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Influence of Age and Gamma Irradiation on the Proliferative Activity in Regenerating Rat Liver

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Summary

The effect of aging (2-14 months) and total body irradiation (5.7 Gy of gamma radiation) on liver regeneration was investigated in rats 30 h after partial hepatectomy. Exposure of rats to irradiation 30 min before partial hepatectomy caused latent injury in the remaining liver cells. During the course of liver regeneration this became manifested as a delay in increasing the nucleic acid concentration and content and liver weight and, furthermore, as inhibition of the increase in the mitotic index and cellularity and pronounced increase in the frequency of chromosome aberrations in the postmetaphase. The pattern of age-related changes during liver regeneratin was the same as that after irradiation, so that the differences between irradiated and nonirradiated animals became smaller with age.

Key words

Cell proliferation – Chromosomal aberrations – Rat liver – Liver regeneration – Age – Gamma irradiation

Introduction

Ionizing radiation causes latent injury in the intact liver and this becomes manifest during stimulated proliferation (e.g. after partial hepatectomy) by inhibiting DNA synthesis and mitotic activity and by increasing the number of chromosome abnormalities (Barbason *et al.* 1983, Curtis *et al.* 1964, Giliyano and Malinovski 1977, Kropatchova and Mishurova 1985, Zorin *et al.* 1975).

The extent to which mitotic activity in the regenerating liver is inhibited after a single dose or continuous irradiation depends on the dose or daily dose rate respectively (Kropatchova and Mishurova 1981, Mišurová *et al.* 1974). With increasing total dose of continuous radiation, inhibition of proliferation becomes enhanced which testifies about the cumulation of damage in the intact liver.

Radiation-induced latent injury has been found to persist in the intact liver after a long postirradiation period (Albert 1958, Witcofski and Pizzarello 1974, Kropatchova and Mishurova 1988).

Besides irradiation and other physical or chemical agents (Albert 1958, Carter *et al.* 1956, Kropatchova *et al.* 1980, Kropatchova and Mishurova 1985, Kropatchova and Mishurova 1989, Šimek 1989), both the rate and extent of liver regeneration is also influenced by the age of the animal. Proliferation in young animals begins earlier and liver regeneration progresses more rapidly than in old ones (Barbason *et al.* 1983, Bucher *et al.* 1964, Witcofski and Pizzarello 1974).

The dynamics of regenerative processes as well as the extent of liver damage owing to ionizing radiation or chemical agents was previously investigated in detail. The aim of this work was to follow age-related changes in the liver regenerating after partial hepatectomy in control and irradiated animals. The effect of these factors was estimated on the basis of quantitative changes in RNA and DNA, cellularity, gain in weight, the mitotic index and frequency of chromosome abnormalities in the regenerating and intact liver of rats.

Material and Methods

Male Wistar rats (SPF, Velaz Prague) 2, 7, 10 and 14 months old, weighing 230–250 g, 480–500 g, 500–520 g and 540–560 g, respectively, were used throughout this study. They were fed a standard pelletized Larson diet (Velaz Prague) and given drinking water.

The rats of all age groups were irradiated with a single whole body dose of 5.7 Gy of gamma radiation from a ^{60}Co source (at a dose rate of 276 mGy.min^{-1}).

Irradiated animals were subjected within 30 min after irradiation together with nonirradiated control animals of corresponding age to partial hepatectomy (two thirds of liver mass – PHE) under ether anaesthesia by a standard procedure (Higgins and Anderson 1931). All animals were operated at 08.00–10.00 h and examined 30 hours after PHE. Along with the hepatectomized rats irradiated and nonirradiated rats with intact livers were also examined.

Squash mounts of liver tissue were stained by the Feulgen method. The squash mounts were preferred over sections because a larger number of complete mitotic figures could be found in them. In each examined group, 50 000–60 000 cells in all phases of the mitotic cycle were evaluated. After this, the mitotic index (MI), i.e. the number of mitotic figures per 1 000 cells was calculated. Genetic damage was estimated on the basis of the incidence of both chromosome bridges (dicentrics) and acentric fragments in the postmetaphase. About 400–500 postmetaphases were evaluated in each group. The extent of damage was expressed in percentage of aberrant cells.

RNA and DNA quantitative changes were determined spectrophotometrically in alkáline or acidic specimen hydrolysates according to Canev and Markov (1960).

Tissue cellularity, i.e. the number of cells per 1 mg of tissue and/or organ cellularity was calculated per liver weight using a Coulter Counter.

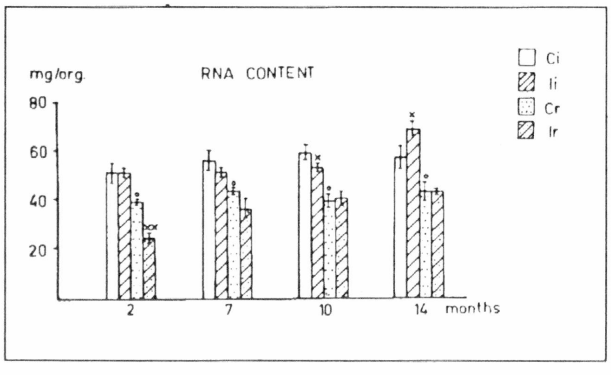

Fig. 1
RNA content in the intact and regenerating rat liver. Ci-control intact; Ii-irradiated (5.7 Gy) intact; Cr-control regenerating (30 h after PHE); Ir-irradiated (5.7 Gy) regenerating (30 h after PHE). The results are given as means ± S.E.M. for six animals per group. Control: Irradiated (Ci:Ii): (Cr:Ir) x $P<0.05$; xx $P<0.01$ Control intact: Control regenerating (Ci:Cr) o $P<0.05$; oo $P<0.01$

The weight gain was determined on the basis of theoretically calculated weight of the remaining liver at the time of operation (depending on the weight of excised liver parts) and weight of the regenerating liver at the time of examination.

Statistical significance was evaluated by the Peritz F-test (Harper 1984). The results are given as means ± S.E.M., for 6 animals in each group.

Results

The total DNA content in the intact liver of control animals (Ci) remained at about the same level in all the groups examined (Fig. 1). Irradiation influenced the RNA content in intact livers (Ii) of older animals. In the regenerating livers of control rats (Cr) of all age groups, the RNA content was decreased, due to reduced liver weight, by 20–25 % 30 h after PHE as compared to preoperation values. A more marked response to irradiation was only seen in the regenerating livers of irradiated 2-month-old rats (Ir).

Besides the DNA concentration, the total DNA content in the liver was also influenced, and depended mainly on the weight of either the intact or regenerating livers. Therefore, the total DNA content (Fig. 2) in regenerating livers of control animals (Cr) was found to be lower in the first two groups examined as compared with the intact controls (Ci).

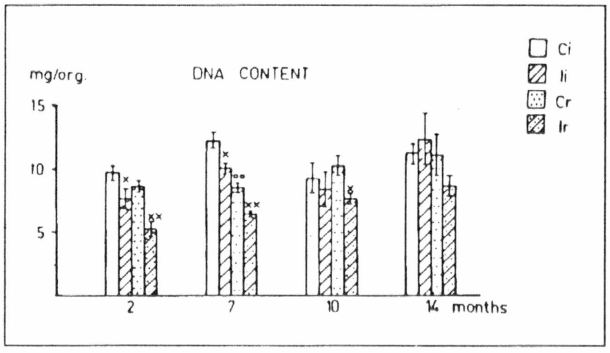

Fig. 2
DNA content in the intact and regenerating rat liver. See Fig. 1 for legend.

Radiation caused marked changes in total DNA content in both regenerating (Ir) and intact livers (Ii) namely in rats of the younger age groups.

The total cellularity in the intact liver of nonirradiated (Ci) as well as irradiated (Ii) animals (Fig. 3) was not influenced markedly by their age.

However, in the regenerating liver of nonirradiated 2 and 7 months old control (Cr) animals, it almost doubled 30 h after PHE as compared to intact controls (Ci). The cellularity in the regenerating livers of 10-month-old rats was similar to the intact liver of animals of the same age and it was even lower in 14-month-old rats.

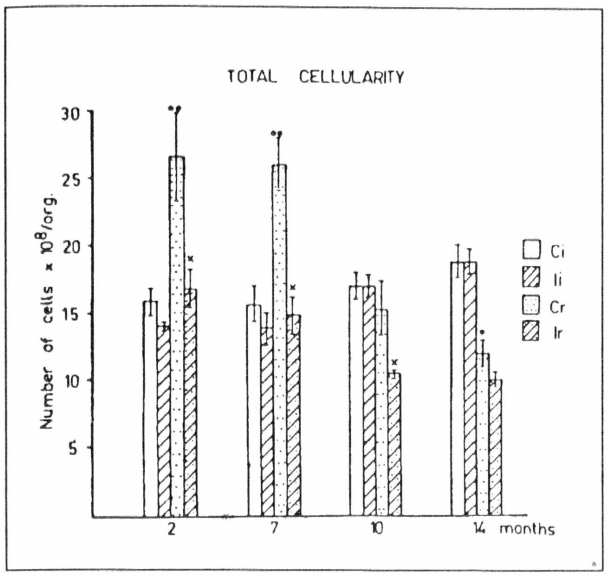

Fig. 3
Total cellularity of liver in the intact and regenerating rat liver. See Fig. 1 for legend.

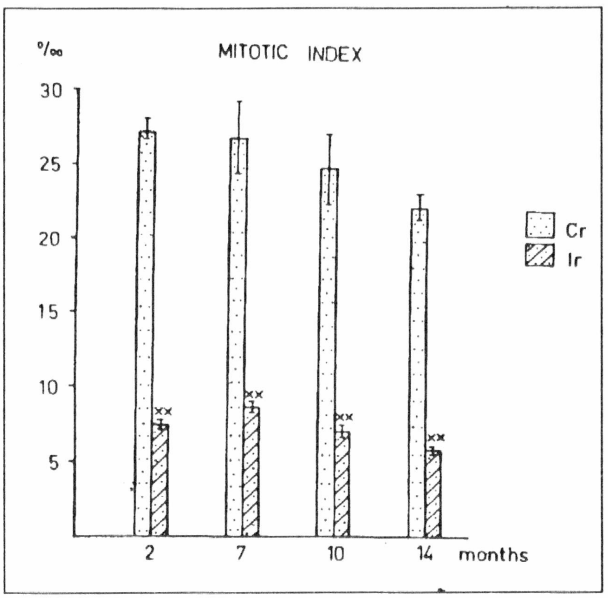

Fig. 5
Mitotic index in the regenerating rat liver. See Fig. 4 for legend.

Irradiation inhibited the increase of cellularity in the regenerating livers namely of 2 and 7-month-old rats. Low total cellularity in the regenerating livers of older control animals (Cr) and of irradiated animals of all age groups (Ir) bears a relation to lower tissue cellularity and also to the lower weight of regenerating livers.

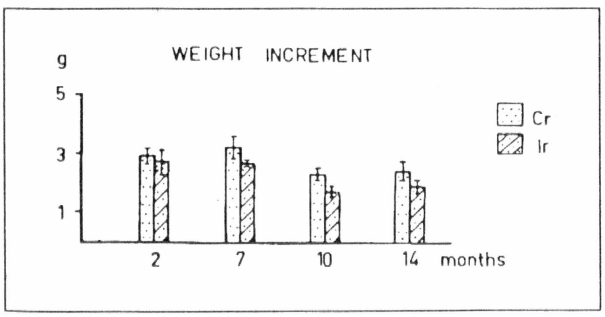

Fig. 4
Weight increment of the regenerating rat liver. Cr-control regenerating (30 h after PHE); Ir-irradiated (5.7 Gy) regenerating (30 h after PHE). The results are given as means ± S.E.M. for six animals per group. Cr:Ir x $P<0.05$; xx $P<0.01$.

The weight gain of regenerating livers (Fig. 4) decreased gradually in control (Cr) and irradiated (Ir) animals, and also depended on the age and irradiation.

The mitotic index (MI) in regenerating livers of 2-month-old controls (Cr) was 27.34±0.47 ‰ 30 h after PHE (Fig. 5).

The MI values decreased mildly with age especially in 14-month-old rats to 22.35±0.94 ‰. A single dose of 5.7 Gy inhibited the mitotic activity which resulted in a pronounced reduction in the number of mitotic figures. In the regenerating livers of irradiated animals (Ir) of all age groups there was about a 3.5 fold decrease of the mitotic index as compared with the corresponding controls.

The proportion of the numbers of metaphases to prophases (M/P ratio) in nonirradiated controls (Cr) increased only moderately with age (Fig. 6). The increase in M/P ratio after irradiation was significant in 2 to 10-month-old rats.

The incidence of postmetaphase chromosome abnormalities in regenerating livers of the controls (Cr) increased with age from 3.66±1.36 % in 2-month-old to 17.67±1.81 % in 14-month-old rats (Fig. 7).

Discussion

Most parenchymal liver cells in adult animals are in the G_0 phase of the cell cycle which is less sensitive to radiation (Lajtha 1964, Fabrikant 1969, Gushchin 1975). Four hours after partial hepatectomy about 90 % of the cells enter synchronously into the presynthetic G_1 phase (Malinovski *et al.* 1973, In the regenerating livers of irradiated (Ir) animals, the frequency of chromosome abnormalities increased in all age groups to 93.60±2.96 – 96.97±2.10 %.

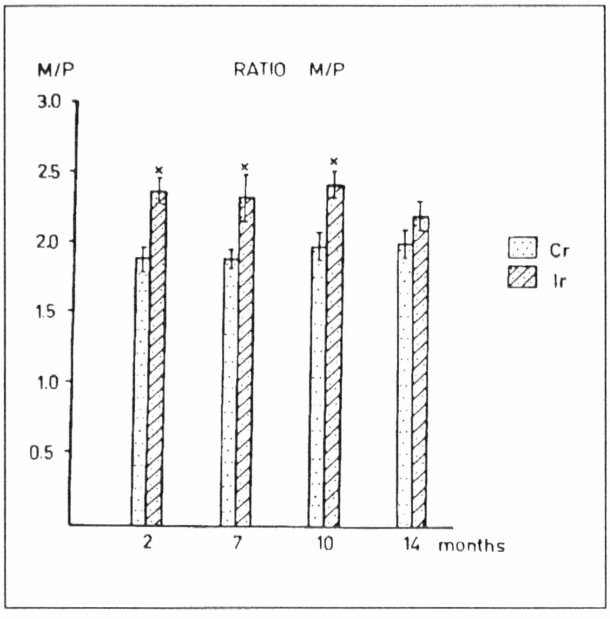

Fig. 6
Metaphase to prophase ratio in the regenerating rat liver. See Fig. 4 for legend.

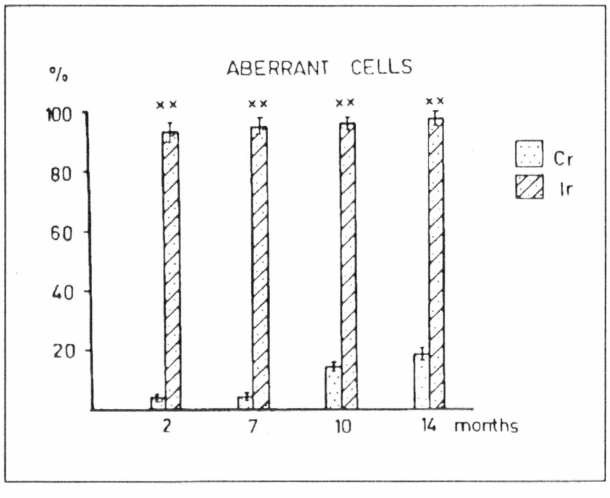

Fig. 7
Aberrant cells in the regenerating rat liver. See Fig. 4 for legend.

Yakovlev 1979) followed by further phases of interkinesis and mitosis. In young rats, almost 95 % of hepatocytes enter synchronously into the first wave of mitosis (Giliyano and Malinovski 1984). With increasing age, synchronization of hepatocyte transition to S phase of the cell cycle diminishes (Obolenskaya 1976) and, consequently, inhibition of liver regenerative activity occurs (Barbason *et al.* 1983, Bucher *et al.* 1964, Witcofski and Pizzarello 1974).

In our experiments, no statistically significant age-related changes were found in the intact liver of rats between 2 and 14 months of age. However, the influence of age became clearly manifest during liver regeneration after partial hepatectomy. Thirty hours after the operation, an abated increase in nucleic acid concentration as well as marked inhibition of the increase in cellularity was found with age. The more profound changes in cellularity than in the DNA content may be considered as a sign of proceeding polyploidization and formation of binuclear hepatocytes due to retarded and attenuated mitosis and cytokinesis (Wheatly 1972, Fabianova *et al.* 1981, Mišurová *et al.* 1987). A gradual diminution of hepatectomy-induced proliferative activity with age in the regenerating liver may also be documented by the lower mitotic index and increased M/P ratio which is probably related to extended duration of metaphases in comparison to prophases (Kropatchova and Mishurova 1985). The higher incidence of chromosome abnormalities (from 3.66 ± 1.36 % in 2-month-old nonirradiated rats to 17.67 ± 1.81 % in 14-month-old rats) which hamper and inhibit mitotic division may also contribute to the decrease in cellularity in the regenerating liver with age. Increase in chromosome abnormalities with age (from 8 % in 4-month-old nonirradiated mice to 22 % in 12-month-old nonirradiated mice) was noted in mice by Stevenson and Curtis (1961) and Curtis and Crowley (1963). The above authors postulated a somatic theory of aging on the basis of this phenomenon. In the regenerating liver of irradiated rats, the following changes were found as compared to nonirradiated animals: a lower concentration and total content of RNA and DNA, a markedly reduced tissue and organ cellularity, a profound decrease of the mitotic index and a pronounced increase in the relative ratio of metaphases to prophases (M/P) and in the incidence of chromosomal abnormalities amounting about 96.97 ± 2.10 % . Curtis and Crowley (1963) also found, by the same method of preparing and evaluating the preparations, 60 % of aberrancies in mice exposed to a single dose of 226 rad (approximately 2.2 Gy) of X-rays before PHE. Furthermore, after a single dose similar to that in our experiment, they found more than 80 % of chromosome abnormalities. Giliyano and Malinovski (1976) found as many as 88 % of chromosome abnormalities after a single dose of 630 rad (approximately 6 Gy) in rats. The pattern of radiation-induced changes was the same as during aging and, therefore, the most pronounced differences between irradiated and nonirradiated rats were seen in young animals. With increasing age they became smaller or even disappeared. Literary data concerning the maximum of mitosis (Barbason *et al.* 1983, Kropatchova and Mishurova 1981) and an increase in chromosome aberrations (Curtis and Crowley 1963, Giliyano and Malinovski 1976, Kropatchova and Mishurova 1981) in the regenerating liver with age or after irradiation are in agreement with this finding.

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Reprint Requests

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