The pregnancy as a inducting factor of intrinsic pathway of apoptosis in kidney's cells of female rats

Fizjologiczna ciąża jako czynnik aktywujący wewnętrzną drogę apoptozy w komórkach nerek samic szczura

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Abstract

The aim of this study was immunohistochemical evaluation of intrinsic pathway of apoptosis in renal epithelial cells in pregnant rats including time.

We evaluated in immunohistochemical way expression of the initiating caspase 9 – activated during apoptosis in protein complex-apoptosome.

Rats used in this experiment was divided into 4 groups – 8 individuals each: I group –female rats which was fertilized and decapitated in 10 day of pregnancy, II group female rats which was fertilized and decapitated in 20 day of pregnancy III group female rats which was fertilized and decapitated in 10 day after delivery VI group – control - female rats which decapitated in 30 day of experiment.

Specimens of kidneys taken to examine we analyzed in immunohistochemical standard three-step method detecting caspse 9 protein.

Immunohistochemical positive reaction increased in all experimental groups compared to control.

Pregnancy – induced hormonal changes in rats body activated apoptosis of renal cells via intrinsic pathway with active caspase 9. This pathway was the most intensive 10th day of pregnancy.

Keywords: Caspase 9, programmed cell death, kidney, pregnancy

Streszczenie

Celem pracy była immunohistochemiczna ocena egzogennej drogi indukcji sygnału do apoptozy w komórkach nabłonka nerek w fizjologicznej ciąży uwzględniająca czynnik czasu.

W pracy oceniono immunohistochemicznie ekspresję białka kaspazy 9 – inicjującej, aktywowanej białkowym kompleksie apoptosomie podczas wewnętrznej drogi indukcji sygnału do apoptozy.

Użyte do doświadczenia samice szczura podzielono na 4 równoliczne grupy – po 8 samic w każdej I grupa – samice, które zapłodniono i dekapitowano 10 dnia trwania ciąży II grupa - samice, które zapłodniono i dekapitowano 20 dnia trwania ciąży III grupa – samice, które zapłodniono i dekapitowano 10 dni po rozwiązaniu IV grupa – kontrolna – samice, które dekapitowano 30 dnia od początku doświadczenia.

Preparaty nerek pobrane do badań analizowano immunohistochemicznie standardowa trójstopniową metoda wykrywając białko kaspaza.9

Odczyn immunohistochemiczny wzrósł we wszystkich grupach doświadczalnych w porównaniu z grupą kontrolną.

Zmiany hormonalne wywołane fizjologiczną ciążą aktywowały apoptozę komórek nerek samic szczura ,w której sygnał do indukcji przebiegał drogą wewnętrzna przez aktywację kaspazy 9 . Droga ta była charakterystyczna szczególnie10 dnia ciąży.

Słowa kluczowe: kaspaza 9, programowana śmierć komórki, nerki, ciąża

Introduction

During normal pregnancy kidneys are changed as a first organ. Differences are seen in their metabolism and morphology.[1]. Renal pelvises and ureters are dilated. This is an influence of progesterone. On the end of pregnancy growing uterus presses ureters and causes low flow of urine. The result is physiological proteinuria (200-300mg/day).

During so big adaptation of kidneys to pregnant hormonal changes, kidneys' cells are damaged and destroyed. The increase of cells' apoptosis is observed.

This is one among other reason of EPH gestosis. Symptoms of gestosis are hypertension, oedemas and proteinuria [1].

The influence of the pregnancy on the evolution of kidney disease is still controversial. During physiological pregnancy could appear hypertension and disorder in kidney function. The pathology of these complication is unclear. When proteinuria and oedemas accompany it the pregnancy is in danger with eclampsia.[2]

In present study we analyzed the influence of pregnancy on cells of mothers' kidneys. We focused our attention on programmed cell death – apoptosis.

There are two general pathways of apoptosis [3]. Extrinsic pathway is stimulated by external factors .This pathway is connected to membranous death receptors. In intrinsic pathway we can distinguish mitochondrial and reticular ways.

The aim of the study was immunohistochemical evaluation of caspase 9 expression in pregnancy induced apoptosis of kidney's cells of female rats. Time was an important factor which was analyzed. We evaluated renal tubular epithelial cells and epithelial and endothelial cells of glomeruli on the beginning of pregnancy, on the end of pregnancy and ten days after delivery.

The aspartic acid specific protease caspase-9 (initiated caspase) has been linked to the mitochondrial death pathway. The active form of caspase-9 goes on to cleave procaspase-3 and procaspase-7.

Those caspases are members of executive phase of apoptosis[4]. They cleave several cellular targets, including poly ADP ribose polymerase. They destroy a genetic material in cellular nucleus (DNA).The cell dies.

Material and methods

The study material consisted of white Wistar female rats of the baseline body weight – 200-250g aged 2.5-3 months.

The rats were randomly selected according to the simultaneity principle of control and experimental groups.

On the beginning of experiment female rats paired with male rats. Every day fluid from vagina was observed under light microscope to detect the beginning of pregnancy (fertilization).

The animals were divided into 4 equal groups - 8 females each.

I group – fertilized female rats, decapitated 10th day of pregnancy.

II group - fertilized female rats, decapitated 20th day of pregnancy.

III group - fertilized female rats, decapitated 10th day after delivery

IV group – control – female rats, decapitated 30th day of experiment.

After decapitation the specimens from the left kidney were collected for further examinations. The kidneys were assessed macroscopically.

The renal specimens collected for immunohistochemical examinations were: fixed in 10% formalin, dehydrated in the alcohol series, subjected to xylene, embedded in paraffin blocks. The blocks were cut into 5µm-sections, which were placed on the silanized glasses. Two specimens from each kidney collected from each animal were used. Next paraffin was removed in xylene and in a graded alcohol series. The specimens were subjected to thermal preparation in the acid medium for antibodies against protein caspase 9.Next endogenous peroxidase was blocked by incubation in 0.3% H_2O_2. After that slides was incubated with mice rabbit primary antibody at room temperature (caspase 9 (Lab Vision RB-1205-PO) in 1% TBS/BSA, dilution 1/100),

Then the DakoCytomation kit was used for immunohistochemical reactions, which included: biotinylated secondary antibody against mice and rabbit antibodies (Biotinylated Link Universal), streptavidin conjugated with horse-radish peroxidase (Streptavidin-HRP), AEC substrate - HRP reaction dye (AEC Substrate chromogen)

After chromatogen staining the specimens were placed in the haematoxylin solution. The specimens were covered with coverslip using the Aquatex fluid.

The photographic documentation was prepared using the computer-guided Colour Video Camera CCD-IRIS(Sony).

For each specimen its negative control without primary antibody was prepared.

The results of immunohistochemical examinations were subjected to qualitative evaluation taking into account the intensity of colour reaction at the antigen-antibody site in rat organs examined in individual groups. And quantitative evaluation using the Analysis-pro software, version 3 (Soft Imaging System GmbH, Germany). The microscopic images, magnification x125 were analysed assessing the protein expression in 3 randomly chosen areas, 781193.35µm^2, each. The surface area of cells with positive reaction (+) was calculated.

The results were presented as means and standard deviation of the mean using the ONE WAY ANOVA test. 5% error risk and statistical significance at $p \leq 0.05$ were accepted.

The statistical analysis involved the differences in mean area with positive immunohistochemical reaction for antibody caspase9 examined in the individual groups.

Results

The immunohistochemical reaction for protein caspase 9 increased in all examined group comparison to control one.

In group II and III was on the same level. Between colour reaction noticed 20th day of pregnancy and 10 day after delivery there was no significant differences. Reaction in those groups was les intensity than in group observed 10 th day of pregnancy and significant bigger than in control group.

Pregnancy – induced hormonal changes in rats body activated apoptosis of renal cells via intrinsic pathway with active caspase 9. This pathway was the most intensive 10th day of pregnancy.

Disscussion

Normal, physiological pregnancy induces apoptosis of cells in some mother's organs and tissues. Apoptosis

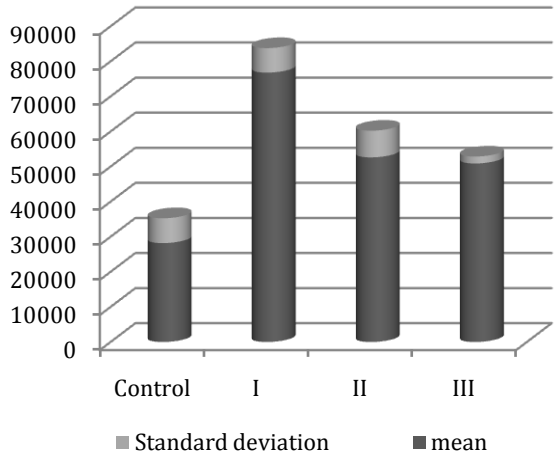
Tab. 1 The mean area [um ^{2}] covered by caspase 9 reaction in examined area: 781193,35 um^2 in all groups. Statistical significance of differences. ONE WAY ANOVA test

	kidney Caspase 9				One way ANOVA
	Control	I	II	III	
mean	28195,1	76794,41	52632,59	50897,63	p<0,0001
Standard deviation	7081,18	6980,91	7599,38	1974,41	

Tab.2 Caspase 9 reaction in rat kidneys. Statistical significance (p) of differences between experimental groups and control as well as of intergroup differences in experimental groups. Student's t-test

	kidney			
	Control	I	II	III
I	p<0,001			
II	p=0.001	p=0.001		
III	p=0.002	p=0.0008	p=0.68	

Table 1. Caspase 9



was described in cell of placenta. [5]. Some authors noticed apoptosis of maternal neutrophil. [6]. The Apoptosis plays an important role in the regulation of trophoblast survival and differentiation during pregnancy [7]. Shynlova et.al [8] described programmed cells death in pregnant rat myometrium. They noticed an activation of caspase cascade.

In our previous studies we showed, that physiological pregnancy induces apoptosis in renal cells of mother's kidneys.

On the end of pregnancy apoptotical signal was transmitted via extrinsic pathway by stimulation of death receptors and activation of caspase 8 [9].

In our present study we described pregnancy-induced apoptosis of mothers' renal cells in which inductive signal lead via intrinsic, mitochondrial pathway.

This pathway was the most intensive on the end of thirst trimester of pregnancy (10th day of pregnancy).In this time activation of caspase 9 increased the most. Caspase-9 is an initiator caspase encoded by the *CASP9* gene [10]. It is activated during programmed cell death (apoptosis).

Destroyed mitochondrial membrane by stress factors releases of cytochrome c from mitochondria. Cytochrome C and activated apaf-1 make protein complex (apoptosome). Apoptosome can transverse pro-enzyme of caspase-9 into the active form.

Results show that early pregnancy induces intrinsic pathway of apoptosis in mother's renal cell.

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