

An interim report on influence of NO on apoptosis in hepatocytes of white mice during aging

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ABSTRACT

Nitric oxide (NO) and its reaction products can either promote or prevent apoptosis in multiple of settings. The ubiquitous distribution of the NO syntheses and the remarkable diffusibility and diverse chemical reactivity of NO in biological systems make this molecule unique among the regulators of apoptosis. The experiments were performed on juvenile, adult and senescent white mice. The animals were killed under ether narcosis. The liver was removed. For the detection of apoptotic nuclei the flow cytometry was used. Concentration of NO in samples was measured by ESR Study. Obtained data show that during aging both, the rate of apoptosis and the intensity of NO production decrease in adults and increase again in senescents. However, the intensity of these changes is not similar in senescents. It can be suggested that the pro- or antiapoptotic influence of NO on hepatocytes is not univocal and it greatly depends on age-related changes in cellular homeostasis.

KEYWORDS: hepatocyte, nitric oxide (NO), apoptosis, aging

Apoptosis, sometimes called “programmed cell death”, the process that goes on continuously throughout the life has received phenomenal attention in the past few years [1,2,7]. Recent data suggest, that apoptosis and elimination of apoptotic cells are crucial factors in the maintenance of liver health [3,10]. It allows hepatocytes to die without provoking a potentially harmful inflammatory response. Liver disease is often associated with enhanced hepatocytes’ apoptosis [6].

In recent years, several studies have established that nitric oxide (NO) and its reaction products can either promote or prevent apoptosis in multiple of settings. The ubiquitous distribution of the NO syntheses and the remarkable diffusibility and diverse chemical reactivity of NO in biological systems make this molecule unique among the regulators of apoptosis [4,8].

The aim of the present work was to investigate the possible influence of nitric oxide (NO) on hepatocyte’s apoptosis in white mice during aging.

MATERIALS AND METHODS

Animals: 36 white mice were used. According to the age the animals were distributed in three groups: I group – juveniles (12 mice, 2-months old, $18,0 \pm 2,0$ g body weight), II group – adults (12 mice, 10-months old, $25,0 \pm 2,0$ g body weight), III group – senescents (12 mice, 18-months old, $30,0 \pm 2,0$ g body weight). The animals were maintained at 18-22°C temperature and light-controlled environment with a 12:12-h light-dark cycle and provided with food and water ad libitum. The animals were

anesthetized with ether narcosis and were sacrificed by the method of decapitation. Liver was removed.

Methods: Flow Cytometry. Samples were homogenized with a glass-Teflon Potter homogenizer in the 2,2 M succrose solution prepared on the phosphate buffer (pH 7,4). Nuclei were collected by centrifugating the homogenate at 18000 x g for 45 min. Received pure of nuclei was suspended in 3 ml TMS solution and centrifugated at 3000 x g during 10 min. 70% ethanol was added to the pellet and samples were incubated during 24-h at 4°C. Then 20 ml of RNase (10 mg/ml) was added and samples were incubated during 30 min at 37°C. After 1 ml of EB staining solution was added, samples were incubated during 30 min at 37°C. Number of apoptotic nuclei were counted by a Becton Dickinson (Mountain View, CA) FACScan flow cytometer.

ESR Study: Diethyldithiocarbamate-Na ($C_5H_{10}NS_2Na$) was used to measure the concentration of NO in samples. ESR spectra of the samples were recorded at liquid nitrogen temperature using an ESR spectrometer RE 1307 (Chernogolovka, Russia).

Quantitative analyses were performed using Student t test for independent samples. Probability (P) values of <0,05 were considered to be significant. All data are expressed as mean (standard deviation).

RESULTS AND DISCUSSION

The obtained quantitative data are presented in Tab.1.

PARAMETERS		Apoptosis %	Nitric oxide (NO) mm/mg
GROUPS			
I group	LIVER	5,05±0,72	21±1,73
II group	LIVER	2,63±0,65*	12,67±2,08*
III group	LIVER	37,3±11,7* *	16,1±0,85*

Note: * P < 0,05 ** P < 0,005

Tab.1 The distribution of apoptotic nuclei and nitric oxide (NO) in hepatocytes during aging.

As it is seen from *Tab.1*, the number of apoptotic nuclei decreases in adults (when compared to the same parameter of juveniles), and significantly increases in senescents. Thus, the lowest values of the parameter is characterized for adults, whereas highest for senescents.

The quantitative analysis of the NO content in hepatocytes indicate, that during aging the intensity of NO production decreases in adults, and, then increases again in senescents, however, this parameter still is significantly low than the analogous parameter in juveniles.

Razavi et al. [2005] have shown, that NO is an important regulator of apoptosis within the mammalian system, capable of both inducing and preventing apoptosis, depending upon the level of NO production and environmental milieu [5,9].

It is reported, that nitric oxide (NO) is a potential inhibitor of apoptosis in many cell types, including hepatocytes [4]. Young-Myeong et al. [1997] have indicated that NO prevents apoptosis in hepatocytes by either direct or indirect inhibition of caspase-3-like activity through protein S-nitrosylation. On the other hand in hepatocytes NO also prevents increases in caspase activity by a c-

GMP-dependent mechanism that does not involve S-nitrosylation.

According to our data the strong correlation between the rate of apoptosis and intensity of NO production is evident (*Fig.1*).

As it is shown from *Fig.1*, during aging the both parameters decrease in adults and increase again in senescents. However, the intensity of these changes is not similar in senescents. The rate of apoptosis becomes extremely high in senescents, in contrast with the intensity of NO production. The latter also increases in senescents, but never reaches the value of the same parameter in juveniles.

It can be suggested that the pro- or antiapoptotic influence of NO on hepatocytes is not univocal and it greatly depends on age-related changes in cellular homeostasis.

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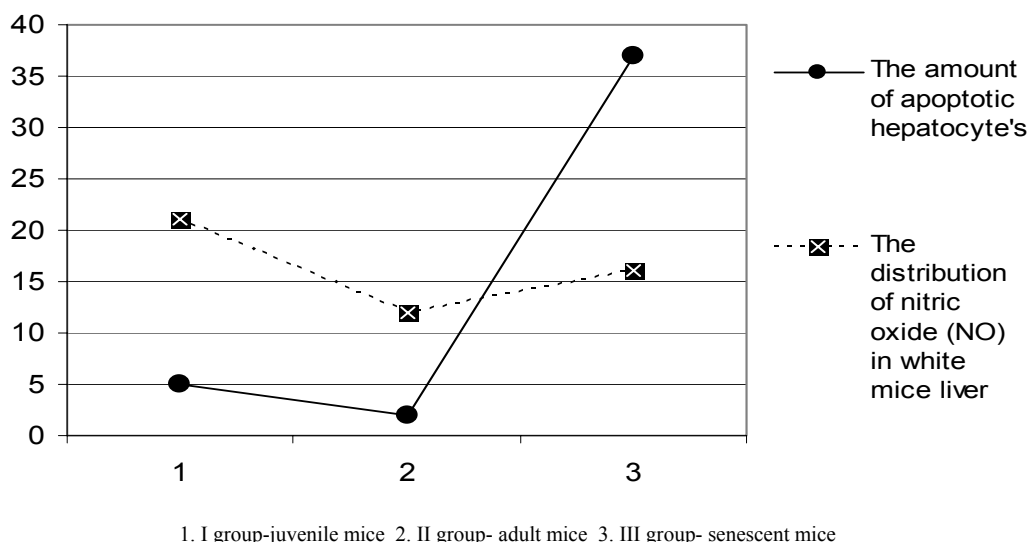


Fig.1 Age related changes in white mice hepatocytes'.

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Возрастные особенности влияния азотного оксида (NO) на интенсивность апоптоза в гепатоцитах белых мышей

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Р Е З Ю М Е

Оксид азота является одним из важнейших регуляторов апоптоза. Целью исследования являлось определение взаимосвязи между интенсивностью апоптоза и синтезом NO в гепатоцитах белых мышей в возрастном аспекте. Эксперименты проводились на ювенильных, зрелых и старых животных. Количество апоптотических ядер определяли методом проточной цитофлуориметрии. Интенсивность синтеза NO изучали с помощью радиоспектрометра РЭ-1307. Установлено, что между возрастными изменениями интенсивности апоптоза и синтезом NO имеется определённая корреляция.

Ключевые слова: *гепатоцит, оксид азота, апоптоз, старение*