Renal mechanisms in the cardiovascular effects of chronic exposure to inorganic mercury in rats

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Abstract

Male weanling Wistar rats received 200 µg/ml of mercury (Hg), as HgCl₂, in drinking water for 180 days. At the end of the treatment, systemic arterial blood pressure was augmented, cardiac inotropism was reduced, and heart rate was unchanged. Light and electron microscopical studies of the kidney showed a mesangial proliferative glomerulonephritis in about 80% of the glomeruli. Tubular cells showed reduction of the acid phosphatase activity, which was related to functional abnormalities of the lysosomes. In the 24 hour urine samples of the Hg exposed rats, there was slight reduction of kallikrein activity, but evident proteinuria was not present in all samples. Plasma renin activity was reduced, that of angiotensin I-converting enzyme was augmented, and plasma aldosterone concentrations were unchanged. Mercury was accumulated mostly in the kidney of the Hg treated animals; and the content of Hg in the heart was higher than in the brain. These data show that chronic exposure to Hg acts on the kidney with complex mechanisms of toxicity; these contribute to modify systemic haemodynamics.

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Epidemiological and clinical studies have shown increased incidence of arterial hypertension and nephropathy with proteinuria and enzymuria in mercury (Hg) exposed workers.¹⁻⁶ Although it was found that the kidney has a greater capacity than other organs to accumulate Hg^{7,8} there is, however, no experimental evidence that renal storage of Hg activates mechanisms of toxicity leading to arterial hypertension or cardiovascular disorders.

Animal studies have found that chronic exposure to inorganic Hg affects some mechanisms regulating cardiovascular homeostasis. Male Sprague-Dawley rats, treated with 50 µg/ml Hg as mercuric chloride (HgCl₂) in drinking water for 320 or 350 days, showed an increase in cardiac inotropism, hyporeactivity of baroreflex mechanisms, and changes in cardiovascular reactivity after stimulation of the peripheral α - or β -adrenoreceptors⁷⁻⁹; arterial blood pressure was significantly increased only in the rats exposed to the metal for 350 days.^{8,9} These findings suggested that Hg interferes with both influx and intracellular availability of calcium ions (Ca²⁺) for contractile mechanisms in cardiac and vascular myocytes by an action on receptor operated Ca²⁺ channels in the plasma membrane and on cyclic nucleotide pathways. Light and electron microscopical observation of the kidneys of these rats showed lysis of lysosomes in the tubular cells and minor morphological alterations in the glomeruli (segmental thickening of the basal membrane with corresponding focal fusion of foot processes).⁹

In a recent experiment, male Wistar rats received 50 or 200 µg/ml of Hg (as HgCl₂) in the drinking water for 350 days from weaning. Blood pressure was increased in the rats exposed to both doses of Hg, whereas cardiac inotropism was augmented only in the animals exposed to the lower dose.^{10,11} Exposure to Hg specifically reduced cardiovascular reactivity to stimulation of either baroreceptors or cardiac and vascular α_1 -adrenoreceptors; there was also an increase of the effects after activation of the cardiovascular DA₂-dopaminergic and β_1 - and β_2 -adrenergic receptors. Light microscopical observation of the kidney showed glomerulonephritic lesions in 30% of the glomeruli of the rats exposed to 50 ppm

Hg and in almost all the glomeruli of the animals treated with 200 ppm Hg; in the glomeruli thickening of the basal membrane and hypercellularity with proliferation of the mesangial matrix were found; in the tubuli, there was hydropic degeneration of the cells and some casts.^{10 11}

Other experimental studies have confirmed that subacute or chronic exposure to Hg, at doses able to affect cardiovascular function, may induce renal alterations. For example, Wistar rats, treated with doses of inorganic Hg that ranged from 50 to 200 µg/100 g body weight by subcutaneous route three times a week for a period of two to 10 months, showed a membranous glomerulonephritis.¹² In another study, Wistar rats that received 150 µg of HgCl₂ per 100 g body weight by subcutaneous injection three times a week for 27 weeks showed a mesangial glomerulonephritis with deposition of IgG, IgM, and complement component C₃ in the mesangium.¹³ Other strains of rats treated with HgCl₂ presented different immunological alterations dependent upon genetic variables.¹⁴ Brown Norway rats, treated with different doses of HgCl₂ for different periods, had autoimmune abnormalities that included lymphoproliferation, production of antglomerular basement membrane and anti-DNA antibodies, increased serum IgE concentrations, and polyclonal increase in total IgG concentration, as well as circulating immune complexes. On the basis of these results, it was suggested that Hg stimulates autoreactive T-helper cells, inhibits T-suppressor cells, and induces polyclonal activation of β-lymphocyte cells. After the initial period of exposure there was spontaneous remission of these changes, which suggests that the T-suppressor cells may recover from inhibition and regulate the Hg induced autoimmune responses. On the other hand, Lewis rats, treated with HgCl₂ in experimental protocols similar to those of the studies on Brown Norway rats, showed impaired regulation of immunological function and immunosuppression.^{14 15} Finally, rabbits and mice treated with HgCl₂ showed several autoimmune disorders and glomerulonephritis.¹⁴

The purpose of this study was to investigate some mechanisms of renal toxicity possibly involved in the dysfunction of cardiovascular function, in rats exposed to doses of Hg known to induce both arterial hypertension and autoimmune lesions in the kidney.

Materials and methods

Sixteen male weanling Wistar rats were randomly divided into two equal groups, housed in stainless steel cages, and fed a standard laboratory diet. One group received 200 µg/ml of Hg (as HgCl₂) in deionised drinking water for 180 days and the other group was kept as a control. At the end of the exposure all the rats were placed in metabolism cages for the collection of 24 hour urine samples.

The animals were anaesthetised with a single intraperitoneal injection of sodium thiopental (50 mg/kg body weight) in order to perform haemodynamic measurements. The trachea was cannulated to allow spontaneous breathing and polyethylene catheters (containing sodium heparin, 100 USP units/ml) were placed in the left femoral artery to record aortic blood pressure. Systolic and diastolic blood pressure were measured by means of a P23Db Statham pressure transducer (Statham Medical Instruments, Los Angeles, CAL) and averaged electronically. A Biotronex BL 620 derivative computer (Biotronex Laboratories, Inc., Kensington, MA) was used for determining the maximum rate of rise of the left ventricular isovolumetric pressure (dp/dt), an index of cardiac inotropism. In this regard, a calibrated 3F catheter tip pressure transducer (Millar Instruments, Houston, TX), inserted in the right common carotid artery and advanced in the left ventricle, was used to determine dp/dt.^{10 11} The computer was adjusted to minimise the expression of preload and afterload according to Crawford *et al.*¹⁶ and Davidson *et al.*¹⁷ Heart rate was measured by a Beckman cardiometer coupler, which was triggered by an R-peak of the lead II electrocardiogram. The cardiovascular parameters were continuously monitored on a Beckman type RM Dynograph recorder (Beckman Instruments, Inc, Schiller Park, ILL) after stabilisation for 30 minutes.

Blood samples were collected from the left common carotid artery for determining plasma renin activity (PRA),¹⁸ plasma angiotensin I-converting enzyme (ACE) activity,¹⁹ and plasma aldosterone concentrations.²⁰ Kallikrein activity,²¹ and creatinine, total protein, sodium, potassium, and calcium concentrations were determined in the 24 hour urine samples.

Samples of renal tissue were snap frozen in liquid nitrogen for histochemical and immunofluorescence examination; cryostat sections were prepared from these samples for demonstrating acid phosphatase activity by the method of Gomori.²² Sections 3 µm thick were cut in a cryostat, acetone fixed, washed in phosphate buffer solution (PBS, pH 7.4), and stained with monospecific antisera to IgA, IgG, IgM, C₃, C_{1q}, albumin, and fibrinogen (Boehringer Mannheim Biochemica, Mannheim, Germany), washed in PBS, and mounted in buffered glycerine.²³ Other renal samples were excised for light and electron microscopical observation, as previously described.²⁴ Specimens of several tissues were also excised for histopathological observation by light microscopy.

Samples of blood, brain, heart, and kidney were prepared for determination of Hg content. Mercury was analysed by flameless atomic spectrophotometry after special digestion of tissues and blood.²⁵ The Hg content was quoted as the wet weight of blood and tissues.

Data were compared by Student's *t* test. Results were considered significant at $p < 0.05$.

Results

Body weight and general appearance of the animals were not affected by Hg exposure. In the Hg exposed rats, systolic and diastolic blood pressure were significantly increased, whereas dP/dt was reduced and heart rate was unchanged (table 1).

In the Hg treated animals plasma renin activity (PRA) was decreased, plasma ACE activity augmented, and plasma aldosterone concentrations were unmodified compared with the control rats (table 2). Moreover, urinary kallikrein activity and urinary creatinine concentration were slightly but significantly reduced after exposure to Hg. Proteinuria was evident only in some of the Hg exposed rats. Concentrations of 24 hour urinary sodium, potassium, and calcium were similar to those of the controls (table 3).

Table 1 Blood pressure, heart rate, and maximum rate of rise of left ventricular isovolumetric pressure (dP/dt) in rats chronically exposed to Hg

	Control (n = 8) (mean (SE))	Exposed (n = 8) (mean (SE))
Systolic pressure (mm Hg)	131 (6)	151 (3)*
Diastolic pressure (mm Hg)	96 (7)	123 (6)*
Heart rate (beats/min)	242 (18)	248 (25)
dP/dt (mm Hg/s)	4421 (332)	3066 (227)

*Significantly different from control, $p < 0.05$.

Table 2 Plasma renin activity (PRA), plasma angiotensin I-converting enzyme (ACE) activity, and plasma aldosterone concentration in rats chronically exposed to Hg

	Control (mean (SE))	Exposed (mean (SE))
PRA (ng/ml/h)	33.3 (3.2)	16.9 (6.0)*
Plasma ACE (nmole/ml)	0.14 (0.1)	0.30 (0.1)*
Plasma aldosterone (pg/ml)	491 (112)	453 (103)

* $p < 0.05$; significantly different from control.
For both groups $n = 6$ for PRA and ACE, and $n = 4$ for plasma aldosterone.

Table 3 Twenty four hour urinary kallikrein activity and creatinine, urea nitrogen, protein, sodium, potassium, and calcium concentrations in rats chronically exposed to Hg

	Control (n = 8) (mean (SE))	Exposed (n = 8) (mean (SE))
Kallikrein (mamidolitic U)	6070 (550)	4500 (370)*
Creatinine (μ g)	17.8 (1.2)	13.0 (1.8)*
Urea nitrogen (μ g)	386 (45)	358 (49)
Protein (mg)	8.4 (1.4)	26.4 (16.2)
Sodium (mEq)	1.2 (0.05)	1.4 (0.1)
Potassium (mEq)	1.3 (0.1)	1.4 (0.1)
Calcium (μ g)	1.4 (0.3)	1.1 (0.2)

* $p < 0.05$; significantly different from control.

Light microscopical observation showed alterations in about 80% of the glomeruli of the Hg exposed rats (fig 1). Glomeruli showed hypercellularity, deposition of amorphous material in the mesangium, and thickening of the basal membrane. Examination with electron microscopy did not show modifications in the glomerular capillary wall, but only deposits of amorphous material in the mesangium; deposition of amorphous material and thickening of the vascular wall were found in the interstitium by light microscopy. The immunofluorescence technique showed deposition of IgM, but not of IgA, IgG, C₃, C_{1d}, albumin, or fibrinogen in the glomeruli of the Hg exposed rats (fig 2). Light focal hydropic degeneration of the tubular cells and amorphous material in some lumens were found (by light microscopy) after Hg treatment (fig 3). At electron microscopy lysosomes of all the tubular cells showed a reduction of electron density and irregular borders, but not lysis. The acid phosphatase activity of these cells, mostly localised in the lysosomes,²⁶ was reduced. Histopathological examination of the arteries and of the kidney showed that exposure to Hg induced thickening of the wall of the renal vessels and focal thickening of the middle layer of the abdominal aorta.

We found that Hg was accumulated much more in the kidney than in the brain and in the heart of the Hg exposed animals. The heart appeared to store more Hg than the brain (table 4).

Discussion

In this study, unlike Sprague-Dawley rats exposed to 50 ppm of Hg in drinking water for 320 or 250 days,⁷⁻⁹ Wistar rats treated with 200 ppm of Hg for 180 days showed a reduction in cardiac inotropism. The high concentrations of Hg found in the heart of Wistar rats treated with 200 ppm of Hg may explain the reduction seen in the present experiment. In this regard, the ratio between the Hg stored in the heart and that accumulated in brain or kidney was three or four times higher in Wistar than in Sprague-Dawley rats.^{9,10} On the other hand, the increase in blood pressure seen in Wistar rats may be explained only by a vasoconstrictor effect of Hg with a consequent rise in the total peripheral resistance. This rise is likely to be related to greater release of noradrenaline from postganglionic adrenergic neurons and to baroreflex hyposensitivity, as suggested in previous studies.⁷⁻¹⁰ On the basis of these experiments, the increase in total peripheral resistance does not seem to depend on effects of Hg causing alterations of sympathetic nerve activity or vascular responsiveness after activation of the α_1 -, α_2 , and β_2 -adrenoreceptors and DA₂-dopaminergic receptors in blood vessels. On the other hand, the cardiovascular alterations found in the Wistar rats exposed to 200 ppm of Hg cannot be explained by increased reactivity to stimulation of

Figure 1 Glomerulus and tubular cells of an Hg exposed rat. The glomerulus presents hypercellularity and deposition of amorphous material in the mesangium with reduction of the vascular space. Tubular cells present hydropic degeneration. (PAS $\times 400$).

Figure 2 Renal glomerulus of an Hg exposed rat showing intraglomerular deposits of IgM. (immunofluorescence $\times 400$).

Figure 3 Proximal tubular cells of an Hg exposed rat showing hydropic degeneration, deposition of ialine material in lumens, and inflammatory cells (mostly lymphocytes) in the interstitium (PAS $\times$ 250).

Table 4 Concentration of Hg in blood, brain, heart, and kidney of rats chronically exposed to Hg

	Control (mean (SE))	Exposed (mean (SE))
Blood ($\mu\text{g/g}$)	0.94 (0.27)	<0.03*
Brain ($\mu\text{g/g}$)	2.27 (0.73)	<0.06*
Heart ($\mu\text{g/g}$)	4.12 (1.16)	<0.07*
Kidney ($\mu\text{g/g}$)	160.25 (27.16)	<0.20*

* $p < 0.05$; significantly different from control.

All values are expressed on a wet weight basis ($n = 4$ in both groups for blood and brain and $n = 6$ in both groups for heart and kidney).

the peripheral β_1 and β_2 -adrenoreceptors, as it was found in the Sprague-Dawley rats exposed to 50 ppm of Hg for 320 or 350 days.⁷⁻⁹ Because Hg was shown to reduce the availability of Ca^{2+} for contractile mechanisms in both cardiac and vascular myocytes (by an interaction with both receptor operated Ca^{2+} channels and cyclic nucleotide pathways), it may be that the decrease of cardiac inotropism found in this study is the result of a local toxic effect of Hg that involves myocardial contractile processes.⁷⁻¹⁰

Both reduction of creatinine excretion and proteinuria found in some of the Hg exposed Wistar

rats seem to depend upon a mild form of glomerulonephritis induced by Hg. In this regard, rats exposed to Hg showed several forms of glomerulonephritis such as membranous,¹² a mesangial,¹³ or an immune complex type of glomerulonephritis.¹⁵ The hypercellularity and deposition of amorphous material in the mesangium, without alterations in the glomerular vessels, indicate that Hg caused a mesangial proliferative glomerulonephritis in the Wistar rats used in this research.²⁷ Differences in duration of exposure, doses, and routes of administration of Hg, strains of animals, and other variables may account for the ability of Hg to induce various types of glomerulonephritis. On the other hand, autoimmune mechanisms involved in these glomerular lesions may also be related to the morphological alterations of lysosomes seen in the tubular cells and to reduction of the acid phosphatase activity, which is localised in these lysosomes.²⁷ Thus as lysis of lysosomes was found in tubular cells of Hg exposed Sprague-Dawley rats, it may be that circulating Hg binding proteins, derived from the lysis of lysosomes, play a part in determining the different Hg induced immunological and pathological alterations found both in humans⁴ and experimental animals.^{9-11 15}

A surprising result of this experiment was the increase of plasma ACE activity in the presence of reduced PRA. It is possible, therefore as in lead exposed humans and laboratory animals,²⁸⁻³⁰ that Hg initially increases PRA and, whenever the exposure is prolonged, reduces the activity of this plasma enzyme. Moreover, considering the biochemical and functional relations between the plasma renin and urinary kallikrein systems,³⁰ the possibility also exists that reduction of urinary kallikrein activity and reduction of PRA are interdependent. On the other hand, reduction of urinary kallikrein alone was not seen in Sprague-Dawley rats chronically exposed to 50 ppm of Hg³¹ and in asymptomatic Hg exposed workers,³² but only in cadmium exposed rats³³ and human subjects.³² Another explanation for the increase in plasma ACE activity and reduction of PRA may be derived from the recent revision of the classic concept of the circulating renin angiotensin system (RAS).³⁴ In this respect, the most important findings are that (a) the major site of production of angiotensin I (A1) and II (A2) is peripheral tissues, (b) the primary role of the circulating RAS is that of delivering renin and angiotensinogen to tissues, and (c) in tissues (including vessel wall and kidney) there is synthesis of angiotensinogen, renin or renin-like enzymes, and ACE. It is apparent that this local production (and degradation) of A1 and A2 is subject to tissue specific mechanisms of regulation, which change local A2 concentration without reference to the plasma concentrations of renin or A2. Thus Hg may interfere with these local tissue specific mechanisms such as the extent of tissue uptake of renin, angiotensinogen A1 and A2, and the local tissue concentration of ACE. In other words, the effects of Hg on plasma ACE activity and PRA may be the result of different actions of Hg on plasma and tissue RAS. According to these concepts, the normal concentrations of plasma aldosterone in Hg exposed rats may reflect the cumulative effects of Hg on the adrenal glands, RAS, and renal kallikrein system (influencing both synthesis and release of aldosterone).³⁰

This study confirms that the kidney is one of the main targets of the toxic effects seen after chronic exposure to Hg. In particular, through different mechanisms (including those of autoimmunity), Hg causes changes in the RAS and the kallikrein-kinin system that contribute to modify systemic haemodynamics. Experimental variables (such as dose of exposure, route of administration, duration of treatment, sex of the animals, and environmental factors) may have influenced or modulated the effects of Hg.

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