averaged" status of processes influencing the injected preparation. The model is valid for a certain volume, covering the entire quantity of the preparation in the body, its mean concentration in the body being equal to its plasma concentration [1]. Using the one-compartment model, it is possible to evaluate the main pharmacokinetic parameters and total picture of the drug penetration into the body.

The pharmacokinetic parameters were determined using the following equation:

$$C(t)=C_0\times e^{-K\times t},$$

where C_0 is the initial concentration of the studied preparation in the plasma, K_e the elimination velocity constant, and t time elapsed after injection.

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C_0 is determined by the point where the curve crosses the ordinate axis and is represented as D/V_d, where D is the dose and V_d is the apparent distribution volume. K_e reflects the rate of drug elimination from the body and is equal to the tangent of the slope of the linear part of the semilogarithmic curve. The half-life period ($t_{1/2}$) characterizes elimination of the preparation from the plasma and is equal to the time during which the concentration of the preparation decreases by half:

$$C_0/2=C_0\times e^{-Ke\times t1/2}.$$

The $t_{1/2}$ and K_e are in a simple relationship:

$$K_e=\ln 2/t_{1/2}=0.693/t_{1/2}.$$

Half-life period reflects a relationship between the volume of distribution and clearance (Cl, a value characterizing the rate of drug elimination from the plasma:

$$t_{1/2}=0.693\times V_d/Cl.$$

There is a simple relationship between Cl, dose, and summary area under concentration curve from the moment of its entry into the body until complete elimination (AUC): Cl=D/AUC. The mean duration of the preparation retention in the body (MRT) is estimated by the ratio: $MRT=1/K_e$. MRT indicates the time of the preparation retention in the body; for a single dose it represents the mean value for all time intervals from the moment of injection until the moment of elimination of each molecule of the preparation. Summary area under the curve (product of time by the preparation concentration from the moment of its entry into the body until complete elimination; AUMC) is calculated as AUMC= MRT/AUC.

In order to obtain objective data on ANG concentrations in the serum and test tissues from experimental animals for each term, the background concentration of endogenous ANG in control animals was deduced from the resultant values.

RESULTS

After intravenous injection of ANG to mice its serum concentration decreased (Fig. 1). Evaluation of the pharmacokinetic parameters of the protein in the plasma showed that ANG $t_{1/2}$ was 52.9 min, C_0 20 μg/ml, V_d 0.029 ml/g, K_e 0.0131 min^{-1}, Cl 0.38×10^{-3} ml/min×g, AUC 1526.72 μg×min/ml, AUMC 11.65×10^4 μg×min^2/ml, and MRT 76.33 min.

The concentration of ANG in the serum rapidly dropped to about 50% of its basal level 5 min after injection. The presence of ANG in the blood was detected for ~90 min after the start of injection. Blood ANG level depends mainly on two factors: elimination and distribution in organs. According to pharmacological values obtained in the study, elimination in this case was not so significant as distribution (total Cl value reflecting the capacity of the organism to elimination proved to be very low).

A variant of one-compartment model was used for evaluation of pharmacokinetic parameters of ANG in the thymus, bone marrow, and brain (Fig. 2); the characteristics of protein absorption were taken into consideration, like in other than extravascular injection. All curves in Fig. 2 reach the maximum at a certain moment (t_{max}). By analyzing the terminal parts of the curves (regression straight lines) it is possible to calculate ANG K_e in the organs by the method of least squares. The parameters describing the ascending slope of the curve (when ANG content in the organ (Q) is still increasing) were evaluated by the equation:

$$Q=Q_0\times\frac{K_a}{K_a-K_e}\times(e^{-Ke\times t}-e^{-Ka\times t}),$$

where Q_0 is the threshold accumulation of ANG in the organ at a certain dose and K_a is absorption constant.

Methods for the search of parameters in the Excel electronic tables medium were used [1]. The parameters of the model were precisely evaluated by the method of coordination by the maximum ordinate value.

Analysis of the pharmacokinetic parameters of ANG in the thymus, bone marrow, and brain of mice (Table 1) characterized the velocity of the protein accumulation, retention time, and velocity of its elimination from the organs.

Increase in ANG content in the organs during the initial period after injection was paralleled by a pronounced drop of its serum concentration, but the distribution of the protein was selective (Fig. 2). For example, the least content of ANG entered the bone marrow (the peak being observed by minute

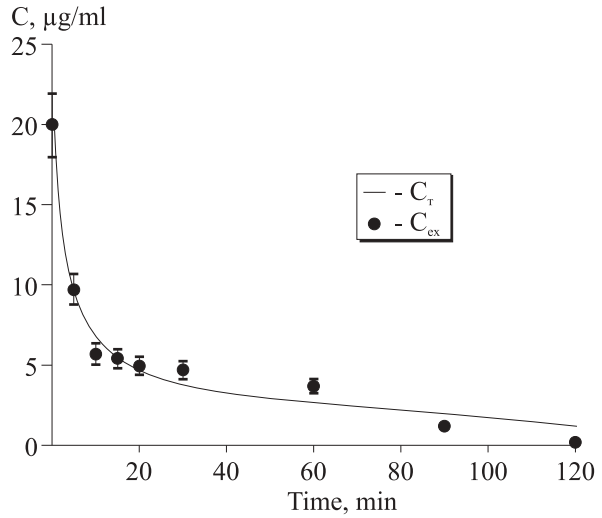

Fig. 1. Dynamics of serum ANG concentration in mice. $C_т$ is theoretical concentration, C_{ex} is ANG concentration in experiment.

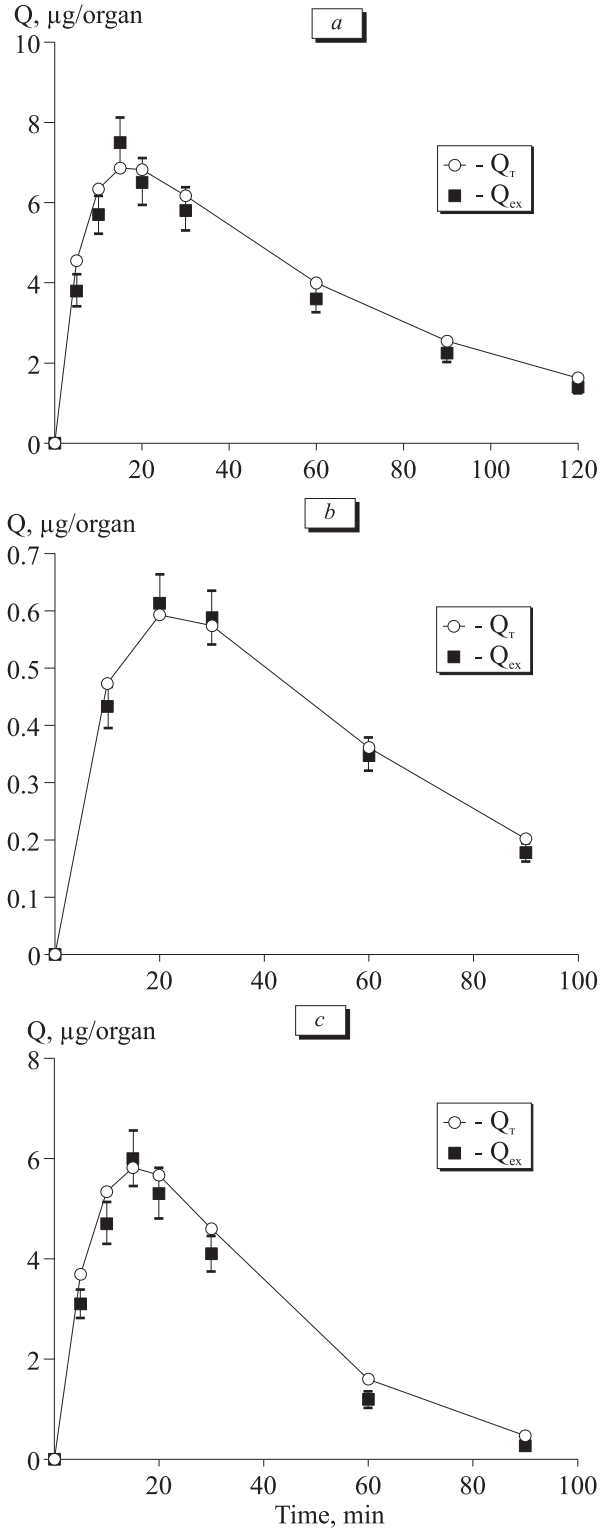

Fig. 2. Dynamics of ANG content in the thymus (*a*), bone marrow (*b*), and brain (*c*). Q_T is the theoretical content, Q_{ex} is ANG content in experiment.

20) and greatest into the brain. Reaching the maximum by minute 15, the protein was rapidly eliminated from the brain. In this case K_e was approximately 2-fold more than K_a. This distribution can mean that these kinetic models can be described by the "flip-flop" phenomenon (when the maximum concentrations of the pharmacokinetic curve change their position at the t_{max} moment, corresponding to equality between elimination and absorption velocities). Retention time in the brain was just 11 min *vs.* 50 and 67 min in the bone marrow and thymus, respectively. This is explained by lower K_e in comparison with K_a in the thymus and bone marrow (by one order of magnitude for the thymus and about 4-fold for the bone marrow).

TABLE 1. Main Pharmacokinetic Values of ANG in the Thymus, Brain, and Bone Marrow of Mice

Parameter	Thymus	Brain	Bone marrow
K_a, min^{-1}	0.153	0.042	0.084
K_e, min^{-1}	0.015	0.090	0.020
$t_{1/2}$, min	46.20	7.70	34.65
Q_0, μg	8.87	24.27	0.93
AUC, min	591.3	269.6	46.5
AUMC, min^2	39 422	2996	2325
MRT, min	67	11	50

Hence, ANG is longer retained in the thymus and slower eliminated from it, which determines its longer contact with thymus-dependent immunocompetent precursor cells, playing an important role in the realization of specific defense mechanisms. On the whole, we should like to emphasize high velocities of ANG absorption and elimination in animals, which should be taken into consideration in creation of drugs based on this protein.

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