ORIGINAL ARTICLE

RADIOPROTECTIVE EFFECT OF HEPARIN

Radioprotektivní účinek heparinu

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Summary

The study was aimed at the experimental assessment of the efficacy of heparin as radioprotector in condition of acute whole body γ -ray irradiation. The efficacy of the product was based on the 30-day survival rate and average life expectancy of dead irradiated animals. To reveal possible mechanisms of radioprotective effects of heparin the thymus mass, the number of leukocytes and colony formation units (CFU) in the peripheral blood and bone marrow were studied. It was shown that radioprotective effect of heparin administered intraperitoneally in a dose of 250 unit/kg was developed within one day after the administration and maintained for periods up to 30 days. Heparin increased the survival rate only in radiosensitive BALB/c and inbred mice. The product exerted stimulating effects on the migration of haemopoietic cells: injection of heparin doubled the number of CFU in the peripheral blood of BALB/c mice and reliably reduced it in the bone marrow.

Key words: heparin – radioprotector – irradiation – survival rate – haemopoiesis

Souhrn

Tato studie byla zaměřena na experimentální hodnocení účinnosti heparinu jako radioprotektivní látky ve stavu akutního celotělového ozáření zářením gama. Účinnost přípravku byla založena na třicetidenním přežívání a průměrné době života uhynulých ozářených zvířat. Pro zjištění možných mechanismů radioprotektivních účinků heparinu byla studována hmotnost thymu, počet leukocytů a jednotek tvořících kolonie v periferní krvi a kostní dřeni. Bylo zjištěno, že radioprotektivní účinek heparinu podaného intraperitoneálně v dávce 250 jednotek na kilogram se dostavil během jednoho dne po podání a udržoval se po dobu do 30 dnů. Heparin zvyšoval přežívání pouze u radiosenzitivních myší BALB/c a inbredních myší. Přípravek vykazoval stimulační účinky na migraci krvetvorných buněk: injekce heparinu zvyšovala počet jednotek tvořících kolonie na dvojnásobek v periferní krvi myší BALB/c a spolehlivě snižovala tento počet v kostní dřeni.

Klíčová slova: heparin – radioprotektivní látka – ozáření – přežívání – krvetvorba

INTRODUCTION

Heparin is a highly sulfated, unbranched glycosaminoglycan (GAG). GAG molecules described previously as “ground substances” and “mucopolysaccharides” were historically thought to serve a spacefilling function necessary for the orientation and organization of the extracellular matrix (Forsberg and Kjellen 2001; Taylor and Gallo 2006). Advances in glycobiology revealed that the active participation of GAG molecules and their proteoglycan backbone is important in a variety of cell communications (Harmer 2006; Mulloy and Rider 2006). Recent data demonstrated GAG to initiate and control the processes of inflammation and repair. The close structural similarity between heparin and the ubiquitous cell-surface GAG heparan sulfate suggested a possibility of potential roles of heparin in the control of molecular events in the extracellular matrix. Heparin can alter cell signalling and adhesion reactions by binding to extracellular matrix proteins, including fibronectin, laminin, thrombospondin, and vitronectin (Taylor and Gallo 2006; Lukashin and Grebenyuk 2010).

Contrary to heparin, which is synthesized exclusively as serglycin proteoglycan in connective tissue-type mast cells, the structurally related heparan sulfate is produced by most mammalian cell types. Important classes of ligands for heparin (heparan sulfates) include growth factors and extracellular matrix macromolecules.

Because of these myriad interactions, the possibility that heparin could be used for therapeutic reasons other than the control of coagulation has been the subject of many inquiries (Beneš and Rotreklova 1970; Lane and Adams 1993; Lukashin and Grebenyuk 2010). Since its discovery in 1916, heparin has been used increasingly as an antithrombotic agent, but its physiologic role has not yet been clearly defined. The study presented here should encourage workers exploring the non-anticoagulant therapeutic possibilities of heparin.

One of perspective approaches to the enhancement of the organism of radioresistance is using of stimulators of haemopoiesis and immunity (Lukashin 2007; Grebenyuk et al. 2010). Heparin/heparan sulfate modulated the biological activity of growth factors and cytokines (Harmer 2006; Mulloy and Rider 2006). These sulfated GAG induce functionally important

changes in growth factors conformation and act as growth regulators.

Thus, the purpose of the research was the experimental estimation of the radioprotective efficacy of heparin in acute irradiation.

MATERIAL AND METHODS

Animals

Experiments were conducted in males of 648 inbred mice, 594 BALB/c mice and 446 hybrid (CBA × C57Bl) F₁ mice up to 90 days old of body weight between 18 and 20 g.

The animals were housed, fed on a standard diet and were given water *ad libitum* until the end of the experiment. The room temperature was controlled at 25±2 °C. The rules of ethical treatment of experimental animals were observed during the experiments.

Irradiation

The animals were exposed to a total single irradiation with γ -rays of Co⁶⁰ at a dose rate of 0.703 mA/kg (1.2 Gy/min) and source to skin distance 1 m. The dosimetry was provided with the ID-11 dose meter.

Chemicals

Heparin dissolved in 0.2 ml of the saline solution was injected intraperitoneally to mice in all the experimental groups in a dose of 250 unit/kg at different times before irradiation. Cystamine as the standard radioprotector (Kuna et al. 2004) was injected intraperitoneally 20 min before irradiation in a dose of 150 mg/kg. The controls were intraperitoneally injected with the same volumes of the saline solution at the same time.

Methods

The radioprotective effect of heparin was studied based on the 30-day survival rate and average life expectancy of dead animals. To reveal possible mechanisms of radioprotective effects of heparin, the thymus mass, number of leukocytes and colony formation units (CFU) in the peripheral blood and bone marrow were studied by standard methods (Till and McCulloch 1961) on the 9th day after irradiation. The results of three similar experiments were summarized.

Statistical analysis

The data obtained in experiments were used to calculate average values and standard errors. Student's test and probit-logarithmic analysis were used to compare mean survival rates and thymus mass, respectively. The statistical significance of differences between particular groups was established with the use of the Student's t-test at a level of $p < 0.05$.

From the lethality date, lethal doses and relative radiation effect with the 95% confidence limits were calculated by the probit-logarithmic

analysis. The dose reduction factors (DRF) of applied radioprotectors for 30-days mortality are reverse values of the relative radiation effect in mice treated with heparin or cystamine before irradiation.

RESULTS

The long-term radioprotective effect of single intercourse of heparin was revealed in inbred mice (Fig. 1).

Fig. 1 The long-term radioprotective effect of single intercourse of heparin on irradiated white inbred mice

* $p < 0.01$ in comparison with control (irradiation with saline)

It was found out that administration of heparin 1–30 days before exposure to γ -rays effectively protect mice irradiated at $LD_{70-80/30}$ against death. As a result it is established that after unitary heparin injection the radioprotective effect developed within 1 day and was maintained for periods up to 30 days.

Comparative study of the influence of heparin injected 3 days before irradiation and cystamine

injected 20 min before irradiation on mice with different degrees of radioresistance showed that radioprotective effect of cystamine in radiosensitive BALB/c mice was similar to that in more resistant hybrid (CBA $\times$ C57Bl) F_1 mice. Heparin increased the survival rate only in radiosensitive BALB/c and inbred mice (Table 1).

Table 1 Comparative analysis of radioprotective efficacy of heparin and cystamine in irradiated mice with different degrees of radioresistance (number of surviving animals/total number of animals in group)

Group	Dose of irradiation, Gy								
	4	5	6	7	8	9	10	11	12
White inbred mice									
Saline	9/17	10/20	2/20	2/20	0/10				
Heparin				18/24	10/24	12/24	4/24	1/12	
Cystamine		15/19	13/20	8/20	4/19	4/19	1/10		
BALB/c mice									
Saline	14/20	8/20	6/20	2/20	0/11				
Heparin	18/20	16/20	10/20	4/20	0/10				
Cystamine		15/19	13/20	8/20	4/19	4/19	1/10		
Hybrid (CBA × C57Bl) F ₁ mice									
Saline		16/16	16/20	10/20	4/20	0/12			
Heparin		15/15	17/20	12/20	8/20	2/20			
Cystamine					19/22	16/20	8/20	2/18	0/15

The results of calculation of DRA by probit-logarithmic analysis are set out in Table 2.

Table 2 Radioprotective efficacy of heparin and cystamine in mice

Line of mice	LD _{50/30} , Gy	DRF	
		Heparin	Cystamine
White inbred mice	5.8	1.16	1.47
BALB/c mice	5.0	1.20	1.50
Hybrid (CBA × C57Bl) F ₁ mice	6.9	1.06	1.48

Sublethal irradiation led to decreases in the thymus mass in BALB/c and hybrid (CBA × C57Bl) F₁ mice (Table 3). Thymus mass changes in mice were biphasic. At the same time, the degree of the secondary decrease in the thymus mass depended on radioresistance of mice. On the 20th day after irradiation, the thymus mass in BALB/c mice and hybrids was reduced by fac-

tors of 3 and 2, respectively. Upon heparin injection from day 5 to day 9 after irradiation, the post-irradiation pattern of the thymus mass recovery in BALB/c mice was the same as in hybrid F₁ mice. The data obtained suggest that heparin activates genetically determined reserves of radioresistance of animals.

Table 3 Influence of heparin on post-irradiation pattern of thymus mass recovery of irradiated mice with different degrees of radioresistance (%)

Group	Dose, Gy	Day after irradiation			
		12	16	20	30
BALB/c mice					
Saline	4.0	68.8±7.1	44.0±7.8	32.0±3.4	59.7±6.2
Heparin		57.0±6.1	53.4±5.6	49.2±5.2 *	82.0±10.0
Hybrid (CBA × C57Bl) F ₁ mice					
Saline	5.0	62.3±5.9	82.8±5.9	50.7±5.8	82.7±9.5
Heparin		63.1±6.1	62.8±6.0	49.0±5.8	80.0±9.5

* p<0.05 in comparison with controls (irradiation with saline)

Heparin exerted a stimulating effect on the cell migration. A single injection of heparin doubled the number of CFU in 1 ml of peripheral

blood of intact BALB/c mice and reliably decreased it in the bone marrow (Table 4).

Table 4 Influence of heparin to the count of leukocytes and number of CFU in peripheral blood and bone marrow of BALB/c mice

Group	Leukocytes, × 10 ⁶ /l	Peripheral blood		Bone marrow
		CFU/10 ⁶ leukocytes	CFU in 1 ml	CFU/10 ⁵ myelocytes
Saline	7.6±0.4	5.2±0.6	39.0±4.0	17.0±0.6
Heparin	12.7±0.6 *	6.4±0.5	81.0±6.0 *	13.1±0.9 *

* p<0.01 in comparison with controls (irradiation with saline)

Further investigation revealed that a 5–6-fold increase in the number of leukocytes in the peripheral blood of mice injected with heparin was observed 24 hrs after irradiation.

DISCUSSION

Four structurally distinct GAG families can be recognized as follows: heparin/heparan sulfate, keratan sulfate, chondroitin sulfate/dermatan sulfate and hyaluronic acid (hyaluronan). Heparin/heparan sulfate participates in a variety of

biological processes, including cell-matrix interactions, cell adhesion, cell recognition and activation of chemokines, enzymes and growth factors. Heparin was shown to regulate acute and chronic inflammation, wound healing and tissue regeneration (Harmer 2006; Taylor and Gallo 2006; Lukashin and Grebenyuk 2010).

The present study indicated that pretreatment with heparin increased post-irradiation survival of radiosensitive BALB/c mice with DRF 1.2 (LD_{50/30} increased from 5.0

Gy to 6.0 Gy). Heparin increased the migration of haemopoietic cells and induced changes in the proliferation and differentiation of the stem cells in the bone marrow: the leukoerythroblastic ratio rose from 4.1 to 7.6 (Micklem and Ogden 1970).

Our finding on radioprotective efficacy of heparin agrees with those of other authors (Argutinskaya and Salganik 1963; Brückner 1976; Chaubal and Codbole 1983), supplement them and increase the sum of knowledge of the mechanism of action of this substance.

CONCLUSIONS

Radioprotective effects of heparin intraperitoneal injection in a dose of 250 unit/kg were developed within 1 day after the administration and maintained for periods up to 30 days.

In condition of whole body γ -irradiation, heparin increased survival rate only in radiosensitive BALB/c and inbred mice.

Heparin exerted stimulating effects on the migration of haemopoietic cells: the injection of the substance doubled the number of CFU in the peripheral blood of BALB/c mice and reliably decreased it in the bone marrow.

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