

THE EFFECT OF X-RADIATION ON RETICULO- ENDOTHELIAL SYSTEM AND ITS TREATMENT WITH RADIODETOXIFIED-ENDOTOXIN AND TRACE ELEMENTS IN RATS

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A new in vivo method has been developed for the precise observation of RES activity. Both *Escherichia coli* endotoxin 100 yg/100 g i.v. (LPS) and radiodetoxified endotoxin 100 pg/100 g body weight i.v. (RD-LPS, TOLERIN) both increased the granulopoietic activity of RES. The RD-LPS was more effective. The preparation containing trace elements also increased the activity of RES. The treatment consisting of the use of both trace elements and RD-LPS proved to be the most effective. The activity of RES was inversely proportional to various doses of X-ray irradiation (7, 8, 9 Gy). Trace elements and RD-LPS even improved the immunity system of animals having deteriorated RES.

Phagocytosis by cells of reticuloendothelial system (RES) is an essential defense mechanism against infection. The fixed macrophages lining the vascular compartment constitute an important group of cells of the RES. The majority of these macrophages are located in the sinusoids of the liver and spleen. Activity of these cells can be investigated with, "clearance-tests" when collides are administered intravenously and the rate of clearance of particles from the blood is measured [1]. The effect of X-ray and ethanol consumption on the activity of RES with clearance-test has been described in the literature. Early studies [2] demonstrated the importance of the RES in prevention of radiation-induced bacteriaemia. Benacerraf et al. [3] reported that exposure of rats to 850 R of X-irradiation inhibited phagocytic recovery from carbon-induced phagocytic blockade. Di Luzio [4] observed that the rate of clearance of a tracer dose of colloidal gold was not affected by radiation, although tissue distribution of the collide was altered. Saba et al. [5] demonstrated that whole body X-irradiation with 800 R markedly impaired the phagocytosis of a gelatinized ¹³¹I-labelled triolein in adult rats. But other studies reported that irradiation stimulates the RES [6, 7] or has no effect at all [8, 9].

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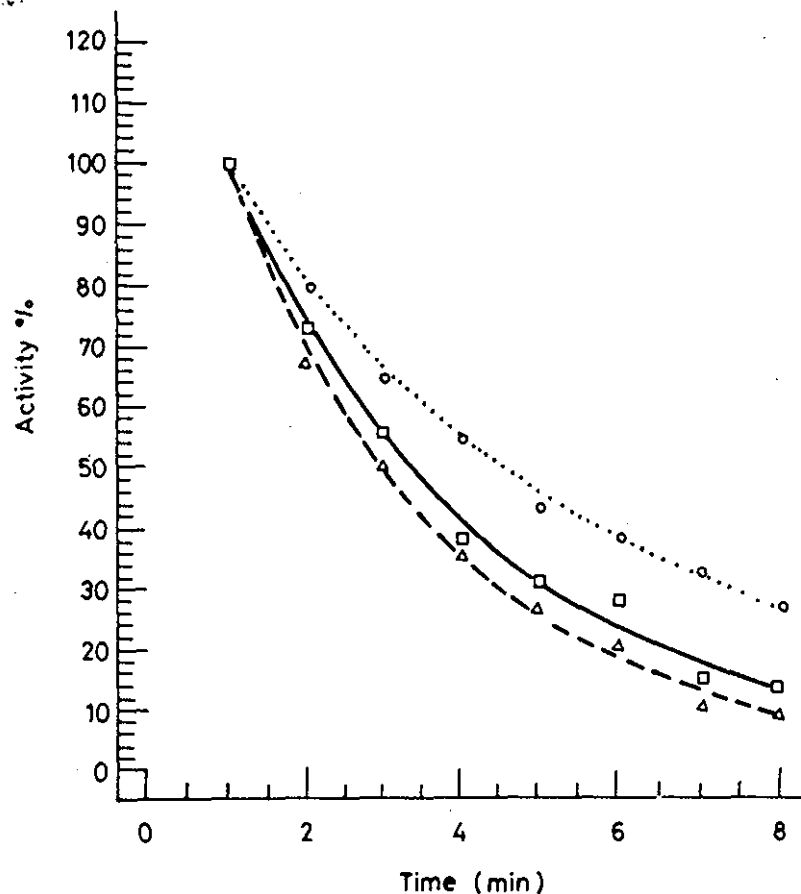


Fig. 1. The effect of endotoxin (LPS) on clearance curve of 50 µg ^{99m}Tc labelled nano-albumin microcolloid. Endotoxin was injected i.v. 24 h before the RES study. □ K_{control} : 0.2928 ± 0.0122 $T_{1/2}$: 2.37 min; △ $K_{\text{LPS } 100 \mu\text{g}}$: 0.3371 ± 0.0124 $T_{1/2}$: 2.05 min; ○ $K_{\text{LPS } 200 \mu\text{g}}$: 0.1883 ± 0.0078 $T_{1/2}$: 3.68 min

In the present study we observed the effect of various materials and interventions on RES in vivo. We used radiodetoxified endotoxin (RD-LPS) [10-12] and/or a special preparation containing trace elements [13] to moderate these damaging effects of whole body X irradiation on RES of rats. There are no publications about the effects of combination of these interventions and treatments on the reticuloendothelial system.

Materials and methods

Animals. We used 170 female Wistar rats from LATI (Gödöllő) weighting 140-150 g. They were fed with granulated chow (LATI, Gödöllő) and tap water ad libitum. Animals used this study were maintained and used in accordance with the "Guide for the Care and Use of Laboratory Animals" (Hungarian Academy of Sciences).

Preparation of microaggregated nanoalbumin. The microaggregated ^{99m}Tc labelled nanoalbumin (NANOALBUMON) was prepared in "Frederic Joliot-Curie" National Research Institute for Radiobiology and Radiohygiene. The particle size was 50-80 nm.

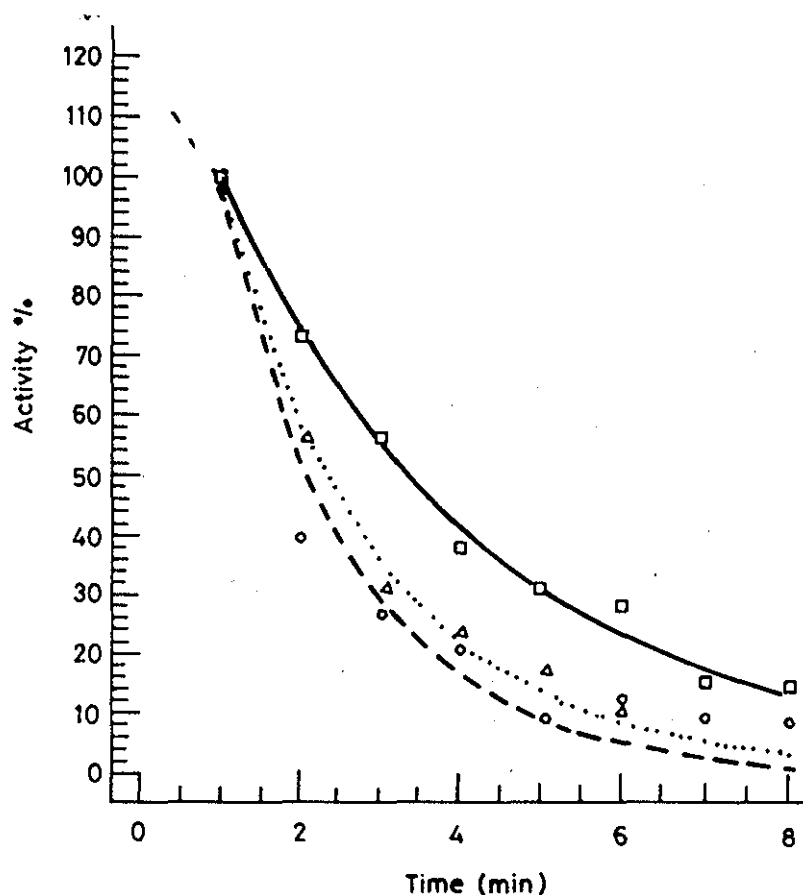


Fig. 2. The effect of radiodetoxified endotoxin (RD-LPS) on clearance curve of 50 µg ^{99m}Tc labelled nano-albumin microcolloid. RD-LPS was injected i.v. 24 hours before the RES study. □ K_{control} : 0.2928 ± 0.0122 $T_{1/2}$: 2.37 min; △ $K_{\text{RD-LPS 100 } \mu\text{g}}$: 0.4928 ± 0.0495 $T_{1/2}$: 1.41 min; ○ $K_{\text{RD-LPS 200 } \mu\text{g}}$: 0.5749 ± 0.0924 $T_{1/2}$: 1.20 min

Preparation of endotoxin. The *B. coli* endotoxin (LPS) was prepared in our laboratory according to a modified method of Westphal et al. [14].

Preparation of radiodetoxified endotoxin. The radiodetoxified endotoxin (RD-LPS, TOLERIN) was prepared in our laboratory from *B. coli* endotoxin irradiated with 150 kGy ^{60}Co gamma-ray [15].

Trace element treatment. A special trace element preparation (Béres Drops Plus; Béres Inc., Hungary) was given to 4 groups of animals through a probe at doses of 28 drops (2 ml) every day for 4 weeks. The trace element preparation contains Fe, Zn, Na, Mg, K, Cu, Mo, V, Ni, B, F, Cl, Co, glycerol, EDTA, L-(+)-tartaric acid, succinid acid, L-(+)-ascorbic acid.

Radiation. The whole body irradiation of the animals was made with a THX-251 X-ray device at 200 kV, 20 mA, 60 cm focus-body-middle distance, 0.5 mm Cu screening. Dose output, 0.498 Gy/min. The groups received 7, 8 or 9 Gy X-ray doses.

Experimental groups. 1.1. Control. 1.2. 100 µg LPS injected i.v. 24 h before the RES activity study. 1.3. 200 µg LPS injected i.v. 24 h before the RES activity study. 1.4. 100 µg RD-LPS injected i.v. 24 h before the RES activity study. 1.5. 200 µg RD-LPS injected i.v. 24 h before the RES activity study.

* 2.1. Control. 2.2. Trace element treatment for 4 weeks before the experiment. 2.3. 100 µg RD-LPS injected i.v. 24 h before the RES activity study. 2.4. Combination of trace element and RD-LPS treatment

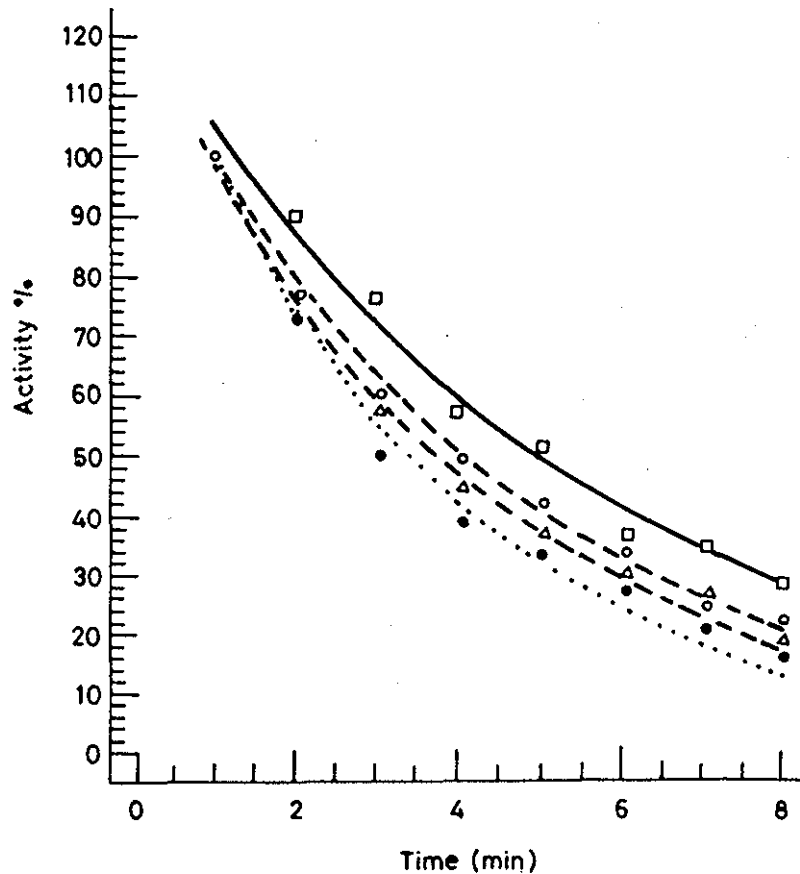


Fig. 3. The effect of RD-LPS and trace elements on clearance curve of 50 µg ^{99m}Tc labelled nano-albumin microcolloid. □ K_{control} : 0.1874 ± 0.0109 $T_{1/2}$: 3.70 min; ○ $K_{\text{RD-LPS } 100 \mu\text{g}}$: 0.2223 ± 0.0072 $T_{1/2}$: 3.12 min; △ $K_{\text{trace elements}}$: 0.2312 ± 0.0098 $T_{1/2}$: 3.00 min; ● $K_{\text{RD-LPS+ trace elements}}$: 0.2670 ± 0.0159 $T_{1/2}$: 2.59 min

3.1. Control. 3.2. 9 Gy X-ray 4 days before the RES activity study. 3.3. 8 Gy X-ray 4 days before the RES activity study. 3.4. 7 Gy X-ray 4 days before the RES activity study.

4.1. 8 Gy X-ray 4 days before the RES activity study. 4.2. 8 Gy X-ray 4 days, 100 µg RD-LPS 1 day before the RES study. 4.3. Trace element treatment for 4 weeks and 8 Gy X-ray 4 days before the RES study. 4.4. Combination of trace element and RD-LPS treatment with 8 Gy X-ray.

Experimental method. Animals were anaesthetized by Nembutal (0.004 g/100 g body weight). Both v. jugularis were prepared, a canule (1 mm I.D.) was led into one of the veins. The 50 µg ^{99m}Tc labelled nanoalbumin microcolloid was injected in the other vein. Eight blood samples were taken through the canule at 1 min intervals.

Calculation of results. The weight and the radioactivity of the blood samples were measured and a calculation was made for 1 g of blood. Regarding the first blood sample as 100% the other samples were compared to this. Data were expressed in diagrams and exponential curves were fitted by STATGRAPHIC computer software. We compared the different Granuloplectic Indices (K) [1]. The difference between these indices was subjected to a standard Mest ($p=0.05$, $T_{j/2}$ = half time).

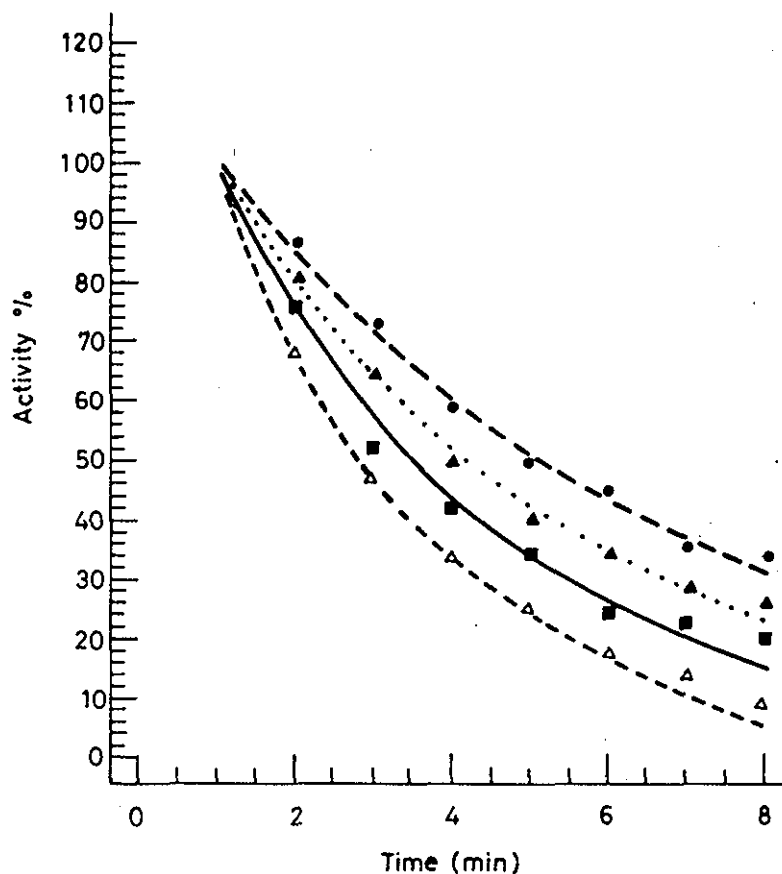


Fig. 4. The effect of X-ray on clearance curve of 50 µg ^{99m}Tc labelled nano-albumin microcolloid. RES was studied 4 days after the radiation. Δ K_{control} : 0.2255 ± 0.0108 control $T_{1/2}$: 3.07 min; \bullet $K_{9\text{ Gy}}$: 0.1193 ± 0.0175 $T_{1/2}$: 5.81 min; \blacktriangle $K_{8\text{ Gy}}$: 0.1381 ± 0.0142 $T_{1/2}$: 5.02 min; \blacksquare $K_{7\text{ Gy}}$: 0.1818 ± 0.0121 $T_{1/2}$: 3.81 min

Results

Comparing the clearance curves of the first main group on Figs. 1 and 2, it can be seen that the whole dose of LPS and RD-LPS had a different effect. All 100 µg and 200 µg RD-LPS doses increased RES activity, but without a significant difference in effect. Whereas the low dose of LPS increased RES activity, the high dose decreased it. Endotoxin had a toxic effect in contrast to RD-LPS. There was a significant difference between these groups.

All three treatments (RD-LPS, trace elements and the combination of them) increased the granulopoietic activity of the RES. There are significant differences between the Granulopoietic Indices of the four groups (Fig. 3).

The effect of the different X-ray doses is shown in Fig. 4. All the 7, 8, 9 Gy X-ray radiation doses decreased RES activity. This effect is directly proportional to X-ray doses.

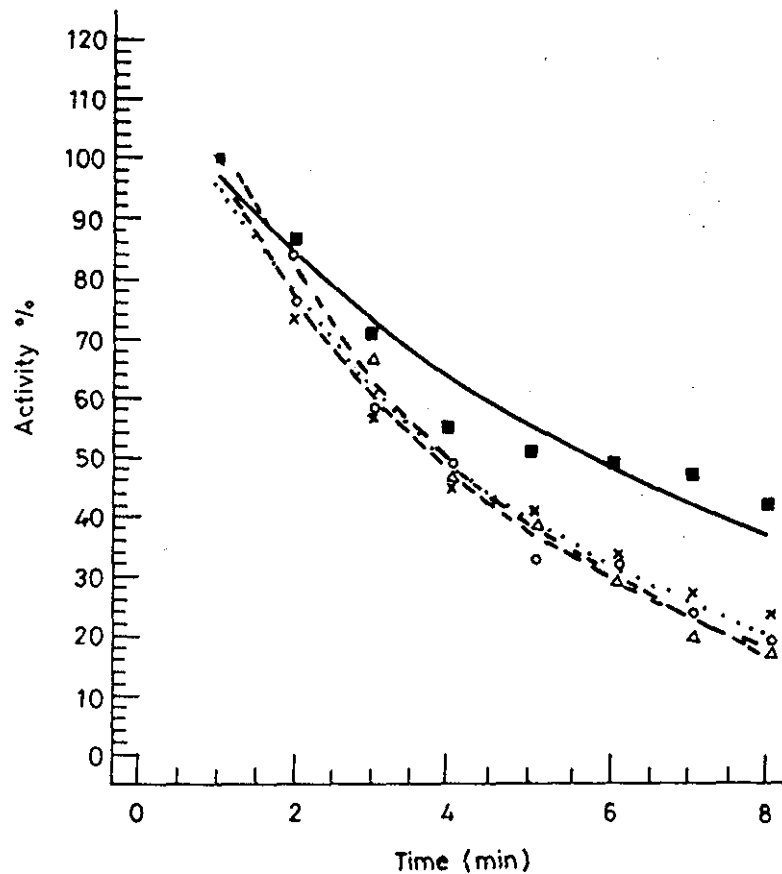


Fig. 5. The effect of X-ray with RD-LPS and trace elements treatment on clearance curve of 50 µg ^{99m}Tc labelled nano-albumin microcolloid. ■ $K_8 \text{ Gy}$: 0.1381 ± 0.0142 $T_{1/2}$: 5.02 min; ○ $K_8 \text{ Gy} + \text{RD-LPS}$: 0.2422 ± 0.011 $T_{1/2}$: 2.86 min; △ $K_8 \text{ Gy} + \text{trace elements}$: 0.2461 ± 0.0108 $T_{1/2}$: 2.82 min; × $K_8 \text{ Gy} + \text{RD-LPS} + \text{trace elements}$: 0.2232 ± 0.0137 $T_{1/2}$: 3.11 min

We tried to moderate the RES-damaging effect of X-ray. Figure 5 shows that both RD-LPS and trace elements preparation had an advantageous effect. They could activate the RES functions in animals weakened by ethanol or X-ray.

Discussion

We developed a new method to investigate the granuloplectic activity of RES. This method causes less pain to the animals but it is very simple and allows the taking a lot of blood sample in contrast to other bloodsampling methods (from tail or sinus retroorbitalis).

We investigated some RES-injuring factors and managed to moderate these damaging effects by RD-LPS and trace element treatment.

The 8 Gy X-ray exerts an effect on the gastrointestinal tract [2] and, as a result, endotoxin enters the circulation. We modelled this effect in our first experiment.

Endotoxin has a membrane-damaging effect so that it decreases the RES activity [11]. The radiodetoxified endotoxin lacks this injurious effect, in fact, it can increase the RES functions [12]. Animals treated with X-ray became weakened, they lost water and had dyspepsia. In contrast to the results of Fred et al. [6] or Antonojevic [7], in our experiments X-ray irradiation impaired the phagocytosis. However, when rats were given the essential trace elements, they could synthesize a lot of important enzymes so their phagocytic cells could function. The effect of the RD-LPS has not exactly known yet but it can also activate phagocytosis. These animals can show more resistance to other infections. Based on our experiments it may assumed that the radiodetoxified endotoxin and trace elements can activate phagocyte cells of reticulo-endothelial system weakened by X-ray irradiation. It could be a possible therapy to protect patients suffering in radiation sickness from other bacterial infections.

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