Toxic Effect of Cisplatinum on the Liver of Adult Albino Rat and the possible Protective Role of Vitamin E: (Light and Electron Microscopic Study)

Thesis

Submitted for the partial fulfillment of M.Sc. degree in anatomy

Submitted by

Tarek Ibrahim Abd El-Galil

M.B.B.Ch.
Faculty of Medicine, Cairo University

Supervisors

Saber Ibrahim Shona

Professor of Anatomy
Faculty of Medicine, Cairo University

Abd Elwakil Essawy

Professor of Anatomy
Faculty of Medicine, Cairo University

Sherif Mohamed Zaky

Assistant professor of Anatomy
Faculty of Medicine, Cairo University

Anatomy Department
Faculty of Medicine
Cairo University

2009

Table of contents

Subject	page
Introduction	1
Aim of the work	1
Review of literature	
Normal structure and function of the liver	2
Susceptibility of the liver to chemical injury	6
Vitamin E	8
Cisplatinum	10
Cisplatinum-induced hepatotoxicity	12
Results	15
Discussion	102
Summary	110
References	112
Arabic summary	

Abstract

Cisplatinum is one of the most effective antineoplastic drugs used in the treatment of ovarian, testicular and head and neck tumors, but it has undesirable toxic effects on the liver at high doses.

The present study was designed to detect the toxic effect of the cisplatinum on the liver in the adult albino rats and the possible protective role of vitamin E in prevention of such toxicity.

Thirty two rats were used in the current study. They were divided into three groups (control group, sham control group treated with vitamin E, cisplatinum treated group and vitamin E and cisplatinum treated group). Each group was subdivided into two subgroups ("1" and "2") according to time of scarification (after 16 hour in subgroup "1" and after 3 weeks in group "2").

The possible mechanism of cisplatinum toxicity in the liver was due to affection of oxidant-antioxidant system in the hepatocytes of rats and this explained the partial improvement with vitamin E administration.

Key words:

Cisplatinum – Vitamin E – Oxidative stress.

Introduction

Cisplatinum (cis-diamminedichloroplatinum II) is an antitumor agent against several types of cancer and has been widely used as a chemotherapy. The activity of the drug has been demonstrated against a variety of tumors, particularly those of head and neck, testis, ovaries, urinary bladder and small cell carcinoma of the lung **(Kuhlmann et al., 1997 and Weijl et al., 1997).** However, high-dose administered to patients produces nephrotoxic and hepatotoxic side effects so the dose of cisplatinum must be the least toxic **(Yoshida et al., 2000).** Administration of cisplatinum causes an increase in lipid peroxidation (malondiadehyde) level and a decrease in the activity of enzymes that prevent or protect against lipid peroxidation in the tissues. Therefore, administration of antioxidants before treatment with cisplatinum has been used to protect against nephrotoxicity in humans and experimental animals such as vitamin C **(Antunes and Darin, 2000)** and selenium **(Caffrey and Frenkel, 2000 and Antunes et al., 2001).**

Vitamin E is a major lipophilic chain-breaking antioxidant present within cell membranes **(Landvik and Packer, 1990).** In contrast to vitamin A and C, vitamin E can be safely used in prevention of diseases such as diabetes and cardiovascular diseases as it suppresses the oxidative stress of the cell membranes **(Czernichow and Hereberg, 2001)**.

The aim of the present study was to detect the histopathological effect of cisplatinum on the liver in adult albino rat and the possible protective role of vitamin E in prevention of cisplatinum-induced hepatotoxicity.

Normal structure and function of the liver

The liver was found to be an important organ for maintenance of vertebrates. It had double blood supply, the hepatic artery and the portal vein. The hepatic artery provided nutrition, while the portal vein was found to deliver substances absorbed by the gastrointestinal tract for elimination and metabolic conversion.

The portal tract consisted of branches emerging from the portal vein, hepatic artery and bile duct. Blood entering the portal tract via the portal vein and the hepatic artery; was mixed in the sinusoids and percolated along the cords of parenchymal cells. It flowed towards the terminal hepatic venules and exited the liver via the hepatic vein propelled by blood pressure gradient **(Marks, 1969).**

Although, the zonal arrangement of the cells with respect to circulation, function and pathology proved that lobulation was an essential structural feature of the hepatic parenchyma, yet such lobulation was a topic of controversy **(Schiff and Schiff, 1982). Buscher et al. (1991) and Ekataksin et al. (1993)** described an elementary unit, termed the hepatic microcirculatory unit (HMC) or the choleohepaton. It was a pyramidal unit with its base at the perimeter of the classic lobule and its apex at the central vein. HMC was supplied by a single inlet venule, feeding a group of approximately 19 sinusoids, co-localized with a canal of Hering that drained the bile from the same pyramidal area. This model highlighted a countercurrent flow between vascular flow and secretory pathways of the liver. HMC appeared to be the basic unit of the liver that could independently subserve its endocrine and exocrine functions **(Ekataksin et al., 1997).**

The liver tissue was provided by a system of two tunnels; the portal tract and the hepatic central canal, which merged in such a way that they never meet each other. As far as possible, the system of two tunnels ran in planes perpendicular to each other **(Dooley and Sherlock, 1997). DeFrances and Michalopoulos (1997)** said that the liver was the most important organ providing optimal nutrition for all the body cells. It consumed 12-20% of the total body energy, so it had to generate this energy to its own cells. For any individual, liver disease was a profound life changing disease as the liver's function affected almost every other organ or system in the body.

Hepatic microstructure:

The hepatocytes were polarized cell consisting of three functionally specialized membrane domains; the basolateral or sinusoidal domain faced the sinusoids and perisinusoidal space of Disse, the canalicular domain formed the bile canaliculus in the intercellular space between adjacent hepatocytes. Whereas the lateral domain faced the intercellular space not related to the bile canaliculus. These domains differed from each other in protein and lipid composition, the fluidity and presence of enzymes, receptors and molecular transport systems **(Wolters et al., 1991).**

The nucleus of the hepatocytes constituted 5-10% of the cell volume. It was spherical in shape and contained one or more prominent nucleoli. Twenty five percent of hepatocytes were binucleated to cope with the extraordinary metabolic activity. All the hepatocytes were loaded with organelles **(Feldmann, 1992).**

The liver was formed of complex network of hepatocytes interpenetrated and ensheathed by supportive connective tissue and permeated by great number of blood vessels perfusing the liver with a rich flow of blood. The hepatocytes which carried out the major metabolic activities of this organ were assisted by additional classes of cells which possed storage, phagocytic and mechanically supportive functions **(Dooley and Sherlock, 1997).**

Hepatocytes were approximately 100 billion in number, constituting 80% of hepatic cell population. They were arranged in plates of a single cell thickness with intervening sinusoids. Hepatocytes were irregular polyhedrals of variable dimensions and geometric shape, ranging in size from 20 to 30µm **(Bioulac-Sage et al., 1999).** Additionally, **Dunkelberg et al. (2001)** demonstrated that, the shape and size of hepatocytes were not fixed but adapted to alternations in sinusoidal blood flow and osmotic load.

The peri-sinusoidal space of Disse lied between the hepatocytes basolateral domain and the interrupted sinusoidal endothelium, constituting 2-4% of the hepatic parenchyma. It contained fat-storing hepatic stellate cells, microvilli projecting from the hepatocytes and extracellular matrix protein. Because neither hepatocytes nor cells lining the sinusoids had basement membranes, movement of fluid occurred freely across the space of Disse. Exchange of substance between blood and hepatocytes was a function of hepatocyte plasma membrane. The space of Disse extended deeply among intercellular spaces between adjacent hepatocytes, creating intercellular recesses, where it was

separated from the bile canaliculus by a narrow gap called zone of minimal distance **(Motta, 1982).** Therefore, the space of Disse formed a labyrinth of interconnecting intercellular channels with the space of Mall, which was an intercellular space between the limiting plate of hepatocytes and fibrous tissue of the portal tract **(Motta, 1982 and Reid et al., 1992).**

Four types of cells populated the hepatic sinusoids; endothelial cells, Kupffer cells, stellate cells and lymphocytes. Morphometric analysis indicated that endothelial cells, Kupffer cells and stellate cells accounted for the vast majority of hepatic non-parenchymal cells **(Ramadoril et al., 1990).**

Sinusoidal endothelial cells accounted for 2-5% of the lobular parenchyma. They had numerous fenestrae and lack a basement membrane **(Arias, 1992).**

Kupffer's cells accounted for 2% of the lobular parenchyma and belonged to the macrophage monocyte system, representing fixed macrophages of the liver and were the largest population of the macrophages any where in the body. They were more numerous in the periportal sinusoids but could migrate along the sinusoids to the areas of liver injury with and against the blood flow **(MacPhce et al., 1992 and Rogoff and Lipsky, 1992).**

The liver contained populations of several types of lymphocytes that provided it with innate immunity **(Wisse et al., 1999 and Doherry, and Farrelly, 2000).**

Stellate cells represented 1.4% of lobular parenchyma and lied between hepatocytes in the space of Disse **(Burt, 1999 and Greets, 2001).** Stellate cells were also known as Ito cells or para-sinusoidal cells or fat storing cells as they stored vitamin A **(Eng and Friedman, 2001).**

Susceptibility of the liver to chemical injury:

Biotransformation reactions had traditionally been considered protective as detoxifier of potentially toxic foreign substances. However, such reaction could also convert non-toxic agents to potentially toxic products. Indeed, it had become clear that the formation of reactive and toxic metabolic intermediates within the hepatocytes accounted for the injury it sustained from many of the known toxic chemicals and drugs **(Mitchell, 1976).**

The side effects of drugs and chemicals could mimic virtually any recognized liver disorder, both clinically and pathologically. Some chemicals might cause one type of injury but with significant individual variation in severity of damage. Other substances could cause a variety of injurious patterns depending on a number of factors such as dose and duration of treatment **(Hall, 1994).**

Some organs were more liable to the adverse effects of drugs than others. The liver was often target organ for injurious effects of xenobiotics as it detoxified most of them. Moreover, the liver had the ability of concentration, biotransformation and excretion of chemicals, irrespective to the route of exposure as it had the highest concentration of xenobiotic metabolizing enzymes **(Larrey and Pageaux, 1997).**

Acute hepatocellular or cytotoxic injury was manifested by necrosis and apoptosis of hepatic parenchymal cells. Hepatocellular necrosis might occur in zonal or non-zonal pattern or both **(Marquardt et al., 1999).**

In the clinical field, the most important form of toxic hepatic injury was caused by therapeutic agents. Drug-induced hepatic injury was the most frequent cause of withdrawal of an approved drug from the market, and it was also responsible for more than 50% of cases of acute liver failure in the United States **(Lee et al., 2003).**

Vitamin E

Chemistry and physical properties

Vitamin E is a fat-soluble vitamin. Four biochemical isomers of naturally occurring nutrients called tocopherols are present; alpha, beta, gamma and delta. The tocopherols differed from each other in position of the methyl group in the ring of the tocopherol molecule **(Bjorneboe et al., 1990).** The alpha tocopherol has the widest natural distribution accounting for 80% of the activity of vitamin E **(Quin and Wang, 2000).**

Physiological role of vitamin E

Vitamin E was considered as a major lipophilic chain-breaking antioxidant present within cell membranes **(Landvik and Packer, 1990).**

Since vitamin E has high affinity to phospholipids, cholesterol and triglycerides (the three main structural elements of the cell membrane, mitochondria and endoplasmic reticulum) due to its hydrophobic lipid solubility. Thus, vitamin E appeared to be the first line of defense against perioxidation of the polyunsaturated fatty acids of the cell membrane **(Vanderveen and Vanderveen, 1990).**

Vitamin E broke free radical chain reactions due to its ability to transfer its phenolic hydrogen to peroxyl free radical of the cell membrane to produce a stable non-radical product finally excreted in bile **(Mayes et al., 1991).**

Many substances were involved in protecting cells from the adverse effects of oxidative stress but vitamin E was the most important

antioxidant. Vitamin E depleted animals were generally more liable to the adverse effects of environmental agents than supplemented animals **(Chow, 1991).**

Wardle (1991), Flagg et al. (1995) and Woodall et al. (1996) concluded that carotenoids present in human diet might have a significant antioxidant activity against oxidative stress being rich in vitamin E.

Quinn and Wang (2000) reported that vitamin E acted as membrane stabilizer by forming complexes with the product of membrane lipid hydrolysis.

Vitamin E had superiority to both vitamins A and C as it could be safely used in prevention of diseases such as diabetes and cardiovascular diseases because it suppressed the oxidative stress of the cell membranes **(Czernichow and Hereberg, 2001)**.

Cisplatinum

Uses of cisplatinum

Cell division was inhibited not only by the electric current but also by the cisplatinum produced from platinum electrodes. Cisplatinum was found to have potent antitumor activity. Cisplatinum was one of the most commonly used drugs in the treatment of a wide spectrum of human malignancies. As a single agent or in combination, cisplatinum was mainstay of treatment for cancer testis, ovaries, urinary bladder, head and neck in addition to small-cell and non small-cell lung cancer **(Chu, 1998).**

Although the response and cure rates with cisplatinum could be high (>90%), as in testicular cancer; in other types of tumors such as ovarian cancer the initial response rate could be up to 70%, but a 5-year survival rate of only 15-20% was recorded **(Ozols, 1991)**

In addition to the serious side effects, the therapeutic efficacy of cisplatinum was also limited by inherent or treatment-induced resistant tumor cell sub-populations **(Perez et al., 1993).**

Pharmacokinetics

Common problems were associated with the clinical use of cisplatinum including nephrotoxicity, ototoxicity and peripheral neuropathy **(Alberts and Noel, 1995).** There had been considerable experience with the intraperitoneal route, particularly in the treatment of ovarian cancer **(Speyer et al., 1990).**

Very high intraperitoneal concentration could be obtained and systemic toxicities could be reduced by concomitant systemic administration of thiosulfate. Cisplatinum had been also administered intra-arterial for treatment of tumors in the extremities, brain tumors and carcinomas of head and neck, liver and urinary bladder **(Bacci et al., 1990).**

Intravesicular instillation of cisplatinum had been used in the treatment of superficial cancers of the urinary bladder. Cisplatinum had been also instilled into pericardial sac in the treatment of malignant pericardial effusions **(Colvin, 2003).**

Intracellular accumulation of cisplatinum

Once cisplatinum entered the cell, the chloride concentration dropped to ≈20 mM and the drug underwent strong hydration to form positively charged active species for subsequent interaction with cellular nucleophiles **(Andrews et al., 1990).**

Cisplatinum cellular uptake was barely understood. The current data indicated that cisplatinum entered cells through transmembrane channels but this data were also consistent with high-capacity facilitated transport. So far, the search for cisplatinum membrane transport system had been unsuccessful **(Gately and Howell, 1993).**

Cisplatinum-induced hepatotoxicity

Generally, the liver toxicity of cisplatinum was characterized by mild to moderate elevation of serum transaminases and less frequently, by a mild elevation of serum alkaline phosphatase, lactate dehydrogenase (LDH), bilirubin and c-glutamyl transpeptidase levels **(Cavalli et al., 1978).**

In spite of the significant anticancer activity of cisplatinum, its clinical use often limited by its undesirable side effects such as nephrotoxicity **(Madias and Harrington, 1978).** Hepatotoxicity could also occur when cisplatinum was administered at high doses and it was known that if cisplatinum was taken in high doses it would produce hepatotoxicity **(Cavalli et al., 1978; Pollera et al., 1987 and Hesketh et al., 1990).**

Cisplatinum-related hepatotoxicity rarely occurred at standard doses. However, at higher doses, hepatotoxicity was frequently observed and could alter the clinical situation of the patients. High doses of cisplatinum, however, produced nephrotoxic and hepatotoxic side effects; therefore, the dose must be the least toxic **(Cersosimo, 1993).**

Clinical evaluation of liver diseases produced by antineoplastic drugs was difficult because cancer patients had many other causes of hepatic dysfunction, including metastasis, viral hepatitis, sepsis and concurrent use of hepatotoxic drugs. A wide range of histopathological changes had been attributed to antineoplastic drugs, but fortunately serious hepatotoxic reactions were infrequent. The liver may be protected by its relative slow rate of cell division and by its abundance of drug-