

Full Length Research Paper

Stimulated Cisplatin on histological changes in renal tissue of rat

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The present study reviews the effect of anticancer drug cisplatin regimens on the renal histopathological and ultrastructure of male rat. The kidney being an essential organ of metabolism and elimination of most of the toxic components, it serves as very important site of attack by various chemical agents including cisplatin, in the form of its activated metabolite. In our study, all the induced alterations revealed acute tubular necrosis along with dilation and sloughing of epithelium seen in its light microscopic structure whereas, ultrastructural changes showed absence of microvilli in few sections. Several rounded cisternae of SER and presence of dense chromatin in the nucleus with increased mitochondrial density is also one of the noticeable changes seen after drug treatment. The damage, which is irreversible at certain dose level, might cause potential damage to the organ, which can be overcome by designing proper dose regimen and monitoring and by giving it in combination with anti-cancer drugs.

Key words: Cisplatin, necrosis, kidney.

INTRODUCTION

Cisplatin (Cis-diamine dichloroplatinum II) is one of the most active anticancer drug (Borch, 1987). However, its significant antitumor action usefulness is often limited by the development of nephrotoxicity, which is evident in various animal species (Devipriya and Shamala, 1999). Various studies have revealed that cisplatin induced renal damage is due to the involvement of oxidative stress via free radical formation which in turn produces impairment in proximal tubular reabsorption of water, sodium ions (Na⁺) and glucose (Dobyn et al., 1980). The mechanism by which the drug exerts its toxic effect is yet not systematically explored. It has been proposed that brush-border membranes are the cellular sites which are responsible for the reduction in the proximal reabsorption

of organic substances (Goldstein et al., 1981).

Previous reports also demonstrate that cisplatin interferes with mitochondrial function and causes injury because it plays an important role in maintaining calcium homeostasis, Na⁺ - K⁺ - ATPase activity and ATP synthesis (Goldstein and Mayor, 1982). The present study was therefore undertaken with an aim to evaluate the effect of cisplatin-induced nephrotoxicity, at both the histopathological and morphological levels in renal tissue of rat.

MATERIALS AND METHODS

Adult 3 month old male albino rats of Wistar strain, weighing about 220 - 250 g of proven fertility were purchased from Rajudyog Biotechnology Division, Talekar Vasti, Wing (Khandala)-Satara-412801, India. The drug cisplatin was procured from Dabur India Ltd. Rats were maintained under identical conditions and received standard food pellets and water ad libitum. The animals were

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acclimatized for a period of two weeks and then treated. They were coded in groups of two per cage and then utilized for the study. Pre-treatment and post treatment hydration was provided with 2.5% dextrose and protein rich diet was also supplied simultaneously. Experimental rats were injected with 0.4 mg of cisplatin per kg i.p in saline daily for the period of 8 weeks. Control rats received 0.5 ml of saline daily along with the treatment group. The change in body weight was monitored per week.

At the cessation of the treatment, rats were given light ether anesthesia and sacrificed. Kidney was rapidly removed and utilized for both histopathological and morphological studies.

Histopathological studies

The kidney sections were stained with hematoxylin and eosin and were observed under light microscope for any histopathological changes.

Morphological studies

Kidneys were removed from the animals immediately after decapitation, sliced into 1 mm pieces in a drop of 3% glutaraldehyde and immersed in fresh ice cold fixative for two hours and then in 0.1 M cacodylate buffer for next 4 h. The tissues were then rinsed briefly in buffer and post osmicated in 1% osmic acid for one to two hours. The tissue were then dehydrated in an ascending series of alcohol, followed by propylene oxide and finally embedded in resin that was polymerized at 600°C. Subsequently the blocks were prepared in araldite and 1 m sections were cut with a glass knife on LKB-2000S, ultramicrotome mounted on glass slides and stained with buffered toluidine blue. Appropriate areas were selected with the light microscope. Finally, ultra thin sections of selected blocks were cut with a diamond knife, picked up on copper grids and stained with uranyl acetate and lead citrate for final viewing. The ultra thin sections were scanned and photographed on JEM-JEOL 100s electron microscope.

RESULTS

Histopathology

Histopathology examination of renal slices from rats treated with cisplatin alone showed different alterations, including acute tubular necrosis (Figure 1). The sections of the cisplatin kidney from rats exhibited marked dilation of proximal convoluted tubules with slogging of almost entire epithelium due to desquamation of tubular epithelium (Figure 2)

Morphology

Morphological alterations observed in brush border microvilli after cisplatin treatment were similar to those described by Kim et al. (1995). Cisplatin regimens induced a total absence of microvilli, with several rounded cisternae of smooth endoplasmic reticulum (SER) (Figure 3) and presence of dense chromatin in the nucleus of the tubular lining and intact but highly increased mitochondrial population were also observed

(Figures 3 and 4). Lumen showed presence of degenerative debris along with increased lysosomal bodies which was noticeable effect observed after cisplatin treatment (Figure 3).

DISCUSSION

Extensive use of cisplatin for the management of oncological therapy is usually accompanied by long-term non-haematological toxicity such as nephrotoxicity (Borch, 1987). Several lines of evidence reported so far states that reactive oxygen species play a deleterious role in causing nephrotoxicity (Devipriya and Shamala, 1999).

It has been recognized that the nephrotoxic effect of cisplatin has been witnessed by the spectrum of cytotoxic injury ranging from mild sublethal changes to a catastrophic necrotic death which lead to an inflammatory response (Dobyn et al., 1980). In the present study, the weight of renal tissue was reported to be non-significantly increased. The overall external appearance of kidney looked weathered and discolored. This is in line with the earlier reports by Devipriya and Shyamala Devi (Kim et al., 1995).

Histological changes of rat kidney after cisplatin treatment revealed acute tubular necrosis which confirms irreversible injury to kidney. Cisplatin intoxication also showed a severe atrophy of glomerulus, which was apparent due to the reduction in its size. Marked dilation of proximal convoluted tubules with slogging of almost entire epithelium due to desquamation of tubular epithelium was evident. Cellular debris in the tubular lumen and increased tissue in the interstium is also an indication of cisplatin-induced renal necrosis.

The changes obtained in the present study run parallel with the report documented by Shirwaikar et al. (2003) where the investigators had demonstrated cisplatin-induced acute renal necrosis with marked congestion of the glomeruli and glomerular atrophy. Cisplatin treatment also showed desquamation of the tubular epithelial cells with casts in the tubular lumen and infiltration of inflammatory cells in the interstitium (Borch, 1987). Goldstein and Mayor (1983) have observed that cisplatin toxicity primarily caused degenerative changes in the proximal tubules (Hanigan and Devaranjan, 2003). They have also revealed findings demonstrating focal acute tubular necrosis primarily affecting the distal and collecting tubules causing dilation of convoluted tubules and formation of casts in autopsy samples (Hanigan and Devaranjan, 2003). Silkensen et al. (1997) showed marked tubular necrosis which is localized to the outer area of the medulla, a region rich in proximal tubules. It is an evidence of cisplatin-induced nephrotoxicity (Borch, 1987). Morphological alterations seen after electron microscopy in the present study showed much profound effect of the drug regimen. It is observed that the drug resulted in a total absence of microvilli of the



Figure 1. Light micrographs of rat kidney treated with cisplatin for 8 weeks. Note the tubules (T) showing acute necrosis. Also note the atrophied glomerulus (G) ($\times 40$).

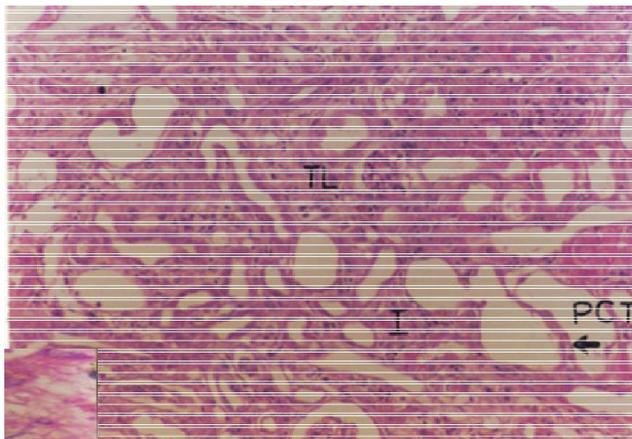


Figure 2. Light micrographs of rat kidney treated with cisplatin for 8 weeks. One of the noticeable features is the dilated Proximal Convoluted Tubule (PCT) with slugged of epithelium (bold arrow). Note the tubular lumen (TL) filled with cellular debris and elevated tissue in interstitium (I) indicative of necrotic cell ($\times 10$).

epithelium in some areas. The changes in the present study are in agreement with the prior observations made by Kim et al. (1995), wherein it was reported that cisplatin treatment in rabbits caused nephrotoxicity, which was evidenced by significant loss of brush-border microvilli which is supposed to be responsible for reducing the area for active glucose reabsorption (Devipriya and Shamala, 1999).

Dobyan et al. (1980) revealed several ultrastructural changes in the pars recta which include profound thinning with focal loss of brush border, cellular swelling, condensation of nuclear chromatin, cytoplasmic vacuolization, rounded mitochondria with swollen cristae, dissociation of mitochondria from basal infoldings, loss of basal infoldings and increased number and size of pinocytotic vesicles and lysosomal bodies in the apical



Figures 3. Electron micrographs of rat kidney treated with cisplatin for 8 weeks. Note the total absence of microvilli (Mv) in the lumen (L) of some of the sections. Note the numerous rounded cisternae of SER with amorphous nucleus (N) and nucleolus (Nu) showing dense chromatin. Rare appearance of electron dense lysosomes (Ly) are noticeable ($\times 5000$).

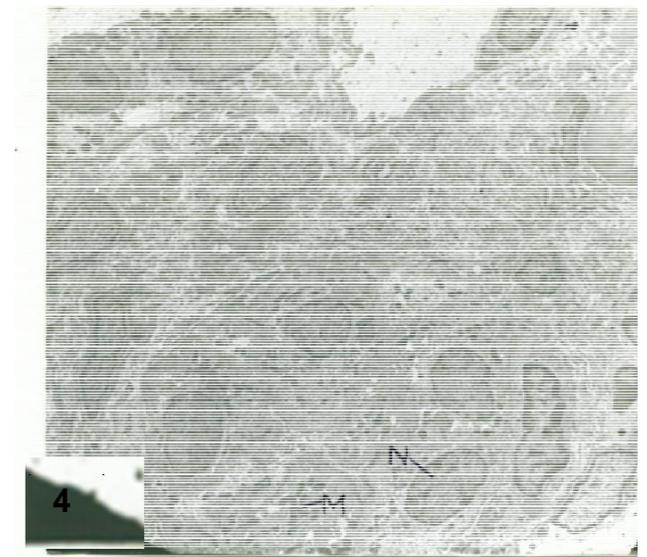


Figure 4. Electron micrographs of rat kidney treated with cisplatin for 8 weeks. The tubular lining shows the presence of tissue with heterochromatic nucleus (N) and dense accumulation of mitochondria (M) ($\times 2500$).

region bordering the lumen (Dobyn et al., 1980).

Furthermore, Vickers et al. (2004) showed acute nephrosis of the tubular epithelium induced by cisplatin *in vivo* and was reproduced in both human and rat kidney slices, while the glomerulus appeared resistant even at high concentrations (Vickers et al., 2004).

Giuseppa et al. (2009) suggested that the matrix remodeling by (MMP-9) Matrix metalloproteinases and (TIMP-3) tissutal inhibitors of metalloproteinases, in the

later stages, can play an important role in the development of glomerular sclerosis and interstitial fibrosis after Cisplatin treatment (Giuseppa et al., 2009).

Meral et al. (2007) Histopathological examination showed widespread tubular necrosis and dilatation in Cisplatin-treated rats which states Cisplatin induced nephrotoxicity (Meral et al., 2007).

Nevertheless, in some of the sections, lumens showed presence of microvilli on either side. Several rounded cisternae of SER are indicative of the increased activity of some of enzymatic antioxidants responsible for carrying out various detoxication reactions. The presence of dense chromatin in the nucleus of the tubular lining and intact but highly increased mitochondrial population was also observed. Lumen showed the presence of degenerative debris. Increased lysosomal activity was also one of the noticeable effects observed after cisplatin treatment.

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