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Supramolecular Enzymatic Systems of the Dog Blood: Clinical Diagnostic Implications

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Abstract—Some enzymatic activities (or the enzyme profile) of the dog blood serum that are used in clinical diagnosis have been estimated. The alanine aminotransferase (ALT), aspartate aminotransferase (AST), γ-glutamyl transferase (GGT), lactate dehydrogenase (LDH), and creatine kinase (CK) activities of the blood of 6- to 11-year-old dogs with chronic heart failure (CHF) have been found to be, respectively, 55–221%, 85–285%, 168–234%, 6.4- to 7.4-fold, and 2.3- to 3.1-fold higher than in healthy animals of the same age. Analysis of the LDH isoenzyme profile has shown the isoenzymatic activities decreased in the order $LDH_1 > LDH_2 > LDH_4 > LDH_3 > LDH_5$ in dogs with CHF, irrespective of their ages.

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The creation, study, and use of supramolecular biochemical systems (SBSs), i.e., highly organized systems of proteins, lipids, and other biologically active substances with desired properties is a topical problem at the interface between biochemistry, bioorganic chemistry, biotechnology, nanotechnology, medicine, and veterinary.

Many enzymes detected in the blood plasma and widely used for diagnosing the physiological and biochemical states of animals may be regarded as "endogenous" SBSs of different organizational levels, because they consist of several polypeptide chains (subunits), which, combined in different ways, form the quaternary structures of the enzymes [1–3]. For example, lactate dehydrogenase (LDH) consists of four subunits of two types (H and M), and creatine kinase (CK), of two subunits of two types (B and M). Moreover, five LDH isoforms and three CK isoforms have been found in animals [1–3]. The characteristic set of all these enzymes and their isoforms in the animal blood (the enzymatic SBS or enzyme profile of the blood) depends on pathological changes in organs and tissues, which was the basic model for our biochemical studies on the animal blood and improvement of the methods of biochemical analysis.

We measured some enzymatic activities of the dog blood serum that are used in clinical diagnosis and estimated the correlation of the resultant enzyme profile of the blood of six-year-old and older dogs with changes in the heart functioning.

MATERIALS AND METHODS

The sample consisted of 37 dogs, which were divided into two groups: group 1 comprised clinically healthy dogs, and group 2, dogs with chronic heart failure (CHF) of functional class III. CHF was diagnosed at the Tsentr Veterinary Clinic (Moscow) [4]. The dogs of both groups were divided three age subgroups: 6–7, 8–9, and 10–11 years. All animals were analogous, namely, male poodles with a body weight of 15–20 kg. Blood serum and plasma were the material of the study. Blood of the dogs of all groups was sampled in the morning on an empty stomach. Plasma was stored at a temperature of 4–8°C for 6 h. If a longer storage was required, it was frozen and stored at –20°C. Blood serum was obtained by settling of whole blood and retraction of the clot followed by centrifugation (if necessary). The serum was centrifuged at 2000 rpm for 10–15 min [4].

We measured the total activities of alanine aminotransferase (ALT), aspartate aminotransferase (AST), γ-glutamyl transferase (GGT), LDH, and CK in the dog blood by the standard biochemical method [5] based on the spectrophotometry of a mixture of the blood serum and the appropriate reagents after it was incubated at 37°C for 1–3 min. We used polyacrylamide gel electrophoresis according to the standard protocol [4–6] to determine the LDH and CK isoenzyme spectra. The proportion of each isoenzyme was determined as the ratio of the peak of the assayed isoenzyme fraction to the sum of all isoenzyme peaks and expressed in percent of the total LDH or CK activity (X, %) [7].

The Statistica for Windows software was used for statistical treatment of the obtained data. The significance of differences of the measured parameters from one another and from the normal level was estimated using Student's t test; the significance level was taken to be 0.95.

Table 1. Enzyme activities (*A*, U/l) in the blood of healthy dogs aged 6–11 years (*n*, number of animals)

Enzyme	*n*	*A* (U/l), 6–7 years	*n*	*A* (U/l), 8–9 years	*n*	*A* (U/l), 10–11 years
ALT		34.6 ± 1.9		26.6 ± 1.7$^{\#}$		16.2 ± 1.7$^{\#\#\#}$
AST		32.4 ± 1.1		24.5 ± 1.4$^{\#\#}$		16.1 ± 1.2$^{\#\#}$
GGT	5	4.7 ± 0.2	5	4.1 ± 0.3$^{\#}$	5	3.5 ± 0.2$^{\#\#}$
LDH		120 ± 9		113 ± 8		116 ± 12
CK		52 ± 5		61 ± 5		55 ± 5

Note: Significance of differences from the enzyme activity at an age of six to seven years: $^{\#}$ $p < 0.05$; $^{\#\#}$ $p < 0.01$; $^{\#\#\#}$ $p < 0.001$.

Table 2. Enzyme activities (*A*, U/l) in the blood of dogs with CHF aged 6–11 years (*n*, number of animals)

Enzyme	*n*	*A* (U/l), 6–7 years	*n*	*A* (U/l), 8–9 years	*n*	*A* (U/l), 10–11 years
ALT		53.7 ± 1.8**		54 ± 2***		52 ± 2***
AST		60 ± 2***		63 ± 3***		62 ± 2***
GGT	7	12.6 ± 0.4***	9	12.6 ± 0.9***	6	11.7 ± 0.7***
LDH		1009 ± 79***		837 ± 76***		965 ± 65***
CK		213 ± 17***		204 ± 14***		226 ± 19***

Note: Significance of differences from the control values (Table 1): * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

RESULTS AND DISCUSSION

The enzyme profile of the dog blood may be considered as an example of an SBS, because the estimation of the physiological and biochemical states in many diseases requires the measurement of the main enzymatic activities of the animal blood, which constitute a specific set or enzyme profile depending on the normal or pathological states of organs and tissues of a given animal. Some enzymes used in veterinary diagnosis have several isoforms, which considerably complicates the characteristic enzyme profile of the animal blood and further confirms that it may be regarded as an SBS of a high organizational level.

Table 1 shows the obtained data on the serum enzyme activities (the blood enzyme profile comprising the AST, ALT, GGT, LDH, and CK activities) in healthy and diseased dogs.

Some enzymatic activities substantially changed with age in healthy (control) dogs (Table 1): the mean ALT, AST, GGT, and LDH activities in eight- to nine-year-old dogs were 23, 24, 13, and 6% lower than in six- to seven-year-old dogs, respectively, and the CK activity was somewhat higher in the older dogs (although the changes in the LDH and CK activities were nonsignificant). The age-related changes were more distinct in the oldest age subgroup (10–11 years). The ALT, AST, and GGT activities of their blood were decreased by 53, 50, and 26%, respectively, compared to dogs aged six to seven years, respectively, whereas the LDH and CK activities were only slightly changed (Table 1). In summary, we may conclude that the serum enzyme profile of healthy dogs changes with age as follows: the ALT, AST, and GGT activities significantly (and substantially) decrease, whereas the LDH and CK activities do not change significantly.

Chronic heart failure of different etiologies was accompanied by a significant increase in all the above enzymatic activities in dogs from all age subgroups studied (Table 2). The ALT, AST, and GGT activities of 6- to 11-year-old dogs with CHF were, respectively, 55–221, 85–285, and 168–234% higher than in control dogs of the same ages (Table 1). The increase in the blood serum LDH and CK activities in CHF was especially pronounced (Table 2): the former was, on average, 7.4, 6.4, and 7.3 times higher and the latter was 3.1, 2.3, and 3.1 times higher in dogs with CHF aged 6–7, 8–9, and 10–11 years, respectively, compared to these activities in healthy dogs from the respective age subgroups (Table 1).

Regarding the age-related changes in the studied enzymatic activities between dogs with CHF, the slight changes in the ALT, AST, and GGT activities practically fell within the measurement error (Table 2); i.e., they were nonsignificant. Nor can we consider significant the larger variations in the LDH and CK activities observed in dogs with CHF (Table 2), which were related to huge "disproportional" changes in the activities of different LDH and CK isoenzymes, as we will demonstrate below.

We believe that correct use of the obtained data in clinical diagnosis also requires detailed analysis of the isoenzyme spectra of the studied enzymes, e.g., LDH, in the blood of healthy dogs (Table 3) and dogs with CHF (Table 4).

The data shown in Table 3 indicate that, in healthy dogs, the activity of the LDH_4 isoenzyme is the highest

Table 3. Activities of LDH fractions (*A*, U/l) in the blood serum of healthy six-year-old and older dogs (*n*, number of animals)

LDH fraction	*n*	*A* (U/l), 6–7 years	*n*	*A* (U/l), 8–9 years	*n*	*A* (U/l), 10–11 years
LDH_1		34.1 ± 2.8		32.6 ± 2.7		31.6 ± 2.9
LDH_2		23.5 ± 2.3		21.7 ± 2.9		22.6 ± 3.1
LDH_3	5	12.3 ± 0.9	5	11.5 ± 0.3	5	12.2 ± 0.8
LDH_4		44.9 ± 2.9		42.4 ± 2.1		44.4 ± 4.8
LDH_5		5.3 ± 0.3		4.6 ± 0.4		4.8 ± 0.7

Table 4. Activities of LDH fractions (*A*, U/l) in the blood serum of six-year-old and older dogs with CHF (*n*, number of animals)

LDH fraction	*n*	*A* (U/l), 6–7 years	*n*	*A* (U/l), 8–9 years	*n*	*A* (U/l), 10–11 years
LDH_1		586 ± 41**		492 ± 43**		485 ± 32**
LDH_2		333 ± 31**		258 ± 28**		379 ± 29**
LDH_3	7	24.6 ± 2.5**	9	20.0 ± 1.9**	6	24.2 ± 2.4**
LDH_4		54.4 ± 3.6**		57.4 ± 3.1**		64.7 ± 4.5**
LDH_5		11.3 ± 0.5**		10.4 ± 0.5**		11.7 ± 0.5**

Note: Significance of differences from the control values (Table 3): * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

(from 42.4 to 44.9 U/l) and that of the LDH_5 isoenzyme is the lowest (from 4.6 to 5.3 U/l) at all ages studied. In general, the data shown in Table 3 demonstrate that the activities of LDH isoenzymes decrease in the order $LDH_4 > LDH_1 > LDH_2 > LDH_3 > LDH_5$ in healthy dogs of all ages; a decrease in these activities with age is small, and the differences between all age subgroups are nonsignificant (Table 3).

Our data demonstrated that the activities of all LDH isoenzymes were considerably increased in dogs with CHF (Table 4); however, there were no age-related differences in the activity of any isoenzyme. In dogs with CHF aged 6–7, 8–9, and 10–11 years, the mean LDH_1 activity was increased, respectively, by factors of 17.2, 15.1, and 15.3 (Table 4); the LDH_2 activity, by factors of 14.1, 11.9, and 16.8; the LDH_3 activity, by factors of 2.0, 1.7, and 2.0; the LDH_4 activity, by factors of 1.2, 1.4, and 1.5; and the LDH_5 activity, by factors of 2.1, 2.3, and 2.4 compared to healthy dogs of the respective ages (Table 3).

The data shown in Table 4 demonstrate that LDH_1 and LDH_2 were the most active LDH isoforms in dogs with CHF (485–586 and 258–379 U/l, respectively), and LDH_5 was the least active (10.4–11.3 U/l) at all ages. In general, the data shown in Table 4 demonstrate that the activities of LDH isoenzymes in CHF dogs of all ages decrease in the following order:

$$LDH_1 > LDH_2 > LDH_4 > LDH_3 > LDH_5.$$

These two series of the activities of serum LDH fractions in healthy and CHF dogs may serve as a basis for a novel diagnostic parameter, the ratio of the total activity of four LDH isoenzymes ($LDH_1 + LDH_2 + LDH_3 + LDH_5$) to the activity of the remaining isoenzyme, LDH_4. This parameter, which we hereinafter refer to as the LDH isoenzyme parameter (LDH–IP), was 1.7 for healthy six- to seven-year-old dogs and 17.6—more than ten times higher—for dogs of the same age with CHF; hence, it is a sufficiently informative indicator suitable for clinical diagnosis of this pathology. We selected the LDH_4 activity as the denominator for the index that we suggest for comparative analysis because this fraction both had the highest enzymatic activity in healthy dogs and was comparatively little increased (by 20–50%) in CHF, whereas the activities of all other LDH fractions were several times higher in CHF.

In the biochemical analysis of the human blood, data on the LDH isoenzyme profile are indicated in percent. Applying the same form of presentation to our data on dogs, we obtain the following isoenzyme profiles for dogs aged six to seven years. Healthy dogs: LDH_1, 28.4%; LDH_2, 19.6%; LDH_3, 10.2; LDH_4, 37.4%; LDH_5, 4.4%; dogs with CHF: LDH_1, 58.1%; LDH_2, 33.0%; LDH_3, 2.4; LDH_4, 5.4%; LDH_5, 1.1%. In our opinion, LDH–IP is the best parameter for comparative analysis of clinical biochemical data on animals with CHF.

Thus, the physiological and biochemical adaptation of dogs to altered cardiac functioning in the course of ontogeny can be estimated by analyzing enzymatic SBSs of the blood, namely, the total ALT, AST, GGT, LDH, and CK activities and their isoenzyme spectra, although assaying other enzymes and compounds may also be useful. This makes it possible to diagnose destructive processes in the myocardium caused by CHF.

The AST : ALT ratio is an important clinical biochemical parameter of the animal blood [5]. In humans,

this ratio is normally about 1.3–1.4. As can be seen in Table 1, this coefficient for healthy dogs is 0.94, 0.92, and 1.0 at ages of 6–7, 8–9, and 10–11 years, respectively. In dogs with CHF, it is 1.1 at 6–7 years of age and 1.2 at ages of both 8–9 and 10–11 years (Table 2). These small age-related differences are nonsignificant, whereas the absolute age-related changes in the AST and ALT activities in dogs with CHF are significantly larger than in healthy dogs. Therefore, we suggest that summary coefficients should be used, e.g., the ratio of the total activity of three serum transaminases (AST, ALT, and GGT) in animals with CHF to that for healthy (control) animals. This ratio for dogs aged 6–7, 8–9, and 10–11 years is 1.8, 2.3, and 3.5, respectively. The ratio of the total activity of all the five enzymes studied here (the SBS coefficient) in CHF to that for healthy (control) dogs is an even better indicator; its values at the respective ages is 5.5, 5.1, and 6.4.

Thus, the data presented here indicate that the enzyme profile of the dog blood should be regarded as an SBS with a high organizational level. The use of the entire set of data on enzymes and their isoforms as an integrated SBS is the most promising for estimating the physiological and biochemical state of dogs with CHF at different ages.

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