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Omeprazole activates the mitogenic cell response in rat kidney: Implication of ERK1/2 signaling cascade

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Abstract: Proton pump inhibitors (PPIs) are a group of drugs that is commonly prescribed worldwide. However, the use of PPIs is accompanied with acute kidney injury and chronic kidney disease by mechanisms not entirely known. In the present study, omeprazole administration was found to cause a rapid phosphorylation of ERK1/2 signaling cascade accompanied with an increase in cyclin B1expression in rat kidney. In addition, administration of omeprazole enhancedthe activity of A disintegrin and metalloproteinase-17 (ADAM-17) ane the phosphorylation ofepidermal growth factor receptor (EGFR).Interestingly, concomitant administration of the pharmacological inhibitor of EGFR, gefitinib along with omeprazole caused an almost complete reduction of the phosphorylation of EGFR and ERK1/2 induced by omeprazole. Moreover, omeprazole administration significantly increased lipid peroxidation and reduced superoxide dismutase (SOD) activity in rat kidney. Collectively, Omeprazole activates ERK1/2 signaling cascade that could be translated to an increase in the expression of the cell proliferation gene cyclin B1inreactive oxygen species (ROS)-dependent manner. These effects may contribute to omeprazole-mediated chronic kidney disease.

Keywords: ERK1/2 signaling cascade; Kidney; Oxidative stress; EGFR.

1. INTRODUCTION

Proton pump inhibitors (PPIs) are the most important drugs that are usually used in management of hyperacidity via blocking gastric acid secretion. They act via inhibition of H+/K+-ATPase enzyme (proton pump) in the parietal cells of the stomach. This enzyme plays very important role in hydrochloric acid secretion in the stomach. One of the most prescribed PPIs is omeprazole. It is commonly used in the management of gastro esophageal reflux disease (erosive esophagitis-healing, erosive esophagitis-maintenance and non-erosive reflux disease), peptic ulcer disease (duodenal ulcer-healing, gastric ulcer-healing and Zollinger-Ellison syndrome) and treatment of Helicobacter pylori (dual therapy and triple therapy).[1] Several studies have reported that administration of PPIs may be accompanied with serious unwanted effects, including hip fractures, kidney injury and chronic kidney disease (CKD) [1] . Interestingly, it has been reported that an initial signal for CKD occurs when the patients cannot fully recover their kidney function after acute interstitial nephritis[2] . In acute interstitial nephritis, acute inflammation and tubulointerstitial damage are usually occurring. However, long term acute interstitial nephritis may progress to interstitial fibrosis and chronic interstitial nephritis.[3] Several studies suggested an association between PPIs and CKD.[1,3] However, the mechanism of these findings is still unclear. It has been reported that the PPI esomeprazole (the S enantiomer of omeprazole) has the ability to induces reactive oxygen species (ROS) through mitochondrial dysfunction and involvement of NADPH oxidase.[4,5] Administration of omeprazole has been shown to generate ROS.[6] Previously, it has been shown that ROS is involved in EGFR-mediated phosphorylation of ERK1/2 and consequent induction of

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proliferation in various cell types[7,8]. Cells proliferation are usually initiated by the heparin-binding EGF (HB-EGF) or epidermal growth factor (EGF) .[9,10]A disinterring and metalloproteinases (ADAMs) have the ability to liberate the active form HB-EGF that activates EGFR resulting in the activation of ERK1/2 signaling cascade.[7,11]The current work was designed to examine the prospective effect of omeprazole upon ERK1/2 signaling cascade that is usually accompanied with an increase in the expression of cell proliferation genes in rat kidney.

2. MATERIALS AND METHODS

2.1. Animals

In this study Male Sprague Dawley rats were used (weighing 180-200g) were provided from Nile Co., Cairo, Egypt. The rats were housed in the animal facility of the Faculty of Pharmacy, Al-Azhar University (twelve-hour dark/light cycle with controlled humidity and constant temperature). Water and a standard diet were provided *ad libitum*. One week before treatments the animals were kept under observation for adaptation.

2.2. Chemicals

Omeprazole was purchased from Gulf Pharmaceutical Industries (Julphar, Ras Al Khaimah, UAE). The EGFR inhibitor gefitinib was purchased from MedChem Express, NJ, USA. Antibodies specifically raised against cyclin B1 p-ERK1/2, and p-EGFR were obtained from Thermo Fisher Scientific (USA). Anti-rabbit HRP linked IgGs and anti-mouse HRP linked IgGs were obtained from Santa Cruz Biotechnology, USA. ADAM-17 ELISA kit was obtained from Lifespan biosciences inc, WA, USA. Superoxide dismutase (SOD) and Thiobarbituric acid reactive substances (TBARS) assay kits were obtained from Bio diagnostics, Egypt.

2.3. Experimental Design

2.3.1. Experiment I

To test the effect of omeprazole on EGFR activation and subsequent ERK 1/2 activation, the animals (6 rats per group) were received a single dose of omeprazole10mg/kg body weight (i.p.)[6](for 1, 2, 4, 6,8 and 24hours). A group of animals received the vehicle (saline) of omeprazole (i.p.) and used as control. At the end of experiment, the rats were sacrificed using cervical dislocation. Immediately after death, the kidney was dissected rinsed with phosphate buffered saline (ice cold) and stored at -20ºC for determination of phosphorylated ERK1/2.

2.3.2. Experiment II

In this experiment, we examined the role of EGFR in ERK1/2 signaling cascade. The rats were divided randomly into four groups (6 rats per group). The first group was given the vehicle of gefitinib (i.p.) and served as control. The second group was administered omeprazole (10mg/kg body weight i.p.). The third group received the EGFR inhibitor, gefitinib (80mg/kg body weight i.p.)[12] one hour before omeprazole administration. The last group was administered the EGFR inhibitor, gefitinib (as previously mentioned). At the end of experiment (4 hours after injection, the rats were sacrificed using cervical dislocation. Immediately after death, the kidney was dissected, rinsed with phosphate buffered saline (ice cold) and stored at -20ºC for determination of p-ERK1/2 and p-EGFR and ADAM 17.

2.3.3. Experiment III

Here we examined first whether ERK1/2phosphorylation induced by omeprazole could be translated to an increase in the expression of cells proliferation genes. Second, the role of EGFR in ERK1/2 signaling cascade and subsequent expression of the cell's proliferation genes induced by omeprazole. Third, the potential modulatory effect of omeprazole on SOD activity and the byproduct of lipid peroxidation malondialdehyde (MDA). The animals were administered either vehicle (control) or omeprazole or the EGFR inhibitor gefitinib or omeprazole in combination with gefitinib for 24h. At the end of experiment, the animals were sacrificed using cervical dislocation. Immediately after death, the kidney was dissected, rinsed with phosphate buffered saline (ice cold) and stored at -20ºC for the analysis of cyclin B1 expression, SOD activity and MDA content.

2.4. Western blot analysis

Phosphorylated ERK1/2, total ERK1/2, p-EGFR, total EGFR, Cyclin B1 and β-actin were detected using Western blotting as previously described.[13]Briefly, after blotting, blocking in 5% bovine serum albumin (BSA) in Trisbuffered saline containing 0.05% Tween (TBST) was done. Afterwards, the membrane was incubated with the primary antibody (1:1000) diluted in 1x TBST-buffer overnight at 4 °C and washed 4 times

for 10 min each with 1× TBST-buffer. Then, the membrane was incubated for 30 minutes with the secondary antibody diluted in 1x TBST-buffer (1:10000), before signal detection using enhanced chemiluminescence (ECL) system

2.5. Assessment of ADAM-17 level

The renal level of ADAM-17 was measured using ELIZA kits (raised against rat ADAM-17) in accordance with the manufacturer's instructions (Lifespan biosciences inc, WA, USA).

2.6. Assessment of SOD Activity

The activity of SOD in kidney tissue was determined as previously described.[14]

2.7. Assessment of lipid peroxides

Malondialdehyde (MDA) content in kidney tissues was measured in accordance with the manufacturer's instructions (Biodiagnostics, Egypt).

2.8. Statistical Analysis

Data are expressed as means ± SD. Multiple comparisons were performed using one-way ANOVA followed by Tukey-Kramer as a post-hoc test. *P*-values less than 0.05 were regarded as indication for statistically significant differences between groups compared.

3. RESULTS

3.1. Omeprazole induces ERK1/2 phosphorylation in rat kidney

Here, we tested the effect of omeprazole on ERK1/2 phosphorylation. It was found that omeprazole administration augments ERK1/2 phosphorylation with maximal effect seen after hours which declined to the normal levels after 24 hours (Figure 1).

3.2. Omeprazole administration induces metalloproteinase activity

Here, we tested the effect of omeprazole on metalloproteinase activity. As demonstrated in Figure 2, omeprazole administration significantly activates ADAM-17 in renal tissue after 4 hours.

3.3. ERK1/2 activation induced by omeprazole is mediated via the EGFR

Omeprazole administration significantly induced phosphorylation of EGFR (Figure 3). On the other hand, the pharmacological inhibitor of EGFR, gefitinib caused a clear reduction of EGFR phosphorylation induced by omeprazole in rat kidney (Figure 3A). In addition, omeprazole induced ERK phosphorylation was significantly reduced in the presence of the EGFR inhibitor, gefitinib (Figure 3B).

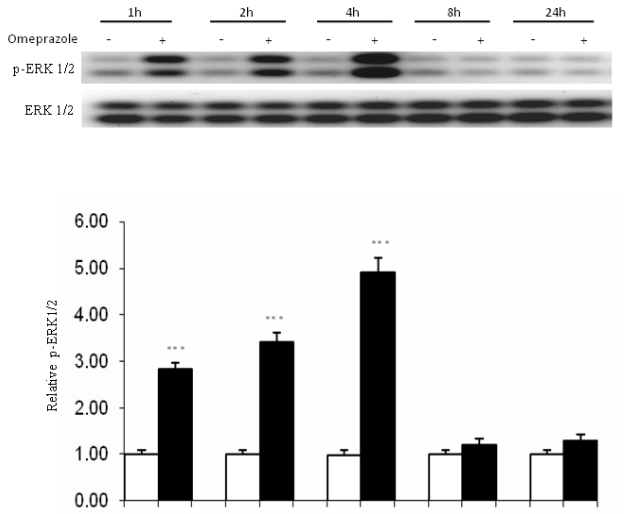

Figure (1): Omeprazole administration causes a rapid phosphorylation of ERK1/2 in rat kidney
A. Total kidney extracts from rats treated with either vehicle (-) or omeprazole (10 mg/kg body weight i.p.) for the indicated time points were subjected to Western blot analysis and probed with anti-p-ERK1/2 and total ERK1/2 antibodies. The lower panel shows a densitometric analysis of p-ERK1/2 relative to the total ERK1/2 level. Data represent means ± S.D. (n=6), * $p < 0.05$, *** $p < 0.001$ versus control.

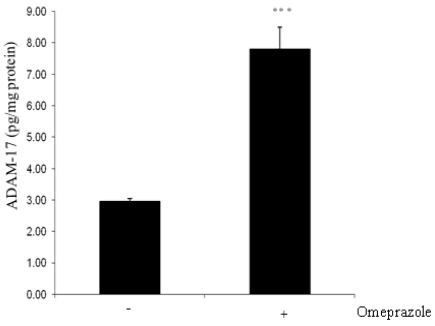

Figure (2): Omeprazole administration induces metalloproteinase activity in rat kidney ADAM-17 activity in kidney tissues from rats treated with either vehicle (-) or omeprazole (10 mg/kg body weight i.p.) was determined by ELISA. Data represent means ± S.D. (n=6), *** $p < 0.001$ versus control.

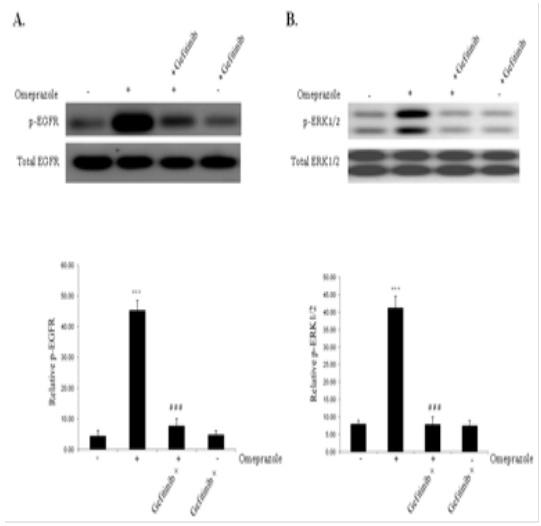

Figure (3): EGFR is critical for ERK1/2 activation induced by omeprazole in rat kidney
A. Total kidney extracts from rats treated with either vehicle (-) or omeprazole (10 mg/kg body weight i.p.) or the pharmacological inhibitor of EGFR gefitinib (80mg/kg body weight i.p.) or omeprazole in combination with the pharmacological inhibitor of EGFR gefitinib were subjected to Western blot analysis and probed with anti-p-EGFR and total EGFR antibodies. The lower panel shows a densitometric analysis of p-EGFR relative to the total EGFR level. Data represent means ± S.D. (n=6), *** $p < 0.001$ versus control, ### $p < 0.001$ versus omeprazole alone-treated animals. **B.** Total kidney extracts from rats treated with either vehicle (-) or omeprazole (10 mg/kg body weight i.p.) or the pharmacological inhibitor of EGFR gefitinib (80mg/kg body weight i.p.) or omeprazole in combination with the pharmacological inhibitor of EGFR gefitinib were subjected to Western blot analysis and probed with anti-p-ERK1/2 and total ERK1/2 antibodies. The lower panel shows a densitometric analysis of p-ERK1/2 relative to the total ERK1/2 level. Data represent means ± S.D. (n=6), *** $p < 0.001$ versus control, ### $p < 0.001$ versus omeprazole alone-treated animals.

3.4. Omeprazole induces oxidative stress in rat kidney

To test the effect of omeprazole on lipid peroxidation and SOD activity, rats were administered omeprazole. As shown in Fig 4, the renal MDA content was highly increased in rats given omeprazole compared with control animals (Fig 4A). Furthermore, SOD activity was highly decreased in animals given omeprazole compared with control animals (Fig 4B).

3.5. Cells proliferation genes induced by omeprazole depends on EGFR tyrosine kinase activity

To examine whether ERK1/2 phosphorylation induced by omeprazole could be translated to an increase in the expression of the cell's proliferation gene cyclin B1, rats were administered either omeprazole or omeprazole in combination with the EGFR inhibitor, gefitinib. As shown in Fig 5, omeprazole can induce the expression of cyclin B1. However, this expression of cyclin B1 was highly attenuated in the presence of the EGFR inhibitor, gefitinib.

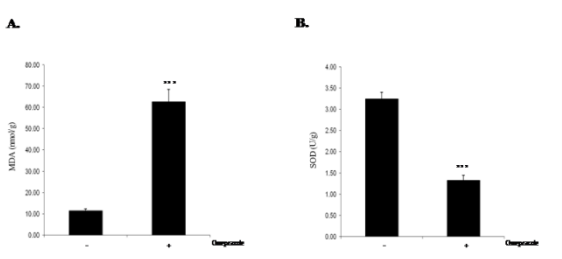

Figure (4): Omeprazole induces oxidative stress in kidney tissues
A. MDA content in kidney tissues from rats treated with either vehicle (-) or omeprazole (40 mg/kg body weight i.p.) was determined using a TBARS assay kit. Data represent means ± S.D. (n=6), *** $p < 0.001$ versus control. **B.** SOD activity in kidney tissues from rats treated with either vehicle (-) or omeprazole (40 mg/kg body weight i.p.) was determined by ELISA. Data represent means ± S.D. (n=6), *** $p < 0.001$ versus control.

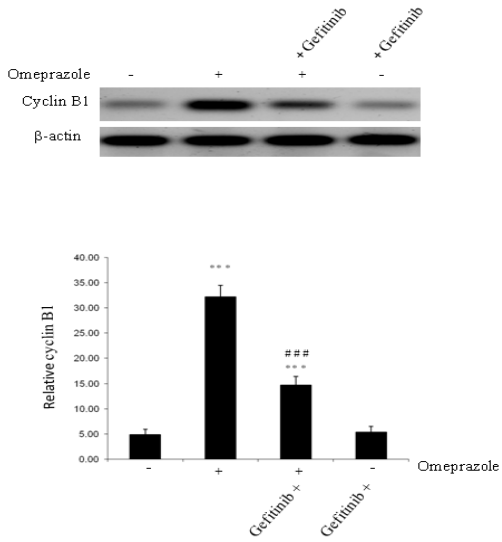

Figure (5): EGFR is critical for cyclin B1 expression in rat kidney
Total kidney extracts from rats treated with either vehicle (-) or omeprazole (10 mg/kg body weight i.p.) or the pharmacological inhibitor of EGFR gefitinib (80mg/kg body weight i.p.) or omeprazole in combination with the pharmacological inhibitor of EGFR gefitinib were subjected to Western blot analysis and probed with anti-cyclin B1 and β-actin antibodies. The lower panel shows a densitometric analysis of cyclin B1 relative to β-actin level. Data represent means ± S.D. (n=6), *** $p <$

0.001 versus control, ### $p < 0.001$ versus omeprazole alone-treated animals.

4. DISCUSSION

Omeprazole is the most prescribed PPIs. However, the use of omeprazole may be associated with serious unwanted effects, including acute kidney injury (AKI) and chronic kidney disease (CKD)[1]. It has been reported that administration of PPIs increases the risk of kidney injury by 20-50%.In addition, it has been demonstrated that the use of PPIs may be responsible for several cases of acute renal failure.[19] Furthermore, the relationship between AKI and the use of PPIs has been reported[15]. Moreover, the relationship between PPIs and CKD has been shown in several studies.[1-3] However the mechanism by which, PPIs may cause CKD is still unclear. Here we demonstrate for the first time that omeprazole administration induces ERK1/2 phosphorylation in rat kidney. An effect which may be attributed to generation of ROS by omeprazole. In line with previous findings demonstrated that ROS activates ADAM-17 and phosphorylate EGFR,[8,16]the renal ADAM-17 level was highly increased in rats treated with omeprazole. This effect may be based on generation of ROS as indicated by an elevation in lipid peroxidation and reduction of SOD activity in animals treated with omeprazole. Moreover, the functional implication of EGFR in ERK1/2 activation induced by omeprazole was investigated using the EGFR inhibitor, gefitinib. It was found that administration of gefitinib along with omeprazole significantly decreased p-ERK1/2 caused by omeprazole. In line with our results, Akool and his colleagues showed a clear reduction of ERK1/2 phosphorylation in renal mesangial cells upon treatment with the EGFR tyrosine kinase inhibitor, AG1478 before treatment with cyclosporin A[8].The Ras-Raf-MEK-ERK1/2 signaling pathway plays an important role in cell cycle regulation.[17] Activation of MAPKs results in G2/M arrest with subsequent induction of cyclin B1.[18] In the present work, it was found that omeprazole administration significantly induced cyclin B1 expression. Interestingly this increase in cyclin B1 expression was highly reduced in animals treated with the EGFR tyrosine kinase inhibitor gefitinib along with omeprazole indicating that EGFR activation and subsequent Ras-Raf-MEK-ERK1/2activation is involved in cyclin B1 expression in kidney tissues. This agree with previous study showed an increase in cyclin B1 expression following ERK1/2 activation in synchronized Hela cells which is reversed when the cells pretreated with UO126 (a selective inhibitor of the ERK1/2 activator, MEK1/2).[19]Our findings show that omeprazole has the ability to activate ERK1/2 signaling cascade that contribute to renal fibrosis.

5. CONCLUSION

Our findings demonstrate for the first time that administration of omeprazole activates ERK1/2 signaling cascade and subsequent expression of the cell proliferation gene cyclin B1 in rat kidney (Figure 6). Furthermore, our study presents explanatory mechanism of this signaling pathway involving ROS as determined by an increase in lipid peroxidation and a clear reduction in SOD activity by omeprazole. Finally, this activation of mitogenic cell responses by omeprazole in rat kidney emphasizes the unwanted effects triggered by these widely used PPIs.

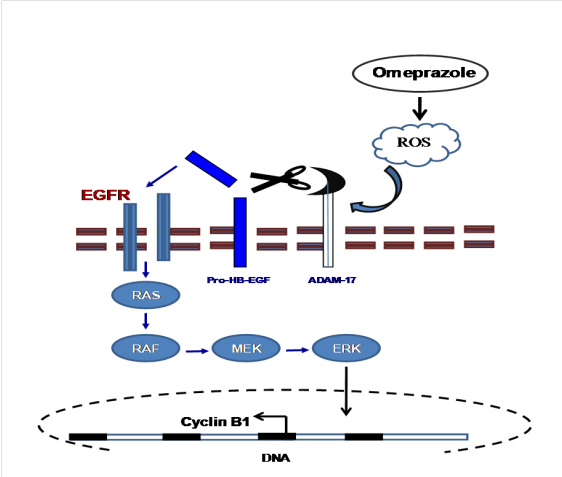

Figure (6): Schematic summary of the modulatory effect of omeprazole on ERK1/2 signaling pathway in rat kidney. Omeprazole induces oxidative stress which in turn activates ADAM-17 resulting in EGFR phosphrylation which in turn activates ERK1/2 signaling pathway and subsequent expression of cyclin B1 in rat kidney.

Conflicts of Interest: Regarding the publication of this article there is no conflict of interest.

Ethical Statement: The protocol used in this study was approved by the Al-Azhar Faculty of Pharmacy (Girls) animal ethics committee (no 80, 2016).

Author contribution

This work was carried out in collaboration between all authors. AA. and AB designed the study. AA performed the experiments. AB wrote the manuscript; NAA, AB, and AA analyzed data and revised the manuscript.

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