Effect of Radioprotector Indralin on the Course of Acute GVH Disease

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The effect of radioprotector indralin on the graft-versus-host reaction was studied on the model of acute GVH disease induced in mice by intraperitoneal transplantation of 40×10^6 semiallogenic splenocytes. The effect was evaluated by animal mortality from GVH disease. Recipients were male F_1(CBA×C57Bl/6) mice exposed to 7 Gy 24 h before transplantation. Donors were male C57Bl/6 mice. Indralin, intraperitoneally injected in a dose of 100 mg/kg 5 min after irradiation attenuated the severity of GVH disease. It eliminated phase I of acute GVH reaction and shifted to the right the dynamics of mortality. Estimated time of 50% mortality (LT_{50}) was prolonged by more than 4 days (the parameter increased by 31.1%). Two (5.7%) animals recovered from acute GVH disease, while all controls died. Indralin treatment after irradiation resulted in a 30% increase in survival of exposed mice.

Key Words: *radioprotector; indralin; GVH disease; spleen; ionizing radiation*

The effects of radioprotectors on GVH disease, a severe complication of bone marrow transplantation in patients with radiation disease, are little studied. The graft-versus-host (GVH) reaction underlies the pathogenesis of this disease. This reaction can be enhanced or attenuated by immunomodulators [3,9,12].

We studied the effect of indralin, an urgent radioprotector [4], on the course of acute GVH disease after pre-exposure of the recipient. As the role of the adrenergic system in the pathogenesis of GVH disease is not studied and as indralin is a direct α_1-adrenoceptor agonist [2], we evaluated the effect of stimulation of this system through α-adrenoreceptors on the manifestation of the acute form of the disease.

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MATERIALS AND METHODS

Experiments were carried out on male F_1(CBA× C57Bl/6) and C57Bl/6 mice. The animals were kept on standard fodder and drinking water. The mice were exposed to whole-body γ-irradiation in a dose of 7.0 Gy on a Chisotrone device (^{60}Co) at a dose power of 32.5-33.7 cGy/min. For exposure, the animals were placed into special polystyrene boxes, 10 per box, and exposed on one side at a distance of 38.5 cm from the γ-source.

Indralin (B-190 agent, Farmzashchita Center) was injected intraperitoneally (100 mg/kg; 2.5% solution) to recipient mice 5 min after irradiation. This regimen of radioprotector administration was selected in order to rule out appreciable attenuation of radiation injury after its preventive injection and retain approximately similar effect of ionizing radiation on the manifestation of acute GVH disease in control and experimental groups.

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The development of acute GVH disease in mice was induced by semiallogenic transplantation of 40×10^6 splenocytes of C57Bl/6 mice to F_1(CBA×C57Bl/6) animals pre-exposed (24 h before transplantation) in a dose of 7 Gy {C57Bl/6-F_1(CBA×C57Bl/6)}. Syngeneic transplantation of splenocytes: F_1(CBA×C57Bl/6)-F_1(CBA×C57Bl/6) served as the control. Donor splenocytes were obtained after careful homogenization of splenic tissue in a glass homogenizer in 5 ml Hanks' solution. After 10-min centrifugation of splenocyte suspension at 1*g* the supernatant was carefully removed with a pipette. A fresh portion of Hanks' solution (2 ml) was added to the precipitate on the bottom of the tube. Splenocytes from 5-6 mice were pooled, cell concentration was evaluated in a Goryaev chamber, adjusted to needed concentration with Hank's solution, and injected intraperitoneally to recipient F_1(CBA×C57Bl/6) mice in a volume of 0.5 ml.

The severity of GVH disease and acute radiation disease were evaluated by body weight changes over the entire period of observation (60 days), by leukopenia, changes in the weight and histology of the spleen on day 11 after irradiation, by the dynamics of animal mortality, and by the mean life span (MLS) of animals and time of 50% mortality (LT_{50}), which was estimated by the probit analysis after Litchfield and Wilcoxon. The spleen for pathomorphological examination was fixed in Bouin fixative for 24 h, embedded in paraffin, histological sections were prepared, stained with hematoxylin and eosin, and examined under a Biomed-6 microscope (×4 eyepiece).

The significance of the results was evaluated by the exact Fisher test, Mann—Whitney *U* test, by Cox—Mantel test (analysis of survival curve by the Kaplan—Mayer method), and by two-way dispersion analysis.

RESULTS

Acute and chronic forms of induced GVH disease in animals are known. Two phases of acute GVH disease in mice are distinguished: early (up to 7 days after bone marrow transplantation; BMT) and the main phase, starting from day 12 after BMT [13]. The presence of two phases is confirmed by two peaks of T lymphocyte activation in the spleen recorded by expression of IL-2 mRNA emerging 4 days before clinical manifestation of GVH disease. Phase I of GVH disease is presumably a response to activation of the host antigen-presenting cells (APC) to donor cell alloantigen associated with the release of cytokines activating donor T-toxic lymphocytes with realization of their damaging effect on the body. The main pathogenetic mechanism of

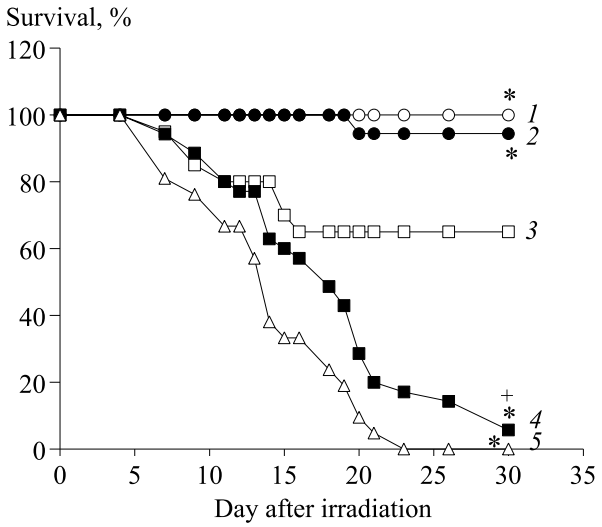

Fig. 1. Mortality of F_1(CBA×C57Bl/6) mice after γ-irradiation in a dose of 7 Gy and semiallogenic and syngeneic transplantation of splenic cells after treatment with B-190 agent after γ-irradiation (Kaplan—Mayer method). Here and in Fig. 2: *1*) syngeneic transplantation; *2*) indralin after irradiation; *3*) irradiation control; *4*) indralin after irradiation+allogenic transplantation; *5*) allogenic transplantation. $p<0.01$ compared to *irradiation control (Cox—Mantel test), +allogenic transplantation control (Cox—Mantel test).

GVH disease is realized later during multiplication of donor APC [13].

The effects of B-190 agent on the course of acute GVH disease in male F_1(CBA×C57Bl/6) mice induced by cytotoxic activity of transplanted allogenic T lymphocytes from C57Bl/6 donors were studied (Fig. 1; Table 1). Semiallogenic transplantation of 40×10^6 splenocytes from C57Bl/6 mice 24 h after γ-irradiation of recipient F_1(CBA×

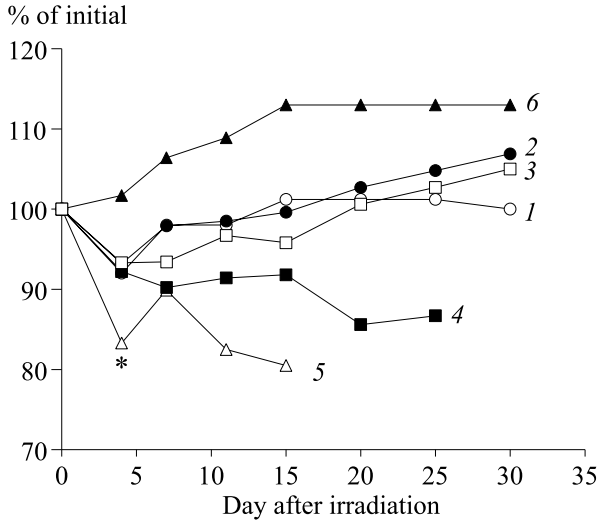

Fig. 2. Changes in body weight of mice after γ-irradiation (7 Gy) after semiallogenic and syngeneic transplantation of splenocytes and treatment by B-190 agent after irradiation. *6*) biological control. *$p<0.05$ compared to indralin+allogenic transplantation group (Duncan test).

TABLE 1. Anti-Radiation Effects of Indralin Radioprotector and Its Effect on the Outcome of GVH Disease in Recipient Mice after γ-Exposure in a Dose of 7 Gy

Group	Drug dose, mg/kg	Number of mice	Survival 30 days after irradiation, %	MLS, days
Irradiation control	—	20	65.0	11.7±1.4
Syngeneic transplantation of 8-40×10^6 splenocytes 24 h after irradiation	—	20	100.0*	—
Semiallogenic transplantation of 40×10^6 splenocytes of C57Bl/6 mice 24 h after transplantation	—	21	0*	14.0±1.1
Indralin 5 min after exposure	100	26	96.1*	20.0
Indralin 5 min after exposure+syngeneic transplantation of 8×10^6 splenocytes 24 h after irradiation	100	10	100.0	—
Indralin 5 min after exposure+syngeneic transplantation of 40×10^6 splenocytes of C57Bl/6 mice 24 h after irradiation		35	5.7	17.2±1.0+

Note. $p<0.05$ compared to control group: *exact Fisher test, +Mann—Whitney U test.

C57Bl/6) mice in a dose of 7 Gy causing 30% mortality of mice over 30 days of observation ($LD_{30/30}$) led to 100% animal mortality within 22 days after transplantation (Fig. 1) with LT_{50}=13.2 (11.1±15.7) days. Two phases can be distinguished by the dynamics of body weight loss in mice with acute GVH disease: phase I during the first 4 days and phase II starting from day 10 after splenocyte transplantation. Pathomorphological study of splenic tissue on day 11 of the experiment showed pronounced lymphoid infiltration of the splenic parenchyma characteristic of GVH reaction. The clinical picture of acute GVH disease in mice was characterized by pronounced exhaustion with more manifest body weight loss on day 4 after irradiation, than in irradiated controls (Fig. 2).

TABLE 2. Anti-Radiation Efficiency of Indralin Radioprotector Used Early after Irradiation and Its Effects on the Manifestation of GVH Reaction, Evaluated by Changes in Spleen Weight, Number of Splenic Colonies, and Leukocyte Level after Irradiation in a Dose of 7 Gy

Group	Period after irradiation	Spleen weight, mg		Leukocyte count on day 30, thousands/μl blood	Number of splenic colonies on day 11
		day 11	day 30		
Biological control	—	86.9±3.8 (7)	86.6±6.2 (8)	10.1±1.1 (8)	0 (7)
Irradiation control (7 Gy)	—	34.7±3.7* (9)	87.0±2.4 (7)	4.3±0.4* (7)	0.33±0.16 (9)
B-190 agent (100 mg/kg intraperitoneally)	After 10 min	42.0±4.6* (8)	112.8±7.5* (8)	7.2±0.7+ (8)	2.8±0.65+ (8)
Syngeneic transplantation of 8×10^6 splenocytes per mouse	After 24 h	42.1±3.2* (9)	92.2±4.1 (11)	6.6±0.8+ (11)	7.8±1.2+ (9)
Semiallogenic transplantation of 40×10^6 splenocytes of C57Bl/6 mice	After 24 h	44.0±7.2* (8)	Death	Death	0 (8)
B-190 agent (100 mg/kg intraperitoneally)+ semiallogenic transplantation of 40×10^6 splenocytes of C57Bl/6 mice	After 10 min+ after 24 h	55.8±4.0+ (7)	Death	Death	0.70±0.33 (7)

Note. The number of mice in experimental group is shown in parentheses. $p<0.05$ compared to: *biological control, +irradiation control group.

Injection of indralin after irradiation completely abolished body weight drop characteristic of GVH disease phase I on day 3 after allogenic transplantation of splenocytes (Fig. 2). Moreover, indralin alleviated the course of phase II of acute GVH disease and delayed its manifestation, which led to a significant shift of mouse mortality curve from acute GVH disease to the right; LT_{50} increased to 17.3 days (14.7±20.4, by 4.1 days or by 31.1% compared to the control, Fig. 1). Moreover, some mice receiving indralin survived for 60 days after allogenic transplantation of splenocytes, whereas 100% controls died (Table 1).

Indralin and syngeneic transplantation alone and in combination exhibited a characteristic positive effect on the course of acute radiation disease, leading to complete recovery of animals exposed in a dose of 7 Gy ($LD_{30/30}$). Pronounced growth of endogenous colonies in the spleen was noted on day 11 after exposure and higher leukocyte count was recorded 30 days after irradiation (Tables 1, 2). These results confirm that in addition to pronounced positive effect of preventive use before irradiation [4], indralin (radioprotector) used early after irradiation, exhibits a therapeutic effect improving by 30% the survival of mice with acute radiation disease. Indralin exhibits similar effect in cases with carboplatinum hemotoxicity [1]. By its pharmacodynamics, the effect did not exceed 30 min after exposure to the damaging factor, but the response of the hemopoietic system to indralin (stimulation of ribonucleotide reductase, involved in DNA synthesis in hemopoietic cells) is observed over 3 days [5].

The mechanism of favorable effect of indralin on acute GVH disease is unclear. It is known that acute GVH disease is augmented by proinflammatory cytokines in case of predominating T_1-immune response, primarily under the effects of TNF-α and IL-12 [7,11, 14]. It was found that the inflammatory reaction in tissues was essential for migration of donor allogenic T cells into recipient nonlymphoid tissues (skin, intestine, liver) [6]. Damage to these tissues in case of their infiltration with allogenic cytotoxic lymphocytes eventuates in the development of GVH disease [15].

The data on the involvement of α_1-adrenoreceptors in immunity regulation are scanty, and no data for T_1 immunity were reported [8]. Since indralin completely eliminates phase I of acute GVH disease developing 3 days after allogenic transplantation and stimulates hemopoiesis by stimulating endogenous colony growth in the spleen after irradiation, it seems that cytokines involved in this process (granulocyte-macrophage (GM-CSF) and granulocyte (G-CSF) colony stimulating factors, *etc.*) can modify the course of acute GVH disease, partially blocking its development. This possibility was proved in previous experiments [10] demonstrating G-CSF suppression of TNF-α_1 production by donor allogenic lymphocytes, which reduced the severity of acute GVH disease.

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