Effect of Usage of the Proton Pump Inhibitor Omeprazole on the Structure of the Kidney of Male Albino Rats

THESIS

Submitted for Partial Fulfilment of Master Degree in Anatomy and Embryology

By

Heba Mohammed Hussein Ali

Demonstrator of Anatomy and Embryology,
Faculty of medicine Ain Shams University

Supervised by

Prof. Dr/ Nagwa Ebrahim El-Nefiawy

Professor of Anatomy and Embryology
Faculty of Medicine –Ain shams University

Ass. Prof. Dr / Haidy Farid Abdel Hamid

Assistant professor of Anatomy and Embryology
Faculty of Medicine –Ain shams University

Dr. / Marwa Abd El Moneim Abo El Fetoh

Lecturer of Anatomy and Embryology
Faculty of Medicine –Ain shams University

Faculty of Medicine
Ain shams University
2019

Acknowledgement

First, thanks are all due to ***Allah*** *for Blessing this work until it has reached its end, as a part of His generous help throughout our life.*

My profound thanks and deep appreciation to ***Prof. Dr/ Nagwa Ebrahim El-Nefiawy,,*** *Professor of Human Anatomy and Embryology, Faculty of Medicine- Ain Shams University, for her great support and advice, her valuable remarks that gave me the confidence and encouragement to fulfill this work.*

I am deeply grateful to ***Prof. Dr/ Haidy Farid Abdel Hamid*** *Professor of Human Anatomy and Embryology, Faculty of Medicine- Ain Shams University for adding a lot to this work by her experience and for her keen supervision.*

I would like to direct my special thanks to ***Dr./ Marwa Abd El Moneim Abo El Fetoh,*** *Lecturer of Human Anatomy and Embryology, Faculty of Medicine- Ain Shams University, for her invaluable help, fruitful advice and continuous support.*

I am extremely sincere to ***my family*** *especially my dear father and mother who stood beside me throughout this work giving me their support.*

Words fail to express my respect and appreciation to ***my husband dr.Tarek Ali Abdullah*** *for his unlimited help and support.*

Lastly, all the love to ***my dear son omar*** *for being patient throughout this work.*

List of Content

List of abbreviations

ACE : Angiotensin converting enzyme.

ACH : Acetylcholine.

ADH : Antidiuretic hormone

AKI : Acute kidney injury.

ANOVA : One-way analysis of variance

ARBs : Angiotensin receptor blockers.

BUN : Blood urea nitrogen.

CARE : Committee of animal research ethics

cAMP : Cyclic adenosine 3′, 5′-monophosphate.

CKD : Chronic kidney disease.

COX-2 : Cyclooxygenase 2.

CYP : Cytochrome P

DCT : Distal convoluted tubule.

ECL : Enterochromaffin like

eGFR : Estimated glomerular filtration rate.

ENRD : Endoscopy-negative reflux disease

ESRD : End stage renal disease

FDA : Food and Drug Administration.

GBM : Glomerular basement membrane.

GERD : Gastro esophageal reflux disease.

GFR : Glomerular filtration rate.

GI : Gastrointestinal.

GRP : Gastrin-releasing peptide.

H_2RAs : Histamine$_2$-receptor antagonists.

I cells : Intercalated cells.

IC : Intracellular canaliculi.

KIM-1 : Kidney injury molecule-1.

NERD : Nonerosive reflux disease.

NGAL : Neutrophil gelatinase-associated lipocalin.

NIH : National Institute of Health.

PCT : Proximal convoluted tubules.

NSAIDs : Nonsteroidal anti-inflammatory drugs.

PGES : Prostaglandin synthase.

PPIs : Proton pump inhibitors.

PUD : Peptic ulcer diseases

SPSS : Statistical Package for Social Studies

UGI : Upper gastrointestinal.

ZES : Zollinger-Ellison syndrome

Introduction

Proton pump inhibitors (PPIs) are one of the most widely used drugs worldwide. They are used for suppression of gastric acid secretion. The standard clinical indications for treatment with PPIs include gastro esophageal reflux disease (GERD), peptic ulcer disease and erosive esophagitis. PPIs are also prescribed as a prophylaxis against peptic ulcer in combination with some medications like steroids, non-steroidal anti-inflammatory drugs (NSAIDs) and with antibiotics for eradicating Helicobacter pylori (**Yadlapati and Kahrilas, 2017**).

On the other hand, it has been reported that 60% of people on PPIs do not have a verified indication to use the drug. PPIs are even available for sale over the counter without prescription in some countries like the United States (**Yadlapati and Kahrilas, 2017**).

PPIs inhibit gastric acid secretion through blocking gastric $H^+\backslash K^+$-ATPase pump responsible for H^+ ions secretion into the gastric lumen **(Shin and Sachs, 2010)**.

Several generations of PPIs are available in the market. All are similar in action with no evidence that one is more effective than another. They differ in how they are broken-down by the liver and in their drug interactions , making the effect of some PPIs may last longer. (**Shin and Sachs, 2010)**.

Literature defines the duration of medications usage either as short term use or long term use of PPIs. Short term

use is limited to a maximum 2-month supply during any 12-month period. One additional month can be approved upon request for tapering purposes until discontinuation. So more than this period is considered as long term usage (**Katz, 2013**).

PPIs are generally well tolerated with minimal side effects. However, high doses and long term use may increase the risk of osteoporosis-related fractures of the hip, wrist, or spine, reduces absorption of vitamin B12 (cyanocobalamin), lower levels of magnesium with increased risk of heart attacks **(Freedberg et al., 2017).**

Recent clinical observational studies claim that PPIs use is associated with an increased risk of acute kidney injury (AKI), chronic kidney disease (CKD), chronic kidney disease progression and end stage renal disease (ESRD). (**Klatte et al., 2017**).

Normal range of glomerular filtration rate (GFR) is 90-120 ml/min/1.73m^2 .

The literatures define AKI as increase in serum creatinine level double normal range, or decrease in GFR about 50% from the basal level .

Chronic kidney disease (CKD) is defined as decreased GFR less than 60 ml/min/1.73m^2 for more than 3 months which could be classified into five stages which start from normal GFR with histopathologial changes and progress till reaching 15 ml/min/1.73m^2. ESRD is described when GFR reaches less than 15

ml/min/1.73m^2 and so replacement therapy is needed either dialysis or renal transplantation (**Klatte et al., 2017**).

Aim of the work

The effect of omeprazole as a proton pump inhibitor on the kidney is still not thoroughly investigated. Thus, the aim of the present study was to show the effects of long term use of the proton pump inhibitor omeprazole on the histological architecture and function of the kidney of male albino rats and to compare between the effects of omeprazole usage upon different periods after two weeks, four weeks and six weeks.

Specific objectives:

1- Detection of histopathological characteristic findings after treatment.

2- Biochemical studies for kidney function before and after treatment.

3- Histomorphometric measurements of renal structures before and after treatment.

4- Statistical analysis of data obtained from histomorphometry and biochemical tests.

Kidney structure and function

Anatomy of the kidney

In human, kidneys are bean shaped organs located retroperitoneally in the posterior abdominal region, in the extraperitoneal connective tissue, just lateral to the vertebral column. kidneys extend from approximately twelvth thoracic vertebra superiorly to third lumbar vertebra inferiorly in the supine position, with the right kidney somewhat lower than the left because of its relationship with the liver (**Singh, 2018) & (Standring, 2015).**

The hilum of the kidney lies on the medial margin of each kidney, through which renal vessels, lymphatics, nerves and ureter can enter and leave the substance of the kidney **(Standring, 2015).**

Each kidney has a smooth anterior and posterior surface covered by a fibrous capsule, which is easily removable except with disease and represent the true capsule of the kidney. There are two more coverings representing the false capsule ; fatty capsule (peri-renal fat); which continues into the hilum and sinus surrounding all structures and has a role in keeping kidney stable in its place; and externally fascial capsule (Zukercandle fascia) (**Verland, 1998).**

The weight of each kidney ranges from 125 - 170 gm in the adult male. The human kidney is approximately 11 - 12 cm in length, 5 - 7 cm in width, and 2.5 -3 cm in thickness **(Standring, 2015).**

Regarding the internal structure, each kidney consists of an outer renal cortex and an inner renal medulla. The

renal cortex is a continuous band of pale tissue which completely surrounds the renal medulla. The renal columns are extensions of the renal cortex project into the inner aspect of the kidney, dividing the renal medulla into discontinuous aggregations of triangular-shaped tissue called the renal pyramids **(Standring, 2015).**

The renal pyramids represent an inverted pyramid with their bases directed outward towards the renal cortex, while the apex of each renal pyramid projects inward toward the renal sinus to form renal papilla. About ten to twenty five small openings, represent the distal ends of the collecting tubules of Bellini, open on the renal papilla which is an apical projection surrounded by a minor calyx. The minor calices receive urine and represent the proximal parts of the tube that will eventually form the ureter. Several minor calices unite to form a major calyx in the renal sinus, and two or three major calices unite to form the renal pelvis, which is the funnel-shaped superior end of the ureters **(Standring, 2015).**

The rat kidney is similar to the human one anatomically except in the mean weight of the kidneys that ranges from 0.96-1.1 gm with average dimensions ; 1.23-1.28 cm in length, 0.85-0.88 cm in width, and 0.79-0.81 cm in thickness. Also, in the fact that the rat kidney has a single renal pyramid (uni-papillate) with only one calyx and specializied fornices which evaginate into of the renal pelvis **(Kiss and Hamar, 2016; Al Samawy, 2012).**

Blood supply

In humans, the blood supply to the kidneys arises from the paired renal arteries at the level of L2. They enter into the renal hilum. The renal artery divides into an anterior and a posterior division, and these divide into 4-5 segmental arteries. Three segmental or lobar arteries arise from the anterior branch and supply the upper, middle, and lower thirds of the anterior surface of the kidney. The posterior branch supplies more than half of the posterior surface and occasionally gives rise to a small apical segmental branch. **(Standring, 2015).**

The segmental arteries branch into interlobar arteries, which travel in a parallel way between the major calyces and then branch into arcuate arteries that run within the cortex across the bases of the renal pyramids. They then radiate into interlobular arteries, which extend into the cortex of the kidney to finally become afferent arterioles, then peritubular capillaries then efferent arterioles **(Standring, 2015).**

The afferent arterioles are short, straight branches of the interlobular arteries. Each divides into multiple capillary branches to form the tuft of vessels in the glomerulus. The capillaries coalesce to form the efferent arteriole, which in turn breaks up into capillaries that supply the tubules (Peritubular capillaries) before draining into the interlobular veins. The arterial segments between glomeruli and tubules are thus technically a portal system, and the glomerular capillaries are the only capillaries in the

body that drain into arterioles. However, there is relatively little smooth muscle in the efferent arterioles. The capillaries draining the tubules of the cortical nephrons form a peritubular network, whereas the efferent arterioles from the juxtamedullary glomeruli drain not only into a peritubular network, but also into vessels that form hairpin loops (the vasa recta). These loops dip into the medullary pyramids alongside the loops of Henle (**Zacchia et al., 2018)**

The descending vasa recta have a non-fenestrated endothelium that contains a facilitated transporter for urea, and the ascending vasa recta have a fenestrated endothelium, consistent with their function in conserving solutes. The efferent arteriole from each glomerulus breaks up into capillaries that supply a number of different nephrons. Thus, the tubule of each nephron does not necessarily receive blood solely from the efferent arteriole of the same nephron. In humans, the total surface of the renal capillaries is approximately equal to the total surface area of the tubules (**Zacchia et al., 2018)**

In rats, the right and left renal arteries emerge from the abdominal aorta. They divide into dorsal and ventral primary divisions. The dorsal and ventral branches are divided into two branches, the cranial and caudal secondary branches (segmental arteries). Segmental arteries give off 5-7 inter lobar arteries which give off arcuate arteries. The interlobular arteries arise from arcuate arteries. No

significant anastomosis exists between any of the sub branches of the renal arteries **(Yoldas and Dayan, 2014).**

Microscopic structure of the kidney:

The nephron:

Each single renal tubule and its glomerulus is a unit called nephron. The size of the kidneys varies between species, as does the quantity of nephrons they contain. Each human kidney has about 1 million nephrons (**Capasso, 2007)**.

Rat kidney contains approximately 30,000 nephrons in each kidney **(Skorecki et al., 2016).**

Bonventre and Yang (2011) mentioned that in both human and rat the components of the nephron include; the renal corpuscle (glomerulus and Bowman's capsule), the proximal convoluted tubule (PCT), the thick and thin limbs of the loop of Henle, the distal convoluted tubule (DCT), and the connecting tubule which leads to the collecting duct which is not a part of the nephron.

A-The renal corpuscle:

The glomerulus, which is about 200 μm in diameter, is formed by the invagination of tuft of capillaries into the dilated, blind end of the nephron which is called Bowman's capsule. The capillaries are supplied via an afferent arteriole and drained through the efferent arteriole, and it is form the glomerulus where the filtrate is formed. The diameter of the afferent arteriole is larger than the efferent

arteriole. There are two cellular layers separate the blood from the glomerular filtrate in Bowman's capsule; the capillary endothelium layer and a layer of specialized epithelium of the capsule **(Skorecki et al., 2016).**

The endothelium of the glomerular capillaries is fenestrated, with pores that are about 70–90 nm in diameter. The endothelium of the glomerular capillaries is totally surrounded by means of the glomerular basement membrane along with specialized cells known as podocytes. Podocytes have multiple pseudopodia that interdigitate to form filtration slits along the capillary wall. The slits are approximately 25 nm wide, and each is closed with the aid of a very thin membrane **(Hu and Jiao, 2013).**

The glomerular basement membrane does not include visible gaps or pores. Stellate cells known as mesangial cells are located between the basal lamina and the endothelium. They are almost similar to cells called pericytes, which are found in the partitions of capillaries elsewhere in the body. Mesangial cells are especially common between two neighboring capillaries, and in these locations the basal membrane forms a sheath shared by both capillaries. The mesangial cells are contractile and play a role in the regulation of glomerular filtration. Mesangial cells secrete the extracellular matrix, take up immune complexes, and are involved in the progression of glomerular disease (**Lu et al., 2017)**