Cell Cycles During Persistence of Liver Cells after Potentially Lethal DNA Damage by Dipin

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The S and G_2 phases of the first cell cycle during which the clastogenic effect of dipin is realized are prolonged. However, further cycles of genetically defective cells are normal, often ending in abortive mitosis.

Key Words: *hepatocyte; genome; injury; cell cycle; apoptosis*

DNA damage by numerous chemical and radiation agents is realized during the first mitosis. Nevertheless, cell death may occur in further generations after repeated DNA replications and mitoses. The mechanisms underlying the remote effects of DNA damage remain obscure.

In our model [14], a single DNA damage in mouse hepatocytes led to death of all hepatocytes and repopulation of the parenchyma from the stem reserves (Fig. 1). From the moment of DNA damage to death of numerous cells within several weeks or months these cells remain viable and functionally active. The first cell cycle that realizes the clastogenic effect of dipin was characterized previously [3,5,10]. In this work we studied the parameters of mitotic cycles and mitoses in genetically defective hepatocytes of other generations.

MATERIALS AND METHODS

Twenty-five male CBA/C57Bl/6 mice weighing 20-22 g were used. According to our model [14], 2 h before standard partial resection of the liver dipin was injected intraperitoneally in a dose of 60 mg/kg. In order to stimulate the proliferative activity, the animals were treated with CCl_4 (inhalation) 8-10 weeks after the initiation. The material for study was fixed by the end of day 3 after the treatment. ^{3}H-thymidine with a specific activity of 1.75 TBq/mmol was injected intraperitoneally in a dose of 37 kBq/g 2, 8, and 9 h before fixation. Colchicine (1 μg/g) was injected in some animals 0.5-1 h before fixation. Cells were isolated by alkaline dissociation of a piece of liver fixed with 10% formalin and stained after Feulgen. The preparations were coated with a NIIKHIMFOTOPROEKT emulsion and exposed for 2 months. The parameters of cell cycle were determined by counting labeled mitotic figures (20-50 figures per animal). The ploidy of mitoses was determined by the content of DNA-fuchsin during prometaphase using a Vickers M86 cytophotometer.

RESULTS

Figure 1 shows that 8-10 weeks after DNA damage the hepatocyte population consisted predominantly of the initial cells, although newly formed cells appeared. These cells actively proliferated before and after treatment with CCl_4. Cytophotometric determination of the DNA content in prometaphasal cells (Fig. 2) showed that the bulk of dividing cells are polyploid initial cells with ploidy levels 8, 16, and 32n. Less numerous mitotic figures containing 4 and 8c DNA represent low-polyploid newly formed hepatocytes and dividing stromal cells. On liver cell autographs, mitoses and ^{3}H-thymidine-labeled hepatocytes were found in all animals; their occurrence varied from solitary cells to 1-2%. Two

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hours after labeling, the mitoses were always unlabeled, and 8 and 9 h later all mitotic figures contained the radiolabel. Thus, investigation of the cell cycle parameters by the two key points on the conventional curve of labeled mitoses led us to conclude that the duration of S and G_2 phases 8-10 weeks after DNA damage is the same as that reported for the majority of somatic cells.

The behavior of cells in our model differed in many aspects from the responses to DNA damage observed in other cellular systems. In cultures of diploid cells it was demonstrated that the induction of one double-strand rupture of DNA, one chromosome fragment in the mitosis, or one micronucleus in the interphase, leads to cell death in the nearest cell cycle [6,11]. The buffer properties of polyploid genome may account for the tolerance of hepatocytes and for their prolonged survival after a massive damage. During exposure to dipin all hepatocytes are in the G_0 phase; therefore, the reaction of the total population is the same [2].

The first cell cycle after damage is characterized by decelerated DNA replication and blocking of the G_2 phase and is completed by an aberrant mitosis (Fig. 3). During the first mitosis the preclastogenic effect of the alkylating preparation manifests itself in chromosomal ruptures. Previously, we showed that the first generation of aberrant cells has solitary micronuclei containing chromosomal fragments, while their nuclei contain 8 or 16c DNA [1]. Cells surviving after the first cycle and aberrant mitosis are still capable of entering further cycles and spontaneously go through at least 1-2 replications. This is confirmed by experiments with continuous thymidine labeling: up to 28% of cells incorporate the label during 4 days, while after 1 month of saturation 92% of the initial population bind the label [4]. The induction of additional cycles of DNA replication and mitoses aggravates DNA damage and provokes cell death. The number of visually detectable disturbances (micronuclei) increases during interphase. Cells become giant and polyploid containing abnormal nuclei and increased number of nucleoli. Mitoses are characterized by multipolarity, three-group anaphases, telophase fusion, and the presence of numerous chromosomes not associated with the spindle. Chromatin condensation typical of the active phase of apoptosis concomitant to the condensation of chromatin in the micronuclei occurs in some mitotic cells during telophase (Fig. 3). Cell death observed in this study is similar to "mitotic catastrophe" and corroborate recent concepts of apoptosis as an abortive mitosis [12,13].

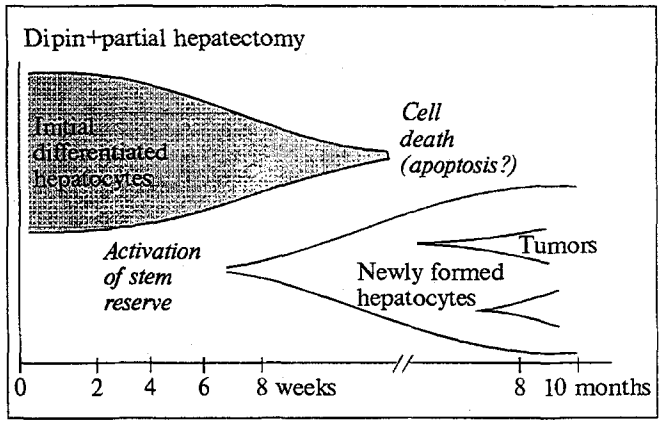

Fig. 1. Complete substitution of murine liver parenchyma cells as a result of a single dipin-induced genome damage and partial hepatectomy ([14], with modifications).

The slowdown of cell growth in response to DNA damage is a normal cellular defense reaction postponing critical events in the cells cycle: DNA replication and mitosis. Recent studies show that these reactions are initiated by p53 protein which was originally characterized as a human tumor suppressing factor [8]. This protein controls the delay in the G_1 and S phases and, according to the recent reports, in the G_2/M phase, but more often it induces apoptosis [13]. In a multicellular organism, apoptosis is preferable to reversible cycle delay, because it protects the organism at the expense of individual cells. This holds true mainly for renewed

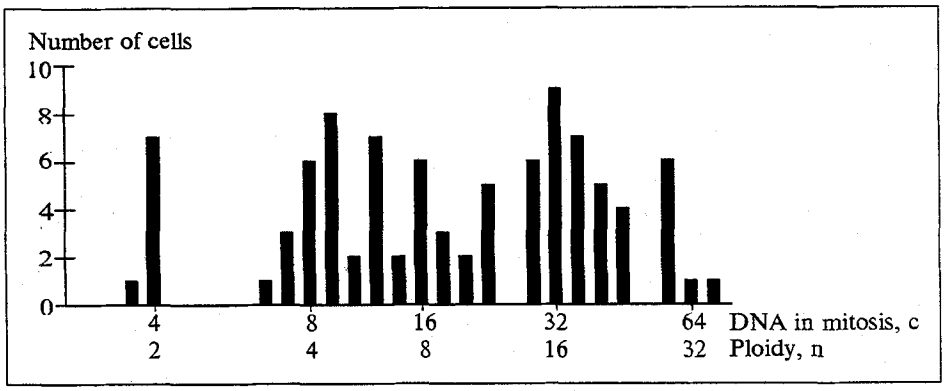

Fig. 2. Distribution of colchicine-blocked prometaphases by the content of DNA-fuchsin.

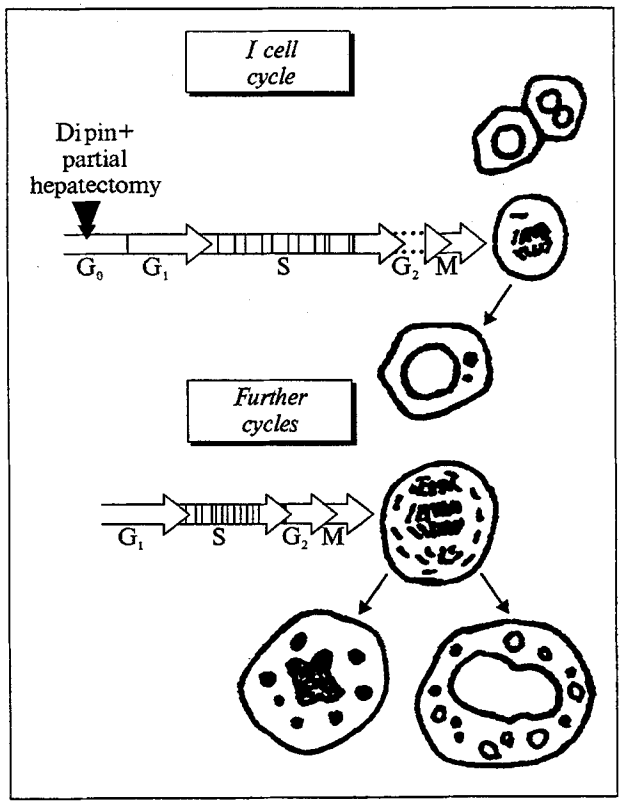

Fig. 3. Cell cycles in consecutive generations of genetically damaged hepatocytes. The first cycle after DNA damage realizing the clastogenic effect of dipin is characterized by decelerated DNA replication and prolonged blockade of the G_2 phase ending in aberrant mitosis. The first generation of aberrant cells has solitary micronuclei consisting of chromosomal fragments. The S and G_2 phases normalize during further cell cycles. Induction of additional cycles of DNA replication and mitoses aggravates DNA damage and provokes cell death similar to that resulting from abortive mitosis.

tissues with a spontaneous rate of programmed death. In our model, synchronous apoptosis of the majority of hepatocytes during the first cell cycle would have caused death of the animals.

Normalization of the S and G_2 phases during further cycles of obviously aberrant cells is of interest (Fig. 3). Presumably, this indicates genome destabilization and elimination of control over the cell cycle due to the loss of the regulatory gene function and, primarily, of p53. Alteration of the type of DNA damage, namely, the absence of double-strand ruptures as a result of chromosomal rearrangements during the first cycle, cannot be ruled out. It was reported that double-strand ruptures are required for induction of the p53 activity [9].

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REFERENCES

1. I. V. Uryvaeva, G. V. Delone, and T. L. Marshak, *Dokl. Ross. Akad. Nauk*, **348**, No. 5, 703-705 (1996).
2. I. V. Uryvaeva and V. M. Faktor, *Byull. Eksp. Biol. Med.*, **105**, No. 1, 77-80 (1988).
3. I. V. Uryvaeva, V. M. Faktor, and V. Ya. Brodskii, *Tsitologiya*, **21**, No. 6, 678-685 (1979).
4. V. M. Faktor, N. A. Eliseeva, and A. Ya. Tamakhina, *Izv. Ross. Akad. Nauk, Ser. Biology*, No. 6, 821-834 (1992).
5. V. M. Faktor, I. V. Uryvaeva, A. S. Sokolova, *et al.*, *Tsitologiya*, **21**, No. 5, 341-347 (1979).
6. M. Abend, A. Rhein, K.-P. Gilbertz, *et al.*, *Int. J. Radiat. Biol.*, **67**, No. 3, 315-326 (1995).
7. M. L. Agarwal, A. Agarwal, W. R. Taylor, and G. R. Stark, *Proc. Natl. Acad. Sci. USA*, **92**, No. 18, 8493-8497 (1995).
8. L. S. Cox and D. P. Lane, *Bioessays*, **17**, No. 6, 501-508 (1995).
9. A. DiLeonardo, S. P. Linke, K. Clarkin, and G. M. Wahl, *Genes Dev.*, **8**, No. 21, 2540-2551 (1994).
10. V. M. Faktor, I. V. Uryvaeva, A. S. Sokolova, *et al.*, *Virchows Arch. [B]*, **33**, 187-197 (1980).
11. G. Iliakis, *Bioessays*, **13**, No. 12, 641-648 (1991).
12. K. L. King and J. A. Cidlowsky, *J. Cell. Biochem.*, **58**, No. 2, 175-180 (1995).
13. W. Meikrantz and R. Schlegel, *Ibid.*, 160-174.
14. I. V. Uryvaeva, *Monogr. Dev. Biol.*, **23**, 233-236 (1992).