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The influence of taurine pretreatment on aluminum chloride induced nephrotoxicity in Swiss albino mice

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Summary. The present study was carried out to investigate (1) the alterations in biochemical parameters, free radicals and enzyme activities induced by aluminum chloride ($AlCl_3$) in kidney of male Swiss albino mice, and (2) the role of taurine in alleviating the nephrotoxic effects of $AlCl_3$. Taurine plays an important role as an antioxidant and is consequently expected to protect tissues from damage caused by reactive oxygen metabolites. The animals were randomized into four groups (n=6/group). Group I was the control group. Group II received a single dose of $AlCl_3$ (25 mg Al^{3+}/kg b.w., ip). Group III received taurine (100 mg/kg b.w., ip) for 5 consecutive days before administration of $AlCl_3$ (25 mg Al^{3+}/kg b.w., ip). Group IV received taurine (100 mg/kg b.w., ip) for 5 consecutive days. 24 h following the administration of compounds, all the mice were assessed using serum and tissue homogenate biomarkers as well as the pathological evaluation. Exposure to $AlCl_3$ led to an increased level of renal lipid peroxidation as measured by malondialdehyde (MDA), while reduced glutathione (GSH), glutathione peroxidase (GPx) and catalase (CAT) decreased. Marked elevation of blood urea and serum creatinine concentrations were also observed in $AlCl_3$ treated mice, thereby indicating renal damage. All these factors were significantly improved by taurine pretreatment. The histological and ultrastructural observations on the kidney tissues also confirmed the renoprotective nature of taurine. Thus these results may indicate that taurine treatment protects against functional, biochemical and morphological damage in $AlCl_3$-induced acute renal failure in mice.

Key words: $AlCl_3$, Taurine, Oxidant stress, Kidney function, Histopathology, Ultrastructure

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Introduction

Aluminum is a ubiquitous metal in nature. Although its role in cellular metabolism remains to be elucidated, aluminum is potentially toxic to humans. Aluminum compounds have many medical implications. They are widely used in antacids, phosphate binders, buffered aspirins, vaccine, antiperspirant, and allergen injections (Lione, 1985; Baydar et al., 1998; Exley, 1998). Furthermore, aluminum contamination has also been reported in parenteral nutrition additives, intravenous solutions, dialysates, lipid emulsions, and infant formulas (Fernandez-Lorenzo et al., 1999; Popinska et al., 1999). Thus, the increasing awareness of the dangers associated with aluminum is generating more and more concerns about unsafe exposure to humans (Forbes et al., 1995; Dawson et al., 2000). Aluminum accumulates in mammalian tissues such as brain, bone, liver and kidney (Wills et al., 1993; Sahin et al., 1994); this accumulation is accompanied by renal failure (Alfrey, 1980; Ecelbarger et al., 1994) or associated with age (Ecelbarger et al., 1994). Moreover, aluminum accumulation in kidney promotes degeneration in renal tubular cells, inducing nephrotoxicity (Ebina et al., 1984; Cacini and Yokel, 1988; Bertholf et al., 1989; Roy et al., 1991; Somova et al., 1997). Aluminum accumulation in renal tissue affects cellular metabolism, promotes oxidative stress, and induces alterations in renal tubular

p-aminohippuric acid transport and renal tubular phosphate reabsorption, together with impairment in sodium and water balance (Mahieu and Calvo, 1998; Mahieu et al., 2003).

Taurine (2-aminoethanesulfonic acid) is the major intracellular free ß-amino acid, which is normally present in most mammalian tissues. It has several functions in cell metabolism including osmoregulation, membrane stabilization, detoxification and regulation of cellular calcium homeostasis (Huxtable, 1992). Taurine has been demonstrated to function as a direct antioxidant by scavenging reactive oxygen radicals, inhibition of lipid peroxidation and as an indirect antioxidant by preventing changes in membrane permeability resulting from oxidant injury in many tissues, including liver (Timbrell et al., 1995; Waters et al., 2001; Hagar, 2004). A number of investigators reported that taurine protects several organs in the body against toxicity and oxidative stress due to heavy metals and other toxins as well as drugs (Hwang et al., 1998; Dogru-Abbasoglu et al., 2001; Gurer et al., 2001; Hagar et al., 2006; Tabassum et al., 2006; Jagadeesan and Sankarsami Pillai, 2007; Manna et al., 2008; Parildar-Karpuzoglu et al., 2008). In a previous study conducted in our lab, we have shown that taurine had anaphylactic and therapeutic activity against hepatotoxicity induced by $AlCl_3$ in mice (El-Sayed et al., 2011). The aim of this study was to test the efficiency of taurine in antagonizing the nephrotoxic effects of aluminum (as chloride). Testing was performed by using an acute experimental model on male mice with taurine given before application of aluminum ions.

Materials and methods

Chemicals

$AlCl_3$, taurine and other routine chemicals (analytical grade) were purchased from Sigma-Aldrich® Chemical Company (St. Louis, MO, USA). Commercially available diagnostic kits and reagents used in the biochemical study were supplied by Cayman Chemical Company, USA.

Animals and treatments

In this study male mice weighing 15-20 g were obtained from the animal house of Faculty of Veterinary Medicine, King Faisal University (Saudi Arabia). The animals were housed throughout the experiment in polypropylene cages (with each cage housing 5 animals) and allowed to acclimatize to the laboratory environment for 10 days. Animals were maintained under controlled conditions of temperature at 25±2°C, relative humidity of 50±15% and normal photoperiod (12-12 h light-dark cycle). The animals were allowed free access to standard dry pellet diet and water ad libitum. All animal procedures were approved by the University of King Faisal Animal Care and Use Committee and were conducted in agreement with NIH guidelines for the humane care of laboratory animals. The animals were randomly divided into four experimental groups (n=6/group). Group I served as the vehicle treated control. Group II received a single intraperitoneal injection of $AlCl_3$ solution (25 mg Al^{3+}/kg b.w.; 0.5 LD_{50}) in physiological saline. Group III received taurine (100 mg/kg b.w.) intraperitoneally for 5 consecutive days before administration of $AlCl_3$ (25 mg Al^{3+}/kg b.w.). Group IV received taurine (100 mg/kg b.w.) intraperitoneally for 5 consecutive days. The doses of chemicals, the frequency and the route of administration were selected according to previous reports by Viezeliene et al. (2006) and Flora et al. (2008).

Tissue and blood collection

Mice from each group were sacrificed by cervical decapitation under ether anesthesia 24 h after administration of the compounds. The kidney tissues were quickly excised and washed immediately with ice-cold physiological saline (0.9% NaCl). Tissues were homogenized in 0.01MTris-HCl buffer (pH 7.4) and aliquots of this homogenate were used for the assays. These tissues were used to measure the oxidative state of renal tissue. Blood samples were drawn by cardiac puncture and centrifuged to harvest the serum, with which urea and creatinine were analyzed. A section of the kidney was set aside for light and electron microscopic studies.

Measurement of oxidative stress markers

Lipid peroxidation (LPO) was measured by thiobarbituric acid (TBA) method (Esterbauer et al., 1991) that determines aldehyde formed by degradation of hydroperoxide, including malondialdehyde (MDA). The reduced glutathione (GSH) level was measured from the reduction of 5,5'-dithiobis (2-nitrobenzoic acid) (DTNB). The chromogen formed is directly proportional to the GSH concentration (Beutler et al., 1963). Cytosolic glutathione peroxidase (selenium-dependent) (GPx) activity was determined from the azide insensitive rate of oxidation of NADPH in the presence of hydrogen peroxide, glutathione and glutathione reductase (Flohe and Gunzler, 1984). Catalase (CAT) activity was measured from the rate of dismutation of hydrogen peroxide (H_2O_2) to water and molecular oxygen in a two-step coupling reaction (Aebi, 1984). Total protein content was determined according to Lowry et al. (1951).

Assessment of renal function

Serum creatinine level was determined from the red-complex formed through reaction with basic picrate solution (Murray, 1984). Blood urea was hydrolysed into

ammonia and CO_2 and the indophenol formed from reaction of ammonia with hypochlorite and salicylate was measured (Kaplan, 1984).

Histological study

After fixation in Bouin's fluid for 48 h, the kidneys were dehydrated in graded ethanol and blocks were made in paraffin. Sections of 4 μm thickness were stained with haematoxylin and eosin (H&E), and examined under an Olympus CX41 light microscope.

Electron microscopic study

For examinations of kidney tissues, primary fixation was done in 3% glutaraldehyde in sodium phosphate buffer (200 mM, pH 7.2) for 3 h at 4°C. Kidney tissues were washed with the same buffer and postfixed in 1% osmium tetroxide (Agar Sci. Ltd.) in sodium phosphate buffer, pH 7.2, for 1 h at 4°C. Tissue samples were washed with the same buffer for 3 h at 4°C and then embedded in Araldite (Agar Sci. Ltd.). Thin sections were cut with LKB ultramicrotome. Samples were stained with 2% uranyl acetate and lead citrate. The sections were viewed and photographed on a Jeol JEM-1011 transmission electron microscope (Jeol Ltd., Japan) at 80 kV.

Statistical analysis

Data were analyzed using SPSS 17.0 for windows. Significance was calculated using one-way analyses of variance (ANOVA) followed by least significance difference (LSD) for multiple comparisons. $P<0.05$ was considered statistically significant.

Results

Biochemical parameters

The results for the estimation of renal LPO, GSH and antioxidant enzymes are presented in Table 1. $AlCl_3$ alone treatment caused enhancement in LPO level by 653% as compared to control group. Pretreatment with taurine at a dose of 100 mg/kg b.w. caused reduction in LPO by 74% as compared with $AlCl_3$-treated group. Treatment of mice with taurine alone did not affect the LPO when compared to the vehicle control group. Treatment with $AlCl_3$ alone resulted in depletion of GSH and reduction in the activities of GPx and CAT by 30%, 61% and 26%respectively, as compared with control group. However, pretreatment of animals with taurine at 100 mg/kg b.w. resulted in recovery of the above-mentioned parameters by 57%, 171% and 51% respectively, as compared with $AlCl_3$-treated group. The data in Table 2 showed that $AlCl_3$ treatment led to about 344% and 89% enhancement in the values of blood urea and serum creatinine, respectively, as compared with control group. Pretreatment with taurine resulted in 54% and 23% reduction in the values of urea and creatinine respectively as compared with $AlCl_3$-treated group.

Histopathological findings

Photomicrographs of kidney sections from various treatment groups are shown in Fig. 1. In the $AlCl_3$ loaded mice, injured glomeruli were hypertrophied and often showed segmental sclerosis characterized by collapse of capillary loops and adhesion of the tuft to Bowman's capsule (Fig. 1B). In addition, all $AlCl_3$-treated mice developed renal lesions characteristic of

Table 1. Effect of taurine pretreatment on renal peroxidative alterations induced by $AlCl_3$ in mice.

Parameter	Control N=3	$AlCl_3$ N=4	Taurine + $AlCl_3$ N=4	Taurine N=5
MDA (μmol/mg protein)	0.55±0.04	4.14±0.42[a]	1.06±0.13[b]	0.56±0.06[b]
GSH (μmol/mg protein)	8.76±0.26	6.10±0.37[a]	9.57±0.19[b]	9.77±0.04[b]
GPx (nmol/min/mg protein)	902.67±63.85	352.00±38.09[a]	954.25±94.60[b]	1457.60±76.65[a,b]
CAT (U/mg protein)	24.58±2.62	18.29±2.21	27.64±2.97	34.02±1.24[b]

Each value represents the mean ± SE. [a]: $P<0.05$ compared with control group, [b]: $P<0.05$ compared with $AlCl_3$ group (LSD-multiple comparison test). Abbreviations: MDA, malondialdehyde; GSH, reduced glutathione; GPx, glutathione peroxidase; CAT, catalase.

Table 2. Effect of taurine pretreatment on serum nephrotoxicity indices induced by $AlCl_3$ in mice.

Parameter	Control N=3	$AlCl_3$ N=4	Taurine + $AlCl_3$ N=4	Taurine N=5
Creatinine (mg/dl)	1.10±0.06	2.08±0.14[a]	1.60±0.13[b]	1.16±0.07[b]
Urea (mg/dl)	56.62±9.40	251.13±16.10[a]	115.21±6.81[a,b]	67.64±3.11[b]

Each value represents the mean ± SE. [a]: $P<0.05$ compared with control group, [b]: $P<0.05$ compared with $AlCl_3$ group (LSD-multiple comparison test).

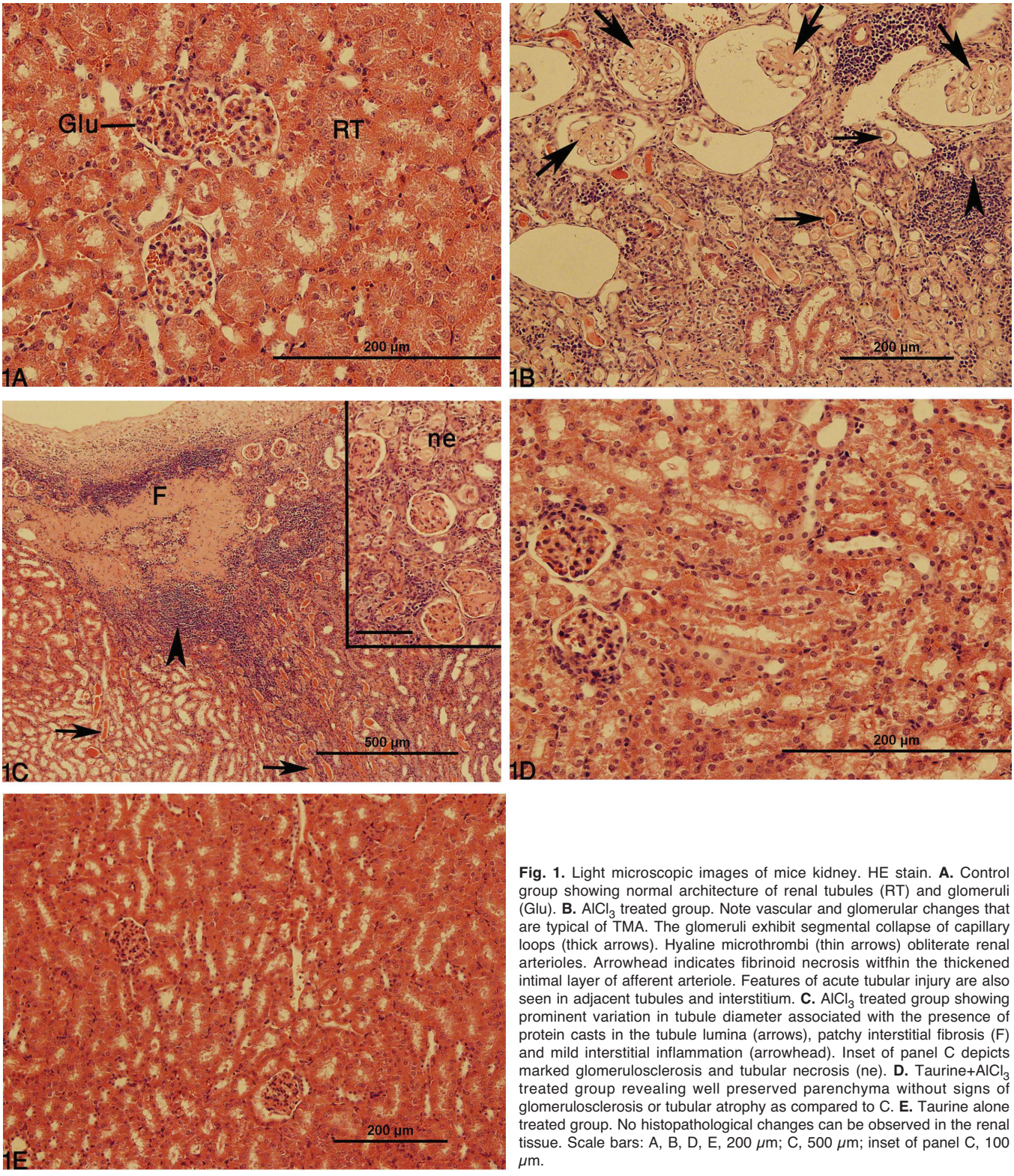

Fig. 1. Light microscopic images of mice kidney. HE stain. **A.** Control group showing normal architecture of renal tubules (RT) and glomeruli (Glu). **B.** $AlCl_3$ treated group. Note vascular and glomerular changes that are typical of TMA. The glomeruli exhibit segmental collapse of capillary loops (thick arrows). Hyaline microthrombi (thin arrows) obliterate renal arterioles. Arrowhead indicates fibrinoid necrosis witfhin the thickened intimal layer of afferent arteriole. Features of acute tubular injury are also seen in adjacent tubules and interstitium. **C.** $AlCl_3$ treated group showing prominent variation in tubule diameter associated with the presence of protein casts in the tubule lumina (arrows), patchy interstitial fibrosis (F) and mild interstitial inflammation (arrowhead). Inset of panel C depicts marked glomerulosclerosis and tubular necrosis (ne). **D.** Taurine+$AlCl_3$ treated group revealing well preserved parenchyma without signs of glomerulosclerosis or tubular atrophy as compared to C. **E.** Taurine alone treated group. No histopathological changes can be observed in the renal tissue. Scale bars: A, B, D, E, 200 μm; C, 500 μm; inset of panel C, 100 μm.

thrombotic microangiopathy (TMA). Kidney sections showed widespread arterial and arteriole myointimal thickening with mucoid degeneration. The thickened myointimal layers within the interlobular and afferent arterioles contained fibrinoid material. Glomerular and vascular changes were accompanied by patchy tubulointerstitial injury characterized by epithelial cell necrosis, tubular atrophy, an acute inflammatory infiltrate, interstitial edema and fibrosis (Fig. 1C). Scattered tubule casts were also observed. In taurine plus $AlCl_3$ group, there were no significant histological abnormalities and the renal tubule cells were of a normal appearance (Fig. 1D), with the lumen clearly visible in most of the cortical tubuli. There were no signs of glomerulosclerosis, although a glomerular congestion was seen to a mild degree. Renal slices from the control (Fig. 1A) and taurine-alone (Fig. 1E) groups showed normal architecture.

Cytopathological observations

In the glomeruli of control mice, blood capillaries

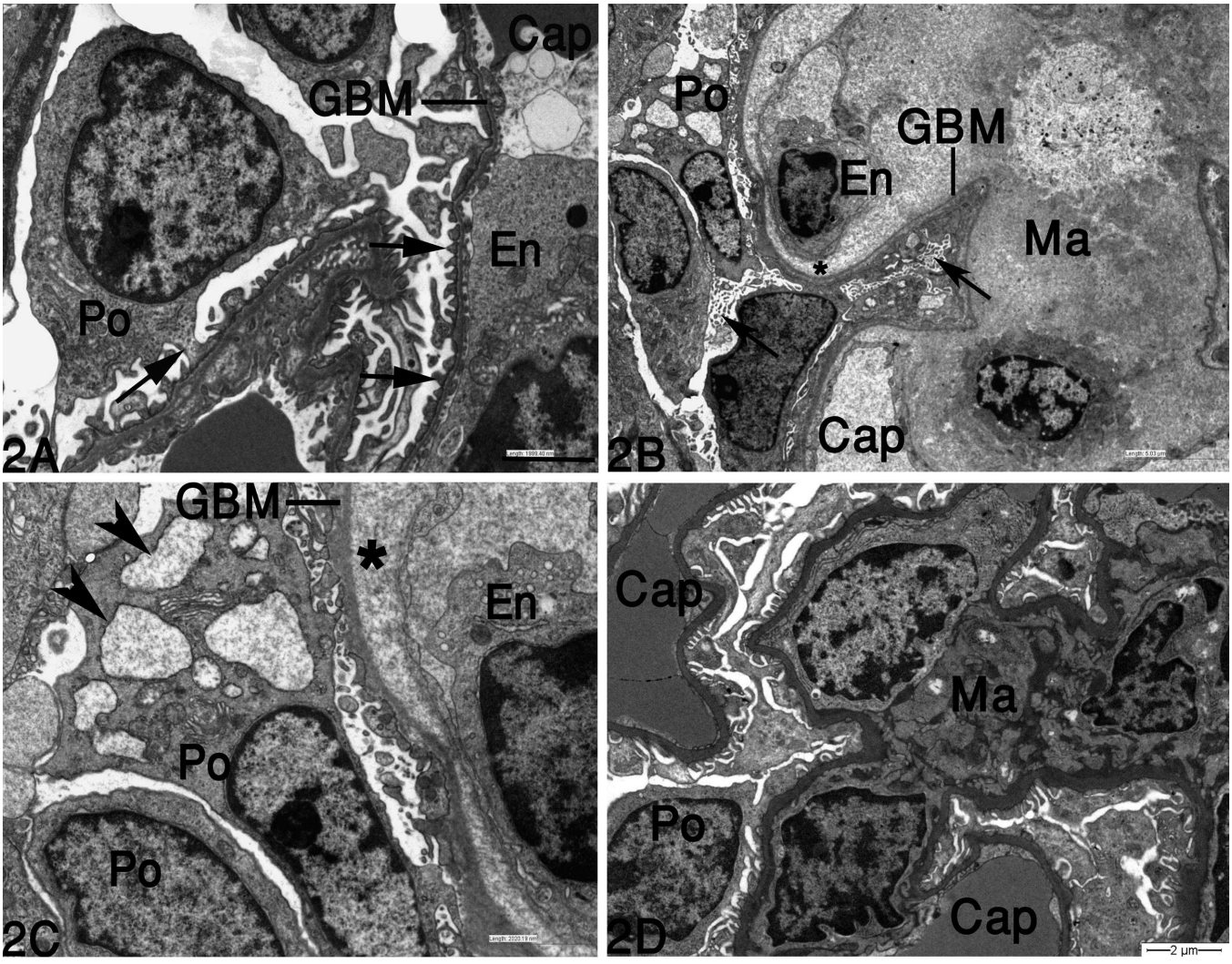

Fig. 2. Transmission electron micrographs of kidney glomeruli from control and experimental groups of mice. Uranyl acetate and lead citrate stain. **A.** Control group. Glomerular basement membrane (GBM) and foot processes (arrows) of podocytes (Po) are normal. Patent capillary lumina (Cap), thin endothelium (En) with abundant and well preserved fenestrations are observed. **B.** $AlCl_3$ treated group. There is increased mesangial matrix (Ma) and evidence of mesangiolysis. A podocyte (Po) exhibits signs of degeneration with widespread effacement and simplification of foot processes (arrows). The glomerular capillaries (Cap) are narrow and contain flocculent materials. Capillary endothelium (En) shows marked loss of fenestrations and there is also a prominent expansion of subendothelial space (*) by electron-lucent debris. GMB: Glomerular basement membrane. **C.** Higher magnification of figure **(B)** showing podocyte detachment with large intracytoplasmic electron lucent vacuoles (arrowheads). **D.** Taurine+$AlCl_3$ treated group. The podocytes (Po) appear better preserved than in B. Mesangial matrix (Ma) is less developed. The glomerular capillaries (Cap) are engorged with blood elements. Scale bars: A, C, D, 2 μm; B, 5 μm.

had fenestrated endothelium and thin glomerular basement membrane (GBM). Podocytes had large vesicular nuclei. Their processes were seen ending on the basement membrane (Fig. 2A). Treatment of adult mice with $AlCl_3$ resulted in diffuse mesangiolysis associated with increased lucency and corrugation of GBM (Fig. 2B). The podocyte feet were irregular and frequently disrupted. Foci with vacuolization were found in the cytoplasm of podocytes, some of which were probably derived from deteriorated mitochondria and/or frequently present swollen endoplasmic reticulum (Fig. 2C). Glomerular capillaries were constricted and the endothelium showed extensive loss of fenestrae and widening of the subendothelial spaces. In mice pretreated with taurine, the alterations mentioned above were less expressed (Fig. 2D). In most cases, the glomerular endothelia showed a regular feature and the degeneration of foot processes was limited, although they were indistinct in some places.

The proximal convoluted tubule (PCT) of control

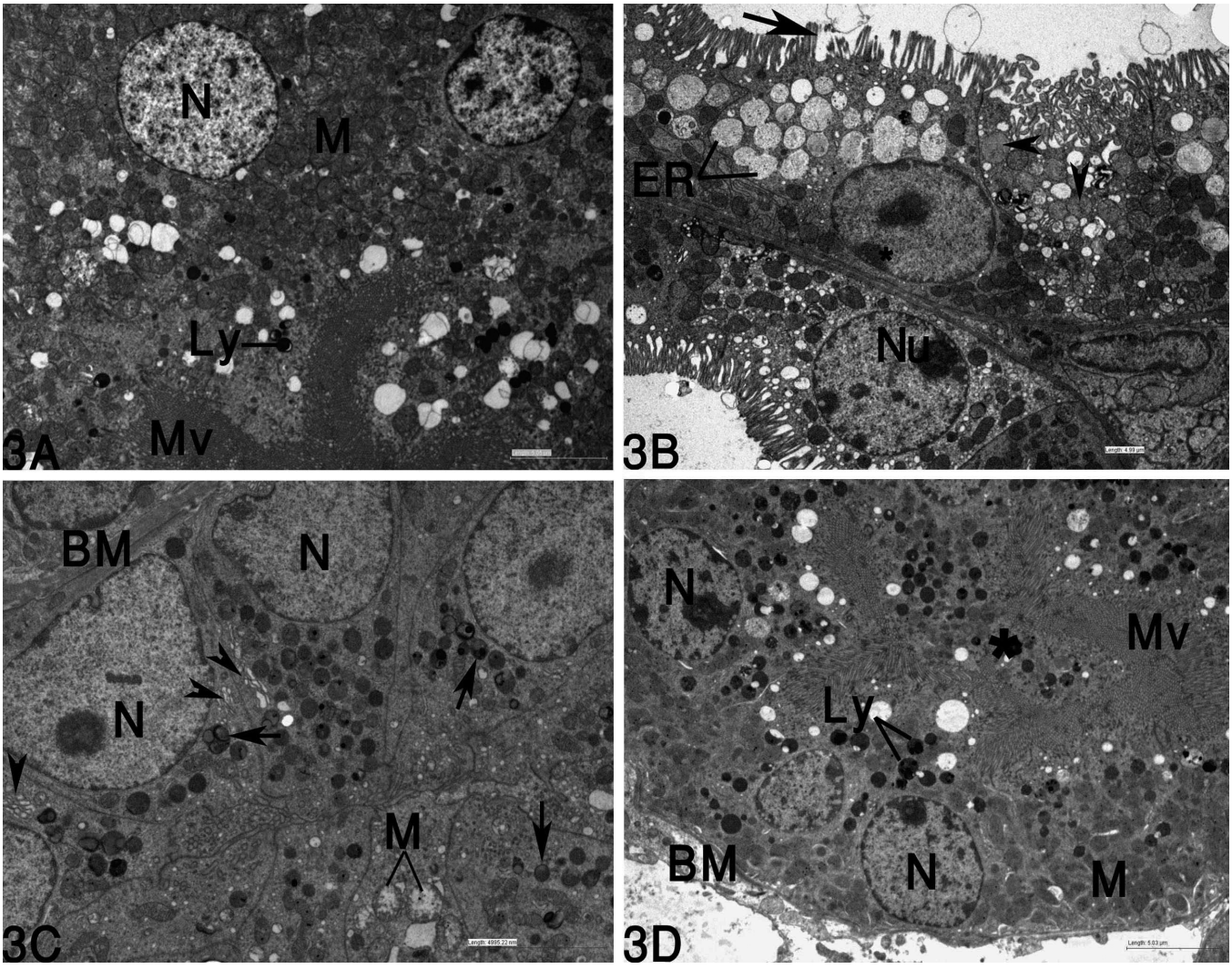

Fig. 3. Transmission electron micrographs of kidney proximal epithelium from control and experimental groups of mice. Uranyl acetate and lead citrate stain. **A.** Control group. Note the normal organelles, including mitochondria (M) and microvilli (MV). Nucleus (N) is of normal size and has evenly distributed chromatin. Ly: lysosomes. **B.** $AlCl_3$ treated group. Deformed apical microvilli (arrow), nuclei with abnormal distribution of peripheral chromatin (*) and eccentric nucleolus (Nu) are discernible. Noteworthy dilated ER membranes. The dilated ER contains fine granular material and it obviously compresses the cell nucleus. Arrowheads point to damaged mitochondria. **C.** $AlCl_3$ treated group. Note irregular nuclei (N), increased presence of secondary lysosomes (arrows), enlarged Golgi bodies (arrowheads). Some mitochondria (M) are swollen and have distorted cristae. Apical microvilli are destroyed. BM: basement membrane. **D.** Taurine+$AlCl_3$ treated group. The degenerative changes in epithelial cells of PCT are minimal as compared to **B** and **C.** Mitochondria (M) and nuclei (N) are generally normal. Brush border microvilli (Mv) extending to the lumen are seen. Indention of parts of cell cytoplasm (*) into tubular lumen can be noted. Ly: secondary lysosomal elements. Scale bars: A, B, C, 5 μm; D, 2 μm.

mice was lined by cuboidal cells showing well developed apical microvilli (Fig. 3A). Alterations in proximal tubular cells were predominant in kidney samples from $AlCl_3$-treated mice with some evidence of single cell necrosis. The majority of nuclei appeared with condensed peripheral chromatin. The brush border microvilli were shorter and either irregular in height or focally absent. The affected renal tubular cells showed dilatation of the endoplasmic reticulum. The dilated rough endoplasmic reticulum (RER) lost the characteristic parallel arrangement of their cisternae and disclosed partial detachment of the bound polyribosomes and accordingly had degranulated appearance. Occasional marked dilatation of RER was discerned and the dilated ER was filled with fine granular material (Fig. 3B). Mitochondria demonstrated dramatic signs of injury in the form of swelling, loss of cristae and membrane disintegration (Figs. 3B and C). Several mitochondria also contained dark matrix granules. Lysosomes increased in number and were full of amorphous materials (Fig. 3C). Tubule lumens frequently contained exfoliated cells and cellular organelles (data not shown). Pretreatment with taurine decreased the tubular pathology in most PCT epithelium. However, some minor alterations (e.g., partial loss of brush border microvilli) could be observed (Fig. 3D).

Discussion

Aluminum is not an essential element in the human. Acute aluminum toxicity can occur when the urinary bladder is irrigated with 1% alum to treat bladder hemorrhage. This acute toxicity is almost always limited to patients who have renal insufficiency (Phelps et al., 1999). The antioxidant enzymes play important protective roles in the kidney (Ichikawa et al., 1994). Primarily because of its transport functions, the kidney has a very active oxidative metabolism that results in the production of reactive oxygen species (ROS). The antioxidant enzymes GSH-Px and CAT are responsible for the protection against ROS. Oxidant injury is now recognized as playing a key role in the induction of experimental renal diseases (Ichikawa et al., 1994; Haugen and Nath, 1999). The present study was undertaken to investigate whether taurine (an antioxidant) could modulate $AlCl_3$ induced cytotoxicity in kidney tissues. In the $AlCl_3$ treated mice, the observed increase in the levels of MDA (a thiobarbituric acid reactive substance; TBARS) and the decrease in antioxidant defenses GSH, GPx and CAT in kidney tissue suggest increased formation of free radicals. These observations are similar to the data reported by Yousef (2004), Yousef et al. (2005, 2007) and Nehru and Anand (2005) who indicated that aluminum intake produces oxidative stress. Although aluminum is not a transition metal and therefore cannot initiate peroxidation, many investigations have searched for a correlation between aluminum accumulation and oxidative damage in the body tissues (Cherroret et al., 1995; Wilhelm et al., 1996; Nehru and Anand, 2005). An *in vitro* study has indicated that aluminum greatly accelerates iron-mediated LPO (Xie and Yokel, 1996). In fact, aluminum, a non-redox-active metal, is a pro-oxidant both *in vivo* and *in vitro* (Exley, 2004). The primary effects of aluminum on the brain, liver and kidney functions are thought to be mediated via damage to cell membranes. LPO of biological membranes results in the loss of membrane fluidity, changes in membrane potential, an increase in membrane permeability and alterations in receptor functions (Nehru and Anand, 2005). Nehru and Anand (2005) also reported a significant increase in brain TBARS after stimulation by aluminum salts. The ionic radii of Al^{3+} most closely resemble those of Fe^{3+}, therefore the appearance of Al^{3+} in Fe^{3+} sites is probable. Aluminum is known to be bound by the Fe^{3+} carrying protein transferrin thus reducing the binding of Fe^{2+}. The increase in free intracellular Fe^{2+} causes the peroxidation of membrane lipids and thus causes membrane damage (Nehru and Anand, 2005). Orihuela et al. (2005) reported that at high doses, aluminum was able to induce oxidative stress in the intestinal mucosa, as indicated by the significant increase in the concentration of both, oxidized glutathione/reduced glutathione (GSSG/GSH) ratio and TBARS levels. Aluminum might affect the GSH synthesis by decreasing the activity of glutathione-synthase (GS), a non-limiting step of the reaction which would result in a reduction of GSH content. On the other hand, it has been demonstrated that aluminum is able to inhibit NADPH-generating enzymes such as glucose 6-phosphate dehydrogenase and NADP-isocitrate dehydrogenase (Zatta et al., 2000). Since NADPH is shown to be a main factor for GSH regeneration, the decreased GSH level could be also ascribed to insufficient supply of NADPH. Therefore, aluminum could indirectly contribute to unbalance redox equilibrium in the renal tissue.

Results from our study revealed significant increases in blood urea and serum creatinine levels (markers of impaired glomerular function) following treatment with $AlCl_3$. The glomeruli of aluminum loaded mice showed evidence of ischemic glomerular collapse. The predominant abnormalities observed by electron microscopy were diffuse mesangiolysis, and endothelial swelling and detachment from the underlying GBM. Mesangiolysis describes a process whereby mesangial cells degenerate and the mesangial matrix dissolves or is attenuated. This form of injury is seen in a variety of glomerular diseases, including thrombotic micro-angiopathies (e.g., preeclampsia, hemolytic uremic syndrome) and toxic glomerulopathy, and in renal transplantation (Antignac et al., 1989; Koitabashi et al., 1991; Paueksakon et al., 2002), in which the primary injury is believed to occur in the endothelial cells. In animal models, mesangiolysis can be induced in one of two ways: direct damage to mesangial cells, as occurs with Habu snake venom (Kubo et al., 2002) and anti-Thy-1 antibody (Morita et al, 1996), or direct damage to the endothelial compartment through injection of

antiendothelial antibodies (Matsuda, 1988) or monocrotaline, an alkaloid from *Crotalaria spectabilis* (Kurozumi et al., 1983). Both pathways lead to mesangiolysis within minutes after injection of the toxin. Intravenous administration of aluminum lactate to rabbits causes deposition of aluminum in the mesangial cells that also results in mesangiolysis and microaneurysm (Hong et al., 2000).

Vascular endothelial growth factor (VEGF) produced by podocytes is necessary for glomerular endothelial growth and maintenance of fenestrae (Esser et al., 1998; Eremina et al., 2003, 2006). In extra-glomerular vasculature, VEGF mediates endothelial cell differentiation/proliferation and vascular tone/permeability (Schrijvers et al., 2003). It is therefore conceivable that by targeting VEGF, $AlCl_3$ could induce endothelial injury. An *in vitro* study of Baek et al. (2012) demonstrated that taurine is associated with an increase in endothelial cell proliferation by promoting cell cycle progression via activation of both MEK/ERK and PI3K/Akt-dependent signaling pathways, as well as elevation of cell migration via the Src/FAK axis. A number of studies have demonstrated that taurine exerts beneficial effects on vascular function through its antioxidant action (Huxtable, 1992; Zulli, 2011). For example, taurine attenuates hyperglycemia-induced endothelial cell apoptosis, as well as leukocyte-endothelial cell interactions and cardiac dysfunction (Casey et al., 2006). Taurine improves vascular-endothelial dysfunction induced by experimental diabetes via its antioxidative property (Wang et al., 2008). Indeed, renal cortical blood flow after $AlCl_3$ induced renal injury was significantly improved in this study with taurine pre-treatment. Taurine preserved the overall glomerular vascular architecture of the kidney, allowing for improved microcirculation and ultimate improved viability of tubular cells, endothelial cells, and podocytes.

Alteration in foot processes is a common response of podocytes to almost every kind of stress, in part, as an adaptive change to improve their attachment to the GBM. Podocytes have been shown to undergo flattening and spreading of major processes in early postischemic renal failure (Racusen et al., 1984). Taurine in this study showed protection of podocytes by maintaining cell integrity and tight foot process attachment to GBM, although the morphological protection was partial. Because the cell replication capability of podocytes is limited in the adult and cannot be replaced once they undergo degeneration (Kriz et al., 1999), prevention of podocyte damage with taurine likely leads to sustained renal function.

In addition, we clearly showed that $AlCl_3$ induced ultrastructural changes on cortical proximal tubules. This was also supported by histopathological observations. Different studies reported that proximal tubules are the target of aluminum (Ebina et al., 1984; Roy et al., 1991; Wills et al. 1993; Sargazi et al., 2001; Kutlubay et al., 2007). In proximal epithelial cells, we observed nuclear and cytoplasmic changes. Necrotic nuclei and detachment of brush border indicated a functional impairment of the urinary reabsorption. Moreover, mitochondria were damaged. Mitochondria are the energy source of the cell and therefore damage of the mitochondria due to aluminum can deplete the ATP of cell (Sood et al., 2011), resulting in necrosis. Mitochondrial damage can lead to intracellular oxidative stress and elevated ROS levels produced by impairment of the mitochondrial electron transport chain (Indo et al., 2007). Taurine might contribute to the amelioration of nuclear and mitochondrial damage probably due to the antioxidant effects of taurine on DNA (Messina and Dawson, 2000), oxidative enzymes (Tabassum et al., 2006, 2007; El-Sayed et al., 2011) and mitochondrial respiratory chain complexes (Lakshmi Devi and Anuradha, 2010). Support for this concept comes from the present results, which showed that taurine produced a remarkably significant increase in renal GPx and CAT activities. The stimulatory effect of taurine on endogenous antioxidants was also reported by others (Erdem et al., 2000).

Proliferation of lysosomes in proximal tubule cells is a typical response to heavy metals (Abdel-Moneim and Said, 2007; Abdel-Moneim, 2009). The accumulation of heavy metals may also damage the lysosomal membrane and lead to cell injury. Lysosomal damage is closely correlated with the amount and duration of aluminum loading (Marzella and Lee, 1998). In a recent experiment, taurine was shown to prevent the leakage of lysosomal enzymes following severe renal impairment (Eldin et al., 2008), possibly by increasing the stability or decreasing vulnerability of lysosomal membrane due to its antioxidant property. When we examined ERs of mice treated with $AlCl_3$, we observed large dilations and vacuolizations of ER membranes in many areas of proximal epithelium. ER is normally composed of tubules, vesicles and sacs forming lamella-like shape (Gartner and Hiatt, 2001). Crucial pathways are related to carbohydrate metabolism, biotransformation, steroid metabolism, and protein processing function in the ER with integration of other organelles (Sato et al., 1999; Csala et al., 2006). Dilated ER is considered a representative of cell damage (Gartner and Hiatt, 2001), causing the cell to stop functioning and leading to cell lysis. Aluminum greatly stimulated interstitial fibrosis (Somova et al., 1997) while the antifibrogenic effect of taurine *in vivo* has been shown (Sato et al., 2002). The reduction of renal degenerative effects of $AlCl_3$ by taurine could be achieved by reducing the overall bioavailability of aluminum or the intracellular availability of absorbed aluminum (Yeh et al., 2009). Taurine possesses functional groups (an amino group and a sulfonate group) that might bind with heavy metals, and then stimulates the excretion of such compounds. Other studies have shown that taurine exerts morphological and functional protection against renal damage induced by several toxins and drugs, such as gentamicin (Erdem et al., 2000), tamoxifen (Tabassum et

al., 2007), methiocarb (Ozden et al., 2009) and acetaminophen (Das et al., 2010).

In summary, the present study brings new information on aluminum toxicity at the cellular and tissue levels. Endothelial damage with subsequent development of TMA resulting in tubular necrosis are the likely pathogenic processes giving rise to acute renal failure in $AlCl_3$ treated mice. Our data showed the renoprotective effect of taurine pretreatment against $AlCl_3$-induced acute nephrotoxicity. This protective effect was associated with the attenuation in oxidant stress and with the preservation of antioxidant enzymes. Taurine should be considered a useful amino acid for various ischemic diseases.

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