J Korean Soc Appl Biol Chem (2015) 58(2):249–256
DOI 10.1007/s13765-015-0021-2

Online ISSN 2234-344X
Print ISSN 1738-2203

ARTICLE

Protective effect *Malus pumila* Mill leaf polyphenols in reserpine-induced gastric ulcer in mice

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Received: 17 October 2014 / Accepted: 20 December 2014 / Published online: 4 February 2015

Abstract The protective effect of polyphenols from *Malus pumila* leaf (MALP) on reserpine (10 mg/kg)-induced gastric ulcer in Kunming (KM) mice was investigated. Gastric juice secretion, pH of gastric juice, serum neuropeptides, including motilin (MOT), substance P (SP), vasoactive intestinal peptide (VIP), and somatostatin (SS) levels, as well as serum levels of inflammatory cytokines (TNF-α, IL-6, IL-12, and IFN-γ) were measured. Gastric occludin and p38MAPK phosphorylation was analyzed by RT-PCR or Western blotting. MALP reduced reserpine-induced gastric juice secretion and increased the pH of gastric juice. MALP increased the serum levels of VIP and SS and reduced MOT, SP, and inflammatory cytokines in serum. It also modulated the gastric expression of occludin and p38MAPK phosphorylation in mice. These results suggest that MALP showed a protective effect against reserpine-induced ulcer in mice by reducing gastric juice secretion, modulating serum neuropeptide levels, attenuating serum inflammatory cytokines, and regulating gastric levels of occludin and p38MAPK phosphorylation.

Keywords *Malus pumila* leaf · Polyphenols · Neuropeptides · Occludin · P38MAPK

Introduction

Chronic peptic ulcer disease (PUD), which is divided into gastric ulcer (GU) and duodenal ulcer (DU), is closely associated with gastric cancer (de Martel et al. 2013). The development of PUD has been associated with an imbalance between offensive (acid, pepsin, and *Helicobacter pylori* infection) and defensive factors (mucin, prostaglandin, bicarbonate, nitric oxide, and growth factors) (Malfertheiner et al. 2009). In addition, unhealthy life-style factors (such as alcohol, smoking, and stress), and long-term intake of drugs such as non-steroidal anti-inflammatory drugs (NSAIDs) and reserpine have been also widely accepted as an important cause in the pathogenesis of PUD (Phillipson et al. 2002; Huang et al. 2002). Especially, long-term use of reserpine as a classic antihypertensive drug used to control high blood pressure could induce the excessive secretion of gastric acids through increasing cholinergic activity which then causes gastric ulcers (Sandor et al. 1996).

Neuropeptides play an important role in the regulation of gastric functions such as gastric juice secretion, motility, and contractions (Gyires 2004). Vasoactive intestinal peptide (VIP), substance P (SP), somatostatin (SS), and motilin (MOT) are widely distributed within the gastrointestinal tract and play an important role in the immunomodulation of the intestinal mucosa (Gyires 2004; Chen and Tsai 2012).

Jia-Le Song and Kai Zhu contributed equally to this study.

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Abnormal levels of neuropeptides have been observed in patients with gastrointestinal mucosal lesions and hemorrhage (Long et al. 1962). In addition, increased serum levels of some inflammatory cytokines such as interleukin (IL)-6, IL-12, tumor necrosis factor (TNF)-α, and interferon (IFN)-γ have been observed in PUD patients (Malfertheiner et al. 2009; Robinson et al. 2008).

Reduction in gastric juice secretion and protection of the gastric mucosa are both a main strategy for PUD treatment in clinic (Mejia and Kraft 2009). However, long-term use of anti-ulcer drugs, such as histamine-2 receptor antagonist (H2RA) (e.g., cimetidine) or proton-pump inhibitors (e.g., lansoprazole) may cause serious side-effects, including hypergastrinemia, gynecomastia in men; galactorrhoea in women, nausea, abdominal pain, constipation, diarrhea, as well as long-term proton-pump inhibitor therapy increasing the risk for hip fractures (Parsons and Keeling 2005; Thomson et al. 2010). Therefore, the development of more effective and safer drugs for treating gastric ulcers has become essential. Medical herbs and dietary plants which contain high levels of natural compounds such as polyphenols have traditionally been used as an agent for the prevention and treatment of PUD, and gastric cancer chemoprevention (Borrelli and Izzo 2000).

The apple, the pomaceous fruit of the *Malus pumila* Mill of the rose family (Rosaceae) tree, is one of the most widely known health fruits. The leaf of apple has been reported to contain high levels of polyphenols including catechin, epicatedchin, chlorogenic acid, quercetin glycosides, and procyanidins (B2, B5, and E-B5) and exhibit great in vitro antioxidant activities (Mayr et al. 1995; Lister et al. 1994; Macheix et al. 1990; van der Sluis et al. 2001). Enhancing antioxidant activity in gastric mucosa was used for gastric ulcer treatment (Repetto and Llesuy 2002). Therefore, the aim of this study was to evaluate the potential gastric protective activity of polyphenol extracts from *M. pumila* leaf, and to elucidate the mechanisms underlying its protective effects in reserpine-induced gastric ulcers in mice.

Materials and methods

Chemical reagents

Trizol reagent, OligodT_{18} primer, murine maloney leukemia virus (MMLV) reverse transcriptase, RNase inhibitor, ethidium bromide (EtBr), and agarose were purchased from Invitrogen Life Technologies (Carlsbad, CA, USA). Reserpine and omeprazole were purchased from Sigma Chemical Co. (St. Louis, MO, USA). All reagents were of analytical grade.

Extraction of *Malus asiatica* leaf polyphenols

Fresh *Malus asiatica* leaves, which purchased form a local Chinese medicinal herb market (Chongqing City, People's Republic of China), were freeze-dried and then ground into a fine powder. A 40-fold volume of ethanol (20 %, vol/vol) was added to the powdered samples and extracted by sonication (20 min, 600 W) at 75 °C twice. After filtering, the *M. asiatica* leaf total polyphenols (MALP) were added three times of diethyl ether to remove chlorophyll, and concentrated by heat evaporation, freeze-dried and stored at −20 °C until further study. The polyphenols content of *M. asiatica* leaf was 15.2 % which was determined by the Folin–Ciocalteu method.

Animal studies

Male KM mice (6-week-old) were purchased from Experimental Animal Center of Chongqing Medical University (Chongqing, China). The mice were housed with a standard 12-h light–dark cycle at room temperature, and had access to food and water ad libitum. Mice were randomly divided into five groups of seven mice each: group 1, mice treated with 0.9 % normal saline; group 2, reserpine (Res)-treated mice; and groups 3–5, Res-treated mice were, respectively, administered omeprazole (30 mg/kg) and MALP (100 and 200 mg/kg) daily via an intragastric route for 28 days. Gastric ulcer was induced by intraperitoneal (i.p.) administration of 10 mg/kg reserpine according to the method described by Garattini et al. (1962) at day 29. After 24 h, all of the mice were sacrificed. Blood samples were collected from the inferior vena cava using a vacuum blood collection tube, then centrifuged (3,000×*g* for 10 min at 4 °C) and stored at −80 °C until further study. The animal protocol used in this study was reviewed by the Animal Ethics Committee of Chongqing Medical University.

Evaluation of anti-ulcer activity

Gastric secretion volume of each mouse was measured with a 10-ml measuring cylinder, and the pH of gastric juice was determined using a SevenEasy pH meter (Mettler Toledo, Schwerzenbach, Switzerland). The isolated stomachs were inflated by injecting 10 ml of 1 % formalin solution for 10 min to fix the tissues, and opened along the greater curvature. The area (mm^2) of hemorrhagic lesions that had developed in the stomach was measured under a Leica MZ7.5 dissecting microscope (Leica, Bensheim, Germany) with a square grid.

Measurement of gastric neuropeptide levels

Serum levels of motilin (MOT), substance P (SP), vasoactive intestinal peptide (VIP), and somatostatin (SS) were measured with radioimmunoassay kits (Beijing Puer Weiye Biotechnology Co., Ltd., Beijing, China) according to the manufacturer's protocols.

Measurement of serum pro-inflammatory cytokine levels

Serum levels of TNF-α, IFN-γ, IL-6, and IL-12 were measured with a commercial ELISA kit (ELISA MAX, Biolegend, San Diego, CA, USA) according to the manufacturer's protocol.

Reverse transcription polymerase chain reaction (RT-PCR) assay

Gastric mRNA expression of occludin was measured with an RT-PCR assay. Total RNA was isolated from 200 mg of gastric tissue using Trizol reagent according to the manufacturer's recommendations and centrifuged at 12,000×*g* for 15 min at 25 °C following the addition of chloroform. Isopropanol was added to the supernatant at a 1: 1 ratio and the RNA was pelleted by centrifugation (12,000×*g* for 15 min at 4 °C). After washing with ethanol, the RNA was solubilized in diethyl pyrocarbonate-treated RNase-free water and quantified by measuring the absorbance at 260 nm using a UV-1750 spectrophotometer (Shimadzu, Kyoto, Japan). Equal amounts of RNA (1 μg) were reverse transcribed in a master mix containing 1× reverse transcriptase buffer, dNTPs (1 mM), oligodT_{18} primers(500 ng), MMLV reverse transcriptase(140 U), and RNase inhibitor(40 U) for 45 min at 42 °C. PCR was then carried out in an automatic thermocycler (Bioneer, Daejeon, South Korea) for 30 cycles (94 °C for 30 s, 55 °C for 30 s, and 72 °C for 40 s) followed by an 8-min extension at 75 °C. The PCR products were separated in 2 % agarose gels and visualized by EtBr staining. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used for normalization.

Protein extraction and western blot analysis

One hundred milligrams of gastric tissue samples were first washed with ice-cold phosphate buffered saline (PBS), homogenized with ice-cold RIPA buffer, and then centrifuged at 13,000×*g* for 30 min at 4 °C. Protein concentrations were determined with a Bio-Rad protein assay kit (Hercules, CA, USA). For Western blot analysis, protein extracts (50 μg) were separated by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) and then electrotransferred onto a nitrocellulose membrane (Schleicher and Schuell, Keene, NH, USA). The blots were incubated with antibodies against occludin, p38, and phosphorylated p38MAPK (p-p38MAPK) obtained from Cell Signaling Technologies (Beverly, MA, USA), and then incubated with horseradish peroxidase-conjugated secondary antibody (Cell Signaling Technologies) for 1 h at room temperature. The blots were washed three times with PBS containing 0.05 % v/v Tween 20 (PBS-T) and antibody binding was visualized by enhanced chemiluminescence (GE Healthcare Life Sciences, Little Chalfont, UK).

Statistical analysis

Data are presented as the mean ± SD. Differences between the mean values for individual groups were assessed by a one-way ANOVA with Duncan's multiple range tests. $p < 0.05$ was considered to indicate a statistically significant difference. The SAS v9.1 statistical software package (SAS Institute Inc., Cary, NC, USA) was used for the analysis.

Results

MALP attenuated reserpine-induced gastric ulcer

As presented in Table 1, reserpine (10 mg/kg) significantly induced formation of gastric ulcers in mice (gastric ulcer area, 6.65 ± 1.38 mm^2). Following 28 days of administration with 100 and 200 mg/kg of MALP, the gastric ulcer inhibition rate was 58.5 % (gastric ulcer area, 2.76 ± 0.56 mm^2) and 86.8 % (gastric ulcer area, 0.88 ± 0.22 mm^2), respectively. These results suggested that MALP showed a significant preventive effect against reserpine-induced gastric ulcers.

Table 1 Effect of *Malus asiatica* leaf total polyphenols (MALP) on the gastric ulcer area and gastric ulcer inhibitory rate in mice treated with reserpine (10 mg/kg)

Groups	Degree of gastric ulcer	
	Gastric ulcer area (mm^2)	Inhibition rate (%)
Normal	0.0 ± 0.0[e,f]	100
Control (reserpine)	6.65 ± 1.38[a]	/
Omeprazole (30 mg/kg)	0.67 ± 0.31[d]	89.9
MALP (100 mg/kg)	2.76 ± 0.56[b]	58.5
MALP (200 mg/kg)	0.88 ± 0.22[c]	86.8

[a–e] Mean values with different letters in the same column are significantly different ($p < 0.05$) according to Duncan's multiple range test

[f] Data are presented as mean ± SD

Table 2 Effect of *Malus asiatica* leaf total polyphenols (MALP) on gastric secretion volume and pH of gastric juice acidity in mice treated with reserpine (10 mg/kg)

Groups	Gastric secretion volume (mL)	pH of the gastric juice
Normal	$0.24 \pm 0.05^{e,f}$	3.47 ± 0.28^{a}
Control (reserpine)	1.11 ± 0.23^{a}	0.77 ± 0.07^{e}
Omeprazole (30 mg/kg)	0.37 ± 0.08^{d}	3.02 ± 0.33^{b}
MALP (100 mg/kg)	0.83 ± 0.13^{b}	1.93 ± 0.28^{d}
MALP (200 mg/kg)	0.49 ± 0.04^{c}	2.64 ± 0.30^{c}

[a–e] Mean values with different letters in the same column are significantly different ($p < 0.05$) according to Duncan's multiple range test

[f] Data are presented as mean ± SD

However, its effect was weaker than that in 30 mg/kg omeprazole (a proton-pump inhibitor which to treat gastric lesion)-treated mice (gastric ulcer area is 0.67 ± 0.31 mm^2; inhibition rate is 89.9 %). Treatment with 10 mg/kg of reserpine significantly ($p < 0.05$) increased the gastric juice secretion (1.11 ± 0.23 ml) compared to normal mice (0.24 ± 0.05 ml). Following administration with low dose (100 mg/kg) and high dose (200 mg/kg) of MALP for 28 days, there was significantly ($p < 0.05$) reduced gastric juice secretion (0.83 ± 0.13 and 0.49 ± 0.04 ml, respectively). In addition, MALP significantly ($p < 0.05$) increased the pH of gastric juice in mice treated with reserpine (10 mg/kg) as shown in Table 2. Furthermore, none of the animals in the five groups died during the experimental periods.

Effect of MALP on serum neuropeptide levels in reserpine-induced gastric ulcer mice

As shown in Table 3, administration with 10 mg/kg of reserpine significantly increased the serum levels of MOT (112.8 ± 7.9 pg/ml) and SP (129.3 ± 4.6 ng/ml) compared with levels in normal mice (MOT: 44.3 ± 2.8 pg/mL and SP: 60.4 ± 1.8 ng/l). Treatment with 100 and 200 mg/kg of MALP significantly ($p < 0.05$) attenuated the reserpine-induced MOT (84.2 ± 3.6 and 66.8 ± 2.1 pg/ml) and SP (122.8 ± 3.9 and 93.5 ± 4.2 ng/l) generation. In addition, we observed MALP dose-dependently increased the levels of SS (63.6 ± 4.1 and 78.3 ± 3.1 ng/l) and VIP (50.3 ± 3.3 and 75.4 ± 2.6 ng/l) compared to that in reserpine-treated mice (50.6 ± 3.0 and 38.2 ± 1.8 ng/l, respectively). These results indicated that MALP may modulate gastric juice secretion in reserpine-induced gastric ulcers by increasing the serum levels of SS and VIP, as well as reducing the levels of MOT and SP in serum.

Effect of MALP on IL-6, IL-12, TNF-α, and IFN-γ in reserpine-induced gastric ulcer mice

Administration of reserpine (10 mg/kg) significantly increased the serum levels of TNF-α, IL-6, IL-12, and IFN-γ than that found in normal groups (Table 4). Following treatment with 100 and 200 mg/kg of MALP, reserpine-induced generation of these inflammatory cytokines were reduced as shown in Table 4. However, MALP showed a weaker activity to reduce the reserpine-induced generation of inflammatory cytokines than that in omeprazole-treated gastric ulcer mice.

Effect of MALP on gastric levels of p38MAPK phosphorylation and occludin in reserpine-induced gastric ulcer mice

As shown in Fig. 2, we found reserpine significantly increased the gastric p38MAPK phosphorylation in gastric ulcer KM mice. Following treatment with 100 and 200 mg/kg of MALP, the reserpine-induced p38MAPK phosphorylation in mice was attenuated. In addition, reserpine significantly decreased the gastric mRNA and protein levels of occludin in gastric ulcer mice. MALP treatment (100 and 200 mg/kg) significantly increased the gastric mRNA and protein levels of occludin in reserpine-induced gastric ulcer mice (Fig. 3).

Table 3 Effect of *Malus asiatica* leaf total polyphenols (MALP) on motilin (MOT), substance P (SP), somatostatin (SS), and vasoactive intestinal peptide (VIP) in mice treated with reserpine (10 mg/kg)

Groups	MOT (pg/ml)	SP (ng/l)	SS (ng/l)	VIP (ng/l)
Normal	$44.3 \pm 2.8^{e,f}$	60.4 ± 1.8^{e}	107.8 ± 1.7^{a}	110.2 ± 6.3^{a}
Control (reserpine)	112.8 ± 7.9^{a}	129.3 ± 4.6^{a}	50.6 ± 3.0^{e}	38.2 ± 1.8^{e}
Omeprazole (30 mg/kg)	60.2 ± 2.8^{d}	67.8 ± 2.8^{d}	83.5 ± 5.7^{b}	92.1 ± 9.2^{b}
MALP (100 mg/kg)	84.2 ± 3.6^{b}	98.3 ± 3.2^{b}	63.6 ± 4.1^{d}	50.3 ± 3.3^{d}
MALP (200 mg/kg)	66.8 ± 2.1^{c}	81.1 ± 2.6^{c}	78.3 ± 3.1^{c}	75.4 ± 2.6^{c}

[a–e] Mean values with different letters in the same column are significantly different ($p < 0.05$) according to Duncan's multiple range test

[f] Data are presented as mean ± SD

Table 4 Effect of *Malus asiatica* leaf total polyphenols (MALP) on tumor necrosis factor (TNF)-α, interlukin (IL)-6, IL-12, and Interferon (IFN)-γ in mice treated with reserpine (10 mg/kg)

Groups	TNF-α (pg/ml)	IL-6 (pg/ml)	IL-12 (pg/ml)	IFN-γ (pg/ml)
Normal	37.8 ± 2.2[e,f]	33.7 ± 2.6[e]	512.3 ± 28.6[e]	27.3 ± 1.2[e]
Control (reserpine)	92.1 ± 6.5[a]	109.3 ± 6.4[a]	972.3 ± 43.2[a]	109.3 ± 2.6[a]
Omeprazole (30 mg/kg)	45.6 ± 3.7[d]	44.2 ± 1.6[d]	589.2 ± 21.2[d]	38.9 ± 2.4[d]
MALP (100 mg/kg)	77.5 ± 1.6[b]	83.2 ± 2.6[b]	772.3 ± 32.2[b]	83.4 ± 3.2[b]
MALP (200 mg/kg)	53.4 ± 2.8[c]	54.2 ± 2.3[c]	634.2 ± 20.7[c]	49.3 ± 2.5[c]

[a–e] Mean values with different letters in the same column are significantly different ($p < 0.05$) according to Duncan's multiple range test

[f] Data are presented as mean ± SD

Discussion

Epidemiological evidence suggests that chronic PUD is closely associated with gastric cancer (de Martel et al. 2013; Molloy and Sonnenberg 1997; Krejs 2010). In recent years, medical herbs and dietary plants have been used for the prevention and treatment of the PUD (Borrelli and Izzo 2000), as well as gastric cancer chemoprevention (Huang et al. 2009). In China, dried *M. pumila* leaf has used as herb tea for a long time. Therefore, we investigated the protective effect of MALP using a reserpine-induced gastric ulcer model in ICR mice.

Excess gastric acid secretion is an important factor in the development of gastric ulcer (Delaney et al. 1970; Guth 1982); reduction in gastric juice secretion was able to protect the gastric mucosa and is a useful strategy in the prevention and treatment of gastric ulcer (de Martel et al. 2013; Long et al. 1962). In this study, we found that MALP significantly reduced the reserpine-induced gastric juice secretion and inhibited the gastric ulcer in KM mice (Table 1; Fig. 1). In addition, we also observed that the pH of gastric juice was increased following pretreatment with MALP in reserpine-treated mice (Table 2). Maintaining a higher pH condition in gastric juice was able to reduce the activity of pepsin which is a risk factor for gastric ulcers (Alphin et al. 1977; Venables 1986), and enhanced the healing of the gastric mucosa (Yamamoto and Okada 1984).

It is well known that gastric juice secretion is regulated by the central nervous system, particularly the vagus nerve system (Sandor et al. 1996). Neuropeptides which include VIP, SS, SP, and MOT were not only involved in the regulation of gastric acid secretion, but also demonstrated an important effect on gastric tissue repair (Xie et al. 2006). In normal physiological conditions, SP stimulated gastric juice secretion (Otsuka and Yoshioka 1993; Geoghegan

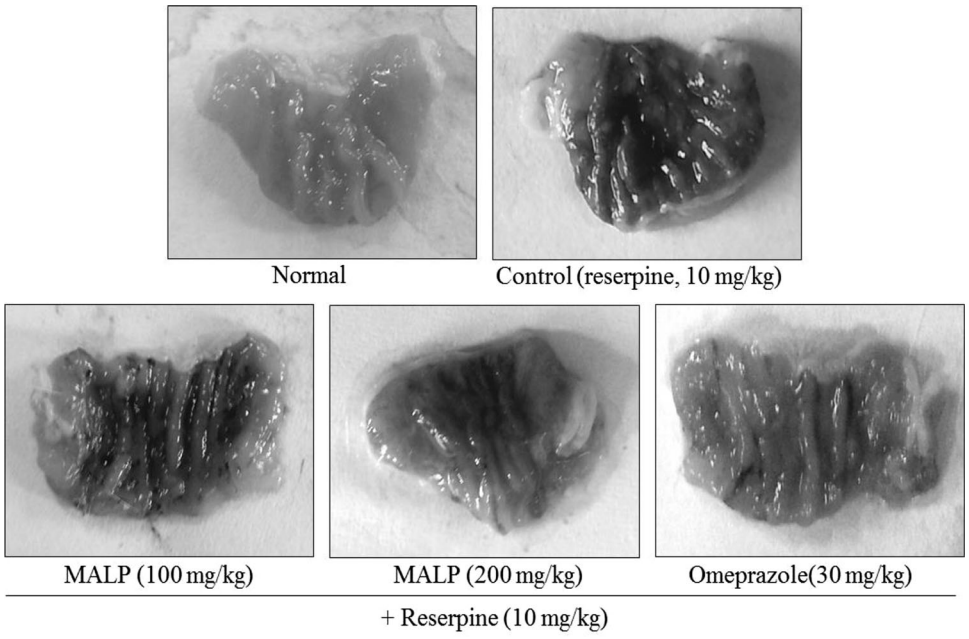

Fig. 1 Stomachs appearance of *Malus asiatica* leaf total polyphenols (MALP) treatment in mice treated with reserpine (10 mg/kg)

and Pappas 1997) and decreased gastric contraction (Tache et al. 1990). The increased levels of SP have been observed in gastritis patients (Erin et al. 2012). It resulted in delayed gastric emptying and aggravated the gastric ulcer (Holzer 1998). MOT, a 22-amino acid residue polypeptide, is a powerful inducer of gastrointestinal motor activity (Brown and Parkes 1967), intestinal contractility (Itoh et al. 1975), and stimulation of human gastric pepsin secretion (Ruppin et al. 1974). The increase in pepsin levels is an important risk factor in the pathogenesis of gastric ulcers (Alphin et al. 1977; Venables 1986). In the present study, pretreatment with 100 and 200 mg/kg of MALP effectively decreased the serum levels of SP and MOT in gastric ulcer mice (Table 3). On the other hand, we observed that pretreatment with 100 and 200 mg/kg of MALP significantly increased the serum levels of SP and VIP in gastric ulcer mice (Table 3). SS is widely distributed in the brain and the periphery and exists in two forms, the 14 and 28 amino acid peptides (Gyires 2004). It is able to inhibit gastric acid secretion stimulated by pentagastrin, bethanechol, or histamine in rats through activation of somatostatin receptor type-2 (sst_2) receptors (Schubert and Peura 2008; Lloyd et al. 1995; Aurang et al. 1997; Martinez et al. 1998). Some studies have reported that peripheral injection of VIP resulted in decreased gastric acid secretion through inhibition of the activation of the vagal nerve in rats (Schubert and Peura 2008; Lloyd et al. 1995; Saadé et al. 1995; Nassar et al. 1995). These results indicated that MALP may modulate gastric juice secretion in reserpine-induced gastric ulcers by increasing the serum levels of VIP and SS, as well as reducing the levels of SP and MOT in serum.

Increased generation of inflammatory cytokines (such as IL-6, IL-12, TNF-α, and IFN-γ) was associated with the pathological process of chronic PUD in humans (Malfertheiner et al. 2009; Robinson et al. 2008). TNF-α is a classic inflammatory cytokine and has been indicated as a key mediator in the regulation of IL-6 in stomach disease (Abdollahi et al. 2011). In this study, we found that MALP treatment significantly reduced the reserpine-induced generation of these inflammatory cytokines in gastric ulcer mice.

The gastrointestinal (GI) epithelial barrier is a physical barrier that reduces the diffusion of macromolecules such as pathogens, toxins, and allergens. Its normal function is regulated by the mitogen-activated protein kinases (MAPKs) family including p38, extracellular regulated protein kinases (ERK)1/2 and c-Jun N-terminal kinase (JNK) (Oshima et al. 2007). Tight junction (TJ) complexes, which contain occludin, claudin, and junction adhesion molecule (JAM), are an important factor to maintain the normal function of GI epithelial barrier (Carrozzino et al. 2009). As one of the most important TJ proteins, occludin was able to regulate the TJ barrier function (Schneeberger and Lynch 2004). Phosphorylation of p38MAPK caused the decrease in occludin and an increase in GI epithelial cell permeability and disruption of the TJ (Oshima et al. 2008). In this study, we found reserpine-induced p38MAPK phosphorylation (Fig. 2). However, MALP treatment significantly reduced p38MAPK phosphorylation (decreased gastric protein levels of p-p38) in reserpine-treated mice. Inhibition of p38MAPK phosphorylation is able to increase the GI mucosal epithelial barrier function (Carrozzino et al. 2009). In addition, treatment with 100 and 200 mg/kg of MALP also significantly increased the gastric mRNA and protein levels of occludin (Fig. 3). Some studies also suggested that inhibition of p38MAPK phosphorylation prevents the disruption of TJ proteins through increasing occludin in vivo and in vitro (Oshima et al. 2007; Wu et al. 2013).

This study has demonstrated that MALP exhibits a great protective effect against reserpine-induced gastric ulcers in

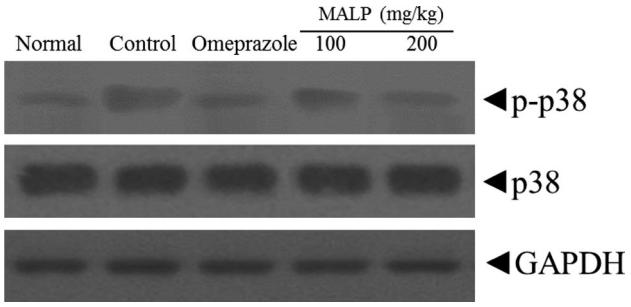

Fig. 2 Effect of *Malus asiatica* leaf total polyphenols (MALP) on gastric levels of p38MAPK phophorylation in mice treated with reserpine (10 mg/kg)

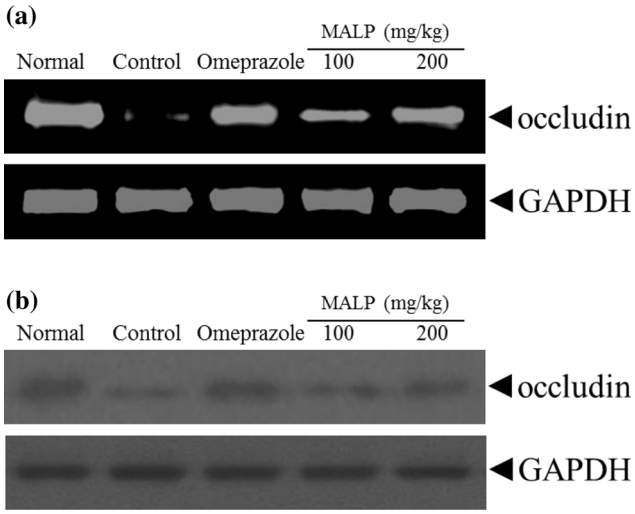

Fig. 3 Effect of *Malus asiatica* leaf total polyphenols (MALP) on **a** mRNA and **b** protein levels of occludin in mice treated with reserpine (10 mg/kg)

KM mice. MALP administration was attenuated reserpine-induced gastric acid secretion and increased the pH of gastric juice. In addition, MALP administration also modulated serum levels of the neroupeptides (MOT, SP, VIP, and SS), and decreased the serum levels of inflammatory cytokines (TNF-α, IL-6, IL-12, and IFN-γ). MALP also reduced the phosphorylation of p38MAPK to increase gastric occludin and maintain the normal function of the gastric mucosa. The results from this study indicate that the potential mechanism of the protective effects of MALP involves reduction of gastric juice secretion, modulation of neroupeptides, and regulation of the gastric levels of occludin and p38MAPK phosphorylation as important mechanisms against reserpine-induced gastric ulcers. It indicated that *M. asiatica* leaf polyphenols represent a potential beneficial agent for the prevention of reserpine-induced gastric ulcer in vivo.

Acknowledgments This work is supported by the Scientific and Technological Research Program of Chongqing Municipal Education Commission (Grant No. KJ1401402) and the Scientific Research Foundation for the Returned Overseas Chinese Scholars, State Education Ministry, People's Republic of China.

References

Abdollahi H, Shams S, Zahedi MJ, Darvish Moghadam S, Hayatbakhsh MM, Jafarzadeh A (2011) IL-10, TNF-α and IFN-γ levels in serum and stomach mucosa of *Helicobacter pylori*-infected patients. Iran J Allergy Asthma Immunol 10:267–271

Alphin R, Vokac V, Gregory R, Bolton P, Tawes JW III (1977) Role of intragastric pressure, pH, and pepsin in gastric ulceration in the rat. Gastroenterology 73:495–500

Aurang K, Wang J, Lloyd K (1997) Somatostatin inhibition of acid and histamine release by activation of somatostatin receptor subtype 2 receptors in rats. J Pharmacol Exp Ther 281:245–252

Borrelli F, Izzo AA (2000) The plant kingdom as a source of anti-ulcer remedies. Phytother Res 14:581–591

Brown J, Parkes C (1967) Effect on fundic pouch motor activity of stimulatory and inhibitory fractions separated from pancreozymin. Gastroenterology 53:731–736

Carrozzino F, Pugnale P, Féraille E, Montesano R (2009) Inhibition of basal p38 or JNK activity enhances epithelial barrier function through differential modulation of claudin expression. Am J Physiol Cell Physiol 297:775–787

Chen CY, Tsai CY (2012) Ghrelin and motilin in the gastrointestinal system. Curr Pharm Des 18:4755–4765

de Martel C, Forman D, Plummer M (2013) Gastric cancer: epidemiology and risk factors. Gastroenterol Clin North Am 42:219–240

Delaney J, Cheng J, Butler B, Ritchie W (1970) Gastric ulcer and regurgitation gastritis. Gut 11:715–719

Erin N, Türker S, Elpek Ö, Yıldırım B (2012) Differential changes in Substance P, VIP as well as neprilysin levels in patients with gastritis or ulcer. Peptides 35:218–224

Garttini S, Giachetti A, Jori A, Pieri L, Valzelli L (1962) Effect of imipramine, amitriptyline and their monomethyl derivatives on reserpine activity. J Pharm Pharmacol 14:509–514

Geoghegan J, Pappas T (1997) Central peptidergic control of gastric acid secretion. Gut 40:164–166

Guth PH (1982) Pathogenesis of gastric mucosal injury. Annu Rev Med 33:183–196

Gyires K (2004) Neuropeptides and gastric mucosal homeostasis. Curr Top Med Chem 4:63–73

Holzer P (1998) Neural emergency system in the stomach. Gastroenterology 114:823–839

Huang JQ, Sridhar S, Hunt RH (2002) Role of *Helicobacter pylori* infection and non-steroidal anti-inflammatory drugs in peptic-ulcer disease: a meta-analysis. Lancet 359:14–22

Huang WY, Cai YZ, Zhang Y (2009) Natural phenolic compounds from medicinal herbs and dietary plants: potential use for cancer prevention. Nutr Cancer 62:1–20

Itoh Z, Honda R, Hiwatashi K, Takeuchi S, Aizawa I, Takayanagi R, Couch EF (1975) Motilin-induced mechanical activity in the canine alimentary tract. Scand J Gastroenterol Suppl 39:93–110

Krejs GJ (2010) Gastric cancer: epidemiology and risk factors. Dig Dis 28:600–603

Lister CE, Lancaster JE, Sutton KH (1994) Developmental changes in the concentration and composition of flavonoids in skin of a red and green apple cultivar. J Sci Food Agric 64:155–161

Lloyd K, Wang J, Aurang K, Gronhed P, Coy D, Walsh J (1995) Activation of somatostatin receptor subtype 2 inhibits acid secretion in rats. Am J Physiol 268:102–106

Long DM, Leonard AS, Story YJ, French LA (1962) Hypothalamus and gastric ulceration: II. Production of gastrointestinal ulceration by chronic hypothalamic stimulation. Arch Neurol 7:176

Macheix JJ, Fleuriet A, Billot J (1990) Fruit phenolics. CRC Press, Boca Raton

Malfertheiner P, Chan FK, Mccoll KE (2009) Peptic ulcer disease. Lancet 374:1449–1461

Martinez VL, Curi AP, Torkian B, Schaeffer JM, Wilkinson HA, Walsh JH, Taché Y (1998) High basal gastric acid secretion in somatostatin receptor subtype 2 knockout mice. Gastroenterology 114:1125–1132

Mayr U, Treutter D, Santos-Buelga C, Bauer H, Feucht W (1995) Developmental changes in the phenol concentrations of 'golden delicious' apple fruits and leaves. Phytochemistry 38:1151–1155

Mejia A, Kraft WK (2009) Acid peptic diseases: pharmacological approach to treatment. Expert Rev Clin Pharmacol 2:295–314

Molloy R, Sonnenberg A (1997) Relation between gastric cancer and previous peptic ulcer disease. Gut 40:247–252

Nassar CF, Abdallah LE, Barada KA, Atweh SF, Saadé NE (1995) Effects of intravenous vasoactive intestinal peptide injection on jejunal alanine absorption and gastric acid secretion in rats. Regul Pept 55:261–267

Oshima T, Sasaki M, Kataoka H, Miwa H, Takeuchi T, Joh T (2007) Wip1 protects hydrogen peroxide-induced colonic epithelial barrier dysfunction. Cell Mol Life Sci 64:3139–3147

Oshima T, Miwa H, Joh T (2008) Aspirin induces gastric epithelial barrier dysfunction by activating p38 MAPK via claudin-7. Am J Physiol Cell Physiol 295:800–806

Otsuka M, Yoshioka K (1993) Neurotransmitter functions of mammalian tachykinins. Physiol Rev 73:229–308

Parsons ME, Keeling DJ (2005) Novel approaches to the pharmacological blockade of gastric acid secretion. Expert Opin Investig Drugs 14:411–421

Phillipson M, Atuma C, Henriksnäs J, Holm L (2002) The importance of mucus layers and bicarbonate transport in preservation of gastric juxtamucosal pH. Am J Physiol Gastrointest Liver Physiol 282:211–219

Repetto MG, Llesuy SF (2002) Antioxidant properties of natural compounds used in popular medicine for gastric ulcers. Braz J Med Biol Res 35:523–534

Robinson K, Kenefeck R, Pidgeon EL, Shakib S, Patel S, Polson RJ, Zaitoun AM, Atherton JC (2008) *Helicobacter pylori*-induced

peptic ulcer disease is associated with inadequate regulatory T cell responses. Gut 57:1375–1385

Ruppin H, Domschke S, Domschke W, Wünsch E, Jaeger E, Demling L (1974) Effects of 13-nle-motilin in man-inhibition of gastric evacuation and stimulation of pepsin secretion. Scand J Gastroenterol 10:199–202

Saadé NE, Abdallah LE, Barada KA, Atweh SF, Nassar CF (1995) Effects of intracerebral injections of VIP on jejunal alanine absorption and gastric acid secretion in rats. Regul Pept 55:269–276

Sandor A, Kidd M, Lawton GP, Miu K, Tang LH, Modlin IM (1996) Neurohormonal modulation of rat enterochromaffin-like cell histamine secretion. Gastroenterology 110:1084–1092

Schneeberger EE, Lynch RD (2004) The tight junction: a multifunctional complex. Am J Physiol Cell Physiol 286:1213–1228

Schubert ML, Peura DA (2008) Control of gastric acid secretion in health and disease. Gastroenterology 134:1842–1860

Tache Y, Garrick T, Raybould H (1990) Central nervous system action of peptides to influence gastrointestinal motor function. Gastroenterology 98:517–528

Thomson AB, Sauve MD, Kassam N, Kamitakahara H (2010) Safety of the long-term use of proton pump inhibitors. World J Gastroenterol 16:2323–2330

van der Sluis AA, Dekker M, de Jager A, Jongen WM (2001) Activity and concentration of polyphenolic antioxidants in apple: effect of cultivar, harvest year, and storage conditions. J Agric Food Chem 49:3606–3613

Venables C (1986) Mucus, pepsin, and peptic ulcer. Gut 27:233–238

Wu HL, Gao X, Jiang ZD, Duan ZT, Wang SK, He BS, Zhang ZY, Xie HG (2013) Attenuated expression of the tight junction proteins is involved in clopidogrel-induced gastric injury through p38 MAPK activation. Toxicology 304:41–48

Xie XZ, Zhao ZG, Qi DS, Wang ZM (2006) Assay of gastrin and somatostatin in gastric antrum tissues of children with chronic gastritis and duodenal ulcer. World J Gastroenterol 12:2288–2290

Yamamoto O, Okada Y (1984) Effects of a proton pump inhibitor, omeprazole, on gastric secretion and gastric and duodenal ulcers or erosions in rats. Dig Dis Sci 29:394–401