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Peculiarities of Bcl-2, p53 and c-Kit protein distribution in endocrinocytes of pancreatic islets in SHR rats with arterial hypertension

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Ключові слова: підшлункова залоза, артеріальна гіпертензія, SHR, інсулін, глюкагон, Bcl-2, p53, c-Kit, автоматизований підрахунок клітин.

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Arterial hypertension is a concomitant disease in type 2 diabetes and the coexistence of both conditions increases the risk of microvascular and macrovascular complications in patients, as well as leads to a threefold increase in the risk of other cardiovascular diseases.

Aim. Identify quantitative distribution patterns of Bcl-2, p53 and c-Kit proteins and the magnitude of their expression in endocrinocytes of pancreatic islets in SHR rats with hereditary arterial hypertension.

Materials and methods. The study was conducted on 10 white Wistar rats and 10 SHR rats with hereditary arterial hypertension. Insulin, glucagon, c-Kit, Bcl-2 and p53 proteins were detected by immunofluorescence using antibodies produced by Santa Cruz Biotecnology (USA). The immunofluorescence reaction was studied using an AxioImager-M2 fluorescence microscope (Carl Zeiss, Germany) equipped with an AxioCam-5HRm camera (Carl Zeiss, Germany), using 38NE and 43NE high emission light filters (Carl Zeiss, Germany).

Results. A distinctive feature of the organization of pancreatic islets in hypertensive SHR rats was characterised by a 30 % ($p < 0.05$) decrease in the average area of pancreatic islets, a 14 % ($p < 0.001$) decrease in the number of beta cells in them, combined with an 18 % ($p < 0.001$) increase in the number of alpha endocrinocytes compared to normotensive Wistar animals. At the same time, insulin concentration in beta-cells of hypertensive animals was decreased by about 20 % ($p < 0.001$), and glucagon concentration in alpha-cells was increased by 36 % ($p < 0.001$). Examination of immunoreactivity to Bcl-2, p53 and c-Kit proteins in normotensive and hypertensive rats showed patterns of high and low expression in endocrinocytes of pancreatic islets. The present study shows that SHR rats develop diverse changes in the functional state of endocrine cells of the pancreas, based on which several pathogenetic aspects can be suggested to explain the remodelling of pancreatic islets in hereditary hypertension: a decrease in the number of beta cells expressing Bcl-2 protein reduces their anti-apoptotic potential and thus facilitates the formation of pro-apoptotic proteins of the BCL-2 family that activate the mitochondrial pathway of apoptosis However, against the background of a decreasing population of beta-endocrinocytes in the pancreas, such a mechanism should be regarded as ineffective.

Conclusions. Formation of hereditary hypertension in SHR rats leads to a reduction in the population of beta-cells in the pancreatic islets, a decrease in insulin concentration in them and an increase in the number of alpha-endocrinocytes. In the beta-cells of hypertensive SHR rats, the expression of proliferation factor c-Kit protein increases, the expression of the anti-apoptotic protein Bcl-2 decreases and the intracellular concentration of pro-apoptotic protein p53 remains at the level of normotensive animals. In alpha-cells of hypertensive SHR rats, the expression of c-Kit protein is suppressed against the background of the increase in the total content of pro-apoptotic protein p53 in islets, while the content of anti-apoptotic protein Bcl-2 in islets remains at the level of normotensive animals.

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Особливості розподілу білків Bcl-2, p53 та c-Kit в ендокриноцитах панкреатичних острівців у щурів лінії SHR з артеріальною гіпертензією

Т. В. Іваненко, Ю. М. Колесник, А. В. Абрамов

Артеріальна гіпертензія є супутнім захворюванням при діабеті 2 типу, і співіснування обох станів збільшує ризик мікросудинних та макросудинних ускладнень у пацієнтів, а також призводить до трикратного збільшення ризику інших серцево-судинних захворювань.

Мета роботи – визначити кількісні закономірності розподілу білків Bcl-2, p53 і c-Kit та величину їх експресії в ендокриноцитах острівців підшлункової залози у щурів лінії SHR зі спадковою артеріальною гіпертензією.

Матеріали і методи. Дослідження проведено на 10 білих щурах лінії Вістар та на 10 щурах лінії SHR зі спадковою артеріальною гіпертензією. Інсулін, глюкагон, білки c-Kit, Bcl-2 та p53 виявляли імунофлуоресцентним методом за допомогою антитіл виробництва Santa Cruz Biotecnology (США). Вивчення імунофлюоресцентної реакції проводили на флуоресцентному мікроскопі AxioImager-M2 (Carl Zeiss, Німеччина), оснащеному

камерою AxioCam-5HRm (Carl Zeiss, Німеччина), із застосуванням високоемісійних світлофільтрів 38HE та 43HE (Carl Zeiss, Німеччина).

Результати. Особливість організації острівців підшлункової залози у гіпертензивних щурів лінії SHR характеризувалася зменшенням середньої площі острівців підшлункової залози на 30 % ($p < 0,05$), зменшенням кількості бета-клітин у них на 14 % ($p < 0,001$) у поєднанні зі збільшенням кількості альфа-ендокриноцитів на 18 % ($p < 0,001$) порівняно з нормотензивними тваринами лінії Вістар. Водночас концентрація інсуліну в бета-клітинах тварин з гіпертензією знизилася приблизно на 20 % ($p < 0,001$), а концентрація глюкагону в альфа-клітинах збільшилася на 36 % ($p < 0,001$). Дослідження імунореактивності до білків Bcl-2, p53 та c-Kit у нормотензивних та гіпертензивних щурів показало закономірності високої та низької експресії в ендокриноцитах острівців підшлункової залози. У цьому дослідженні показано, що у щурів лінії SHR розвиваються різноманітні зміни функціонального стану ендокринних клітин підшлункової залози, на основі яких можна запропонувати кілька патогенетичних аспектів для пояснення ремоделювання панкреатичних острівців при спадковій артеріальній гіпертензії: зменшення кількості бета-клітин, що експресують білок Bcl-2, знижує їх антиапоптотичний потенціал і тим самим сприяє утворенню проапоптотичних білків родини BCL-2, які активують мітохондріальний шлях апоптозу. Однак на тлі зменшення популяції бета-ендокриноцитів у підшлунковій залозі такий механізм слід розглядати як неефективний.

Висновки. Формування спадкової гіпертензії у щурів лінії SHR зумовлює зменшення популяції бета-клітин в острівцях підшлункової залози, зниження концентрації інсуліну в них та збільшення кількості альфа-ендокриноцитів. У бета-клітинах гіпертензивних щурів SHR експресія фактора проліферації – білка c-Kit збільшується, експресія антиапоптотичного білка Bcl-2 зменшується, а внутрішньоклітинна концентрація проапоптотичного білка p53 залишається на рівні нормотензивних тварин. В альфа-клітинах гіпертензивних щурів лінії SHR експресія білка c-Kit пригнічується на тлі збільшення загального вмісту проапоптотичного білка p53 в острівцях, тоді як вміст антиапоптотичного білка Bcl-2 в острівцях залишається на рівні нормотензивних тварин.

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Arterial hypertension is the most common form of cardiovascular disease in the adult population of the world and is often the main cause of other cardiovascular diseases, chronic renal failure, cerebral stroke and premature death worldwide [1]. As a rule, arterial hypertension is a concomitant disease in type 2 diabetes and the coexistence of both conditions increases the risk of microvascular and macrovascular complications in patients, as well as leads to a threefold increase in the risk of other cardiovascular diseases [2]. Although hypertension and type 2 diabetes share common risk factors, particularly obesity, dyslipidaemia, sedentary lifestyle, and common pathogenetic mechanisms such as excessive sympathetic nervous system activation, insulin resistance and endothelial dysfunction, according to most researchers both pathological conditions are independent in their occurrence, although mutually aggravating clinical pathologies [2,3,4].

An earlier study of metabolic status in hypertensive SHR rats showed, that when fasting blood glucose, triglycerides and insulin levels were normal, after glucose loading the animals showed impaired glucose tolerance test [5,6], and diet with added sucrose led to even higher systolic blood pressure and increased insulin resistance [5]. Morphological study of the pancreas of normoglycemic hypertensive SHR rats revealed signs of pancreatic islets remodelling, characterised by a decrease in the pool of insulin-synthesising beta-endocrinocytes [7] and an increase in the pool of glucagon-synthesising alpha-cells [8]. At the same time, a significant decrease in the concentration of anti-apoptotic protein Bcl-2 and an increase in the apoptosis index of endocrinocytes in pancreatic islets of SHR rats were found [9].

The above data suggest that the formation of genetically determined arterial hypertension in SHR rats is accompanied by functional and morphological disorders of the endocrine apparatus of the pancreas, based on a decrease in the pool of cells synthesising insulin. In this connection, it seems important for us to evaluate the potential ability of pancreatic endocrinocytes to differentiation and proliferation (c-Kit protein), as well as the balance of pro-apoptotic and anti-apoptotic molecular markers (p53 and Bcl-2 proteins).

Aim

To identify quantitative distribution patterns of Bcl-2, p53 and c-Kit proteins and the magnitude of their expression in endocrinocytes of pancreatic islets in SHR rats with hereditary arterial hypertension.

Materials and methods

The study was conducted on 10 white Wistar rats and 10 SHR rats with hereditary arterial hypertension. Systolic blood pressure in rats was measured using a non-invasive recording system Blood Pressure Analysis Systems TM BP2000 Series II (Visitech Systems, USA). Blood glucose levels were measured in the morning on an empty stomach using a GlucoCard-II glucometer (Japan). Wistar rats constituted the group of 'normotensive animals' (control) with an average weight of 232 ± 7 g, fasting glycaemia of 3.94 ± 0.09 mmol/l and systolic blood pressure of 105.0 ± 1.1 mm Hg. SHR rats constituted the group of 'hypertensive animals' with an average weight of 305 ± 6 g, fasting glycaemia level of 4.73 ± 0.10 mmol/l and systolic blood pressure of 155.7 ± 0.9 mm Hg. Under thiopental anaesthesia (50 mg/kg), the pancreas was harvested, fixed in Bouin's solution for 20 hours, and, after standard histological processing, embedded in paraffin

Table 1. Distribution of endocrinocytes in pancreatic islets

Animal groups	Area of islets, μm²	Relative number of endocrinocytes in islets, %		Hormone concentration in endocrinocytes, Uif/μm²	
		beta cells	alpha cells	insulin	glucagon
Normotensive	5260 ± 686	78.80 ± 1.05	21.20 ± 1.05	2.03 ± 0.03	1.51 ± 0.05
Hypertensive	3589 ± 423*	67.82 ± 0.48**	31.42 ± 1.48**	1.62 ± 0.02*	2.06 ± 0.04**

*: significance of differences to the indicators of normotensive animal $p < 0.05$, **: significance of differences to the indicators of normotensive animal $p < 0.001$.

(MkCormick, USA). Serial 5 μm thick histological sections of the pancreas were deparaffinised and demasked in citrate buffered saline (pH = 9.0) in a PT module (Thermo Scientific, USA). Insulin, glucagon, c-Kit, Bcl-2 and p53 proteins were detected by immunofluorescence, using antibodies from Santa Cruz Biotechnology (USA). For this purpose, a mixture of antibodies to insulin or glucagon conjugated to AlexaFluor-546 and to Bcl-2, p53 or c-Kit conjugated to FITC, respectively, was prepared at a dilution of 1:200, followed by incubation in a humid chamber (T = + 4 °C, 24 hours). Sections washed in phosphate buffer (pH = 7.4) were fixed in UltraCruz™ Mounting Medium in a mixture of phosphate buffer and glycerol with DAPI (Santa Cruz Biotechnology, USA) and covered with glass slides (Menzel-Glaser, Germany). The specificity of antibody binding was tested in the same way, except for incubation with primary antibodies.

The immunofluorescence reaction was studied using an AxioImager-M2 fluorescence microscope (Carl Zeiss, Germany) equipped with an AxioCam-5HRm camera (Carl Zeiss, Germany), using 38NE and 43NE high emission filters (Carl Zeiss, Germany). For fluorescence imaging, the AxioVision-4.8.2 digital image analysis system (Carl Zeiss, Germany) was used according to the method [10]. The digital image analysis system ImageJ version 2.1.0/1.53c (public open license) was used for image analysis.

For each pancreatic islet, the area of the material immunoreactive to the studied biomarkers was measured automatically. For insulin and glucagon, the area of immunoreactive material was calculated in relation to the total area of the islet and this parameter was further considered an indicator of the relative number of beta- and alpha-endocrinocytes in the pancreatic islet (%). For Bcl-2, p53, and c-Kit proteins, the area of immunoreactive material in the pancreatic islets was calculated in relation to the area of immunoreactive material to insulin or glucagon, respectively, and this parameter was considered an indicator of the relative number of Bcl-2/p53/c-Kit-expressing beta or alpha cells (%). The concentration of insulin, glucagon, Bcl-2, p53, and c-Kit in pancreatic cells was measured in arbitrary units of immunofluorescence (Uif/μm²) relative to nonspecific background fluorescence. At least 5 cm² of the total area of pancreatic sections from each animal was examined. At least 100 pancreatic islets were analysed for each marker.

The results were statistically processed in Excel Office365. Differences between the compared parameters were considered significant at $p < 0.05$ by Student's t-test. The data in the tables are presented in the form of the mean value and its error (M ± m). The data in the text are presented as mean and confidence interval.

Results

The peculiarities of pancreatic islet organisation in hypertensive SHR rats were a 30 % ($p < 0.05$) decrease in the average area of pancreatic islets, a 14 % ($p < 0.001$) decrease in the number of beta cells in them, combined with an 18 % ($p < 0.001$) increase in the number of alpha endocrinocytes compared with normotensive Wistar animals (*Table 1*). At the same time, insulin concentration in beta-cells of hypertensive animals was decreased by about 20 % ($p < 0.001$) and glucagon concentration in alpha-cells was increased by 36 % ($p < 0.001$).

Examination of immunoreactivity to Bcl-2, p53 and c-Kit proteins in normotensive and hypertensive rats showed patterns of high and low expression in endocrinocytes of pancreatic islets (*Fig. 1–3*).

The pattern of endocrinocytes with a high level of expression of regulatory proteins in normotensive animals was characterised by a predominance of c-Kit-expressing endocrinocytes and a significantly lower number of Bcl-2- and p53-expressing beta- and alpha-cells (*Table 2*). This was also combined with higher c-Kit protein concentrations in the cells compared to Bcl-2 and p53 proteins.

Formation of hereditary hypertension in SHR rats resulted in almost 2-fold increase ($p < 0.001$) in the number of beta-endocrinocytes with a high level of Bcl-2 protein expression, combined with a slight, by 7.8 % ($p < 0.05$), increase in its concentration in insulin-synthesising cells (*Table 2*). On the part of the pool of glucagon-synthesising alpha-cells, there was a 3.7-fold increase in the number of cells with a high level of Bcl-2 protein expression, combined with an 82 % increase in its concentration ($p < 0.001$). At the same time, a rather significant, by 2 orders of magnitude, decrease in the number of endocrinocytes with a high level of c-Kit protein expression was observed in the alpha-cell population.

In contrast to cells with a high level of expression of regulatory proteins, the pattern of endocrinocytes with a low level of expression of these proteins in normotensive animals was characterised by a predominance of Bcl-2- and p53-expressing beta- and alpha-cells with a much lower number of c-Kit-expressing endocrinocytes (*Table 3*). The number of alpha-cells with low level of Bcl-2 protein expression was twice as high as the corresponding number of beta-endocrinocytes.

In contrast to normotensive animals, in SHR rats with hereditary hypertension there was a significant, order-of-magnitude reduction in the number of endocrinocytes of both types with low level of Bcl-2 protein expression. At the same time, only in alpha cells an increase in Bcl-2 protein concentration was observed

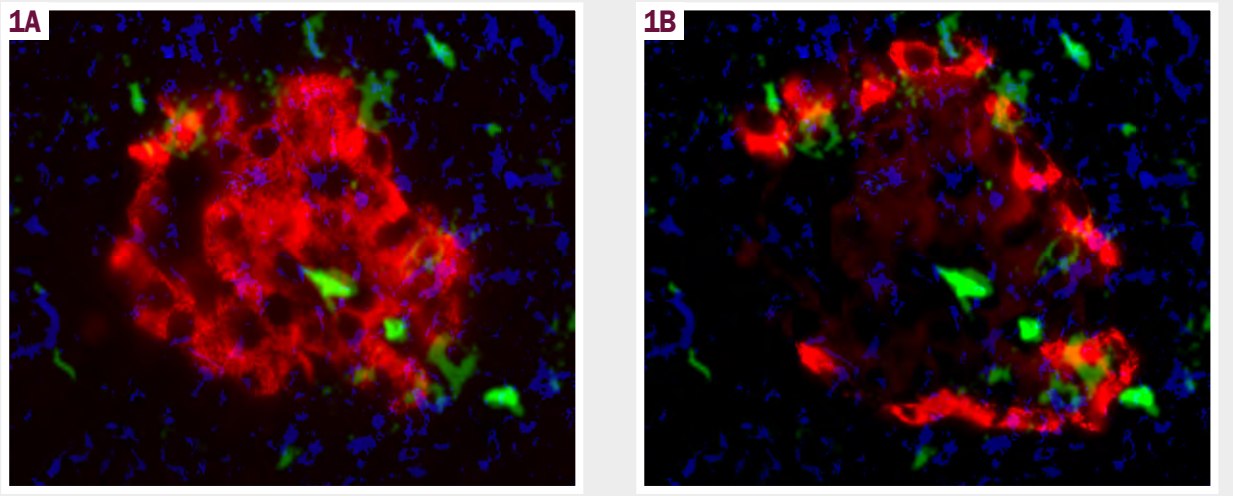

Fig. 1. Immunoreactivity with high (**green fluorescence**) and low (**blue fluorescence**) expression levels to c-Kit protein in pancreatic islets stained (**red fluorescence**) for insulin (**A**) and glucagon (**B**).

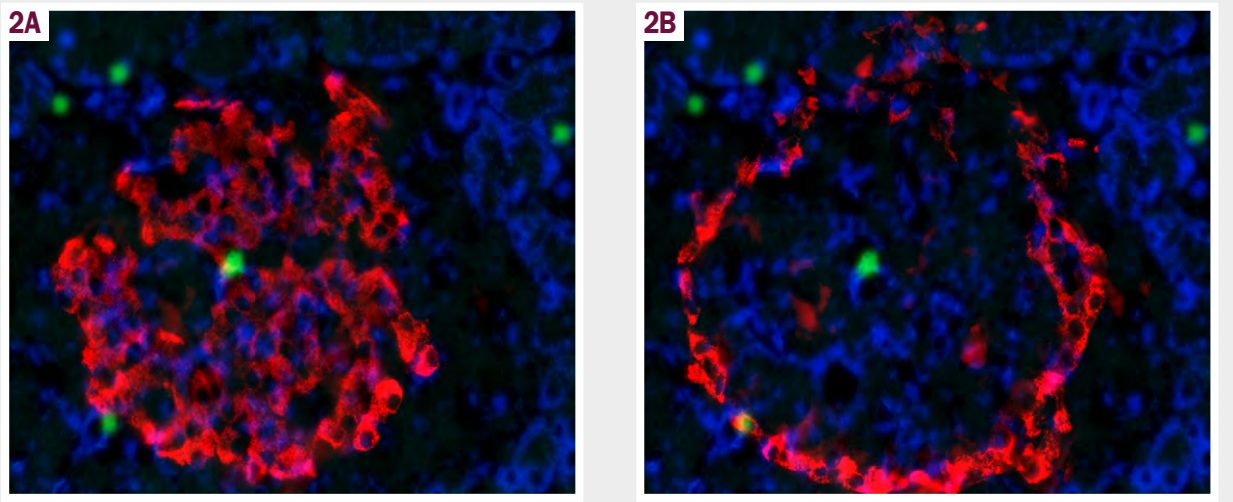

Fig. 2. Immunoreactivity with high (**green fluorescence**) and low (**blue fluorescence**) expression levels to p53 protein in pancreatic islets stained (**red fluorescence**) for insulin (**A**) and glucagon (**B**).

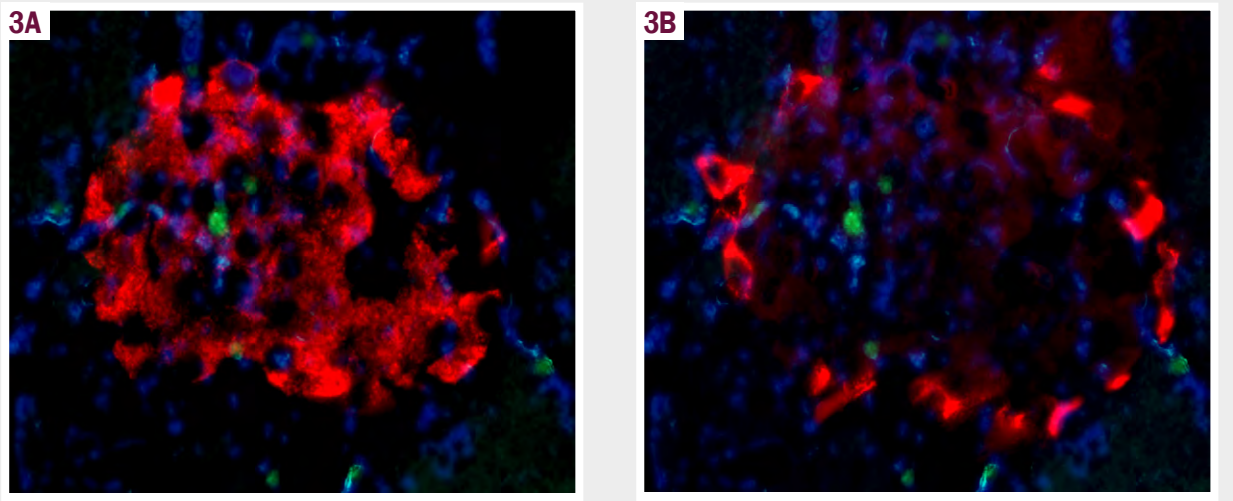

Fig. 3. Immunoreactivity with high (**green fluorescence**) and low (**blue fluorescence**) expression levels to Bcl-2 protein in pancreatic islets stained (**red fluorescence**) for insulin (**A**) and glucagon (**B**).

Table 2. Parameters of endocrinocytes with high expression of Bcl-2, p53 and c Kit proteins

Protein	Animal groups	Beta cells		Alpha cells	
		number of cells expressing the protein, %	protein concentration, Uif/μm²	number of cells expressing the protein, %	protein concentration, Uif/μm²
Bcl-2	Normotensive	0.199 ± 0.065	1.214 ± 0.279	0.159 ± 0.046	1.258 ± 0.311
	Hypertensive	0.383 ± 0.052*	1.676 ± 0.168	0.595 ± 0.087**	2.295 ± 0.288*
p53	Normotensive	0.803 ± 0.242	1.438 ± 0.215	0.629 ± 0.190	1.228 ± 0.222
	Hypertensive	0.833 ± 0.263	1.848 ± 0.276	1.120 ± 0.421	1.785 ± 0.264
c-Kit	Normotensive	5.689 ± 0.480	2.335 ± 0.042	2.926 ± 0.466	3.577 ± 0.177
	Hypertensive	4.855 ± 0.606	2.516 ± 0.046*	0.027 ± 0.011**	3.663 ± 0.236

*: significance of differences to the indicators of normotensive animal $p < 0.05$, **: significance of differences to the indicators of normotensive animal $p < 0.001$.

Table 3. Parameters of endocrinocytes with high expression of Bcl-2, p53 and c Kit proteins

Protein	Animal groups	Beta cells		Alpha cells	
		number of cells expressing the protein, %	protein concentration, Uif/μm²	number of cells expressing the protein, %	protein concentration, Uif/μm²
Bcl-2	Normotensive	5.586 ± 1.367	0.554 ± 0.064	12.568 ± 1.604	0.293 ± 0.061
	Hypertensive	0.594 ± 0.171**	0.55 3± 0.101	0.971 ± 0.187**	0.911 ± 0.121**
p53	Normotensive	7.267 ± 0.702	0.826 ± 0.059	9.753 ± 0.750	0.681 ± 0.069
	Hypertensive	8.378 ± 0.784	0.710 ± 0.070	10.189 ± 0.925	0.692 ± 0.080
c-Kit	Normotensive	0.782 ± 0.263	0.224 ± 0.062	0.780 ± 0.320	0.505 ± 0.145
	Hypertensive	5.028 ± 0.291**	1.674 ± 0.068**	0.044 ± 0.011*	1.301 ± 0.041**

*: significance of differences to the indicators of normotensive animal $p < 0.05$, **: significance of differences to the indicators of normotensive animal $p < 0.001$.

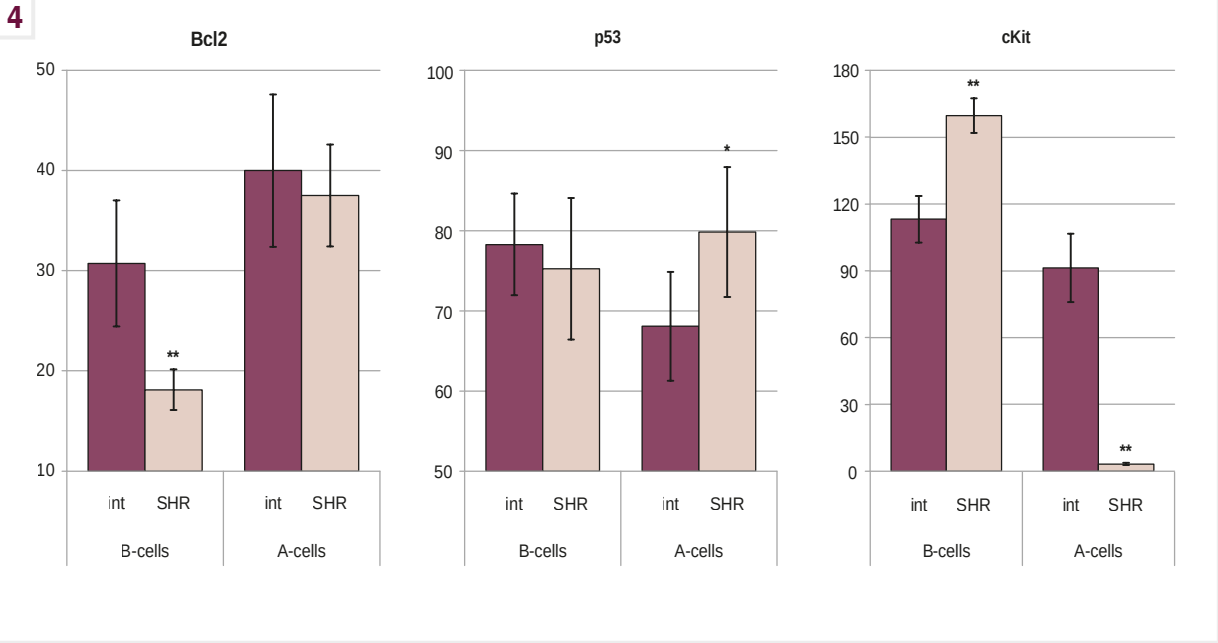

Fig. 4. Total content of regulatory proteins in pancreatic islets (Eif/1000 μm²); *: significance of differences to normotensive animals $p < 0.05$; **: significance of differences to normotensive animals $p < 0.001$.

(3-fold, $p < 0.001$). In the beta-cell population in hypertensive rats, the number of endocrinocytes with a low level of c-Kit protein expression increased by an order of magnitude in combination with a significant increase in its concentration (7.5-fold, $p < 0.001$). At the same time, in the population of alpha-endocrinocytes in hypertensive rats, there was a significant reduction in the pool of cells with a low level of c-Kit protein expression with a twofold increase in their c-Kit protein concentration ($p < 0.001$). It should be noted that hypertensive state of SHR rats had no effect on the pattern of endocrinocytes with low level of p53 protein expression *(Table 3)*.

Taking into account the presence among endocrine cells of the pancreas of patterns with different levels of expression of regulatory proteins, we calculated the integral characteristic of their content in pancreatic islets calculated per 1000 μm2 of islet cross-section (*Fig. 4*). On the basis of the obtained results we can generalise that in the pool of insulin-synthesising beta-endocrinocytes the formation of hereditary hypertension in SHR rats is accompanied by an increase in the content of c-Kit protein ($p < 0.001$) and a decrease in the content of Bcl-2 protein ($p < 0.001$). At the same time in the pool of glucagon-synthesising alpha-cells in hypertensive animals there is an increase in the content of p53 protein ($p < 0.05$) in combination with a significant reduction in the content of c-Kit protein ($p < 0.001$).

Discussion

The study of SHR rats has shown, that these animals are one of the representative models combining genetically determined arterial hypertension and insulin resistance [10,11]. Characteristic features of insulin resistance in SHR rats include decreased sensitivity of adipose [12] and muscle tissue to insulin [13], retardation of tyrosine phosphorylation in insulin receptor and insulin substrate receptor IRS-1 [14], and suppression of GLUT-4 glucose transporter membrane translocation in adipose tissue [15]. Our studies have shown, that the formation of hereditary hypertension in SHR rats is accompanied by remodelling of the endocrine apparatus of the pancreas, which is characterised by a decrease in the average size of pancreatic islets, a reduction in the pool of beta-cells combined with a decrease in insulin concentration in them, and opposite changes in the alpha-cell pool. Our data are in agreement with the results of earlier studies [7,8] and indicate a disturbance of pancreatic islets histogenesis in hereditary hypertension under normoglycemic status.

It should be understood that maintaining a physiologically optimal number of insulin-synthesising cells in the pancreas is controlled by the interaction of molecular factors that stimulate cell proliferation, survival and differentiation on the one hand, and apoptosis on the other.

One of the factors, controlling the population of insulin-synthesising cells is the membrane protein c-Kit, which is a tyrosine kinase receptor and by means of which various regulatory molecules stimulate the differentiation of beta cells from pancreatic progenitor cells and control the subsequent migration and invasion of endocrinocytes with the formation of new pancreatic islets [16,17]. The expression of c-Kit protein in endocrine cells increases the production of VEGF-A, which is the most important regulator of pancreatic islets angiogenesis [18], which is necessary to ensure the survival of beta cells and physiological regulation of islets function. Our data showed, that in normotensive rats among c-Kit-immunopositive beta-endocrinocytes the cells with high level of protein expression dominate, and in hypertensive animals in the pool of beta-cells the number of endocrinocytes with low level of c-Kit protein expression significantly increases, in which the concentration of c-Kit protein itself increases, as well as in endocrinocytes with high level of c-Kit expression.

At the same time, the total content of c Kit protein in beta-cells of pancreatic islets increases, which can be regarded as stimulation of regenerative potential of beta-endocrinocytes in hypertension. It seemed, that this should activate the proliferation of beta cells and lead to an increase in their number, but we obtained the opposite result: the number of beta cells in the pancreas of hypertensive rats significantly decreased, as well as the concentration of insulin in them. This differs significantly from the reaction of alpha-endocrinocytes to the formation of hypertension in SHR rats, the number of which in the islets increased together with the concentration of glucagon in them, despite a significant reduction in the number of c-Kit-immunopositive alpha-endocrinocytes and a decrease in the total content of c-Kit protein in pancreatic islets. The obtained data indicate the formation of dysregulatory disorders in the endocrine apparatus of the pancreas in hereditary hypertension. The peculiarities of pancreatic islets remodelling in hypertensive SHR rats may also depend on the expression parameters of other molecular regulators of histogenesis, in particular, apoptosis regulator proteins Bcl-2 and p53.

The p53 protein is a member of the family of pro-apoptotic cellular proteins, which has the ability to bind to sensitive DNA elements and thus induce or repress gene expression [19]. Intracellular activation of the p53 protein leads to an immediate anti-proliferative programme through a series of cellular events such as apoptosis, irreversible cell growth arrest and senescence, and cell cycle arrest with the ability to perform DNA repair after undergoing cellular stress [20]. Thus, one of the main functions of p53 protein is to monitor the functional state of cells and prevent the proliferation of damaged cells.

We previously reported, that in hypertensive SHR rats, the level of immunoreactivity to p53 protein in pancreatic islets does not differ from similar indicators in normotensive Vistar rats. In this study, it was established that despite the change in the numerical balance between beta and alpha cells in the islets, there are no interspecies differences in the intensity of p53 protein expression in endocrinocytes. Consequently, it can be assumed that the reduction in the number of beta-endocrinocytes in hypertension is not determined by the level of expression of the pro-apoptotic protein p53. On the other hand, it is necessary to take into account the need to maintain the physiological concentration of insulin in beta cells as an important factor in cell survival, since insulin, by activating the PI3K/AKT mechanism, ensures the degradation of the pro-apoptotic protein p53 in the cytoplasm and nucleus of the cell [20,21]. At the same time, stabilization of the nuclear fraction of the p53 protein can activate the transcription of pro-apoptotic genes, such as *bax* and *puma*, and suppress the expression of anti-apoptotic genes, such as *bcl-2* and *bcl-xL* [20]. Stabilized cytoplasmic p53 can be translocated into mitochondria and inter-

act with proteins of the BCL-2 family, suppressing the activity of anti-apoptotic proteins Bcl-2, Bcl-xL, and MCL-1, and enhancing the activity of pro-apoptotic proteins BAX and BAK [19,22]. In addition, the mobilization of the mitochondrial fraction of the p53 protein leads to the destabilization of the mitochondrial membrane and the release of cytochrome C into the cytosol, which leads to an increase in apoptosis and a decrease in the mass of beta cells in vitro [20,22].

It is obvious, that in order to understand the mechanisms of remodeling of pancreatic islets and changes in the number of endocrinocyte population in hereditary hypertension, it is necessary to take into account the nature of the expression of other cellular proteins involved in cell survival, for example proteins of the BCL-2 family.

The Bcl-2 protein is the main anti-apoptotic protein of the BCL-2 family and promotes cell survival. The second representatives of anti-apoptotic proteins of the BCL-2 family are BCL-xL, BCL-w, BCL2-A1/BFL-1 [19,23]. In addition, the BCL-2 protein family also includes a group of proteins that have the opposite, pro-apoptotic effect on cells: BAD, BAK, BAX, BID, BIK, BIM. There is a dynamic interaction between individual representatives of the BCL-2 protein family, which determines the strategy for implementing an anti- or pro-apoptotic effect in the event of cellular stress caused, for example, by the accumulation of reactive oxygen species in the cytosol or the action of pro-inflammatory cytokines on the cell membrane [22,23,24,25]. At the same time, the anti-apoptotic effect of the Bcl-2 protein is associated with the blocking of the synthesis of the complex of pro-apoptotic BAX/BAK proteins in the cytosol, as well as the interruption of the internal, mitochondrial, pathway of apoptosis due to the retranslocation of the active BAX/BAK complex from the mitochondria to the cytosol with its subsequent transformation into an inactive form [22,23,].

We found, that in the pancreatic islets of normotensive animals, alpha- and beta-endocrinocytes with a low level of Bcl-2 protein expression dominate, and the formation of chronic hypertension significantly reduces their number. Despite the fact that hypertension increases the number of endocrinocytes with a high level of Bcl-2 protein expression, they still constitute a minor fraction of Bcl-2-immunopositive cells of pancreatic islets. At the same time, the total content of Bcl-2 protein in the population of alpha cells in hypertensive rats does not differ from the similar indicator in normotensive rats. That is, in hypertension, the anti-apoptotic potential of alpha cells is not impaired, which cannot be said about beta-endocrinocytes, in whose population the total content of the Bcl-2 protein is significantly reduced.

Thus, the present study shows, that various changes in the functional state of endocrine cells of the pancreas are formed in SHR rats, on the basis of which we can hypothesize several pathogenetic aspects explaining the remodeling of pancreatic islets in hereditary hypertension:

1) A decrease in the number of beta-cells expressing the Bcl-2 protein reduces their anti-apoptotic potential and thus facilitates the formation of pro-apoptotic proteins of the BCL-2 family, which activate the mitochondrial pathway of apoptosis of beta-endocrinocytes.

2) A decrease in the formation of insulin in beta cells contributes to the intracellular stabilization of p53 protein and the activation of both its own pro-apoptotic activity and potentiation of the action of pro-apoptotic proteins of the BCL-2 family.

3) A decrease in the number of beta cells in the islets and a decrease in the formation of insulin in them removes the paracrine suppressive effect on the function of alpha cells.

4) Increased expression of the c-Kit protein in beta cells reflects compensation mechanisms aimed at preserving cell mass in case of increased apoptosis. However, against the backdrop of a reduction in the population of beta-endocrinocytes in the pancreas, such a mechanism should be considered ineffective.

In conclusion, it should be stated, that the problem of preserving the cell population is determined by the balance of cell proliferation stimulators and factors promoting cell survival, on the one hand, and molecular activators of cell apoptosis, on the other. Since genetically determined arterial hypertension and insulin resistance are combined in SHR rats, and with a normal level of fasting glycemia, the glucose tolerance test is impaired, the reduction in the number of insulin-synthesizing endocrinocytes in the pancreas may be the premorbid background, which precedes the first clinical manifestations of type 2 diabetes with hereditary hypertension.

Conclusions

1. The formation of hereditary hypertension in the SHR rat line leads to a reduction in the population of beta cells in the pancreatic islets, a decrease in the concentration of insulin in them, and an increase in the number of alpha-endocrinocytes.

2. In beta cells of hypertensive SHR rats, the expression of the proliferation factor – protein c-Kit increases, the expression of the anti-apoptotic protein Bcl-2 decreases, and the intracellular concentration of the pro-apoptotic protein p53 remains at the level of normotensive animals.

3. In the alpha cells of hypertensive SHR rats, the expression of the c-Kit protein is suppressed against the background of an increase in the total content of the pro-apoptotic protein p53 in the islets while maintaining the content of the anti-apoptotic protein Bcl-2 in the islets at the level of normotensive animals.

Ethical approval
The research programme was reviewed and approved by the Bioethics Commission of Zaporizhzhia State Medical and Pharmaceutical University (Protocol No. 10 dated 18 September 2025). The document was prepared on the basis of the "General Principles of Animal Experiments" (III National Congress on Bioethics, Kyiv, 2007) and brought into line with the "European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes" (Strasbourg, 1986), Directive 86/609/EEC and the Law of Ukraine "On the Protection of Animals from Cruel Treatment" No. 3447-IV of 21 February 2006.

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