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Effects of Immunomodulators on Postirradiation Recovery in the Thymus

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Summary

The effect of immunomodulatory agents on reparation processes in the thymus was studied in mice injured by a single sublethal or lethal dose of ionizing radiation ranging between 6.5–9.5 Gy. Reparation of thymus weight was not influenced by pretreatment with immunomodulators. Furthermore, the morphological picture did not exhibit appreciable differences between non-protected and protected groups, except for greater proliferation of fibroblasts and macrophages in protected animals.

Key words
Thymus – Irradiation – Immunomodulators – Reparation

Introduction

Increasing knowledge of the functional importance of the mammalian thymus has caused an increased interest in the morphology of this organ in general.

Studies of histopathological changes in the thymus following acute X-irradiation have emphasized the rapid and extensive destruction of lymphocytes in the cortex and to a lesser extent in the medulla. Several investigators have described a biphasic pattern of thymic regeneration as measured by changes in thymic weight (Takada *et al.* 1969, Decleve *et al.* 1972, Sharp and Thomas 1975) and quantitative cell counting techniques (Petrakis 1956, Blomgren and Revesz 1968, Sato and Sakka 1969).

The thymus has been shown to have several important functions. It is well established that the thymus is required for the normal development of the immune system. Numerous papers have dealt with the influence of T cells on the differentiation and proliferation of the haematopoietic tissue (Hršák 1973, Burek *et al.* 1977, Goodman *et al.* 1978, Lepault *et al.* 1981). The effect of thymus and T-lymphocytes as well as that of their extracts on erythropoietic and granulopoietic activity were also documented (Roland 1964, Albert *et al.* 1965, Yokke and Sainte-Marie 1965, Goodman and Shinpock 1968, Goodman and Grubbs 1970).

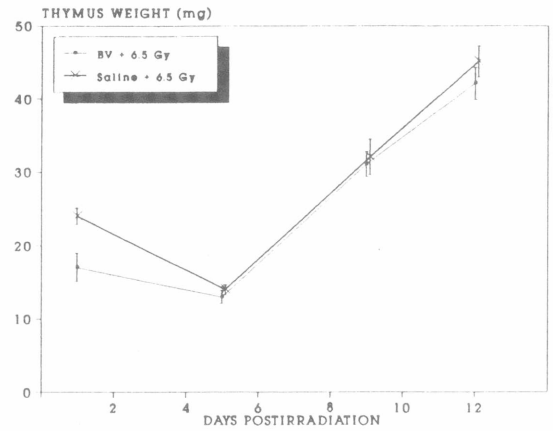
The data about the effects of pre- or postirradiation application of immunomodulatory agents on reparation processes in the thymus are sporadic (Pospíšil *et al.* 1986, Morrissey *et al.* 1988).

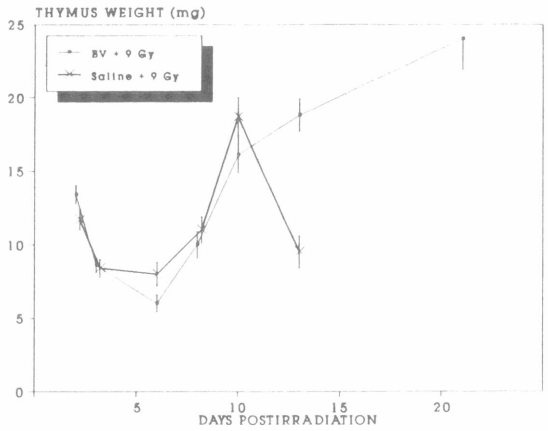
The present paper deals with reparation processes in the thymus of irradiated animals after application of immunomodulatory agents.

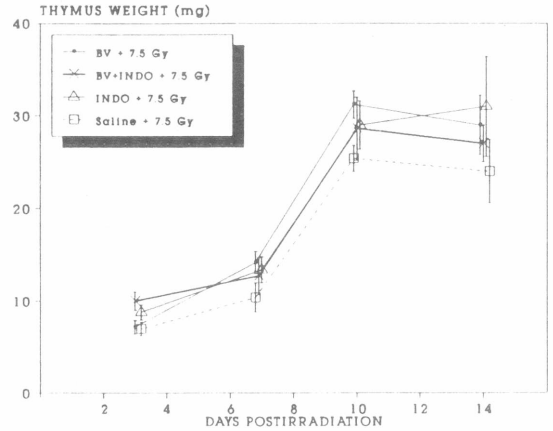
Materials and Methods

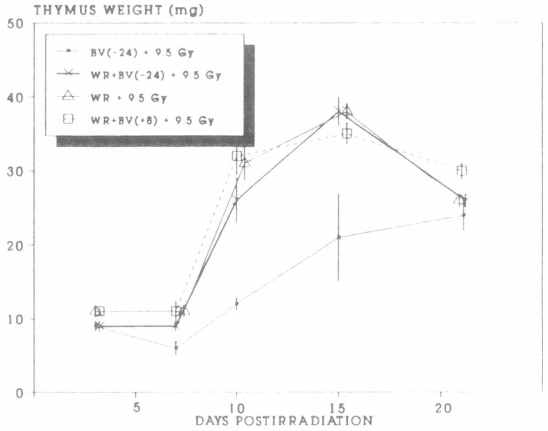
Mice

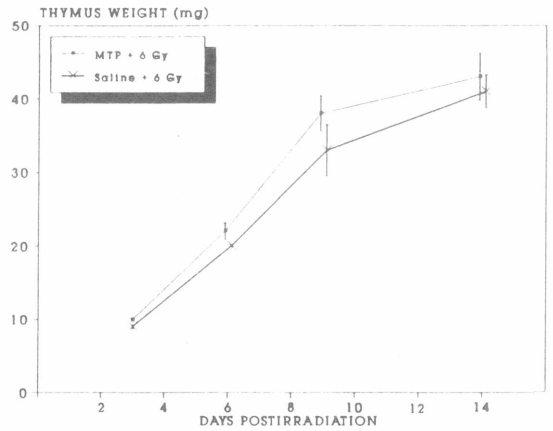
Female C57Bl/6 mice, 8–10 weeks old, were obtained from Velaz (Prague, Czech Republic). Animals were quarantined for a period of 2 weeks. They were housed in rodent cages, five to seven animals per cage at about 22 °C, and were given Velaz/Altromin 1320 St laboratory chow and tap water acidified to pH 2.4 *ad libitum* (Toropila *et al.* 1996). Research was conducted according to the principles enunciated in the "Guide for the Care and Use of Laboratory Animals", prepared by the State Veterinary Office of the Slovak Republic, Bratislava.

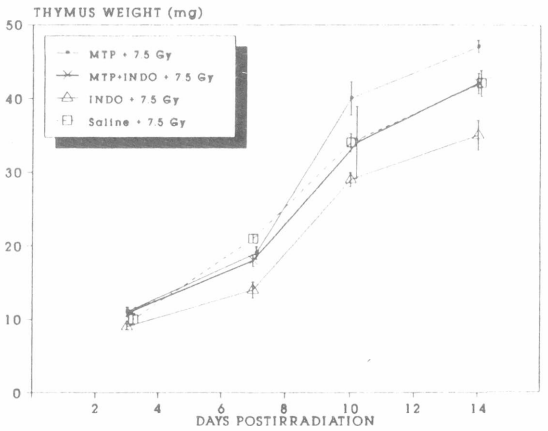












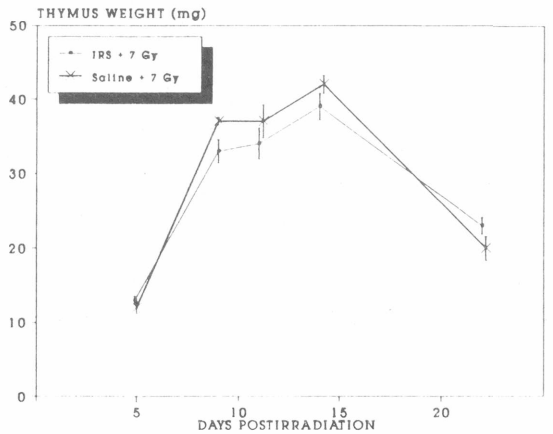


Fig.1 *Effects of various immunomodulators alone and their combinations with indomethacin (INDO) or WR-2721 on thymus weight in irradiated mice. Values represent the mean weight ± S.E.M. from two different experiments of 7 – 10 mice/group/experiment.*

Irradiation

Mice were exposed to 6.5–9.5 Gy of single whole body gamma rays (^{60}Co source) at a dose rate of 0.3 Gy/min. The Chisostat (Chirana, Czech Republic) therapeutic apparatus was used for all irradiations.

Treatment with drugs

Broncho-Vaxom (BV) (Biogal Pharmaceutical Works, Debrecen, Hungary, under licence from OM Laboratoires, Geneva, Switzerland) is a lyophilized extract of the eight most common bacteria of the upper respiratory tract (Fedoročko *et al.* 1992) and free of endotoxins (less than 0.0002 % by Limulus and pyrogenicity tests; Bottex *et al.* (1988)). Immediately before use, the drug was resuspended in saline in a volume of 0.4 ml and administered intraperitoneally (i.p.) 24 h before or 8 h after irradiation in a dose of 25 mg/kg.

Liposomal muramyl tripeptide phosphatidylethanolamine (MTP-PE/MLV, CGP 19835A; Ciba-Geigy Ltd., Basel, Switzerland) is a synthetic muramyl tripeptide coupled to dipalmitoylphosphatidylethanolamine and it is N-acetylmuramyl- L-alanyl- D-isoglutaminyl- L-alanine- 2-[(1,2-dipalmitoyl- sn-glycero-3-(hydroxy-phosphoryloxy)] ethylamide monosodium salt encapsulated in a multilamellar liposome. The drug was administered i.p. 24 h before irradiation in a dose of 10 mg/kg MTP-PE encapsulated in liposomes.

IRS-19 (Laboratoires de Thérapeutique Moderne, L.T.M. - Sarbach, Suresnes Cedex, France) is antigenic bacterial lysate of eight bacteria (*Diplococcus pneumoniae, Streptococcus, Micrococcus pyogenes/Staphylococcus, Gafkya tetragena, Neisseria, Klebsiella pneumoniae, Moraxella, Haemophilus influenzae*). The drug was administered i.p. 24 h before irradiation in a volume 0.6 ml per mouse.

WR-2721 was synthesized by C. Krajčovič (Kuna *et al.* 1983) according to Piper *et al.* (1969). Approximately 30 min prior to irradiation, mice were i.p. injected with 200 mg/kg WR-2721 in a volume of 0.5 ml.

Indomethacin (INDO; Sigma, St. Louis, USA) was prepared by dissolving 10 mg in 1 ml of 95 % ethyl alcohol. This solution was then diluted to a working concentration with Dulbecco's phosphate-buffered saline and injected i.m. at 2 mg/kg (in a volume of 0.2 ml) 24 and 3 h before irradiation.

Control animals received saline in the same volume and at the same time as the treated groups.

After decapitation of the mice, the thymus was removed, weighed, and then processed using routine histological methods. Slides of the thymus were stained with haematoxylin-eosin. The statistical significance of the differences between thymus weight of protected and non-protected irradiated groups was evaluated by the t-test.

Results and Discussion

Ionizing radiation induces the development of various morphological and physiological changes. The lymphoid and haematopoietic tissue are two systems which are at the greatest risk from radiation damage.

In the non-protected and protected groups, thymus weight decreased to almost 75–80 % of the weight of control non-irradiated animals (52±3 mg) by days 3–6 (Fig. 1). Histologically, the well-known initial phase of destruction and depletion of thymocytes was seen in all the experimental animals. The only difference was that clusters of fibroblasts in the subcapsular zone. Macrophages in the cortex and medulla of protected animals were more frequently seen on days 5–6 after irradiation. Regeneration started on subsequent days and the thymus weight increased to a maximum on days 10–14 after irradiation. The pattern of the repopulated parenchyma was very similar to that of the normal thymus. Subsequently, as judged by both weight and histological appearance, the thymus undergoes secondary atrophy from 14 to 23 day after IRS 19 and combined administration of WR-2721 with Broncho-Vaxom (Fig. 1). The cortex and the medulla were again found to be poorer in thymocytes. The rapid decrease of thymus weight from days 10 to 15 in single irradiated mice with 9.0 Gy (saline-treated mice) is the result of irreversible damage of total haematopoiesis. This was one of the reasons causing gradual mortality of all mice by day 15.

As follows from this study that the reparation of thymus weight was not influenced by pretreatment with immunomodulators. Similar findings concerning the response of thymus weight were observed by Pospíšil *et al.* (1986) after postirradiation administration of the non-steroid anti-inflammatory drug indomethacin, a potent inhibitor of prostaglandin synthesis. Morrissey *et al.* (1988) observed a similar response of lymphoid tissues of the thymus and spleen after prolonged administration of interleukin 1 (IL-1) in irradiated animals. This IL-1 therapy retarded the regeneration of thymic cellularity.

It has been well recognized that various cell types, including fibroblasts, release colony-stimulating factors (Zucali *et al.* 1986, 1987). It is generally believed that thymic macrophages also have more functions than just elimination of dying cells. These cells as well as the thymus epithelial cells (Le *et al.* 1987) release soluble factors such as IL-1, which is known to be active in promoting thymocyte proliferation (Gery and Waksman 1972). As follows from our other results, analysis of the morphological picture did not reveal appreciable differences between non-protected and protected groups except for greater proliferation of fibroblasts and macrophages in protected animals.

Since the factors produced by macrophages, fibroblasts, endothelial and reticular cells play an important role in thymus physiology, it can be assumed that the thymus could also be affecting reparation processes of haematopoiesis in other organs *via* a humoral mechanism.

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