Morphological Changes in the Liver, Thymus, Spleen, and Small Intestine of Animals after Leucine Treatment

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Leucine treatment (100 mg/kg daily for 5 days) leads to activation of the hepatocyte nuclear system and granular endoplasmic reticulum and to a drastic increase in the number of mitochondria, characterized by polymorphism. In the spleen, Malpighian bodies and periarterial lymphoid sheaths are enlarged, lymphocytes infiltrate the periarterial zone, and the mantle zone is enlarged. In the thymus, the width of the cortical matter shrinks, while that of the medulla increases. The content of lymphocytes in the medulla decreases, while that of Hassal's bodies increases. Unambiguous effects of leucine on the small intestinal morphology (mainly on the villous epithelium) were shown. Goblet cells in the villous epithelium were sharply stenosed because of decreased secretory granules in them.

Key Words: *leucine; liver; thymus; spleen; intestine*

Effective drugs with target metabolic effects can be created on the basis of amino acids. Highly purified amino acids are actively used in pharmaceutical industry as nutrient additives and as substances for the manufacture of many drugs. Free amino acids and their derivatives are the most universal natural regulators and endogenous modifiers of biological reactions. *In vivo* they are presented by a wide spectrum of compounds of related chemical structure; their transport, intermediate metabolism, synthesis, and utilization are unified by the main metabolic streams in the cell. Their content in the cell is the most important regulatory factor in protein biosynthesis and formation of highly active biological compounds (mediators, hormones), determines the rate of the main metabolic flows, specifically, of the Krebs cycle and urea formation cycle.

Evaluation of the consumption of specific nutrients, to which amino acids belong, should include specification of their maximum safe volume. A scale should be developed with the maximum level showing the quantity beyond which a negative (toxic) effect can be expected. Determination of the highest tolerable dose should include evaluation of the probability of specific negative effects, response to the dose determined as the maximum tolerable, characterization of risk and the population at risk, and identification of this population.

There are many published data on the safety of amino acid-based drugs, but amino acid metabolites (ketoacids) formed during leucine, isoleucine, and valine oxidation can be toxic. Treatment with some amino acids inhibits animal growth, presumably disordering other amino acid metabolism, including the competition for transporting or common metabolic pathways. For example, arginine excess causes hypotension because of the formation of NO [4] directly modulating the blood vessels. For glutamine, one of the main metabolizing systems is gluconeogenesis with subsequent glucose formation

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and release into the blood. Increased activity of this system during long-term treatment with glutamine can promote the development of diabetes. Modulation of other metabolic components in case of amino acid overdosage is possible, which can lead to cardiovascular (hyperlipemia) or immune diseases [3]. Similarly as glutamine, addition of arginine or ornithine to the ration stimulates the immune system [2,3]. Effects of high-dose amino acids on secretion of specific hormones are known. For example, leucine and arginine stimulate insulin and growth hormone secretion, respectively, which can lead to leucine-induced hypoglycemia or potential activation of cell division. Amino acids in high doses can cause electrolyte disorders: hyponatremia in glycine treatment, acidosis and hyperkaliemia in treatment with arginine hydrochloride. Free amino acids are absorbed very rapidly and cause osmotic disorders. Development of diarrhea after oral arginine intake was described [3]. Behavioral and psychological tests are a very important aspect in the studies of high-dose amino acid effects, because some of them are characterized by neurological effects.

The most pronounced metabolic effects can develop after treatment with essential amino acids or amino acids with low endogenous biosynthesis. Amino acids with branched carbohydrate chain (ABCC), such as leucine, isoleucine, and valine, attract special attention. Excessive doses of leucine impair utilization of two other amino acids. The mean need in ABCC is 1.53 g/kg for parenteral and 2.64 g/kg for enteral treatment. Safe level of ABCC consumption is 1.99 g/kg/day for parenteral and 3.13 g/kg/day for enteral treatment. Up to 40% ABCC is utilized by the small intestine. Up to 57% leucine taken enterally appears in the portal bloodflow. Insufficient content of leucine in the ration results in limitation of protein biosynthesis and increase in the plasma concentrations of other essential amino acids [2]. Leucine excess modulates neutrophil phagocytosis [1].

We analyzed morphofunctional changes in this tissues after additional enteral leucine treatment.

MATERIALS AND METHODS

Male albino Wistar rats (180-220 g) received leucine intragastrically through a tube in a daily dose of 100 mg/kg for 5 days. The rats were kept in large cells at natural illumination and room temperature with free access to water and food. The animals were sacrificed by ether overdosage. Changes in the thymus, spleen, small intestine, and liver were studied by histological methods. Ultrastructural changes in the liver were studied by electron microscopy. Liver specimens for electron microscopy were fixed in 1% osmium tetroxide in 0.1 M Millonig buffer (pH 7.4) at 4°C for 2 h. After dehydration in ascending alcohols and acetone the specimens were embedded in epon and araldite mixture. Semithin sections were sliced from blocks on an MT-7000 ULTRA ultramicrotome and stained with methylene blue. The preparations were examined under an light microscope and sites for subsequent study of ultrastructural changes were selected; in order to provide standard results, similar sites of the hepatic lobules were selected during the final preparation of liver samples. Ultrathin sections were then prepared and contrasted with 2% uranyl acetate in 50% methanol and with lead citrate. The preparations were examined in a JEM-100 CX electron microscope at 7000-24,000 magnifications. Fragments of the thymus, spleen, small intestine, and liver for microscopic examination were fixed in Carnoy fluid, 5-μ sections were stained with hematoxylin and eosin.

RESULTS

Light microscopy showed that 5 doses of leucine did not cause appreciable changes in the structure of the liver tissue. Only moderate uneven dilatation of the sinusoidal capillaries in the peripheral and central compartments of the liver lobules was seen.

Leucine caused reversible ultrastructural changes in the liver. Enlargement of the nucleoli and increase in their number were noted in the hepatocyte nuclei, the granular component predominating in the nucleoli, which were located close to the membrane. This status of the nucleoli, decondensed chromatin, and clearly seen pores in the nuclear membrane indicate increased biosynthetic activity, which was confirmed by increased number of mitochondria with numerous cristae and increased activity of the granular endoplasmic reticulum (GER) with numerous ribosomes fixed to its cisterns (Fig. 1). A close contact of the mitochondria with the GER membranes was noted; this fact indicates processes requiring much energy. In parallel, the cytoplasm of many hepatocytes more often than in control animal liver contained Golgi complex components and high content of lipid incorporations (Fig. 1). These morphological signs indicate intensification of the compensatory and repair processes in response to partial damage to the liver tissue. Activation of cell-cell exchange processes was observed, which was confirmed by numerous depressions and protrusions of the hepatocyte membrane lateral surfaces. On the other hand, the content of lysosomes increased in the cytoplasm of many

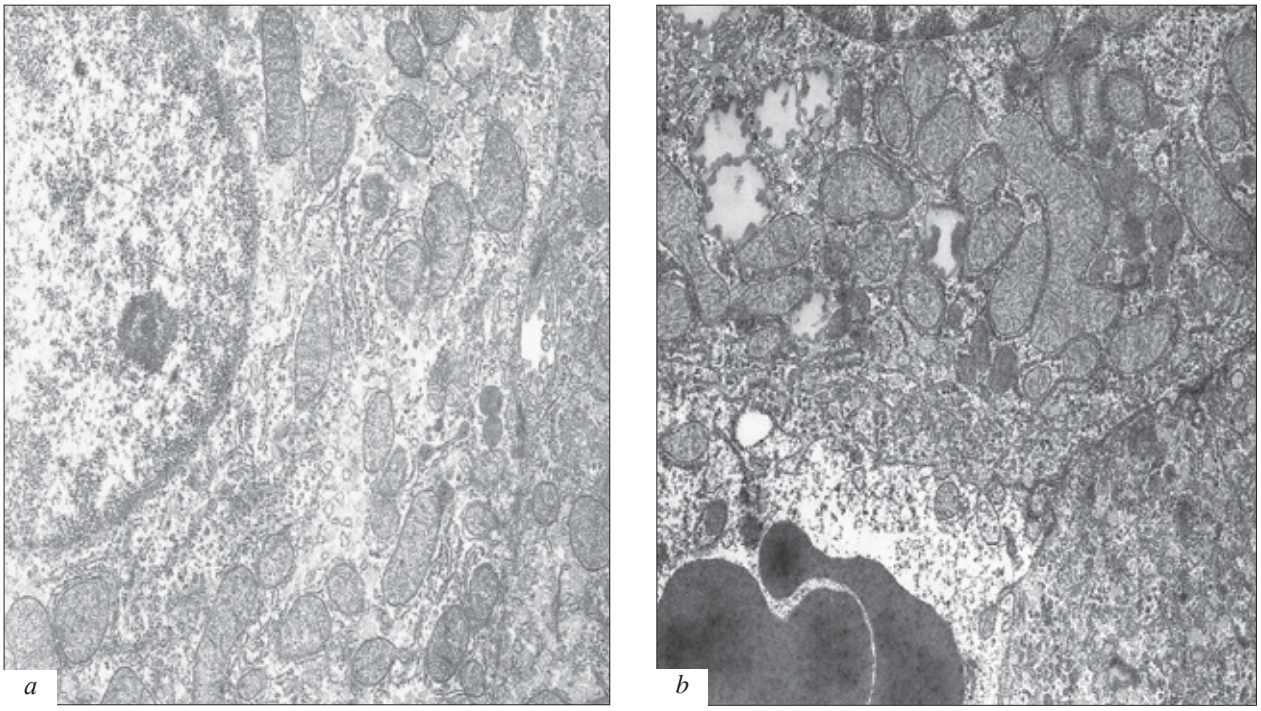

Fig. 1. Ultrastructural changes of hepatocytes. Contrasting with uranyl acetate and lead citrate. *a*) control animals (×10,000); *b*) animals after leucine treatment (×7200).

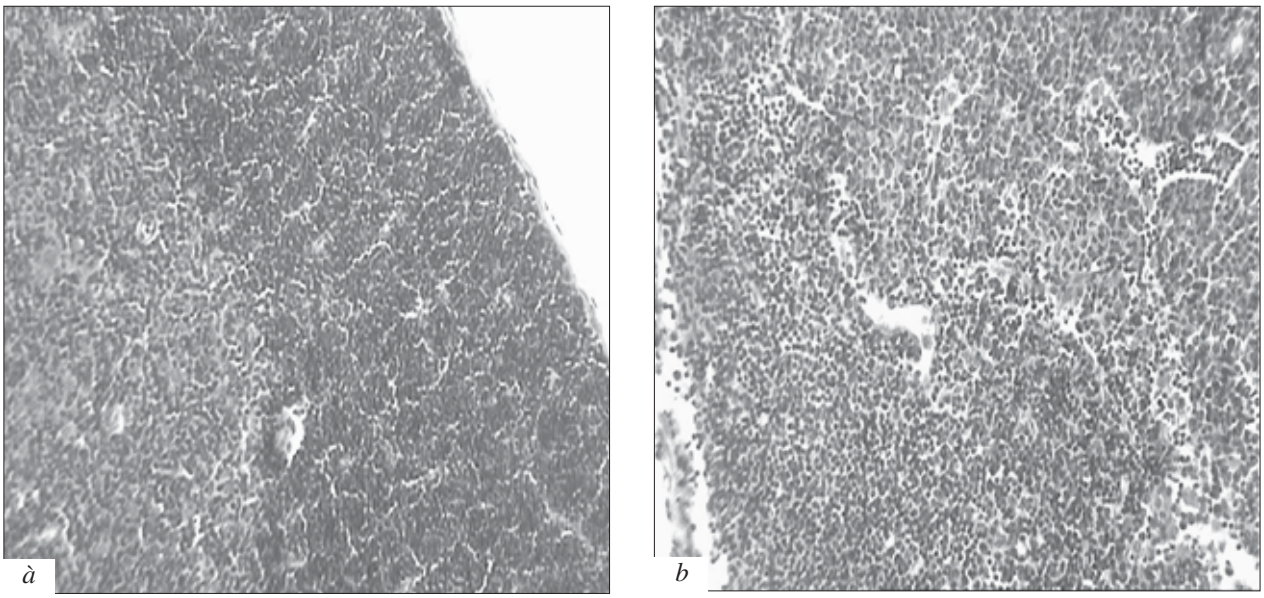

Fig. 2. Morphologic changes in the thymus. Hematoxylin and eosin staining (×10,000). *a*) control animals; *b*) animals after leucine treatment.

hepatocytes, which, depending on their contents, looked like peribiliary bodies and granules, multivesicular bodies and vesicles, lamellar structures, enveloped in double membrane, phagolysosomes. The increase in the number of lysosomes was presumably caused by increased functional loading of cells in response to pharmacological effect of leucine.

No appreciable destructive changes in capillaries were detected: sinusoidal capillaries were moderately dilated, filled with fine granular substance containing solitary macrophages, fibroblasts, and few blood cells. Bile capillaries had typical structure, contained numerous microvilli directed into slightly narrowed (at the expense of the villi) lumen. Hepatocytes of the same lobule were hetero-

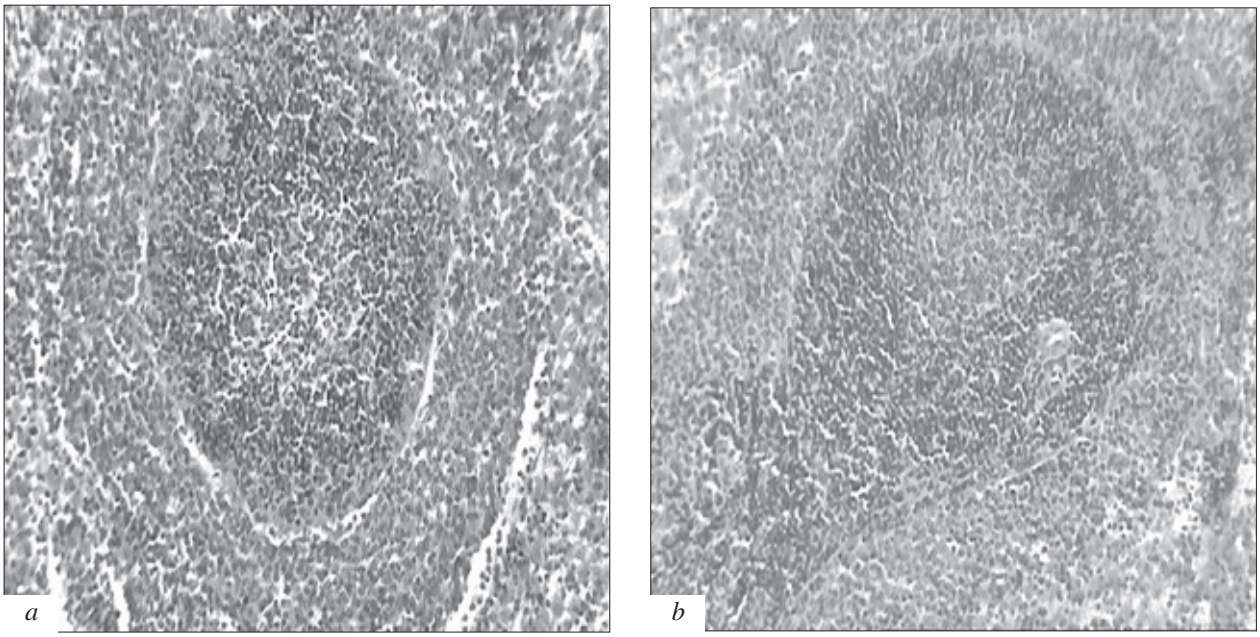

Fig. 3. Morphologic changes in the spleen. Hematoxylin and eosin staining (×10,000). *a*) control animals; *b*) animals after leucine treatment.

geneous by the density of the cytoplasmic matrix and number of organelles (Fig. 1, *b*). "Dark" hepatocytes were characterized by greater number of mitochondria, which reflected higher functional activity of these cells.

The thickness of the cortical matter of the thymus decreased after leucine treatment, while the width of the medulla increased in comparison with control animals (Fig. 2). Vascular lumens were dilated, the number of lymphocytes decreased, that of Hassal's bodies and plasma cells increased in the medulla. Macrophage accumulation was observed among lymphocytes in the thymic cortical matter. Large lymphocytes were more often seen in the subcapsular zone than in controls.

Malpighian bodies and periarterial lymphoid sheaths increased in size in the spleen after leucine treatment (Fig. 3). Lymphocyte infiltration of the periarterial zone increased in the Malpighian bodies and the mantle zone increased in size. Moderately dilated venous sinuses were seen in the red pulp and accumulation of hemolytic erythrocytes was noted among stromal reticular cells.

Five intragastric doses of leucine had a variety of effects on the small intestinal structure. An unfavorable effect on villous epithelium was noted. Epitheliocytes decreased in height and their cytoplasm was vacuolated, its oxyphilic characteristics sharply decreased, and the nuclei were pyknotized. Many of epitheliocytes were desquamated, denuding connective tissue base of the villi, abundantly infiltrated by lymphocytes. The small intestinal mucosa was covered with a thick mucous film with numerous desquamated epitheliocytes. Goblet cells in the villous epithelium were sharply narrowed because of decrease in the number of secretory granules in them. On the whole, goblet cells were decreased presumably because of intense secretion.

Hence, five daily enteral doses of leucine (100 mg/kg) led to morphofunctional changes in the organs. Further studies will help to define the pathophysiological indications for monotherapy with this amino acid. It is obvious that the creation of amino acid-based drugs and treatment with amino acids or their combinations should be carried out with due consideration for the risk of morphofunctional changes in some organs and tissues. These effects should be evaluated and described at the stage of preclinical trials of the drugs.

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