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SBA AND PNA LECTIN RECEPTORS IN RAT LIVER STRUCTURAL COMPONENTS ON THE BACKGROUND OF EXPERIMENTAL STREPTOZOROCIN-INDUCED DIABETES MELLITUS

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РЕЦЕПТОРИ ЛЕКТИНІВ SBA ТА PNA У СТРУКТУРНИХ КОМПОНЕНТАХ ПЕЧІНКИ ЩУРА НА ТЛІ ЕКСПЕРИМЕНТАЛЬНОГО СТРЕПТОЗОТОЦИН-ІНДУКОВАНОГО ЦУКРОВОГО ДІАБЕТУ

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РЕЗЮМЕ

З використанням лектинів PNA, SBA, дослідити вуглеводні детермінанти βDGal, NAcDGal у складі структурних компонентів печінки на тлі експериментального стрептозотоцин — індукованого діабету, провести порівняльний аналіз з експресією рецепторів інших лектинів, на основі попередньо проведених досліджень. Дослідження проводили на 20 щурах-самцях лінії Вістар масою 110–120 г, які були розділені на дві групи (10 контрольних і 10 дослідних). Експериментальний цукровий діабет викликали дочеревним уведенням тваринам стрептозотоцину фірми “Sigma” (США) з розрахунку 7 мг на 100 г маси тіла. Розвиток діабету контролювали за рівнем глюкози, яку визначали глюкооксидазним методом з використанням реактивів фірми “LaChema” (Чехія). На 14 день розвитку діабету після евтаназії тварин забивали. Кусочки печінки фіксували у 4 %-ному нейтральному формаліні з наступною заливкою у парафін за стандартною методикою. Для отримання оглядових препаратів зрізи товщиною 5–7 мкм фарбували гематоксиліном і еозином. Вуглеводні детермінанти досліджували з використанням двох лектинів, мічених пероксидазою хрону; візуалізацію здійснювали в системі 3’3 діамінобензидину тетрагідрохлориду (“Sigma”, США) в присутності H2O2. На тлі стрептозотоцин-індукованого діабету спостерігали розширення просвіту синусоїдних гемокапілярів і перисинусоїдного простору Діссе, інфільтрацію лейкоцитами портальних трактів, внутрішньочасточкову інфільтрацію лімфоцитами і плазмоцитами, зернисту та жирову дистрофію гепатоцитів, каріопікноз. Утворення лімфоцитарних інфільтратів всередині печінкових часточок, розширення центральних вен, збільшення кількості стромальних елементів навколо портальних трактів. Окрім морфологічних змін збільшується кількість клітин Купффера та перисинусоїдних ліпоцитів з експонуванням у них глікополімерів βDGal, NAcDGal.

РЕЦЕПТОРЫ ЛЕКТИНОВ SBA И PNA В СТРУКТУРНЫХ КОМПОНЕНТАХ ПЕЧЕНИ КРЫСЫ НА ФОНЕ ЭКСПЕРИМЕНТАЛЬНОГО СТРЕПТОЗОТОЦИН-ИНДУЦИРУВАННОГО САХАРНОГО ДИАБЕТА

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РЕЗЮМЕ

С использованием лектинов PNA, SBA, исследовали углеводные детерминанты βDGal, NAcDGal в составе структурных компонентов печени на фоне экспериментального стрептозотоцин — индуцированного диабета, провели сравнительный анализ с экспрессией рецепторов других лектинов на основе предварительно проведенных исследований. Исследования проводили на 20 крысах-самцах линии Вистар массой 110–120 г, которые были разделены на две группы (10 контрольных и 10 опытных). Экспериментальный сахарный диабет вызывали внутрибрюшным введением животным стрептозотоцина фирмы “Sigma” из расчета 7 мг на 100 г массы тела. Развитие диабета контролировали за уровнем глюкозы, которую определяли глюкооксидазным методом с использованием реактивов фирмы “LaChema” (Чехия). На 14 день развития диабета после эутаназии животных забивали. Кусочки печени фиксировали в 4 %-ном нейтральном формалине с последующей заливкой в парафин. Для получения обзорных препаратов срезы толщиной 5–7 мкм окрашивали гематоксилином и эозином. Углеводные детерминанты исследовали с использованием двух лектинов, меченных пероксидазой хрена; визуализацию рецепторов лектинов осуществляли в системе 3’3 диаминобензидина тетрагидрохлорида (“Sigma”, США) в присутствии H2 O2.На фоне стрептозотоцин-индуцированого диабета наблюдали расширение просвета синусоидных гемокапилляров и перисинусоидного пространства Диссе, инфильтрацию лейкоцитами портальных трактов, внутридольковую инфильтрацию лимфоцитами и плазмоцитами, зернистую и жировую дистрофию гепатоцитов, кариопикноз. Образование лимфоцитарных инфильтратов внутри печеночных долек, расширение центральных вен, увеличение количества стромальных элементов вокруг портальных трактов. Кроме морфологических изменений увеличивается количество клеток Купффера и перисинусоидных липоцитов с экспонированием у них гликополимеров βDGal, NAcDGal.

Key words: experimental diabetes, liver, lectin histochemistry, Kupffer cells, Ito cells.

Diabetes mellitus (DM) — one of the most abundant endocrine diseases, currently it is the medical and social problem, which occupies one of the numerous places along with cardio-vascular and oncologic pathology. World specialists in diabetology acknowledge, that DM is noninfectious epidemic, which belongs to the 5 main population mortality reasons in most countries [2, 9]. According to the prognosis of International diabetes institute (Australia), till 2010 increase of DM patients amount in the world is expected to be 239.3 millions,

and insulin-dependent form — 23.7 millions, in particular. The amount of DM patients in Ukraine is 1.5–3 % of the whole population [2, 7, 9]. Insulin-dependent DM development is caused by combined or isolated effect of genetic tendency, environmental factors, immune regulation violations. These factors can explain antibody-dependent cytotoxicity, that suggests possibility of this mechanism in liver cells damage in patients with insulin-dependent DM [1, 6, 12]. The stated facts indicate, that different types of liver damages — inflammatory and exchange ones, develop at DM. Liver plays important role in normal physiology and homeostasis, and Kupffer cells are the keys in understanding of liver damage mechanisms in these various processes [5, 13]. The most widespread DM model is streptozotocin-induced diabetes, which replaced alloxan model, which has been used previously [4, 9]. Streptozotocin, natural broad-spectrum antibiotic, is produced by Streptomyces achromogens actinomycetins, possesses oncogenic, diabetogenic properties and represents a 2-deoxy-2 ({[methyl (nitroso) amino]carbonyl)amino)-β-D-glucopyranose, which leads to specific toxic effect on B cells [8, 11].

Considering, that insulin receptors are on the hepatocytes surface, liver is directly involved in carbohydrate exchange and DM beginning, we propose the given research.

Aim: By means of PNA and SBA lectins to investigate βDGal and NAcDGal carbohydrate determinants in liver structural components on the background of experimental streptozotocin-induced diabetes, to conduct comparative analysis with other lectin receptors expression on the basis of preliminary done research.

MATERIALS AND METHODS

Research was performed on 20 Wistar male rats with mass 110–120g, which were divided into two groups. Control group included 10 animals, experimental group — 10 rats, which were kept in standard vivarium conditions. Experimental DM was caused by intraabdominal streptozotocin (company "Sigma", USA) injection in the rate of 7 mg/100 g body mass. Diabetes progress was controlled by the glucose level, which was detected by glucooxydase method by use of "LaChema" (Czech Republic) company reagents in accordance with producer instructions. Animal maintenance and manipulations were performed according to the positions "General ethical principles of the experiments on animals", approved by I National Congress of Bioethics (Kyiv, 2001).

In 14 days after streptozotocin injections animals, which got glucose level in blood in between 10–18 mmol/l, were scored by decapitation after ether narcosis overdose. Liver pieces were fixed in 4 % neutral formalin with subsequent embedding in paraffin according to the standard techniques. For getting panoramic slides, sections 5–7 μm thick were stained by hematoxylin and eosin. Carbohydrate determinants were examined by lectins, labeled with horseradish peroxidase; visualization was conducted in 3'3-diaminobenzidine tetrahydrochloride ("Sigma", USA) system in H_2O_2 presence, as described by [10]. The used lectins: peanut lectin (PNA, specific to βDGal (β1–3)DGalNAc), soy lectin (SBA, specific to DGalNAc). Lectins were purified and labeled by horseradish peroxidase at Histology Department of Danylo Halytsky Lviv National Medical University by Doctor of Pharm. Sciences V. Antoniuk [3]. For specificity control of histochemical reactions were used: 1) lectin-peroxidase conjugates exclusion from staining protocol; 2) before lection solution application, preincubation of histological slides was conducted during 60 min. in 1 % HIO_4 (Reanal, Budapest, Hungary), for oxidation of glycopolymers carbohydrate determinants. In the 1st case histochemical reaction results were completely negative, in the 2nd — essentially reduced. Besides abovementioned, negative staining of separate cell compartments on the base of lectin-reactive structures served as peculiar control of reaction specificity. Slides were analyzed by means of Carl Zeiss Jena Ng microscope, for photographing digital camera Canon IXUS 700 and photo-system Olympus on the base of BX-41 microscope were used.

RESULTS AND DISCUSSION

Panoramic slides showed, that control animals liver has lobular structure. In centers of lobules central veins are located, from them liver plates go, which are formed by hepatocytes. The latter have lightly acidophilic cytoplasm, separate hepatocytes have two nuclei, also hepatocytes with polyploid nuclei are detected. Sinusoidal blood capillaries are often filled with erythrocytes. Portal triads are surrounded by connective tissue with large amount of cellular elements, among which fibroblasts prevail.

Sinusoid lumen and space of Disse enlargement, portal triad infiltration by leukocytes, intralobular infiltration by lymphocytes and plasma cells, granular and adipose dystrophy of hepatocytes, karyopyknosis and absence of nuclei in separate hepatocytes, sometimes areas of necrosis were observed under conditions of glucose high concentration in blood in rat liver of experimental animals. Formation of lymphocyte infiltrations inside liver lobules, central veins enlargement, increase of stromal elements amount around portal triads were observed as well.

In control animals PNA lectin receptors, localized near central veins, were identified in separate hepatocytes, while at DM increased expression of specific PNA lectin βDGal (β1–3)DGalNAc receptors in all hepatocytes around central veins was observed.

Besides, PNA lectin receptors were detected in cytoplasm of Kupffer cells, amount of which increased at DM. Tiemeyer M. et al., 1992, [15] have showed, that on the surface of Kupffer cells there are oligosaccharides terminal residues in the form of galactose, N-acetylgalactoseamine, and fucose.

Kupffer cells belong to the mononuclear phagocytic system and play phagocytic function. Increase of their amount with βDGal (β1–3)DGalNAc expression indicate an activation of immune processes on early stages of streptozotocin-induced DM passing, also, Takeishi T. et al., 1999, [14] have indicated, that Kupffer cells have the potential to exert both stimulatory and inhibitory influences on hepatocyte proliferation.

Lectin histochemical investigations, conducted earlier by us, detected specificity in different carbohydrate specificity lectins binding with liver structural components in both control and experimental animals. So, at experimental streptozotocin-induced diabetes reduction of Con A lectin Man-specific receptors was observed in cytoplasm of central lobular hepatocytes with simultaneous reactivity expression of nuclear and cytoplasmic glycoconjugates of central veins endothelial cells, nuclei of adjacent hepatocytes, and also within hepatocytes cytoplasm of lobules periphery and endothelial cells of portal triads vessels. At DM WGA lectin receptors reduction was observed in the direction from central veins to the periphery of lobules, however the highest reactivity to that lectin hepatocytes around central vein and sinusoids endothelial cells showed.

Hepatocytes destructive changes, presence of intralobular inflammatory areas, detected signs of apoptosis or necrosis may be the reason of activation, and, possibly increasing of macrophages amount at diabetes, exhibiting of their surface βDGal (β1–3)DGalNAc is accompanied by intensification of their functional properties.

In control animal liver moderate activity of SBA lectin receptors was noted on the hepatocytes vascular surface and on nuclear membrane of their nuclei. In experimental animal liver high activity of SBA receptors was observed in cellular elements of connective tissue around portal triads and in their blood vessels, with their gradual reduction in hepatocytes in the direction from central veins to lobular periphery, with their simultaneous expression in Kupffer cells cytoplasm. Epithelial cells of cholangioles and bile ducts exhibited affinity to NAcDGal-SBA specific lectin.

Besides abovementioned, high activity of SBA lectin receptors was in perisinusoidal lipocytes (Ito cells) at DM. Since at streptozotocin-induced DM adipose dystrophy of hepatocytes is observed, increase in amount of connective tissue elements around portal triads is observed with high activity in them SBA receptors. Probably, this is connected with Ito cells activity, lectin SBA exhibiting in Ito cells stimulate their functional activity and collagen genesis. Lectin histochemical investigations, conducted earlier, showed that receptors expression of HPA lectin, which has similar carbohydrate specificity to HPA lectin in endothelial cells of portal triads vessels, increased, while in control high expression of that lectin was in cytoplasm of hepatocytes, reticular fibers of space of Disse, endothelial cells of central veins tunica intima.

CONCLUSION

Besides morphological changes in liver, amount of Kupffer cells and perisinusoidal lipocytes, with exhibiting in them glycopolymers βDGal, NAcDGal, increase on 14th day after streptozotocin injection.

In perspective liver investigation is planned in more remote terms of experimental streptozotocin-induced diabetes passing, by using wider panel of new lectins with similar carbohydrate specificity.

Table 1

Lectin histochemical characteristics of rat liver structural components in the norm and after streptozotocin injection

Name of lectin, its c.h. s.	Control group							Experimental group						
	Liver lobule					P.t.	S.e.	Liver lobule					P.t.	S.e.
	H	S	B.c.	K.c.	C.v.			H	S	B.c.	K.c.	C.v.		
Lectin of peanut seeds PNA (αNAcDGlc)	+	-	-	++	-	-	+ c.f.	+	++	++	-	-	-	+ c.f.
Lectin of soybean seeds SBA (NAcDGal)	++	-	-	++	+	+++ t.i.	+ c.f.	++	++	++	-	-	+++ t.i.	+ c.f.

Note: c.h. s.- carbohydrate specificity,

H-hepatocytes, S-sinusoids, B.c.-bile capillaries, K.c.- Kupffer cells, C.v.— central vein,

P.t.-portal triads, S.e.-stromal elements,

+ light lectin binding; ++ moderate; +++ intensive lectin binding;

— absence of binding;; t.i.— vascular tunica intima; c.f.– collagen fibers

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