IFN and protein kinase R during regeneration of rat liver after partial hepatectomy

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The paper is devoted to validation of our hypothesis concerning an obligatory involvement of innate immune response in a liver transition from quiescence to proliferation. Our research is focused on the expression of IFN , its receptor (first subunit) and its target – protein kinase R (PKR) during first 12 hours after regenerative stimulus. Two models were used – rat liver after partial hepatectomy (PHE) and after laparatomy, that imitate transition of inactive liver cells to proliferation and acute phase response as a component of liver response to PHE, cor- respondingly. After PHE a short-term increase in IFN expression is revealed in Kupffer cells. In hepatocytes PKR – mRNA up-regulation takes place, followed by an increase in IFN – mRNA production. Taking into account the dual function of PKR as a target and inducer of IFN , we suggest that secretion of IFN by Kupffer cells leads to the activation of PKR gene expression in hepatocytes, in which the product of PKR gene in its turn provokes an increase in the IFN gene expression. After laparatomy the IFN expression is down-regulated in Kupffer cells and hepatocytes. The expression of PKR – gene is opposite to that, observed after PHE – the level of PKR-RNA decreases in hepatocytes and transiently increases in Kupffer cells. PKR protein is detected in nuclei and cytoplasm in hepatocytes in intact liver and liver after laparatomy while it is concentrated in cytoplasm after 6 hours post-PHE, releasing nuclei from antigen. The expression of all investigated genes is cell-specific. It reveals respectively the activation and inhibition of IFN system that is characteristic of the liver restoration and acute phase reaction.

Keywords: interferon , protein kinase R, liver regeneration.

Introduction. Liver regeneration after mechanical or toxic injury is a complex multi-component process, which is regulated in time and space and leads to the restoration of organ's mass and function. Our previous study has revealed for the first time that the synthesis of interferon alpha (IFN) increases at the early stage of liver regeneration [1]. This fact, in concordance with

the activation of complement system [2], points to the essential role of innate immunity in the triggering of liver regeneration.

The aim of this study was to unravel the specific involvement of hepatocytes and sessile macrophages, Kupffer cells, in IFN and IFN -mediated gene expression after partial hepatecomy (PHE) and laparatomy as the respective models of liver transition from quiescence to proliferation and acute phase response. The latter is a component of liver response for PHE and serves as a control. The research was carried out during the first 12 hours after both operations, which correspond and embrace transitional and prereplicative periods of the first cell cycle in hepatocytes after PHE.

The hepatocytes are mainly responsible for the work of "biochemical factory" of the body and the Kupffer cells - for the synthesis of multiple regulatory molecules including cytokines and eicosanoids [3]. We focused our investigation on the genes that encode IFN , IFN -regulated protein kinase R (PKR), and gene that encodes the first chain of IFN receptor (IFNAR1), as indispensable mediator in signal transduction from IFN to PKR promoter.

PKR (EC 2.7.11.1) is encoded by a single gene copy in 6q11 locus of the 6th chromosome in rat genome. The promoter of *PKR* gene contains a putative interferon-stimulated response element (ISRE) with a similarity score of 0.77 to ISRE-matrix, as ascertained by COTRASIF program [5]. Therefore, *PKR* gene expression can be induced by IFN . Reciprocally, PKR protein mediates the induction of *IFN* gene expression [4]. The PKR enzyme possesses dual specificity, as serine/threonine kinase with protein substrates and thyrosine kinase in autophosphorylation [4]. PKR, originally inactive, may be activated by double-stranded RNA, intracellular proteins like PACT/RAX, and 5 -untranslated regions of several messenger RNAs. The resulting autophosphorylation is indispensible for the enzymatic activity. In addition to the kinase activity, PKR acts as an adaptor for some intracellular proteins. It regulates translation and intracellular signal transduction that points to its ability to essentially amplify the inducer signal, particularly that from IFN [4].

Materials and Methods. The research was carried out on female nonlinear rats (200–250g). Operations of PHE and LAP were conducted according to the standard procedure under ether anesthesia [6]. Hepatocytes and Kupffer cells were isolated by the modified method of Berry and Friend in 1, 3, 6 and 12 hours after both operations using anesthesia by ketamine and xylasine [7]. Gene expression was assessed at mRNA level by quantitative reverse transcriptase - polymerase chain reaction in real time (qRT-PCR). RNA was isolated by standard Chomczynski method [8]. RNA samples were subjected to DNAse treatment, followed by reverse transcription with random hexamer primers [9]. Primer design for PCR and verification of their quality were described previously [1]. To construct standard curve for qRT-PCR the specific amplicons were produced, purified and used for calibration either directly or being cloned into vector DNA.

The expression of *PKR*-gene at the protein level was assessed by Western-blot analysis with polyclonal anti-rat antibodies (Sigma-Aldrich, Germany). The results were normalized to the total protein load after Ponseau S staining and subsequent densitometry. The localization of the PKR protein in the liver was detected by standard immunohistochemical procedure with polyclonal anti-rat antibodies (Sigma-Aldrich, Germany).

The time-dependent level of the PKR protein in response to PHE was compared to its level in cultivated hepatocytes subjected to IFN treatment during corresponding time. After isolation the hepatocytes were cultivated for 24 hours in Williams' medium E (Sigma-Aldrich, Germany) without serum, the medium was changed for the same one with an addition of the rat recombinant IFN (Sigma-Aldrich, Germany) to the final concentration of 250 units/ml or without it. The cells were incubated for 3, 6 and 12 hours. Afterwards the cells were washed with PBS, RNA was extracted with Trizol (Qiagen, USA) and qRT-PCR was carried out as described above.

Results and discussion. *The levels of IFN -, IFNAR1- and PKR-specific mRNAs in Kupffer cells and hepatocytes after PHE and LAP.* As it is shown in Fig. 1a, the definite content of IFN -specific mRNA is detected in Kupffer cells isolated from quiescent liver.

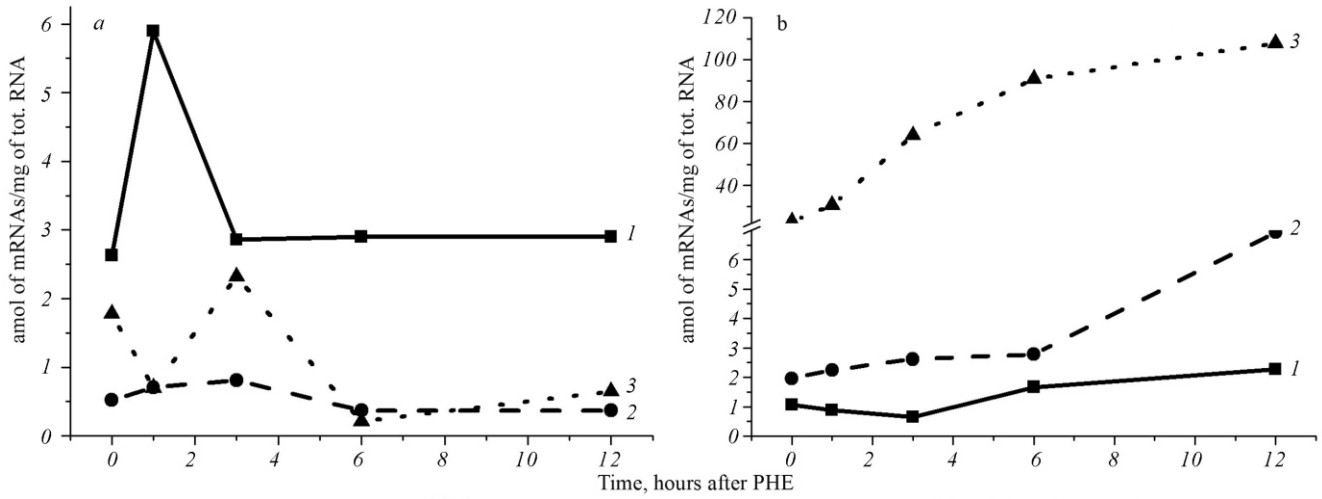

Fig.1. The level of mRNAs in Kupffer cells (a) and hepatocytes (b), isolated from regenerating rat liver. *1* – IFN ; *2* – IFN*AR1; 3* – PKR

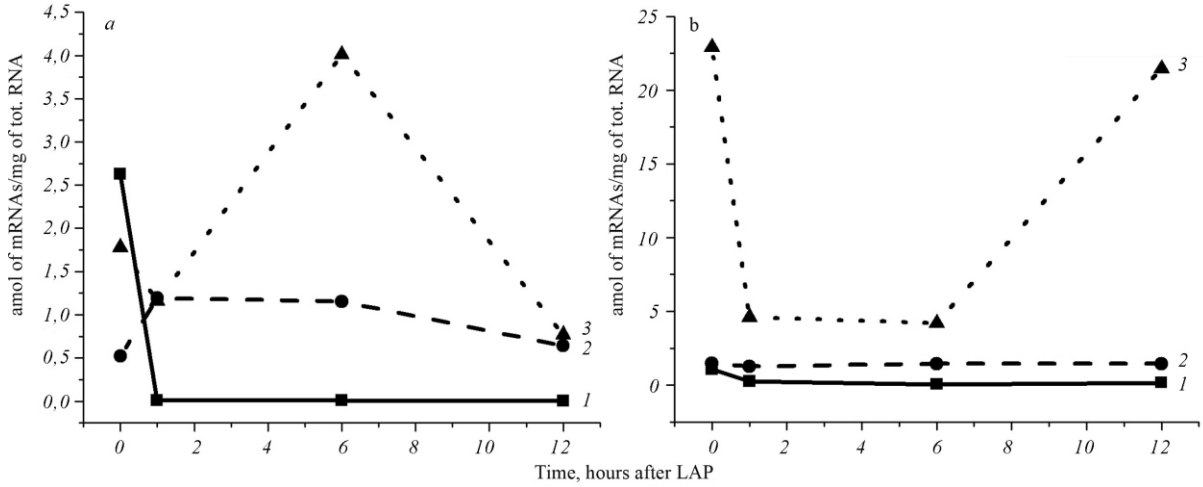

Fig.2. The level of mRNAs in Kupffer cells (a) and hepatocytes (b), isolated from rat liver after laparatomyï: *1* – IFN ; *2* – IFN*AR1; 3* – PKR

It substantially increases immediately after operation and returns to the initial level thereafter.

It is known that Kupffer cells in quiescent rat liver are in the primed state even when obtained from germ-free rats. It means that they produce IFN at a low level being not activated and thus do not require like e.g. mouse macrophages the two-stage activation. They need only an elicitor to become fully active [10]. Induced by PHE rapid up-regulation of initially low IFN expression in Kupffer cells illustrates this statement.

As we have shown previously with a biological test the production of IFN protein increases at the first hours after PHE in the whole liver [11] and corresponds to the up-regulation of IFN mRNA in this study. We presume that initially synthesized by Kupffer cells IFN is secreted. It binds to cognitive receptors particularly on Kupffer cells and hepatocytes in autocrine and paracrine manner correspondingly, and transmits the signal inside the cells.

It is highly probable that IFN , as a typical inducer of *PKR* gene, causes substantial increase of PKR-specific mRNA in hepatocytes and small fluctuation in Kupffer cells that are observed in this study (Fig. 1a, b). To find out to what extent the increase in PKR-specific mRNA in hepatocytes may be caused by the IFN action, we cultivated the primary hepatocytes in the presence and absence of IFN for 3, 6 and 12 h. The content

of PKR-specific mRNA in cultivated hepatocytes increases under IFN treatment, reaches its maximum to 6 h and returns to the initial level to 12 h of incubation. It does not change noticeably in the cells not treated with IFN (data not shown). We assume that after PHE besides IFN other agents - most likely signals from Toll-like receptors [4] - may support more prolonged up-regulation of the *PKR* expression in hepatocytes.

PHE induces also the up-regulation of *IFN* - and *IFNAR1*- genes expression in hepatocytes but both are less pronounced than that of *PKR-gene. The elevation of IFN -specific mRNA content in hepatocytes may be partly explained by putative activity of* PKR protein that can stimulate IFN transcription via signaling pathways [4]. The increase of *IFNAR1*- mRNA content in hepatocytes may be connected with the increased turnover of IFNAR1-receptor due to the binding of elevated amount of IFN , receptor internalization with its subsequent degradation [12].

The expression of the investigated genes after LAP substantially differs from that after PHE. The content of IFN mRNA dramatically declines in Kupffer cells and slightly declines in hepatocytes (Fig.2 a, b). These data are in accordance with the decrease in content of IFN protein previously revealed by biological testing [11].

The liver response to the injury of abdomen wall and peritoneum is a significant part of a systemic response or acute phase reaction to any kind of injury. At the same time it is a component of a liver response to PHE that includes a local and systemic response to the injury and adaptive response leading to the restoration of liver mass and function. The dramatic down-regulation of *IFN* gene expression after LAP gives a clue to consider it as a negative regulator of acute phase reaction and emphasizes the importance of *IFN* up-regulation in response to PHE.

After LAP the level of IFNAR1 mRNA decreases in Kupffer cells and does not undergo substantial changes in hepatocytes. These data correspond to the down-regulated expression of *IFN* . The level of PKR mRNA temporarily increases in Kupffer cells while it decreases and returns to the initial level in hepatocytes 12 h after operation (Fig.2). An effector of the negative regulation of *PKR* expression after LAP is unknown.

Content and subcellular localization of PKR protein in liver cells after PHE and laparatomy. Western blot analysis has revealed that PHE induces the up-regulation of total PKR in Kupffer cells in 3h after operation that is simultaneous with the up-regulation of PKR mRNA. The level of PKR protein in hepatocytes after both operation and in Kupffer cells after LAP does not change significantly. In contrast to nearly pertinent amount of the PKR protein after both operations its intracellular distribution is not similar (data are not presented). According to the immunohistochemical analysis, the PKR protein is detected in hepatocytes of intact rats as dispersed staining nearly equally distributed between nuclei and cytoplasm that correlates with existing information about protein compartmentalization in the cells [13]. In Kupffer cells PKR is detected in the form of intensively brown granules. After laparatomy the protein distribution between the nuclei and cytoplasm in hepatocytes does not change substantially. However, at 6 h after PHE the nuclei are nearly completely free from the protein while cytoplasm contains the major amount of it. At 12 h after PHE the initial compartmentalization of PKR is restored.

According to the existing data, the PKR protein may interact with 40S subunit of ribosomes and regulate the process of translation by phosphorylation of eukaryotic initiation factor 2 (eIF2). Phosphorylated eIF2 partially inhibits the mRNA translation and changes the profile of translated RNAs [4]. Therefore, the transient accumulation of PKR in the cytoplasm may be an important event in the development of regenerative process and provides a challenge for further investigation. Besides phosphorylation of eIF2 , PKR may interact with different adaptor proteins and thus interfere in different signaling pathways [4].

Conclusions. For the first time we have shown that the IFN gene expression is up-regulated at the early steps of liver regeneration induced by PHE. Kupffer cells and hepatocytes are involved in this process in the given order. In hepatocytes the expression of PKR, the target gene of *IFN* , is up-regulated at the background of less manifested increase in the *IFNAR1* expression. The up-regulation of PKR expression in Kupffer cells in 3h after PHE is supported by similar changes at the protein level at the background of slight fluctuations of *IFNAR1* expression. The PKR protein initially equally distributed between nuclei and cytoplasm is temporally

redistributed after PHE with the majority of the protein in cytoplasm. In contrast to PHE LAP induces dramatic decrease in content of IFN -specific mRNA in Kupffer cells and slight decrease in hepatocytes. The content of PKR mRNA in hepatocytes is down-regulated with the minimal value in between 1 and 6h postoperatively. The content of PKR protein in Kupffer cells and hepatocytes is slightly fluctuates and the initial nearly equal distribution between nuclei and cytoplasm retains. Taking into account the opposite changes of *IFN* expression after PHE and LAP inducing the transition of liver cells from quiescence to proliferation and acute phase response, respectively, we suggest that the IFN plays a special role in the triggering of regenerative process.

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Інтерферон та протеїнкіназа Р у процесі відновлення печінки щурів після часткової гепатектомії

Резюме

Після часткової гепатектомії (ЧГЕ) у клітинах Купфера спостерігається короткочасне виражене зростання експресії гена ІФН , а в гепатоцитах – підвищення рівня РНК протеїнкінази Р (ПКР), за яким збільшується вміст ІФН -РНК. Лапаратомія спричиняє в клітинах Купфера та гепатоцитах стрімке зниження концентрації ІФН -РНК. Вміст мРНК ПКР після операції зменшується. Білок ПКР ви- значається в ядрах і цитоплазмі гепатоцитів в інтактній печінці та печінці після ЧГЕ і лапаратомії в усі досліджувані строки, окрім 6 год після ЧГЕ, коли він присутній у цитоплазмі і майже не виявляється у ядрі. Експресія досліджуваних генів у клітинах обох типів має виражений клітиноспецифічний характер і свідчить про активацію та інгібування системи ІФН відповідно в процесі відновлення печінки після ЧГЕ та реакції гострої фази після лапаратомії.

Ключові слова: інтерферон , протеїнкіназа Р, регенерація печінки.

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Интерферон и протеинкиназа Р в процессе восстановления печени крыс после частичной гепатэктомии

Резюме

После частичной гепатэктомии (ЧГЭ) в клетках Купффера наблюдается кратковременный выраженый подъем экспрессии гена ИФН , а в гепатоцитах повышается количество РНК протеинкиназы (ПКР), вслед за чем возрастает уровень ИФН -РНК. Лапаратомия в клетках Купффера и гепатоцитах приводит к резкому снижению содержания ИФН -РНК. Реакция ПКР на операцию является противоположной таковой на ЧГЭ. Белок ПКР определяется в ядрах и цитоплазме гепатоцитов в интактной печени и печени после лапаратомии во все исследованные сроки, кроме 6 ч после ЧГЭ, когда он присутствует в цитоплазме и не выявляется в ядре. Экспрессия исследуемых генов в клетках обоих типов имеет выраженный клеточноспецифический характер и свидетельствует об активации и ингибировании системы ИФН соответственно в процессе восстановления печени и реакции острой фазы после лапаратомии.

Ключевые слова: интерферон , протеинкиназа Р, регенерация печени.

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