RADIOPROTECTIVE ACTION OF THE SYNTHETIC GLUCOCORTICOID ANTAGONIST RU 38 486

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UDC 615.357:577.175.53].017:615.849.1.015.25].07

KEY WORDS: irradiation; RU 38 486; programmed cell death

Death of lymphoid cells under the influence of radiation and glucocorticoid hormones, and also in the process of specific T-cell killing is an example of programmed cell death [6]. We know that it is interphase death of erythroblasts, lymphoblasts, small lymphocytes, and thymocytes that largely determines the specific character of radiation sickness in mammals [6]. Accordingly, in recent years special attention has been paid to the study of the effect of ionizing radiation on lymphoid cells.

Progress in modern molecular radiobiology has led to a closer understanding of the biological mechanisms of irradiation-induced interphase cell death. Convincing proof has now been obtained of the existence in lymphoid cells of a genetic program of death, which can be triggered by various lympholytic agents. Immediately after exposure to ionizing radiation and glucocorticoids, silent or weakly expressed genes in the cells become activated. This is followed by induction of RNA-polymerase II activity [7], and synthesis and phosphorylation of specific proteins [14, 15]. The final stage of development of the program is connected with activation of nucleases, leading to internucleosomal degradation of chromatin and death of the cells. So far, however, the "messenger," triggering operation of the death genes, is still unknown. Meanwhile we do know that during glucocorticoid-induced death of lymphoid cells the role of such a "messenger" is performed by cytoplasmic receptors of glucocorticoid hormones. It was shown previously in the writer's laboratory that thymocytes can be protected against the action of ionizing radiation with the aid of the hydrocortisone antagonist, progesterone [1]. We therefore decided to study the effect of the synthetic antiglucocorticoid RU 38 486 on development of radiation-induced death of lymphoid cells.

EXPERIMENTAL METHOD

The antiglucocorticoid RU 38 486 was generously provided by D. Martini ("Roussel-Uclaf," France). Noninbred male albino mice weighing 20-25 g and rats weighing 110-120 g were used. RU 38 486 was injected intraperitoneally into the animals 1 h before irradiation by ^{137}Cs γ-rays in a dose of 6.5 Gy on the "Igur-1" apparatus (dose rate 1.56 Gy/min). The cell suspension was subjected to x-ray irradiation in a dose of 4 Gy on the RUM-1 apparatus under the following conditions: focal distance 30 cm, tube voltage 200 kV, current 15 mA, filters 0.5 mm Cu + 1.0 mm Al, dose rate 0.316 Gy/sec. Irradiation was carried out in open vessels (height of the layer of suspension 1 cm) under conditions guaranteeing maximal backscatter. Thymocytes were isolated in medium 199 at 20°C and the cells were preincubated in the presence of $5 \cdot 10^{-6}$ M RU 38 486 for 1 h at 25°C. Further incubation of the cells was carried out in a gas-flow thermostat (5% CO_2 + 95% O_2) at 37°C for 6 h. The survival rate of the cells was determined on the basis of inability to take up trypan blue. Thymocyte nuclei were isolated by the method in [3].

Central Roentgeno-Radiologic Research Institute, Ministry of Health of Russia. Academician N. N. Petrov Research Institute of Oncology, Ministry of Health of Russia, St. Petersburg. Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 114, No. 7, pp. 70-73, July, 1992. Original article submitted November 15, 1991.

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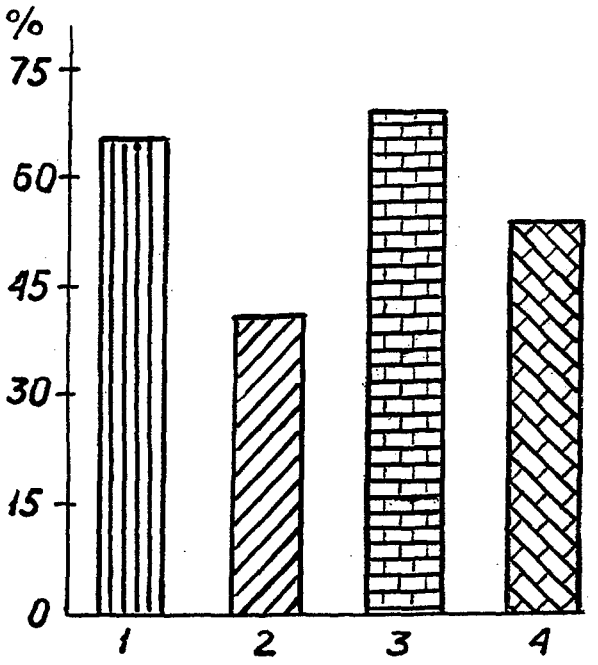

Fig. 1. Effect of RU 38 486 on survival of irradiated rat thymocytes: 1) control, 2) irradiation, 3) preincubation with RU 38 486, 4) preincubation with RU 38 486 + irradiation. Ordinate, number of surviving cells (in %).

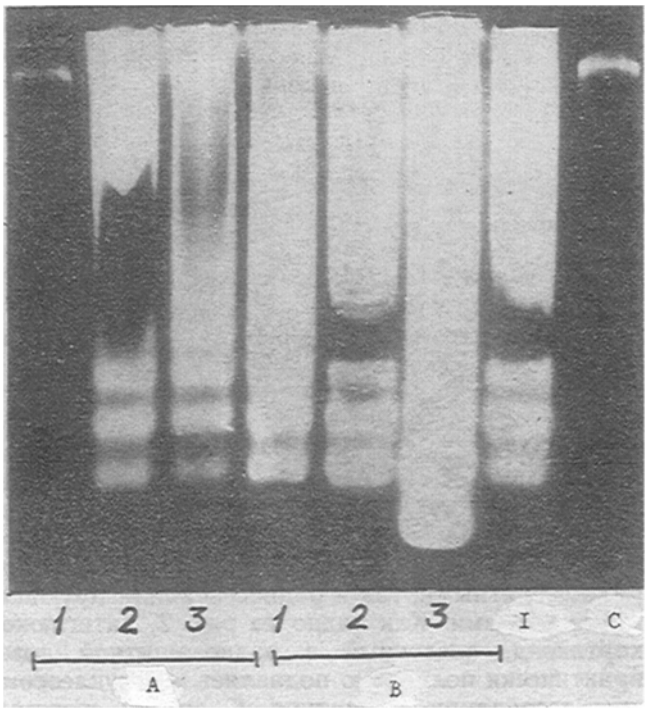

Fig. 2. Effect of RU 38 486 on formation of postradiation breakdown products of chromatin. Antiglucocorticoid injected in dose of 3.125 (A) and 6.5 (B) mg/kg body weight; 1, 2, and 3) time (in h) after injection of RU 38 486. C) Control, I) irradiation.

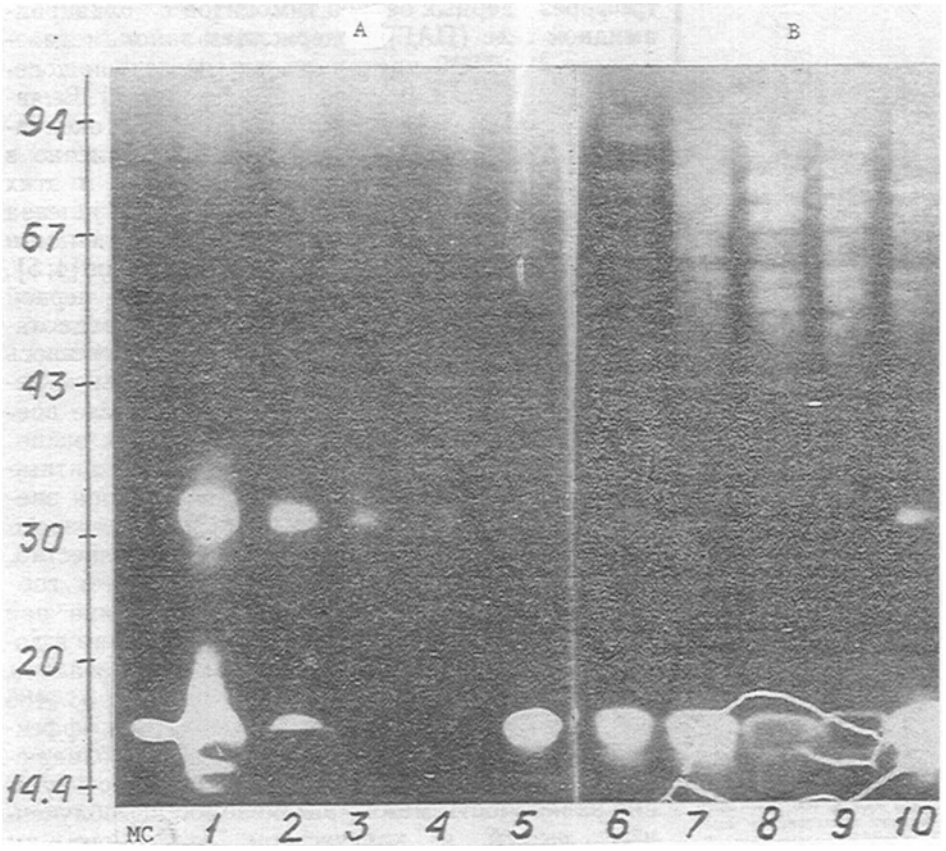

Fig. 3. Effect of RU 38 486 on activity of nuclear nucleases of rat thymocytes. A) Irradiation, B) RU 38 486 + irradiation. (1-4) – Proteins isolated 1, 2, 3, and 4 h after irradiation; (6-9) – proteins isolated 1, 2, 3, and 4 h after administration of RU 38 486 and irradiation. 5, 10) Control. MC) Micrococcal nuclease (10 pg).

Polydeoxyribonucleotide (PDN) was extracted from the nuclei with 0.7 mM EDTA, pH 8.0. DNA was estimated quantitatively by the method in [9]. Extracts of thymocyte nuclei were obtained as described in [4, 5]. Electrophoretic separation of PDN in the presence of sodium sarcosylate was carried out as in [11]. Thymocyte nuclear proteins were subjected to electrophoresis in polyacrylamide gel (PAG) containing polymerized ^{3}H-DNA, in the presence of SDS, as in [13]. Nuclease activities in the gel were determined at the end of electrophoresis as described in [4, 5]. Micrococcal nuclease (10 pg) served as the positive control in these experiments. Nuclease activity was assayed quantitatively as described in [4, 5].

EXPERIMENTAL RESULTS

In the first series of experiments to study the radioprotective properties of the antiglucocorticoid, the survival rate of mice irradiated with the minimal lethal dose (6.5 Gy) of ^{137}Cs γ-rays after receiving RU 38 486 was investigated. Preliminary experiments showed that the radioprotective properties of RU 38 486 are exhibited only if given to animals 1 h before irradiation (data not given). We know that this antiglucocorticoid blocks hormone-induced degradation of chromatin if given to animals by intraperitoneal injection in a dose of 12.5 mg/kg body weight [10]. It was found, however, that RU 38 486, if injected into mice in this dose, had no marked radioprotective action. To determine the dose of the preparation giving a radioprotective effect, the survival rate of irradiated mice was studied as a function of the quantity of RU 38 486 injected. RU 38 486 was found to exhibit radioprotective properties when injected into animals in a dose of 3.125 mg/kg body weight 1 h before irradiation, and under these circumstances 64% of the mice survived.

In the next series of experiments the effect of RU 38 486 was studied on survival of rat thymocytes in vitro. The results showed that this compound increases survival of both irradiated cells and nonirradiated but preincubated cells (Fig. 1).

The leading biochemical manifestation of interphase cell death is internucleosomal degradation of chromatin. We therefore decided to study the effect of RU 38 486 on the process of lympholytic degradation of chromatin. For this purpose the antiglucocorticoid was given to animals in different doses and at different times before irradiation. The results showed that RU 38 486, depending on its dose, possesses both radioprotective and radio-sensitizing properties. As will be clear from Fig. 2, the antiglucocorticoid, if injected in a radioprotective dose, suppressed internucleosomal degradation of chromatin virtually completely. On the other hand, if given to animals in a dose of 6.25 mg/kg body weight 3 h before irradiation, RU 38 486 doubled the intensity of chromatin degradation. Moreover, among the degradation products there was a DNA fragment smaller than nucleosomal DNA. This bidirectional dose-dependent effect of RU 38 486, synthesized as an abortifacient, was noted by its discoverer [12]. This compound, if administered under chronic conditions, had no antiovulatory effect, but increased the serum lactate dehydrogenase and progesterone levels and increased the mass of the ovaries.

In the study of interphase death of lymphoid cells particular attention is paid to the study of endogenous enzymes involved in orderly degradation of chromatin. By SDS-electrophoresis in PAG containing uniformly labeled high-molecular-weight DNA, several polypeptides possessing DNase activity were identified in rat thymocyte nuclei [4, 5]. It was shown that 1 h after lympholytic action (irradiation or dexamethasone) activity of enzymes with mol. wt. of 32, 17.7, 17.2, and 16.4 kD was stimulated. In our subsequent experiments we therefore studied the effect of RU 38 486 on activity of these nucleases. It was found (Fig. 3) that the antiglucocorticoid prevents post-radiation activation of the above-mentioned enzymes.

Hence, as a result of these experiments to study the effect of the antiglucocorticoid RU 38 486 on radiation-induced death of thymocytes it was found that the compound possesses radioprotective properties both in vivo and in vitro, and also prevents postradiation activation of nucleolytic enzymes with, as a result, the prevention of internucleosomal degradation of chromatin.

The antiglucocorticoid properties of RU 38 486 are based on its ability to compete effectively with glucocorticoids for binding sites with their receptors. The antihormone forms a stable complex with receptors and prevents transformation of the receptor, keeping it in a high-molecular-weight form [7]. It has been shown that similarity exists, in principle, in the development of events in lymphoid cells in response to both irradiation and glucocorticoids. It can therefore be postulated that hormonal receptors of the cytosol may be involved in the realization of processes of interphase death of lymphoid cells [1, 2]. The results of the present investigation provide further proof of the existence of a single program for death of lymphoid cells.

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