Therapy of combined radiation injuries with hemopoietic growth factors

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Abstract.

Radiation accidents of the 5-7th levels according to IAEA scale lead to life-threatening acute radiation syndrome and many patients will probably suffer from additional thermal burns. These combined injuries (CI) will be among the most difficult to achieve survival. Present therapeutic means need to augment with new approaches to stimulate host defence mechanisms, blood system recovery and to enhance survival. The evaluation of therapeutic properties of human recombinant G-CSF, IL-1, IL-2 and other so called "biological response modifiers" on survival and blood recovery after CI was the purpose of this work. Experiments carried out with mice CBA x C57BL6 receiving 7 Gy total body irradiation followed by a full-thickness thermal burn of 10% of body surface. It established that G-CSF does not exhibit a positive modifying action on the damage level and on hematopoietic recovery. I.p two-four/fold infusion of IL-2 during the initial 2 days has provided a significant statistically survival increase from 40% (untreated mice with CI) to 86%. Single s.c IL-1 injection resulted in abrupt deterioration of the outcome when dealing with CI; three/fold administration of IL-1 in 2,4 and 6 days after CI did not increase survival. Extracellular yeast polysaccharides resulted only a 15 to 30% increase in survival if given 1 h after CI. The best results obtained when mixture of heat-killed L.acidophilus injected s.c immediately after CI - survival has increased from 27% (untreated mice) to 80%. Revealed beneficial effects of IL-2 and biological response modifiers did not accompany by a corresponding correction of depressed hematological parameters.

1.Introduction.

It is well known that in the event of radiation accidents many patients will probably suffer from thermal burns in addition to acute radiation syndrome (ARS). The outcome of these combined injuries (CI) is worse than for ARS alone. Even benign by themselves thermal burns become hazardous when a combination of trauma occurs in conjunction with total body irradiation at minimal-lethal or midlethal dosages. Exposed patients with additional thermal burn die during the first two or three weeks after irradiation mainly due to sepsis. In spite of long standing study of CI pathogenesis, management of infectious complications after CI remain as very hard medical problem [1-4]. Early supportive therapies of patients who have been exposed to high doses of radiation and trauma include antimicrobial therapy, platelet transfusion, fluids, electrolytes, immunoglobulin therapy, and surgical debridement or cleansing of wounds [5]. Recently several authors have reported that so called "immunoregulatory biological response modifiers" (glucan, trehalose dicorynomycolate, heat-killed Lactobacillus casei, recombinant cytokines et al.) can enhance hematopoietic cell recovery and increase survival when given after gamma or fission neutron irradiation alone [6-10]. Published data proved that agents of this group may increase macrophage's activity and secretion of cytokine such as hemopoietic growth factors. Efficacy of this new

therapeutic approach for CI treatment did not study as a matter of fact. Madonna et al.[11] showed that injection of immunomodulator significantly augments resistance to infection and increases survival of irradiated mice. However, this treatment did not increase survival of mice with sepsis following irradiation and wound trauma. Recent work with mice in our laboratory showed that bacterial polysaccharide pyrogenal, thymus preparations, heterologic immunoglobulines don't modify in fact the low values of 30-day survival under CI. Single injection of prodigiozan, zymozan and some other yeast polysaccharides in 1 h after CI resulted at unimportant increase of survival [12-13]. The evaluation of therapeutic properties of human recombinant IL-1, IL-2, G-CSF and other biological response modifiers on survival and blood system state after CI was the purpose of this work.

2. The effectiveness of the hemopoiesis regulatory cytokines in CI models.

Recombinant IL-1-β was kindly supplied by Dr Ketlinski (Institute of High-Purity Biopreparation, St.-Petersburg, Russia). IL-2 (Biotech, St.-Petersburg, Russia) was a generous gift from Dr Jurkevich. G-CSF was supplied by Institute of Radiation Medicine (Chine).

CBA x C57BL6 male mice used in all studies. Animals held in quarantine for 2 weeks then irradiated from ^{60}Co source at a dose-rate 0.45 Gy/min. The midline absorbed dose was 7.0 Gy. Non-lethal per se full-sickness thermal burn 10% of body surface inflicted immediately after irradiation by means of powerful light of halogen lamps. This model of CI characterised by sharp decrease of 30-day survival in compare with only irradiated mice; untreated animals died mainly from sixth to twelfth days after CI.

The most attention we spared to therapeutic use of IL-1 in murine model of CI. It was taken into account that IL-1 act as an essential molecular master switch for secretion of GM-CSF, G-CSF, M-CSF, IL-3, IL-6 and other hemopoietic growth factors [14,15]. Several authors [16,17] have reported that IL-1 given 1-4 hour's postradiation (50-100-200 micrograms/kg, subcutaneously or intraperitoneally) increased survival of mice exposed to radiation alone. Our studies showed that rIL-1-β given once in 4 hr after CI (100 micrograms/kg, s.c.) caused a higher and earlier rate of mortality in 2-3 day after CI. In particular, 28 from 40 "treated" mice died instead of 100% survival untreated animals. Analogous results obtained when IL-1 dose reduced to 150 ng/mouse (40% of "treated" mice died at early phase of CI). Single s.c injection 150 ng IL-1 accelerated lethal outcomes of CI even followed 24 hr after CI. Repeated i.p. injection of smaller dose IL-1 (100 pg/mouse) in 2-4 and 6 days after CI did not influence on disease development and outcomes. It should be stressed that single administration of "high" dose IL-1 (100 micrograms/kg) and repeated small dose injection of this cytokine to only irradiated mice did not modify the early phase of ARS. Moreover slightly therapeutic effect took place and survival rate in 30-day period increased up to 15 or 30%. Aggravating effect of IL-1 on outcome of CI when injected in 4 or 24 hr are apparently connect with "burn component" of CI. Really, IL-1 administration in 4 h after burn alone resulted in 55% dearth rate during the first 2-4 days after non-lethal and non-shockogenic trauma per se. Possibility exist that at early phase of thermal burn or CI exogenous IL-1 interacts synergistically with endogenously produced TNF-α and IL-1-β. As result severe hypotension or other toxic effects of these cytokines may occur and potentially beneficial action of IL-1 on the radiosensitive systems (immunity and hemopoiesis) proves masked.

The role of interleukin-2 has been shown to be of great significance in the modulation of immune response and host resistance to sepsis in thermally injured mice [18]. On the other hand IL-2 may increase survival of irradiated mice and dogs [19]. These data lead us to investigate therapeutic properties of recombinant IL-2 for combined injury's treatment. Animals received intraperitoneal injections of rIL-2 in dose 5000 U/mouse. Three experimental schemes used: a) 4 injections in 15 min, 4, 24 and 48 hours after CI; b) 2 injections in 15 min and 4 hour after CI; c) 2 injections in 24 and 48 hours after CI. Results showed that IL-2 provides statistically significant survival increase from 40% (untreated control group) accordingly to 86%, 82% and 89%. Comparative study effects of "unstriking" cytokine IL-1 and "beneficial" cytokine IL-2 on hematology made too (Tabl. 1). Both cytokines did not correct severe cell devastation of bone marrow, leucopenia and anemia during the critical phase of CI. Only IL-2 administration increased E-CFU number per spleen and slightly raised blood platelets count.

Table 1

The influence of IL-1 and IL-2 treatment on mice blood system state after combined injury

Parameters	*CI + saline*	*CI + IL-1*	*CI + saline*	*CI + IL-2*
Number of bone marrow cells	2.25 ± 0.25	2.29 ± 0.36	2.00 ± 0.20	2.70 ± 0.46
Endogenous CFU/spleen	0.95 ± 0.18	1.95 ± 0.23	2.89 ± 0.74	6.40 ± 1.00
White blood cells	289 ± 44	329 ± 55	200 ± 28	183 ± 24
Granulocytes	50 ± 11	79 ± 29	45 ± 16	85 ± 22
Platelets	41 ± 4.4	51 ± 3.0	42 ± 2.2	79 ± 7.2
Erythrocytes	3.67 ± 0.12	3.95 ± 0.08	3.64 ± 0.10	3.67 ± 0.15

IL-1 injected in 2-4 and 6 days after CI. IL-2 injected in 15 min and 4 hour after CI. Blood system state registrated in 8 day after CI. Mean ± standard error of values presented. Bone marrow cells - x 10^6 per femur, erythrocyte - x 10^6 µl, platelets - x 10^3 µl.

Recombinant human G-CSF was investigated for the ability to accelerate bone marrow regeneration and to decrease the severity of leukopenia after irradiation only or CI (we thank O.Semina and T.Semenets for the participation in this experiments carry out). Mice were exposed to sublethal dose 4 Gy. G-CSF (2.5 micrograms/day) or saline administered on days 0-4 post-irradiation. Bone marrow cellularity, exogenous CFUs and white blood cell's number evaluated in 8 days after irradiation or CI. Results demonstrated that therapeutic G-CSF increased number of CFUs per femur from 640 ± 91 (untreated only irradiated mice) to 1030 ± 165. When animals exposed to CI this parameter changed insignificantly from 517 ± 71 (untreated mice with CI) to 541 ± 54. G-CSF did not strongly modify lowered bone marrow cellularity and leukocytes score in 8 day after irradiation or CI.

3. Therapeutic properties of new biological response modifiers in CI models.

Extra-cellular yeast polysaccharides of Bullera alba (B-678) and Sporobolomyces alborubescens (Sp-50) prepared by Prof. Elinov et al. (St.-Petersburg, Russia) and heat-killed Lactobacillus acidophilus prepared by Dr. Pospelova et al. (Moscow, Russia) have been investigated for CI treatment. Single i.p. injection of B-678 and Sp-50 (20 mg/kg) in 1 h after CI increased 30-day survival from 3% (untreated mice) accordingly to 23 and 20%. Heat-inactivated L.acidophilus (10^8 microbes per 1 ml growth media) injected s.c. following CI in volume of 0.1 ml/mouse. In this experimental group survival increased from 27% to 80%. None of the studied preparation rendered any beneficial action on the scores of bone marrow nucleated cells, white blood cells and CFUs as compared to control untreated groups (Tabl.2).

Therefore our studies demonstrated that short course of rIL-2 therapy or single subcutaneous injection of heat-killed L.acidophilus may increase survival of irradiated mice inflicted with thermal burn. Curative efficacy does not accompany by corresponding correction of depressed blood system state. Further research needs to establish the mechanisms of revealed beneficial effect on survival under combined injuries.

Table 2

Effects of biological response modifiers on some hematological parameters after CI

Experimental group	*Bone marrow cells, x 10^6*	*Endogenous CFU/spleen*	*Leukocytes*
1. CI + saline	1.10 ± 0.09	0.8 ± 0.33	292 ± 50
CI + B-678	1.27 ± 0.13	2.5 ± 0.94	345 ± 60
CI + Sp-50	1.44 ± 0.22	1.70 ± 0.39	291 ± 50
2. CI + saline	1.80 ± 0.21	2.24 ± 0.86	200 ± 41
CI + L.acidophilus	1.35 ± 0.20	1.14 ± 0.53	240 ± 49

Hematological parameters registrated in 8 day after CI. Mean ± standard error of values presented.

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