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The effect of spironolactone pretreatment on the biliary excretion and renal accumulation of inorganic mercury in the rat

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A discrepancy has been noted in the literature on the effect of spironolactone (Sp) pretreatment on the biliary excretion of inorganic mercury [1–5]. Haddow *et al.* [1] and the authors [2, 3] reported that the biliary excretion of i.v. administered mercury in Sp pretreated rats was more than ten times higher than control rats. Conversely, Garg *et al.* [4], and more recently Klaassen [5], reported that Sp pretreatment did not produce a significant increase in mercury excretion in rats. Klaassen injected pure Sp material i.p., while Haddow *et al.* and the present authors administered oral Aldactone tablets. Because of the pharmacokinetic differences reported between the oral administration and the i.v. injection of Sp [6, 7], the difference in drug administration or the material used could be one possible cause for this discrepancy. Another difference in the procedure is the dose of mercury administered. Klaassen injected only 30 μg/100 g body weight of mercury, while Haddow *et al.* and the present authors administered a dose more than five times higher than the dose used by Klaassen. In order to find out the true cause for the discrepancy in Sp effect on the biliary excretion of mercury, we performed several tests under different experimental conditions.

Male SPF Sprague Dawley rats weighing 250–350 grams were used. Commercial Sp tablets (Aldactone A. G. D. Searle & Co., Chicago, IL) were ground into powder and suspended in distilled water or ethylene glycol. A water suspension of powdered Aldactone was given orally through a stomach tube, or injected i.p. Ethylene glycol suspension was given i.p. Pure Sp material was purchased from Sigma Chemical Company (St. Louis, MO). This Sp material was suspended in ethylene glycol or propylene glycol and was administered i.p. The Sp dose was 5 mg/100 g B.W. In control rats, only ethylene glycol or propylene glycol was administered i.p. Mercury excretion studies were performed on rats 1–2 hr after the pretreatment. Under pentobarbital anesthesia (4.5 mg/100 g i.p.), the common bile duct was cannulated (PE-10 tubing) and a saline solution of mercuric chloride containing $^{203}HgCl_2$ (RCC, Amersham, England) was injected i.v. Two different mercury doses (0.2 mg/100 g, 30 μg/100 g) were tested in separate experiments. Four 30 min cumulative bile samples were collected during the following 2 hr. The rectal temperature was maintained between 37° and 38° throughout the experiment. Thereafter, rats were exsanguinated and the liver and both kidneys removed. The recovery of the i.v. administered mercury in the bile and the mercury content in these organs were then measured for their radioactivity. The means of the treated groups were compared with the control value by Student's *t* test.

The biliary recovery of i.v. administered mercury and the mercury contents of the liver and kidneys expressed as a percent of the administered dose are summarized in Table 1.

Table 1. Biliary recovery and organ content of intravenously administered inorganic mercury in control and spironolactone pretreated rats (mean ± S.D., per cent of the injected dose)

Spironolactone pretreatment	Biliary recovery of mercury				Total recovery for 2 hr	Mercury content 2 hr after mercury injection	
	0–30 min	30–60 min	60–90 min	90–120 min		Liver	Kidneys
			Mercury dose 0.2 mg per 100 g body weight				
Control* (4)	0.32 ± 0.05	0.46 ± 0.08	0.39 ± 0.05	0.28 ± 0.03	1.45 ± 0.12	14.78 ± 0.92	34.77 ± 4.18
Oral (W-Al) (4)	5.53 ± 1.49§	5.15 ± 1.31§	2.17 ± 0.43§	1.22 ± 0.21§	13.13 ± 3.08§	13.30 ± 1.61	28.52 ± 4.00¶
IP (W-Al) (3)	3.71 ± 0.98§	3.77 ± 0.49§	1.98 ± 0.43§	1.03 ± 0.23§	10.49 ± 1.16§	22.07 ± 7.32¶	17.68 ± 1.50§
IP (EG-Al) (3)	5.26 ± 0.79§	4.11 ± 0.69§	2.36 ± 0.11§	1.26 ± 0.12§	12.99 ± 1.61§	15.25 ± 2.19	13.73 ± 3.67§
IP (EG-Sp) (4)	5.11 ± 1.02§	3.92 ± 0.80§	2.20 ± 0.33§	1.35 ± 0.85§	12.58 ± 1.39§	14.81 ± 1.39	6.58 ± 2.65§
			Mercury dose 30 μg per 100 g body weight				
Control† (3)	0.21 ± 0.03	0.29 ± 0.05	0.25 ± 0.03	0.18 ± 0.06	1.27 ± 0.59	7.89 ± 1.39	20.63 ± 3.50
IP (PG-Sp) (6)	1.92 ± 0.57§	1.54 ± 0.27§	0.87 ± 0.16§	0.54 ± 0.06§	4.87 ± 0.84§	12.41 ± 2.25¶	4.81 ± 1.43§
IP (EG-Sp) (3)	2.64 ± 0.50§	1.84 ± 0.19§	1.00 ± 0.02§	0.45 ± 0.17§	5.93 ± 0.54§	12.61 ± 0.88¶	4.16 ± 0.70§

* i.p. injection of ethylene glycol only † i.p. injection of propylene glycol only.
§ Significantly different from respective control value ($P < 0.01$).
¶ Significantly different from the control value ($P < 0.05$).
All pretreatments were done 1–2 hr prior to mercury study.
Spironolactone doses were all 5 mg/100 g body weight as Sp weight.
Number in parenthesis indicates the number of rat studied.
IP: intraperitoneal injection, Oral: oral administration, W: water suspension, EG: ethylene glycol suspension, PG: propylene glycol suspension, SP: pure spironolactone material, Al: powdered Aldactone A tablet.

In the higher mercury dose (0.2 mg/100 g) studies, the four experimental groups pretreated with Sp in different ways showed, significant increases in the biliary excretion of mercury in comparison to the controls, which were given ethylene glycol only. This is in agreement with the previous reports by Haddow *et al.* [1] and ourselves [2, 3], in which Sprague–Dawley or Wistar male rats were pretreated with orally administered Aldactone tablets. Since the i.p. injection of Sp (either powdered Aldactone or pure Sp) suspension increased the biliary excretion of mercury in the present study, the difference in the administration of Sp does not appear to explain the absence of the significant enhancement of biliary excretion of mercury reported previously by Klaassen [5]. When the lower dose used by Klaassen was tested, Sp also increased significantly the biliary excretion of mercury. However, the percent recovery of mercury in Sp pretreated rats was one half of that when the higher dose of mercury was used, while the control biliary excretion value was approximately the same for the two different mercury dose studies. The biliary excretion of mercury in 2 hr was 4–5 times higher than control value in the lower mercury dose study. This is approximately the same as in one of Klaassen's experiments although the difference between control and Sp treated rats was not significant in his study. Thus the difference in the effect of Sp on the biliary excretion of mercury between Klaassen's study and the studies by Haddow *et al.* and ourselves appear to be due to the difference in the mercury dose used and not to the difference in the administration or the material used as was suspected previously [3].

Table 1 further shows a significant decrease in the kidney content of mercury in pretreated rats compared with control rats. Interestingly, an i.p. injection appears to be more effective in decreasing the mercury content in the kidneys in comparison with oral administration ($P < 0.01$, t test). Furthermore, an i.p. injection of pure Sp was more effective than Aldactone injection ($P < 0.025$) in decreasing the kidney content of mercury. However, as far as the biliary excretion of mercury is concerned, the four methods of administration were approximately equally effective. Thus, the pharmacokinetic difference reported previously [6, 7] could possibly affect the effect of Sp on the mercury accumulation in the kidney, although it may not affect the Sp effect on the enhancement in biliary excretion of mercury. The significant difference in the mercury content of the kidney between orally administered and i.p. injected rats and between rats given Aldactone and pure Sp suggests that the difference in the administration and the material used might cause a difference in the degree of protection against mercury poisoning [8] despite similar biliary excretion of mercury.

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