Effect of Reticuloendothelial Stimulation and Depression on Rare Earth Metal Chloride-Induced Splenic Calcification and Fatty Degeneration of the Liver

The calcifying effect of rare earth metals is well-known[1-4]. According to SNYDER et al.[5], intravenous administration of some of these metals, particularly cerium chloride, causes fatty degeneration of the liver.

Rare earth metals, injected parenterally, are localized in the liver, spleen and other organs rich in reticuloendothelial cells[6-8]. Since these metals markedly depress the activity of the RES[9-11], it seemed of interest to study the effect of reticuloendothelial stimulation and depression on the splenic calcification caused by gadolinium chloride and on the fatty infiltration induced by cerium chloride in the liver.

Materials and methods. For all our experiments, we used female Sprague-Dawley rats (Canadian Breeding Farm & Laboratories Ltd., St. Constant, Que.) averaging 110 g (range 100–120 g) and maintained ad libitum on Purina Lab Chow (Ralston Purina Co. of Canada) and tap water.

Reticuloendothelial stimulation was induced by 2.5 mg of zymosan (Fleischmann Laboratories) in 0.25 ml 0.9% NaCl, 2.5 mg of triolein (N.B.C.) in 0.25 ml 5% dextrose (containing 0.5% Tween 20 homogenized in a Waring Blender), and by 1 mg of BCG (Institut de Microbiologie et d'Hygiène, Université de Montréal) in 1 ml 0.9% NaCl. BCG was given on the 1st day, while the other 2 compounds were administered on the 1st and 2nd day, all through the tail vein. RES depression was induced by 100 mg of methylpalmitate (Eastman) in 1 ml 5% dextrose (containing 0.1% Tween 20 homogenized in a Waring Blender) and by 0.5 mg of gadolinium chloride in 0.5 ml water. Methylpalmitate was injected once on the 1st and twice on the 2nd day, whereas gadolinium chloride was given once on the 2nd day only, both through the tail vein (Table I).

Reticuloendothelial activity was estimated on the 3rd day in groups of 10 zymosan-, triolein-, methylpalmitate- and gadolinium chloride-treated rats, and on the 15th day in the BCG-treated batch. For this purpose, all the animals received 16 mg/100 g body weight of carbon (C11/1431a, Gunther Wagner, Hannover, Germany), intravenously, under pentobarbital anesthesia. Blood was withdrawn from the retro-orbital plexus at regular intervals, after which the logarithm of the carbon concentration was plotted against time and the value of the phagocytic index (K) calculated according to the method of BIOZZI et al.[12].

Rare earth metals (K. & K. Laboratories) were given to additional groups of zymosan-, triolein-, methylpalmitate- or gadolinium chloride-treated rats on the 3rd day, and to a BCG-treated batch on the 15th day. The chlorides of gadolinium and cerium were administered i.v. in 1 ml water at dose levels of 12 mg and 1 mg/100 g body weight, respectively. The animals of the gadolinium chloride group were killed with chloroform 3 days after the injection (Table II). At autopsy, splenic calcification was judged by loupe inspection and expressed in terms of an arbitrary scale of 0 = no lesion, 1 = just detectable, 2 = moderate and 3 = severe lesions[1]. For histologic examination, specimens of the spleen were fixed in alcohol-formol, embedded in paraffin and stained according to the von Kóssa technique for the demonstration of calcium salts. All cerium chloride-treated rats were killed 2 days after the injection. Here, specimens were taken from the liver, fixed in Ca-formol, and stained with Oil red O. Lipids were extracted according to the method of FOLCH et al.[13]. Total lipid content was determined gravimetrically.

Our results were biometrically evaluated by Student's *t*-test. In the Tables and in the Figure, the SD is presented as an expression of the observed variations. The 'Exact Probability Test' of Fisher and Yates was used to compare the incidence of splenic calcification.

Table I. Effect of reticuloendothelial stimulation and depression on the splenic calcification induced by gadolinium chloride

Treatment	Splenic calcification	
	Positive/total	Scale 0–3
$GdCl_3$	18/19	2.5 ± 0.1
Zymosan + $GdCl_3$	1/10 [b]	0.2 ± 0.2 [b]
Triolein + $GdCl_3$	0/9 [b]	0 [b]
BCG + $GdCl_3$	0/10 [b]	0 [b]
Methylpalmitate + $GdCl_3$	7/10 NS	1.3 ± 0.3 [a]
$GdCl_3$ + $GdCl_3$	10/10 NS	2.4 ± 0.2 NS

[a] $P < 0.01$, [b] $p < 0.001$; NS, not significant.

Table II. Effect of reticuloendothelial stimulation and depression on the cerium chloride-induced fatty degeneration of the liver

Treatment	Number of rats	Total lipid content (mg/g wet tissue)
None	9	48.97 ± 1.28
$CeCl_3$	10	74.38 ± 6.24 [b]
Zymosan + $CeCl_3$	10	76.74 ± 2.80 [c] (NS) [d]
Triolein + $CeCl_3$	10	72.09 ± 5.59 [b] (NS) [d]
BCG + $CeCl_3$	10	85.80 ± 1.06 [c] (NS) [d]
Methylpalmitate + $CeCl_3$	10	50.92 ± 3.47 NS(a) [d]
$GdCl_3$ + $CeCl_3$	10	85.72 ± 2.45 [c] (NS) [d]

[a] $p < 0.05$; [b] $p < 0.01$; [c] $p < 0.001$; [d] compared with group 2; NS, not significant.

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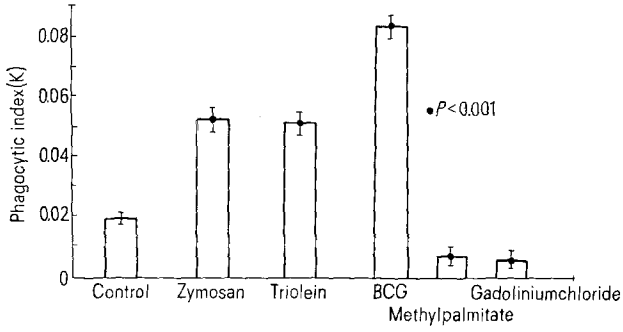
Results. In accordance with other investigations[14-17], zymosan, triolein and BCG markedly increased the granulopectic activity of the RES (Figure). Methylpalmitate[18] and gadolinium chloride caused the same degree of RES depression.

Intense splenic calcification was noted in the controls 4 days after treatment with gadolinium chloride (Table I). Histologically, calcified material was found in the marginal zone and in the red pulp of the spleen.

No calcification was evident in the triolein- or BCG-treated rats, ond only one positive von Kóssa reaction was noted among the animals given zymosan. The cerium chloride-induced fatty infiltration in the rat liver was prevented only by methylpalmitate (Table II).

Discussion. The mechanism through which reticuloendothelial stimulation protects against gadolinium chloride-induced splenic calcification is not yet fully understood. RES stimulation probably alters the distribution of gadolinium chloride. Apparently, the Kupffer cells, not the spleen, play a decisive role in reticuloendothelial stimulation by tumor growth and partial hepatectomy[19-22]. Di Luzio[23], during graft versus host reactions, and Munson et al.[24], during zymosan-induced reticuloendothelial stimulation, noted a depressed splenic uptake and a markedly enhanced hepatic uptake of I^{131}-lipid emulsion.

That the RES participates in lipid metabolism is well-known[25,26]. The prevention by methylpalmitate of cerium chloride-induced fatty livers is in accordance with the investigations of Gaillard et al.[27], who found that the increase of triglycerides and hepatic fatty acids, elicited in rats by ingestion of sodium selenite, ethanol, isopropanol, carbon tetrachloride, ethionine or DDT, is less marked when the RES is partially blocked by ethyl stearate. However, further investigations are needed to determine why gadolinium chloride did not protect against cerium chloride-induced fatty livers[28].



Reticuloendothelial activity in rats treated with zymosan, triolein, BCG, methylpalmitate or gadolinium chloride.

Résumé. Chez le rat, la calcification de la rate produite par le chlorure de gadolinium peut être prévenue par des activateurs de SRE tels que le zymosan, la trioléine et le BCG. Le chlorure de cérium produit dans le foie une dégénérescence graisseuse empêchée par le méthylpalmitate qui est un inhibiteur du SRE.

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The Effect of Semicarbazide Pretreatment on the Depressant Action of Barbiturates

The depressant action of barbiturates on spinal synaptic activity is well studied. Transmission across both monosynaptic and polysynaptic reflex arcs is depressed[1], while the amplitude and duration of the segmental dorsal root potential (DRP) is increased after smaller doses, and the time for half-decay further prolonged by larger doses[2]. The contribution of the barbiturate-induced increase in DRP to the depression of the ventral root discharge is difficult to assess. Furthermore, it is not known whether the action of barbiturates is mediated through some spinal inhibitory substance or whether it results from a direct action on neuronal elements. Since γ-aminobutyric acid (GABA) is present in the spinal cord[3] and exerts a potent depressant action on spinal neuronal activity when applied microelectrophoretically[4], while depolarizing afferent presynaptic terminals when added to the perfusing medium[2], the effects of pentobarbital (Nembutal) on spinal transmission were examined after depletion of GABA by semicarbazide, a compound which blocks the synthesis of this inhibitory amino acid[5].

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