Physiol. Res. 40: 377–380, 1991

RAPID COMMUNICATION

Hemopoiesis Stimulating and Radioprotective Effects of Carboxymethylglucan

M. POSPÍŠIL, J. ŠANDULA[1], I. PIPALOVÁ, M. HOFER, Š. VIKLICKÁ

Institute of Biophysics, Czechoslovak Academy of Sciences, Brno and
[1]Institute of Chemistry, Slovak Academy of Sciences, Bratislava

Received February 13, 1991
Accepted May 21, 1991

Summary

Carboxymethylglucan, a novel soluble derivative of beta-1,3-glucan, was found to enhance hemopoietic recovery in sublethally gamma-irradiated mice and to increase survival in lethally irradiated animals when given 24 hours prior to irradiation. Postirradiation treatment with carboxymethylglucan also induced favourable effects in terms of survival when used in combination with preirradiation cystamine administration.

Key words:
Carboxymethylglucan – Gamma-irradiation – Hemopoiesis – Radioprotection – Cystamine

Extensive studies have shown that glucan, a beta-1,3-linked polyglucose, which is derived from the yeast *Saccharomyces cerevisiae*, is a broad-spectrum enhancer of host defence mechanisms against bacterial, viral, fungal and parasitic infections, and neoplasia (Di Luzio 1985). Glucan administration is followed by the proliferation of macrophages as well as by the release of various mediator factors producing an increase in all aspects of hemopoiesis, i.e. pluripotent hemopoietic stem cells, and granulocyte and erythroid progenitor cells (Patchen and MacVittie 1983). For these reasons glucan exhibits radioprotective ability (Patchen and MacVittie 1985, Pospíšil *et al.* 1982). Various preparations of glucans, differing according to the source of isolation, route of preparation and chemical structure, were already used. For practical reasons, soluble fractions of glucan may be preferable to particulate glucan in view of the inherent ease of parenteral administration and the lack of granulomatous reactions within the reticuloendothelial system, which are observed after particulate glucan administration (Di Luzio *et al.* 1979). The purpose of this study was to determine the feasibility of the novel soluble derivative carboxymethylglucan (CMG) to enhance recovery from radiation-induced hemopoietic depletion, and the ability of this glucan to increase survival of irradiated mice.

Conventional male mice (CBA x C57BL)F_1, aged three months, with an average body weight of 30 g were used. Pelleted sterilized standard diet (DOS–2 ST

Velaz) and HCl-treated tap water (pH 2–3) were given *ad libitum*. The mice were irradiated with single whole-body doses from a ^{60}Co gamma-ray source, at a dose rate of 0.4 Gy/min.

Beta-1,3-D-glucan was prepared from the cell walls of the yeast *Saccharomyces cerevisiae* at the Institute of Chemistry of the Slovak Academy of Sciences, Bratislava. The extraction of the yeast biomass was made with diluted alkalis and the subsequent treatment with diluted acids according to the method of Manners *et al.* (1974). Insoluble glucan was solubilized by carboxymethylation using monochloroacetic acid (Pastýr *et al.* 1986). A crude sample of the CMG–Na salt obtained was fractionated by stepwise precipitation with acetone. In these experiments the main fraction II was used which had a molecular weight of 7.63×10^5 Da estimated by gel permeation chromatography (Horváthová *et al.* in press). The degree of substitution with carboxymethyl group per anhydroglucose unit was 0.91. CMG–Na (1 or 2 % solution in saline) was administered intraperitoneally, in volumes of 0.2–0.5 ml. Saline was used for control injections.

Cystamine dihydrochloride was diluted in saline and administered by means of a probe perorally 30 min prior to irradiation, at a dose of 7.5 mg per mouse, in a volume of 0.3 ml.

Nucleated cells in the bone marrow and spleen were determined by a Coulter Counter after washing off the marrow of femoral diaphyses and crushing the spleen tissue. For histological examination the spleens were embedded in paraffin, the sections were stained with hematoxilin-eosin, and microscopic evaluation of endogenous spleen colonies was performed. For differential counting of cell lines bone marrow smears were stained by the May-Grünwald and Giemsa-Romanowski method. In lethally irradiated mice deaths were recorded up to the 30th day after exposure.

Statistical significance was evaluated using the distribution-free sequential test and the χ^2 test.

The results of experiments evaluating the effects of 5 mg CMG administered 24 h prior to irradiation on the femoral bone marrow and spleen cellularity on days 4–12 after a sublethal exposure (7 Gy) are given in Fig. 1. The interval of 24 h for preirradiation CMG administration was chosen in accordance with previously achieved results suggesting the suitability of this treatment protocol for evaluating the radioprotective action of glucans (Patchen and MacVittie 1985, Pospíšil *et al.* 1982). The postirradiation time intervals used reflect the phase of recovery of the radiation-induced dip in bone marrow and spleen cellularity (femoral cellularity of unirradiated mice being approximately 20×10^6, that of the spleen 140×10^6). As shown, CMG treatment enhanced cell repopulation of both organs. An earlier manifestation of the stimulatory effect in the femoral bone marrow could be conditioned by the higher ability of bone marrow stem cells to undergo radiation repair as compared to that of the spleen (Lewis *et al.* 1977). Such a situation can lead to an earlier effectiveness of proliferation pressure in the bone marrow as opposed to the spleen (Pospíšil *et al.* 1981). Morphological examination of the spleen on day 10 after 6 Gy revealed enhanced endogenous erythroid colony formation in glucan-treated animals. Differential counting of femoral bone marrow cells at the same interval has shown elevation of all the elements of erythroid, granuloid and lymphoid lines in glucan-treated mice (data not given).

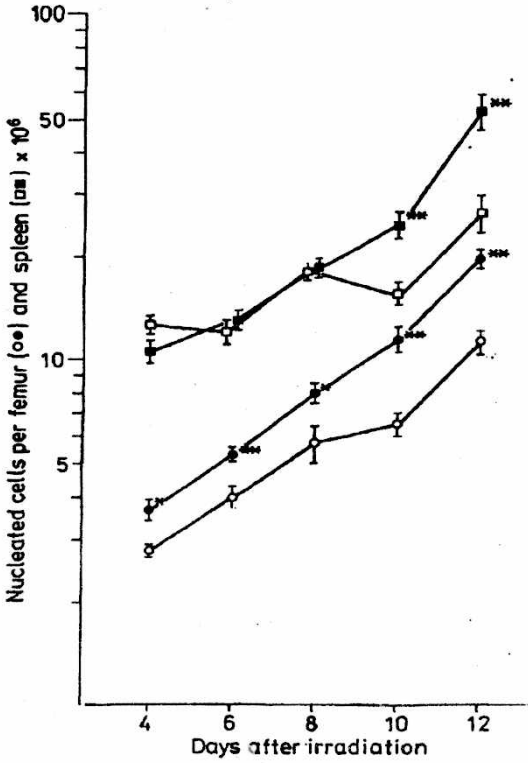

Fig. 1
Nucleated cells per femur and spleen at various intervals after irradiation (7 Gy) in control mice (open symbols) and mice treated with 5 mg CMG 24 h before irradiation (full symbols). Ten animals per group were used. Data are means ± S.E.M. Statistical significance as compared to controls: * $p<0.05$, ** $p<0.01$

Table 1
Survival of irradiated mice treated with carboxymethylglucan (CMG)

Radiation dose	Cystamine administration	CMG administration	n	30-day survival	Significance (vs controls)
9.5 Gy	–	–	80	2.5 %	–
9.5 Gy	–	2 mg – 24 h before irradiation	40	10.0 %	n.s.
9.5 Gy	–	4 mg – 24 h before irradiation	40	20.0 %	$p<0.01$
10 Gy	7.5 mg – 30 min before irradiation	–	118	30.5 %	–
10 Gy	7.5 mg – 30 min before irradiation	2 mg – 2 and 24 h after irradiation	39	48.7 %	$p<0.05$
10 Gy	7.5 mg – 30 min before irradiation	2 mg – 2, 24 and 48 h after irradiation	40	62.5 %	$p<0.001$

Hemopoiesis-stimulating action of CMG could be of importance in overcoming the lethal consequences of the radiation hemopoietic syndrome. The effects of the preirradiation CMG treatment on the survival of mice subjected to a lethal radiation dose of 9.5 Gy are given in Tab. 1. A statistically significant increase in the 30-day survival was achieved using 4 mg CMG. Previous observations indicate that postirradiation glucan treatment is less effective (Pospíšil *et al.* 1982), probably due to its action at the level of depleted stem cell population, and thus a lower effectiveness of the stimulatory action. Such unfavourable situation can be partially overcome by the simultaneous use of thiol compounds, acting *via* mechanisms such as free-radical scavenging and hypoxia, thus decreasing the radiosensitivity of hemopoietic stem cells and inducing their higher postirradiation survival (Patchen *et al.* 1989). Our results, given in Tab. 1, are in accordance with such expectation. They demonstrate a slight protective effect of cystamine given alone (10 Gy is the absolute lethal dose). A statistically significant increase of 30-day postirradiation survival, as compared to mice protected with cystamine alone, was observed when administering two or three injections of CMG after irradiation.

The results of the experiments performed thus suggest that the novel soluble glucan derivative tested exhibits favourable action in conditions of a radiation hemopoietic syndrome. Radioprotective effects of CMG seem to be comparable with the efficacy of glucan preparations of other provenience (Pospíšil *et al.* 1982, Patchen and MacVittie 1985, Patchen *et al.* 1989).

References

DI LUZIO N.R.: Update on the immunomodulating activities of glucans. *Springer Semin. Immunopathol.* **8**: 387–400, 1985.

DI LUZIO N.R., WILLIAMS D.L., MCNAMEE R.B., EDWARDS B.F., KITAHAMA A.: Comparative tumor-inhibitory and anti-bacterial activity of soluble and particulate glucan. *Int. J. Cancer* **24**: 773–779, 1979.

HORVÁTHOVÁ M., ŠOLTÉS L., LUTÓNSKÁ H., ŠANDULA J., MISLOVIČOVÁ D.: Study of water soluble biopolymer beta-1,3-glucan behaviour under dimethylsulfoxide denaturation. *J. Appl. Polymer Sci.* (in press).

LEWIS J.P., O'GRADY L.F., GREEN R.A.: Patterns of exogenous and endogenous hematopoietic repopulation following radiation injury. *J. Lab. Clin. Med.* **89**: 229–239, 1977.

MANNERS D.J., MASSON A.J., PATTERSON J.C.: The heterogeneity of glucan preparations from the walls of various yeasts. *J. Gen. Microbiol.* **80**: 411–417, 1974.

PASTÝR J., ŠANDULA J., MASLER L., TRNOVEC T., BENEŠ L.: *Czechoslov. Patent Appl.* PV 3945/86, 1986.

PATCHEN M.L., MACVITTIE T.J.: Dose-dependent responses of murine pluripotent stem cells and myeloid and erythroid progenitor cells following administration of the immunomodulating agent glucan. *Immunopharmacology* **5**: 303–313, 1983.

PATCHEN M.L., MACVITTIE T.J.: Stimulated hemopoiesis and enhanced survival following glucan treatment in sublethally and lethally irradiated mice. *Int. J. Immunopharmacol.* **7**: 923–932, 1985.

PATCHEN M.L., MACVITTIE T.J., JACKSON W.E.: Postirradiation glucan administration enhances the radioprotective effects of WR–2721. *Radiat. Res.* **117**: 59–69, 1989.

POSPÍŠIL M., NETÍKOVÁ J., KOZUBÍK A.: Comparison of cell proliferation in the regenerating bone marrow and spleen of irradiated mice with the use of ^{125}I–iododeoxyuridine. *Acta Radiol. Oncol.* **20**: 373–378, 1981.

POSPÍŠIL M., JARÝ J., NETÍKOVÁ J., MAREK M.: Glucan-induced enhancement of hemopoietic recovery in gamma-irradiated mice. *Experientia* **38**: 1232–1234, 1982.

Reprint Requests
Dr. M. Pospíšil, Institute of Biophysics, Czechoslovak Academy of Sciences, CS–612 65 Brno, Královopolská 135.