

The potential targets of Kupffer cells activity during liver regeneration

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This paper addresses possible causal and kinetic correlations between production of biologically active molecules by Kupffer cells and biochemical events in regenerating liver after partial hepatectomy. The expression and activity of some genes and enzymes were evaluated that are potential targets for regulation by Kupffer cells, e. g. transcriptional factors coded by the nuclear protooncogenes, c-fos and c-myc, a tissue-specific gene P450IIE1 and 2'5'oligo(A) synthetase. The results were compared with data about Kupffer cell activity during regeneration. Kupffer cells manifest their specific activities during transition of hepatocytes from quiescence to the first cell cycle and during the promotion of the last one. After termination of these functions the macrophages themselves enter into a cell cycle. These phenomena indicate a regulatory role of mesenchymal cells in triggering and promoting liver regeneration.

Introduction. An intriguing question of contemporary research on liver regeneration is the particular role of sinusoidal non-parenchymal cells. They not only participate in the growth processes by increasing their number but also must be seen as participants in the metabolism of the whole regenerating organ and in the cross-talk with hepatocytes. This relation has not yet been well elucidated. Several points argue in favor of a participation of Kupffer cells (KC) that produce numerous mediators [1]. With regard to the regenerating process, their activation prior to partial hepatectomy (PHE) accelerates, their inhibition delays the restoration of the parenchyma [2]. Animals with genetically altered sensitivity to lipopolysaccharide (LPS) manifest weaker response to PHE [3]. The pattern of produced mediators changes in the course of regeneration, e. g. medium conditioned by KC obtained during the first 12 h after PHE stimulates DNA synthesis in hepatocytes [4] while that from nonparenchymal cells obtained on the second day after operation delays it [5]. The expression and activity of some genes and enzymes were evaluated that are potential targets for regulation by Kupffer cells [6]. The results were compared with data about Kupffer cell activity during regeneration.

C-Fos is a ubiquitous nuclear phosphoprotein that together with *c-Jun* forms stable heterodimer known

as the transcriptional activator protein (AP-1). AP-1 possesses a dual function activating not only transcription but DNA replication [7]. AP-1 activity is regulated by wide spectrum of stimuli, including cytokines and growth factors.

C-Myc is a nuclear phosphoprotein that together with *c-Max* forms a transcriptional factor. The functional activity of *c-Myc* is regulated at transcriptional, posttranscriptional and posttranslational levels. *c-Myc* is critical for proliferation/differentiation balance and cell cycle progression especially during G1 and S-to-G2/M phase [8].

P450IIE1 is a microsomal enzyme involved in metabolism of foreign compounds. Cytokines IL-1, IL-6, TNF- α and IFN α/β downregulate its expression [9]. 2'5'oligo(A)synthetase is a limiting enzyme of 2'5' oligo(A)synthetase/RNase L system negatively regulating the amount of RNA at the pretranslational level. The *c-myc* RNA is a potential target of the system. Cytokines IFN α/β and TNF- α positively regulate enzymes expression [6, 10].

The most reliable time marker of regenerating process is the DNA synthesis in the liver cells. Hepatocytes, KC and sinusoidal endothelial cells enter their first cell cycles in the given order [11]. The S phase of hepatocytes (ca. 12–25 h after PHE) is preceded by prereplicative period conveniently subdivided into immediate-early response (ca. 0.5 h after PHE), delayed reaction with metabolic

reorientation providing competence to proliferate (ca. 0.5–3 h after PHE) and a G1-like period (ca. 3–12 h after PHE) [12]. The G2 and M phases in hepatocytes have standard periods of 4.5 h and 1 h, respectively.

Materials and Methods. *Pretreatment of the animals.* Male Wistar rats (150–200 g) were used throughout. Partial (2/3) hepatectomy and sham operation were performed by standard procedure. Livers were perfused *in situ* at the time indicated with cold physiological saline and were processed either immediately or frozen and stored at -70°C before usage.

Detection of relative amount of specific RNA. Total RNA from nuclear and cytoplasmic (S10) liver fractions was isolated by a slightly modified guanidinium isothiocyanate method. Poly(A)⁺cytoplasmic RNA was separated on oligo(dT) cellulose, the relative amount of specific RNAs was registered by dot-, blot hybridizations in the presence of formamide. The probes were radioactively labeled with [³²P]-dCTP in nick-translation or randomly primed reactions. The intensity of hybridization signals was registered in autographs with a laser scanner. The following probes were used: the *Pst*I fragment (1100 bp) of the *v-fos* gene, the *Xba*I-*Sac*I fragment of murine *c-myc* cDNA and the *Pst*I fragment (300 bp) of murine P450IIE1 cDNA.

Detection of 2'5'oligo(A)synthetase activity. Enzymatic activity of the nuclear (S12) and cytoplasmic (S15) fractions was detected using the specific binding activity of polyI-polyC paper and catalytic activity of bound enzyme. 2'5'oligo(A) adenylates were synthesized in the presence of [¹⁴C]-ATP and were separated by chromatography on PEI-cellulose. Their radioactivity was measured [13].

Results. *The relative amounts of c-fos, c-myc and P450IIE1 specific RNAs.* Nuclear and poly(A)⁺cytoplasmic RNAs from intact, regenerating and sham operated liver produced hybridization signals with each of these probes. The nuclear RNAs existed as precursor molecules of greater molecular weight (data not shown). The time course of changes is specific for each RNA. The kinetics of nuclear and poly(A)⁺cytoplasmic P450IIE1 RNA are similar—the up-regulation during the first three hours after PHE is followed by down-regulation during 6–24 h interval and slight increase later on (fig. 1, 4). This coincidence suggests the prevalence of transcriptional over posttranscriptional control of gene expression. Down-regulation during late G1–S phase of hepatocytes correlates with resistance of regenerating liver to hepatotoxins [14]. Nuclear *c-fos* and *c-myc* specific RNAs are up-regulated with the maxima at 3 h and ca. 18 h after PHE (fig. 2). Their counterparts in poly(A)⁺cytoplasmic RNA do not follow the same time course (fig. 3), especially during the prere-

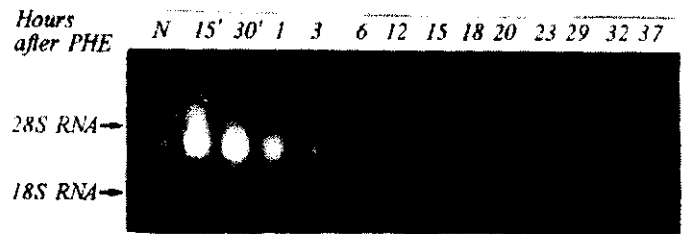


Fig. 1. Hybridization signals of P450IIE1 cytoplasmic RNA in regenerating liver

pliative period; this raises the questions about an involvement of posttranscriptional events in the regulation of these genes. Both poly(A)⁺RNAs are down-regulated after their immediate increase after PHE, later on, the relative amount of *c-myc* RNA is elevated during the G1-to-G1/S and S-to-G2/M transitions (fig. 3), that of *c-fos* RNA during the S phase of hepatocytes (fig. 4). Immediate-early transient expression of *c-fos* and *c-myc* transcripts is well documented for different cells in particular for mitogen-stimulated hepatocytes [15]. The expression of these genes during the later periods of regeneration remains to be clarified. The increase of *c-fos* transcripts during the S phase of hepatocytes implicates *c-Fos* participation in DNA synthesis or related

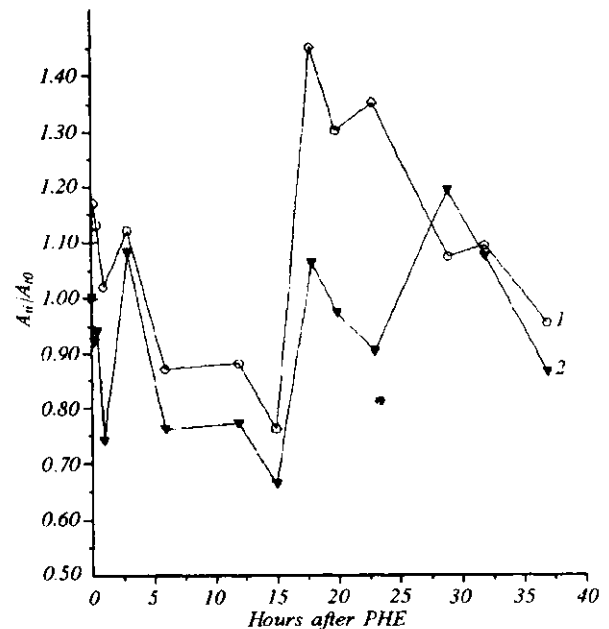


Fig. 2. Relative amount of *c-fos* (1) and *c-myc* (2) RNA in nuclear RNA from regenerating liver

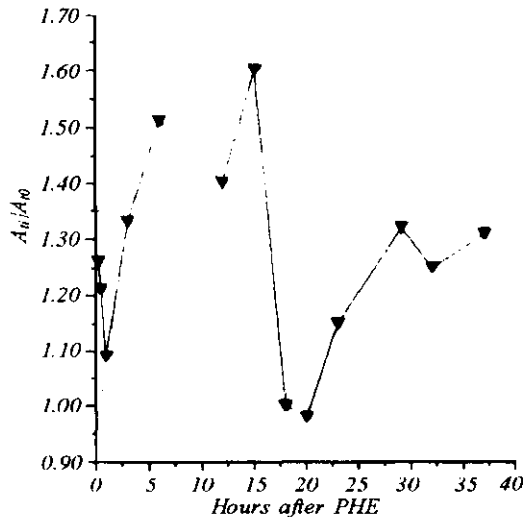


Fig. 3. Relative amount of *c-myc* RNA in cytoplasmic RNA from regenerating liver

processes and correlates with activating role of AP-1 in DNA replication [7]. The time-course of *c-myc* transcripts in regenerating liver resembles the similar changes of *c-Myc* — *c-Max* transcriptional activity and phosphorylation pattern of *c-Myc* in proliferating cells [8].

2'5'oligo(A)synthetase activity after PHE. Enzyme activity was detected in nuclear and cytoplasmic fractions, the nuclear specific activity being higher by

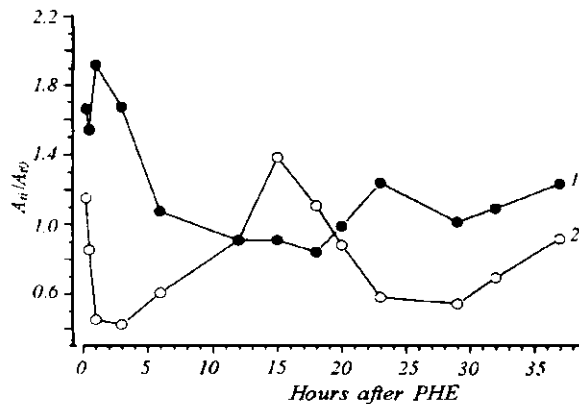


Fig. 4. Relative amount of P450IIE1 (1) and *c-fos* (2) RNA in cytoplasmic RNA from regenerating liver

one order (fig. 5). Taking into account the distribution of the cellular protein between both fractions, their input to the total cellular enzymatic activity is nearly equal. PHE induced a temporal decrease in nuclear activity with minimum at 3 h (compare the up-regulation of *c-fos*, *c-myc* and P450IIE1 nuclear transcripts at this time (fig. 2), and an increase in the cytoplasmic activity with a maximum at 0.5 h after PHE (compare the simultaneous decline of *c-myc* transcripts in cytoplasm) (fig. 3). It should be noted that the reorganization of ribosomal machinery occurs also at 0.5 h after operation. The ribosomes transiently lose attachment to the rough endoplasmatic reticulum. The restoration of the typical structure of the latter begins 1 h after PHE [12].

Discussion. The biological activities of mediators, their availability in each period of the process and the corresponding biochemical events in regenerating liver have been analyzed. The available evidences about KC activity during liver transition from quiescence to proliferation and during their first cell cycle are summarized in table. The production of thromboxane A_2 (TxA_2) was up-regulated during the immediate-early response (see table); this eicosanoid is produced in the liver mainly by KC from the endogenous pool of bound arachidonic acid in response to KC stimulation by phagocytosis or intracellular Ca^{++} elevation [1]. The ability of thromboxane to induce glycogenolysis, platelet activation and sinusoids constriction [1] is in line with the processes occurring at this time in the liver [16]. The nature of the earliest up-regulation of nuclear protooncogenes expression is not yet clear.

During the delayed-early response, the action of potent cytokines, e. g. $TNF-\alpha$ and $IL-1$ [17—20], and

Kupffer cells activity in regenerating liver

Periods	Mediators
Immediate-early	TxA_2
Delayed-early	TxA_2 , PGE_2 , $PGF2\alpha$, $TNF-\alpha$, $IL-1\alpha$, $IL1-\beta$, $IFN\alpha/\beta$
Cell cycle of HC	
G1	PGE_2 , $PGF2\alpha$, $TNF-\alpha$, $INF\alpha/\beta$, $TGF\alpha^*$, $TGF\beta^*$
S	PGE_2 , $IL-6^*$
G2-G2/M	PGE_2 , $TGF\beta^*$
Cell cycle of KC	
G1	PGE_2 , $TGF\beta^*$, NO

*The mediators potentially produced by KC. The role of KC in their production is not yet proved for regenerating liver.

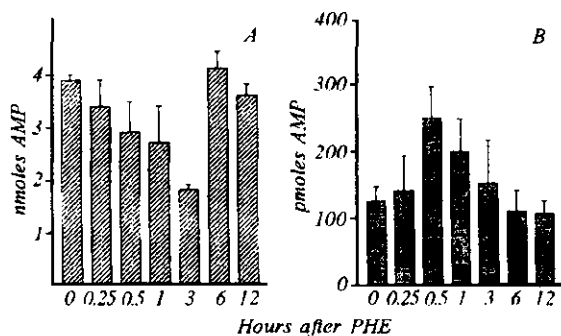


Fig. 5. 2',5'-oligo(A)synthetase activity in nuclei (A) and cytoplasm (B) in regenerating liver

the increased responsiveness to them due to elevated expression of TNF receptors [17] may define the whole set of observed events, e. g. up-regulation of *c-fos*- and *c-myc*- and down-regulation of P450IIE1 expression, as demonstrated here, NO production in hepatocytes as shown previously [21, 22], proinflammatory and hepatocyte-specific promitogenic reactions, and autostimulation of PGE₂ synthesis [23, 24]. Prostaglandin E₂ is instrumental in the regulation of cytokine production [23], the increase of intracellular cAMP [23], in relaxation of sinusoids and consequently enhanced blood flow in the liver [25]. IFN α/β and TNF- α are potential regulators of 2',5'-oligo(A)synthetase activity [10], but differential regulation of enzyme in nucleus and in cytoplasm points to the complexity of this regulation.

During the G1-like period, the set of acting cytokines enlarged by the two complete mitogens, transforming growth factor α and hepatocyte growth factor (HGF); counteracting is TGF- β , an inhibitor of DNA synthesis [15]. They originated in regenerating liver from hepatocytes, endothelial cells and hepatic stellate cells, respectively [15]. Whether and to what extent KC are implicated in this synthesis is not yet clear. Their role as stimulators of HGF expression via TNF- α , however, is established [25]. The up-regulation of *c-fos* and *c-myc* expression, the sustained down-regulation of P450IIE1 gene expression and the enhanced production of NO by hepatocytes are consistent with an involvement of KC.

During the S phase of hepatocytes KC activity slowly declines. The progressively increasing production of PGE₂ seems to inhibit TNF- α and IL-1 syntheses by KC [1]. The liver macrophages obtained at this time synthesize *in vitro* less cytokines than the cells from sham-operated animals while blockade of cyclooxygenase pathway greatly activates them [24]. The involvement of KC in stimulation of IL-6

synthesis at this or previous stage is also likely [19]. IL-6 is the most potent regulator of the acute phase response that temporally coincides with the S phase [27]. Despite the fact that stimulated KC produce IL-6 [1], their involvement in synthesis occurring in regenerating liver has not yet been proved.

Two other sets of evidences complete the scheme of regenerating process. Previously, we have shown that NO production coincides with the entrance of hepatocytes into the cell cycle [21]. While NO production in hepatocytes is under cytokines control [22], NO synthesis in activated KC is under PGE₂ control [1]. It seems likely that the high level of PGE₂ triggers NO production in KC manifesting their entrance into the cell cycle. Endogenous NO is known as inhibitor of cyclooxygenase pathway and IL-6 production in KC [28]. If it is also the case in the regenerating liver, the paracrine activity of KC is suppressed giving the way for the cell cycle-connected processes.

Thus, the cell-specific actions of Kupffer cells provide the regulatory network in the regenerating liver. The synergism between cell-specific functions of Kupffer cell and non-parenchymal cells in general and proliferative activity of hepatocytes is a key mechanism of liver regeneration. This statement is compatible with the general idea about the regulatory role of mesenchymal cells in the functioning of epithelial cells.

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М. Ю. Оболенська

Взаємозв'язок активності клітин Купфера та біохімічних процесів у регенеруючій печінці

Резюме

Цю роботу присвячено аналізу причинно-наслідкових зв'язків та часових співвідношень між синтезом клітинами Купфера біологічно активних молекул і біохімічними процесами, що проходять у регенеруючій печінці після часткової гепатектомії. Досліджували експресію і активність ряду генів і ферментів, які потенційно регулюються клітинами Купфера, а саме: протоонкогенів *c-fos* і *c-myc*, кодуючих транскрипційні фактори, тканиноспецифічного гена P450IIE1 і ферменту 2',5'-оліго(A)синтетази. Синтез біологічно активних молекул в клітинах Купфера є найвиразнішим у період переходу гепатоцитів від стану спокою до ділення. По мірі проходження гепатоцитами фаз клітинного циклу набір біологічно активних молекул звужується. Клітини Купфера переходять до проліферації після завершення гепатоцитами їх першого

клітинного циклу. Пропонується гіпотеза щодо регулюючої ролі клітин Купфера в запуску і розвитку регенераційного процесу у печінці.

М. Ю. Оболенская

Взаимосвязь активности клеток Купфера и биохимических процессов регенерирующей печени

Реферат

Настоящая работа посвящена анализу причинно-следственных связей и временных соотношений между синтезом клетками Купфера биологически активных молекул и биохимическими процессами, происходящими в регенерирующей печени после частичной гепатэктомии. Исследовали экспрессию и активность ряда генов и ферментов, потенциально регулируемых клетками Купфера, а именно: протоонкогенов *c-fos* и *c-myc*, кодирующих транскрипционные факторы, тканеспецифического гена *P450IIE1* и фермента 2',5'-олиго(А)синтетазы. Результаты исследований сравнивали с данными об активности клеток Купфера во время регенерации. Синтез биологически активных молекул в клетках Купфера наиболее выражен в период перехода гепатоцитов от покоя к делению. По мере прохождения гепатоцитами фаз клеточного цикла набор биологически активных молекул сужается. Клетки Купфера переходят к пролиферации после завершения гепатоцитами их первого клеточного цикла. Предлагается гипотеза о регулирующей роли клеток Купфера в запуске и развитии регенерационного процесса в печени.

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