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CERAMIDE AS A MARKER OF SEVERITY AND ACTIVITY OF DIABETES MELLITUS TYPE 2 AND NONALCOHOLIC FATTY LIVER DISEASE.

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Sphingolipids are biological active components of all cell membrane. They play a great role in cell interconnections and take part in such process as a proliferation, maturation and cell apoptosis. Ceramide deserve special an attention, because they could be precursors of apoptosis and lead to development of diabetes mellitus type 2 (DM type 2) and nonalcoholic fatty liver disease (NAFLD). The aim of the research was to investigate dependence between ceramide levels in plasma, level of insulin and HOMA indices in patients with DM type 2 and activity of AST, ALT in patients with NAFLD.

Materials and Methods. The study was performed in three groups. The total number of patients was 60. Two of groups consisted of patients with DM type 2 and NAFLD (n=20, n=20 respectively) and the third one included healthy persons. The level of insulin, HOMA indices and activity of AST and ALT were assessed by using common biochemical blood analyses. Plasma ceramides (C14:0, C16:0, C18:0, C18:1, C20:0, C24:0 and C24:1) were quantified using electrospray ionization tandem mass spectrometry after separation with HPLC.

Results. As result of researches, we got data that show high levels of ceramide in the first and second group comparing with healthy persons. This result is tightly correlate with the high level of insulin, HOMA indices and activity of AST and ALT in patients with DM type 2 and NAFLD respectively.

Conclusion During our researches, we found out dependence between high ceramide levels with severity of DM type 2 and activity rate of NAFLD. So, further investigation of ceramide is needed for taking them as parameters for prognosis DM type 2 severity or as development NAFLD complications such as nonalcoholic steatohepatitis.

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FEATURES OF DISTRIBUTION OF VICIA SATIVA AGGLUTININ (VSA) RECEPTORS IN THE INTERCELLULAR MATRIX OF THE MENISCI OF RAT KNEE JOINT AFTER INTRAFETAL INJECTION OF ANTIGENS

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The results of previous investigations at the Department of Human Anatomy of Zaporizhzhia State Medical University have shown that for the modeling of the syndrome of undifferentiated connective tissue dysplasia in rats it is possible to use the intrauterine antigenic effect that leads to changes in the rates of morphogenesis of fetal organs and tissues [3]. Lectins are informative molecular probes that can detect glycoconjugates in cells and tissues. Lectins and their receptors provide intercellular, cell-matrix interactions, participate in the regulation of proliferation, differentiation and apoptosis of cells [1, 2]. Aim of the work was to establish the features of distribution of Vicia sativa agglutinin (VSA) receptors in the intercellular matrix of the menisci of rat knee joint after intrafetal injection of antigens.

Material and Methods. Menisci of knee joints were studied in 160 white laboratory rats from the 1st to the 90th days of life. Group I – 60 intact rats. Group II – 60 experimental rats – the offspring of female rats, which on the 18th day of the dated pregnancy underwent the injection of purified staphylococcal toxoid (1:10, 0.05 ml) according to the method of professor N.A. Voloshyn (1981). 40 rats of group III after injection of saline solution served as control. When working with animals we were guided by «European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes» (Strasbourg, 18.03.86) and Law of Ukraine «On the protection of animals against cruel treatment» (№ 3447-IV). Receptors for Vicia sativa agglutinin in the histological sections were detected using standardized sets of VSA-HRP (RPC «Lectinotest»). The imaging was carried out in the diaminobenzidine-hydrogen peroxide system. The intensity of the deposition of benzidine label was assessed semi-quantitatively.

Results. On the 1st day after birth the intensity of VSA receptors distribution in the intercellular matrix of both inner (+/++) and outer (+) zones is low. The visceral part of the joint capsule covering the menisci in all groups of animals is stained in yellow-brown (++) color and is not changed during three months of observation in the group of animals after intrafetal injection of antigens.

On the 5th day in all groups of rats, the intensity of the benzidine label deposition in the inner zone decreases to the level of outer one (+). This level of α-D-mannose residues in the intact and control groups persists until the end of the first month inclusive in the inner zone and up to the 14th day in the outer one. In the group of antigen-injected rats, in contrast to the control ones, there is a temporary increase in the number of VSA receptors in the outer zone on the 7th day of observation. On the 21st day after birth, the level of VSA receptors in the inner zone of the menisci of experimental rats increases (++), followed by a decrease to the previous level on the 30th day (+). In the outer zone of the menisci of rats of all groups the content of α-D-mannose residues increases (++), which remains until the end of the first month inclusive.

Subsequently, starting from the 45th day, the decrease in the intensity of the benzidine label deposition (0/+) in both zones of the menisci of rats in all the studied groups is determined until the end of the third month of postnatal life.

Thus, in animals of the intact and control groups it was found that during the first month of life there is insignificant content of the VSA receptors in the meniscus structures with a slight increase in the outer zone at the end of the second month. Intrafetal antigens injection results in a relative increase in the content of a-D-mannose residues in the outer zone on the 7th day and in the inner zone on the 21st day. The revealed changes between the observation groups are leveled after the 30th day.

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Key words: meniscus, rat, antigen, Vicia sativa agglutinin (VSA).

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ROLE OF ADIPOKINES AND INTERLEUKIN-17 IN THE REGULATORY MECHANISM OF BONE REMODELING IN RAT MODEL OF KIDNEY FUNCTION DISORDER

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Cytokines and adipokines take an active part in regulatory mechanism of bone remodeling which is involved in the higher level mechanism of regulation uniting bone with the kidney. Role of these substances in the mechanisms is not clearly understood. Increased adiponectin levels are characteristic of the chronic kidney disease and associated with its progression. It promotes renal fibrosis through inducing monocyte-to-fibroblast transition [1]. In normal kidney it has anti-inflammatory properties and supposed to have renal protecting effect. Adiponectin signaling plays an important role in endocrine mineral regulation. Visfatin level is also increased in kidney disease and it was supposed that visfatin may mediate inflammatory responses inducing pro-inflammatory cytokines [2]. IL-17 is considered as proinflammatory cytokine which augment renal injury [3]. The aim of the study was to investigate the effects and interrelations of adiponectin, visfatin and IL-17 in bone remodeling regulation in kidney function disorder model in rats.

Materials and Methods In this study two groups of 9-month-old female rats (control (20 intact animals) and experimental (20 animals)) weighing 210 ± 30 g were used. Renal excretory function impairment was created by a single intramuscular 50% glycerol injection in dose of 1.0 ml / 100 g weight of the animal. Experiment was carried out in 12 weeks after glycerol injection. Blood samples were collected through heart puncture of the anesthetized with chloroform rats. Serum adiponectin, visfatin and interleukin-17 (IL-17) levels were measured using ELISA. The impairment of bone remodeling was controlled by direct measurements of the bone density which was calculated as a ratio between the bone mass (g) and the bone volume (cm3) was measured by the liquid replaced. The statistical analyses were performed by Statistica 6.0 programmes. The significance was considered at $p<0.05$.

Results. In rats with kidney function disorder model all the cytokines have elevated levels ($p<0.05$). These data correlate with literature [4-7]. Analysis of their interrelations showed that in the intact rats it was no correlation between adiponectin and visfatin levels, weak positive correlation between adiponectin and IL-17 and weak negative correlation between visfatin and IL-17 was shown. This may be the evidence of the same direction of adiponectin and IL-17 regulating action and opposite one of visfatin and IL-17 in the intact animals. In kidney function disorder model these cytokines change their interrelations. It was high negative correlation ($r=-0.79$) of adiponectin with visfatin levels, medium strength negative correlation ($r=-0.43$) of adiponectin with IL-17 levels and high positive correlation ($r=0.84$) of visfatin with IL-17 levels, demonstrating unidirectional regulatory effects of visfatin and IL-17 and opposite adiponectin with visfatin and adiponectin with IL-17 directions of their effects. This may give evidence of these three cytokines compensatory effects in impaired functional regulatory cytokine network in rats with kidney function disorder. Kidney function disorder model results in inflammatory processes and this involve certain links of integrative regulatory mechanisms which unite regulation in bone and kidney. Increased levels of proinflammatory visfatin and IL-17 may demonstrate their role in bone and kidney integration.

Conclusion. Adiponectin, visfatin and IL-17 can be identified as a link in regulatory mechanisms of bone remodeling in model of kidney function disorder.

Perspectives These adipokines and IL-17 may serve as a promising therapeutic target for correction of kidney disease.