Morphological and Immunohistochemical Study of the Hepatic Changes Following Cisplatin Administration in Adult Male Albino Rat and the Possible Protective Role of L-Carnitine

Thesis Study

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ABSTRACT

Background: Cisplatin is one of the most effective chemotherapeutic agents used in the treatment of several human cancers, but its usefulness is limited by its hepatotoxicity which sometimes requires discontinuation of the treatment. A number of drugs were used to ameliorate the injurious effect of cisplatin on liver. One of the most important of these drugs is L-carnitine.

Aim of the work: The present study was designed to investigate the different pathological effects and the extent of apoptosis induced by cisplatin in liver tissue, and the potential protective effect of L-carnitine against it. This in turn, may affect the treatment regime of cisplatin.

Material and methods: Eighty adultmale albino rats were divided into 5 groups. Group I (control group) contained 20 rats,whereas each of the other groups contained 15 rats. These experimental groups were cisplatin group (group II) which received saline injection 5 days prior and after a single cisplatin injection (7 mg/kg IP), cisplatin recovery group (group III) which received saline 5 days before and 21 days after a single cisplatin injection of the same dose. Cisplatin and L-carnitine group (group IV) and cisplatin and L-carnitine recovery group (group V) corresponded to groups II and III respectively in their time design, but L-carnitine was used instead of saline. Liver specimens were subjected to hematoxylin and eosin, Masson trichrome, silver staining as well as immunohistochemical Caspase-3 examination and measurements utilizing image analysis technique.

Results: The histological results revealed the injurious effect of cisplatin on the liver. The groups treated with cisplatin alone showed degeneration featured by cytoplasmic vacuolization, ghost figures, karyolysis, pyknosis, mononuclear cellular infiltration, hypochromatic nuclei, multiple nucleoli, intranuclear vacuolization, portal congestion and oedema and necrotic cells. The lesions affected mainly the pericentral and midzonal areas. The groups treated with cisplatin and L-carnitine showed mild or no affection, with preservation of normal hepatic architecture. The central vein diameter, the stromal framework disruption and apoptotic cells increased in the cisplatin groups, but it was much less affected in the cisplatin and L-carnitine groups. The recovery groups showed statistically higher values as regards the area percent of fibrous tissue, with partial privilege to the L-carnitine combined treatment

Conclusion: The present work revealed that L-carnitine provided an excellent protective factor against the damaging effects of cisplatin on the liver as regards general histological picture, stromal framework integrity, central vein diameter and apoptosis, but it partially protected the liver against fibrosis. The safeguarding effect of L-carnitine increased with its administration for a longer period before and after cisplatin.

Key words: liver – cisplatin – L-carnitine - apoptosis – immunohistochemistry – rat

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Introduction

Cisplatin is one of the most commonly used compounds for the treatment of a wide spectrum of human malignancies; testicular, ovarian, bladder, cervical, head and neck and lung cancers **(Chu, 2012).**

Common problems associated with the clinical use of cisplatin include hepatotoxicity, nephrotoxicity, ototoxicity, and peripheral neuropathy **(Alberts and Noel, 1995).** Cisplatin-related hepatotoxicity rarely occurs at standard doses. However, at higher doses, hepatotoxicity is frequently observed and can alter the clinical situation of cancer patients **(Cersosimo, 1993 and Jie et al., 1998).** Although apoptotic lesions seem to characterize the damaged liver parenchyma, the histological analysis of liver tissue revealed cytoplasmic changes especially cells around central vein with hepatocellular vacuolization and sinusoidal dilatations (**Koc et al., 2005)**. **Lu and Arthur (2006**) have reported that cisplatin induced hepatotoxicity is enhanced by the increase in generation of reactive oxygen species and oxidative stress. **Durak et al. (2003)** ensured the role of antioxidants in cisplatin-induced hepatotoxicity in guinea pigs and reported that treatment with antioxidants (a combination of vitamin C and E) activated the liver antioxidant system, eliminated oxidation reactions and prevented cisplatin-induced liver cell failure.

L-Carnitine is an amino acid derivative. It was initially considered to be an essential vitamin, it was later discovered that it could be manufactured in the liver, kidney and brain from amino acid precursors (lysine and methioine) **(Vaz and Wander, 2002)**. In

human, 75% of carnitine intake comes from dietary sources **(Carter et al., 1995)**. In liver, L-carnitine, has been proved to ameliorate the oxidative stress and free radicals production in gentamicin induced hepatotoxicity in rat even if L-carnitine was started after the gentamycin administration **(Kopple et al., 2002).** It has been also proven to ameliorates methotrexate induced oxidative injury in the liver and inhibits leukocyte apoptosis **(Sener et al., 2006)**. The oxidative injury induced by cisplatin in rat liver was successfully inhibited by L-carnitine without decreasing the chemotherapeutic action of cisplatin **(Chang et al., 2002 and Pisano et al., 2003). Sayed-Ahmed et al. (2004)** stated that Cisplatin treatment significantly decreases the level of total carnitine in liver tissues and carnitine deficiency is an additional risk factor in cisplatin induced liver dysfunction. L-carnitine supplementation attenuated liver alteration induced by cisplatin through its antioxidant activity.

Aim of study

The aim of the present work is to study the adverse effect of Cisplatin induced lesions on the histological structure of the liver in adult male albino rat. Moreover, the possible protective role of L-carnitine in the treatment of this effect.

ANATOMY OF RAT LIVER

The rat liver is multilobulated as in other mammals. In rats, the liver mass represents approximately 5% of the total body weight, while in adult humans it represents 2.5%. In rats weighing between 250 and 300 g, the liver mean weight is 13.6 g and the liver transverse diameter measures from 7.5 to 8.0 cm. The superior-inferior diameter measures from 3.8 to 4.2 cm, while the anterior-posterior diameter ranges from 2.2 to 2.5 cm **(Zanchet and Monteiro, 2002)**.

The rat liver, when the rat is in the decubitus position, has basically three surfaces: superior, inferior and posterior. A sharp, well-defined margin divides the inferior from the superior surface. Different from the human liver, the other margins are also sharp. Although the rat liver is lobated, it has rather uniform surfaces as lobes lie flat against each other. The only exception to this is the posterior caudate lobe (CL), which is separated from the remainder of the liver by the stomach **(Kongure et al., 1999)**.

Ligaments

Similar to the human liver, the rat liver is connected to the undersurface of the diaphragm and to the anterior wall of the abdomen by five ligaments: the falciform, the coronary, and the two laterals are peritoneal folds; the fifth, the round ligament, is a fibrous cord, the obliterated umbilical vein. The liver is also attached to the lesser curvature of the stomach by the hepatogastric ligament,, and to the duodenum by the hepatoduodenal ligament **(Martins and Peter, 2007)**

Liver lobes

The middle or median lobe (ML) is the largest, accounting for approximately 38% of the liver weight. It has a trapezoidal shape and is fixed in the diaphragm and abdominal wall by the falciform ligament. It is in continuity with the left lateral lobe (LLL) and is subdivided by a vertical fissure (main fissure or umbilical fissure) into a large right medial lobe (RML) (2/3 of the volume of the medial lobe) and a smaller left medial lobe (LML; 1/3 of the volume). The RML has both left and right hepatic vascular components **(Kongure et al., 1999).**

The right lobe (RL) is located on the right of the vena cava and posteriorly in the right hypochondrium and is almost completely covered by the medial lobe. It comprises about 22% of the liver weight and is divided by a horizontal fissure Into two pyramidal-shaped lobules: the superior (SRL, also called the right posterior lobe) and inferior (inferior right lobe, IRL, also called the right anterior lobe) lobules (**Martins and Peter, 2007)**.

The caudate lobe (CL) is situated behind the LLL and on the left of the vena porta and inferior cava vein. It comprises 8-10% of the liver weight **(Kongure et al., 1999)**.

Liver vasculature

The origin and course of the major vessels are similar to those of humans, and no variability in vessel origin is identified in rats. **(Casting et al., 1990; Wu et al., 2005)**.

They constitute hepatic artery, portal vein and the hepatic heins **(Lorente et al., 1995).**

About 75% of the liter blood in supplied by the portal vein and the remaining 25% in supplied by the hepatic artery **(Douglas, 2000).**

Biliary tract

The rat does not have a gallbladder. Most commonly, each the lobe is drained by his own bile ducts **(Lorente et al., 1995).**

The CBD is formed by the junction of the main hepatic ducts. The main hepatic ducts join together on the caudate process, in this series, the CBD ranged from 12 to 16mm in length and from 0.6 to 1mm in diameter, but it can be upto 45mm long **(Hebei and Stromberg, 1996)**.

Histology of the liver

The appearance and structure of the adult mammalian liver have been well established **(Ham and Cormack, 2001 and Fawcett, 2002)**. In general, the main structural component of the liver is the liver cell or the hepatocyte. The hepatocytes are grouped in plates forming lobules which are separated from each other, in certain animals, by a layer of connective tissue. The space between the plates of hepatocytes is filled by sinusoid capillaries, called liver sinusoids **(Junqueira and Carneiro, 2005)**.

The stroma of the liver:

The liver is covered by a thin connective tissue capsule (Glisson's capsule) that becomes thicker at the hilum, where the portal vein and the hepatic artery enter the organ and where the right ducts are surrounded by connective tissue, all the right and left hepatic ducts and lymphatics exit. These vessels and ducts are surrounded by connective tissue all the way to their termination (or origin) in the portal spaces between the liver lobules. At this point, a delicate reticular fiber network that supports the hepatocytes and sinusoidal endothelial cells of the liver lobules is formed **(Junqueira and Carneiro, 2005)**.

Lobulation of the liver:

The classic hepatic lobule is a roughly hexagonal mass of tissue. It consists of stacks of anastomosing plates of hepatocytes. One cell thick, separated by the anastomosing system of sinusoids that perfuse the cells with the mixed portal and arterial blood. Each lobule measures about 2.5 X 0.7 mm. at the center of the lobule is a

relatively large venule, the terminal hepatic venule (central vein), into which thesinusoids drain. The plates of cells radiate from the central vein to the periphery of the lobule, as do the sinusoids **(Michael, 2006)**.

In some species e.g., the pig, the classic lobule is easily recognized because the portal areas are connected by relatively thick layers of connective tissue. In humans, however, there is normally very little interlobular connective tissue, and it is necessary, when examining histologic sections of liver, to draw imaginary lines between portal areas surrounding a central vein to get some sense of the size of the classic lobule **(Douglas, 2000)**.

A portal triad occupies a potential space (portal space) attach of the six corners of the classic lobule. Each triad contains three main elements surrounded by connective tissue: a portal venule (a branch of the hepatic artery), and a bile ductule (tributary of a bile duct). A lymphatic vessel also may be seen **(Douglas, 2000)**.

A single vein marks the center of each lobule. It is easily distinguished from those in the portal triad by its lack off a connective tissue sheath **(Ham and Cormack, 2001)**.

Hepatocyte plates and hepatic sinusoids: Many such plates radiate from the central vein toward the lobule periphery (like the spokes of a wheel). The plates are separated by hepatic sinusoids, which receive blood from the vessels , in the triads, converging on the lobule center to empty directly into the central vein **(Michael, 2006)**.

The portal Lobule:

The portal lobule is the mass of liver cells that drains its secretion into a common bile duct; it is triangular area of hepatocytes which is delineated by drawing imaginary lines connecting three central veins of three adjacent classical lobules. A portal tract lies in its center **(Ham and Cormack, 2001)**.

The portal lobule emphasizes the exocrine function of the livers which is bile secretion:

The Hepatic Acinus:

It is a diamond-shaped mass of liver tissue, formed of 2 adjacent hepatic lobules, surrounding a central vascular core (containing branches of the hepatic artery and the portal vein). The explains why the peripheral hepatocytes are richly supplies with blood, while the central hepatocytes are poorly supplied with blood **(Michael, 2006)**.

Parenchyma of the Hepatic Lobules:

The main structural component of the liver is the liver cell or hepatocyte. The peculiar arrangement of the hepatocytes is described by almost all histology textbooks **(Michael, 2006; Junqueira and Carneiro, 2005; Fawcett, 2002 and Ham and Cormack, 2001)**.

Hepatocytes:

Hepatocytes make up the anastomosing cell plates of the liver lobule. It is large, polygonal cells measuring between 20 and 30 mm in each dimension. They constitute about 80% of the cell population of the liver. Hepatocytes nuclei are large and spherical and occupy

the center of the cell. Many cells in the adult liver are binucleated **(Junqueira and Carneiro, 2005)**.

In sections stained with hematoxylin and eosin (H&E), the cytoplasm of the hepatocyte is eosinophilic, mainly because of the large number of mitochondria and some smooth endoplasmic reticulum. Hepatocytes located at different distances form the portal space, show differences in structural, | histochemical, and biochemical characteristics. The surface of each hepatocyte is in contact with the wall of the sinusoids, through the space of Disse, and with the surfaces of other hepatocytes. Wherever two hepatocytes abut, they delimit a tubular space between them known as the bile canaliculus **(Michael, 2006)**.

Numerous organelles can be recognized in the cytoplasm. They differ according to the location within the lobule. These organelles include mitochondria, endoplasmic reticulum, Golgi apparatus, lysosomes and microbodies **(Ham and Cormack, 2001)**.

Each hepatocyte contains about 1000 mitochondria. They show distinctmorphological changes associated with glycogen storage, being small andspherical in peripheral cells and more slender and elongated in central cells **(Fawcett, 2002)**.

The cytoplasm of the hepatocytes has an endoplasmic reticulum both in its smooth and granular varieties. The granular variety forms aggregates dispersed in the cytoplasm that constitute the basophilic bodies. The smooth variety ofendoplasmic reticulum is composed of irregular tubes and vesicles without surrounding ribosomes. **(Junqueira and Carneiro, 2005)**.

Feldmann (1982) observed that the rough endoplasmic reticulum forms a series of thin parallel channels usually arranged in several clusters uniformly distributed throughout the cytoplasm. The outer aspect of these channels is studded with small ribosomal serves as the site of synthesis of protein primarily for secretions.

Lysosomes are organelles specialized for digestion and storage and are commonly observed in the hepatocytes. Primary lysosomes are available without preceding stimuli, but secondary lysosomes are formed around materials to be acted upon, and are therefore indication of proceeding exogenous or endogenous stimuli.

The number of lysosomes increases in a variety of pathologic conditions, ranging from simple obstructive stasis to viral hepatitis and anemia **(Michael, 2006)**.

In addition to the organelles, the cytoplasm of the hepatocytes contains two prominent cell inclusions which are glycogen and fat droplets **(Junqueira and Carneiro, 2005)**.

The Hepatic Sinusoids:

They are vascular spaces found between hepatic plates. They carry blood from the portal tract to the central vein. They are lined partially by fenestrated endothelial cells and partially by the Von kupffer cells. They could be demonstrated by gelatin carmine injection **(Michael, 2006)**.

Von-kupffer cells: They are large branched phagocytic cells, lining the liver blood sinusoids. They are rich in lysosomes. They can be stained by vital stains, e.g. trypan blue. They play a role in

destruction of senile RBCs and formation of bile pigments **(Junqueira and Carneiro, 2005)**.

Space of Disse: It is the space that separates the hepatocytes from the wallsof blood sinusoids (perisinusoidal space). It contain the microvill projecting from the surface of hepatocytes, plasma and reticular fibers which support the walls of the sinusoids it contains also stellate-shaped fat-storing cells (lipocytes),which store vitamine A **(Junqueira and Carneiro, 2005)**.

The Biliary Passage:

The daily basal secretion of bile is approximately 500 ml. The bile produced by the hepatocyte flows through the bile canaliculi, bile ductules and bile ducts. These structures gradually merge, forming a network that converges to from the right and left hepatic ducts, which unite to form the common hepatic duct. The common hepatic duct, after receiving the cystic duct from the gallbladder, continues to the duodenum as the common bile duct (ductuscholedochus) **(Fawcett, 2002)**.

Blood flows through the sinusoids from portal to central venous regions. Distribution of hepatocytes along sinusoids has thus been described in both anatomic and functional terms. Anatomically, hepatocytes are distributed in three indistinctly separate areas of a lobule, periportal, mid zonal and central lobular. Functionally, hepatocytes are considered to reside in acini composed of two metabolic zones, zone I reflects greatest proximity to vascular supply of substances and oxygen; this roughly approximates the periportal area. Zone II is an intermediate region that receives second-class blood as far as the central vein; its hepatocytes have

to depend on blood that is relatively low in nutrients and oxygen. The difference in anatomic disribution and metabolic function results in subtle morphologic variations in hepatocytes ***(Cormack, 1997)***.

Cisplatin

The platinum drugs represent a unique and important class of antitumor agents. The clinical development of the neutral, square planar, coordination complex cis-diamminedichloroplatinum (II) (cisplatin), is considered as a landmark in the treatment of cancer. Cisplatin is widely used for the treatment of many malignancies, including testicular, ovarian, bladder, cervical and small cell and non–small-cell lung cancers ***(Rosenberg et al., 2000).***

Despite the great efficacy at treating certain kinds of cancers, cisplatin, carboplatin, and other cisplatin analogs introduced into clinics have major problems, such as several side effects and the acquisition or presence of resistance to these drugs that undermines their curative potential ***(Kelland, 2003).***

Chemistry:

The platinum compounds that are active antitumor agents can have either four or six ligands, with a square planar or hexahedral configuration, respectively ***(Grunberg et al., 1989).***

In some cisplatinum compounds in clinical use, the chloride leaving ligands are replaced with carboxyl ester groups, as in carboplatin and oxaliplatin ***(Colvin, 2003).***

Mechanism of action:

Cisplatin is a well-known DNA-damaging agent and the current thinking is that DNA plastination is an essential first step in