A STUDY OF N,N´-(ETHANE-1,2-DYYIL)BIS(QUINOLINE-2-CARBOXAMIDE), DIAKAMPH, AND METFORMIN INFLUENCE ON THE HISTOLOGICAL STRUCTURE OF THE LIVER IN THE ACUTE PERIOD OF ALLOXAN-INDUCED DIABETES IN RATS

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Introduction. Diabetes mellitus (DM) is the most acute medical and social problem relating to the priorities of the national health systems of all countries. DM is the third place most common cause of morbidity and the most common endocrine disease. The rapid increase in the prevalence, heterogeneity of patients, and continuous progression of DM determine the need in the development of new effective drugs. On the way of these drugs search, our attention is attracted by N,N'-(ethane-1,2-dyyil)bis(quinoline-2-carboxamide) – a compound with an original mechanism of action, which, according to our data, involves I_1 and I_2 imidazoline receptors stimulation. Another approach consists in drugs development on the basis of water-soluble diacamph derivative (diacamph hydrochloride), which combines antihyperglycemic and cerebroprotective properties. As the liver plays a crucial role in the mechanisms of DM development, it is expedient to determine the influence of the investigated drugs on its functional state and morphological structure.

Aim. The objective of this study is to investigate the effect of N,N'-(ethane-1,2-dyyil)bis(quinoline-2-carboxamide) and diacamph hydrochloride in comparison with the reference drug metformin on the histological structure of the liver and its glycogen content in the acute period of alloxan-induced diabetes in rats.

Materials and methods. White random-bred male rats with the body mass equal to 0.20±0.02 kg were divided into the groups of intact control, untreated (diabetic animals), and three groups of diabetic animals receiving the aforementioned drugs. DM was modeled by subcutaneous alloxan monohydrate (Sigma, USA) administration once at a dose of 150 mg/kg as a 5% solution in acetate buffer, pH 4.5. The animals previously were deprived of food for 24 h, but had free access to water. N,N'-(ethane-1,2-dyyil)bis(quinoline-2-carboxamide) was administered intragastrically at a daily dose of 11.64 mg/kg (ED_{50} for hypoglycemic effect) as an aqueous suspension stabilized by polysorbate-80. Diacamph hydrochloride and metformin were administered intragastrically at daily doses of 25 mg/kg and 100 mg/kg respectively, for three days after alloxan injection. Animals of the intact control and the untreated group received an equivalent amount of drinking water. The animals were taken out of the experiment under anesthesia and liver samples for further light microscopy studies were collected at the beginning of the fourth day after alloxan injection, at which time β-cell destruction is considered to reach its

maximal level. Liver samples were taken for the determination of glycogen content with anthrone reagent. Fragments of the liver were fixed in 10% formalin solution, dehydrated in increasing concentrations of ethanol, and embedded in paraffin. The sections were stained with hematoxylin and eosin. The microscope "Granum" and the digital video camera "Granum DCM 310" were used, and the photographs were processed using computer Pentium 2,4GHz through a ToupView program.

Results and discussion. The histological structure of the liver of intact animals remained normal. Hepatocytes were arranged in radiating cords. The cells retained their characteristic shape and size, the cytoplasm was evenly painted and optically dense, it contained no inclusions visible under light microscopy. The nulcei of hepatocytes were normochromatic, centrally located, containing one, and sometimes two nucleoli. Pool of binuclear cells was sufficient. The boundaries of the lobules were determined by triads. Triades zones were narrow. The normal state of epithelium of triads and other vessels was registered. Intralobular sinusoidal hemocappilaries were moderately dilated and contained moderate number of lymphoid cells. Kupffer cells (stellate reticuloendotheliocytes) demonstrated normal structure. No disorders of beam-lobule structure were seen in the liver samples of the untreated group. In certain animals, fibrinoid swelling of arterial vessel wall and protein effusion in whole triad zone were observed. Increase in apoptosis of hepatocytes, mild mononuclear cell infiltration of the bile ducts in some of the triads. The histological structure of the liver of diabetic animals receiving N,N'-(ethane-1,2-dyyil)bis (quinoline-2-carboxamide), was not changed, still moderate edema, fibrinoid swelling of arterial vessel wall in some of the triads were present. The histological structure of the liver of diabetic animals treated with diacamph hydrochloride was not changed. Slight focal vacuolation of the cytoplasm of hepatocytes was observed in one sample. In some of the triads fibrinoid swelling of the arterial vessel wall was seen. In the liver of rats receiving metformin the changes of histological structure of hepatocytes and vessel wall in the triads, visible under light microscopy, were practically absent, a very fine droplet vacuolization of hepatocytes was observed in one sample. Glycogen content in the liver of the untreated diabetic rats reduced almost 3 times. Diacamph hydrochloride and metformin did not change this value, while it even tended towards increase against a background of N,N'-(ethane-1,2-dyyil)bis (quinoline-2-carboxamide).

Conclusions. N,N'-(ethane-1,2-dyyil)bis (quinoline-2-carboxamide) reduces the toxic effect of alloxan on the liver in rats, that is evidenced by the absence of hepatocyte apoptosis, the decrease in morphological signs of the degenerative changes of the vascular wall, and prevention of glycogen depletion. This drug, as well as diacamph hydrochloride, is almost as effective as the well-known drug metformin.