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**THE CELL CYCLE NUCLEI PHASES
IN THE LIVER OF IMMATURE RATS
WITH CHRONIC MEDICINAL HEPATITIS**

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Introduction. The attention of researchers has been drawn to the study of molecular mechanisms of drug-induced liver damages, in recent years. It is well known that the regeneration of the liver structure and function in the case of its damage can be carried out due to proliferation, polyploidization and hypertrophy of hepatocytes [1, p. 148]. Mature hepatocytes have been shown to have a higher Hayflick limit than other somatic cells and to exhibit more than 100 replicative cycles. Unipotency of hepatocytes is associated with a polyploid set of chromosomes that are normally stable in the G0 resting phase, however after loss of the parenchyma, they may return to the G1 phase of the cell cycle, followed by DNA synthesis and mitosis. Further proliferative activity may be insignificant or very pronounced, depending on the conditions in which regeneration occurs [4, p. 285]. Polyploidy, on the one hand, can be considered as a sign of severe damage to

hepatocytes, on the other hand – it is a kind of protective mechanism in response to hepatotoxins, as polyploid cells are more resistant to pathogens [5, p. 2].

Along with proliferation and polyploidization, hepatocyte hypertrophy plays an important role in the reparative regeneration of the liver, which can be caused either by an increase in cell ploidy or the size of their cytoplasm. Hepatocyte hypertrophy, which is not associated with polyploidization, occurs due to increased intracellular regeneration, which leads to an increase in the number and hypertrophy of various intracellular structures and organelles [6, p. 153].

Goal. To investigate the phases of the cell cycle (CC) in the liver cells nuclei of immature rats in experimental chronic drug-induced hepatitis (HMG) caused by rifampicin and isoniazid and in the pathogenetic correction of quercetin and thiotriazoline.

Materials and methods. An experimental study was performed on 50 nonlinear white laboratory sexually immature male rats with an initial body weight of 60 – 70 g. The choice of immature animals is due to the need to maximize the approximation of experimental pathology studied in children. HMG modeling was performed according to our own method [2, p. 284] by intragastric administration of rifampicin and isoniazid three times a week for 29 days. Experimental animals were divided into 4 groups: 1st – intact animals (n = 12), 2nd – HMG modeling (n = 14), animals of the 3rd (n = 12) and 4th groups (n = 12).) in parallel with rifampicin and isoniazid for 29 days daily intragastric injected respectively quercetin («Quertile», CJSC NPC Borschagovsky Chemical-Pharmaceutical Plant at a rate of 75 mg / kg and thiotriazoline (JSC «Galichpharm», corporation «Arterium» – 22.5 mg / kg. ED50 recalculation for quercetin and thiotriazoline was performed according to the method of Yu.R. Rybolovlev [3, p. 15–14]. After completion of the experiment, the animals under euthanasia under thiopental anesthesia were decapitated and liver tissue was collected.

The DNA content in the nuclei of liver cells was determined by flow cytometry (MPC). Suspensions of nuclei from liver cells were obtained using a special solution for nuclear DNA testing CyStain DNA from Partec, Germany according to the protocol of the manufacturer's instructions. Cyclic analysis (phases of the cell cycle) was performed using FloMax software in full digital compliance. The digital correspondence to the experimental data was performed according to the mathematical model.

Statistical analysis of the obtained results was performed using non-parametric methods of evaluation of the obtained data.

Results and discussion. The largest percentage of nuclei that were in the range of G0-G1 was registered with the use of synthetic hepatoprotector

thiotriazoline in immature rats with HMG. This indicates an increase in differentiation and functional specialization of hepatocytes that did not have irreversible damage. A significantly higher percentage of diploid hepatocyte nuclei in the G1 phase compared to untreated animals, which reached or even exceeded that in intact animals, in our opinion, should be regarded as a process of compensation of specialized function, when dystrophically altered hepatocytes cannot fully perform it. We consider this fact to be positive effect of thiotriazoline.

Slightly different results were observed with respect to the percentage of liver cells in the S-phase: in the treatment of animals with HMG quercetin, this figure was lower than in untreated animals ($3.85 \pm 1.16\%$ vs. $6.46 \pm 0.96\%$, $p < 0.05$), but did not reach the norm, as it significantly exceeded that intact ($p < 0.05$), which, in our opinion, indicates an insufficient level of repair in the liver on the background of the introduction of this drug. However, when using thiotriazoline, this figure did not differ from that in the group of intact, but was significantly lower than in animals with HMG without drug correction, which indicates a sufficiently high hepatoprotective effect of this drug (see Table 1).

Table 1

**Phases of the cell cycle and fragmentation of nuclear DNA
of immature rats liver cells with HMG and its treatment
with hepatoprotectors, (M \pm m)**

Groups	Indicators			
	G1, %	S, %	G2M, %	IP, %
1. Intact	67,90 \pm 4,27	2,53 \pm 0,74	29,57 \pm 4,73	32,10 \pm 4,27
2. HMG	61,70 \pm 5,56*	6,46 \pm 0,96*	31,84 \pm 5,21	38,38 \pm 5,57*
3. HMG + quercetin	62,18 \pm 1,90*	3,85 \pm 1,16*#	33,97 \pm 2,63*	37,81 \pm 1,90*
4. HMG + thiotriazoline	70,02 \pm 4,91	2,81 \pm 1,23#	27,18 \pm 4,15	29,98 \pm 4,91
p ₃ – p ₄	<0,05	>0,05	<0,05	<0,05

Notes:

1. * – significant difference compared to intact ($p < 0,05$).
2. # – significant difference compared to HMG ($p < 0,05$).
3. p₃ – p₄ – significant difference between the groups HMG + quercetin and HMG + thiotriazoline ($p < 0,05$).

It was found that the postsynthetic and mitotic phases (G2M interval) of the CC of liver cell nuclei under HMG conditions and its treatment with hepatoprotectors are as follows: in the HMG + thiotriazoline group this indicator did not differ as such in intact, exceeded that in intact animals ($33.97 \pm 2.63\%$ vs. $29.57 \pm 4.73\%$, $p < 0.05$) (see table 1). In our opinion,

this may indicate incomplete reparative regeneration of hepatocytes on the background of therapeutic and prophylactic administration of quercetin.

Similar dynamics was observed with respect to the proliferation index (RI). Thus, thiotriazoline significantly reduced this figure by 22% compared with that in untreated animals, and brought it closer to that of control animals. Whereas in animals of the HMG + group, quercetin RI did not differ from that in untreated animals with HMG and was $(37.81 \pm 1.90)\%$ versus $(32.10 \pm 4.27)\%$ ($p < 0.05$) in the control (see Table 1).

Conclusions. Thus, the results of experimental studies obtained with the help of MPC showed that the studied hepatoprotectors have different effects on the cellular mechanisms of reparative regeneration of hepatocytes. Based on the results of research, the choice of hepatoprotector should take into account in each case the degree of damage, the state of reparative regeneration and the predominance of regenerative hypertrophy or proliferation in the damaged liver.

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