Biochemical Pharmacology, Vol. 30, No. 16, pp. 2353–2355, 1981.
Printed in Great Britain.

0006-2952/81/162353-03 $02.00/0

SHORT COMMUNICATIONS

Alterations of essential metal levels and induction of metallothionein by carrageenan injection

(*Received* 28 *January* 1981; *accepted* 7 *March* 1981)

Since metallothionein (MT) was first isolated as a Cd- and Zn-binding protein from equine renal cortex [1], much attention on the protein has been focused on the protective role from toxic metals [2]. However, since it was reported that MT also plays an important role in Zn homeostasis [3], the protein has been widely investigated from this point of view and clarified to be induced as Zn-thionein not only by Zn loading but also by stresses [4], bacterial infections [5], alkylating agents [6], dexamethazone [7], indomethacin [8], endotoxin [9], hypersensitivity reaction [10], hepatectomy [11], and adrenalectomy [12]. Recently, it has been suggested that the induction of MT may be associated with an inflammatory reaction [13]. The last report prompted us to investigate the changes of essential metal levels and the induction of MT by an inflammatory drug.

Carrageenan is a sulfated polygalactan. Among the several biological effects of the drug, one of the most frequently used properties is its ability to induce inflammation [14].

The present paper deals with the changes of essential metal levels in tissues by the injection of λ-carrageenan, which is a non-gelling form and is most potent in inflammatory activity [15]. The metal concentrations were determined in a simultaneous multi-element analytical mode by a new spectrometer, inductively coupled plasma-atomic emission spectrometer (ICP-AES) [16]. The induction of hepatic MT was examined by the new analytical method consisted of a high speed liquid chromatograph equipped with a gel permeation column and an atomic absorption spectrophotometer (HLC-AAS) [17].

Materials and Methods

Ten-week-old female ICR mice were injected i.p. with a 0.25 ml solution of 1 per cent (w/v) λ-carrageenan (Sigma) and sacrificed by cardiac puncture 3, 6, 12, 18 hr, 1, 2, 3, and 4 days after the injection (6 mice/group). The liver (about 0.3 g portion), both kidneys, and whole spleen from each mouse were digested with 1 ml of mixed acid (HNO_3:$HClO_4$ = 5:1, v/v), and the solutions were diluted to 10, 10 and 5 ml with doubly distilled water, respectively. Metal concentrations were determined simultaneously by ICP-AES (Jarrell-Ash Model 975 plasma Atomcomp).

A 0.2 g portion of each liver from mice sacrificed 1 day after injections of λ-carrageenan and saline solutions were combined in each group and homogenized in 3 vol. of 0.1 M Tris-HCl buffer solution (pH 7.4, 0.25 M glucose) using a polytron homogenizer under ice-water cooling and nitrogen atmosphere. The homogenates were centrifuged at 170000 × *g* for 60 min. To detect the induced MT as Cd-thionein, $CdCl_2$ solution (1000 ppm, 10 μl) was added to 200 μl of each supernatant and the excess Cd was removed as Cd-containing denatured high mol. wt proteins by heat-treatment (80°, 5 min) and centrifugation (10000 × *g*, 1 min).

The outlet of a high speed liquid chromatograph (Toyo Soda HLC 803A) equipped with a gel permeation column (TSK GEL SW 3000 column, Toyo Soda, 7.5 × 600 mm with a precolumn (7.5 × 100 mm)) was directly connected to a nebulizer tube of a flame atomic absorption spectrophotometer (Hitachi 170-50A). A 100 μl portion of heat-

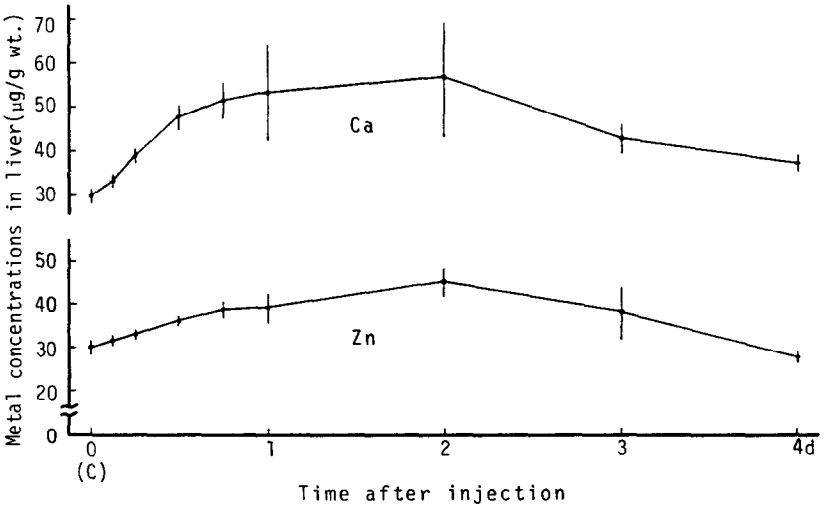

Fig. 1. Changes of Zn and Ca concentrations in liver after injection of λ-carrageenan. The animals were sacrificed 3, 6, 12, 18 hr, 1, 2, 3, and 4 days after the injection. Control animals were injected with saline solution and killed 1 day after the injection. Vertical bars indicate S.D. of the mean.

treated supernatant was applied and the column was eluted with 50 mM Tris-HCl buffer solution (pH 8.0) at a flow rate of 1 ml/min as reported previously [18].

Results and Discussion

Injection of λ-carrageenan induced the marked changes of essential metal levels especially in the liver and spleen. As shown in Fig. 1, Zn and Ca concentrations in the liver increased with time to maxima at 1 or 2 days after the injection and thereafter decreased progressively. The transitory increase of Zn concentration can be ascribed to the induction of MT, which was analyzed by HLC-AAS (Fig. 2). Recently, Sobocinski *et al.* have suggested that the synthesis of MT may occur as a sequela to inflammation [13]. Since carrageenan is a well-known inflammatory drug [14], the induction of MT in this study seems to occur due to the induced pathologic changes. When, in general, MT is induced by the single injection of heavy metals or organic chemicals, Zn concentration in the liver reaches its maximum within 1 day or so after the injection [19]. The delayed maximum observed in this study may be due to the viscosity of λ-carrageenan solution. Although it is not clear as yet whether or not the disturbed Ca homeostasis is a symptom of pathologic damages, the correlation between Zn and Ca suggests that the alteration in Ca concentration may result from the inflammation as the cause of MT induction.

Figure 3 shows the time-courses of the weight and four metal contents in the spleen after λ-carrageenan injection. The weight started to increase steeply from day 2, and Zn and Mg contents exhibited almost the same pattern as the weight. On the other hand, Ca content increased progressively up to day 1 and then showed a constant level (in concentration, the level showed almost the same pattern in the liver). Fe content decreased markedly after 6 hr post-injection and then stayed at a constant low level throughout despite the steep increase of the weight (in concentration, the level decreased continuously till day 4).

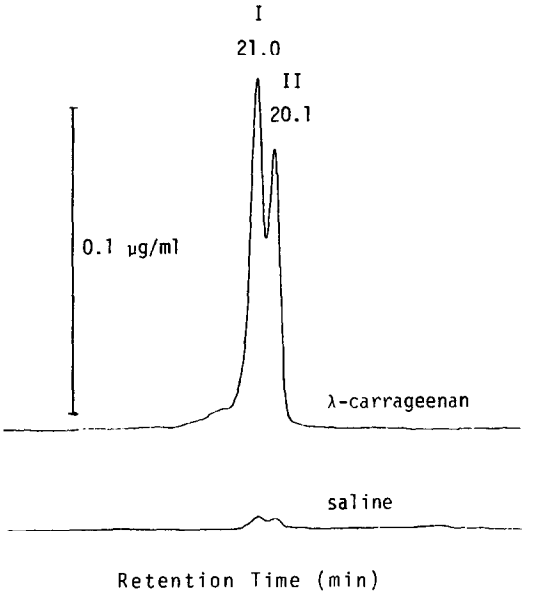

Fig. 2. Gel permeation-Cd atomic absorption chromatograms of liver supernatants after injections of λ-carrageenan and saline solutions. Mice were sacrificed 1 day after the injections. Livers were homogenized in three volumes of 0.1 M Tris-HCl buffer solution and the homogenates were centrifuged at 170,000 × *g* for 60 min. $CdCl_2$ solution was added to the original supernatants and the excess Cd was removed by heat-treatment and centrifugation. The detector level of atomic absorption spectrophotometer was set as indicated by a vertical bar. I and II indicate MT-I and -II, respectively [18].

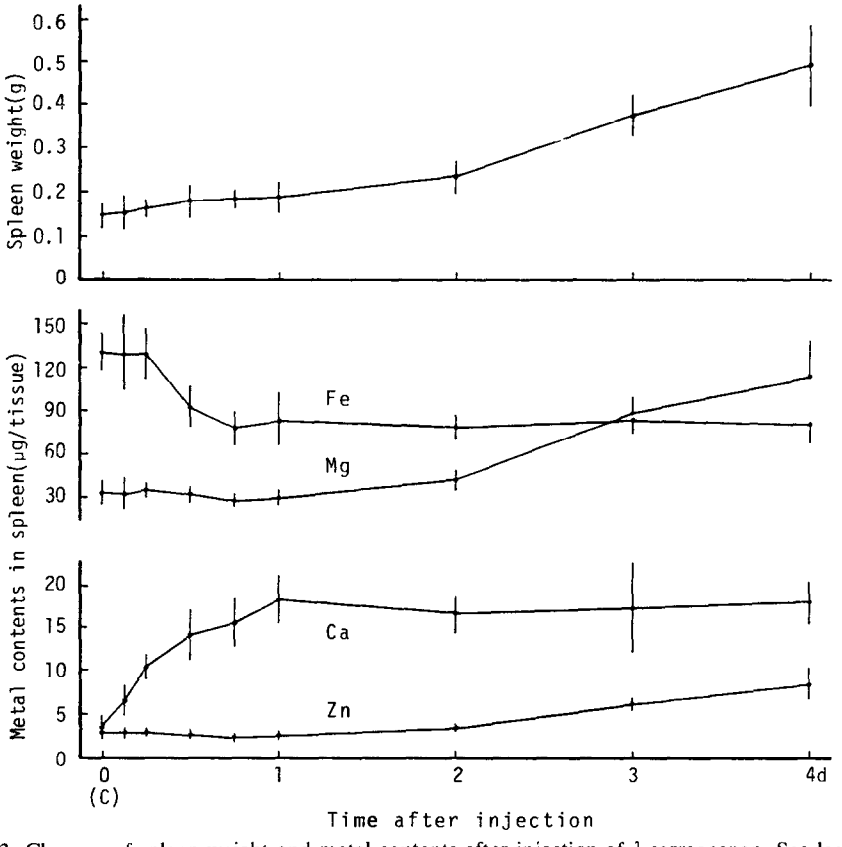

Fig. 3. Changes of spleen weight and metal contents after injection of λ-carrageenan. See legends in Fig. 1.

The above-mentioned marked alterations of essential metal levels are supposed to be closely related to the well-known immunosuppressive character of carrageenan [20]. Recently, we have reported the changes of essential metal levels and splenomegaly induced by Cd loading [21]. The shifts of metal levels were quite similar to those in this study. Therefore, it may be considered that the decreased Fe and increased Ca levels are common features in splenomegaly. Although the three metal (Zn, Ca, Mg) concentrations were almost recovered by day 4 despite the progressive increase of spleen weight, Fe level did not show any recoveries in concentration and content by the time. Since a large part of Fe in spleen arises from ferritin as the storage form of the metal [22], it is supposed that Fe level need not be recovered in a short period for animals.

Cu concentrations in the liver, kidneys, and spleen decreased by the injection (data not shown). Cu is also related to the inflammation processes. The increase of plasma Cu concentration as a result of accelerated synthesis and release of ceruloplasmin in liver is a commonly observed feature during various infectious or inflammatory diseases [23]. Therefore, the decreases of Cu concentration in the tissues may be, at least in part, attributable to the accelerated ceruloplasmin synthesis and release on inflammatory disease.

In summary, the injection of λ-carrageenan induced transitory increases of Zn (due to the induction of MT) and Ca levels in the liver. Both changes were almost parallel in time-course. In the spleen, Ca level increased successively up to day 1 and then showed a constant value in content or a progressive decrease in concentration, while Fe decreased and remained at a low level both in concentration and in content even at day 4, accompanying the progressive increase of the weight.

Acknowledgements—We thank Dr. K. Kubota for encouragement and Mr. M. Nishikawa for technical assistance on ICP-AES measurements.

National Institute for Environmental Studies
Yatabe Tsukuba
Ibaraki 305
Japan

TAMIO MAITANI
KAZUO T. SUZUKI

REFERENCES

1. M. Margoshes and B. L. Vallee, *J. Am. Chem. Soc.* **79**, 4813 (1957).
2. J. H. R. Kägi and B. L. Vallee, *J. biol. Chem.* **235**, 3460 (1960).
3. I. Bremner and N. T. Davies, *Biochem. J.* **149**, 733 (1975).
4. S. H. Oh, J. T. Deagen, P. D. Whanger and P. H. Weswig, *Am. J. Physiol.* **234**, E282 (1978).
5. P. Z. Sobocinski, W. J. Canterbury, Jr., C. A. Mapes and R. E. Dinterman, *Am. J. Physiol.* **234**, E399 (1978).
6. F. N. Kotsonis and C. D. Klaassen, *Toxicol. Appl. Pharmacol.* **51**, 19 (1979).
7. K. R. Etzel, S. G. Shapiro and R. J. Cousins, *Biochem. biophys. Res. Commun.* **89**, 1120 (1979).
8. P. Z. Sobocinski, G. L. Knutsen, W. J. Canterbury, Jr. and E. C. Hauer, *Toxicol. Appl. Pharmacol.* **50**, 557 (1979).
9. K. T. Suzuki and M. Yamamura, *Biochem. Pharmac.* **29**, 2260 (1980).
10. P. Z. Sobocinski, W. J. Canterbury, Jr., E. C. Hauer and F. A. Beall, *Proc. Soc. exp. Biol. Med.* **160**, 175 (1979).
11. H. Ohtake and M. Koga, *Biochem. J.* **183**, 683 (1979).
12. F. O. Brady and P. C. Bunger, *Biochem. biophys. Res. Commun.* **91**, 911 (1979).
13. P. Z. Sobocinski, W. J. Canterbury, Jr., C. A. Mapes, R. E. Dinterman, E. C. Hauer and F. B. Abeles, *Fedn Proc.* **37**, 890 (1978).
14. C. A. Winter, E. A. Risley and G. W. Nuss, *Proc. Soc. exp. Biol. Med.* **111**, 544 (1962).
15. E. Moore and R. W. Trottier, Jr., *Res. Comm. Chem. Path. Pharmacol.* **7**, 625 (1974).
16. V. A. Fassel and R. A. Kniseley, *Anal. Chem.* **46**, 1110A (1974).
17. K. T. Suzuki, *Analyt. Biochem.* **102**, 31 (1980).
18. K. T. Suzuki, M. Yamamura, Y. K. Yamada and F. Shimizu, *Toxic. Lett.* **7**, 137 (1980).
19. K. T. Suzuki and M. Yamamura, *Toxic. Lett.* **6**, 59 (1980).
20. L. Aschheim and S. Raffel, *J. Reticuloendothel. Soc.* **11**, 253 (1972).
21. K. T. Suzuki, Y. K. Yamada and F. Shimizu, *Biochem. Pharmac.* **30**, 1217 (1981).
22. K. T. Suzuki, T. Motomura, Y. Tsuchiya and M. Yamamura, *Analyt. Biochem.* **107**, 75 (1980).
23. W. R. Beisel, *Med. Clin. N. Am.* **60**, 831 (1976).