Tropical Journal of Pharmaceutical Research December 2021; 20 (12): 2527-2532
ISSN: 1596-5996 (print); 1596-9827 (electronic)

Available online at http://www.tjpr.org
http://dx.doi.org/10.4314/tjpr.v20i12.10

Original Research Article

Effect of omeprazole on gastric mucosal damage and PI3K-Akt signaling pathway in infant mice infected with *Helicobacter pylori*

Xiaoyan Fan[1], Si Zhang[1], Dan Yin[1], Ali Pang[2]*
[1]Department of Pediatric Gastroenterology, Jiujiang Maternal and Child Health Hospital, Jiujiang 332000, Jiangxi Province, China, [2]Department of Student Management, Jiujiang University, Jiujiang 332000, Jiangxi Province, China

For correspondence:* ***Email:* *m1303p@163.com*

Sent for review: 30 August 2021 *Revised accepted: 29 November 2021*

Abstract

Purpose: *To determine the impact of omeprazole on gastric mucosa injury in young mice with Helicobacter pylori (Hp) infection, and underlying mechanism.*
Methods: *The young mice were assigned to control, model and drug groups. The control group was not treated, while model and drug groups were mice with Hp infection. Mice in the model group were given omeprazole. The mRNA and protein expression levels of PIEN, PI3K, AKT and P-Akt were assayed with real-time PCR and Western blotting, respectively.*
Results: *The mRNA levels of PI3K, AKT and P-Akt were significantly increased in model mice, relative to controls, while PIEN mRNA levels were lower ($p < 0.05$). The mRNA and protein expressions of PI3K, AKT and P-Akt were significantly down-regulated in drug group, relative to model mice ($F = 131.750$, $p < 0.05$; $F = 268.440$, $p < 0.05$; $F = 91.560$, $p < 0.05$).*
Conclusion: *Omeprazole reduces inflammatory response, improves oxidative stress response, and alleviates gastric mucosal damage in young mice infected with Helicobacter pylori, probably through a mechanism related to the regulation of mRNA and protein expressions in PI3K-Akt signal route. This finding may be useful in the development of new drugs for protection against gastric mucosal lesion.*

Keywords: *Omeprazole, Helicobacter pylori infection, Gastric mucosal injury, PI3K-Akt signaling pathway*

Tropical Journal of Pharmaceutical Research is indexed by Science Citation Index (SciSearch), Scopus, International Pharmaceutical Abstract, Chemical Abstracts, Embase, Index Copernicus, EBSCO, African Index Medicus, JournalSeek, Journal Citation Reports/Science Edition, Directory of Open Access Journals (DOAJ), African Journal Online, Bioline International, Open-J-Gate and Pharmacy Abstracts

INTRODUCTION

Helicobacter pylori (Hp) is a gram-negative, aerobic microorganism which changes the physiology of its host and destroys host immune response [1]. *Helicobacter pylori* (Hp) not only leads to chronic gastritis and peptic ulcer, but is associated with the pathogenesis of gastric adenocarcinoma, gastric mucosa-associated lymphoma, and other diseases [2]. Chronic gastritis (CG) is a disease that seriously compromises the digestive system and adversely affects human health [3]. Prilosec is a proton pump inhibitor that inhibits gastric acid secretion, alleviates gastric mucosal damage and protects the gastric mucosal barrier. It is often used in clinical treatment of gastric ulcer and duodenal ulcer, but the mechanism involved in its effects

remains poorly understood [4]. The present research on 60 Balb/C pups was carried out to study the influence of omeprazole on *Helicobacter pylori*-mediated damage in gastric mucosa, as well as the underlying mechanism.

EXPERIMENTAL

Materials

Sixty (60) Balb/C baby mice aged 6-8 weeks, and weighing 17-20 g, were employed in this investigation, and were kept at temperature range of 19-26℃and humidity of 40 - 70 % under a 12-h light/12-h dark cycle. The young mice received laboratory diet and drinking water *ad libitum*. The animal experiments were carried out in keeping with regulations on the use and care of experimental animals. Omeprazole was provided by Zhejiang Pharmaceutical Co. Ltd, Xinchang Pharmaceutical Factory (National drug approval number: H20030309; specification: 20 mg).

Cell culture

Helicobacter pylori (Hp) was cultured in an incubator at 37 ℃. The monoclonal pin-like transparent colonies were inoculated in a new petri dish and cultured for 3 days, and transferred to Brinolet broth. The absorbance of the bacterial broth was measured at a wavelength of 660 nm. A bacterial suspension of concentration of 1×10^9 CFU/mL was prepared.

Animals and treatments

Sixty Balb/C mice were allotted to control, model and drug groups. The control group was not treated, while model and drug groups were model mice with Hp infection. The Hp suspension was administered intragastrically for 14 days, and the model group was given omeprazole (10 mg/kg) *via* intragastric administration for 1 week, once a day after the successful establishment of the Hp mouse model. This research was approved by the Animal Ethical Committee of Jiujiang University (approval no. 20200891), and performed according to the guidelines of "Principles of Laboratory Animal Care" (NIH publication no. 85-23, revised 1985) [5].

Evaluation of test indices

Morphology of gastric tissues

The gastric antrum tissues of the young mice were excised and cut into 0.5 cm × 0.2 cm sections which were subjected to fixation in 4 % paraformaldehyde for 24 h, dehydration in increasing alcohol concentrations, clearing and embedding in wax. Slices of 5-μm thickness were cut using a microtome. The slices were dried in an incubator at 38 ℃, and stained with H & E, followed by microscopic observation.

Degree of inflammation in gastric mucosa

The degree of inflammation was graded according to the extent of infiltration of chronic inflammatory cells such as lymphocytes, plasma cells and eosinophils, based on the new Sydney system standard. In this grading system, gastric mucosa without inflammatory cell infiltration is grade 0, while the presence of a small amount of scattered inflammatory cell infiltration on the surface or bottom of gastric mucosa is grade 1. Grade 2 is for increased infiltration of inflammatory cells in all parts of the gastric mucosa, while the presence of a high population of inflammatory cells is grade 3.

Oxidative stress

The gastric tissue homogenates were prepared using a glass homogenizer with 400 μL of normal saline, and the homogenate was centrifuged at 2500 rpm for 10 min. The ROS levels in the supernatant were measured using DCFH-DA method, while MDA and GSH concentrations were estimated with TBA method and Beutler modified method, respectively.

Measurement of inflammatory response

Blood was taken from the eyeball of each mouse and left at room temperature for 20 min, followed by centrifugation at 3000 rpm for 10 min, and the resultant serum was refrigerated at -20℃ to avoid repeated freezing and thawing. The serum levels of IL-1 β, IL-18 and IL-33 were assayed with ELISA.

Expressions of protein levels

The mRNA levels of PIEN, PI3K, AKT and P-Akt were determined using real-time PCR. Total RNA was extracted from gastric tissues using TRIzol method. The RNA was reverse-transcribed to cDNA and amplified, followed by real-time fluorescence quantitative PCR. The circulation threshold (CT value) of each gene was calculated to determine its expression level.

Western blotting

The relative protein amounts of p-AKT, PIEN, PI3K, and AKT were determined using Western blotting assay. Gastric tissue total protein was

extracted, and protein concentration was determined using the BCA method. The protein concentration was adjusted to 30 μg/μL. The proteins were resolved using 10 % SDS-PAGE, followed by electro-transfer to PVDF membranes which were subjected to incubation overnight at 4 °C with 1° primary antibodies, followed by incubation with 2° antibody at room temperature. The blots were subjected to ECL and imaged. Gray scanning was carried out with Quantity-One software.

Statistical analysis

The SPSS20.0 software was employed for statistics. Measured data that conformed to normal distribution are expressed as mean ± SD. Multiple groups were compared with 1-way ANOVA, while SNK-q test was used for paired comparison. Counting data are presented as %, and comparison amongst groups was done with χ^2 test. Statistical significance of difference was assumed at $p < 0.05$.

RESULTS

Morphology of gastric mucosa

The morphology of each layer of gastric mucosa in control mouse was intact, and the epithelial cells and glands were of consistent size and shape, and orderly arranged. Inflammatory cells in the mucosa lamina propria were rare. In the model group, gastric mucosa was thinner, epithelial cells and glands were disordered, and the number of glands was significantly reduced. Mucosal muscular layer was thickened and extended to lamina propria, showing a large amount of inflammatory infiltration. In the treatment group, the structure of each layer of gastric mucosa was basically intact, the glands were arranged orderly, and the interstitial inflammatory cells were lower in population. These results are shown in Figure 1.

Graded degree of chronic inflammation of gastric mucosa

The degree of inflammation was significantly higher in model mice than in control mice, but the degree of inflammation in drug-treated mice was markedly decreased, relative to model mice. These results are shown in Table 1.

Serum inflammatory cytokine levels

As shown in Table 2, the levels of IL-1β, IL-18 and IL-33 in the model group were significantly higher than those in the control group, and the levels of IL-1β, IL-18 and IL-33 in the treatment group were significantly lower than those in the model group ($p < 0.05$).

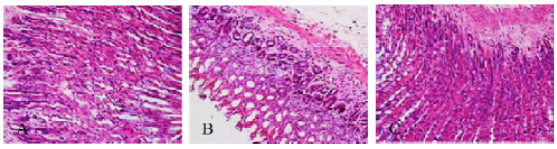

Figure 1: Gastric mucosa morphology in each group. H&E staining image of gastric mucosa of young rats in control group (A), model group (B), and treatment group (C)

Table 1: Degree of chronic inflammation in gastric mucosa in each group (n, %)

Group	0	Grade 1	Grade 2	Grade 3
Control	20 (100)	0 (0)	0 (0)	0 (0)
Model	0 (0)	0 (0)	14 (70)	6 (30)
Drug	2 (10)	5 (25)	12 (60)	1 (5)
χ^2	65.178			
P-value	＜0.001			

Serum oxidative stress response

Table 3 shows that the ROS and MDA levels of the pups were markedly higher in model mice than in control mice, while GSH level was markedly decreased, relative to control mice. However, there were lower ROS and MDA concentrations in the pups in drug-treated group than in model pups, while GSH level was markedly higher than values in model pups ($p < 0.05$).

MRNA levels of genes associated with PI3K-Akt signal route

The mRNA expression levels of PI3K, AKT and P-Akt were markedly up-regulated in model mice, relative to control mice, while mRNA level of PIEN was markedly lower than the control value. However, mRNA expressions of these parameters (except for PIEN mRNA) were markedly down-regulated in the treatment group, when compared to model mice (Table 4).

Protein levels of factors associated with PI3K-Akt signal route

As shown in Figure 2, the protein levels of PI3K, AKT and P-Akt were markedly up-regulated in model mice, relative to controls, while protein level of PIEN was markedly lower. In contrast, the protein levels of these factors (except for PIEN) were markedly down-regulated in the treatment group, relative to model mice, while the protein level of PIEN was markedly up-regulated, relative to model mice.

Table 2: Levels of serum inflammatory cytokines in each group (mean ± SD)

Group	IL-1β (pg/ml)	IL-18 (pg/ml)	IL-33 (pg/ml)
Control	10.26±2.53	56.59±13.58	42.38±10.58
Model	36.52±5.64[a]	136.52±20.54[a]	125.64±20.16[a]
Drug	20.19±4.85[ab]	76.85±14.38[ab]	80.23±16.52[ab]
F	170.870	127.410	131.770
P	＜0.001	＜0.001	＜0.001

[a]*P* < 0.05, vs control; [b]*p* < 0.05, vs the model mice

Table 3: Serum oxidative stress responses of young mice in each group (mean ± SD)

Group	ROS (mg/L)	MDA (nmol/g)	GSH (μmol/kg)
Control	2.71±0.16	0.15±0.03	3.26±0.19
Model	3.58±0.21[a]	0.38±0.05[a]	2.06±0.15[a]
Drug	3.06±0.21[ab]	0.24±0.03[ab]	2.71±0.23[ab]
F	101.040	187.440	194.170
P-value	＜0.001	＜0.001	＜0.001

[a]*P* < 0.05, vs control pups; [b]*p* < 0.05, vs model pups

Table 4: mRNA expression levels of PI3K-Akt signaling pathway in each group

Group	PIEN	PI3K	AKT	p-AKT
Control	1.62±0.52	0.48±0.06	0.41±0.06	0.59±0.12
Model	0.36±0.05[a]	1.25±0.24[a]	1.25±0.12[a]	1.35±0.26[a]
Drug	1.03±0.16[ab]	0.89±0.08[ab]	0.95±0.15[ab]	0.88±0.12[ab]
F	79.890	131.750	268.440	91.560
P	＜0.001	＜0.001	＜0.001	＜0.001

[a]*P* < 0.05, vs control; [b]*p* < 0.05, vs model

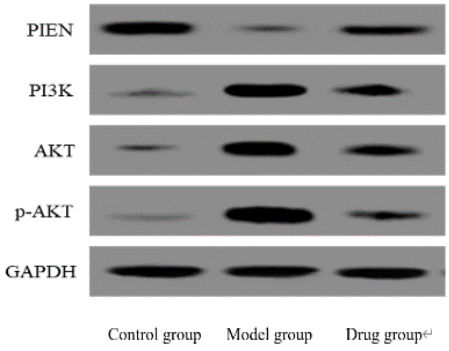

Figure 2: Relative protein expressions of PI3K-Akt signal route-associated factors in each group

DISCUSSION

Heliobacteria pylori (Hp) infection can lead to degeneration of the gastric mucosa epithelium, inflammatory cell infiltration, intestinal metaplasia, gland atrophy and atypical hyperplasia. This infection is an important factor that influences the pathogenesis of chronic gastritis, peptic ulcer and gastric mucosa-associated lymphoid tissue lymphoma, and is closely related to the occurrence of gastric cancer. The World health Organization (WHO) has classified Hp as a class I carcinogenic factor [6,7]. Malfertheiner *et al* showed that patients with Hp infection complicated with chronic gastritis had a significantly increased risk of gastric cancer [8]. Therefore, studies on the mechanism of pathogenicity of Hp infection, and the search for drugs to treat Hp infection complicated with gastritis have attracted a lot of attention from researchers.

Omeprazole is a fat-soluble weak base sub-pump inhibitor that is easily concentrated in acidic environment. It inhibits the activities of H^+ - K^+-ATPase and suppresses the secretion of gastric acid, thereby reducing the acid content of gastric juice. It is often employed for treating gastric ulcer, duodenal ulcer and sundry stomach ailments [9-11]. In this study, the gastric mucosa of the model group was thin, the epithelial cells and glands were disorganized, and the mucous muscle layer was thickened, with a lot of inflammatory infiltration. However, in the treatment group, the structure of each layer of gastric mucosa was basically intact, and there were fewer interstitial inflammatory cells. These results suggest that omeprazole mitigated gastric mucosal damage and protected gastric mucosal barrier in Hp-infected pups.

Earlier investigations have demonstrated that induction of IL-1β production in mouse stomach produced spontaneous gastritis and gastric cancer. It has been shown that Hp infection and chronic gastritis led to abnormal expression level of IL-18 [12]. A study by TuHongfei *et al* demonstrated that serum levels of IL-33 in peptic

ulcer subjects were highly expressed [13]. In this study, the degree of inflammation was markedly higher in model mice than in control mice, while degree of inflammation was markedly lower in omeprazole-treated mice than in model mice. There were markedly higher expressions of IL-1β, IL-18 and IL-33 in model mice than in control mice, but these were markedly decreased in the drug-treated mice, relative to model mice. Thus, omeprazole reduced the inflammatory response and decreased the damage to gastric mucosa caused by inflammatory response in Hp-infected young mice.

It is known that ROS are strong oxidants. High levels of ROS damage cells through lipid peroxidation and aggravate tissue damage, leading to a variety of diseases. Qin Jin *et al* showed that Hp infection led to increased intracellular ROS contents [14]. Reduced glutathione (GSH) is an important physiological antioxidant. During oxidative stress, GSH plays an antioxidant role as a free radical scavenger. Malondialdehyde (MDA) is typical index of lipid peroxidation: changes in MDA level are indicative of the degree of oxidative damage caused by ROS in cells [15]. In this study, ROS and MDA levels were markedly higher in pups in model group than in control pups, while GSH was markedly decreased, relative to controls. However, ROS and MDA levels of pups in drug group were markedly decreased, relative to model mice, but GSH concentration was markedly higher. Thus, omeprazole mitigated oxidative stress response, protected normal cell function, and inhibited gastritis in Hp-infected young mice.

Signal through the PI3K/Akt route is crucial for intracellular conduction of membrane receptor signals: it controls cell viability, apoptosis, metastasis and other cellular processes [16]. Recent studies have found that Hp infection can activate PI3K/Akt signaling pathway [17]. Sun Yuesheng *et al* found that gastric cancer due to Hp infection is relatively severe, and is related to Hp-induced up-regulation of PI3K/AKT signal route and its influence on the expression of apoptosis and invasion genes related to cancer cells [18]. In this study, there were markedly up-regulated mRNA levels of genes associated with PI3K/AKT route in model mice, while PIEN mRNA was markedly down-regulated, relative to controls. However, mRNA and protein expressions of P13K/AKT-associated factors were markedly down-regulated, relative to model mice, while levels of PIEN mRNA and protein were markedly up-regulated. Thus, omeprazole-mediated mitigation of gastric mucosa injury in Hp-infected young mice may be related to the regulation of mRNA and protein expressions of genes associated with the PI3K-Akt signaling pathway.

CONCLUSION

Omeprazole reduces inflammatory response, decreases oxidative stress response, and alleviates gastric mucosal damage in infant mice infected with Hp via a mechanism related to the regulation of mRNA and protein expressions of PI3K-Akt signal route-associated genes. This finding may be useful in the development of new drugs aimed at protection against gastric mucosal injury.

DECLARATIONS

Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

We declare that this work was performed by the authors named in this article, and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. Xiaoyan Fan and Ali Pang designed the study, supervised the data collection, and analyzed the data. Si Zhang and Dan Yin interpreted the data and prepared the manuscript for publication. Ali Pang supervised the data collection, analyzed the data, and reviewed the draft of the manuscript.

Open Access

REFERENCES

1. *Santos MLC, de Brito BB, da Silva FAF, Sampaio MM, Marques HS, Oliveira E Silva N, de Magalhães Queiroz DM, de Melo FF. Helicobacter pylori infection: Beyond gastric manifestations. World J Gastroenterol 2020; 26(28): 4076-4093.*
2. *Ford AC, Yuan Y, Forman D, Hunt R, Moayyedi P. Helicobacter pylori eradication for the prevention of*

gastric neoplasia. Cochrane Database Syst Rev 2020; 7(7): CD005583.

3. *Wang L, Chen W, Lin X, Zhang Z, Wang N, Lv Y, Wang X, Gao Y. Protective Effect of Ultraviolet C Irradiation on the Gastric Mucosa of Rats with Chronic Gastritis Induced by Physicochemical Stimulations. Evid Based Complement Alternat Med 2021; 2021: 5189797.*
4. *Mahawish KM, Leung A. Failure of dabigatran following idarucizumab and omeprazole administration. Intern Med J 2021; 51(1): 134-135.*
5. *World Health Organization. Principles of laboratory animal care. WHO Chron 1985; 39: 51-56.*
6. *Sheikh AF, Yadyad MJ, Goodarzi H, Hashemi SJ, Aslani S, Assarzadegan MA, Ranjbar R. CagA and vacA allelic combination of Helicobacter pylori in gastroduodenal disorders. Microb Pathog 2018; 122: 144-150.*
7. *Teng G, Dai Y, Chu Y, Li J, Zhang H, Wu T, Shuai X, Wang W. Helicobacter pylori induces caudal-type homeobox protein 2 and cyclooxygenase 2 expression by modulating microRNAs in esophageal epithelial cells. Cancer Sci 2018; 109(2): 297-307.*
8. *Malfertheiner P. Helicobacter pylori Treatment for Gastric Cancer Prevention. N Engl J Med 2018; 378(12): 1154-1156.*
9. *Anagnostopoulos GK, Tsiakos S, Margantinis G, Kostopoulos P, Arvanitidis D. Esomeprazole versus omeprazole for the eradication of Helicobacter pylori infection: results of a randomized controlled study. J Clin Gastroenterol 2004; 38(6): 503-6.*
10. *Abed MN, Alassaf FA, Jasim MHM, Alfahad M, Qazzaz ME. Comparison of Antioxidant Effects of the Proton Pump-Inhibiting Drugs Omeprazole, Esomeprazole, Lansoprazole, Pantoprazole, and Rabeprazole. Pharmacology 2020; 105(11-12): 645-651.*
11. *Girod M, Allerton F, Vangrinsven E, Tutunaru AC, de Marchin J, Gómez-Fernández-Blanco C, Ruiz-Nuño A, Wojnicz A, Farnir F, Gommeren K, et al. CSF omeprazole concentration and albumin quotient following high dose intravenous omeprazole in dogs. Res Vet Sci 2019; 125: 266-271.*
12. *Muhammed ZU, Bunyamin G, Sukru C. Evaluation of gastric inflammatory parameters caused by pyloric ring failure. Acta Med Mediterr 2019; 35: 1481-1488.*
13. *Catalan-Dibene J, McIntyre LL, Zlotnik A. Interleukin 30 to Interleukin 40. J Interferon Cytokine Res 2018; 38(10): 423-439.*
14. *Wong JH, Wang YS, Nam S, Ho KH, Chang CM, Chen KC, Chen YF, Chang WC. Phthalate plasticizer di(2-ethyl-hexyl) phthalate induces cyclooxygenase-2 expression in gastric adenocarcinoma cells. Environ Toxicol 2019; 34(11): 1191-1198.*
15. *Iwasaki M, Manz MC, Taylor GW, Yoshihara A, Miyazaki H. Relations of serum ascorbic acid and α-tocopherol to periodontal disease. J Dent Res 2012; 91(2): 167-72.*
16. *Jianyong W, Hongliang S, Xinxin Y, Xiaoli W, Li W, Jianying L, Aimin L. Am80 increases chemosensitivity to doxorubicin in a human neuroblastoma sk-n-be2 cells via downregulation of mrp1 expression by targeting mycn gene. Acta Med Mediterr 2019; 35: 2685-2690.*
17. *Pistritto G, Trisciuoglio D, Ceci C, Garufi A, D'Orazi G. Apoptosis as anticancer mechanism: function and dysfunction of its modulators and targeted therapeutic strategies. Aging (Albany NY) 2016; 8(4): 603-619.*
18. *Matsuo Y, Kido Y, Yamaoka Y. Helicobacter pylori Outer Membrane Protein-Related Pathogenesis. Toxins (Basel) 2017; 9(3): 80-101.*