EXPERIMENTAL STUDY OF THE ROLE OF L - CARNITINE IN PREVENTION AND TREATMENT OF CISPLATIN–INDUCED NEPHROTOXICITY. A HISTOLOGICAL AND ULTRASTRUCTURAL STUDY IN ALBINO RAT

Thesis

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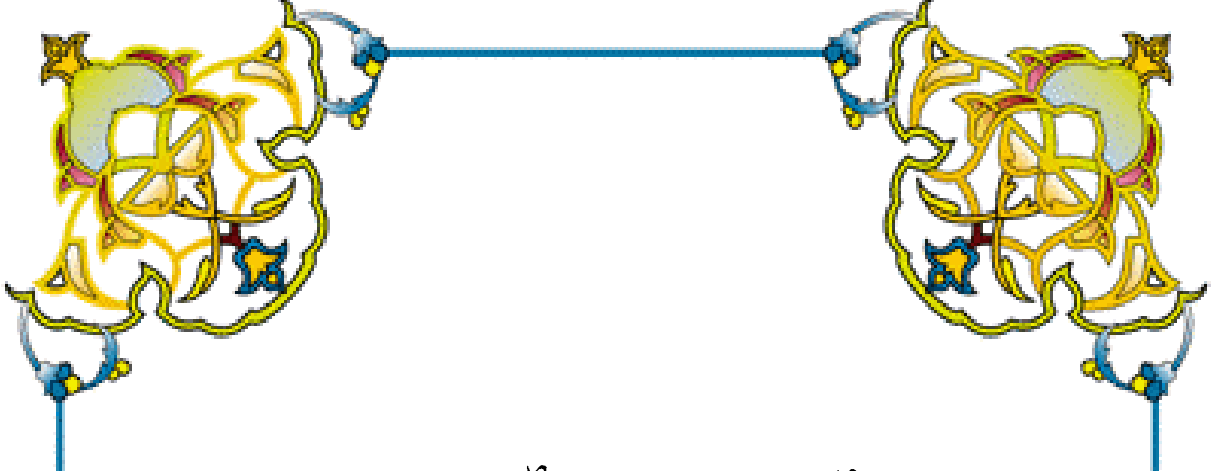

بسم الله الرحمن الرحيم

(وَقُل رَّبِّ زِدْنِي عِلْماً)

صدق الله العظيم

[سورة طه: آية 114]

DEDICATION

To my Parents

To My Wife

To My Brothers

With Love

Contents

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Abstract

Cisplatin is one of the most effective chemotherapeutic agents used in the treatment of several human cancers and nephrotoxicity is the major toxicity of this compound. The present work demonstrated that cisplatin produced variable histological alterations in the kidneys of rats. These changes might be due to oxidative stress and generation of reactive oxygen radicals. The administration of L-carnitine was found to reduce the observed harmful effects of cisplatin on the kidneys.

Key words:

cisplatin toxicity- kidenys of rats – L- carnitine

Cisplatin is an effective chemotherapeutic agent used in treatment of solid tumors of the lung, ovary, testis and bladder **(lebwohl and Canetta, 1998 and Calabresi and Chabner, 2001).**

Cisplatin is beneficial in treatment of carcinoma of the ovary particularly when used with cyclophosphamide. Combination of chemotherapeutic agents as bleomycine and vinblastine with cisplatin was curative for 85% of patients with advanced testicular cancer **(Williams and Einhorn, 1987)**.

Nephrotoxicity is the commonest side effect frequently observed after administration of cisplatin (**Pabla and dong, 2008)**. **Atasoyu et al. (2005), Atessahin et al. (2005)** and **Aly et al. (2009)** who studied the effect of cisplatin on the kidney of rats, found that cisplatin induced functional and structural alteration in the kidney manifested by elevation in urea and creatinine, as well as vacuolization, degeneration and necrosis of the epithelial cells lining the proximal convoluted tubule with tubular cast formation.

Information about the mechanisms leading to cisplatin-induced nephrotoxicity is insufficient. Various studies had reported that free oxygen radicals resulted from cisplatin administration play an important role in inducing nephrotoxicity **(Baliga et al., 1999 ; Davis et al., 2001; Ulubas et al., 2003; Chirino and Pedraza-Chaverri, 2009**).

Free oxygen radicals induced by cisplatin produce cellular injury and necrosis via several mechanisms including peroxidation of membrane lipids and proteins and damage of DNA **(Mora et al., 2003)**. **Salmon and Sartorelli (2001)** revealed that cisplatin destroyed the living cells at all stages of their cycle by inhibition of DNA biosynthesis and by binding DNA forming interstrand cross links.

The administration of antioxidants, such as vitamin C, selenium and carnitine, with cisplatin has been used to protect against nephrotoxicity **(Martinis and Bianchi, 2001; Chang et al., 2002; Naziroglu et al., 2004).**

L-carnitine is a natural compound, primarily located in mitochondria of kidney and liver cells and is proven to exert a protective effect against mitochondrial toxic agents **(Arrigoni-Martelli and Caso, 2001).** L- carnitine has been used as an antioxidant against oxidative stress produced by toxic agents in many organs such as liver, heart, stomach and kidney **(Alvarez et al., 2005; Chang et al., 2005; Derin et al., 2005; Sener et al., 2006)**. **Chang et al. (2002) and Sayed -Ahmed et al. (2004)** demonstrated that L-carnitine administration before cisplatin had inhibited the oxidative stress produced by cisplatin in kidney tissue.

The detailed histopathological effect of cisplatin on the kidney tissue as well as the possible protective and curative role of L- carnitine aganist nephrotoxicity induced by cisplatin is not fully studied in the literature.

Aim of the work

The aim of this work is to study the adverse effects of cisplatin on the histological structure and ultrastructure of the kidney of adult male albino rat. In addition to shed some light on the role of L-carnitine in prevention and treatment of the possible renal histological alterations induced by cisplatin.

REVIEW OF LITERATURE

The nephron is the structural and functional unit of the kidney. Each kidney is composed of 1-4 million nephrons. Each nephron consists of a dilated portion, the renal corpuscle; the proximal convoluted tubule; the thin and thick limbs of the loop of Henle; the distal convoluted tubule and the collecting tubules and ducts **(Kritz and kaissling, 1992).**

Each renal corpuscle is about 200 um in diameter and has a vascular pole, where the afferent arteriole enters and the efferent arteriole leaves and a urinary pole where the proximal convoluted tubules begins. After entering the renal corpuscle, the afferent arteriole divides into 2 to5 primary branches, each subdivides into capillaries that form renal glomerulus. The glomerulus is surrounded by a double –walled epithelial capsule called glomerular (Bowman's) capsule and between the two layers of Bowman's capsule is the capsular space. The external layer forms the outer limit of the renal corpuscle and is called the parietal layer of Bowman's capsule which is formed of a simple squamous epithelium. The internal layer (the visceral layer) of the capsule envelops the capillaries of the glomerlus. With the aid of the electron microscope, the cells of this internal layer the- podocytes- have a cell body from which arise several primary processes. Each primary process gives rise to numerous secondary processes called pedicels. Between the fenestrated

endothelial cells of the glomerular capillary and the podocytes that cover their external surface is the basement membrane. This basement membrane is formed of a central electron –dense layer (lamina densa) and on each side a more electron – lucent layer (lamina rara). In addition to the endothelial cells and podocytes, the glomerular capillaries have mesangial cells adhering to their walls. Mesangial cells are contractile and have receptors for angiotensin II (**Junqueira and Carneiro, 2005).**

The proximal convoluted tubules occupy the cortex and are lined by tall cuboidal or columnar epithelial cells resting on a tubular basement membrane. The cell borders are indistinct and the round nuclei lie at the base of the cell near the basement membrane. The luminal surface possesses a brush border, which is usually well seen in PAS preparations. Ultrastructurally, the brush border is seen to consist of series of microvilli, which increase the cellular surface area to 40 times. The mitochondria are concentrated at the base of the cell and arranged parallel to the long axis of the cell**. (Veroma, 2001).**

Henle's Loop is described as a U-shaped structure consisting of descending and ascending limbs with each limb formed of two parts arranged as follows: a thick part of descending limb follows by a thin part of descending limb, then, a thin part of ascending limb follows by a thick part of ascending limb. In the outer medulla the thick part of the descending limb with the outer diameter of about 60um, suddenly narrows to about 12um

and continues as the thin part of descending limb. The thin part of descending limb and the thin part of the ascending limb are lined by flattened epithelial cells lacking a brush border. Ultrastructurally, the epithelial cells have their cytoplasm contains scanty, small mitochondria, a few profiles of endoplasmic reticulum and occasional polyribosomes. The thick limbs are similar in structure to the distal convoluted tubules, which are lined by cuboidal cells without brush borders **(Cormack, 1997).**

The distal tubule is lined with simple cuboidal epithelial cells. It differs from the proximal convoluted tubule in having no apical brush border, no apical microvilli and in having more lining epithelial cells than the proximal convoluted tubule **(Gartner and James, 2001).**

The collecting tubules are lined with cuboidal epithelium and have a diameter of approximately 40 um and as they penetrate deeper into the medulla their cells increase in height until they become columnar. The diameter of the collecting duct reaches 200 um near the tips of the medullary pyramids **(Junqueira and Carneiro, 2005).**

The renal interstitium is defined as the space between the uriniferous tubules, the blood and the lymph vessels. It occupies a very small volume in the cortex but increases in the medulla and contains connective tissue with fibroblasts and some

collagen fibers. In the medulla, there are secreting cells called interstitial cells which contain cytoplasmic lipid droplets and are implicated in the synthesis of prostaglandins and prostacyclin **(Gartner and James, 2001).**

Cisplatin is a chemotherapeutic drug formed of amonia groups with platinum and chlorine atoms (diamine-dichloroplatinum) **(Roberts et al., 1999).** It has been found to have potent antitumor activity which is commonly used for the treatment of wide spectrum of human malignancies as a single agent or in combination **(Calabresi and Chabner, 2001).**

Cisplatin combined with radiotherapy has proved to achieve long term survival rates in cases of localized carcinoma of the cervix superior to those reported with radiation alone **(Salmon and Sartorelli, 2001)**. Intravesicular instillation of cisplatin had been used in the treatment of superficial cancers of the urinary bladder. cisplatin had been also instilled into pericardial sac in the treatment of malignant pericardial effusion **(Colvin, 2003).**

Nowadays, Cisplatin -based combination therapy regimens are used in the treatment of many types of cancer such as testicular, ovarian and head and neck cancer **(Delord *et al*., 2009)**.

High concentrations of cisplatin were found in the kidney, liver, intestine and testes after its intravenous administration but there was poor penetration of cisplatin into the central nervous

system **(Bajorin et al., 1986).**

Once the cisplatin entered the cell, the drug undergo strong hydration to form positively charged active species for subsequent interaction with cellular nucleophiles **(Andrews et al., 1990).**

Only a small portion of the cisplatin was excreted by the kidney during the first 6 hours and 25% was excreted after 24 hours while 43% of the administered dose was eliminated in the urine after 5 days. The authors added that billiary or intestinal excretion of cisplatin appeared to be minimal **(Calabresi and Chabner, 2001).**

Cisplatin has been accumulated in the renal tissue after its intraperitoneal injection **(Aly et al., 2009)**.

The toxic effects of cisplatin in man and animals include nephrotoxicity, ototoxicity, neurotoxicity and bone marrow suppression but the chief factor limiting its use is nephrotoxicity **(Ali and Al- Moundhri, 2006; Pabla and Dong, 2008).**

Cisplatin caused degenerative changes in the kidney in the form of shrunken glomeruli showed that cisplatin produced severe glomerular congestion, degeneration and dilation of the Bowman's space (**Sayed- Ahmed et al., 2004**) **and Sevgin et al., 2007)**. However, **Atasoyu et al. (2005), Aly et al. (2009)** and **Khan et al. (2009)** reported that the glomeruli were not affected in the cisplatin treated rats.

The effect of cisplatin on the rat kidneys, revealed that cisplatin injection caused necrosis and degeneration of renal tubular epithelial cells at the corticomedullary junction and developed fibrotic areas around the affected tubules (**Yamate et al., 2002)**. **Tagnchi et al. (2005**) showed that tubular epithelial cell deletion following cisplatin treatment was a major cause of renal injury. The authors added that the renal changes following cisplatin treatment was a complex process and could be categorized into three main pathological events, which might overlape each other; initial cytotoxic event, inflammatory event and fibroproliferative event. **Aly et al. (2009)** reported that the cisplatin treated rats showed diffuse acute tubular necrosis. There were swelling, vacuolization and sloughing of tubular cells into the tubular lumen. Tubular distention with esosinophilic necrotic material was seen in the majority of renal tubules. Many apoptotic cells were seen within the lumen of many renal tubules. **Guerrero-Beltrán et al. 2010)** revealed that most of cortical tubules showed apoptosis, necrosis and vacuolization of their epithelial lining cells with intraluminal cast formation. **Tanihara et al. (2009)** studied the effect of cisplatin on rat kidney and revealrd that cisplatin induced degeneration of the epithelial lining cells of renal tubules in the form of intraluminal exfoliation, cytoplasmic vacuolization and partial loss of the apical brush border.

Cisplatin induced nephrotoxicty in rats. Histological