

RADIOPROTECTIVE EFFECTS OF MADIOL AND LEUCOTROFINA STUDIED BY THE DYNAMIC CHANGES OF SOME METABOLIC PROCESSES IN THE THYMUS AND THE LIVER OF X-IRRADIATED RATS

BY

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Male Wistar rats were treated with Madiol, irradiated with a single dose of 400 R(X) and injected p.r. with Leucotrofina (active polypeptides isolated from the calf thymus). Dynamic changes of thymus and liver glycogen, lipid and protein biosynthesis showed remarkable radioprotective effects of these substances, which stimulate the recovery processes in whole-body X-irradiated rats.

Anabolic steroids enhance resistance to total body X-irradiation and have moderate radioprotective effects [3], [6]. Some thymus extracts, such as thymosin and leucotrofina (a cell-free calf thymus extract containing active polypeptides), which were able to stimulate lymphocytopoiesis and to increase the immunological competence, were also found to be effective radioprotective agents [9], [11], [12], [13], [14].

The aim of this study was to investigate the effect of treatment with Madiol and Leucotrofina on the metabolism of the thymus and the liver by whole body X-irradiated rats. In an earlier work [6] we reported that some changes in nucleic acid and protein metabolism induced by X-irradiation of mice whole body could be prevented by a.r. administration of Madiol.

MATERIAL AND METHODS

Male Wistar rats weighing 130–160 g were fed with standard food at room temperature. The animals were exposed to whole body X-irradiation with a single dose of 400 R and killed on the 3th, 8th and 15th day after exposure. Other group of animals received a.r. 30 mg Madiol (Biofarm, Bucharest) per 100 g body weight (*per os*) for a period of 30 days.

After irradiation the animals were treated with 1 ml Leucotrofina (Ellem-Spa, Milano) per 100 g body weight. Leucotrofina was injected i.m. after 1 hr and every second day until 3, 8, 15 days.

Glycogen content was determined with Montgomery's technique [7]. Protein concentration was determined with Lowry's method [5]. Some animals received 1 hr before killing 2 μ Ci of (2-¹⁴C) acetate (sodium salt) and the rate of incorporation of radiocarbon into proteins and lipids was determined after extraction of these substances from tissues with the aid of Folch's technique [4], dissolving the proteins in 30 per cent

KOH and in Bray's solution and the lipids in T-fluor solution. The specific radioactivity of these substances was determined at 10°C using a liquid scintillation spectrometer (BF-5003). The results were evaluated by Chauvenet's and Student's statistical methods.

RESULTS

The results obtained showed that thymus glycogen content increased significantly after 3 and 15 days of exposure (Table 1). Liver glycogen

Table 1

Changes of glycogen content in the thymus and the liver of X-irradiated and treated rats. (Concentration of glycogen is expressed in mg per 1 g fresh tissue)

	Control	X-irradiated group			M-X-L-group		
	Days	3	8	15	3	8	15
X	0.96	1.26	1.06	1.60	1.14	1.25	1.07
±SE	0.05	0.11	0.08	0.14	0.07	0.12	0.06
D %	—	+31.25	+10.42	+66.67	+18.75	+30.21	+11.46
P	—	<0.01	>0.25	<0.001	>0.10	>0.5	>0.25
X	12.85	39.49	30.78	69.26	25.58	24.75	31.04
±SE	1.35	3.45	4.96	9.88	6.04	2.76	1.60
D %	—	+207.32	+139.53	+438.99	+99.07	+92.61	+141.56
P	—	0.001	0.01	0.001	0.05	0.001	0.001

content increase after exposure was more accentuated in comparison with the thymus glycogen under the influence of irradiation, suggesting a strong stress effect of the ionizing radiation.

The treatment with Madiol and Leucotrofina caused no change of glycogen content in the thymus and only a moderate increase in the liver after irradiation in comparison with the non-irradiated control (Table 1).

The rate of ($2\text{-}^{14}\text{C}$) acetate incorporation into the thymus lipids showed the same dynamic change as observed in the case of thymus glycogen: after 3 days the rate of incorporation of radiocarbon into lipids increased with 198.57 per cent, after 8 days the value was appropriated to the control value, and after 15 days an increase of 176.81 per cent was observed (Fig. 1). It is important to note that the rate of biosynthesis of lipids in the liver increased only after 8 days of exposure with 130.32 per cent (Fig. 1). Lipid biosynthesis in the thymus of treated animals did not change in comparison with the control, but the rate of incorporation of radiocarbon into lipids in the liver enhanced about five times after 8 days. After 15 days no significant difference of lipid biosynthesis in the liver in comparison with the control (fig. 1) was noticed.

Proteins concentration and their biosynthesis in the thymus and the liver of irradiated and treated animals were studied by the determination of the total protein concentration and of the rate of conver-

sion of (2-¹⁴C) acetate into proteins synthesized *de novo*. The results obtained showed that X-irradiation caused a significant decrease of protein concentration in the thymus of irradiated animals. In the thymus after 3 days of exposure a significant decrease (−22.91 percent) was observed, and the concentration of proteins remained under the control

Fig. 1. — Dynamic changes of the incorporation rate of (2-¹⁴C) acetate into lipids of the thymus and the liver of rats after X-irradiation (a) and treatment with Madiol + Leucotrofina (b) in comparison with the control (c).

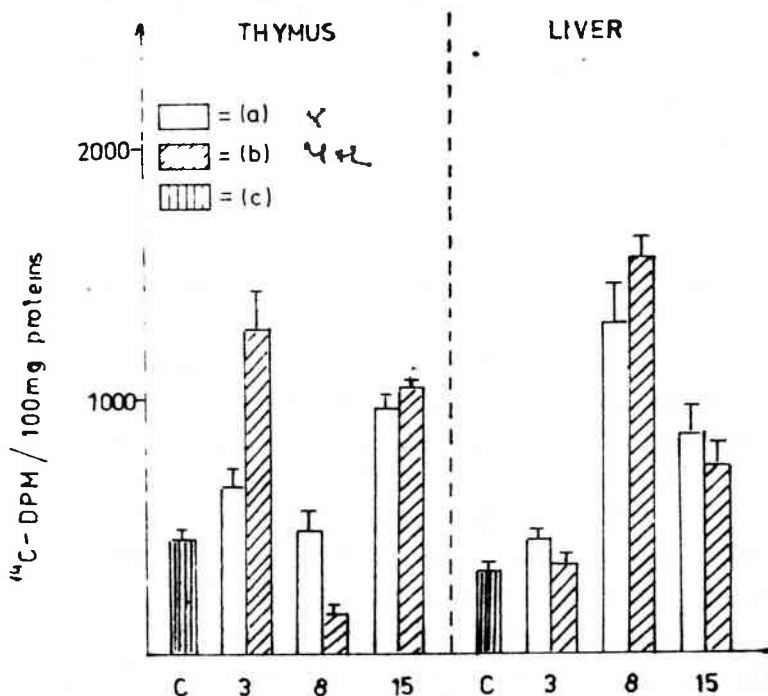
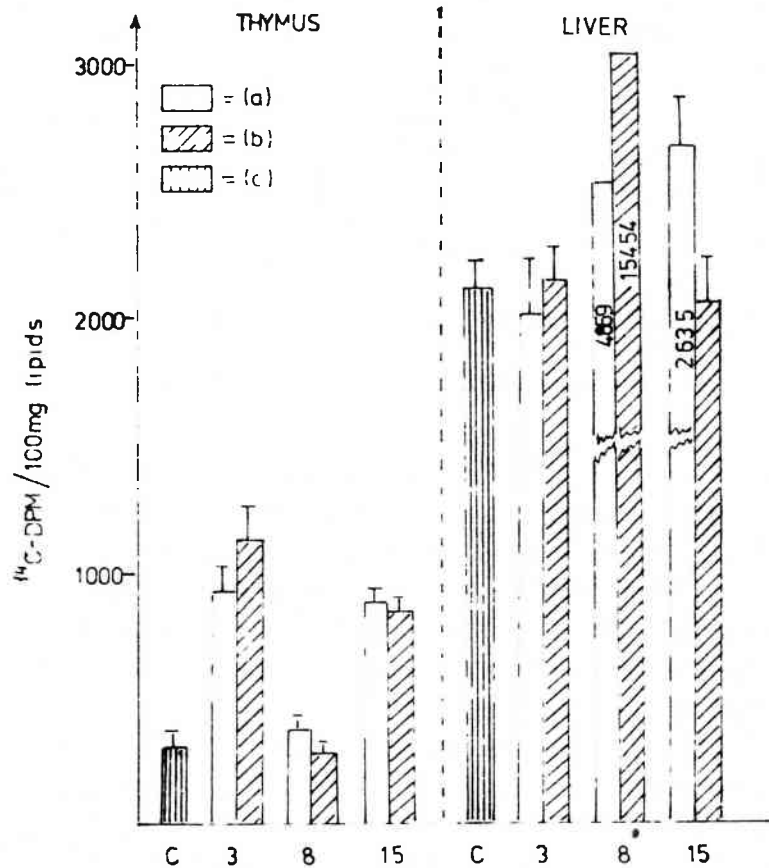


Fig. 2. — Dynamic changes of the conversion rate of (2-¹⁴C) acetate into proteins of the thymus and the liver of rats after X-irradiation (a) and treatment with Madiol + Leucotrofina (b) in comparison with the control (c).

Table 2

Changes of protein concentration in the thymus and the liver of X-irradiated and treated rats. (Protein concentration is expressed in mg per 1 g fresh tissue)

	Control	X-irradiated group			M-X-L-group		
	Days	3	8	15	3	8	15
X	186.34	143.65	169.95	171.03	162.35	186.23	226.45
± SE	2.01	5.46	2.44	5.09	8.89	11.38	11.66
D %	--	- 22.91	- 8.80	8.22	- 12.87	- 0.06	+ 21.53
P	-	< 0.001	< 0.001	< 0.01	< 0.02	> 0.5	< 0.001
X	258.78	244.77	232.91	204.40	228.91	241.68	259.53
± SE	7.96	12.08	7.16	19.59	13.21	13.67	15.96
D %	--	- 5.41	- 9.99	21.01	11.54	- 6.61	+ 0.29
P	-	> 0.5	< 0.02	< 0.02	> 0.5	> 0.25	> 0.5

X = arithmetical media, SE = standard error, D % = per cent difference as against control, P = Student test

M-X-L-group = Madiol treated, irradiated and Leucotrofina treated rats.

value after 8 and 15 days of exposure too (Table 2). A significant decrease of protein concentration in the liver of irradiated rats was observed after 8 and 15 days. No change of protein concentration in the liver was registered by the Madiol + Leucotrofina treated and irradiated animals. In the case of the thymus after 3 days the value of protein concentration remained under the control value, but after 15 days an increase of 21.53 per cent was observed.

The conversion rate of (2-¹⁴C) acetate *via* ¹⁴C-labelled amino acids into proteins of the thymus and liver showed similar dynamic changes as it was observed in the case of lipid biosynthesis, after whole body X-irradiation of rats (Fig. 2). Madiol + Leucotrofina caused stimulation of protein biosynthesis especially on the 3th day of exposure and a decrease on the 8th day. 15 days after exposure the stimulation of protein biosynthesis in comparison with non-irradiated controls is also noticed.

DISCUSSIONS

Several investigators claimed that anabolic steroids offer some protection against X-irradiation. The pretreatment with these substances is allegedly much more efficacious in this respect than the treatment after exposure [2]. In rats, anabolic steroids reduce the body weight loss and increase survival following X-irradiation [1], [3], [6], [10]. Following the treatment with more radioprotectors, their additive effects were observed. With the view to increase the moderate radioprotective effects of anabolic steroids, we proposed that after a pretreatment with Madiol, the administration of a lymphostimulatory biological extract (Leucotrofina) after exposure could have stimulatory action on the recovery systems [11], [12], [13]. This calf thymus non proteic extract, rich

in active polypeptides, stimulates the maturation of bone marrow cells and the increase of leukocytes under circulation [15].

Our results showed an evident stimulation of the recovery processes in the thymus and the liver of X-irradiated animals, and demonstrated clearly that Madiol + Leucotrofina administered a.r., respectively p.r., prevented the changes of glycogenoneogenetic pathways and the protein and lipid biosynthesis induced by irradiation.

Some authors elaborated hypotheses concerning the action of anabolic steroids which may be mediated through the adrenals [10] and inhibition of the catabolic action of endogenous glucocorticoids which are secreted in elevated concentrations after exposure [10]. Our results showed that administration of an anabolic steroid and a lymphostimulatory factor to irradiated animals could prevent some biochemical alterations induced by radiation energy at the level of the lymphatic system and of the liver, by their stimulatory action exerted on the cellular recovery systems and by the inhibition of catabolic steroids action.

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