PREVENTIVE TREATMENT OF COMBINED RADIATION INJURIES

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INTRODUCTION

The risk of sepsis development increases when thermal burns and other trauma occur in conbination with exposure to radiation. Only surgical correction of the life-threatening state recommends within 48 hours after irradiation. All other arrangements have to carry out when hemopoiesis recovery will complete (1). However exposed patients with combined injuries (CI) die during the first two or three weeks mainly due to sepsis. Therefore prophylaxis and preventive therapy of infectious complications are need early. Actual difficulties in choice of valid treatment procedure for acute radiation syndrome (ARS) exhibit additional aggravation under CI. The available facts prove decreasing early therapy efficiency for rather high dose exposure and wound trauma occurrence (2,3). The own results showed that bacterial polysaccharide pyrogenal, glycopin (synthetic analogue of muramil-dipeptide), thymus preparations (thymozin, thymotropin, thymogen), tuftsin, heterologic human and bovine immunoglobulines did not modify the low values of 30-day survival under CI (irradiation + thermal burn). Single injection of prodigiozan, zymozan and some other yeast polysaccharides in 1 hr after CI resulted at moderate increasing of survival (4,5). The main purpose of this study, which bases upon our understanding of CI pathogenesis (6), was search more effective means for preventive treatment of combined radiation injuries. Two groups of remedies were under study. The first group included so called "biological response modifiers" (BRM). These agents may increase host defences to infection, macrophage's activity and hemopoietic growth factor's secretion (7). The second group included antibiotics that should be directed against the potential gram-negative as well as gram-positive pathogens and simultaneously be useful for selective decontamination of gastrointestinal tract (8).

MATERIALS AND METHODS

Experiments performed using CBAxC57BL6 male mice and Wistar rats. Animals irradiated with a ^{60}Co gamma source at 0.45 Gy/min. The midline absorbed dose was 7 Gy for mice and 7.5 Gy for rats. Non-lethal per se full-sickness thermal burn 10% of the total body surface for mice or 15% - for rats inflicted immediately after irradiation by means of powerful lamp's light. Both animal models of CI characterised by sharp decrease of 30-day survival in compare with only irradiated mice and rats. The main increment of lethality observed during the second week after irradiation.

Human recombinant IL-1-β was kindly supplied by Dr S. Ketlinski (Institute of High-Purity Biopreparation, St.-Petersburg, Russia). IL-2 (Biotech, St.-Petersburg, Russia) was a generous gift from Dr J. Jurkevich. Extra-cellular yeast polysaccharides of Bullera alba (B-678), Sporobolomyces albo-rubescens (Sp-50) and Ronasan (sulphated mannan) prepared by Prof. N.Elinov et al. (Chemical and Pharmaceutical Institute, St.-Petersburg, Russia). Heat-killed Lactobacillus acidophilus have prepared in collaboration with Dr V.Pospelova et al. (Institute of Microbiology and Epidemiology, Moscow, Russia). Increase of survival during 30 days after CI and beneficial effects on blood system state used as indexes of BRM's efficiency.

The next broad-spectrum antibiotics were under study: ampicillin, doxiciclin, rifampicin, rifametoprim, sulacillin, ciprofloxacin and pefloxacin. We thank Prof. I.Fomina and Dr M.Vyadro, Russian National Research Institute of Antibiotics, who provided us with medicines for this experimental study. Increase of survival, absence of side effects to hematopoietic system and lymphoid organs, correction of intestine microbiocenosis, increase of host resistance to exogenous infection used as indexes of antibacterial therapy efficacy.

THERAPEUTIC PROPERTIES OF BIOLOGICAL RESPONSE MODIFIERS

Single i.p. injection of B-678, Sp-50 and Ronasan (20 mg/kg) in 1 hr after CI increased 30-day survival from 3% (untreated mice) accordingly to 23%, 20% and 34%. Intraperitoneal two or four injection of rIL-2 within the first or second days after CI (single dose was equal to 5000 U/mouse) provided statistically significant increase of survival up to 42-49% as compare with untreated mice. The best results obtained when mixture of heat-inactivated L.acidophilus used (base concentration 10^8 microbes per 1 ml growth media). Single s.c injection of this remedy in 15 min after CI (0.1 ml/mouse) provided survival increasing from 27% (untreated mice) to 80%. The own results are according to early published data (9) that single subcutaneous injection of heat-killed L.casei in 10 min after irradiation may increase survival.

The opposite data revealed when therapeutic properties of hrIL-1-β investigated in the murine model of CI. This multy-functional cytokine did not improve 30-days survival. Moreover, when given once in 4 hr after CI (100 micrograms/kg, s.c., dose recommended for ARS treatment) IL-1 caused a higher rate of mortality in early stage of CI. In particular, 28 from 40 "treated" mice died in 2-3 days instead of 100% survival untreated animals during the first 5 day after CI. 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 even followed 24 hr after CI accelerated lethal outcomes. It should be stressed that single administration of "high" dose IL-1 (100 micrograms/kg) to only irradiated mice did not modify the early phase of ARS in our experiments. Moreover, slightly therapeutic effect took place and survival rate in 30-day period increased up to 15 or 30%. Effects of repeated i.p. injections of smaller dose IL-1 (100 pg/mouse in 2, 4 and 6 days after irradiation and CI) studied too. Such therapeutic scheme slightly increased 30-day survival of irradiated mice. IL-1 repeatedly injected after CI did not improve survival rate during observation period. The influence of the most effective two BRMs (IL-2 and L.acidophilus) on blood system state studied in 8 days after CI. Results showed that number of bone marrow nucleated cells, white blood cells, neutrophyls end erythrocytes in peripheral blood did not differ in treated and non-treated groups. Statistically significant differences revealed only in platelet's number. However the most effective remedy (L.acidophilus) decreased blood platelet's number as compare to control group. Thus, revealed increase of 30-days survival did not accompany by corresponding correction of cytopenia under CI.

Increase survival rate of irradiated mice receiving BRMs may be connect with enhanced antimicrobial activity of relatively radioresistant macrophages, because protective effect has been seen before significant bone marrow regeneration could occur (10). Probably, BRM-induced increase of macrophages' activity has some "limits of possibility" to enhance antimicrobial host resistance under CI. Because in this situation microbial burden increases due to radioinduced translocation of microorganisms from gastroenteric tract and bacterial invasion from the contaminated burned wound. Macrophage's activation as affected by BRMs may prove to be insufficient for overcoming the infection under CI. An additional need appears in supportive therapy with antibiotics and replacement therapy with selective hemopoiesis regulatory cytokines.

THERAPEUTIC PROPERTIES OF ANTIBIOTICS

Selection of optimal antibiotics for preventive therapy made in rats and murine models of CI. According to obtained results beta-lactams, rifampicin, rifametoprim (rifampicin + trimetoprim) and ciprofloxacin did not modify the low value of survival as compare with non-treated animals. Moreover, ciprofloxacin's administration (the daily dose 20 mg/kg) aggravated bone marrow and thymus devastation, increased level of leukopenia and thrombocytopenia. New combined antibiotic sulacillin (ampicillin+β-lactamase inhibitor sulbactam) and pefloxacin has rendered strong prophylactic action during the first 8-10 days after CI but did not increase 30-day survival rate when given as single agent therapy. Results of the preventive therapy was the best when animals treated during 7-10 days simultaneously using sulacillin (the daily dose 500 mg/kg) and pefloxacin (the daily dose 50 mg/kg). Treatment course started from the first day after CI. Percentage of survival following such antimicrobial therapy increased from 7% (untreated group) to 53%. Selective decontamination effect occurred. There were not find side aggravating effects of sulacillin and pefloxacin to the radiosensitive haemopoietic system and lymphoid organ's state. Selected antibiotics increased host resistance to exogenously inoculated Ps.aeruginosa under CI.

Presented data indicate that some medicines recommended for ARS treatment provide rather insufficient effects under CI or completely fail to improve survival rate. Moreover, single injection of rIL-1-β within the first 4-24 hs after CI is very dangerous and increases death rate. Biological response modifiers such as IL-2 and heat-killed L.acidophilus as well as antibiotics sulacillin and pefloxacin may be recommend for CI preventive treatment.

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REFERENCES

1. D. Browne, J. F. Weiss, T. J. MacVittie, and M. V. Pillai, *Adv. Space Res.* 12, 165-168 (1992).
2. G. D. Ledney, G. S. Madonna, T. B. Elliott, M.M. Moore, and W. E. Jackson, *Rad. Res.* 128, S18-S28 (1991).
3. G. S. Madonna, G. D. Ledney, M. M. Moore, T. B. Elliott, and I. Brook, *J. Trauma* 31, 316-325 (1991).
4. R. Boudagov, V. Nesterenko, G. Makarov, L. Uljanova, and E. Khlopovskaya, *Medical Radiology* 9-10, 41-44 (1992). In Russian.
5. E. Khlopovskaya, R. Boudagov, N. Suchareva, V. Urinjuk, *Science Academy's news. Biological series*, 2, 227-234 (1993). In Russian.
6. R. Boudagov, I. Rolevich, *Combined Radiation Injuries: pathogenesis, clinic and treatment* (Eds A. F. Tsyb and M. N. Farshatov), Moscow, "Medicine", 37-58 (1993). In Russian.
7. M. A. Chirigos and C. D. Simone, *Mediators of Inflammation* 2, S5-S10 (1993).
8. I. Brook, *Rad. Res.* 115, 1-25 (1988).
9. K. Nomoto, T. Yokokura, K. Tsuneoka, and M. Shikita, *Rad. Res.* 125, 293-297 (1991).
10. M. L. Patchen, M. M. D'Alesandro, I. Brook, et al., *J. Leukoc. Biol.* 42, 95-105 (1987).