REVIEWS

Repopulation of the Liver after Dipin-Induced Damage

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The alkylating preparation dipin in combination with partial hepatectomy induces genomic damage and apoptosis of hepatocytes with total replacement of parenchyma at the expense of the stem reserve cells. This model was employed to study the responses of the cell cycle to genetic damage, mechanisms of cell survival and death, and the sources of cells repopulating liver parenchyma.

Key Words: *liver; dipin; genome damage; apoptosis; stem cells*

Repopulation of liver parenchyma by new cells was discovered when the responses of proliferating cells to DNA-damaging agents were estimated [11,12]. According to model [32], a single injection of the alkylating preparation dipin with subsequent (after 2 h) partial resection of the liver induces a process leading to death of hepatocytes and repopulation of the parenchyma by cells from the stem reserve. Dipin (1,4-bis-[N, N'-di(ethylene)-phosphamide]piperazine) was synthesized at the Chemico-Pharmaceutical Institute (Moscow) [23]. In hepatocytes, dipin produces unrepairable damage to the genetic material, which after stimulation of mitoses manifests itself in chromosomal breaks and rearrangements. Cells with this damage are unviable and eventually die. Liver parenchyma is restored by outgrowth of *de novo* hepatocytic nodes that gradually form new tissue by replacing the damaged hepatocytes.

Figure 1 is a schematic illustration of processes occurring in the liver after administration of dipin and partial resection. Aberrant mitoses and postmitotic cells with karyologic anomalies and micronuclei are observed within the first 4 weeks with formation of highly ploidal cells by aberrant hepatocytes. This coincides with proliferation of oval cells, indicating activation of the stem reserve in the liver. Apoptosis of giant hepatocytes occurs on the 8th-10th week. The foci of small *de novo* hepatocytes are indicative of liver regeneration. The giant cells gradually disappear within 4-8 months, being replaced by young hepatocytes from the foci. Since the renewed parenchyma is formed by fusion of individual foci, it preserves atypical arrangement of the lobuli and specific orientation of blood vessels. After 8-10 months, some foci produce large adenomas and malignant hepatomas.

Macroscopically, regenerated liver is also changed. It is discolored in comparison with normal liver and looks heterogeneous, consisting of numerous small round formations. Liver surface remains smooth, and typical elasticity and consistence of the organ are preserved. Mice with regenerated liver did not differ from the control in body weight and appearance.

In this review we describe the dipin model of liver damage which can be employed in the investigation of key problems of cell biology.

Cell Cycle Disturbances

Cells of normal adult mammalian liver do not proliferate and remain in the G_0-phase of the cell cycle. Partial hepatectomy causes their transfer into the G_1-phase, i.e., hepatocyte begin proliferating. Treatment with dipin in the G_0-phase disturbs the entire cell cycle [9,18,29].

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A decrease in the rate of DNA replication with elongation of the S-phase and prolonged blockade of the G_2-phase are the major responses of the cells with damaged genome [13,20,29].

Irrespective of disturbances in DNA replication, its duplication is not impaired, as evidenced by cytofluorimetric measurements of DNA content in the S-phase [13,34]. Duration of the G_1-phase and, hence, initiation of DNA-replication are not changed in damaged cells. The growing fraction including almost the entire population of hepatocytes is not reduced [11,20]. Electron microscopy shows that prolonged cell blockade before mitosis is associated with dipin-induced damage to centrioles and microtubules [1]. It has been established that under conditions of premitotic blockade the expression of G_1-cycline, a cell cycle regulating protein, is considerably increased in mouse liver cells [30].

Elongation of the replication time (about 3-fold) and cell blockade in the G_2-phase underlie many interesting phenomena observed in the liver regenerating after dipin damage. As a result of specific synchronization, more than 50% liver cells are in the S-phase by the end of the second day after operation and by the end of the third day 80% of them are blocked in the G_2-phase [29]. Owing to decelerated DNA synthesis, the index of labeled nuclei increases, and repeated injections of thymidine result in a rapid saturation of the cell population with the label [11, 20]. While in normal regenerating liver the major wave of mitoses is observed within the first 3 days after operation, in the liver in a dipin-treated mouse mitoses were absent during the first 2-4 days of regeneration [18,20,29]. Since the polyploidizing mitoses are absent [31], there is no shift in the cellular classes of ploidy, and the content of binuclear cells [29] does not decrease.

Survival and Death of Cells with Damaged Genome

Being a radiomimetic, dipin causes double-strand DNA breaks. Mitotic stimulation realizes unrepairable DNA damage in chromosomal breaks and rearrangements. Nevertheless, before dying from dipin damage after several weeks or months, liver cells remain viable and perform the tissue functions.

The true triggering event of the process eventuating in cell death may be the termination of blockage in the G_2-phase of the first cycle and the first mitosis realizing the clastogenic effect of dipin [32]. Accumulation of postmitotic cells occurs after the first aberrant mitoses; these cells contain micronuclei with chromosomes fragmented during mitosis [10]. Cells survived after the first mitosis do not lose their reproductive potential and pass through 1-2 cell cycles, as evidenced by ^{3}H-thymidine incorporation, increased ploidy, and the presence of mitotic figures [6-8,10,15]. It was demonstrated that radiolabel (thymidine) is incorporated in 28% cells on the 4th day after damage and by 92% of cells after 30 days [15].

Giant highly ploidal hepatocytes with abnormal nuclei and nucleoli and 10-20 visually identified nuclei are formed after repeated cycles [6-8]. Abnormal mitoses of these cells are not completed by division of the cytoplasm; therefore, cell number does not increase. Cell ploidy increases from 8-16c DNA after the first aberrant mitosis to 32-64c DNA with the development of cytomegaly [6]. Irrespective of obvious nucleolar and cytoplasmic pathologies, metabolic activity of DNA remains high, which was confirmed by the method of nic-translation *in situ* with ^{3}H-thymidine 5'-triphosphate [33]. The high level of nic-translation may also indicate the initial stage of apoptotic degradation of DNA.

Although genomic damage is aggravated with each cell cycle, the rate of replication in the repeated cycles is normal, i.e., it lasts about 8 h. In contrast to the first cycle after dipin damage, cells are not blocked in the G_2-phase [7,8]. Induction of additional premature cell cycles provokes reproductive cell death of abortive mitosis type ("mitotic" catastrophe) [8]. This type of cell death is a variety of apoptosis. Chromatin condensation typical of the active phase of apoptosis often occurs in the telophase and is synchronous to condensation of micronuclear chromatin.

Chromosomal apparatus of surviving cells undergoes radical changes. In the first mitosis, metaphasal plates are polyploidal, all of them bearing chromosomal aberrations such as paired acentric fragments, rings, and multicentric chromosomes [10,29]. Further cycles lead to the development of compensatory processes of genomic reparation and reorganization, probably including selective replication of genes. We have observed telomeric associations and atypical chromosomes in giant cells, which are generally present in cells with amplified genes. Presumably, the long survival of damaged cells and development of adaptive reactions are to a great extent determined by the buffer properties of the polyploidal genome of hepatocytes [31].

Complete Replacement of Hepatocytes by New Cells

Complete replacement of dipin-damaged hepatocytes by new cells was demonstrated by autoradiography [11,12]. Practically the entire population of pre-

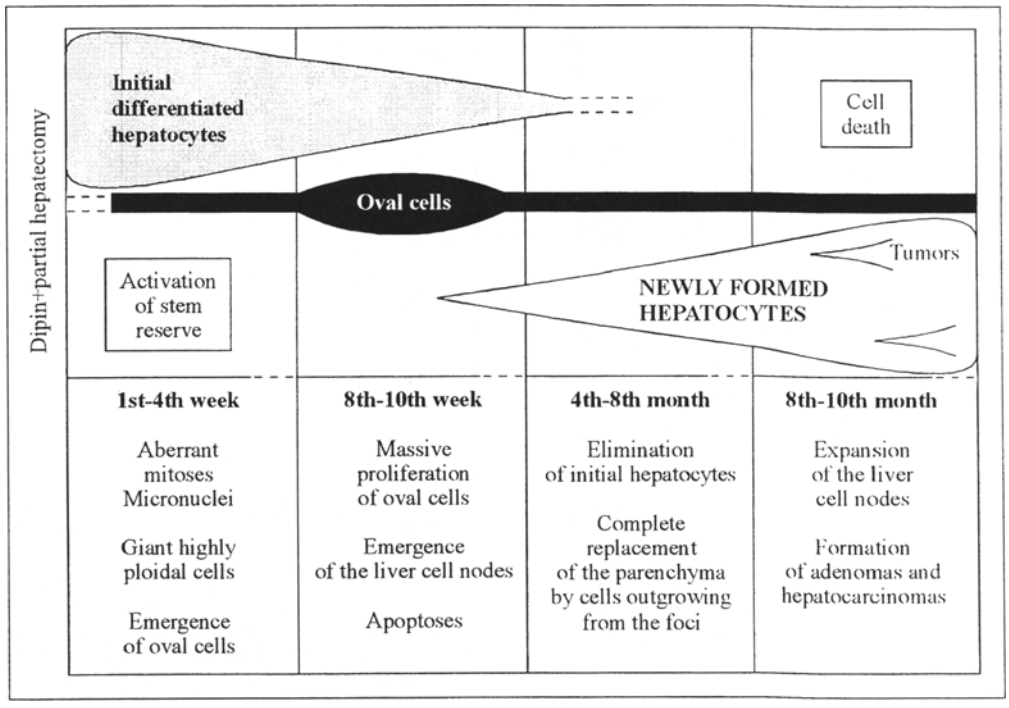

Fig. 1. A schematic drawing illustrating regenerative processes in the liver after treatment with dipin and partial hepatectomy ([32] with modification).

existing cells in liver parenchyma was radiolabeled with thymidine. Owing to the above-mentioned specific features of proliferation kinetics after dipin-induced damage (prolongation of DNA synthesis and proliferation of all parenchymal cells), eight thymidine injections during 5 days of regeneration were sufficient for this purpose. No labeled hepatocytes were detected after 8 and 10 months, indicating that all initial cells were replaced by new ones.

Sources of Renewal of Parenchymal Cells

Differentiated hepatocytes have an extraordinary high ability for reproduction and clonal growth. After any cell damage or loss, the reproductive function is performed by the remaining mature hepatocytes, including highly ploidal and mono- and binuclear cells. According to alteration of polyploidizing mitoses [31], binuclear cell can give a rise to an unlimited clone of mononuclear polyploidal cells.

Numerous researchers have identified another source of regenerative processes in the parenchyma. The stem reserve of the liver is activated when proliferation of mature hepatocytes is suppressed [16,19]. In the liver, this reserve has been associated with oval or ductular cells, the number of which is known to increase upon induction of neoplastic processes, hepatocarcinogenesis, and some other liver pathologies. The population of oval cells contains committed precursors differentiating to hepatocytes and cholangiocytes [14,16,27].

A potent stimulation of the stem reserve has been demonstrated in a liver repopulated after dipin damage. The stem cells differentiate into hepatocytes and produce multiple growth centers in the parenchyma [16,17,21,24]. The stem cell compartment includes epithelial cells of the terminal bile ducts (Hering cells), oval cells, young proliferating hepatocytes, and transitive forms between them. The emergence and replacement of different cell types have been investigated [4,5]. With the use of autoradiographic label it was shown that oval cells originate from the cells of terminal bile ducts [17,27,28]. The relationship between oval cells and bile duct epithelium, on the one hand, and transitive forms of young differentiated hepatocytes, on the other, was demonstrated by immunochemical methods. A monoclonal antibody reacting with cholangiocytes, oval cells, small hepatocytes, and cells derived from some hepatomas have been generated [21,22,24-26]. These studies showed that the facultative stem reserve of the liver serves as a source of clonogenic cells giving rise to the foci of proliferating hepatocytes in the liver damaged by dipin.

Nevertheless, massive proliferation of oval cells and their differentiation into hepatocytes for a long time occur in parallel to intense proliferation of preexisting initial hepatocytes, which also can enter the stage of clonal growth. Recent data demonstrate a complex relationship between the reserve sources of liver regeneration [2,3].

It was found that hepatocytes forming the foci differ considerably from the adjacent parenchymal

cells and normal liver cells by the structure of interphase nuclei. The newly formed tetra- and octaploid cells contain two times less nucleoli, which are larger than in normal hepatocytes of the same ploidy. The origin of these cells remains unclear as well as the mechanisms responsible for changes in their nuclei. Radical changes occurring in genomic organization of newly formed hepatocytes can be attributed to altered association of chromosomal nucleolus-forming centers due to their homologous association or endoreduplication. Now we are trying to identify chromosomal equivalents of the known changes in the nucleolar apparatus in the interphase nuclei of proliferating hepatocytes after treatment with dipin.

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