EFFECT OF FLAVOBION ON TISSUE NUCLEIC ACIDS OF RATS IRRADIATED WITH GAMMA RAYS

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Flavobion ("Spofa") is a hepatoprotective agent whose active moiety is formed by a complex of flavonoids (silybin, silydianim, and silychristin), under the general name of silymarin. The hepatoprotective and antihepatotoxic effects of silymarin can be attributed to direct protection of the liver cells by stabilization of their membranes (lowering of membrane permeability, changes in the normal lipid content of the membranes) and homeostasis of metabolism [7, 13].

Silymarin has been shown to stimulate rRNA synthesis in cell nuclei. In rats, CCl_4 damages important structural elements of the liver cells, leading to hydropic degeneration of the cells, abolition of their lipotropic capacity, and damage to the mitochondria and endoplasmic reticular system, but if silymarin is given at the same time, these changes do not appear [12].

Experiments to study the pharmacologic action of silymarin have been conducted mainly in its target organ, namely the liver. The aim of this investigation was to study the effect of silymarin on nucleic acids in the liver (intact and regenerating) and in the spleen and bone marrow of rats after gamma-ray irradiation and partial hepatectomy. This study is a continuation of our previous work to study the effect of silymarin (in the form of flavobion) and of x-ray irradiation on some cytologic parameters in the intact and regenerating rat liver [2].

EXPERIMENTAL METHOD

Experiments were carried out on adult male Wistar rats weighing 270-300 g. Flavobion was given in suspension form through an oral tube in a single dose of 70 mg/kg 1 h before whole-body irradiation in a dose of 5.7 Gy of gamma-radiation (^{60}Co). The animals underwent partial hepatectomy in the 30 min after irradiation by the standard method, when 68-72% of the mass of the liver was removed. Quantitative changes in nucleic acids were studied in the intact liver (in tissue removed at operation) and in the regenerating liver, spleen, and bone marrow (30 h after the operation). The RNA and DNA concentrations were determined spectrophotometrically in digests of the samples [4]. Statistical significance was determined by Peritz' F test [8].

The animals were divided into four groups: 1) control animals undergoing partial hepatectomy, 2) control animals receiving flavobion 90 min before partial hepatectomy, 3) animals irradiated in a dose of 5.7 Gy 30 min before partial hepatectomy, and 4) animals receiving flavobion and irradiated before partial hepatectomy in the same doses as in groups 2 and 3.

EXPERIMENTAL RESULTS

Flavobion had virtually no effect on the RNA and DNA levels in the intact liver of the control unirradiated rats. In irradiated animals (group 3) the RNA and DNA concentrations were raised compared with the control, unirradiated animals, by about 20% (Fig. 1a). In irradiated animals protected by flavobion, changes in the nucleic acid concentrations

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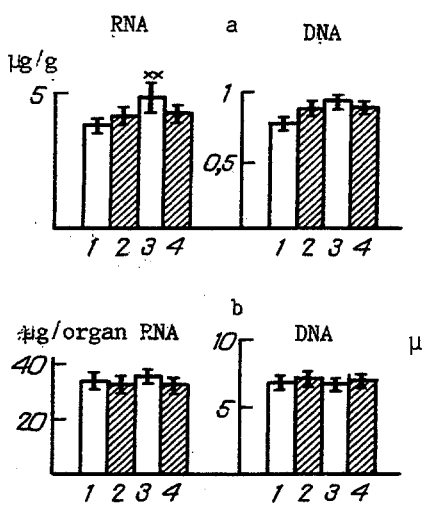

Fig. 1

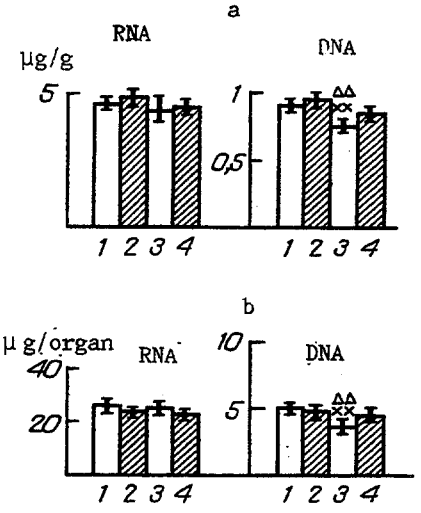

Fig. 2

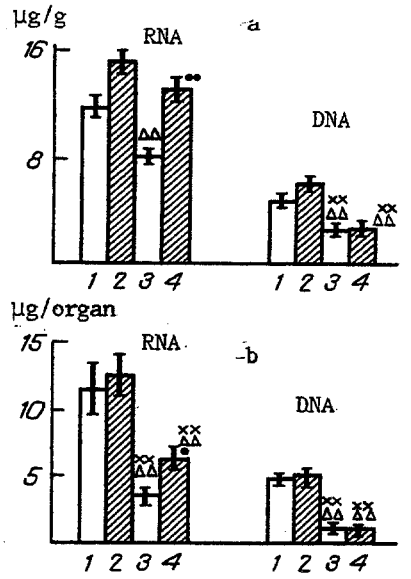

Fig. 3

Fig. 1. Concentration (a) and content (b) of RNA and DNA in intact liver of rats 30 min after irradiation in a dose of 5.7 Gy and administration of flavobion: 1) control, 2) flavobion, 3) irradiation, 4) flavobion + irradiation. Asterisk indicates values for which $p < 0.05$ compared with control.

Fig. 2. Concentration (a) and content (b) of RNA and DNA in regenerating liver 30 h after irradiation in a dose of 5.7 Gy and administration of flavobion.

Fig. 3. Concentration (a) and content (b) of RNA and DNA in rats' spleen 30 h after irradiation in a dose of 5.7 Gy and administration of flavobion.

were less marked than in the irradiated unprotected animals. The hepatoprotective agent had no effect on the total RNA and DNA content after irradiation in the intact liver (Fig. 1b).

In the regenerating rat liver, flavobion alone had no effect on the concentration and total content of RNA and DNA compared with the control (Fig. 2a, b). The DNA concentration and content in the regenerating liver of the irradiated animals were significantly lower than in rats of the control groups (to 28%, $p < 0.05$). In irradiated animals protected with flavobion, postradiation losses of DNA were smaller and the differences between groups 1 and 4 were not significant. More significant differences likewise were not found in the concentration and total content of RNA in the regenerating liver after administration of the hepatoprotective agent.

Flavobion had virtually no effect on the concentration and total content of RNA in the spleen of the control rats. In irradiated animals protected with flavobion postradiation losses of RNA were 1/3-1/2 less than in unprotected animals. Quantitative changes in DNA after the use of flavobion alone were less marked than changes in RNA, and in the irradiated animals flavobion did not abolish the decrease in concentration and total content of DNA (Fig. 3a, b).

In the bone marrow of the unirradiated control rats flavobion likewise caused an increase in the concentration and content of RNA and DNA. The protective effect of flavobion on irradiated animals was not significant, and the concentration and content of the nucleic acids were reduced by 30-15% of the control values.

In the control rats, flavobion (silymarin) thus caused no definite changes in nucleic acids in either the intact or the regenerating liver or in the spleen and bone marrow. In the liver, during flavobion administration most cells were in stage G_0 [1]. After partial hepatectomy most cells entered phase G_1 after 4 h, and phase S after 16-18 h, and DNA synthesis reached a maximum after 20-24 h [3]. The highest silymarin concentration in the liver was found 1 h after its administration. It can accordingly be postulated that the preparation acted also in the regenerating liver, on cells of which most were still in phase G_0 (meaning that dividing cells were not affected), whereas in the spleen and bone marrow, which possess

high proliferative activity, silymarin acted on cells in all phases of the cell cycle. In irradiated rats the use of flavobion restored to normal the early changes in the RNA concentration in the intact liver and reduced the severity of changes in nucleic acids in the regenerating liver and spleen 30 h after partial hepatectomy. An increase in the RNA concentration in the intact liver 30 min after irradiation was accompanied by a decrease in weight of the liver, and for that reason the total content of RNA (and DNA, changes in which were less marked) remained at the level of the control values. Changes in weight of an organ with low proliferative activity, while the total DNA content remained the same, were associated with changes in the blood supply of the organ and in the migration of fluid. These results, like those relating to the effect of hepatoprotective agents [7, 13], indicate the possibility that flavobion may have a stabilizing effect on cell membranes after irradiation. In the regenerating liver and spleen of the rats 30 days after irradiation a protective effect of flavobion was found, and which was evidently also attributable to membrane stabilization, but also to general activation of cell metabolism.

The stimulating effect of silymarin on the level of rRNA synthesis has been described by many investigators [6, 11]. An increase in the concentration and content of RNA may be connected with the action of silymarin on template activity of the chromatin or with activation of RNA-polymerase [10], processes which lead to increased RNA synthesis, although slowing of RNA degration likewise cannot be ruled out.

The results of clinical investigations [5, 9], which demonstrated besides the positive effect of silymarin on liver tissue, intensification of pathological changes in the spleen in the majority of cases, are interesting when considered in connection with the results now obtained.

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