Effect of Colostrum Factor on Structure and Functional Activity of Rat Liver Nuclei Late After γ-Irradiation

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A radioprotective effect of cow colostrum factor in rats injected subcutaneously in a dose of 1 mg/g body weight 30 min before and for 4 days after whole-body γ-irradiation (8 Gr) is demonstrated. The factor improves survival rate and accelerates postirradiation recovery of nuclear structures and RNase and ATPase activities of the nucleus.

Key Words: *ionizing radiation; cell nuclei; colostrum factor; ribonuclease; adenosine triphosphatase*

Cell nucleus regulates cell growth and development in the organism [2]. Ionizing radiation damages the genetic apparatus, impairs nucleus-cytoplasm interaction, and alters receptor function and permeability of the nucleoplasm [6,7]. Most of the currently used radioprotectors produce toxic effects and induce structural and functional changes in membranes [3]. In light of this, we investigated the effects of colostrum factor (CF) a natural radioprotector [5,11].

MATERIALS AND METHODS

Colostrum factor isolated from cow colostrum in the early period of lactation contains low-molecular-weight components, including polypeptides (about 10 kD), lipids, carbohydrates, nucleotides, and inorganic compounds. Experiments were carried out on random-bred male rats (120-140 g body weight) divided into 2 groups. The rats were exposed to γ-radiation (^{60}Co, 8 Gy, 0.0233 Gy/sec power), and group 2 rats were subcutaneously injected with CF (1 mg/g body weight) 30 min before and for 4 days after irradiation. Survival was evaluated during 3 months postirradiation. Nuclear structure and enzyme activity were assessed 1 and 24 h, 5, 10, 15, and 20 days, and 1, 2, and 3 months postirradiation. Liver sections and isolated structures were fixed with 2.5% glutaraldehyde in 0.1 M phosphate buffer (pH 7.4) and postfixed with 1% OsO_4. The dehydrated material was embedded in Epon-Araldite. In some experiments ^{3}H-CF [12] was subcutaneously injected in a dose of 1 μCi/g body weight. Cell nuclei were isolated [4] and lipids were extracted [8] as described elsewhere. Mg^{2+}-dependent ATPase [13] and RNase [10] activities were measured.

RESULTS

The rats irradiated in a lethal dose of 8 Gy died on days 3-9 postirradiation (50% animals died over 6 days, Fig. 1), while CF-protected rats died on days 10-11 postirradiation (62% survival rate).

Subcutaneously injected ^{3}H-CF rapidly propagated in organs and tissues. The protective effect developed 30-40 min postinjection. During this period, ^{3}H-CF accumulates in the nuclear matrix, nuclei, and microsomal fractions (Table 1). These findings agree with published data that radioprotectors are predominantly accumulated in cell nuclei and protect the cell from radiation-induced damage [1,9].

Electron microscopy showed that γ-irradiation induced marked alterations in the nuclear structures. Maximum abnormalities were found in isolated liver

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nuclei from group 1 rats: invaginations, loosening of the outer nuclear membrane, enlargement of the perinucleal zone, chromatin condensation, and very dense nucleoprotein structure. Changes in the nuclear morphology were also seen on ultrathin liver sections (Fig. 2, *a*, *b*).

In group 2 rats 1 h postirradiation the nuclei retained their shape. The outer nuclear membrane had no clear contours. In some cells invaginations of the nuclear membrane were noted.

The later postirradiation period was characterized by complete recovery of the nucleus morphology. On day 20 postirradiation, the nuclei were round-shaped, the nuclear membrane was clearly contoured (Fig. 2, *c*). However, round light structures were still present in some nucleoli. Nuclear structures completely recovered 90 days postirradiation (Fig. 2, *d*). On the ultrathin sections, liver nuclei had regular round shape and

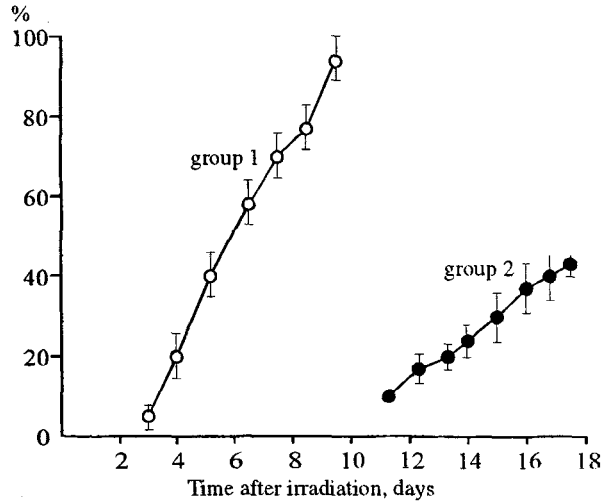
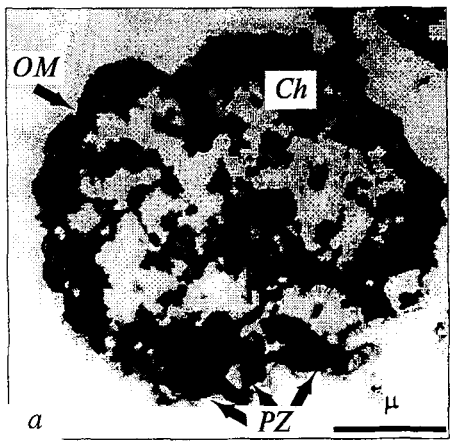
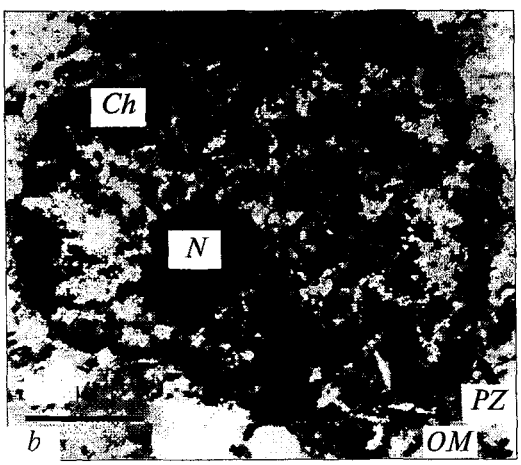
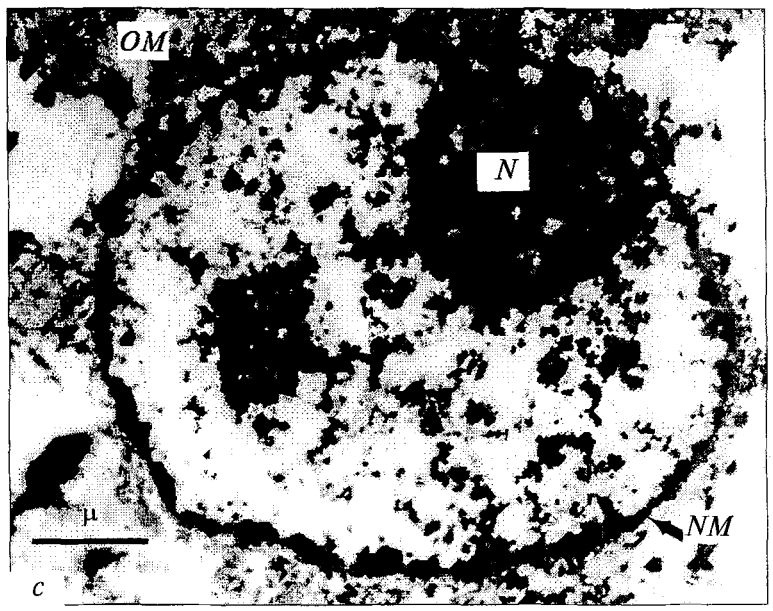


Fig. 1. Radioprotective effect of colostrum factor on rat survival. Ordinate: % of survival.







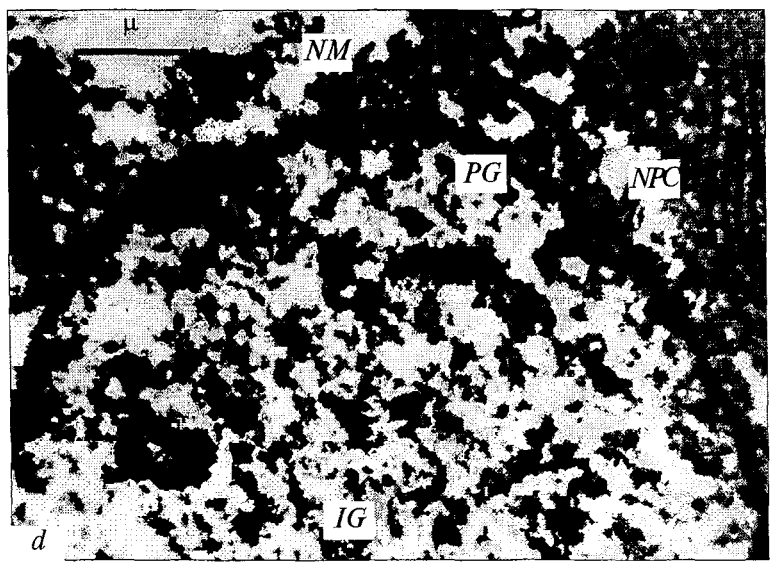


Fig. 2. Ultrathin sections of isolated liver nuclei and tissue. *a*) isolated nuclei 1 h after whole-body irradiation in a dose of 8 Gy; *b*) nucleus fragment of liver section 1 h postirradiation; *c*) the same 20 days postirradiation in rat injected with colostrum factor; *d*) the same 3 months postirradiation. OM: outer membrane; Ch: chromatin; PZ: perinuclear zone; N: nucleolus; NM: nuclear membrane; NPC: nuclear pore complex; PG and IG: peri- and interchromatin granules.

TABLE 1. Subcellular Distribution of ^{3}H-CF in Rat Liver ($M\pm m$, n=5)

Subcellular structures	^{3}H-CF, cpm/mg protein
Nuclei	2670±125
Nuclear matrix	10154±498
Mitochondria	944±101
Microsomes	2221±213
Cytosol	1906±201

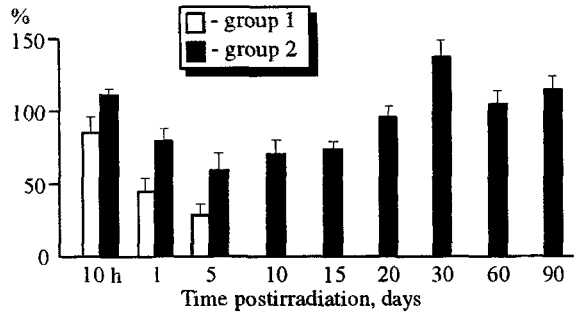


Fig. 3. RNase activity in isolated rat liver nuclei. RNase activity in intact rats 3.6 A_{260}/h×mg protein is taken as 100%.

well-defined ultrastructures: nucleoli, chromatin network, nuclear membrane with nuclear pore complexes, and inter- and perichromatin granules. These findings suggest that CF exhibits pronounced radioprotective activity and accelerates reparation processes.

Single γ-irradiation in a dose of 8 Gy (group 1) inhibits enzymes associated with the nuclear membrane, in particular, Mg^{2+}-dependent ATPase and RNase. One and 5 days postirradiation, RNase activity decreased by 45 and 56%, respectively (Fig. 3). In group 2 rats, RNase activity was considerably less suppressed (by 24 and 22% on days 15 and 20, respectively) and returned to normal 30 days postirradiation (Fig. 3).

One hour after whole-body γ-irradiation in a dose of 8 Gy, Mg^{2+}-dependent ATPase activity in the liver nuclei decreased by 45%, while 24 h postirradiation 66% of enzyme activity was inhibited. In CF-treated rats, ATPase activity 1 and 24 h postirradiation decreased by 35 and 40%, respectively. On day 5 and 10 after exposure, enzyme activity was inhibited by 27 and 25%, respectively, and returned to normal 60-90 days postirradiation.

Irradiation considerable activate LPO in cell nuclei: the content of dienes 1 and 24 h postirradiation increased 2.8- and 2.6-fold, respectively. On day 5, this parameter 3.1-fold surpassed the normal.

In CF-injected rats, maximum increase in the diene content (30%) was noted on day 5 postirradiation. On day 20, parameters of lipid peroxidation returned to normal.

Thus, CF from cow colostrum in a dose of 1 mg/g body weight injected 30 min before and for 4 days after whole-body γ-irradiation (8 Gy) possesses pronounced radioprotective activity, increases survival rate and promotes recovery of the nuclear structure and function.

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