Triage and Treatment of Combined Injury in Mass Casualty Situations

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Acute radiation sickness, which originates from high dose exposures following an accident or nuclear explosion, may be associated with thermal burns as well as different types of mechanical traumas. Actual difficulties of early-stage diagnosis and choice of valid therapy procedure for overexposed patients increase if combined injuries occur. Owing to previous numerous experimental studies, that mainly have fulfilled in USA, Germany, former USSR and recently in Russia, many problems of pathophysiology and management of combined injuries have been solved. However, physicians and especially surgeons need a concrete information about common principles of the earliest diagnosis, outcomes' prediction and the content of emergency medical aid. They need easy reading recommendation how to act when increased work loads on medical personnel in mass casualty situations. Scientists have to recommend them a course of action and effective utilisation of available resources during overloaded burn and surgical centres.

I am afraid we won't be able to give a concise information concerned to all of these physician's needs today, but we should really try to do it. At the same time I would like to stress our unsolved problems as concerns to combined injuries.

As you know outcome of CI is worse than that of ARS, thermal burns or mechanical traumas separately. Burns render the most apparent change for the worse. On the other hand, such variety of combined injury is expecting more frequently. For this reason my presentation will address mainly to radiation and burn injuries.

Considering known data on the severity of ARS or thermal burns, and taking into account theirs mutual aggravating action, combined injuries classified into four categories.

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Grade I includes superficial non-extensive burns (B-1) combined with total body irradiation up to 2 Gy (R-1). Prognosis for health is favourable for victims of Grade I.

The same burns combined with acute total body exposure to 2 or 3 Gy (R-2) concern to Grade II. Full-thickness burns up to 10% of body surface or extensive superficial burns from 10 to 40% (B-2) combined with mild irradiation less than 2 Gy applies to Grade II too.

Prognosis for health and survival is favourable as rule but life-threatening complications may occur in 20% of victims if emergency medical aid not rendering in time.

Severe combined injuries (Grade III) consist from the next components:

a) Superficial non-extensive burns plus 4 or 5 Gy irradiation (R-3);

b) Full-thickness burns up to 10% of body surface or 10-40% superficial burns plus 2 or 3 Gy irradiation;

c) Full-thickness burns from 10 to 40% of body surface or more than 40% superficial burns (B-3) plus irradiation up to 2 Gy.

Prognosis for health and survival is doubtful, about 50% of patients have chance to recovery if complete surgical and therapeutically therapy is available in time.

Other combinations of acute radiation with thermal burns apply to extremely severe irreversible types of combined injuries - Grade IV. All patients died in spite of the most modern therapy. These victims need only symptomatic medical aid.

The presented classification may be useful mainly for medical triage in mass casualty situations. The immediate estimation of the radiation damage extent becomes some more importance for a successful treatment strategy as concerns to combined injuries. Emphasis should put on to sort out severe burned patients who need an emergency medical care. The most difficult question for surgeons will be a distinction of such patients from those who additionally exposed to high doses of radiation. Anorexia, nausea, vomiting, diarrhoea, salivation, dehydration, fatigue, headache and hypotension are using commonly to define the severity of the exposure. However, predominant manifestation of burn shock can mask these clinic symptoms. Recommended biological markers of exposure to ionising radiation, such as chromosome aberrations of blood lymphocytes or dynamics of blood cell counts, are not practicable for urgent triage of patients with combined injury.

Our experimental study revealed that initial post-burn leukocytosis did not depend of absorbed radiation dose within prodromal phase. Erythrocyte's number, haemoglobin's and hematocrite's levels increased significantly in 3-6 hours after CI. However, the same degree of hemoconcentration observed after thermal burn without additional irradiation.

By other words, known early-stage burn-induced haematological features, such as leukocytosis and hemoconcentration, can not be useful for triage and outcomes' prediction of thermal trauma if combined radiation injuries occur.

Therefore, an approximate early estimation of radiation damage degree in mass casualty situations more probably will base:

a) On the anamnethis data and duration of victims being in radiation area;

b) On the data of individual physical dosimeters (if possible);

c) On the non-accordance of more severe common state of patients to relatively mild degree of trauma.

I think we have to answer the next questions in the future for triage improving:

-Which simple methods of biological dosymetry would to enable a rapid estimate of the radiation damage degree in patients with severe burn or other type of shocking trauma that is need of urgent surgical aid?

-How we really would to sort out victims with severe trauma and shock "which killed already (because simultaneous high dose irradiation) but not died yet"?

Management of victims following a nuclear disaster has to include the successive complex of surgical and therapeutically treatment. Life-saving standard emergency surgical procedures for nonradiation injuries and therapy of burn or traumatic shock are the first important step within 24-48 hours after CI. Antiemetic drugs, such as Ondansetron, Granisetron, Dimetcarb or Dixaphen, are useful for nausea and vomiting removal. Single tetanic anatoxin injection is compulsory too. Antimicrobial therapy should start from the first day after CI of Grade II or Grade III.

All essential special surgical operation should be carry out before the beginning of the third phase of acute radiation syndrome - manifest illness. Operative treatment of extensive full-thickness thermal burn not recommended until hemopoiesis recovery will complete. However, previous experiences and experimental studies indicate that patients

with severe CI die during the first two or three weeks mainly due to bacterial endotoxicosis and gram-negative sepsis. Unfortunately, the available facts prove decreasing efficiency of antimicrobial therapy and some biological response modifiers for rather high dose exposure and open wound trauma occurrence (publications of G.D.Ledney, G.S.Madonna, I.Brook and co-workers from the Armed Forces Radiobiology Research Institute, Bethesda).

The main scope of our recent experimental research was search and selection effective means for the preventive therapy of toxic and infectious complications of combined injury. Three groups of remedies were under study, which given at early stage of disease, would be useful to improve efficacy of following standard treatment schemes and to increase survival. The first group included biological response modifiers. Published data proved that these agents may increase macrophage's activity and secretion of cytokines such as hemopoietic growth factors. Mean of the second group used for decreasing of the initial "aseptic" phase of bacterial enteroendotoxemia and early post-burn intoxication. The third group included antibiotics for prophylaxis and preventive therapy of infectious complications.

Mice and rats irradiated at minimal lethal doses. Non-lethal per se full-sickness thermal burn inflicted immediately after irradiation by means of powerful lamp's light. Combined injuries characterised by sharp decrease of 30-day survival of animals in compare with separate ARS.

The next biological response modifiers used in our work: pyrogenal, prodigiozan, zymozan (10 mkg/mouse, i.p.), synthetic analogue of muramyldipeptide glycopin (5 or 50 mkg/mouse, i.p.), thymozin, thymotropin, thymogen (20 mkg/mouse, i.m.), different kinds of new yeast polysaccharides (20 mg/kg, i.p.) and other. Increase of survival and beneficial effects on blood system state used as indexes of this drug's efficacy.

According to obtained results bacterial polysaccharide pyrogenal, glycopin and thymus preparations did not modify the extremely low value of mice's survival.. Single injection of prodigiozan, zymozan and three extra-cellular yeast polysaccharides in 1 h after CI resulted in moderate increasing of survival as compare with untreated mice. The best results obtained when recombinant IL-2 or heat-killed Lactobacillus acidophilus used (10^8 microbes per 1 ml growth media, s.c.). Increase of 30-day survival accordingly composed 42% and 53%.

Recombinant human G-CSF investigated for the ability to accelerate bone marrow regeneration and to decrease the severity of leucopenia after irradiation only or CI. Results demonstrated that G-CSF increased number of bone marrow's CFUs in the group of separately irradiated mice. When animals exposed to CI this index did not change significantly. G-CSF did not modify lowered number of bone marrow nucleated cells and leukocytes score within manifest illness.

We spared the most attention to therapeutic use of rhIL-1-β. It taken into account that IL-1 act as essential molecular master switch for secretion of GM-CSF, G-CSF, IL-3, IL-6 and other important hemopoietic growth factors. Several authors reported that IL-1 given in 1-4 hour's (50-100-200 mkg/kg, s.c or i.p.) increased survival of mice exposed to radiation only.

Single injection of IL-1 in amounts proposed for acute radiation syndrome treatment (100 mkg/kg) in 4 h resulted in abrupt prognosis deterioration when dealing with CI. About 60% of "treated" mice died during the first 2-3 days after CI. The same results obtained if dose of IL-1 reduced to 150 ng/mouse. Analogous data we received also when single IL-1 injection made in 24 h after CI.

Experiments in rats' model made to study the ability of natural and artificial enterosorbents to eliminate gut derived bacterial endotoxins and to decrease burn toxaemia. All medicines injected into stomach in 1 h and then in 24 hours. Indexes of absorbent efficacy measured in 48 hours after CI. The next drugs were under study: enterodes, phosphalugel, kaolin, polymethylsillocsan, polyphepan, almagel and different kinds of carbon-contained absorbents.

The best results in the prevention of the early endogenous toxic syndrome obtained when artificial carbon-mineral sorbent used. Level of bacterial endotoxemia and blood's total toxicity significantly decreased following two-fold administration of this remedy. It's important that use of enterosorbent increased efficacy of antibiotics doxycyclin or ciprofloxacin. More strong decrease of blood toxicity, correction of intestinal disbacteriosis revealed when antimicrobial therapy combined with enteric toxins' absorption. Survival of treated rats increased up to 80% while all rats from control (untreated) group died during the first two weeks after combined injury.

Selection of optimal antibacterial medicines for preventive therapy has made among broad-spectrum antibiotics. Survival rate, possible side effects to hematopoietic system and lymphoid organs, enteric microbiocenosis and resistance to exogenous infection registered. According to obtained results, beta-lactams, aminoglycoside and rifampicins did not modify low survival value. The complex antibiotic sulacillin, which consists of ampicillin and beta-lactamase inhibitor sulbactam, rendered strong prophylactic action during the first 8-10 days after CI but did not increase 30-day survival. Successful preventive therapy was the best when animals treated with two antibiotics: pefloxacin + sulacillin (treatment course 7-10 days, starting with the first day after CI). Percentage of survival following such antimicrobial therapy increased from 7% (untreated group) to 53%. Selective decontamination effect observed. There was not side aggravating action of this antibiotic complex to the radiosensitive systems.

Therefore, presented data indicate, that early recommended treatment schemes may complete by some new effective means of preventive therapy. On the other hand, one can say that direct extrapolation of treatment techniques, recommended early for the acute radiation sickness alone, to combined injuries may be incorrect. Future investigations are need for pathogenetically based improving of supportive and restorative therapy.

I think we should know much more about the mechanisms of early aggravating effect of thermal burn or wound trauma on ARS severity and outcome (oxidative stress, generation of free oxygen radicals, metallothioneins' synthesis, "cytokine cascade'" response, pathogenesis of early endotoxemia).

I would like to express the hope that we'll be able jointly work out much more effective strategy for the CI therapy. In particular, by search of the most optimal combinations and dose scheduling of haemopoietic growth factors or other biological response modifiers.

I would like to express the hope that our workshop will start more active scientific co-operation between Medical Radiological Research Centre in Obninsk and Armed Forces Radiobiological Research Institute in Bethesda.

CLASSIFICATION AND PROGNOSIS OF COMBINED INJURIES

Categories of CI severity	Component of burn	Component of radiation	Prognosis for health and survival
Grade I	B-1	R-1	Favourable
Grade II	B-1	R-2	Favourable as rule
	B-2	R-1	(Life-threatening complications may occur in 20% of patients)
Grade III	B-1	R-3	Doubtful
	B-2	R-2	(About 50% of patients have chance to recovery)
	B-3	R-1	
Grade IV	B-1 - B4	R-4	Unfavourable
	B-2	R-3	
	B-3	R-2	(All patients die)
	B-4	R-1	

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THE PATHOGENESIS AND THERAPY OF COMBINED RADIATION INJURY

Participating Institutions and Laboratories

- Department of Expermental Hematolofy, Armed Forces Radiobiology Researh Institute, Bethesda, Maryland, USA
- Laboratory Modelling of Radiation and Non-Radiation Effects, Medical Radiological Research Centre, Obninsk, Russia

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Research activity

- Health consequences of high doses radiation exposure owing to nuclear accidents
- The optimal management of severe accidental irradiation and combined injury victims in emergency situations

Objectives

- Analysis the pathophysiology of thermal burns aggravating effect on the acute radiation syndrome outcomes (with special emphasise to hematopoietic system damages and recovery)
- Development of the new treatment techniques for the overexposed persons and those with combined radiation injuries

Project Duration: 1997 - 1999

Background

Two types of "severe accidental irradiation" can be schematically described: high dose localised irradiations and accidental total body overexposure. For total body overexposure, haematological problems are at the forefront [1]. Radiation accidents of 5-7th levels according to IAEA scale lead to life-threatening acute radiation syndrome, and some patients will suffer from additional thermal burns. The mortality and morbidity of

such combined injury (CI) victims are higher than that of the injuries separately. The existing data about aggravating action of thermal burns on the radio-induced damages and following recovery of hemopoietic system are incomplete yet. Recommendations for management of CI do not take into account the modern experience of hemoregulatory cytokines usage [2,3]. Meanwhile, use of growth factors may be a new effective way of treating these patients.

Experimental study of the IL-1 beta, IL-2 and other biological response modifiers for the treatment of CI made in Medical Radiological Research Centre (Obninsk, Russia). Results showed that direct extrapolation of treatment techniques, recommended for irradiation alone, to CI may be incorrect. Future investigations are need for pathogenesis based correction of hematopoietic system failure under CI.

Scope of research

Thermal injury induces significant physiologic responses of acute inflammation and acute phase reaction, mediated by different cytokines. Any physiologic modulation of the thermal injury by biological modifiers must be adapted to extent of burn wound size and phase of injury for optimal benefit and result [4]. The analysis of recent publications showed that thermal injuries may induce essential changes of different cytokines.

Burns can contribute to the development of inflammatory and cytotoxic macrophages from BONE MARROW PROGENITOR CELLS. LPS-stimulated macrophages from burned animals produced different amounts of TNF, IL-1 and IL-6 compared with unburned animals at certain culture times, that may be important in host defence [5]. At 2 or 24 h macrophages primed to produce in vitro different and sometimes large amounts of cytokines [6].

There is functional heterogeneity of macrophages for IL-1, IL-6 and TNF production obtained from different tissues of the same animal (Kupffer cells, alveolar, splenic or peritoneal macrophages) [7]. Increasing evidence shows that cells other than immune cells (hepatocytes, enterocytes) have the potential for increase producing cytokines in 24 h after thermal injury [8]. Inflammatory cytokines and acute-phase proteins

are closely interrelated, and their levels of production by various cells are increased by thermal injury [9].

The systemic cytokine response to burn injury mainly represented by interleukin 6. IL-6 is a key mediator of variations in protein metabolism following burn injury. IL-6 plasma levels increased in all burn patients throughout the study period (day 2 to 21 post-injury). Maximum concentration reached on day 4 and correlated positively with the extent of burn injury, protein turnover and catabolism [10]. In rat model of burn's serum IL-6 levels showed a constant time-dependent change with a single peak 6 hours after the burn. The liver, spleen, lymph node and skin harvested after the burn secreted significantly higher amounts of IL-6 after tissue culture when compared with those taken from sham rats. Serum IL-6 level elevation correlated with the ratio of burn area to total body surface area [11]. Plasma samples with detectable amounts of IL-6 were significantly more frequent in burned patients than in controls. All nonsurving burned patients had higher levels than those of surving patients. IL-6 concentration was highest during the first week after injury and declined over time. IL-6 may influence metabolic and immunologic responses in the first few weeks following thermal injury [12]. Forty-eight hours after thermal injury IL-6 was significantly present in systemic circulation, lung, and skin of the burned patients [13]. An increased expression of IL-6 mRNA in liver tissue from animals of the burned group accompanied by an evaluation of IL-6 released from cultured Kupffer cells and by increased serum levels of this cytokine. Burn-mediated increase in the production of IL-6 and the production of acute phase proteins by hepatocytes paralleled by increases in the corresponding message RNA levels in these cells [14].

The data about post-burn changes of TNF are contradictory. Plasma TNF alpha concentration not increased on the day of admission in patients with thermal injuries compared with control [15]. TNF alpha was undetectable in most plasma samples following thermal injury. TNF transiently elevated in a small subpopulation of burned patients with no obvious relationship to burn size or time postburn [12]. TNF alpha plasma levels elevated significantly on day 7 in the patients who developed sepsis than in the other patients [10]. In the early period after injury (including

the period of burn shock) 24 patients from 42 had detectable TNF-alpha levels in their plasma. However, the plasma TNF levels at the time of admission were very low and did not correlate with the extent of the burn or the prognosis. In contrast, the maximum plasma TNF level over THE WHOLE CLINICAL COURSE significantly correlated with the area of the burn and the prognosis [16]. Forty-eight hours after thermal injury TNF was significantly present in systemic circulation, lung, and skin [17]. The results showed an evident increase in serum TNF activity in severely burned patients. The changes correlated significantly with those of myocardial and hepatic enzymes in multiple organ failure (MOF) patients. TNF play important role in the development of MOF [18]. TNF-alpha mRNA expression in splenocytes of burned mice increased in 4-21 days after injury [19]. LPS-stimulated TNF production by splenic macrophages increased after thermal injury in murine model [20].

IL-1 is present in the blood of burn patients but its pathophysiologic role not fully understood. Serum IL-1 levels increased following thermal injury in rats, there was no apparent relationship between IL-1 levels and infection [21]. Plasma IL-1-beta concentration not increased on the day of admission in patients with thermal injuries compared with control [22]. IL-1 beta rarely detected in burn patients at day 2 to 21 post-injury [10]. IL-1 beta concentration elevated during the first week after injury and positively correlated with burn size, may influence metabolic and immunologic responses following thermal injury [12]. IL-1-beta mRNA expressions in mice's splenocytes consistently increased at intervals from 4 to 21 days after burn [23].

Thus, thermal burns *per se* may induce significant changes of very important cytokines (IL-1 beta, IL-6 and TNF alpha) which play key role in host defence, inflammation, protein turnover, catabolism, immunologic responses, and other homeostasis regulatory processes. IL-1 beta, TNF alpha and IL-6 are the most important endogenous mediators, which responsible for the pathophysiologic changes and the mortality associated with bacterial endotoxemia and sepsis. At the same time IL-1 and TNF induces the production of GM-CSF, G-CSF, M-CSF, IL-3 and other

cytokines that render beneficial effects on the postradiation hematopoiesis recovery [24-28]. Unfortunately, nothing known about the systemic cytokine response to combined thermal injury and irradiation.

Comparative experimental study of cytokines response to burn injury, irradiation and combined injury may be useful for understanding of burn's aggravate effects on the acute radiation syndrome outcomes, and for the treatment improvement.

Cytokine-mediated restoration of hematopoiesis is prerequisite for survival after irradiation. Recently several authors showed that treatment with any cytokine alone had very limited capacity to improve survival. The strongest synergistic effect observed when irradiated animals treated with a combination of cytokines such as stem cell factor, IL-1 and IL-3 [30], GM-CSF and IL-6 [31], GM-CSF and IL-3 [32].

Comparative experimental study of these therapeutic schemes (including GM-CSF, IL-3, IL-6 and c-kit-ligand) for irradiation alone or combined injuries are advisable.

Sublethal irradiation induces transient, splenic cytokine gene expression that can be differentially amplified and prolonged by biological response modifiers (BRMs). Therapy by BRMs presumably accelerates hematopoietic recovery by enhancing expression of cytokines and plasma levels of CSF within 24 h after irradiation. BRMs, that sustained and/or enhanced irradiation-induced expression of specific cytokines genes, improved survival of irradiated mice additionally challenged with Kl. pneumonia [33]. BRMs act as cytokine-inducer on various epithelial cells. Cytokines stimulate multipotential hematopoietic progenitors and their progeny as growth and/or differentiation factors [34]. Because cytokine toxicity remains a significant concern, the clinical application of BRMs, which have no toxicity, is particularly valuable [35]. Results of our previous investigation revealed that single subcutaneous injection of heat-killed Lactobacillus acidophilus may considerably increase survival of irradiated mice that additionally inflicted thermal burn.

These data give us the reason to continue preclinical studies of L.acidophilus preparation as a remedy for early preventive therapy of acute radiation syndrome and combined injuries.

Oxygen free radicals (OFR) may involved in the initiation and propagation of free radical chain reactions and are potentially highly damaging to cells. Production of OFR, exceeding the ability of the organism to mount an antioxidant defence, result in oxidative stress. The ensuing tissue damage may involve in certain disease processes. Evidence that OFR involved in primary pathological mechanisms is a feature of extraneous physical or chemical perturbations of which radiation is perhaps the major contributor. One of the important radiation-induced OFR is the hydroxyl radical. There is a significant increase of blood oxygen free radicals content in burn patients too. OFR may play a role in morbidity and mortality of burn shock. The biological response to radiation and burn may be modulated by alterations in factor affecting the cellular antioxidant status [36-37].

Metallothionein (MT), the synthesis of which can be induced by metalloelement or by IL-6 in response to inflammation, is a known strong radical scavenger [38]. The increase in the content of bone marrow and hepatic MT in mice with maximum at 30 hr after whole-body irradiation shown. The MT level then correlated with the exposure dose [39]. Some agents that increased endogenous MT content also exogenous MT protected biological object from irradiation and agents that caused oxidative stress [40]. A single s.c injection of preparation of heat-killed L.casei significantly increased the survival rate in mice received 8.5 Gy whole-body gamma-irradiation. Effect based on enhanced recovery of hematopoietic tissues and increased MT in the hematopoietic tissues of the treated mice [41].

Based at these data, experimental study the role of MT in the pathogenesis and management of combined injury is advisable.

Objective of research

- *Comparative experimental study of cytokines response to burn injury, irradiation and combined injury*

  - Determination of IL-1 beta, IL-3, IL-6, TNF alpha and GM-CSF serum levels at different phase of thermal burn, irradiation only and combined injury in murine models;

  - Measurement of in vitro IL-1, IL-6 and TNF production by peritoneal, splenic and bone marrow macrophages

  - Comparative and correlative study of blood system state, hepatic and myocardial enzymes, blood toxicity, and survival rate.

- *Comparative experimental study efficacy of therapeutic schemes including combination of GM-CSF, IL-3, IL-6 and c-kit-ligand for irradiation alone or combined injuries*

  Determination of the capacity these schemes to improve survival and to enhance recovery of hemopoietic parameters (bone marrow cellularity, endogenous CFUs, blood cells count) after irradiation alone or combined injury.

- *Preclinical studies of L.acidophilus preparation as a remedy for early preventive therapy of acute radiation syndrome and combined injuries*

  - Experimental study efficacy and possible radioprotector mechanisms of new preparation in murine and rat's models of acute radiation syndrome and combined injuries (the systemic cytokine response at acute phase of injuries, resistance to exogenous infection, intestinal decontamination, measurement of phagocytosis and serum opsonic capacity by chemiluminescence, metallothionein content in bone marrow);

- Investigation therapeutic efficacy of this new preparation when given in combination with antibiotic or well-known radioprotectors.

- **Experimental study the role of metallothionein in the pathogenesis and management of combined injury**

- Measurement of MT content in bone marrow and liver of mice at different phase of acute radiation syndrome and combined injury;

- Experimental study of MT induced synthesis before or after combined injury on the mouse's blood system state and survival.

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Approximate expenditures for project

- Equipment:

Multiwell Plate Reader with accessories (1)

Multiwell Plate Washer for use with 96-well multiwell plates (1)

Multichannel [8-channel] Pipets (2)

pH Meter (1)

multiwell plates, culture dishes, pipet tips, centrifuge tubes

- Reagents and Kits:

ELISA MiniKits for the quantitation of mouse IL-1-β, IL-3, IL-6,

GM-CSF, and TNF-α ("Endogen" firm, USA)

special reagents for the automatical hematological analyser MINOS STX ("IDEAL Products S.A.", France)

reagents for the measurement of cellular chemiluminescence (Luminol, Lucigenin, Zymosan, "Sigma Chemical Company")

rIL-3, IL-6, GM-CSF, and c-kit-ligand for experimental therapy in vivo

- Local assistance to individual participants: Project scientific Head and coordinator (1), Senior Scientist (2), scientists (2), and Laboratory assistants (3).