COMBINED ACTION OF MUTAGENS AND PHENOBARBITAL ON MITOTIC ACTIVITY OF HEPATOCYTES IN THE REGENERATED RAT LIVER

G. R. Mutovin and L. E. Nemirovskii

UDC 612.6.03:612.35.014.3]014.46

After administration of noncytostatic doses of dipin to rats or their x-ray irradiation, followed by administration of phenobarbital, an inadequate regenerative reaction of the liver is observed, expressed as a marked decrease in mitotic activity 28 h after resection. An increase in the interval between administration of dipin and of phenobarbital from 24 h to 60 days gave the same result, with only very slight quantitative changes. A similar effect was obtained by irradiating rats with a dose of 400 R and administering phenobarbital 30 days later.

Investigations in the writers' laboratory [2] have demonstrated inhibition of the first mitotic cycle in the rat liver after partial hepatectomy when preceded by combined administration of mutagens and inducers of gene activity in the G_0 phase. Inhibition of the proliferative response of the hepatocytes was connected with delay in their entry into the phase of DNA synthesis. It was accordingly postulated that inhibition of this type is due to changes in the genetic apparatus of the cell induced by mutagens.

In the investigation described below an attempt was made to determine the relationship between inhibition of regenerative proliferation of the rat liver, on the one hand, and the time between treatment with the mutagen and administration of phenobarbital and the dose of the mutagen on the other hand.

EXPERIMENTAL METHOD

Experiments were carried out on noninbred male albino rats weighing 140-160 g. From 6 to 10 animals were used in each variant of the experiment. The mutagen tested was the polyfunctional alkylating compound dipin (tetraethylenimide-piperazine diphosphate) which, in a dose of 5-20 mg/kg, does not delay the proliferative response after partial hepatectomy [1]. Phenobarbital (the soluble sodium salt), in a dose

TABLE 1. Effect of Dose of Dipin (M ± m) on Mitotic Activity of Rat Liver Cells after Partial Hepatectomy

Variant of experiment	Dose of dipin (in mg/kg)				
	5	10	15	25	50
Mitotic index (in %) in liver of rats receiving dipin	2,28±0,03	3,32±0,86	4,40±0,70	2,79±0,85	1,87±0,62
Mitotic index (in %) in liver of rats receiving dipin and phenobarbital	0,56±0,09	0,62±0,03	1,00±0,23	1,04±0,14 $P>0,05$	0,31±0,21 $P>0,05$
Mitotic activity in experiment relative to control (in %)	24,5	18,6	22,7	—	—

Note: Mitotic index in liver of rats receiving phenobarbital only was 6.56 ± 0.20.

Central Research Laboratory, N. I. Pirogov Second Moscow Medical Institute. (Presented by Academician of the Academy of Medical Sciences of the USSR S. S. Debov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 74, No. 7, pp. 95-98, July, 1972. Original article submitted April 20, 1971.

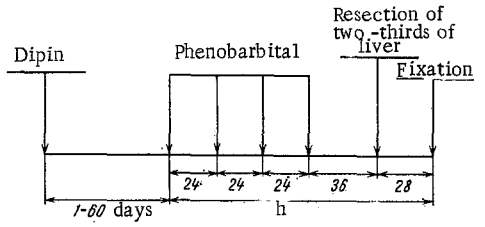

Fig. 1. Scheme of experiment: 1-60) Time interval between administration of mutagen and inducer; 24 h) time between successive injections of phenobarbital; 36 h) time between last injection of phenobarbital and hepatectomy; 28 h) time of fixation of material.

of 60 mg/kg, which is a powerful inducer of gene activity of hepatocytes [6, 7], was used as a modifier of the mutagenic effects. All the agents were injected intraperitoneally in distilled water. Hepatectomy was performed on the animals by the method of Higgins and Anderson, and the operation was carried out at 4 A.M. so that fixation of the material, planned to take place 28 h after resection, could be done at 8-9 A.M. (the diurnal peak of mitotic activity in the rat liver); the mitotic index was counted in per cent (10,000 cells per animal) in temporary squash preparations stained with aceto-orcein.

EXPERIMENTAL RESULTS

In preliminary experiments an attempt was made to determine the level of inhibition of regenerative proliferation in the liver cells after administration of various doses of dipin followed by phenobarbital. For this purpose, dipin in doses of 5, 10, 15, 25, and 50 mg/kg was injected once during the G_0 phase (i.e., into intact rats) in different groups of animals, after an interval of 5 days four injections of phenobarbital were then given at intervals of 24 h, and 36 h after the last injection two-thirds of the liver was resected. Phenobarbital increase mitotic activity in both the intact and the regenerating liver of rats [4, 5]. The present writers have also observed this effect previously [2, 3].

As Table 1 shows, after doses of dipin of 5, 10, and 15 mg/kg had been given an inadequate regenerative response was observed, with a marked decrease in mitotic activity by comparison with the control. After doses of 25 and 50 mg/kg the decrease in the level of proliferation was not significant despite a large absolute difference between the experimental and control series. This can be explained by the wide differences in individual sensitivity of animals to the cytoxic effect of these doses of dipin. In the experiments with doses of 5, 10, and 15 mg/kg no significant differences were found in the level of decrease of the proliferative response. It can accordingly be concluded that dipin is adequately effective in small doses, i.e., a "saturation effect" was observed with a dose of 5 mg/kg.

In the experiments to determine the duration of this phenomenon of inhibition of the proliferative response the

TABLE 2. Effect of Time Intervals between Administration of Dipin and Phenobarbital (M±m) on Mitotic Activity of Rat Liver Cells after Partial Hepatectomy

Variant of experiment	Dose of dipin (in mg/kg)									
	1	3	5	10	15	20	25	30	60
Mitotic index (in %) in liver of rats receiving dipin	3,22±0,13	1,72±0,55	4,40±0,70	2,81±0,25	2,47±0,15	2,41±0,33	2,55±0,11	3,41±0,20	2,00±0,16
Mitotic index (in %) in liver of rats receiving dipin and phenobarbital	0,71±0,12	0,33±0,09	1,00±0,23	1,04±0,24	0,23±0,06	0,68±0,71	0,66±0,03	1,01±0,10	0,60±0,05
Mitotic activity in experiment relative to control (in %)	22,0	19,1	22,7	37,0	9,3	28,3	25,8	29,6	30,0

Note: Mitotic index of hepatocytes of rats weighing 140-160 g and receiving phenobarbital only was 5.60±0.10, mitotic index of hepatocytes of rats weighing 300-350 g tested after 60 days was 3.95±0.15.

effect of dipin was tested in a dose of 15 mg/kg. Mitotic activity was analyzed for the following time intervals between administration of dipin and phenobarbital: 1, 3, 5, 10, 15, 20, 25, 30, and 60 days. The scheme of the experiments is illustrated in Fig. 1. The number of mitoses in animals receiving combined treatment with dipin and phenobarbital was 9.3-37% of the control level, and with an increase in the time interval there was no change in the intensity of inhibition of proliferation. The results of the experiments given in Table 2 demonstrate the prolonged nature of the changes lying at the basis of inhibition of the mitotic cycle of the injured hepatocytes.

Another series of experiments was carried out by a similar scheme, but with the use of x-ray irradiation in a dose of 400 R, itself not inhibiting mitotic activity in the regenerating liver, as the mutagen. Phenobarbital was injected 30 days after irradiation. In that case, just as in the experiments with dipin, considerable delay in the regenerative response was observed: the mitotic index of the hepatocytes of the rats treated with x-rays and phenobarbital was 0.92±0.05%, the mitotic index of the hepatocytes of rats irradiated only was 2.36±0.08%. The phenomenon of inhibition of the proliferative response in the rat liver after partial hepatectomy, as established previously, was thus confirmed in cases when the animals were treated with a combination of mutagens and phenobarbital.

On the assumption that changes in the cell leading to inhibtition of proliferation are explained by alkylation of the cytoplasmic proteins, the phenomenon observed probably would not be so permanent in character because of the possibility of intensive renewal of the cytoplasmic structures of the hepatocyte. However, in the present experiments a decrease in mitotic activity was observed in all parts of the liver even when an interval of 60 days elapsed between administration of the dipin and the inducer. It can accordingly be postulated that the after-effect of the dipin and radiation which were used was due in this case also to changes in the genetic apparatus of the cell.

Fourfold administration of phenobarbital in conjunction with resection of two-thirds of the liver in the writer's opinion is a powerful stimulus for the genetic apparatus of the liver cell including, most probably, a stimulus for the general cellular genes during maximal stimulation of mitotic activity. This load on the gene leads to decompensation if it is preceded by treatment with mutagen. Although the molecular nature of the changes lying at the basis of this phenomenon is unknown, the possiblity of their accumulation in slowly renewed cell populations can be postulated. A basis for this assumption is the duration of these changes, which is at least 1/12 of the mean life span of the rat.

Further study of the nature of this phenomenon is desirable both from the standpoint of broadening ideas regarding the spectrum of mutagenic after effects and also from the standpoint of protection of genetic structures against harmful agents.

LITERATURE CITED

1. M. V. Movsesyan and A. P. Akif'ev, Dokl. Akad. Nauk SSSR, 188, No. 3, 685 (1969).
2. L. E. Nemirovskii, Modification of Potential Genetic Injuries in Somatic Cells. Author's Abstract of Candidate's Dissertation, Moscow (1970).
3. L. E. Nemirovskii and V. V. Klimenko, in: Morphological and Physiological Bases of Regulation and Restoration of the Functions of the Organism [in Russian], Moscow (1970), p. 83.
4. P. C. Burger and P. B. Herdson, Am. J. Path., 48, 793 (1966).
5. M. Japudzic, B. Knezevic, V. Djordjevic-Camba, et al., Exp. Cell Res., 48, 163 (1967).
6. S. Orrenius, I. L. Ericsson, and L. Ernster, J. Cell. Biol., 25, 627 (1965).
7. R. T. Schimke, in: Current Topics in Cellular Regulation, Vol. 1, New York (1969), p. 77.