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SERUM BIOCHEMICAL CHANGES IN MERCURY CHLORIDE-INDUCED RENAL DAMAGE IN RATS

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SUMMARY

Eighty Sprague-Dawley rats, weighing between 200 to 230g and aged between eight to ten weeks, were divided into two groups of 40 rats each. Group 1 (treated) received intravenous injection of 1mL solution containing mercury chloride at the rate of 0.5 mg/kg body weight in 0.85% NaCl through the tail vein. Group 2 (control) was similarly injected with 1mL of 0.85% NaCl. The respective treatment was repeated every alternate day for 10 days before five rats from each group were sacrificed on day 0 and on every four day-interval thereafter, beginning 2 weeks after the first injection. Blood samples were analysed for blood urea nitrogen (BUN), serum creatinine, serum total protein and serum albumin. Urine samples were analysed to determine the albumin level. The concentrations of blood urea nitrogen and serum creatinine increased significantly ($p < 0.05$) compared to controls. Serum creatinine, serum and urine albumin concentrations showed biphasic responses with the first response in serum creatinine concentration was observed as early as 14 days after the first injection. The results suggested that the mechanism of renal damage in mercury chloride toxicity occurred in two phases.

Keywords: Mercury chloride, renal damage, blood urea nitrogen (BUN), creatinine, total protein, albumin

INTRODUCTION

The kidney is a vital organ, which regulates blood volume, chemical composition and internal body environment. It is a major excretory pathway for breakdown products of tissue metabolism, drugs and toxic chemicals. However, many toxic chemicals can cause injury to renal tissues resulting in either partial or total loss of renal functions (Robinson and Hesketh, 1976; Allen and Masters, 1985; Moalli *et al.*, 1996).

The kidney has a high compensatory ability. Thus, damages resulting in severe functional loss can only be detected at a very late stage of renal disease, after at least 80% of the kidney tissue are irreversibly damaged (Wilson, 1986). Although much progress have been made to prolong the live of patients with end-stage renal damage, it is still difficult to diagnose renal damage at a stage early enough to save lives.

The routine laboratory tests used to determine the kidney function profiles include the serum analysis to determine the concentrations of blood urea nitrogen (BUN) and creatinine, and urinalysis to determine the concentration of albumin.

Mercury and mercuric compounds are commonly used in drugs, disinfectants, fungicides and antiseptics, herbicides, and preservatives (Neathery and Miller, 1975; Von Burg and Greenwood, 1991). It is also a common environmental pollutant. Thus, exposure to mercury has become inevitable. Mercury compounds can cause kidney injury in several ways. It can cause tubular lesions and induce biphasic nephropathy in rats (Sapin *et al.*, 1977; Druet *et al.*, 1978; Bellon *et al.*, 1982). Electron microscopic observations of renal biopsies revealed diffuse membranous changes and thickening of glomerular membrane due to the deposition of materials between the epithelial cells and the basement membrane proper.

This report describes the effect of multiple intravenous injections of mercury chloride on serum and urine biochemical parameters.

MATERIALS AND METHODS

Animals

Eighty Sprague-Dawley rats, weighing between 200 to 230g and aged between eight to ten weeks, were divided into two equal groups. The rats in group 1 were injected with 1mL solution containing mercuric chloride ($HgCl_2$) in 0.85% NaCl at the rate of 0.5 mg/kg body weight. Injections were carried out intravenously via the tail vein. Rats in group 2 were similarly injected with 1mL of 0.85% NaCl. The respective treatments were repeated every alternate day for ten days.

All rats were housed in cages (4 rats/cage) fitted with urine collection trays, fed and given water *ad libitum*. They were monitored throughout the study period for clinical abnormalities, particularly those pertaining to renal dysfunction. Five rats from each group were sacrificed at every four-day interval,

commencing from day 4 after the last injection following blood collection by cardiac puncture.

Urine and serum samples

Urine samples were collected in ice-cooled beaker 24 h prior to the sacrifice and centrifuged at 500xg for 10 min. The supernatant was stored frozen at –20°C. Blood samples were collected by cardiac puncture and the serum was separated and stored at –20°C. The urine and serum samples were analysed to determine the concentrations of albumin, while the serum samples were further analysed to determine the concentrations of blood urea nitrogen (BUN), creatinine and total protein. All analyses were conducted on the Cobas Mira Chemistry Analyser (Roche) using standard diagnostic kits (Roche).

RESULTS

Serum analysis

The BUN concentration increased significantly ($p < 0.05$) by day 22, reaching peak on day 26 before decreasing to low values on day 38 (Fig. 1). Serum creatinine concentration showed a significant ($p < 0.05$) increase as early as 14 days after mercury injection, decreased significantly thereafter before it gradually increased to a new peak on day 34 (Fig. 1).

The serum protein concentration showed a significant ($p < 0.05$) increasing pattern from day 14, reaching peak on day 26 before it started to decline four days later (Fig. 1). Similar, but biphasic increasing pattern was observed in the serum albumin concentrations. The albumin concentration reached peak on day 26 before declining and reached a second peak on day 38 post-injection (Fig. 1).

Urine analysis

The urine albumin concentration showed a significant ($p < 0.05$) increasing pattern with two peaks on days 26 and 38 post-injection. The increasing urine albumin concentration pattern paralleled those of serum albumin (Fig. 1).

DISCUSSION

Nephrotic syndrome (NS) is sometimes referred to as a protein-wasting disease (Coggins and Maffly, 1985), in which proteinuria due to albuminuria is a predominant feature. Renal damage due to drugs, heavy metals, infections and autoimmune processes leads to the loss of serum albumin into urine, which decreases plasma albumin concentration and oncotic pressure, leading to oedema, ascites, and pleural effusion (Sapin *et al.*, 1977; Shull *et al.*, 1981; Coggin and Maffly, 1985).

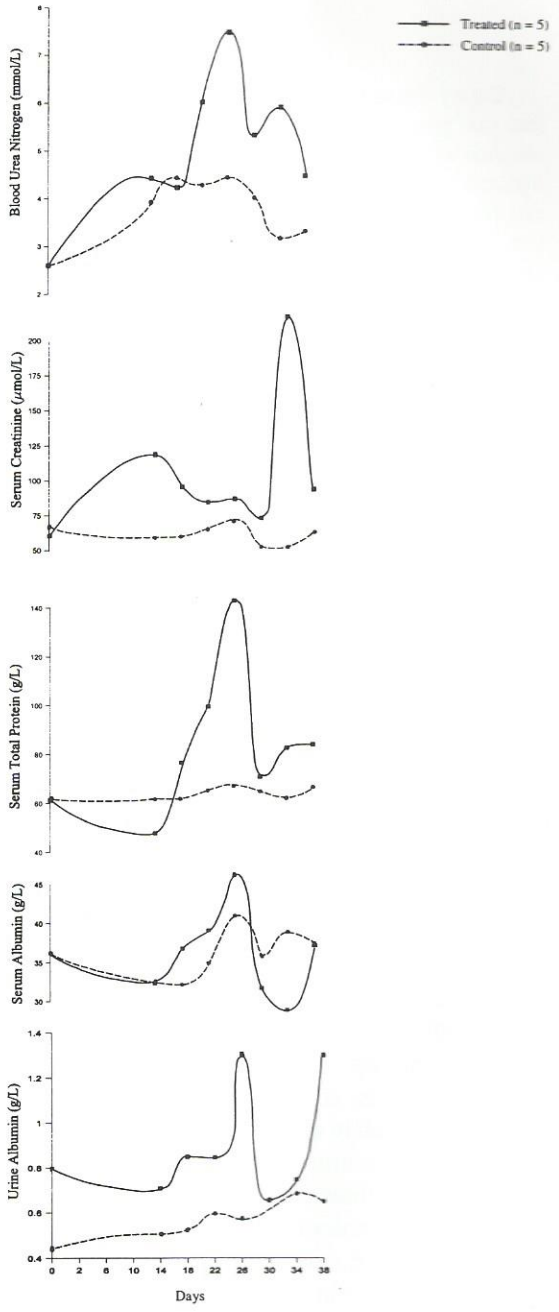

Fig. 1. The concentrations of serum and urine biochemical parameters following repeated exposures to mercury chloride in rats.

In this study, renal damage was induced in rats by multiple intravenous injections of mercury chloride. The damage was later detected using the parameters routinely used in the assessment of renal function, which include the blood urea nitrogen (BUN), serum creatinine, total serum and urine protein, and serum and urine albumin concentrations. As expected, serum creatinine and BUN levels showed significant increase, suggesting that the kidneys have been compromised by mercury chloride. Both the serum and urine albumin concentrations reached low levels at approximately the same time since the source of urine albumin in renal damage is the blood circulation. These changes were the results of renal damage, which occurred in two phases (Sapin *et al.,* 1977; Druet *et al.,* 1978; Hinglais *et al.,* 1979; Bellon *et al.,* 1982).

Although changes in serum and urine parameters were not absolutely conclusive at this stage, they suggested a biphasic insults by mercury chloride on the kidneys. It seemed that the kidneys recovered from an early renal damage only to be later subjected to another bout of renal damage. It has been suggested that anti-glomerular antibodies mediate the first phase of renal damage while the second phase is the result of immune complex deposition in the glomerular membrane, leading to persistent nephritis.

Mercury is reported to bind to metallothionein, a protein of moderate molecular weight found in the renal tissues. Localisation of mercury in renal tissues encourages methylation of the compound to its more toxic form, the methylmercury. While in plasma, mercury is reported to couple with serum proteins to form a protein complex that may induce an autoimmune nephritis (Bariety *et al.,* 1971). Metallothionein-mediated damage might be the mechanism that leads to early renal damage, while the immune-mediate renal damage might occur in a later phase. This observation agrees with earlier studies using Brown Norway rats compromised with mercury chloride, which showed biphasic nephropathy (Hinglais *et al.,* 1979; Bellon *et al.,* 1982).

The serum and urine manifestations of renal damages are greatly dependent on the extent of renal damage. Serum creatinine and BUN concentrations do not reflect early or minimal damage but there are no other serum or urine parameters that can be used reliably to detect early renal damage. However, this study concluded that multiple intravenous injections of mercury chloride produced a biphasic renal damage, reflected by the changes in serum creatinine, serum and urine albumin concentrations.

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RINGKASAN

PERUBAHAN BIOKIMIA SERUM DALAM KEROSAKAN RENAL TERARUH MERKURI KLORIDA DALAM TIKUS

Lapan puluh ekor tikus Sprague-Dawley berat badan di antara 200 hingga 230g dan berumur di antara lapan hingga sepuluh minggu telah dibahagikan kepada dua kumpulan 40 ekor setiap satu. Kumpulan 1 (terperlaku) menerima suntikan intravena 1 mL larutan mengamdungi merkuri klorida pada kadar 0.5 mg/kg berat badan dalam 0.85% NaCl menerusi vena ekor. Kumpulan 2 (kawalan) disuntik secara sama dengan 1ml 0.85% NaCl. Masing-masing perlakuan diulang selang sehari untuk selama 10 hari sebelum lima ekor tikus daripada setiap kumpulan dimatikan pada hari 0 dan pada setiap selang empat hari seterusnya, bermula dua mingu selepas suntikan pertama. Sampel darah dianalisiskan untuk nitrogen urea darah (BUN), kreatinin serum, protein sepenuh serum dan albumin serum. Sampel urin dianalisis untuk albumin. Kepekatan BUN dan kreatinin serum meningkat secara tererti ($p < 0.05$) berbanding kawalan. Kepekatan kreatinin serum, albumin serum dan urin menunjukkan gerakbalas dwifasa dengan gerakbalas pertama kepekatan kreatinin serum dicerapkan seawal 14 hari selepas suntikan pertama. Hasil kajian menyarankan mekanisme kerosakan ginjal dalam ketoksikan merkuri klorida berlaku dalam dua fasa.