



NINTH INTERNATIONAL
CONFERENCE ON RADIATION
IN VARIOUS FIELDS OF RESEARCH
June 14-18, 2021 | Hunguest Hotel Sun Resort | Herceg Novi | Montenegro

S-HETERYL MODIFIED CYSTEAMINE PROTECTS TOTAL BODY IRRADIATION-INDUCED HEMATOPOIETIC SYSTEM IN JURY IN RATS

N. Uzlenkova¹, M. Kornet², N. Skorobogatova¹, O. Brazhko², A. Krivko¹, O. Maslennikova¹, M. Zavhorodnii²

¹ SI “Grigoriev Institute for Medical Radiology of National Academy of Medical Sciences of Ukraine” Kharkiv;

² Zaporizhzhia National University, Zaporizhzhia, Ukraine.

This work was supported by MES of Ukraine, “Rational design of S, N-modified aminothiols as potential anti-radiation agents” (№ 0119U000226).



Introduction

Radiation exposure poses a significant threat to human health, especially the hematopoietic system. Protection and/or mitigation of hematopoietic system from radiation injury is an important goal in the development of medical countermeasure agents.

In this study the effectiveness of S-heteryl modified cysteamine (S-HMC) as a novel agent in enhancing hematopoietic reconstitution in rats following a lethal dose of irradiation was investigated.

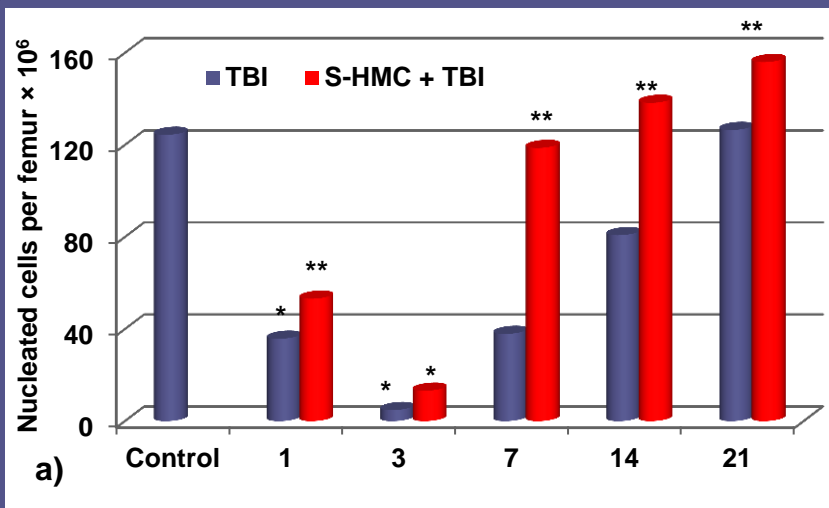
Methods



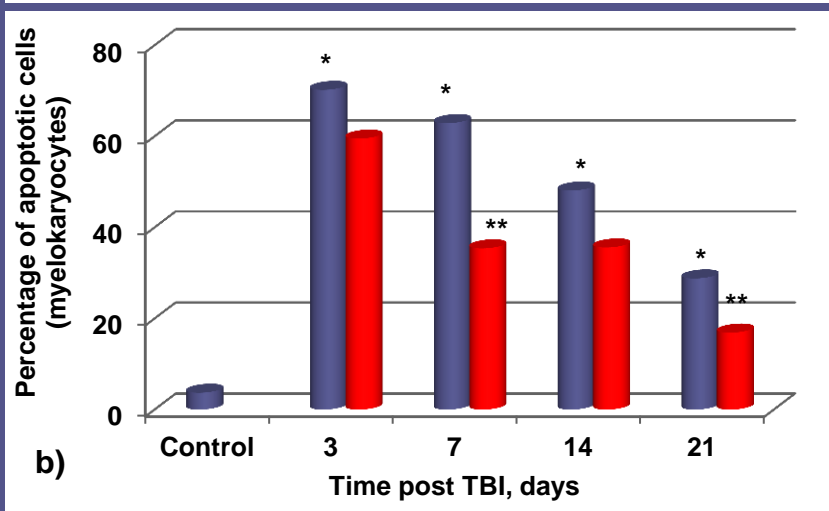
- All animal studies were conducted in accordance to the international principles of "European Convention for the protection of vertebrate animals used for experimental and other scientific purposes" (Strassbourg, 1998) and the Law of Ukraine "On protection against the rude treatment" (Kyiv, 2006). The white female rats, 160-180 g, were used.
- Total body irradiation (TBI) of the rats was carried out on CLINAC (6 MeV) at a dose 7.0 Gy with dose rate 0.76 Gy/min.
- S-HMC was administrated intraperitoneal (i.p.) at a dose 150 mg/kg in 30 min before irradiation.
- Peripheral blood cells were counted by hematology analyzer RT-7600 (Rayto, China).
- The clonogenic pool of mesenchymal stromal cells (MSCs) in bone marrow (BM) was assayed by counting of colony forming unit (CFU-f) under culture conditions *in vitro*.
- The myelokaryocytes apoptosis was determinate with acridine orange (AO).
- The BM cellularity were counted by standard calculation in Goryaev's chamber.
- Hematoxylin and eosin staining was used to BM analysis.
- Kaplan–Meier analysis of rats survival for 30 days was used.

Results: Bone marrow responses in rats with TBI (7Gy) and prophylaxes with S-HMC

Figure 1. a - BM nucleated cells number;
b - percentage of apoptotic cells (myelokaryocytes).



- S-HMC (150 mg/kg) significantly minimized BM cells depletion and accelerated recovery of total BM cellularity in TBI rats



- S-HMC (150 mg/kg) caused profound reduction the apoptotic cell death in BM of TBI rats after exposure

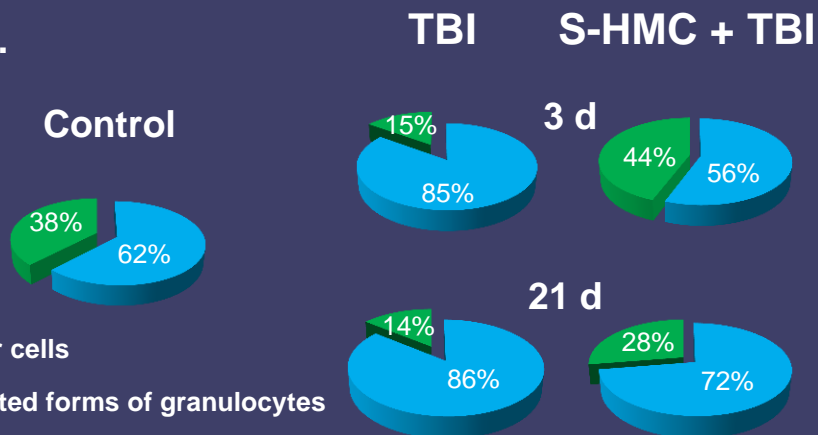
S-HMC (150 mg/kg) was delivered via i.p. injection in a single dose of 150 mg/kg 30 min prior to TBI. Control rats were sham-irradiated; The data are shown as the mean \pm SEM (n=10).

* $P < 0.05$ vs control; ** $P < 0.05$ vs TBI



Results: BM hematopoietic lineages in rats with TBI (7 Gy) and prophylaxes with S-HMC

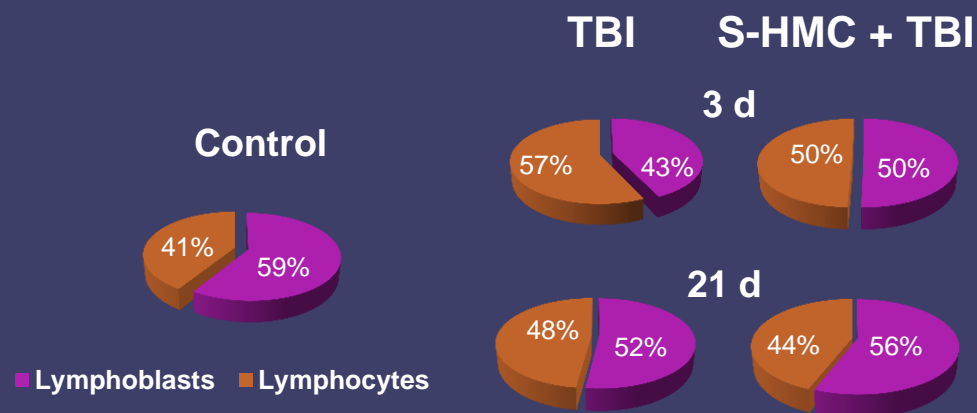
Figure 2.



a) the ratio percentage of progenitor cells and differentiated forms of granulocytes lineage

- S-HMC (150 mg/kg) provides exceedance of the number of differentiated granulocytes in BM compared to TBI rats

- S-HMC (150 mg/kg) saves the ratio of progenitor and differentiated cells in lymphoid lineage and prevents the lymphopoiesis depletion



b) the ratio percentage of progenitor cells and differentiated forms of lymphocytes lineage

- S-HMC (150 mg/kg) attenuates TBI induced disorders of hematopoietic cells proliferation and differentiation in BM and maintains hematopoietic homeostasis.

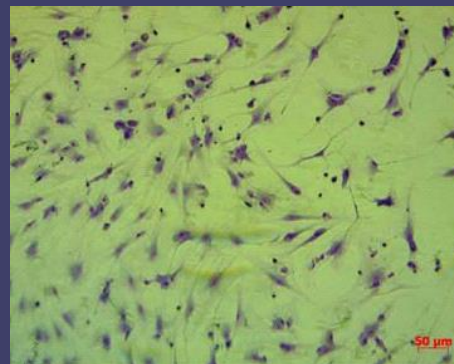
Results: Colony-forming BM MSCs pool in rats with TBI (7 Gy) and prophylaxes with S-HMC

Experiment	CFU-f number in BM obtained from femur		
	Time after TBI, days		
	3	7	14
Control	704,8 ± 133,7		
TBI	29,3 ± 3,6*	69,5 ± 28,6*	31,3 ± 11,1*
S-HMC + TBI	77,7 ± 16,1*#	175,3 ± 58,7*	59,3 ± 33,7*

* p < 0,05 vs control;
 *# p < 0,05 vs TBI



a



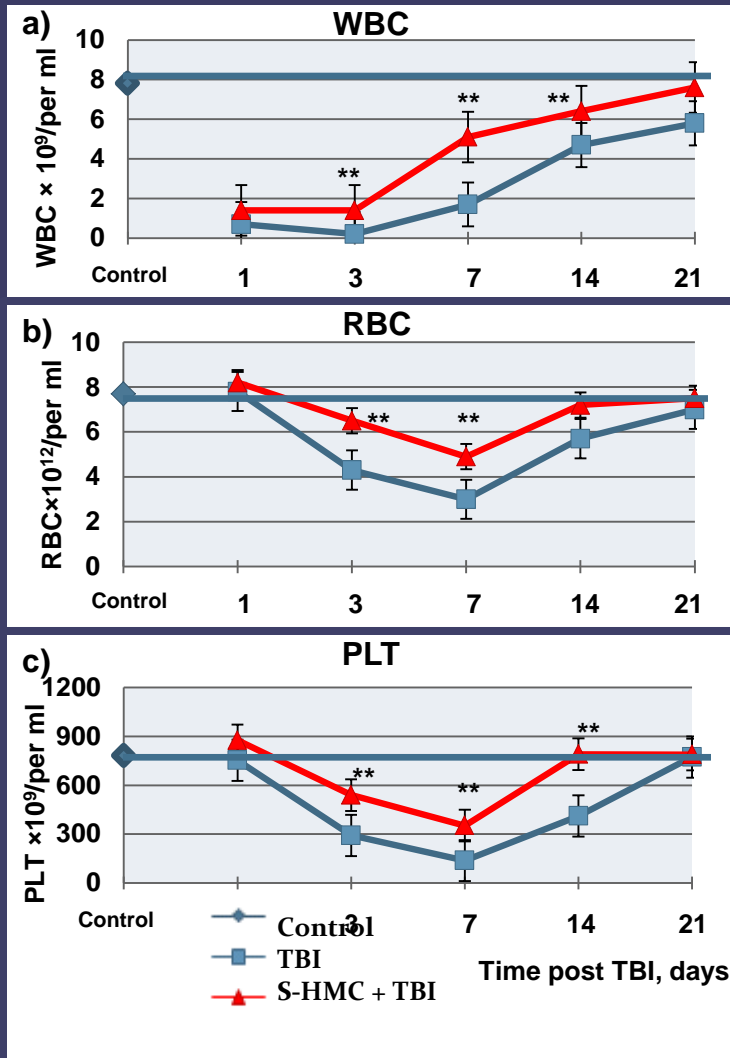
b

Figure 3. Colonies of rat BM MSCs in primary culture *in vitro*. BM was obtained on day 7 after TBI (a) and S-HMC + TBI (b)

- reduction of CFU-f pool in BM after TBI (7 Gy)
- S-HMC (150 mg/kg) provides saving a larger amount of the colony-forming MSCs (in 2,7 times) compared to TBI rats

Results: Peripheral blood cells responses in rats with TBI (7 Gy) and prophylaxes with S-HMC

Figure 4.



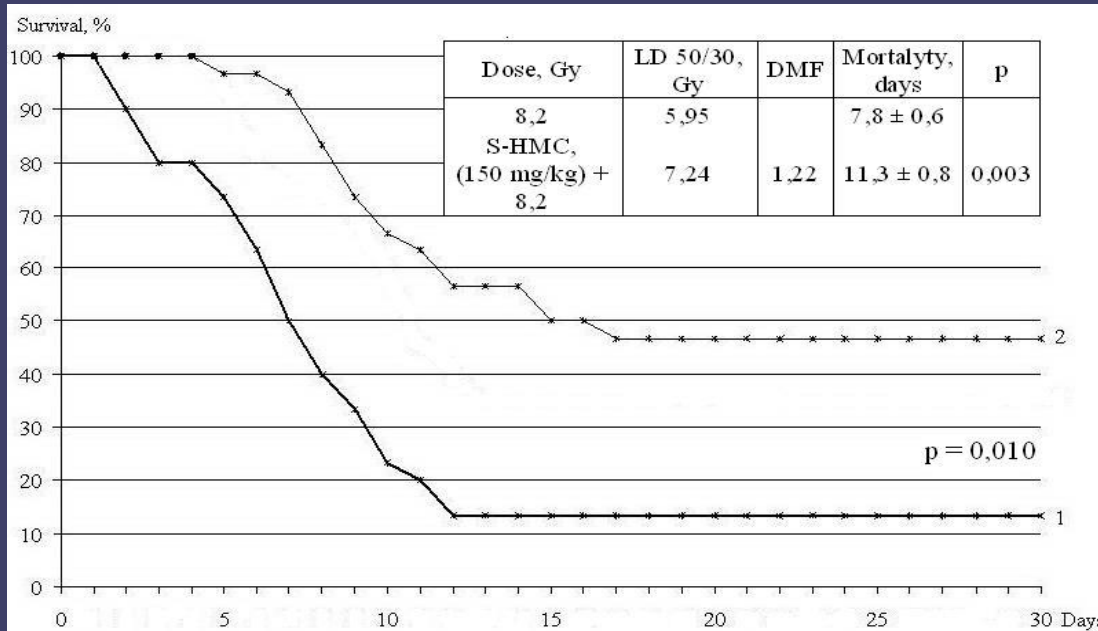
a - the numbers of white blood cells (WBC×10⁹/per ml);
b - the numbers of red blood cells (RBC×10¹²/per ml);
c - the numbers of platelet (PLT ×10⁹/per ml)

• S-HMC demonstrated the protective potential in preventing of TBI-induced decreasing peripheral blood cells number

S-HMC (150 mg/kg) was delivered via i.p. injection in a single dose of 150 mg/kg 30 min prior to TBI. Control rats were sham-irradiated; The data are shown as the mean ± SEM (n=10).
* P < 0.05 vs control; ** P < 0.05 vs TBI

Results: Survival and mean survival time in rats with lethal dose of TBI (8.2 Gy) and prophylaxes with S-HMC

Figure 5.



- S-HMC (150 mg/kg) increased the survival and mean survival time (days) of rats following a lethal dose of TBI.

S-HMC was delivered via i.p. injection in a single dose of 150 mg/kg 30 min prior to TBI. The survival rate was monitored for 30 days after TBI. Kaplan-Meier analysis of rats survival after exposure to the lethal dose (8.2Gy) of TBI (n = 19 rats/group)

Conclusion:

This study suggests that S-HMC could be used as a potent effective agent to protect the hematopoietic system against TBI-induced bone marrow suppression.