

The Changes In Cell Cycle Of Rat Hepatocytes During Aging

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ABSTRACT

The resilience of the liver to aging makes it an intriguing target for investigation. Whether aging enhances apoptosis in liver is still controversial. The effect of age on hepatocyte turn-over in male rats (newborns, young, adult and senescent) in vivo was studied. Number of apoptotic, diploid, tetraploid and polyploid nuclei was counted by a Becton Dickinson (Mountain View, CA) FACScan flow cytometer. It has been considered, that aging influences the distribution of hepatocyte DNA into cell cycle, increases the susceptibility of hepatocytes to apoptosis and inhibits the total proliferative activity of liver cells.

KEYWORDS: *hepatocyte, cell cycle, apoptosis, aging*

Aging is accompanied by a myriad of changes in cell structure, function and composition. The cells undergo transformation and acquire a constellation of abnormal characteristics such as multiple chromosomal abnormalities, genetic mutations, and changes in morphology and growth rate. The senescent cells are removed and replaced by newly generated cells to maintain homeostasis by the process of programmed cell death (PCD), the most frequent form of which is termed apoptosis. Apoptosis is highly involved throughout the aging process from early developmental changes to senescent declines in function. Although many hypothesis have been proposed to explain the aging process, the exact mechanisms are not well defined. Recent accumulating evidence indicates, that dysregulation of the apoptotic process may be involved in some aging processes. However, it is still debatable how exactly apoptosis is expressed during aging in vivo [5].

It is emerged from studies of apoptosis that liver is privileged system. According to some authors under normal conditions apoptosis occurs at negligible rate in the liver. Whether aging enhances apoptosis in liver is still controversial. The resilience of the liver to aging makes it an intriguing target for investigation.

With regard to liver biology, many studies have focused on the model of liver regeneration after partial hepatectomy. However, relatively few studies have focused on mechanisms of cell cycle control under normal physiological conditions [1,3,7].

The present study has been designed to evaluate the effects of age on hepatocyte turn-over in male rats under normal physiological conditions in vivo.

MATERIALS AND METHODS

The study was performed on 12 white male rats. According to the age the animals were distributed in four groups (I group – newborns, II – youngs, III – adults, IV – senescent rats).

The rats were killed under ether narcosis. liver tissue was 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 18000xg for 40 min. Received pure of nuclei was suspended in TMS solution and centrifuged at 600xg during 10 min. 70% ethanol was added to the pellet and samples were incubated overnight at 4°C. Than 10 µl of RNase (10 µg/ml) was added and samples were incubated during 30 min at RT. 1 ml of EB staining solution was added, samples were incubated during 30 min at RT.

Number of apoptotic, diploid (G_0/G_1), tetraploid (G_2/M) and polyploid nuclei was counted by a Becton Dickinson (Mountain View, CA) FACScan flow cytometer.

Statistical analysis was performed using Student's t-test for independent samples, and p values <0,05 were considered significant. Data are presented as mean (standard deviation).

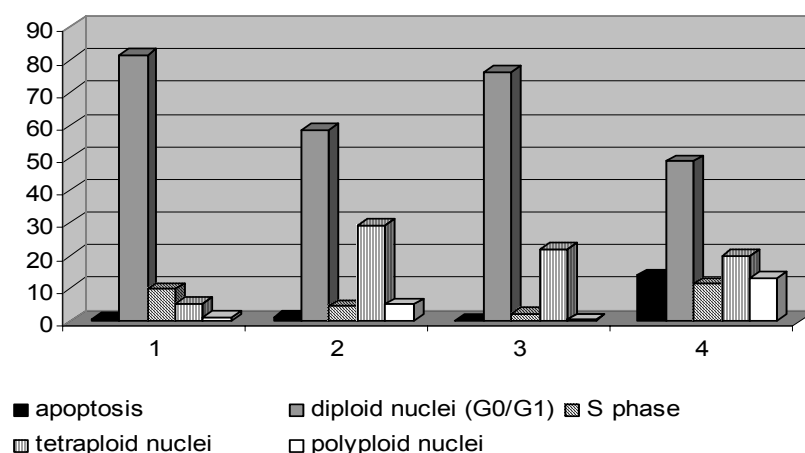


Fig.1 *Distribution of hepatocytes' nuclei in cell cycle during aging (1- Newborn, 2 --young, 3- adult, 4- aged) .*

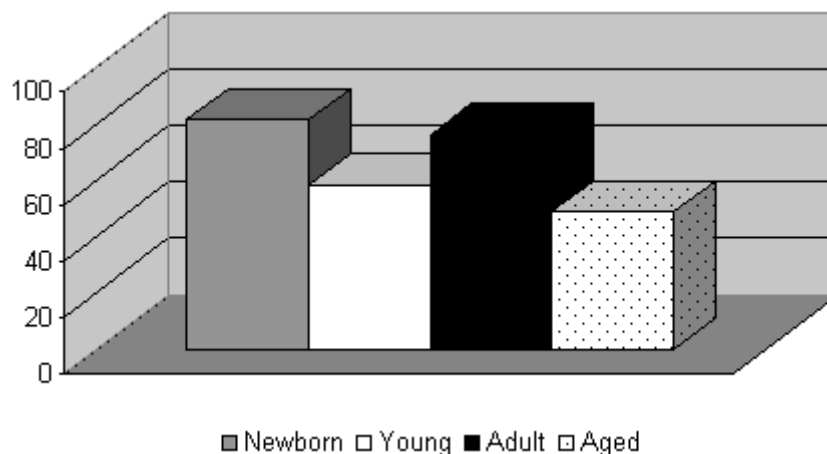


Fig.2 Age related changes in amount of rat hepatocytes' diploid nuclei.

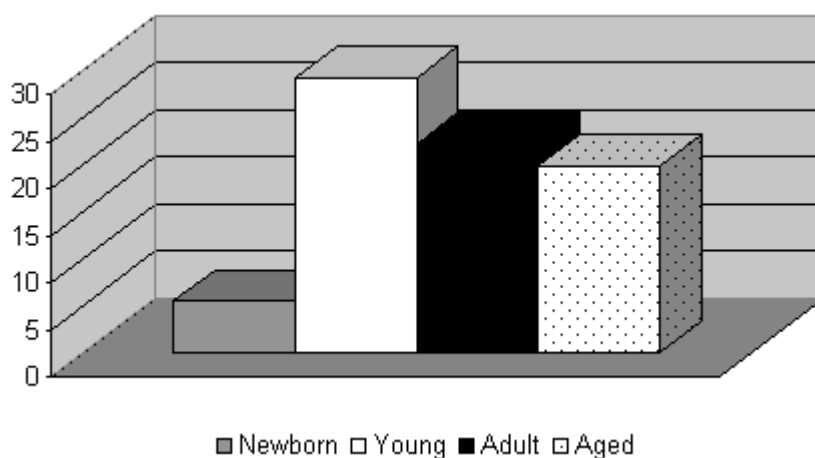


Fig.3 Age related changes in amount of rat hepatocytes' tetraploid nuclei.

RESULTS AND DISCUSSION

Obtained data indicate, that in newborns the $81,6 \pm 4,74\%$ of nuclei are diploid, $51,0 \pm 0,23\%$ – tetraploid and only $1,11 \pm 0,79\%$ – octaploid. the amount of nuclei in S phase of the cell cycle is approximately $9,83 \pm 3,9\%$. The amount of apoptotic nuclei is not high and is $0,57 \pm 0,11\%$. In young rats $58,7 \pm 3,84\%$ of nuclei are diploid, $29,32 \pm 4,31\%$ – tetraploid, $5,12 \pm 2,19\%$ – octaploid and $4,67 \pm 0,7\%$ in S phase. Amount of apoptotic nuclei increases up to $1,40 \pm 0,4\%$. In adult rats about $76,12 \pm 4,23\%$ of nuclei are diploid, $22,24 \pm 0,6\%$ – tetraploid, $43 \pm 0,122\%$ – octaploid, $2,13 \pm 0,01\%$ in S phase and $0,08 \pm 0,07\%$ are apoptotic. In aged rats about $48,9 \pm 8,2\%$ of nuclei are diploid, $19,85 \pm 3,15\%$ – tetraploid, $13,04 \pm 0,07\%$ – octaploid, $11,34 \pm 0,9\%$ in S phase and $13,98 \pm 0,85\%$ – apoptotic.

Existing data, about distribution of hepatocytes of different ploidy at different stages of age are controversial. Guidotti et al. (2003) have shown that in adult rats about 10% of hepatocytes are diploid, 70% are tetraploid and 20% octaploid. According to Gandillet et al. (2003) in young rats most numerous are tetraploid hepatocytes ($56,2 \pm 3,2\%$). On the other hand

Awad, Gruppuso (1999) indicate that, the term liver, obtained 6 h after birth, as well as adult liver demonstrate a high proportion of cells in G_0/G_1 phase.

According to our experimental data in all age groups the liver demonstrate a high proportion of cells in G_0/G_1 phase (Fig.1).

However, the amount of diploid nuclei varies greatly from one age group to another. This parameter is significantly high in newborns ($p < 0,01$). A sharp decrease in diploid nuclei ($p < 0,02$) was observed in senescent rats (fig.2).

In all experimental groups there was a large proportion of cells that were identified as G_2/M . This finding could be accounted for by the tetraploid nature of nonproliferating adult hepatocytes.

As seen from fig. 3. the amount of tetraploid nuclei is low in newborns, but it increases soon in young rats ($p < 0,01$) and remains high in adult and senescent rats. Beginning from young age, in all following age groups, tetraploid nuclei proportionally are most numerous after diploid nuclei. This is attributable to the increased ploidy. Guidotti et al. (2003) that 20% of mononuclear $2n$ hepatocytes failed to undergo cytokinesis. Recent studies in different cell types have shown that tetraploidy causes all cells to arrest in G_1 (2;8).

The amount of nuclei in S phase (fig.4) was high in newborns, and similarly decreased in young and adult rats ($p < 0,01$). Thus, in young and adult ages decrease in diploid population and pronounced increase in hepatocyte polyploid population was observed. However, liver cell population involved in S phase was unchanged. So, it seems that proliferative potential of liver declines with age. However, in the liver of senescent rats the amount of liver cells involved in S phase was significantly high, than in previous two age groups ($p < 0,05$).

At the same time the senescence was accompanied by rapid increase in polyploid nuclei (fig.5). Polyploid nuclei were revealed in all age groups, but their amount was dramatically high in senescent rats ($p < 0,05$). Polyploidy is a general physiological process, that prevails in many cellular systems including plants, insects and mammals (10)

and indicates a terminal differentiation of cells. Polyploidization is a general strategy of cell growth that enables an increase in metabolic output, cell mass, and cell size and may constitute an alternative to cell division (10). The biological significance of hepatic polyploidy remains unclear. Hepatic polyploidy accompanies late fetal development and postnatal maturation (11) and its onset in the adult liver is well recognized. The entire process of hepatocyte polyploidization is considered to be a mechanism of evolutionary adaptation, reflecting an increasing degree of irreversible hepatocellular differentiation adopted to decrease the high risk of genomic damage to which the liver is exposed.

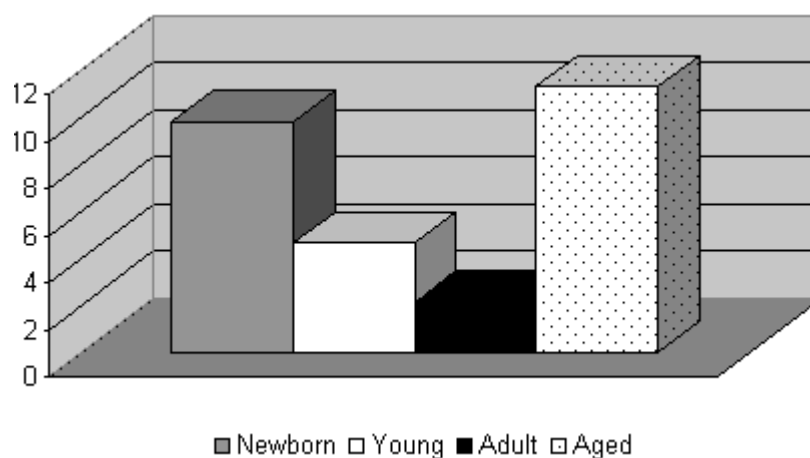


Fig.4 Age related changes in amount of rat hepatocytes' nuclei in S phase.

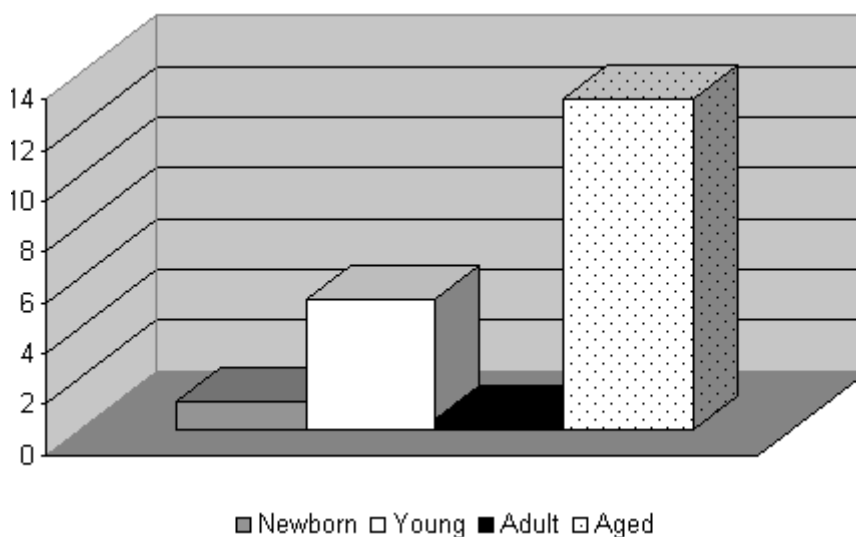


Fig.5 Age related changes in amount of rat hepatocytes' octaploid nuclei

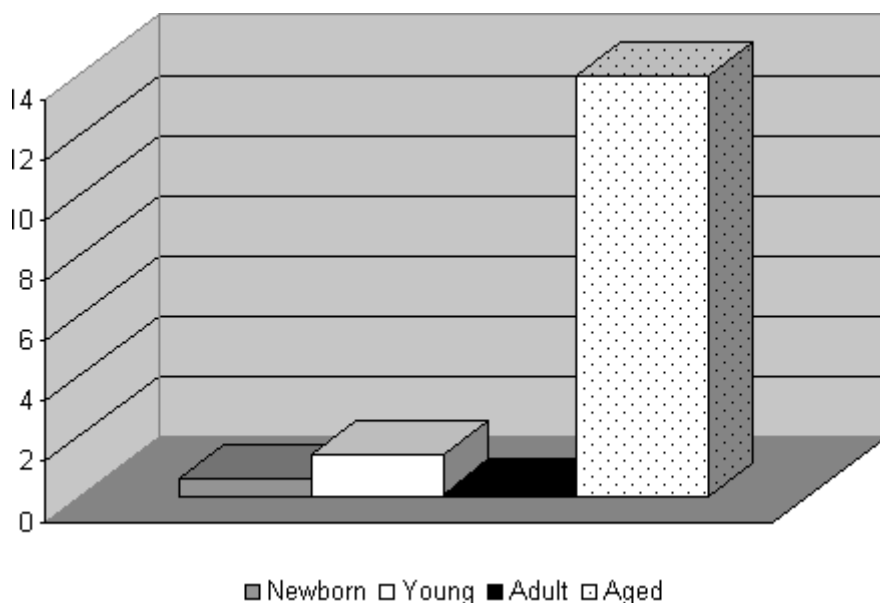


Fig.6 Age related changes in amount of apoptotic nuclei.

The extreme polyploidy would be expected to be associated with cell senescence and eventual cell loss. One consideration is that if an organ contains excessive numbers of polyploid cells with depletion of renewing cell units, organ failure may occur in the setting of continuing liver injury, because polyploid cells with exhibit survival disadvantage. To escape this fate nonpolyploid cell clones may emerge and confer greater propensity for proliferation and regeneration. The revealed high amount of nuclei in S phase in senescent rats confirms this consideration.

Our experiments have shown that in all age groups liver contains the different amount of apoptotic nuclei. This

parameter was significantly high in two - young and senescent age groups (Fig.6). In both groups these changes were accompanied by increase in polyploid nuclei. As insights into significance of polyploidy accumulate gradually it is becoming clear that cells belonging to high ploidy classes exhibit advancement toward terminal differentiation and cellular senescence with greater probabilities of apoptosis.

Thus, according to our experimental data we can consider that aging influences the distribution of hepatocyte DNA into cell cycle, increases the susceptibility of hepatocytes to apoptosis and inhibits the total proliferative activity of liver cells.

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Возрастные особенности течения клеточного цикла гепатоцитов белых крыс

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РЕЗЮМЕ

Изучены возрастные особенности распределения гепатоцитов в различных фазах клеточного цикла. С помощью динамического цитофлюориметра определяли количество ядер - диплоидных, тетраплоидных, полиплоидных, апоптотических, а также находящихся в синтетической (S) фазе клеточного цикла. Особенности течения клеточного цикла изучались у новорожденных, молодых, зрелых и старых животных. Установлено, что возраст влияет на изменение плоидности ядер гепатоцитов, увеличивает их подверженность апоптозу и значительно уменьшает пролиферативную активность печеночных клеток.

Ключевые слова: *гепатоциты, клеточный цикл, апоптоз, старение*