Research Article

Neutrophil gelatinase-associated lipocalin – A novel biomarker for mercury-induced nephrotoxicity

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ABSTRACT

Introduction: The metal which has a relatively high density and toxic at low quantity is referred as heavy metals. The common heavy metals are arsenic, lead, mercury, cadmium, chromium, and thallium. **Aim and Objective:** The aim of this study was to analyze the mercuric chloride toxicity in the kidneys of Wistar albino rats. **Materials and Methods:** Female Wistar rats with an average weight of 180 ± 20 g and age of 120–140 days were assimilated for a week. Mercuric chloride (5 mg/kg b.w) and normal physiological saline (2 ml/kg/b.w) were administrated through intraperitoneal and oral route, respectively, for 5 days. Collection of blood and tissue was done to investigate serum biomarkers and histopathology for nephrotoxicity. **Results:** A significant change was observed in the levels of serum urea, creatinine, and neutrophil gelatinase-associated lipocalin (NGAL) and atypical changes such as tubular necrosis and atrophy of renal tissues. NGAL is a reliable novel biomarker for identifying the mercuric chloride-induced nephrotoxicity in rats.

KEY WORDS: Biomarkers, Mercuric chloride, Nephrotoxicity, Neutrophil gelatinase-associated lipocalin, Serum creatinine

INTRODUCTION

Mercury (Hg) is an environmental toxic substance that produces a wide range of adverse effects in living organisms. Hg exists in environment in three different forms (elemental, inorganic, and organic). Of three different forms, Hg found in the environment such as metallic Hg, mercuric sulfide (cinnabar ore), mercuric chloride, and methyl Hg.[1] Their solubility, reactivity, biological effects, and toxicity vary among these forms. Microorganisms such as bacteria and fungi and natural processes can change the Hg in the environment from one form to another. Hg exposure is the second most common cause of metal poisoning which is quite stable and biotransformed to highly toxic metabolites, thus eliciting biochemical alterations and oxidative stress. In biological systems, heavy metals have been reported to affect cellular organelles and components such as cell membrane, mitochondrial, lysosome, endoplasmic reticulum, nuclei, and some enzymes involved in metabolism, detoxification, and damage repair.[2] Hg is in our air from the combustion of diesel, jet fuel, and heating oil. It deposits on land and water and then concentrates in the food chain. Hg is also emitted by coal-burning power plants and oil refineries. Microorganisms in the environment convert elemental and inorganic Hg into organic Hg or vice versa. Hg is excreted almost through the kidney; thus, kidneys' exposure to mercuric chloride is unavoidable and subsequent toxicity commonly evolves in the kidney.[3,4] It is also reported that Hg induces damage at the molecular level by interacting with components such as DNA and nuclear proteins that may lead to cell cycle modulation, carcinogenesis, and finally into cell death.[5] Even though the existing biomarkers are reliable, a more accurate biomarker for Hg toxicity is in high demand. Hence, the aim of this study is to identify a novel biomarker for effective diagnoses.

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MATERIALS AND METHODS

Chemicals

All chemicals and reagents used in this study were of molecular and analytical grade; they were purchased from Sigma Chemical Company, St. Louis, MO, USA; Amersham Biosciences, Little Chalfont,

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Buckinghamshire, United Kingdom; and Sisco Research Laboratories, Mumbai, India.

Animals

The study was done in six female albino Wistar rats weighing 180 g ± 20 g. The animals were caged and maintained under standard laboratory conditions (temperature 25 ± 2°C) 12 h light and 12 h dark cycle with free access to a standard commercial diet and water *ad libitum* throughout the experimental period. The rats were acclimatized to laboratory conditions for 7 days before commencement of the experiment. The experiment was done according to the CPCSEA guidelines after getting approval from the Institutional Animal Ethical Committee (Saveetha Medical College and Hospital, Thandalam) with reference number: (*SU/CLAR/RD/025/2017)*.

Experimental Protocol

The animals were divided randomly into two groups, each group having three animals.

- Group I: Animals received 2 ml/kg body weight/day of distilled water orally for 5 days.
- Group II: Animals received 5 mg/kg body weight/day of mercuric chloride through intraperitoneal injection for 5 days.

At the end of the experiment and 24 h after the last dose, collection of blood was done in ethylenediaminetetraacetic acid tube through retro-orbital vein. Serum analyses were done for investigating the levels of biochemical parameters for Hg toxicity. All the animals were sacrificed with Isoflurane using desiccator, and then all the animals were dissected for the procurement of tissues. Tissue samples were stored in 10% formalin for histopathological study.

Procedure

Estimation of neutrophil gelatinase-associated lipocalin (NGAL)

The NGAL levels were determined using stored sandwich-type assay based on anti-NGAL antibodies. Microtiter plates were coated overnight at 4°C with monoclonal NGAL antibody dissolved in phosphate-buffered saline (PBS). Plates were blocked for 2 h at room temperature with assay buffer (1% [w/v] bovine serum albumin and 0.05% [v/v] Tween 20 in PBS [PBST]) and washed 3 times with 0.05% (v/v) PBST. A 100 µl of sample was added to the coated plates. Between each step of the assay, the plates were washed 3 times with PBST. Samples and controls were diluted 1:200 in assay buffer and incubated overnight at 4°C. Plates were then incubated with biotinylated monoclonal NGAL antibody in assay buffer and then incubated with streptavidin solution. Enhancement solution was added, and ELISA-horseradish peroxidase substrate and tetramethylbenzidine with hydrogen peroxide were added to the vials. ELISA stop solution was added and plates were measured in an ELISA reader. The samples were analyzed in duplicates.

RESULTS

Biochemical Analysis

The levels of serum urea and serum creatinine were significantly elevated in experimental group when compared with control group. Serum urea and creatinine were elevated up to 5.14 and 7 folds, respectively, than the control group. Similarly, the NGAL was also elevated nearly to 6.67 folds than the control group [Table 1].

From Chart 1, it is understood that the percentage increase of the serum creatinine and NGAL is nearly the same. Hence, NGAL can be a novel biomarker for Hg-induced nephrotoxicity.

Histopathological Analysis

The kidneys of mercuric chloride-induced rats show atrophy in glomeruli and multifocal congestion, followed by tubular necrosis and atrophy is seen more in cortex compared to medulla and there are multifocal congestion in glomeruli are observed in microscopic examination.[10] The kidneys of control animals show parenchyma with well-defined glomerulus and tubules [Figure 1].

DISCUSSION

Various environmental and industrial toxicants can induce toxicity through the metabolic activation to highly reactive free radicals including superoxides and oxygen reactive species.[6] One among them is Hg and their existence in the biological tissues leads to many alterations in the health of living organisms.[7] Hg exists in different forms and is a widespread pollutant that affects the structure and functions of several organs by producing oxidative stress. Hg ions combine with sulfhydryl compounds, such as a thiol group of amino acids, which carry Hg ions to different tubules of the kidney.[8] Among the different forms of Hg, our current study is on mercuric chloride toxicity. Mercuric chloride produces an excessive synthesis of reactive oxygen species and reduces the antioxidant level which leads damage of cells. The primary organ that is affected by mercuric chloride is the liver and kidney. Among these, the kidney is more prone for toxicity mainly due to drug and heavy metals and their identification in early stage is difficult to identify; we used a novel biomarker for identifying the acute kidney injury. In the present study, rats were administrated with mercuric chloride and their serum samples were analyzed with standardized biomarker such as urea and creatinine due to lack of early sensitivity; we adjoin a novel biomarker such as NGAL[9] which was also used

Table 1: Correlation of mean value of different biomarkers between mercury and control groups

S. No	Parameters	Control group	Mercury group	Percentage increase
1.	Serum urea (mg/dl)	35±5	215±8	5.14
2.	Serum creatinine (mg/dl)	0.3±0.2	2.4±0.3	7
3.	NGAL (ng/ml)	2.15±0.35	16.5±0.75	6.67

NGAL: Neutrophil gelatinase-associated lipocalin

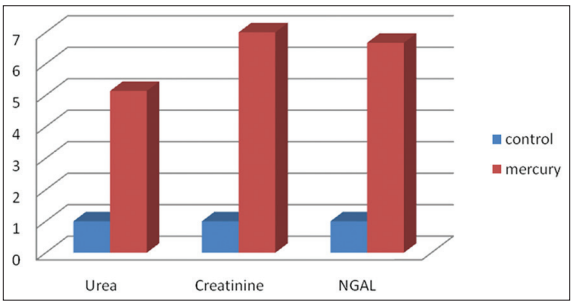

Chart 1: Percentage increase of the mean value of different biomarkers between mercury and control groups

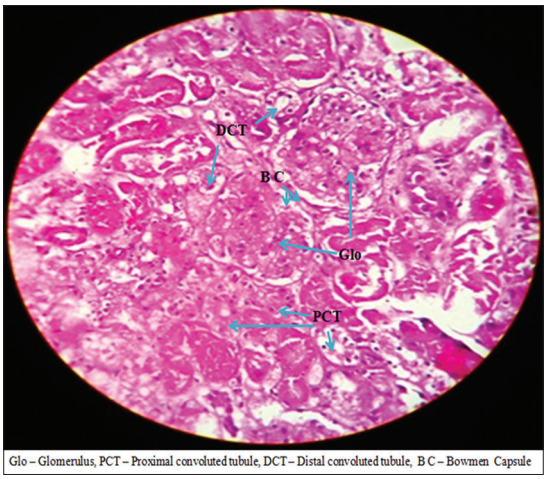

Figure 1: Control animals show the normal architecture of the kidney

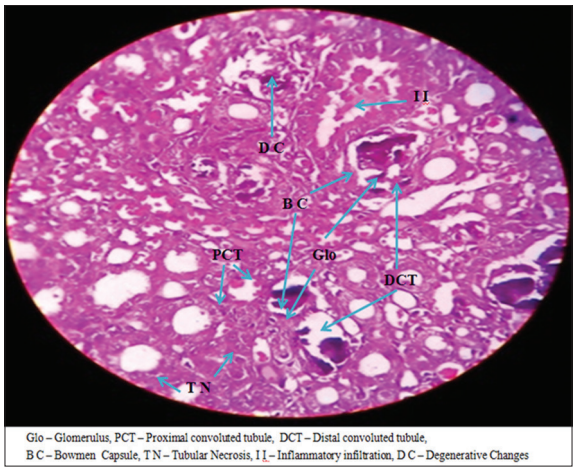

Figure 2: Mercury-induced animal shows loss of normal architecture of the kidney

to analyze and identify the early renal damage. The serum urea and creatinine were increased indicating the renal damage. NGAL is protein-based biomarker also upregulated which absolutely shows early damage in the kidney. As per previous literature, the Hg reduces the antioxidant level and produces oxidative damage of cells with the help of synthesis of free radicals and lipid peroxidation. Lipid peroxidation alters the structural integrity of cell membrane and functions. The changes like tubular inflammatory infiltration, necrosis, and atrophy are predominately seen in the proximal tubules of the kidney and minimally there is multifocal congestion in glomeruli are observed in microscopic examination.[10]

Previous studies have found that the level of serum NGAL rises significantly after 24 h of acute kidney injury and much earlier than the serum creatinine level.[11] Serum NGAL starts to rise within 6 h after kidney injury and it continues to raise up to 48 h, whereas the rise of serum creatinine is significant only after 48 h.[12] NGAL, apart from being more accurate biomarker for acute kidney injury, it can be used as a differential diagnostic tool between various renal diseases such as acute kidney disease and hepatorenal syndrome-related kidney injury.[13] Similarly, the level of NGAL in chronic kidney injury also plays a significant role in estimating the onset, diagnosis, severity, and prognosis of it. In chronic kidney injury, the active renal tubular injury leads to increased NGAL synthesis, which results in increased NGAL concentrations in urine.[14] Renal replacement therapy is becoming a much needed procedure for patients with kidney failure. Plasma NGAL <400 mg is used as an essential parameter to rule out renal replacement therapy.[15] Hence, plasma and urinary NGAL is an accurate tool for acute and chronic kidney injury including nephrotoxicity and renal replacement therapy [Figure 2].

Serum creatinine and urea concentrations are two of the traditional screening indices for kidney functions and renal structural integrity. It is well known that the Hg accumulates more in renal epithelium (Franciscato *et al*., 2011). In the present study, enhanced creatinine and urea concentrations in HgCl 2-treated rats indicate nephrotoxicity. This elevation in creatinine and urea might be due to damage produced in kidney tubules and this was confirmed by marked alterations in renal tissues when compared to the control group [Figure 1]. Oriquat *et al*. (2012), Glaser *et al*. (2010), and Gado and Aldahmash (2013) have also reported

similar biochemical and histopathologic alterations in Hg-induced nephrotoxicity.

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