The Possible Protective Role of Folic Acid versus Taurine on Methotrexate Induced Hepatotoxicity in Albino Rats

Thesis

Submitted for Partial Fulfillment of MD Degree

In Anatomy and Embryology

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2019

Acknowledgements

*First, all praises to **Allah,** the most gracious, the most merciful and blessing and peace to his messenger.*

*My words fail to express my sincere thanks and deepest gratitude to **Prof. Dr. El Erian Youssef Ekladious,** Professor of anatomy and embryology, Faculty of medicine Ain Shams University, for his patience and valuable scientific guidance and support through this work.*

*I am also, greatly honored to express my highest appreciation and gratitude to **Prof. Dr. Hemmat Abdel Kader Abdel Hamid,** Professor of anatomy and embryology, Faculty of medicine Ain Shams University, for her professional co-operation, meticulous supervision, generous support and encouragement in every step in this work.*

*My sincere thanks and deepest gratitude to **Assit. Prof. Dr. Reham Fathi Tash,** Assistant professor of anatomy and embryology, Faculty of medicine Ain Shams University, for her encouragement and support during the work and for continuous guidance enabled me to begin and complete my work successfully.*

*Special thanks to **Dr. Mary Refaat Isaac,** Lecturer of anatomy and embryology, Faculty of medicine Ain Shams University, for her generous help, continuous guidance and great support.*

*Special thanks to **Dr. Mohamed Fathi Mohamed,** Lecturer of anatomy and embryology, Faculty of medicine Ain Shams University, for his encouragement and support during the work.*

*Special thanks to **Prof. Dr. Shahira Youssef Mikhail,** Head of Anatomy and Embryology Department, Faculty of medicine Ain Shams University, for her generous help, continuous guidance and great support.*

I am really indebted to all ***my staff members and colleague*** *in the Anatomy and Embryology Department for the great support and help offered throughout this work.*

No words could ever express my extreme thanks to my ever-giving family specially ***my mother and father,*** *for their endless help, patience, care, support and encouragement. To them, I owe all the success I've reached.*

Words fail to express my love, respect to ***my dear husband*** *for his support and total understanding.*

Lastly, all the love to my sons ***Mohamed, Reda and Malek*** *for being patient and cheerful throughout this work.*

List of Content

List of abbreviations

5-MTHF	: 5-methyltetrahydrofolate
AICAR	: 5 aminoimidazole-4-carboxamide ribonucleotide
ALT	: Alanine transaminase
CoH	: Canals of Hering
CCl4	: Carbon tetrachloride
DHFR	: Dihydrofolate reductase
ECM	: Extracellular matrix
ER	: Endoplasmic reticulum
GSH	: Glutathione
GSH-Rd	: Glutathione reductase
H&E	: Hematoxylin and eosin
HSC	: Hepatic stellate cells
MDA	: Malondialdehyde
MTHFR	: Methylene-tetrahydro folate reductase
Mtx	: Methotrexate
NADP	: Nicotinamide adenine dinucleotide phosphate
PN	: Parental nutrition
RA	: Rheumatoid arthritis

rER	: Rough endoplasmic reticulum
RNS	: Reactive nitrogen species
ROS	: Reactive Oxygen Species
SAM	: S-adenosyl methionine
sER	: Smooth endoplasmic reticulum
SOD	: Superoxide dismutase
THF	: Tetrahydrofolate
TS	: Thymidylate synthase

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ABSTRACT

Background: Methotrexate (MTX) is widely used in the therapy of various types of diseases. It is used as a chemotherapeutic agent for many cancer types and also used for the treatment of multiple sclerosis, dermatomyositis, sarcoidosis, psoriasis, and rheumatoid arthritis. Methotrexate exerts its primary toxic effects against the rapidly replicating cells of the bone marrow and gastrointestinal epithelium. Hepatotoxicity is one of the most common side effects of methotrexate. Taurine is an essential β-amino acid. It is present at high concentrations in many tissues. It plays important roles in numerous physiological functions. Hepatoprotective feature of taurine is attributed to its inhibitory activity on generation of reactive oxygen species (ROS), which are known to play an important role in hepatic injury.

Aim of the work: The aim of the present work is to study the protective role of taurine on methotrexate induced hepatotoxicity.

Material and methods: Thirty albino rats were used in the current study. They were divided into control (group I) and experimental groups. The experimental group was group II (rats were given methotrexate at a dose of 0.5mg/kg by intra-peritoneal injection twice weekly for six weeks) and group III (rats were given methotrexate at a dose of 0.5mg/kg by intra-peritoneal injection twice weekly for six weeks and at the same time were given taurine at a dose of 1000mg/kg daily) using rat gavage needle. The liver was extracted and processed for light and electron microscopic examination.

Results: Examination of Group II showed marked distortion of hepatic cords in their arrangement around the central veins which also showed congestion and dilatation, with pericentral necrosis and inflammatory cells infiltration. Examination of group III showed preservation of control pattern of hepatocytes minor changes were observed some hepatocytes nuclei have abnormal appearance with loss of their nuclear membrane; the cytoplasm of some hepatocytes was vacuolated.

Conclusions: Methotrexate is capable of producing injury to the liver. There is marked improvement of the liver in association with the use of taurine. The present study suggests that the use of taurine with methotrexate had a protective effect on the liver.

Key words: metotrexate, taurine, hepatotoxicity, protection

Introduction

The use of chemotherapeutics is known to cause acute toxic effects in multiorgan systems. Methotrexate (Mtx) is an effective cytotoxic agent and has been widely applied in chemotherapeutic-based treatments for malignancies ***(Erdogan et al*., 2015*).***

It is used as a chemotherapeutic agent for many cancer types such as leukaemia, lymphoma, head and neck tumors, and others. ***(Hytiroglou et al., 2004).***

Mtx is also an effective immunosuppressive and anti-inflammatory agent and used for treatment of some chronic inflammatory disease including psoriasis, rheumatoid arthritis, Crohn's disease ***(Uraz et al., 2008 and Vardi et al., 2010).***

Side effects of Mtx limit its clinical uses. The most common adverse effects include hepatotoxicity, ulcerative stomatitis and decreased white blood cell count. Mtx has toxic side effect on liver particularly in high dose or long-term administration ***(Sener et al., 2006 and Erdogan et al., 2015).***

High-dose as well as low dose therapy can cause hepatotoxicity, high-dose therapy results in elevated liver enzymes and low dose therapy produces a different type of hepatotoxicity which includes cirrhosis ***(Hall et al., 1991).***

It has been postulated that the most serious side effect of Mtx therapy is hepatic toxicity ***(Mardini and Record, 2005),*** Moreover Mtx has been found to increase

mortality in patients with primary biliary cirrhosis ***(Gong and Gluud, 2005).***

Clinically hepatotoxicity which occurs in long-term use of Mtx remains one of the significant restrictions on its use in the doses desired ***(Sener et al., 2006).***

The most common adverse effect for which Mtx was discontinued was elevation of transaminases, twice the value of baseline ***(Rai and Singh, 2016).***

Studies concerned with Mtx side effects highlighted the role of oxidative stress as a leading cause of toxicity induced in the liver and other organs. Levels of both enzymatic and non-enzymatic anti-oxidants were found to be decreased and that of oxidants were increased in liver, kidney, and gut tissues of laboratory animals given Mtx ***(Jahovic et al., 2003).*** It is therefore thought that anti-oxidant therapy may be useful in preventing or reducing Mtx induced hepatotoxicity.

Folic acid (vitamin B9) is essential for numerous body functions including DNA synthesis, repair and methylation as well as acts as a cofactor in certain biological reactions. In addition it is especially important in aiding rapid cell division and growth in infancy and pregnancy. Children and adults both require folic acid to produce healthy red blood cells and prevent anemia. However, humans cannot synthesize folate, therefore it has to be supplied through the diet to meet their daily requirements ***(Weinstein et al., 2003; Lan et al., 2007).***

Experimental evidence suggested that folic acid exert a hepatoprotective effect and attributed this to its antioxidant property as reflected by the evident reduction of lipid peroxidation in the liver ***(Woo et al., 2006).***

Taurine, 2-aminoethanesulphonic acid is an essential amino acid. It is present at high concentrations in many tissues. It plays important roles in numerous physiological functions including conjugation with bile acids, modulation of calcium levels and maintenance of osmolarity, antioxidation and stabilization of membranes ***(Huxtable, 1992; Schrader et al., 2009).***

It was reported to have beneficial effects in various physiological and pathological conditions ***(Ahn et al., 2001; Chiba et al., 2002; Ozturk et al., 2003)*** by mainly diminishing production of Reactive Oxygen Species (ROS). At physiological concentrations, taurine prevents DNA damage ***(Messina and Dawson, 2000; Heibashy et al., 2008).***

It also helps mitochondria to maintain their membrane potential, suppresses superoxide synthesis and subsequent oxidation damage. In addition it maintains healthy skin, protects against diabetes**,** heart disease and arrhythmias. Low blood taurine level has been associated with many cancers ***(Wesseling et al., 2009).***

Regarding the effect of taurine on liver it has been found to improve liver functions in fatty liver disease of children ***(Obinata et al., 1996)*** and induce hepatoprotective effects such as inhibition of extracellular matrix

accumulation in experimental liver fibrosis ***(Balkan et al., 2001).***

It also preserves morphology of major organelles of hepatocytes and delays the development of fibrosis in carbon tetrachloride treated rats ***(Tasci et al., 2008),*** and greatly protected the hepatocytes through different mechanisms in monosodium glutamate treated rats ***(El-Agouza et al., 2010).***

Taurine has a protective action against hepatotoxins and it may find clinical application against a variety of toxins where cellular damage is a consequence of reactive oxygen species ***(Issabeagloo, et al., 2011).***

Taurine has antioxidant and antifibrotic properties, few studies were made to investigate its role to protect against the ultrastructural changes and fibrosis induced by MTX. Hence, the aim of our present study was to investigate the histological changes induced by MTX and to clarify the possible protective role of taurine and folic acid.

Aim of the work

The aim of the present work was to study the histological changes in the liver that result from Mtx administration and to clarify the possible protective role of folic acid and taurine.

Objectives:

The target of the present study is concerned with:

1. The possible changes that may occur in rats liver under the effect of low dose of methotrexate using light and electron microscope.
2. To compare between the possible protective role of folic acid and taurine on methotrexate induced hepatotoxicity.

Review of Literature

Liver is the main organ involved in metabolism of food and drugs and its major functions are carbohydrate, protein and fat metabolism, detoxification, secretion of bile and storage of vitamins. Thus, maintaining a healthy liver is a crucial factor for the overall health and well- being ***(Subramaniam and Pushpangadan, 1999).***

The liver is responsible for transforming metabolites and eliminating toxic substances. The liver also produces plasma proteins such as albumin, coagulation factors and growth factors ***(Fawcett and Jensh, 2002).***

Anatomy of human liver:

The liver is situated in the right hypochondrium and epigastric region, reaching the left hypochondrium, and is almost entirely protected by the ribcage. It is the largest internal organ in the human body. Its size when compared to the total body size is greater in the feotus than in the adult, constituting, in the former, about one eighteenth, and in the latter about one thirty-sixth of the entire body weight ***(El-Shabrawi et al., 2012).***

Glisson capsule envelops the liver from which extensions pass into the liver as connective tissue septa and trabeculae ***(Fawcett and Jensh, 2002).*** Classically, the liver is divided in to two large lobes (right and left) and two small central ones (quadrate and caudate lobes). Its external surfaces can be divided into superior, inferior and posterior.

The superior surface lies under the diaphragm. The diaphragm separates it from the ribs and the costal cartilages on the lateral and posterior sides and the lungs with pleurae and the heart with the pericardium on the superior side. The superior and inferior surfaces are demarcated on the anterior side of the liver by a well-defined margin, the inferior border. In children and women, this border projects below the ribs unlike in adult males where it generally corresponds with the lower margin of the thorax in the right mammillary line ***(El-Shabrawi et al., 2012).***

The liver has a dual blood supply, the portal vein and the hepatic artery. The hepatic artery supplies oxygenated blood. Approximately 75% of the blood is delivered to the liver via the portal vein that drains the spleen, stomach, intestines, and pancreas ***(Thoolen et al., 2010).***

The hepatic artery in the foetal and early postnatal life is the largest branch of the celiac axis, its calibre decreasing with age to an intermediate size artery in adults. At the porta hepatis, it divides into right and left hepatic arteries. They branch out progressively to terminal hepatic arterioles, which communicate with the hepatic sinusoids. The hepatic artery provides the blood supply to the biliary tree through a peribiliary plexus. Hepatic artery thrombosis can cause ischemic necrosis of the biliary tree due to its dependence on the arterial supply. The portal vein enters through the portahepatis and subdivides into right and left branches and then progressively down to the terminal portal

venules and inlet venules to open into the sinusoids. The sinusoidal blood drains into the centrilobular venules, and through the hepatic veins into the inferior vena cava. Segments II, III and IV are drained by the left hepatic vein, the middle hepatic vein gathering blood from segments IV, V and VIII, while the right hepatic vein serves segments V–VIII. One small inferior hepatic vein drains the caudate lobe (segment I) directly into the inferior vena cava. Liver biopsy through the transjugular route is an established procedure to sample liver parenchyma via the hepatic veins ***(Dohan et al. 2014).***

Anatomy of rat liver:

In rats liver mass represents about 5% of the total body weight, while in adults human it represent 2.5%. In rats weighting 250-300 g, the liver mean weight is 13.6 g ***(Zanchet and Monteiro, 2002)***.

Similar to the human liver, the rat liver is connected to the diaphragm and abdominal wall by 5 ligaments and also it is attached to the lesser curvature of the stomach by the hepatogastric ligament and to the duodenum by the hepatoduodenal ligament ***(Martins and Peter, 2007)***.

The rat liver has basically three surfaces superior, posterior and inferior. Different from the human liver all the borders of the liver are sharp ***(Kongure et al., 1999)***.

The origin and course of the major vessels are similar to human and no variation in the origin of the vessels is identified in rats ***(Wu et al., 2005)***.