FLAVOBIONE AND THIOCTACIDE DIMINISH LATENT RADIATION-INDUCED DAMAGE TO THE RAT LIVER

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KEY WORDS: irradiation; rat liver; flavobione; thioctacide; regeneration

The proliferative activity of the adult liver is very low, and in rats it is found in the phase of mitosis of 0.15 ± 0.03 ‰ of cells [2, 5, 7]. Proliferation increases during regeneration of the liver, which can be induced experimentally by any acute intervention, surgical or chemical. After partial hepatectomy (two-thirds) the residue of liver tissue is subjected to a considerably functional and metabolic load. Loss of parenchyma directly causes regeneration, which begins to develop rapidly in the residue of the liver as early as on the first postoperative day. Most of the hepatocytes (up to 90%) pass from the G_0 stage into the presynthetic G_1 phase synchronously 4 h after partial hepatectomy, and this is followed by phases of interkinesis and mitosis. Regeneration of the liver increases most rapidly during the first three days, and the regeneration process terminates 14-21 days after the operation [7, 9, 14, 15].

Some agencies accelerate regeneration (bacterial lipopolysaccharides, for example) and accelerate DNA synthesis and mitosis by 4-6 h [1], whereas others have no effect on the temporal course of the process, but reduce the intensity of regeneration (for example, the use of parathyroid extract and of intracellular protease inhibitors) or inhibit the onset and intensity of regeneration (ionizing radiation, for example).

Irradiation causes the development of latent damage in the intact liver, manifested in the course of induced proliferation, mainly as delay of DNA synthesis and of mitotic activity by several hours, and a lower intensity of proliferation, with a simultaneous increase in the number of chromosomal abberations, depending, moreover, on the dose of irradiation [4, 7, 10, 12]. The development of latent damage in the intact liver persists throughout a long postirradiation period [8] and it can be weakened by means of various radioprotectors (cysteamine, adeturon, or gammaphos, especially if they are used before irradiation).

Some radioprotective agents have a beneficial effect on liver cell metabolism and thus stimulate repair processes in the liver [13]. These substances are used for protection or for the treatment of hepatic lesions caused by various pathogenic agencies and, correspondingly, in the prevention of radiation sickness.

The aim of this investigation was to study the effect of hepatoprotectors (flavobione or thioctacide) and of irradiation on regeneration of the rat liver after partial hepatectomy.

EXPERIMENTAL METHOD

Experiments were carried out on Wistar rats weighing 270-300 g. All the animals were divided into six groups: 1) animals undergoing surgery but not irradiation; 2) animals receiving thioctacide 90 min before the operation but not irradiated; 3) animals receiving flavobione 90 min before the operation but not irradiated; 4) animals irradiated 30 min before the operation; 5) animals receiving thioctacide 60 min before irradiation (i.e., 90 min before the operation); 6) animals receiving flavobione 60 min before irradiation (i.e., 90 min before the operation).

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The animals were irradiated in a single whole body dose of 5.7 Gy of gamma rays (source ^{60}Co, dose rate 481 mGy/min).

Thioctacide ("Astra Pharmaceuticals AG," thiocto-α-lipoic acid) was injected intraperitoneally in a dose of 15 mg/kg.

Flavobione ("Spofa") contains silymarin, a combination of flavonoids, and was used in a dose of 70 mg/kg by oral tube.

The animals underwent two-thirds partial hepatectomy under general ether anesthesia, always in the morning (from 8 to 10 a.m.) and they remained under observation during the period of maximal mitotic activity 30 h after the operation.

Evaluation (by Feulgen's method) of 50,000-60,000 cells in each experimental group revealed all mitotic figures and chromosomal aberrations in postmetaphase, and the mitotic index, i.e., the number of mitotic figures per 1000 cells, and the percentage of chromosomal aberrations among the total number of postmetaphase figures, found among all the cells studied, were determined.

The number of cells in 1 mg tissue was determined with the aid of a "Coulter Counter."

EXPERIMENTAL RESULTS

The proliferative activity of the regenerating rat liver reaches a maximal 30 h after two-thirds partial hepatectomy. The effect of hepatoprotectors and of irradiation on regeneration of the liver is therefore studied at that time. The number of cells in the regenerating liver of the control animals rose rapidly compared with their number in the intact liver (from 0.72 ± 0.01 to $1.22 \pm 0.04 \cdot 10^5$ cells/mg tissue). An increase in the number of cells in the tissue is evidence of a decrease in the mean volume of the cells, as a result of previous mitotic division of some hepatocytes. An even more striking increase in the number of cells was noted in the regenerating liver of animals receiving thioctacide. Irradiation retarded the increase in the number of cells in the regenerating liver of animals protected with thioctacide, and the increase in the number of cells was the same as in unirradiated animals. The use of flavobione had no effect on the number of cells in the tissues of the animals, whether irradiated or not.

The mass of the regenerating liver of the control rats increased by 1.74 ± 0.49 g.

The increase in mass of the liver under the influence of irradiation and hepatoprotectors was virtually unchanged.

The mitotic index in the regenerating liver of the control animals 30 h after the operation was increased by 27.14 ± 0.47 ‰. The mitotic index in the regenerating liver of animals receiving flavobione and, in particular, thioctacide before the operation also was greater than in the corresponding control animals. The increase in the mitotic index after administration of flavobione in the rat liver regenerating after two-thirds partial hepatectomy was described in the literature [13].

Irradiation in a dose of 5.7 Gy delayed mitotic activity, as was shown by a decrease in the number of mitotic figures by about 3.5 times compared with the corresponding control. The use of thioctacide (but not of flavobione) diminished the fall of mitotic activity induced by irradiation.

Aberrant postmetaphases in the regenerating liver of the control animals and of animals receiving the hepatoprotectors before the operation were observed in 3-5% of dividing cells. Irradiation caused a sharp increase in the number of chromosomal aberrations, which varied on average about 95%. Similar changes were described previously in the literature [3, 4]. The use of both hepatoprotectors considerably reduced the increase in the frequency of chromosomal aberrations compared with irradiated, unprotected animals.

When using radioprotectors (cysteamine, adeturon, and gammafos), chiefly before irradiation, previously we also noted that modification of latent liver damage was possible, as shown by changes in MI and in the frequency of chromosomal aberrations compared with irradiated, unprotected animals. When the dose of irradiation used was comparatively similar, so also was the modifying effect, although the action of hepatoprotectors and radioprotectors was based on different mechanisms.

According to our results, thioctacide is more effective than flavobione. After use of flavobione, unlike thioctacide, no increase was observed in the gain in weight of the regenerating liver in the irradiated rats, nor of the number of cells in the liver, but changes in the concentration and total content of DNA and histones were significantly less than in unprotected animals. These results suggest that flavobione has a greater effect on the structure of chromatin and on DNA and histone synthesis than on mitosis.

These results are evidence that hepatoprotective agents (thioctacide and flavobione) diminish latent damage to the genetic material of the liver caused by irradiation, and they thus stimulate regeneration.

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ULTRASTRUCTURAL CHANGES IN INTERNEURONAL JUNCTIONS IN INTRAMURAL GANGLIA OF THE SMALL INTESTINE OF SUCKLING RABBITS WITH EXPERIMENTAL CHOLERA

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The secretory and motor activity of the small intestine is regulated by intramural ganglia of the autonomic nervous system, which consists of assemblages of neurons involved in the mechanism of various lesions arising the gastrointestinal tract [5].

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