Radiobiological Effects of Olipifat during Radiation Exposure

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Preventive injections of olipifat to rats (intramuscularly in a single dose of 250 mg/kg 24 h before γ-exposure in a dose of 7.6 Gy) decrease animal mortality from 70 to 50%, the mean life span of dead rats remained unchanged. Injection of olipifat before exposure in a dose of 5.2 Gy did not change animal mortality in comparison with irradiated controls, but stimulated postradiation recovery of leukocyte count (mainly the granulocytic component and less so the increase in lymphocyte count). No appreciable effects of olipifat on the postradiation changes in individual fractions of blood leukocytes were observed in animals exposed in a dose of 7.6 Gy.

Key Words: *olipifat; radiobiological effects*

Olipifat is a lignin derivative exhibiting antitumor activity in experiments [3]. Pronounced antioxidant and antiradical effects [1] were detected, but we failed to find reports about radiobiological effects of olipifat.

Our preliminary studies showed that olipifat (50 or 100 mg/kg intramuscularly 24 h before γ-exposure) possessed certain radioprotective activity only if used preventively in exposed rats; no therapeutic effect was found. Injection of olipifat 24 h before exposure in a dose of 4.75 Gy modified the effect of radiation and stimulates postradiation recovery of leukocyte count. Olipifat induced virtually no appreciable modification of radiation injury after exposure in doses of 7.65 or 9.50 Gy. However, antiradical activity [1] implies a radioprotective effect, presumably with higher concentrations of the drugs in radiation injury of different degree.

We evaluated the efficiency of preventive injection of olipifat in the maximum nontoxic dose in nonlethal and medium lethal γ-exposure.

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MATERIALS AND METHODS

The study was carried out on male rats (160-180 g) from Breeding Center of Central Roentgenoradiological Institute. The animals were divided into 6 groups: 1) intact control (n=8); 2) 250 mg/kg olipifat 24 h before irradiation (n=4); 3) exposure in a dose of 5.2 Gy (n=10); 4) 250 mg/kg olipifat 24 h before γ-exposure in a dose of 5.2 Gy (n=10); 5) irradiation in a dose of 7.6 Gy (n=10); and 6) 250 mg/kg olipifat 24 h before γ-irradiation in a dose of 7.6 Gy (n=10).

Single whole-body γ-irradiation in doses of 5.2 or 7.6 Gy was carried out on an IGUR-1 device (^{137}Cs) at a dose power of 0.33 Gy/min. The drug was injected intramuscularly. Changes in the total and differential count of blood leukocytes and animal survival 30 days after irradiation were recorded. Hematological and biochemical values were measured in live animals; the blood was collected from the caudal vein 1 and 30 days after irradiation.

Changes in the blood leukocyte DNA content was expressed as the index of leukocyte DNA (ID) estimated as the ratio of DNA concentration (μg/ml) to leukocyte count/ml blood. DNA content in samples was evaluated using 4',6-diamidino-2-phenylindole

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(DAPI; Serva) fluorescent stain in a final concentration of 0.1 μg/ml after lysis of blood samples under conditions [2]. Peripheral blood leukocyte ID in intact rats corresponded to diploid content of DNA in white blood cells. ID above diploid value attested to elevated count of aneuploid cells in the sample; ID below diploid value indicated increased count of apoptotic cells. These assumptions were verified by the analysis of blood samples by the above method in parallel with flow cytometry (the cytometer was graciously offered by Prof. A. S. Yagunov, Central Roentgenoradiological Institute). Fluorescence was measured on a Hitachi Model-850 fluorescent spectrophotometer. Excitation and emission wavelengths for DAPI were 350 and 450 nm, respectively.

RESULTS

A significant reduction of the total count of blood leukocytes (Table 1) and a trend to a decrease in ID in comparison with intact control were observed early (24 h) after irradiation in doses of 5.2 or 7.6 Gy (irradiated controls). These results of γ-exposure were explained by postradiation interphase apoptotic death of some leukocytes.

Injection of olipifat to animals exposed to different doses did not change the total count of leukocytes in comparison with irradiated controls, while ID decreased significantly after γ-exposure in a dose of 7.6 Gy.

Injection of olipifat without irradiation did not change the total leukocyte count (Table 1); however, differential blood count in these animals showed increased count of blood granulocytes mainly at the expense of segmented neutrophils (Table 2). The decrease in the total leukocyte count in irradiated animals was usually at the expense of lymphocytes and to a lesser extent at the expense of monocytes and eosinophils (Table 2).

Injection of the drug before γ-exposure in a dose of 7.6 Gy did not lead to appreciable changes in differential leukocyte count in comparison with the respective exposed control (Table 2). On the other hand, injection of the drug before exposure in a dose of 5.2 Gy significantly increased the count of segmented granulocytes in comparison with intact and irradiated controls. This probably attests to more potent radiomodifying effect of olipifat with lower exposure dose.

Evaluation of postradiation survival of animals 30 days after γ-exposure showed that irradiation in a dose of 5.2 Gy did not cause rat death, while exposure in a dose of 7.6 Gy caused death of 70% animals. The mean life span of animals died after exposure to 7.6 Gy was 14.1±2.9 days, which corresponds to mortality values during the development of the bone marrow irradiation syndrome (days 6-25 [4]).

After preventive injection of olipifat mortality of animals exposed in a dose of 7.6 Gy was 50% (lower than in irradiated control). Injection of the drug did not change the mean life span of dead rats (14.6±2.9 days) in comparison with irradiated controls. The time of rat deaths after irradiation in a dose of 7.6 Gy corresponded to the mortality period in the bone marrow irradiation syndrome. This can indicate that the death of animals in groups 5 and 6 was due to bone marrow irradiation syndrome.

TABLE 1. Effect of Olipifat on Postradiation Changes in Leukocyte Count and DNA Content in Blood Leukocytes of Rats (*M*±*m*)

Group	24 h after irradiation		30 days after irradiation	
	total leukocyte count, $\times 10^9$ cells/liter	ID, pg/cell	total leukocyte count, $\times 10^9$ cells/liter	ID, pg/cell
Intact control	10.2±0.9 (*n*=8)	7.14±0.28 (*n*=8)	14.9±0.5 (*n*=8)	7.14±0.60 (*n*=8)
Olipifat (250 mg/kg)	11.9±1.4 (*n*=4)	7.25±0.80 (*n*=4)	17.6±4.5 (*n*=4)	9.36±0.27** (*n*=4)
Irradiation (5.2 Gy)	4.2±0.7** (*n*=10)	6.51±0.91 (*n*=10)	11.8±1.2* (*n*=10)	6.27±0.70 (*n*=10)
Olipifat (250 mg/kg) +irradiation (5.2 Gy)	5.4±0.5** (*n*=10)	6.40±0.46 (*n*=10)	15.9±1.4$^+$ (*n*=10)	9.52±0.60**$^+$ (*n*=10)
Irradiation (7.6 Gy)	4.0±0.6** (*n*=10)	5.83±0.74 (*n*=10)	21.3±8.9 (*n*=3)	9.30±0.86 (*n*=3)
Olipifat (250 mg/kg) +irradiation (7.6 Gy)	3.8±0.5** (*n*=10)	4.86±0.80* (*n*=10)	14.0±3.8 (*n*=5)	9.35±1.94 (*n*=5)

Note. Here and in Tables 2, 3: *n*: number of animals. *$p<0.05$, **$p<0.01$ compared to intact control; $^+p<0.05$ compared to irradiated control.

No appreciable changes in the total count of leukocytes or individual fractions of white blood cells were detected 30 days after injection of olipifat to rats, while ID increased (Tables 1, 3).

The total count of blood leukocytes was not completely restored in experimental animals 30 days after exposure in a dose of 5.2 Gy, while after 7.6 Gy exposure the total count of blood leukocytes in surviving rats reached the level of intact controls (Table 1). ID in animals exposed in nonlethal dose and in $MD_{70/30}$ did not change significantly in comparison with the intact control. Injection of olipifat to animals irradiated in a dose of 5.2 Gy increased ID in comparison with intact and irradiated controls (Table 1). Injection of olipifat did not change significantly ID in rats exposed to medium-lethal γ-irradiation in comparison with irradiated control (Table 1). Compared to the early effects (24 h after exposure), these results can indicate olipifat-mediated initiation of aneuploidy in blood leukocytes.

Differential blood count 30 days after exposure in doses of 5.2 and 7.6 Gy showed decreased lymphocyte count in comparison with intact controls (Table 3). The most active recovery was observed for the lymphocyte fraction: ~10-fold increase in cell count and appreciable increase in monocyte and eosinophil counts (Tables 2, 3).

Injection of olipifat before irradiation in a dose of 5.2 Gy appreciably increased granulocyte count on day 30 after γ-exposure (mainly at the expense of segmented neutrophils) (Table 3). Olipifat injection before exposure in a higher dose (7.6 Gy) had just a normalizing effect: lymphocyte count changed and the count of eosinophils somewhat decreased (Table 3). This indicated a hemostimulating effect of olipifat mainly in mild radiation injury.

Our results are in line with the data on antiradical effects of olipifat [1], which can exhibit a radioprotective effect. On the other hand, this drug in the maximum nontoxic concentration (250 mg/kg) exhibited a low dose modification factor, judging from the survival criterion (1.12). We previously showed that the drug in concentrations of 50 and 100 mg/kg did not change survival of irradiated rats. Olipifat is not so effective radioprotector as well-known cistafos, mexamine, aminoethylisothiuronium with dose modification factors 1.50-1.55 [4]. On the other hand, the drug can be active in less severe radiation injuries. Due to the absence of pronounced toxicity, olipifat presumably can be used in highly prevalent combined radiation and chemical exposure (in low doses), which take

TABLE 2. Effect of Olipifat on Changes in Individual Fractions of Blood Leukocytes ($\times 10^9$ Cells/Liter) 24 h after Irradiation ($M \pm m$)

Group	Lymphocytes	Monocytes	Granulocytes (total)	Stab neutrophils	Segmented neutrophils	Eosinophils
Intact control (n=8)	7.64±0.66	0.19±0.06	2.50±0.32	0	1.94±0.19	0.52±0.12
Olipifat, 250 mg/kg (n=4)	7.80±1.31	0.12±0.05	4.03±0.38*	0.07±0.04	3.19±0.32**	0.74±0.16
Irradiation, 5.2 Gy (n=10)	0.64±0.07**	0.04±0.02*	2.59±0.39	0.03±0.01	2.46±0.37	0.10±0.02**
Olipifat, 250 mg/kg+ irradiation, 5.2 Gy (n=10)	0.63±0.09**	0.06±0.02*	4.72±0.55**+	0.02±0.01	4.62±0.55**+	0.07±0.03**
Irradiation, 7.6 Gy (n=10)	0.38±0.06**	0.02±0.01*	3.62±0.61	0.06±0.04	3.55±0.61*	0.04±0.01**
Olipifat, 250 mg/kg+ irradiation, 7.6 Gy (n=10)	0.51±0.11**	0.010±0.006**	3.32±0.59	0.10±0.05	3.17±0.59	0.06±0.02**

TABLE 3. Effect of Olipifat on Changes in Individual Fractions of Blood Leukocytes ($\times 10^9$ Cells/Liter) 30 Days after Irradiation ($M \pm m$)

Group	Lymphocytes	Monocytes	Granulocytes (total)	Stab neutrophils	Segmented neutrophils	Eosinophils
Intact control (n=8)	9.76±0.42	0.77±0.15	4.37±0.26	0.06±0.03	3.65±0.20	0.61±0.13
Olipifat, 250 mg/kg (n=4)	10.87±2.97	0.95±0.36	5.81±1.79	0	5.23±1.74	0.44±0.05
Irradiation, 5.2 Gy (n=10)	4.99±0.65**	0.62±0.11	5.74±0.93	0.25±0.10	4.51±0.77	0.92±0.18
Olipifat, 250 mg/kg+ irradiation, 5.2 Gy (n=10)	6.43±0.91**	0.89±0.25	8.74±0.93**+	0.07±0.03	7.52±0.70**+	1.05±0.28
Irradiation, 7.6 Gy (n=10)	5.99±0.06*	0.88±0.61	9.77±4.32	0.28±0.11	8.36±4.14	1.08±0.23**
Olipifat, 250 mg/kg+ irradiation, 7.6 Gy (n=10)	6.91±1.57	1.38±0.34	4.52±1.41	0.27±0.07*	3.76±1.13	0.67±0.24

place in ecologically hazardous regions contaminated with radioactive cesium as a result of Chernobyl accident in our country and in foreign countries, but the use of olipifat under these conditions requires special studies.

Preventive injection of olipifat to rats promoted the development of aneuploidy in leukocytes as a result of postradiation recovery after irradiation in nonlethal doses and induces apoptosis of blood leukocytes during early periods after medium-lethal radiation injury, reducing animal mortality from 70 to 50%.

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