Original Article

Histopathological Analysis of Renal Tissue to Evaluate the Effects of Proton Pump Inhibitors at Cellular Level

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ABSTRACT

Objective: To evaluate the cytopathological effects of proton pump inhibitors on renal tissue in different doses by using an animal model

Study Design: Randomized control trial.

Place and duration of study: This study was conducted at Post Graduate Medical Institute Lahore for a period of 4 weeks.

Materials and Methods: The sample size was 60 mice. The sampling technique was Simple Random Sampling. The mice were obtained from National Institute of Health Islamabad. The animals were kept in labeled cages under standard environmental conditions. Animals were divided into three groups. One group served as control and the other two served as treated groups. Micrometry was done to evaluate the effects of drug.

Results: It was observed on study of histological sections that proton pump inhibitors induce toxic effects on cells of uriniferous tubules of kidney. The tubular cells showed varying degree of tubular atrophy.

Conclusion: The observations of the study revealed that it damages the uriniferous tubules by causing their atrophy leading to renal insufficiency. It is desired that these result will generate awareness about the careful use of the proton pump inhibitors by the people and the clinicians.

Key Words: Cytopathology, Renal toxicity, Atrophy

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INTRODUCTION

Proton pump inhibitors are a group of drugs which have the main function of prominent and long lasting decrease in the production of gastric acid. The primary role in the disorders of gastrointestinal tract is played by the altered gastric acid secretion. Acidic environment exerts a negative effect on the homeostasis. [1]Therefore an intragastric pH above 6 is maintained by the use of acid inhibitors. Proton pump inhibitors act on the gastric H/K ATPase pump to achieve this target. Within this class of drugs, there is no clear difference of efficiency in their function. [2,3]

For the purpose of study, the drug used was Omeprazole which belongs to the class of proton pump inhibitors. It was introduced in 1989. It is available as capsule and powder form. Its introduction led to the self directed use of the proton pump inhibitors. They are considered as safe medicines but nowadays, people are using them on long term basis. This has attracted the attention to evaluate their adverse effects.[4,5]

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The use of proton pump inhibitors as treatment for the gastrointestinal disorders is associated with the development of renal impairment. It is evident in almost 15% of the cases of acute renal failure. Drugs are commonly associated with kidney injury. Mostly drugs exert nephrotoxic effects by various mechanism including altered glomerular hemodynamics, micro-angiopathy and tubular cell injury. [6, 7]Tubulointerstitial diseases of the kidney may be primary in nature involving tubules basically. Secondary form of disease involves the glomeruli or the blood vessels. Variable drugs can cause this condition but symptoms and the findings will be different according to the drug used. [8, 9]

Almost one quarter of the cardiac output undergoes circulation through the renal vasculature so the structural cells of the kidney are at risk of exposure to the toxic effects of the drugs. Medicine induced toxic effects are often encountered possibly because of primary role of the kidneys in the filtration of plasma.[10,11] As a result, renal tubular cells bear significant amount of drug and also its metabolites which can damage the renal tissue. Acute renal damage is manifested as edema and infiltration of inflammatory cells. If the process of damage continues then pathology is observed as tubular necrosis, tubular atrophy with dilatation of lumen and basement membrane disruption.[12,13,14]

MATERIALS AND METHODS

A total of 60 mice were obtained from the National Institute of Health Islamabad. They were divided into three equal groups by random number table. One group served as control, whereas other groups were used as treated group. The animals were handled according to international and ethical guidelines for the animal care. They were kept in separate cages which were labeled. Omeprazole used in this experiment was a product of GETZ Pharmaceuticals with the brand name of RISEK having Omeprazole as 20 mg and 40 mg. Equivalent animal dose of omeprazole was calculated. It was dissolved in distilled water and given orally to the animals of treated group. Controls were given same quantity of normal saline at the same time. Animals were sacrificed after 4 weeks of drug administration. Kidneys were dissected out and tissue blocks prepared. Slides were stained for histological study. The duration of administration is chosen according to the previous studies which reflect the nephrotoxic effects of drug. [15] Micrometry was done with the help of oculomicrometer to measure the size of tubular cells. It was attached to the microscope eyepiece. The stage micrometer was placed on the microscope slide stage. The microscope was adjusted and the cell size of the uriniferous tubules was measured in different fields of vision. Mean size was recorded. [16]

RESULTS

The observations of the study were recorded using the MS word and Excel data sheet. The data was entered and analyzed statistically using MedCal for Windows, version 12.5.0.0 (MedCal Software, Ostend, Belgium). ANOVA (Analysis of Variance) test and t- test were used to calculate the quantitative difference between the groups.

Table No.1: Table showing detail of animal groups

Group	Status	Dose	Duration of therapy
1	Control	Normal saline	4 weeks
2	Experimental	20 mg	4 weeks
3	Experimental	40 mg	4 weeks

Table No.2: Comparison of the cell size of uriniferous tubules

Kidney Tubules	Group 1 n=20 mean ± SD	Group 2 n=20 mean ± SD	Group 3 n=20 mean ± SD
Proximal convoluted tubule	15.50 ±0.54	14.25 ± 0.60	13.30 ± 2.50
Distal convoluted tubule	15.00 ±0.56	14.28 ± 0.75	13.55 ±0.74
Collecting ducts	15.00 ±0.56	14.28 ± 0.75	13.55 ±0.74

n = number of animals

The interpretation of the p- value was done as follows.
p > 0.05 difference insignificant
*p < 0.05 difference significant
**p < 0.05 difference considerably significant
***p < 0.01 difference highly significant

Table No.3: Significance (p- value) of cell size difference using t- test

Group	Proximal convoluted tubule		Distal convoluted tubule		Collecting ducts	
	Mean difference	p- value	Mean difference	p- value	Mean difference	p- value
Control Vs G2	1.25	0.017*	0.7250	0.011*	1.13	<0.001***
Control Vs G3	2.20	<0.001***	1.4500	<0.001***	1.95	<0.001***

Table No.4: Comparison of the nucleus size of uriniferous tubules

Kidney Tubules	Group 1 n=20 mean ± SD	Group 2 n=20 mean ± SD	Group 3 n=20 mean ± SD
Proximal convoluted tubule	5.8 ±0.5	4.5 ± 0.9	4.0 ± 0.7
Distal convoluted tubule	5.75 ±0.5	4.55± 0.84	3.85 ±0.73
Collecting ducts	5.78 ±0.47	4.25 ± 0.66	3.65 ±0.52

n = number of animals

Table No.5: Significance (p- value) of nucleus size difference using t- test

Group	Proximal convoluted tubule		Distal convoluted tubule		Collecting ducts	
	Mean difference	p- value	Mean difference	p- value	Mean difference	p- value
Control Vs G2	1.255	0.001***	1.200	0.001***	1.525	<0.001***
Control Vs G3	1.825	<0.001***	1.900	<0.001***	2.125	<0.001***

DISCUSSION

Proton Pump Inhibitors are commonly utilized for the treatment of gastrointestinal disorders. Omeprazole belongs to this group of drugs. They are known as proton pump inhibitors because they act by inhibiting the H/K ATPase pumps found in the lining cells of the stomach that make gastric acid.[17,18] Literature review

shows that risks are present with their use like Clostridium difficile associated diarrhea, community acquired pneumonia and antiplatelet therapy. In this study, dose related cytopathological effects of proton Pump Inhibitors on the uriniferous tubules were studied. The adverse effects were studied in different groups of animals. These groups were treated with omeprazole and compared with the animals of control group. Acute kidney injury was observed in treated animals. Most of the previous studies are in favor of adverse effects of proton pump inhibitors on kidney.[19, 20]

It was found that proton pumps are also located in uriniferous tubules of kidney which are sensitive to them. It binds to these pumps and selectively inhibits the H/K ATPase pumps showing that omeprazole has organ specificity. So an immunological basis is suspected for the renal effects of proton pump inhibitors.[21,22] The drug binds to the tubular basement membrane component and acts as happen causing the formation of antimembrane antibodies. Endothelial cell activation by the inflammatory mediators promotes leukocyte infiltration. This inflammatory process commonly leads to the tubular damage and acute kidney injury.[23]

The renal tubules of the animals treated with drug showed tubular atrophy as compared to the animals of control group which was evident by the significant results. Numerous other studies also reported a positive relationship between their use and acute kidney injury. The most common etiology of kidney damage is drug induced disease, which underlie almost 60-70% of cases. Proton Pump Inhibitors are among the commonly responsible ones and they are gaining attention now as a cause of acute renal damage because of their widespread and continuous use.[24,25]

CONCLUSION

Omeprazole belongs to the class of Proton Pump Inhibitors. It is commonly used for the relief of gastrointestinal disorders. The present study was conducted to evaluate the adverse effects on the renal tissue when administered in different doses. The observations of the study revealed that it damages the uriniferous tubules by causing their atrophy leading to renal insufficiency. It is desired that these result will generate awareness about the careful use of the proton pump inhibitors by the people and the clinicians.

Conflict of Interest: The study has no conflict of interest to declare by any author.

REFERENCES

1. Baldwin RM, Ohlsson S, Pedesen RS, Mwinyi J, Ingelman-Sundberg M, Eliasson E, et al. Increased Omeprazole mrtabolism in carriers of the CYP2C19*17 allele; A pharmacokinetiocs study in healthy volunteers. Br J Clin Pharmacol 2008;65 (5):767-774.
2. Boussery K, Smet DJ, Cock DP, Velde VS, Mehuys E, Paepe P, et al. Pharmacokinetics of two formulation of omeprazole administered through a gastrostomy tube in patients with severe neuro developmental problems. Br J Clin Pharmacol 2011;72 (6):990-996.
3. Devendran A, Uppugunduri SC, Sundaram, R, Gopal D, Krishnamoorthy, Chandrasekaram A. Relative copy number variation of CYP2C19 in south Indian population. Mol Biol Int 2012; 643856.
4. Ezquerra PR, Morillas SL, Martinez LJ, Fernandez, GD, Tembleque MG, Avarez AS, Sanz ML. Anaphylaxis to omeprazole. Cross reactivity with other proton pump inhibitors. Allergol Immunopathol 2011;39(54): 01.
5. Halbritter J, Mayer C, Rasche FM, Amann K, Lindner TH. Interstitial Nephritis Internist 2009; 50(9):1111-25.
6. Holzer P. Acid sensing by visceral afferent neurons. Acta Physiol 2011;201(1): 63-75.
7. Heidelbaugh JJ, Kim HA, Chang R, Walker CP. Overutilization of proton pump inhibitors: what the clinician needs to know. Therap Adv Gastroenterol 2012;5(4): 219-232.
8. Iida A, Kaneko H, funaki YK, Tokudome K, Iawa S. Inhibition of gastric perception of mild distension by Omeprazole. World J G astroenterol 2012;18(39):5576-5580.
9. James Y, Lau Wai K, Leung Justin CY, Wu Francis KL, Chan Vincent WS, Wong Philip WY, et al. Omeprazole before endoscopy in patients with gastrointestinal bleeding. N Engl J Med 2007; 356:1631-1640.
10. Li J, Shentu ZJ, Wu L, Dou J, Xu Q, Zhou H, Wu G, et al. Relative bioavailability and pharmacokinetic comparison of two different enteric formulations of Omeprazole. J Zhejiang Univ Sci B 2012;13(5):348-355.
11. Lobera T, Navarro B, Pozo D, Gonzalez I, Blasco A, Escudero R. Nine cases of Omeprazole allergy. Cross reactivity between proton pump inhibitors. J Investing Allergol Clin Immunol 2009;19(1): 57-60.
12. Leonard CE, Freeman CP, Newcomb CW, Reese PP, Herlim M, et al. Proton pump inhibitors and traditional nonsteroidal anti-inflammatory drugs and the risk of acute interstitial nephritis. Pharmacoepidemiol Drug Saf 2012;21(11): 1155-72.
13. Mann PC, Vahle J, Keenan CM, Baker JF, Bradly AE, Goodman DJ. International harmonization of toxicologic pathology nomenclature: an overview and review of basic principles. Toxicol Pathol 2012;40(4):7-13.

14. Monzani A, Oderda G. Delayed release oral suspension of omeprazole for the treatment of erosive esophagitis and gastro esophageal reflux disease in pediatric patients, a review. Clinical and Experi Gastroenterol 2010;3:17-25.
15. Michael D, Drepper Spaher L, Frossard LJ. Clopidogrel and proton pump inhibitors- where do we stand in 2012. World J Gasteroenterol 2012; 18(18): 2161-2171.
16. Michelle L, Gumz I, Megan M, Greenlee Brain D, Wingo SC. The renal H+ -K+-Atpases: physiology, regulation and structure. Am J Physiol Renal Physiol 2010;298(1):12-21.
17. Perazella MA, Markowitz GS. Drug induced acute interstitial nephritis. Nat Rev Neph., 6(8): 461-70.
18. Rose, C., Baker, T. and Nicholas, T., 2004. Drug induced tubule-interstitial nephritis secondary to proton pump inhibitors. Experience from a single UK renal unit. Nephrol Dial Transplant 2010;19: 1441-6.
19. Romano C, Chiaro A, Comito D, Loddo L, Ferrau V. Proton pump inhibitors in pediatrics: Evaluation of Efficiency in GERD Therapy. Curr Clin Pharmacol 2011;6(1):41-7.
20. Sujin RM, Subin RM, Mahesh R, Vinolyia J. The effect of Anti Diabetics in Kidney of Rats. Ethnobotanical Leaflets 2009;13:689-701.
21. Shin MJ, Sachs G. Pharmocology of proton pump inhibitors. Curr Gastoenterole Rep 2008;10(6): 528-534.
22. Shi S, Klotz U. Proton pumps inhibitors: an update of their clinical use and pharmacokinetics. Europ J Clin Pharm 2008;64(10):935-951.
23. Sach G, Shin JM, Hunt R. Novel Approaches to inhibition of gastric acid secretion. Curr Gatroeneterol Rep 2010;12(6):437-447.
24. Tamura T, Sakaeda T, Kadoyama K, Okuno Y. Omeprazole and Esomeprazole-associated Hyomagnesaemia: Data mining of public version of the FDA advers eventreporting system. Int J Med Sci 2012;9(5): 322-326.
25. Tsuzuki T, Okada H, Kawhara Y, Takennaka R, Nasu J, Ishioka H, et al. Proton pump inhibitor step-down therapy for GERD: A multi-center study in Japan. World J Gastroenterole 2011;17(11): 1480-1487.

Corrigendum

Name of author in the article titled "Predict the Possibility of Esophageal Varices in HCV Patients on the Basis of Fibro Scan Scoring System" printed in Med Forum volume 2017;28(2) at pages 138-141 has been typographically written as Dr. Haris Ali which may now be read as **"Dr. Haris Alvi."**

Editor