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Changes In The Level Of Free Amino Acids In Blood Plasma Of Rats With Experimental Allergic Contact Dermatitis

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Aim of the study: Allergic contact dermatitis (ACD) is one of the most common among inflammatory skin diseases. Modern diagnostic methods are imperfect, therefore one of the developing methods is metabolic profiling - simultaneous analysis of a large number of metabolites, their concentrations and ratios. The purpose of this work is to determine the differential metabolic profiles of blood plasma amino acids in animals with experimental allergic contact dermatitis.

Material and Methods: The experiment conducted on male Wistar rats weigh 280-300 g (n=7). The model of allergic contact dermatitis was carried out according to a standard procedure. The active ingredient was 2,4-Dinitrofluorobenzene. The basis is a mixture of acetone: olive oil (4:1). The animals were divided into 3 experimental groups: - control group, control group with basis application, experimental group with application of active substance. The material for the study was a deproteinized blood plasma containing an internal standard. The concentration of free amino acids was determined by the HPLC method with pre-column derivatization with o-phthalaldehyde. Chromatographic separation of amino acids derivatives was achieved using an LCMS-2020 liquid chromatograph (Shimadzu, Japan) with an automatic injector and a fluorimetric detector. Column - Zorbax Poroshell 120 EC-C18. Solvent A: 0.15 M Na-acetate buffer, pH 6.0; Solvent B: Methanol/acetonitrile/water 45/45/10 (v/v). The elution was in a gradient mode from 5 to 100% B. The time of analysis was 45 min. (Flow rate 1 ml/min); Column temperature was 35 °C. The OPA-amino acid derivatives were measured with fluorescence (338/445 nm). Statistical analysis and calculations were performed using Statistica 6.0.

Results: In the group with induced contact dermatitis, the level of some amino acids increased in blood plasma: aspartic acid (1,5 times), glutamic acid (1,5 times), tyrosine (1,51 times), α-aminobutyric acid (2 times), valine (1,56 times), leucine (1,6 times), lysine (1,5 times). At the same time, the level of a number of amino acid derivatives decreased compared to the control group: anserine (3,5 times), carnosine (10 times), 3-methylhistidine (1,7 times). The significant decrease in the levels of carnosine and anserine was interesting. These substances are dipeptides, derivatives of amino acids of histidine and β-alanine. Anserine is a derivative of the methylated form of carnosine. The physiological role of carnosine is not completely determined, but it has been established that it possesses immunostimulating properties, participates in the hypersensitivity reaction in the development of photodermatitis. Carnosine has antioxidant activity and involved in a number of biochemical reactions, such as oxidative modification of proteins and glycation of proteins. The glycation products take a direct part in the reactions of oxidative stress, the activation of inflammatory reactions, including in the development of inflammatory skin diseases (ACD). The findings suggest that the development of ACD can cause a significant imbalance in the spectrum of free amino acids of blood and their derivatives. The results are of interest for assessing the extent of metabolic abnormalities, the specific features of metabolic shifts, and also in the identification of metabolic markers that can be used to study the pathogenesis of ACD and the development of effective treatment technologies.

Keywords: Allergic contact dermatitis, metabolomics, amino acids, carnosine, anserine