Biochemical Pharmacology, Vol. 26, pp. 279–282. Pergamon Press, 1977. Printed in Great Britain.

THE EFFECTS OF SPIRONOLACTONE ON THE BILIARY EXCRETION OF MERCURY, CADMIUM, ZINC, AND CERIUM IN RATS

KENICHI KITANI, YOSHIKO MORITA and SETSUKO KANAI
First Laboratory of Clinical Physiology, Tokyo Metropolitan Institute of Gerontology, Tokyo, Japan

(*Received* 18 *June* 1976; *accepted* 16 *August* 1976)

Abstract—The effect of spironolactone (Sp) pretreatment on the biliary excretion of intravenously injected heavy metals (mercury, cadmium, zinc and cerium) was investigated in rats, using five metal compounds (four inorganic metals in chloride form, and methyl mercuric chloride). The oral administration of Sp (5 mg/100 g) 1–3 hr before the bile excretion study increased the biliary recovery of mercury more than ten times over a period of 4 hr in rats injected with mercuric chloride, but did not increase the biliary excretion of the other three metals (Cd, Zn and Ce). Multiple-dose pretreatment (2 doses a day for 3 days) also increased the biliary excretion of mercury but much less than in the acutely treated rats. Cadmium excretion was significantly decreased by multiple dose pretreatment. Sequential nuclear imagings after intravenous injection of $^{197}HgCl_2$, demonstrated clear differences in the tissue distribution of mercury between control and Sp-treated rats.

Spironolactone (Sp) has been reported to protect animals against lethal damage caused by mercury [1], cerium [2], and cadmium [3] intoxication. It has been postulated [4] that a possible mechanism for the protective effect of Sp against mercury poisoning is the change of mercury distribution in the body tissues, particularly the decrease of mercury concentration in the kidney. Another possible mechanism is the enhancement of biliary excretion of mercury reported by Haddow *et al.* [5]. However, Garg *et al.* [6] and more recently Klaassen *et al.* [4] reported that the enhancement of mercury excretion did not occur in Sp-pretreated rats.

Thus, there is a clear discrepancy in the literature on the effect of Sp on the biliary excretion of mercury. Therefore, the present work was designed to determine if the biliary excretion of mercury can be enhanced by Sp. Bjondahl *et al.* [7] examined the fecal excretion of cerium in the rats chronically treated with Sp, but did not see any increase in the fecal excretion of cerium. However, they did not report on the effect of acute pretreatment with Sp. Since information for the other metals is lacking, the effect of Sp on the biliary excretion of cerium, cadmium, and zinc was also investigated.

MATERIALS AND METHODS

Male Wistar rats weighing 200–350 g were used throughout the study. Administration of Sp was performed in two ways.

1. Single dose administration: rats were given orally 5 mg/100 g body weight of Sp through a stomach tube in the form of a water suspension of Sp prepared from commercial tablets (Aldactone, G. D. Searle & Co., Chicago, Ill.). The injection of mercury and other heavy metals was performed 1–3 hr later.

2. Multiple dose administration: rats were given the same dose twice daily for three days and the biliary excretion study was performed on the fourth day, 16–20 hr after the last administration. $^{197}HgCl_2$, $^{203}HgCl_2$, $CH_3{}^{203}HgCl$, $^{141}Ce(NO_3)_3$, $^{115m}CdCl_2$, $^{65}ZnCl_2$ were purchased commercially. Rats were studied under pentobarbital anesthesia (4.5 mg/100 g ip) with additional small doses added as needed during the study. The common bile duct was cannulated (PE-10 tubing) through an abdominal incision and a saline solution of each metal compound (in chloride form) with its respective radioactive tracer (1–5 μCi) was injected intravenously (i.v.) through a femoral vein catheter. Each animal received 0.5 mg of metal. Bile was continuously collected in a polyethylene tube at 30-min intervals for the first hour and then continuously on the hour for the next 3 hr. The rectal temperature was maintained between 37–38° throughout the experiment by a heating lamp. The recovery of the injected metals in the bile was calculated from the radioactivities of the bile and the standard solution prepared from the isotope solution for injection. Radioactivities of isotopes for ^{203}Hg, ^{197}Hg, ^{141}Ce, and ^{65}Zn were determined according to their specific energy peak using a well-type γ-scintillation counter (Aloka, Tokyo). For the measurement of ^{115m}Cd, 20 μl of bile or standard solution containing ^{115m}Cd was dried in a metallic planchette, and the activity was measured by a gas flow counter. Sequential changes in the hepatic content of mercury in the control and Sp-treated rats were studied by the following two procedures. The liver was taken at 15 min, 2 hr and 4 hr after mercuric chloride injection. The liver was immediately perfused with saline through a portal vein catheter to eliminate the blood in the hepatic vessels. The sequential change in the mercury content in the liver was studied by counting the radioactivity of liver tissue. The sequential changes in the distribution of mercury in the liver and kidney was also studied in an *in vivo* system using a nuclear imaging device (Phogamma HP, Nuclear Chicago). One

For reprints contact: K. Kitani, Tokyo Metropolitan Institute of Gerontology, 35-2 Sakaecho, Itabashi-ku, Tokyo, Japan.

Table 1. Biliary recovery of metal compounds after spironolactone (Sp) pretreatment in rats

Metal compound	Sequential recovery in the bile (mean ±S.D., per cent of the injected dose) 0–30 min	30–60 min	60–120 min	120–180 min	180–240 min	Total
$HgCl_2$						
Control (3)	0.11 ± 0.04	0.14 ± 0.04	0.26 ± 0.06	0.18 ± 0.03	0.13 ± 0.02	0.82 ± 0.17
Sp(S) (8)	3.52 ± 1.74*	2.98 ± 1.07*	2.78 ± 0.86*	0.98 ± 0.23*	0.47 ± 0.14*	10.73 ± 3.89*
Sp(M) (5)	0.25 ± 0.10*	0.32 ± 0.10*	0.47 ± 0.11*	0.24 ± 0.03*	0.16 ± 0.02*	1.45 ± 0.30*
CH_3HgCl						
Control (3)	0.28 ± 0.06		0.35 ± 0.14	0.37 ± 0.15	0.35 ± 0.12	1.37 ± 0.46
Sp(S) (3)	0.34 ± 0.02		0.48 ± 0.10	0.55 ± 0.17	0.67 ± 0.12*	2.06 ± 0.38
$CdCl_2$						
Control (6)	3.71 ± 0.67	4.08 ± 0.35	5.34 ± 0.47	2.31 ± 0.24	0.63 ± 0.13	15.70 ± 1.49
Sp(S) (5)	3.59 ± 0.49	3.86 ± 0.67	5.09 ± 0.67	1.97 ± 0.35	0.40 ± 0.19*	14.91 ± 1.93
Sp(M) (4)	2.14 ± 0.81*	2.65 ± 1.85*	4.97 ± 0.89	1.78 ± 0.16*	0.41 ± 0.23*	11.84 ± 1.61*
$ZnCl_2$						
Control (3)	0.38 ± 0.11	0.44 ± 0.13	0.60 ± 0.19	0.35 ± 0.10	0.19 ± 0.05	1.96 ± 0.55
Sp(S) (3)	0.36 ± 0.16	0.42 ± 0.11	0.73 ± 0.27	0.36 ± 0.08	0.20 ± 0.06	2.10 ± 0.66
$CeCl_3$	($\times 10^{-2}$)†					
Control (3)	0.63 ± 0.07	1.87 ± 0.52	4.29 ± 0.99	3.78 ± 1.09	2.76 ± 0.72	13.33 ± 3.02
Sp(S) (3)	0.36 ± 0.07*	1.11 ± 0.25*	3.92 ± 0.23	3.75 ± 0.43	2.85 ± 0.76	11.99 ± 1.21

Sp(S) Single dose of Sp (5 mg/100 g) 1–3 hr prior to metal study. Sp(M) Multiple dose of Sp (2 doses a day for 3 days, the last dose was administered 16–20 hr prior to metal study). Number in parenthesis indicates the number of rats studied.

* Significantly different from respective control value ($P < 0.05$), by Student's t-test.

† Values for cerium are expressed at 10^{-2} (i.e., 0.63 = 0.0063).

hundred μCi of $^{197}HgCl_2$ was injected i.v. into a femoral vein in control and Sp-pretreated rats and the mercury distribution was sequentially monitored over a period of 2 hr by serial imagings.

RESULTS

Table 1 summarizes the recovery of the five metal compounds in the bile in the first 4 hr in control rats, rats pretreated with a single dose, and rats pretreated with multiple doses (only for $HgCl_2$ and $CdCl_2$) of Sp. In control rats, the 4-hr recovery rates varied widely, depending upon the injected metal compound; from 0.13 per cent for cerium chloride to 15 per cent for cadmium chloride. A significant increase in biliary excretion of mercury was observed in rats pretreated with either a single or multiple dose of Sp. Compared with control rats, the excretion rate of i.v. injected mercury (as $HgCl_2$) in rats pretreated with a single dose, was 35 times higher in the first 30 min, 21 times in the next 30 min and 11 times in the second hour. The total recovery of $HgCl_2$ in the first 4 hr was more than 12 times higher than the controls (10.73 ± 3.89 per cent vs 0.82 ± 0.17 per cent). The 3 days' successive treatment of Sp also produced a significant ($P < 0.01$) increase for biliary excretion of mercury. However the excretion rate (1.45 ± 0.30 per cent in 4 hr) was much less than that observed in rats immediately pretreated. The biliary excretion of mercury injected as methyl mercuric chloride was also increased by Sp administration. However, the difference from control value was not significant ($P > 0.05$). Sp pretreatment, did not increase the biliary excretion of the other three metals (cadmium, zinc and cerium). In fact, multiple-dose administration of Sp for 3 days even decreased the biliary excretion of cadmium, even though the bile flow rate and liver weight were both significantly increased in this group of rats compared with control rats. Table 2 indicates the hepatic mercury content after mercuric chloride injection expressed as per cent of the mercury dose in control rats and rats with acute Sp-pretreatment. The control group showed a gradual and continuous increase in mercury content in the liver for the first 4 hr, while the mercury content in the liver of rats given acute pretreatment of Sp rose very rapidly in the first 15 min and then decreased gradually thereafter. Thus, the greatest difference between control and acutely treated rats was observed in the 15-min liver sample (control; 7.34 ± 2.99 per cent vs Sp; 24.94 ± 4.49 per cent, $P < 0.01$). The bile to liver mercury concentration ratio was approximately 1.6 in the first hour in the rats with acute pretreatment, while it was 0.20 in the control rats.

Figure 1 shows the sequential imagings obtained with ^{197}Hg in the control and pretreated rats. The intensities of the imaging of the kidney and liver in control rats continuously increased during the observation period of 2 hr. However, at 15 min post injection, the intensity in the control liver imaging was lower than in the treated rat. In the treated rats, the liver contour was clearly observed with higher intensity even in the picture taken 15 min after injection.

Table 2. Sequential changes in mercury content in rat liver after i.v. injection of mercuric chloride expressed as per cent of the dose

	Time after mercury injection 15 min	2 hr	4 hr
Control rats	7.34 ± 2.99 (5)	17.90 (2)	21.07 (2)
Sp-pretreated rats*	24.94 ± 4.49 (4)	19.24 (2)	15.22 (2)

* Sp treated rats received a single dose of spironolactone (5 mg/100 g) 1–3 hr prior to mercury study.

Number in parenthesis indicates the number of rats studied.

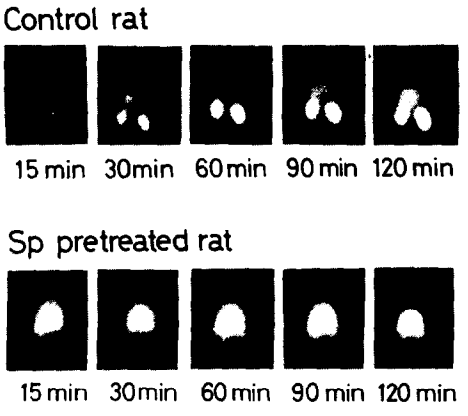

Fig. 1. Sequential nuclear imagings of liver and kidney with $^{197}HgCl_2$, in control and Sp-pretreated rats. In control rats, the liver and kidney uptake of ^{197}Hg was low at 15 min post i.v. injection but gradually increased for 2 hr after injection. At 2 hr post injection, the renal intensity became quite high compared with the treated rats. In Sp-pretreated rats, the liver intensity was much higher than control liver at 15 min post injection. Kidney intensity was lower than in the control rat throughout the period. This difference in kidney intensity was most marked in the pictures taken 2 hr after mercury injection.

The intensity of the liver image in treated rats was much higher than the control throughout the observation period. Conversely, the kidney image intensity was much lower in pretreated rats in comparison to the kidney images at corresponding time intervals in control rats. The biliary excretion of mercury in treated rats could not be clearly seen in the imagings in this study.

DISCUSSION

A 7 per cent liver accumulation of inorganic mercury at 15 min post injection and a 20 per cent accumulation at 4 hr observed in the control study, agree well with the values reported previously [4, 5]. According to the past literature [7–10], it seems that cadmium, zinc and cerium are also to a large extent taken up by the liver shortly after administration (20–50 per cent in the first few hr). Furthermore, there are evidences that a common binding protein in the liver, metallothionein, is involved in the metabolisms of mercury, cadmium and zinc [10–14]. However, the biliary excretion rate for these four metals ranged very widely (100 times as 4 hr biliary recovery). This result indicates that, at least quantitatively, there are great differences in the metabolisms of these metals in the biliary excretion system.

A single-dose Sp-pretreatment increased the biliary excretion of inorganic mercury more than ten times, while it did not increase the biliary excretion of the other three metals (Cd, Zn and Ce), which indicates that the action of Sp is specific to inorganic mercury only. The increase in biliary excretion of mercury by Sp is consistent with the report by Haddow *et al.* [5]. However, it does not agree with the results by Garg *et al.* [6] and Klaassen [4]. Garg *et al.* ligated the bile duct 24 hr before bile duct cannulation, and perhaps, this difference in the experimental procedure may explain why their results are different from ours. Klaassen [4] injected i.p. a propylene glycol suspension of pure Sp*, instead of oral administration of Aldactone tablet used by Haddow *et al.* [5] and ourselves. According to Sadée *et al.* [15], when Sp was injected i.v. in a polyethylene glycol suspension, most of the fluorogenic substances found in the plasma were Sp metabolites, namely canrenone and canrenoate, and unchanged Sp initially present in the plasma in a small amount disappeared within 30 min after injection. Sadée *et al.* also demonstrated [16] that with oral administration of Aldactone tablet unchanged Sp stayed in the plasma in much higher concentration for the first few hours. Therefore, Klaassen's results could be interpreted as revealing that none of these Sp metabolites, canrenone, canrenoate, or free thioacetate increases biliary excretion of mercury. If unchanged Sp in the plasma is assumed to play a significant role in the increase of the biliary excretion of mercury, then the discrepancy between the enhanced excretion observed by Haddow *et al.* [5] and by the authors in the present study and unchanged excretion reported by Klaassen [4] appears to be reasonably explained. In this regard, we confirmed that an i.p. injection of an ethylene glycol (or water) suspension of Aldactone was effective in increasing biliary excretion of mercury as an oral administration†. Therefore it is possible that the difference in the effect of Sp in the mercury excretion is not due to the difference in the procedure (injection vs oral administration) but in the material used. If it is, the results reported by Garg *et al.* [6] appear to be also explained on this basis, since they seem to have administered pure Sp orally.

Repeated administration of Sp is known to increase activities of various hepatic microsome enzymes and the bile flow rate (17–19). Such effects of Sp are considered to accelerate hepatic metabolisms of many drugs, such as cardiac glycosides [19, 20], and indomethacin [20], reducing the toxic effect of these drugs in Sp-pretreated animals [19–21]. However, in the case of inorganic mercury, repeated administration of Sp effected the biliary excretion of mercury to a much smaller extent than acute single-dose administration, indicating that the increased excretion is not due to the results of repeated administration of Sp.

The enhanced biliary excretion of mercury as demonstrated in the present study, might contribute to the protective effect of this drug has against mercury poisoning, as Haddow *et al.* [5] suggested. However, the decrease in mercury concentration in the kidney was produced by an i.p. injection of Sp without an appreciable increase in biliary mercury excretion [4]. Therefore, the decrease in mercury concentration in the kidney *per se* might be playing a significant role in the protection against mercury poisoning.

Biliary excretion of cadmium, zinc or cerium was not increased by Sp-pretreatment. Chronic treatment of Sp even caused some decrease in biliary excretion of cadmium. The decrease in fecal elimination of cerium in mice pretreated with repeated administration of Sp was also reported [7]. Therefore, for cadmium and cerium, other mechanisms might be responsible for the protective effect of Sp.

*Confirmed by personal communication.
†K. Kitani unpublished observation.

The projection of the protective effect of Sp on mercury poisoning documented for rats [1] to other species and ultimately to man requires further work. Nuclear imaging study not only agreed well with the *in vitro* measurement of the concentration of mercury in the liver but clearly demonstrated the decrease in renal accumulation of mercury in the Sp-pretreated rats, which also agrees with the previous *in vitro* studies [4, 5]. Thus, the nuclear imaging could be a simple and perhaps the only practical procedure to test this effect of Sp in man, where the rapid changes in the organ distribution of mercury can be only monitored with this kind of approach.

Acknowledgements—The nuclear imaging study in this work was performed in the Department of Nuclear Medicine and Radiological Sciences, Tokyo Metropolitan Geriatric Hospital. We wish to express our gratitude to Drs. M. Iio, H. Yamada, and K. Chiba, for their helpfulness in placing their facilities at our disposal. Dr. M. Togo kindly helped a part of this work. Mr. J. Ek who carefully reviewed the manuscript, and Miss Y. Ozawa who typed the manuscript are also gratefully appreciated.

REFERENCES

1. H. Selye, *Science* **169,** 775 (1970).
2. M. Bjondahl, M. Möttönen and L. Niemine, *Experientia* **29,** 685 (1973).
3. H. Abe, S. Urakabe, M. Shichiri, T. Suematsu, Y. Orita and A. Ando, *Sogorinsho* **23,** 123 (1974).
4. C. D. Klaassen, *Toxic. appl. Pharmac.* **33,** 366 (1975).
5. J. E. Haddow, C. A. Fish, P. C. Marshall and R. Lester, *Gastroenterology* **63,** 1053 (1972).
6. B. D. Garg, B. Solymoss and B. Tuchweber, *Arzneim. Forsch.* **21,** 815 (1971).
7. K. Bjondahl, B. Isomaa and L. Nieminen, *Biochem. Pharmac.* **23,** 1509 (1974).
8. A. H. Methfessel and H. Spencer, *J. appl. Physiol.* **34,** 63 (1973).
9. G. Magnusson, *Acta pharmac. toxic.* **20,** Suppl. 12, 1 (1963).
10. G. F. Nordberg, M. Piscator and B. Lind, *Acta pharmac. toxic.* **29,** 456 (1971).
11. I. Bremner, N. T. Davies, *Biochem. J.* **149,** 733 (1975).
12. Z. A. Shaikh, O. J. Lucis, *Archs environ. Health* **24,** 419 (1972).
13. J. K. Piotrowski, B. Trojanowska, J. M. Wiśniewska-Knypl and W. Boldnowska, *Toxic. appl. Pharmac.* **27,** 11 (1974).
14. J. K. Piotrowski, B. Trojanowska and A. Sapota, *Archs Toxic.* 32, 351 (1974).
15. W. Sadée, S. Riegelman and S. C. Jones, *J. pharm. Sci.* **61,** 1129 (1972).
16. W. Sadée, M. Dagcioglu and R. Schröder, *J. Pharmac. exp. Ther.* **185,** 686 (1973).
17. B. Solymoss, H. G. Classen and S. Varga, *Proc. Soc. exp. Biol. Med.* **132,** 940 (1969).
18. K. Bergmann, H. P. Schwarz and G. Paumgartner, *Naunyn Schmiedebergs Arch. Pharmac.* **287,** 33 (1975).
19. C. D. Klaassen, *J. Pharmac. exp. Ther.* **191,** 201 (1974).
20. B. Solymoss, S. Toth and S. Varga, *Toxic. appl. Pharmac.* **18,** 586 (1971).
21. H. Selye, M. Krajny and L. Savoie, *Science* **164,** 842 (1969).