Involvement of HLDF Protein and Anti-HLDF Antibodies in the Mechanisms of Blood Pressure Regulation in Healthy Individuals and Patients with Stable Hypertension and Hypertensive Crisis

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 153, No. 5, pp. 618-620, May, 2012
Original article submitted March 2, 2011

We studied the relationships between the blood serum levels of human leukemia differentiation factor HLDF, idiotypic and anti-idiotypic antibodies to HLDF, and clinical indicators of cardiovascular function in apparently healthy individuals and patients with essential hypertension and cerebral hypertensive crisis. Markedly reduced HLDF levels and anti-HLDF antibody titers were found in the blood of the examined patients. Correlations between HLDF levels, duration of hypertension, and systolic and diastolic BP were revealed. These findings suggest that the studied molecular factors are involved in the mechanisms of BP regulation under normal conditions and during hypertension development. The protein HLDF and anti-HLDF antibodies can be considered as biomarkers for early diagnosis of hypertension and its cerebral complications.

Key Words: *peptide HLDF; autoantibodies; hypertension; hypertensive crisis*

High prevalence of essential hypertension (EH; in Russia, 39% men and 41% women above 18 years suffer from EH) and its role as a dominant risk factor of acute and chronic cerebrovascular diseases explains constantly increasing interest to the studies of the mechanisms of blood pressure regulation and to the search for adequate biomarkers of EH [5,7,12].

According to modern concepts, the formation and development of EH and cerebrovascular diseases including cerebral hypertensive crisis [1,9,15] are largely determined by impaired microcirculation, dysfunction of the endothelium and the renin-angiotensin-aldosterone system, and disorders in cell survival and death processes in the nervous and immune systems [10,14]. Autoimmune reactions against factors regulating the above processes are of great importance [3,6].

Human leukemia differentiation factor HLDF and anti-HLDF autoantibodies are currently regarded as regulators of the development and cell death in the nervous and cardiovascular systems [2,7]. Experiments on normotensive Wistar and WKY rats and spontaneously-hypertensive stroke-prone (SHR-SP) rats showed that 24- and 8-membered peptide fragments of HLDF (HLDF-24 and HLDF-8) are active contributors to homeostasis of the cardiovascular system [4]. HLDF protein and anti-HLDF autoantibodies were also shown to be involved in the molecular pathogenetic mechanisms of acute and chronic cerebral circulation disturbance [8].

Here we studied the relationships between the blood levels of the protein HLDF, idiotypic antibodies, and anti-idiotypic antibodies to HLDF and clinical indicators of the cardiovascular function in patients with EH and cerebral hypertensive crisis.

MATERIALS AND METHODS

We performed a comparative clinical and immunobiochemical study of three groups of patients treated at

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the Department of Cardiology of the P. V. Mandryka 2nd Central Military Clinical Hospital. Group 1 (n=43, mean age 63.4±10 years) included patients with a 0.5-34-year history of uncomplicated stable EH; mean systolic BP (SBP) and diastolic BP (DBP) in this group were 144±16 and 89±9 mm Hg, respectively. Group 2 (n=34, mean age 59.3±13.5 years) comprised patients with diagnosed hypertensive crisis and transient neurological EH-related symptoms (EH history 0.5-40 years). During hypertensive crisis, mean SBP and DBP were 210±23 and 117±9 mm Hg, respectively. Group 3 (n=27, mean age 64.0±8.8 years) included individuals undergoing planned medical examination without cardiovascular and neurological pathologies (control, age norm).

Immunobiochemical indicators were determined by ELISA in original modification [2] in serum samples of peripheral blood taken from the cubital vein by the standard technique. The study was carried out once in the control group and in patients with uncomplicated EH. In patients with hypertensive crisis, immunobiochemical indicators were determined in dynamics on days 1, 3, 7, and 21 after admission to hospital. The concentration of HLDF protein was expressed in ng/ml, the levels of idiotypic and anti-idiotypic antibodies to HLDF were expressed as the titer. Statistical analysis was performed using Statistica 6.0 software; nonparametric Kruskal–Wallis analysis of variance with subsequent evaluation of statistical significance of intergroup differences by Mann–Whitney U test and Spearman rank correlation (R) test were applied. The critical significance level (p) at testing of the null hypothesis was taken to be 0.05. The data are presented as arithmetic means (M)±standard error of the mean (m).

RESULTS

Elevated BP in patients with EH and hypertensive crisis (groups 1 and 2) was associated with reduced serum level of HLDF (by 160 and 9 times, respectively) and 2-2.5-fold reduced levels of idiotypic antibodies to HLDF in comparison with those in apparently healthy individuals. Hypertensive crisis was characterized by 2.6-fold reduced titer of anti-idiotypic antibodies to HLDF (Table 1) in comparison with uncomplicated EH.

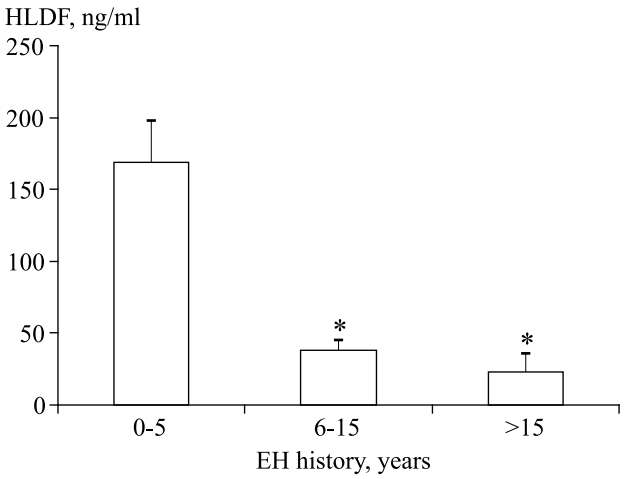

Fig. 1. Relationships between blood serum levels of HLDF protein in all examined patients and the duration of EH. *p<0.05 in comparison with 0-5-year history of EH.

Analysis of variance revealed that the blood serum level of the HLDF protein in all patients inversely correlated with the duration of EH (Fig. 1). The duration of EH in the third group was herewith assumed to be zero. Correlation analysis confirmed that HLDF level inversely correlated with EH duration (R=-0.56 p<0.001).

The relationship between HLDF serum level and BP values was documented in all patients. HLDF level was found to directly correlate with SBP (R=0.45 p<0.001) and DBP (R=0.45 p<0.001; Fig. 2).

Thus, the obtained results suggest that peptide HLDF and anti-HLDF autoantibodies are involved in the mechanisms of BP regulation. A possible mechanism is participation of HLDF in the regulation of endothelial function, which is confirmed by homology of a HLDF amino acid sequence motif and endothelin-converting enzyme [4]. Endothelium is known to be a part of the blood-brain barrier regulating the exchange of substances between the blood and brain tissues [11]. The presence of HLDF in various brain structures was also demonstrated [13]. These data suggest that HLDF can participate in the central regulation of cardiovas-

TABLE 1. Blood Serum Levels of HLDF, Idiotypic and Anti-Idiotypic Antibodies to HLDF in Examined Patients (M±m)

Group	HLDF, ng/ml	idiotypic antibodies to HLDF, titer	anti-idiotypic antibodies to HLDF, titer
Control (age norm)	435.8±21.2	1:4250±982	1:2350±800
Hypertonic disease	2.7±0.9*	1:1965±545*	1:3509±845
Cerebral hypertensive crisis (day 1)	46.4±10.0*+	1:1922±592*	1:1316±293+

Note. p<0.05 in comparison with: *controls, +EH.

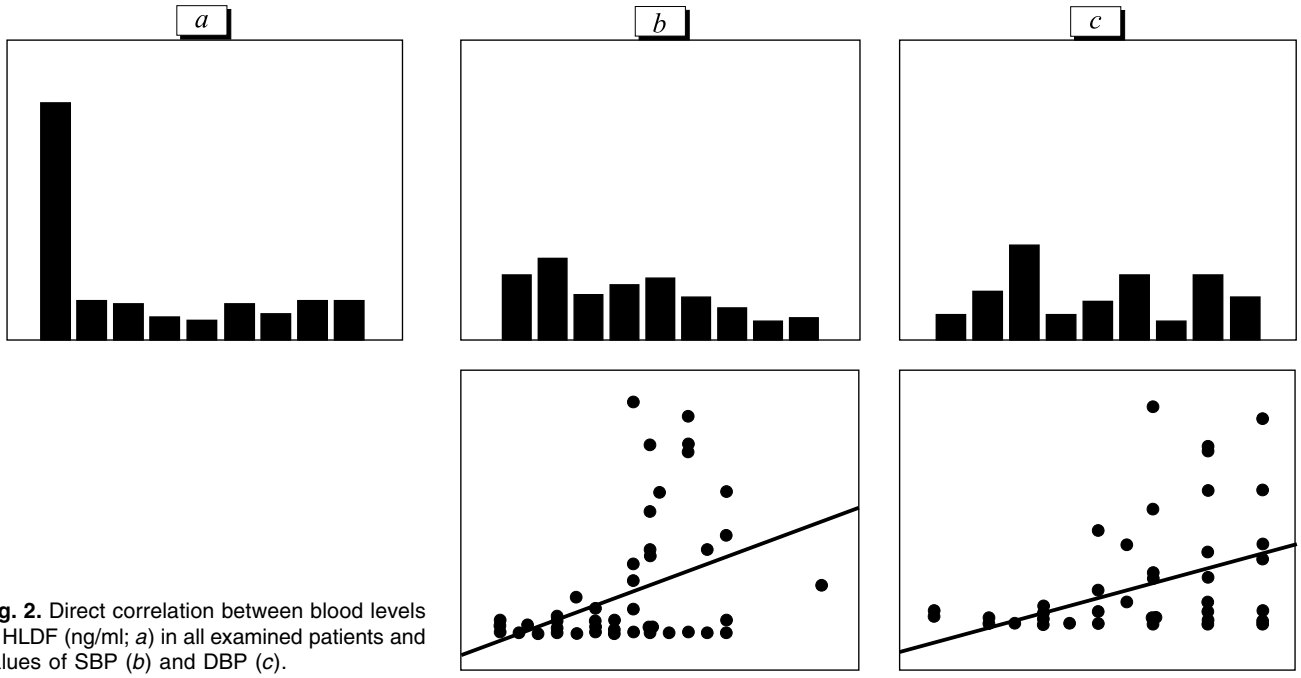

Fig. 2. Direct correlation between blood levels of HLDF (ng/ml; *a*) in all examined patients and values of SBP (*b*) and DBP (*c*).

cular processes providing blood supply to the brain. The observed hemodynamic activity of HLDF peptide fragments [4] suggests their involvement in peptidergic cascade regulation of BP.

Documented relationships between the blood serum levels of HLDF, idiotypic and anti-idiotypic antibodies to HLDF, and clinical indicators of cardiovascular function in patients with stable hypertension and hypertensive crisis suggest that the analyzed molecular factors can be considered as biomarkers of EH and its cerebral complications.

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