Original Article

REPEATED INTRAVENOUS MERCURY CHLORIDE ADMINISTRATION INDUCES REGENERATION AND BIPHASIC RENAL DAMAGE IN RATS

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ABSTRACT

Mercury and its compounds are well known to be very toxic to kidneys. Forty Sprague-Dawley rats treated with intravenous injection of 0.5 mg/kg of body weight mercuric chloride ($HgCl_2$) in 1.0 mL 0.85% NaCl through tail vein. Another group served as a control and received 1.0 mL 0.85% NaCl. The treatments were repeated every other day for ten days. Renal tissue Hg concentration of the treated group increased significantly on day 14 with a value of 2064.5 ppb/g compared to control value of 19.16 ppb/g. The renal Hg content reached 2116.89 ppb/g on day 22 and kept decreasing to its lowest value on day 38 post-treatment; 310.47 ppb/g. The necrotic cells increased significantly with time reaching peak on day 42; 6007.67 damaged cells, compared to the control count of 50.75 damaged cells. The necrosis process was accompanied by regeneration of young cells which appeared bluish in colour and could be seen as early as on day 14 with a cell count of 58 cells/10 fields. The number decreased significantly on day 22 and 26. By day 30, these young cells were no longer seen. No evidence of tissue regeneration was observed in all control samples. Repeated intravenous mercury chloride administration was observed to cause biphasic renal damage. The early damaging phase was accompanied by a high reparative epithelisation process and the severe tubular necrosis began on day 18 as soon as the reparative phase has getting waned off.

INTRODUCTION

Mercury and its compounds have been used in human history for at least 3000 years. Mad hatter's and Minamata disease are the classical examples of mercurial poisoning. It's commonly found in various sources e.g. fish, poultry, insecticides, fungicides, pesticides, disinfectants, dental amalgam, thimerosal-containing vaccines and petroleum and its derivatives [1,2]. Urban discharges, agricultural materials, mining, hydrocarbon combustion and industrial discharges are major anthropogenic sources of Hg emissions into the environment [1,3,4]. It was reported in 2007 that 20,000 tons of mercury are annually released to the environment due to many human activities [5]. In many cases, the contamination chain of Hg follows closely the cyclic order: industry, atmosphere, soil, water, phytoplankton, zooplankton, fish and human [6].

Mercury and its compounds are highly toxic to kidneys causing necrosis [7,8]. It is suggested to bind to sulfhydryl group of enzymes inhibiting mitochondrial respiration [9]. Mercury intoxication has been observed to cause gross tubular lesion and thickening of glomerular basement membrane [10]. This study was designed to determine the pattern damaging effect of renal tissue following repeated intravenous mercury administration on rats.

MATERIALS AND METHOD

Animals**:** A total of 80 Sprague-Dawley rats aged between eight and ten weeks were divided into two groups. Treated group was given intravenous injection of 0.5 mg/kg of body weight mercuric chloride ($HgCl_2$) in 1.0 mL 0.85% NaCl through tail vein. Control group received 1.0 mL 0.85% NaCl. The treatments were repeated every other day for ten days. All rats were housed in cages fitted with urine collection trays, fed and given water *ad libitum*. Five rats from each group were sacrificed every four days commencing from the last day of the treatment (Day 14).

***Mercury Standard solutions*:** The content of 1.0 g Mercury titrisol ampoule (Merck, Germany) was transferred to chemically sterile Erlenmeyer containing 0.5 g potassium dichromate ($K_2Cr_2O_2$) and 50.0 mL concentrated HNO_2. The solution was later on made up to 1.0 L with distilled water. This solution was used as a stock mercury solution (1.0 mg/mL). Standard solutions of 5.0, 10.0, 15.0, and 20.0 µg Hg/ mL were prepared from the stock solution.

***Trace Element Analysis*:** Kidneys and livers of sacrificed rats were removed and kept in chemically sterile sealed plastic bags at -20°C until used. Approximately 0.5 – 1.0 g (wet weight) of kidney tissue samples were placed in 50.0 mL borosilicate test tubes. 4.0 mL of concentrated H_2SO_4 and 1.0 mL HNO_2 were added slowly to sample while in ice. The tubes containing sample were placed in a water bath and maintained at 50.0 – 60.0°C until all tissue samples were digested and solutions became clear. While in ice, 20.0 mL of 6.0% (w/v) potassium permanganate ($KmnO_4$) were added slowly to the solution. The samples were then left overnight at room temperature for further digestion. A blank solution was prepared in the same way except no sample was added to the preparation. After being left overnight, 5.0% (w/v) of hydroxylamine hydroxide was added slowly to all sample tubes to remove excess permanganate ions. All samples were then diluted to 20 times and made up to a final volume of 100.0 mL. The mercury content of the solutions were analysed using a cold-vapour atomic absorption spectrophotometer (Perkin Elmer 3100).

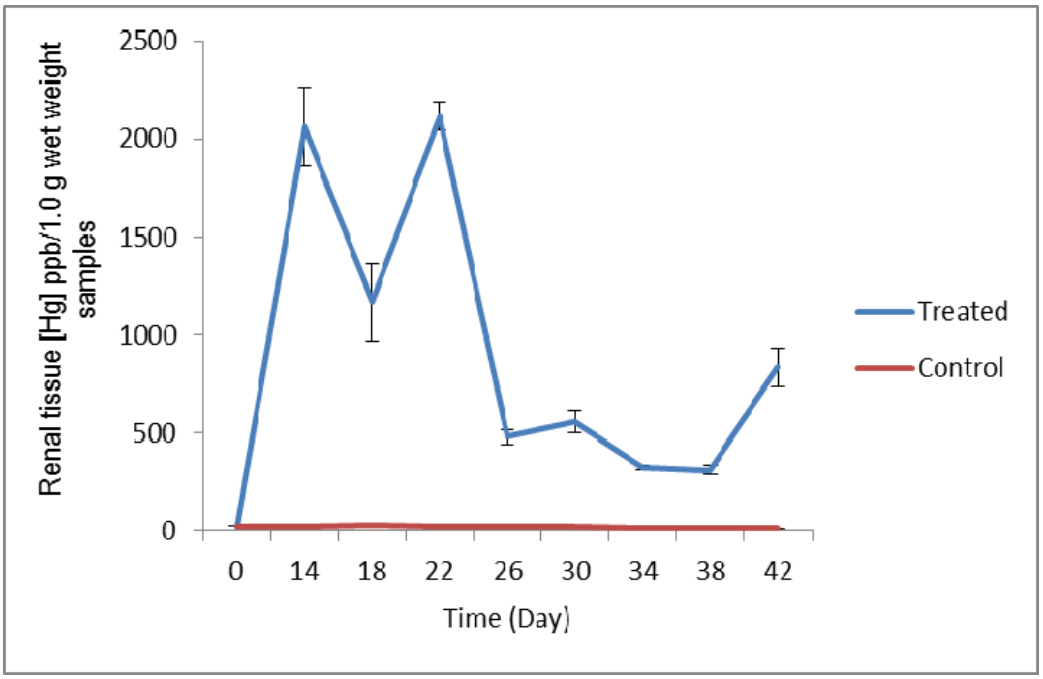
***Histopathology*:** Kidney samples from both treated and control rats were fixed in 10.0% formalin, embedded in paraffin wax and sectioned at 5.0 µm thickness. The sections were then stained with haematoxylin and eosin (H&E) and observed under light microscope (200x) for abnormal cells in 10 randomly selected fields per slide.

***Statistical analysis*:** All the data collected were analysed using analysis of variance (ANOVA) and Duncan test. Value of $p \leq 0.05$ were considered to be statistically significant and all results were expressed as mean ± standard error.

RESULTS

***Renal tissue mercury*:** The renal tissue Hg concentration of treated group increased significantly on day 14 with a value of 2064.5 ppb/ g compared to control value of 19.16 ppb/g (Figure 1). The renal Hg content of treated group significantly decreased on day 18 to 1167.8 ppb/g. The Hg content remained high on day 22; 2116.89 ppb/g, before kept decreasing to its lowest value on day 38 post-treatment; 310.47 ppb/g.

***Gross and histopathology*:** The kidney samples of treated group were grossly enlarged and pale in colour (Figure 2a). Gross observations noted pin-point lesions; resulted from necrosis and healing process, were noted on the surface of the



*Error bars represent standard error calculated from a triplicate samples

Figure 1: Analysis of results showed a renal tissue mercury concentration in rats sacrificed every 4 days following repeated exposure to $HgCl_2$.

treated kidney samples from day 26 onward. The lesions were not clearly seen on day 14, 18, and 22 on organ samples.

Light microscopic observation revealed evidence of tubular epithelial cell damage (Figure 2b). For purpose of quantification, cells which were described as vacuolated cytoplasm (hydropic changes), pyknotic, karyorrhectic, kayolytic and necrotic sloughed cells in the lumen of tubules were considered as damaged cells. Renal tissue samples of the treated group showed a significant early damage evidence by increased number of damaged cells on day 14 post-treatment; 367.2 damaged cell count/10 fields, compared to samples of control group; 22.33 damaged cell count/10 fields. In fact, the number increased significantly with time (Figure 3) reaching peak on day 42; 6007.67 damaged cells, compared to the control count of 50.75 damaged cells. Tissue samples of treated group showed increase of karyolysis and vacuolation. Also by day 26 sloughed off cells began to appear in the lumen of the tubules and the number increased by day 38 post-treatment.

Light microscopic observations also revealed evidence of tubular tissue regeneration through epithelisation in samples of treated group (Figure 4). These regenerative cells appeared bluish in colour in H&E tissue sections. The cells could be seen as early as on day 14 with a cell count of 58 cells/10 fields and the number significantly decreased to 15.67 and 20.67/ 10 fields on day 22 and 26 respectively. However, the cells were totally absent on day 18 in all tissue samples (Figure 5). By day 30 they were no longer seen. No evidence of tissue regeneration was observed in all control samples.

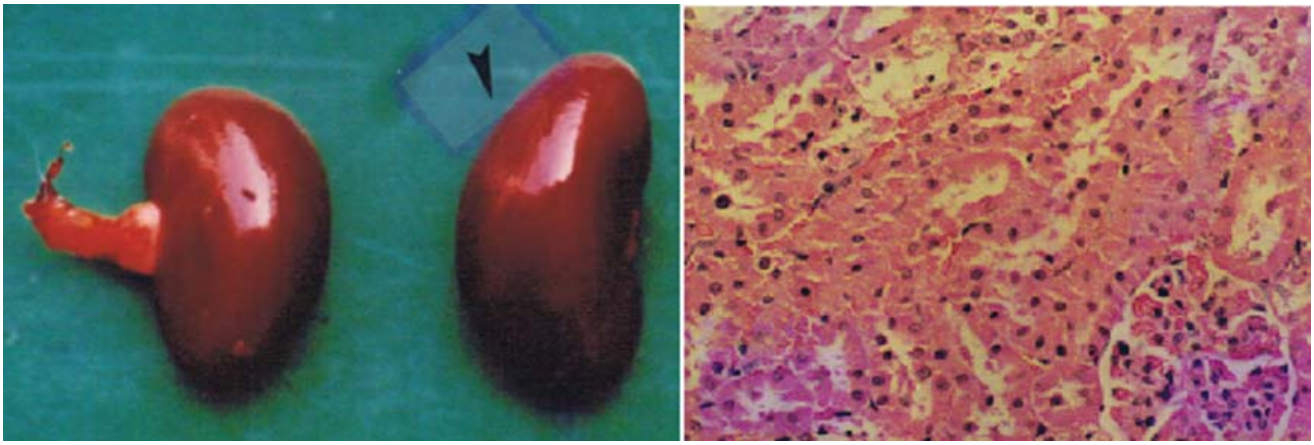
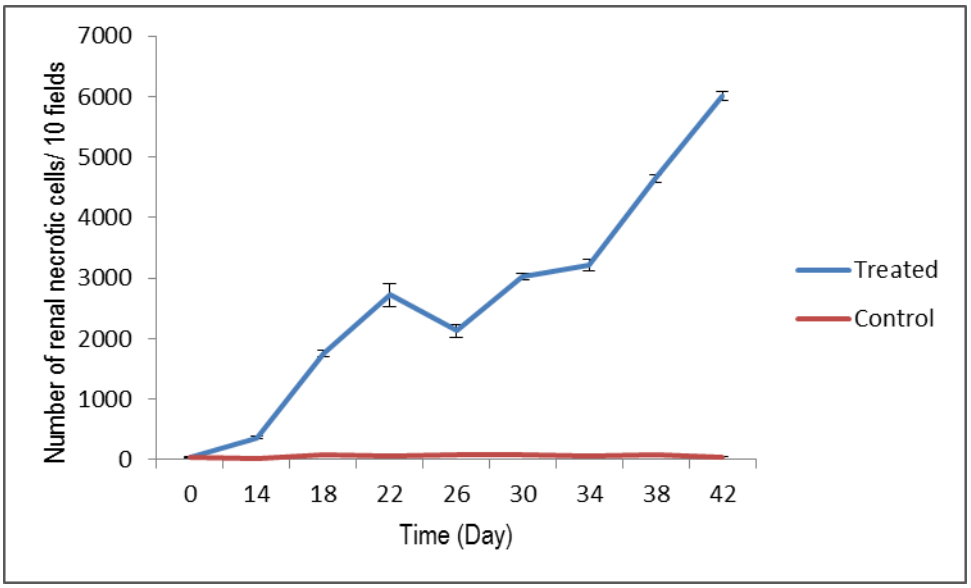


Figure 2: Kidney sample of treated group was enlarged (arrowhead; a) and photomicrograph showed an acute tubular necrosis (b); 200x magnification.



*Error bars represent standard error calculated from a triplicate samples

Figure 3: Light microscopic observation revealed numbers of damaged renal tubular cells in rats sacrificed every 4 days following repeated exposure to $HgCl_2$.

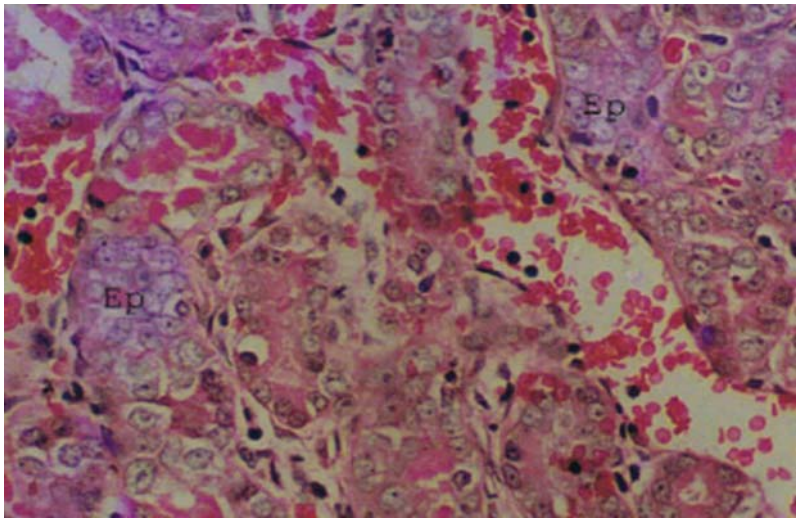
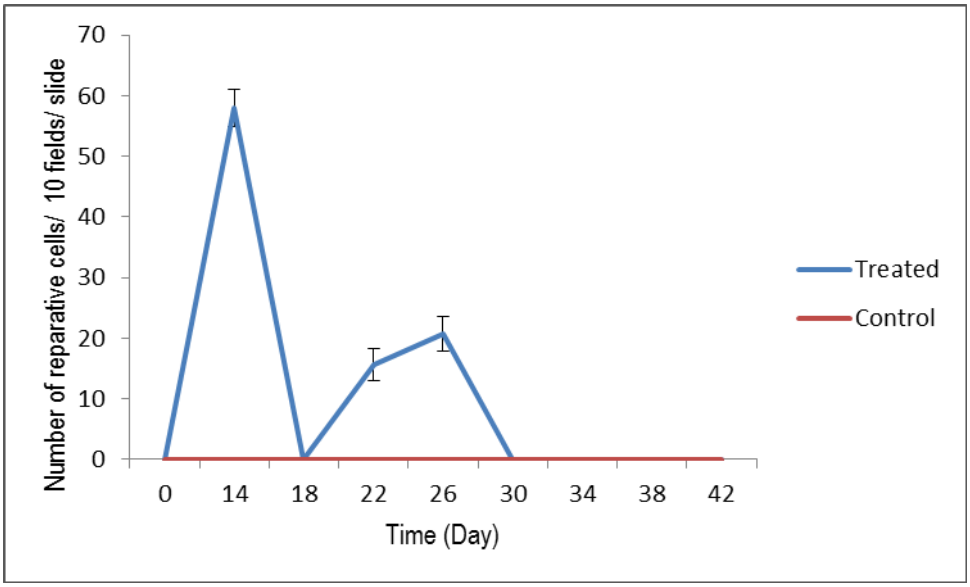


Figure 4: Light microscopic observation revealed epithelisation of renal tubular cells in rats sacrificed every 4 days following repeated exposure to $HgCl_2$. The young cells were bluish in colour (Ep); 200x magnification



*Error bars represent standard error calculated from a triplicate samples

Figure 5: Light microscopic observation revealed numbers of regenerative renal tubular cells in rats sacrificed every 4 days following repeated exposure to $HgCl_2$.

DISCUSSIONS

Mercury chloride has been recognised to be nephrotoxic reagent causing renal tubular vacuolation, interstitial inflammation and cell degeneration [11]. In this study, Sprague-Dawley rats were injected with $HgCl_2$ to induce acute nephrotic syndrome. The data showed a significant steady increase in tubular epithelial cell damage beginning on day 14 till the end of study period. The damage seemed to be more severe in the outer cortex region. This phenomenon could be due to higher accumulation of Hg in the region as suggested by Clarkson, 1972. It is believed that tubular secretion is the main process of Hg elimination from the body [5,13] and lysosome was observed to be the major site of Hg deposition in the renal tubules [14,15]. This may well be true since the characteristics associated with the damaged cells observed in this study; karyolysis, vacuolated cytoplasm, sloughing-off cells into the tubular lumen and swollen cells, are of the tubular cells.

Mercuric cations; Hg^{+} and Hg^{2+}, have high electro-negativity of 2.0 on the Pauling scale and large ionic radius of 0.111 - 0.116 nm. Owing to those characteristics, Hg-cations belong to “soft” acids, and thus having a strong binding affinity to “soft” bases like thiols and selenols.

Many proteins; enzymes and hormones, and cofactors feature thiols or so called sulfhydryl groups, -SH. Thiols appear to be not only of importance for oxidation-reduction mechanisms of mitochondrial respiration but also for the proper functioning of all thiol-featured proteins and cofactors [16]. Therefore, binding of Hg to thiol residues would result in activation of sulphur and functional attenuation or blockage of related enzymes, cofactors and hormones [2,17]. The injury of renal tubules in this study was suggested to be due to ischaemia and also direct nephrotoxicity of Hg. Mercury, as in previous studies, was observed to inhibit the mitochondrial electron transport chain (ETC) leading to cell death [9,18]. Production of reactive oxygen species (ROS) was also reported to be increased [19,20] and activity level of endogenous antioxidants; GSH, SOD, GPx, GR, was decreased [21,22] following administration of organic Hg; thus, putting the cells in oxidative stress.

In this study, there was a drastic regeneration of new cells observed following repeated exposure to $HgCl_2$. Those newly formed cells of tubular epithelium observable as early as on day 14 post-treatment but disappeared completely by day 30 post-treatment. However, those cells were absent in all samples on day 18; giving rise to a biphasic pattern. A previous study showed that regenerative response to Hg-induced injury was as early as 3 to 5 days following $HgCl_2$ administration [23]. Chen *et. al.* (2016) suggested $HgCl_2$ exposure stimulates the cell division of stem cells. The dying cells sent signal to the surrounding stem cells to initiate tissue regeneration by accelerating the proliferation and differentiation of the stem cells, which led to the generation of new-born cells.

The second bout of cell regeneration on day 22 and 26, even the number of newborn cells was significantly much lower, could be due to high compensatory effort of the kidney to go for repair against the injury. Mercury was observed to induce metallothionein and has a strong binding to it [12,25,26]. This ability contributed to longer retention of Hg in tissue and longer exposure of tissue to its toxicity. The excess exposure was suggested giving rise to severe injuries that beyond the cure by tissue regeneration.

CONCLUSION

In conclusion, this study showed that repeated intra-venous mercury chloride administration caused biphasic renal damage. The first phase of the damage was accompanied by a high reparative epithelisation phase. The second phase started as severe tubular necrosis began on day 18, which was as soon as the repair phase has getting waned off. This study suggested that it was possible for the kidney to repair the damage if the injury was well controlled and properly treated.

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