

# The Protective Effect of Capsaicin Receptor-Mediated Genistein Postconditioning on Gastric Ischemia–Reperfusion Injury in Rats

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## Abstract

**Background** No published study has addressed the effect of genistein postconditioning on gastric ischemia–reperfusion (GI–R) injury in rats.

**Aim** To examine whether capsaicin receptor-mediated genistein postconditioning protects against gastric ischemia–reperfusion injury via the PI3K/Akt signal pathway.

**Methods and Results** Chloralhydrat-anesthetized rats underwent occlusion of the celiac artery for 30 min, followed by 60 min of reperfusion. Based on this animal model of gastric ischemia–reperfusion injury, genistein at doses of 100, 500 or 1,000 µg/kg was administered via peripheral vein 5 min before reperfusion. The dose of 500 µg/kg was optimal for postconditioning, at which the severity of I–R-induced gastric injury significantly decreased. Immunohistochemistry also showed that gastric mucosal cell apoptosis decreased. Capsazepine (CPZ), a specific antagonist for the capsaicin receptor, was administered (1,000 µg/kg, i.v.) just before ischemia. Capsaicin (50 mg/kg, s.c.) once a day for 4 days reversed the

protective effects of genistein. Reverse transcription-polymerase chain reaction (RT-PCR) and Western blotting showed increased calcitonin gene-related peptide (CGRP) expression in genistein group but not in capsazepine or capsaicin group. CGRP inhibitor CGRP8-37 also prevented the effects of genistein in decreasing gastric mucosal injury index. In addition, PI3K inhibitor LY294002 (1.5 mg/kg) reversed the protective effect of genistein. Compared with genistein group, Western blots also demonstrated decreased Akt phosphorylation in LY294002 group.

**Conclusion** Our data suggest that capsaicin receptors mediated the protective effects of genistein postconditioning. CGRP secreted by activated capsaicin-sensitive neurons played an important role in the protective effects of genistein. PI3K/Akt pathway was also involved in the protective effects of genistein.

**Keywords** Gastric ischemia–reperfusion · Genistein postconditioning · Capsazepine · Capsaicin · CGRP · PI3K/Akt

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## Introduction

Genistein is a biologically active substance, found at high content in soybean. It has weak estrogen-like activity. Many authors have suggested the use of natural estrogens derived from plants, i.e., phytoestrogens, as a therapeutic alternative to classical estrogens [1, 2]. The main compound of this family is genistein, which is able to exert a vasodilation effect with similar potency to 17β-estradiol in human forearm vasculature [2]. Accumulating evidence suggests that genistein can reduce cholesterol, heart disease, and cancer incidence [3, 4]. Moreover, it has been demonstrated that genistein can inhibit I–R-induced

myocardial apoptosis and has a cardioprotective effect in rabbits [5]. Previous studies also showed that genistein can affect the relaxation of mouse isolated stomach [6] and has a protective effect against stress-induced gastric mucosal lesions [7]. Because of its extensive presence in and close relation to soybean-rich diet, its healthcare and medicinal values are attracting increasing attention.

Related studies [8, 9] found that phytoestrogenic isoflavon or phytoestrogen genistein could affect sensory neuron excitability and attenuate neuronal apoptosis. Recent studies have shown that many noncholinergic, nonadrenoceptor (peptidergic) afferent neurons are distributed in gastrointestinal tract. Their cell membrane and the nerve endings are rich in capsaicin receptor subtype 1 (VR<sub>1</sub>) [10–12]. By using immunohistochemical staining and Western blot analysis, Faussone et al. [13] also revealed that VR<sub>1</sub> is distributed in nerve endings in gastric mucosal layer, submucosa, and parietal cells, all of which are sensitive to thermal, mechanical, chemical, and ischemic stimuli, etc. Capsaicin receptor is believed to play an important role in maintenance of normal functions of gastrointestinal mucosa against injury caused by stimuli [14–16]. Thus, it could be hypothesized that genistein might inhibit I–R-induced gastric mucosal injury by activating VR<sub>1</sub>. The PI3K–Akt signaling pathway plays an important role in regulating gastrointestinal tract function. Some reports [17, 18] showed that the PI3K–Akt pathway mediated antiapoptosis effect of gastric carcinoma cell and tumor necrosis factor–related apoptosis-inducing ligand (TRAIL)- and radiation-induced gastrointestinal cell apoptosis.

First, this study aims to ascertain whether low doses of genistein have protective effects on ischemia–reperfusion injury in rat gastric mucosa. Then, further exploration was performed to investigate whether the capsaicin receptor mediated the protective effects of genistein postconditioning. Genistein was administered during 30 min ischemia, prior to reperfusion, to induce pharmacological postconditioning. From a methodological point of view, pharmacological postconditioning was employed in GI–R injury, which not only avoided the defect of ischemia preconditioning but also the defect of ischemia postconditioning that might cause mechanical injury to the organism. Therefore, acute postconditioning with genistein could be proposed as a therapeutic strategy for treatment of gastric ischemia–reperfusion injury.

## Methods

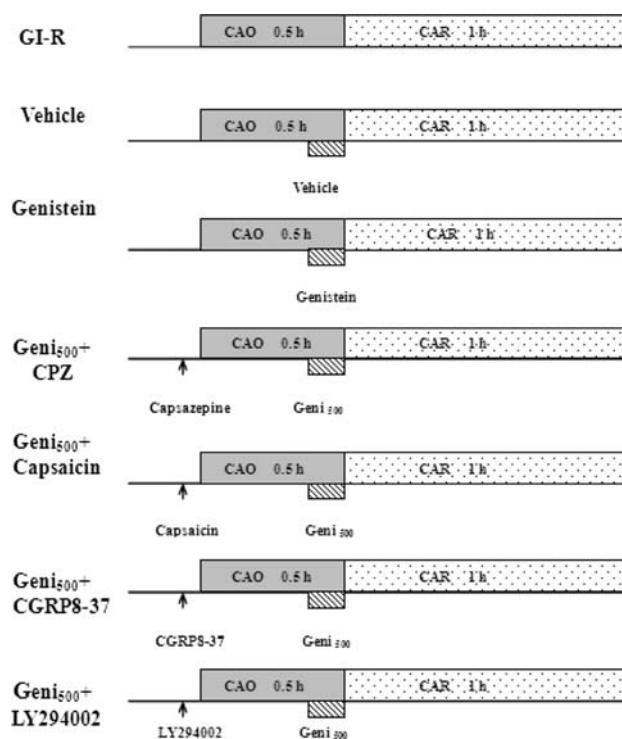
### Animals

Adult male Sprague–Dawley rats, weighing 200–240 g, were provided by the Experimental Animal Centre of

Xuzhou Medical College. All experiments were performed in accordance with the National Institutes of Health *Guidelines for the Care and Use of Laboratory Animals*. These rats were deprived of food but allowed access to tap water ad libitum for 24 h before experiments. Each experiment was performed with six to eight rats under 10% intraperitoneal chloralhydrat anesthesia.

### Experimental Protocol

Acute gastric mucosal injuries were induced by I–R [19]. Briefly, under chloralhydrat anesthesia (10%, i.p.), the celiac artery was clamped with a small clamp, and 30 min later, reperfusion was achieved by removal of the clamp for 60 min. The animals were included in different protocols as illustrated in Fig. 1. They were all subjected to celiac artery occlusion for 30 min and then reperfusion for 60 min. In order to determine whether the capsaicin receptors and the PI3K/Akt pathway were involved in the protective effects of genistein, various doses of genistein (100, 500, and 1,000 µg/kg, i.v.) were used for postconditioning, 5 min prior to reperfusion. Capsazepine (CPZ) (1,000 µg/kg, i.v.), CGRP8-37 (500 µg/kg, i.v.), and LY294002 (1.5 mg/kg, i.v.) were administered before ischemia. Capsaicin (50 mg/kg, s.c.) was administered



**Fig. 1** Experimental protocol. CAO, celiac artery occlusion; CAR, celiac artery reperfusion. Vehicle and genistein were administered 5 min prior to CAO; Capsazepine, CGRP8-37, and LY294002 were administered before ischemia. Capsaicin was administered once a day for 4 days

once a day for 4 days, the other procures with the same as genistein postconditioning.

After 60 min of reperfusion, the animals were sacrificed and their stomachs were excised. The gastric mucosal injury index was recorded and each of the mucosa was divided into halves. One half of the mucosa was immersed in 4% formalin-saline, embedded in paraffin, and sectioned at 5  $\mu$ m for terminal dUTP nick end labeling (TUNEL) detection. The other half was stored at  $-80^{\circ}\text{C}$  for Western blotting. In such groups as sham, I-R, vehicle, genistein, capsazepine, and capsaicin, dorsal root ganglia (T5-L2) was excised for RT-PCR of CGRP.

#### Assessment of Gastric Mucosal Injury Index

At the end of reperfusion, gastric mucosal injury index was measured as described by Guth et al. [20] with slight modification. The stomach was incised along the greater curvature and rinsed with ice-cold phosphate-buffered saline (PBS; 0.1 mol/L). The stomach was spread out on a cold plate and a paned counteracting slab (with 1 mm<sup>2</sup> panes) for injury index count. The index is based on a cumulative length scale, in which individual lesions limited to the mucosal epithelium (including pinpoint erosions, ulcers, and hemorrhagic spots) are scored according to their length as follows: 1 for a lesion  $\leq 1$  mm; 2 for a lesion  $> 1$  mm and  $\leq 2$  mm; 3 for a lesion  $> 2$  mm and  $\leq 3$  mm; etc. For lesions with width  $> 1$  mm, the lesion score was doubled. The sum of these scores is the gastric mucosal injury index. To avoid researcher bias, the gastric mucosal damage index was determined by a researcher who was unfamiliar with the experiment.

#### Terminal dUTP Nick End Labeling (TUNEL) Immunohistochemistry

In order to observe gastric mucosal apoptosis and proliferation after reperfusion, detection of apoptosis using TUNEL technique was performed on paraffin tissue sections with a TUNEL ApopTag kit (CHEMICON, USA). The percentage of TUNEL-positive nuclei within the infarcted area was determined at random. The percentage of positive cells (positive cells/total cells  $\times 100$ ) was calculated.

#### Analysis of Calcitonin Gene-Related Peptide (CGRP) mRNA Expression by RT-PCR

Animals were sacrificed under deep anesthesia after reperfusion, and the dorsal root ganglia (DRG) on both sides of the spinal cord were removed, frozen in liquid nitrogen, and stored at  $-80^{\circ}\text{C}$  until use.

Tissue samples were pooled from two or three rats for extraction of total RNA, which was prepared by a single-step method of acid phenol–chloroform extraction. The sequences of sense and antisense primers for rat CGRP are shown in Table 1. An aliquot of the reverse-transcription reaction product served as a template in 30 cycles of polymerase chain reaction (PCR) with 0.5 min denaturation at  $94^{\circ}\text{C}$ , 0.5 min annealing at  $55^{\circ}\text{C}$ , and 2 min extension at  $72^{\circ}\text{C}$  on a thermal cycler. A portion of the PCR mixture was electrophoresed in 1.5% agarose gel in Tris–ethylenediamine tetraacetic acid (EDTA)–acetic acid buffer, and the gel was stained with ethidium bromide and photographed.

#### Western Blotting

After reperfusion, gastric mucosal was scraped and stored at  $-80^{\circ}\text{C}$ . Tissue lysates were prepared with cold 1% Nonidet P-40 in 50 mM Tris–HCl pH 7.4 containing 120 mM NaCl, 1 mM EDTA, 50 mM NaF, 0.1 mM Na<sub>3</sub>VO<sub>4</sub>, and protease inhibitor cocktail. Total protein levels were quantified by Lowry assay. Lysates (100  $\mu$ g of total protein) were then subjected to sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoresis (PAGE) and transferred onto a nitrocellulose membrane (pore size 0.45  $\mu$ m) according to the method of Towbin et al. [21]. Blotting filters were incubated with 3% bovine serum albumin (BSA) in TBST [10 mmol/L Tris (pH 7.5), 150 mmol/L NaCl, 0.05% Tween-20] at  $4^{\circ}\text{C}$  for 6 h and probed with monoclonal phospho-Ser473 specific anti-active Akt (1:200) and anti-CGRP antibody (1:100) at  $4^{\circ}\text{C}$  overnight. Detection was performed with alkaline phosphatase-conjugated goat anti-rabbit immunoglobulin G (IgG; 1:10,000) or goat anti-mouse IgG (1:10,000) and developed with 5-bromo-4-chloro-3-indolyphosphate *p*-toluidine salt (BCIP)-nitroblue tetrazolium chloride (NBT) color substrate. Immunoblotting bands were scanned

**Table 1** PCR primer sequences and product sizes

Molecule	Primer sequence	Product size (bp)
CGRP	5'-GTGTCAGTCCAGAGAAGATC-3'	102
	5'-CAAAGTTGTCCTTCACCACACC-3'	
$\beta$ -Actin	5'-CCTGTGGCATCCATGAAACTAC-3'	217
	5'-CTTCTGCATCCTGTCAGCGAT-3'	

and analyzed by image analyzer (LabWorks Software; UVP, Upland, CA, USA).

### Preparation of Drugs

The drugs used were chloralhydrat (SCRC, China), genistein (Sigma–Aldrich), capsazepine (Sigma–Aldrich), capsaicin (Sigma–Aldrich), CGRP8-37 (Sigma–Aldrich), and LY294002 (Beyotime, Haimen, China). Chloralhydrat and CGRP8-37 were dissolved in saline, while genistein, capsazepine, capsaicin, and LY294002 were dissolved in dimethyl sulfoxide (DMSO).

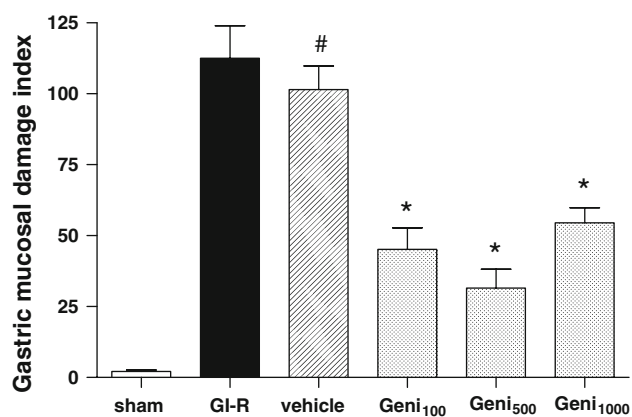
### Statistics

All results are expressed as mean  $\pm$  standard deviation (SD) for six to eight rats per group. Data were compared by one-way analysis of variance and Student's *t* test. All calculations were performed with SPSS 13.0 (SPSS, Chicago, IL, USA) for Windows.  $P < 0.05$  was considered significant.

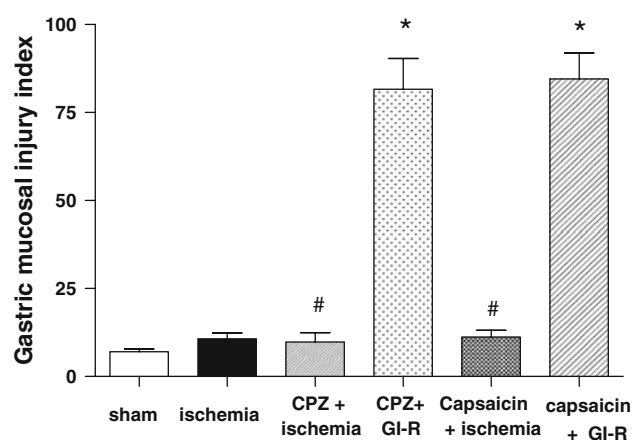
## Results

### Effects of Genistein Postconditioning on I–R-Induced Gastric Injuries in Rats

Laparotomy without clamping the gastric artery (sham operation) did not produce any damage in the gastric mucosa, while treatment of 30 min ischemia and 60 min reperfusion induced gastric injury. In order to ascertain the protective effects of genistein postconditioning and avoid tyrosine kinases inhibition, low doses of genistein (100,



**Fig. 2** Effects of different doses of genistein on I–R-induced gastric injuries in rats. Animals were all subjected to 30 min ischemia and 1 h reperfusion. Vehicle and genistein (100, 500, 1,000  $\mu\text{g}/\text{kg}$ , i.v.) was given 5 min before reperfusion. Gastric mucosal injury index was significantly reduced by genistein but not by vehicle. \*  $P < 0.05$  and #  $P > 0.05$  compared with GI–R group. Data presented as mean  $\pm$  standard deviation (SD)

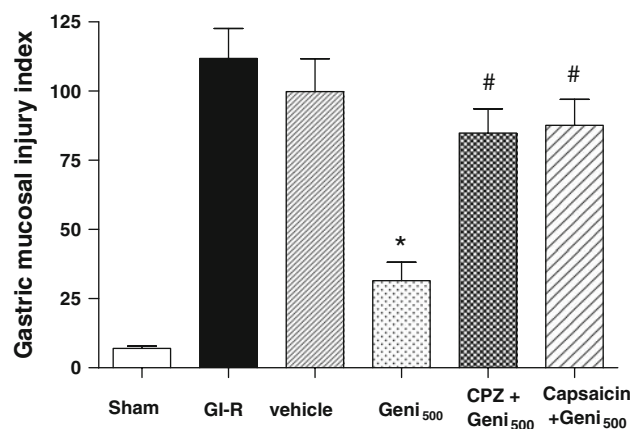


**Fig. 3** Effects of capsazepine and capsaicin on ischemia. Ischemia could not cause observable mucosal injury. Pretreatment with CPZ and excess capsaicin before experiment aggravated mucosal injury. #  $P > 0.05$  compared with ischemia. \*  $P < 0.05$  compared with CPZ + ischemia and capsaicin + ischemia, respectively

500, 1,000  $\mu\text{g}/\text{kg}$ , i.v.) were administered for postconditioning. As shown in Fig. 2, different doses of genistein had significant protective effect against I–R-induced gastric mucosal injury, particularly the dose of 500  $\mu\text{g}/\text{kg}$ .

### Effects of Capsaicin and Capsazepine on Genistein Postconditioning in Rats

After reperfusion, gastric mucosal injury index was measured and the sectioned paraffin tissue was prepared for TUNEL. As shown in Fig. 2, genistein at a low dose (500  $\mu\text{g}/\text{kg}$ , i.v.) significantly reduced I–R-induced gastric injury. However, this protective effect was significantly reversed by prior administration of capsazepine or capsaicin. The



**Fig. 4** Changes of gastric mucosal injury index with capsaicin, capsazepine, and genistein treatment in rats. Pretreatment with CPZ and excess capsaicin before experiment reversed the protective effect of genistein. \*  $P < 0.05$  compared with CPZ and capsaicin. #  $P > 0.05$  compared with GI–R

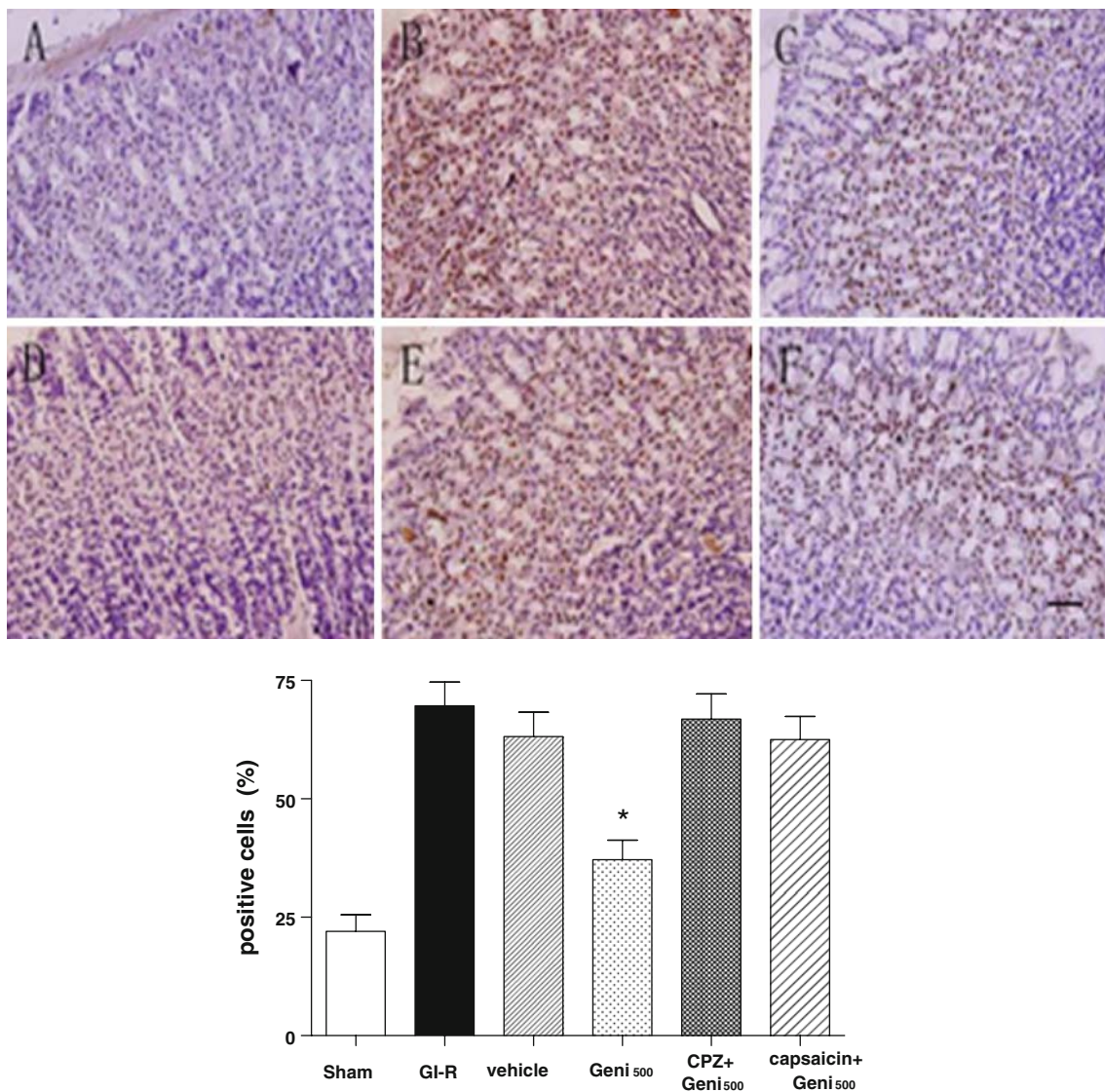
severity of I-R-induced gastric injury was reduced by genistein but was increased by capsazepine and capsaicin. It was firstly demonstrated that prior administration of capsazepine or excess capsaicin did not increase mucosal injury alone, as shown in Fig. 3. Figures 4 and 5 show that genistein reduced tissue apoptosis and injury index, while capsazepine and capsaicin reversed these protective effects.

#### Upregulated Expression of CGRP in Genistein Postconditioning

In addition to the examination of gastric mucosal injury index and tissue apoptosis, expression of CGRP in DRG

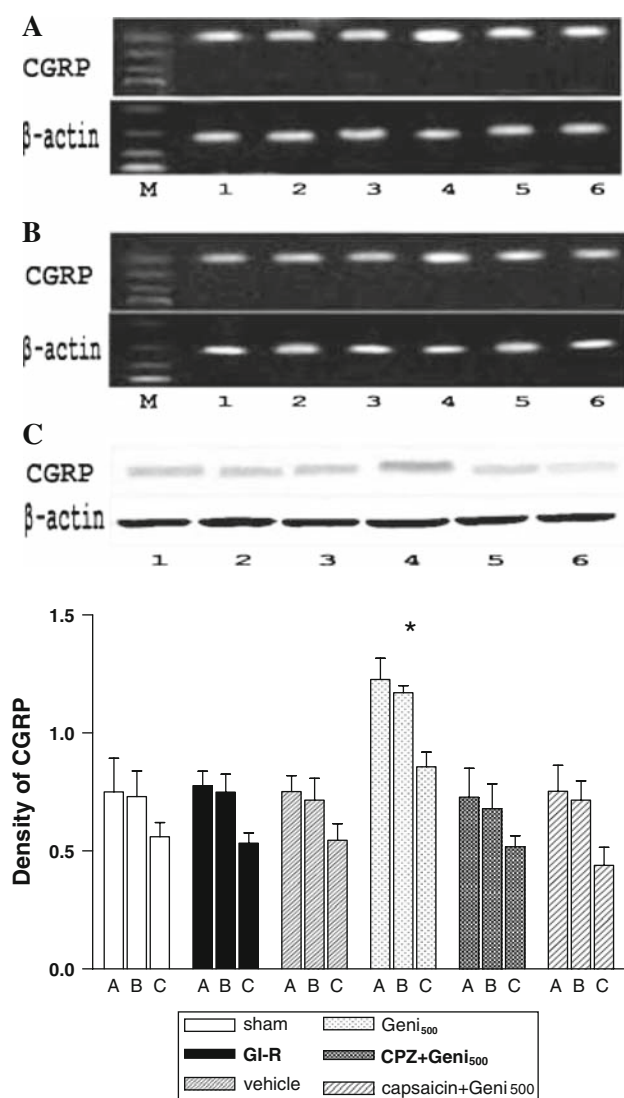
and gastric mucosa was determined. As illustrated in Fig. 6a and b, genistein postconditioning upregulated expression of CGRP messenger RNA (mRNA), which actually reflects CGRP protein, in both DRG and gastric mucosa, while capsaicin and capsazepine prevented the upregulation of CGRP mRNA. As illustrated in Fig. 6c, genistein postconditioning upregulated expression of CGRP protein in gastric mucosa, but this effect could be reversed by capsaicin and capsazepine.

In addition, CGRP8-37, a selective antagonist of CGRP, was administered (500 µg/kg, i.v.) before ischemia. As shown in Fig. 7, the severity of gastric injury increased compared with genistein group.



**Fig. 5** Effects of capsazepine, capsaicin, and genistein on I-R-induced gastric mucosal cellular apoptosis. A: sham; B: GI-R; C: vehicle; D: genistein; E: CPZ + Geni<sub>500</sub>; F: capsaicin + Geni<sub>500</sub>. Values shown are percentages of TUNEL-positive cells counted in ten

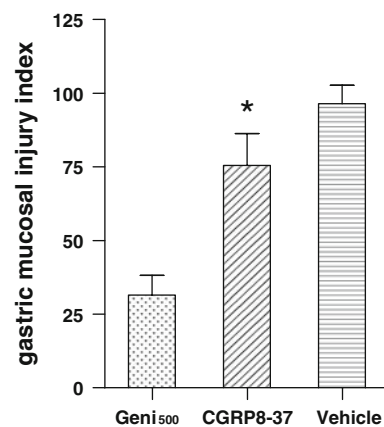
microscopic fields. \*  $P < 0.05$  compared with other groups except sham. There was no significant difference between groups of GI-R, vehicle, CPZ + Geni<sub>500</sub>, and capsaicin + Geni<sub>500</sub>. (Scale bar = 100 µm)



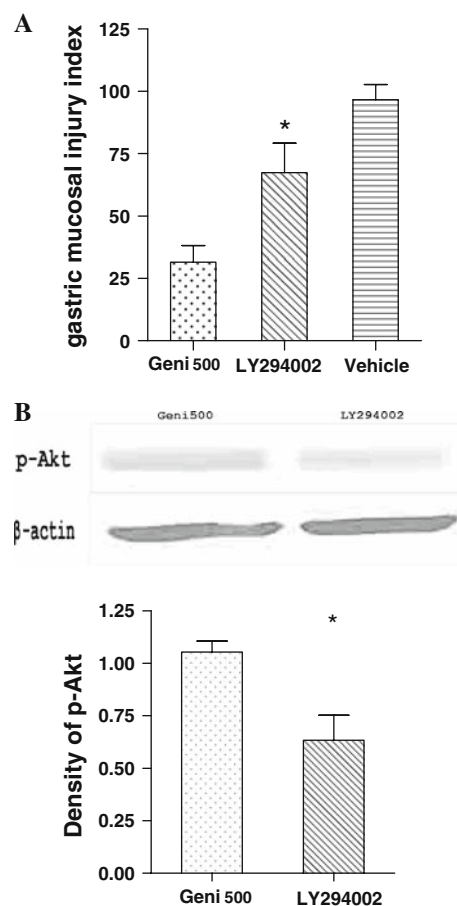
**Fig. 6** M: mark; 1: sham; 2: GI-R; 3: vehicle; 4: genistein; 5: capsazepine; 6: capsaicin. **a.** CGRP mRNA expression in DRG. **b.** CGRP mRNA expression in gastric mucosa. **c.** Content of CGRP in gastric mucosa. CGRP expression was upregulated by Geni<sub>500</sub> in DRG and gastric mucosa. \*  $P < 0.05$  compared, respectively, with the other groups

#### PI3K/Akt Pathway Involvement in the Protective Effects of Genistein Postconditioning

This experiment was intended to investigate whether genistein postconditioning could activate the phosphatidylinositol-3-kinase (PI3K)/Akt pathway, which is central to organ protection by ischemic postconditioning. LY294002 (1.5 mg/kg, i.v.) was administered prior to genistein postconditioning. As shown in Fig. 8, the severity of gastric injury increased, compared with genistein. Western blotting also demonstrated that activity of p-Akt increased in genistein group but not LY294002 group.



**Fig. 7** Effects of CGRP8-37 on genistein postconditioning. Compared with genistein group, the gastric mucosal injury index significantly increased. \*  $P < 0.05$  compared with Geni<sub>500</sub> group



**Fig. 8** **a** Effects of LY294002 on genistein postconditioning in gastric mucosal injury index. \*  $P < 0.05$  compared with Geni<sub>500</sub> group. **b.** Western blots of phosphor-Akt (p-Akt) and  $\beta$ -actin from the Geni<sub>100</sub> and the LY294002 groups. \*  $P < 0.05$  compared with Geni<sub>500</sub> group

## Discussion

Ischemia–reperfusion is known to lead to tissue injury [22–24]. As the experiment results showed, treatment of 30 min ischemia followed by 60 min reperfusion resulted in gastric mucosal injury. The mechanisms of tissue injury caused by I–R remains an important issue, despite there are numerous research reports about I–R injury. There is much evidence that ischemic postconditioning has a protective effect on organs subjected to ischemia-reperfusion [25–27]. The present study demonstrates that administration of pharmacological doses of genistein via peripheral vein attenuated GI–R injury in a dose- and time-dependent manner. However, the dose of 1 mg/kg of genistein exerted less protection than the dose of 0.5 mg/kg, which may be due to the exertion of a pleiotropic effect such as inhibition of tyrosine kinases by the higher genistein dose. These results clearly demonstrate that genistein postconditioning played a protective role in gastric mucosal defense.

To our knowledge, there are some reports that genistein has pleiotropic effects: high doses of genistein produced an effect similar to tyrosine kinase inhibitor [28], but myocardial infarct sizes and cardiocyte apoptosis in rabbits subjected to ischemia–reperfusion were significantly reduced by low doses of genistein (0.1–1.0 mg/kg, i.v.) [5, 29]. Also, a study showed a protective effect of genistein on gastric mucosal integrity in stress-induced lesion formation [30]. Furthermore, similar to  $17\beta$ -estradiol, genistein was shown to be cardioprotective and relied on the activation of estrogen receptor for its protective effect [31].

However, our study established that capsaicin receptor subtype 1 ( $VR_1$ ) could be activated by genistein postconditioning. In order to avoid tyrosine kinases inhibition, three doses of genistein (100, 500, and 1,000  $\mu$ g/kg, i.v.) were administered 5 min before reperfusion. As the results showed,  $Geni_{500}$  had a significant effect against I–R-induced injury. Interestingly, in subsequent study, it was found that capsazepine and excess capsaicin could prevent the gastric protective effects of genistein. The percentage of TUNEL-positive nuclei within the infarcted area was decreased by genistein. The finding that genistein activates  $VR_1$  was novel. In fact, there are many reports that capsaicin-sensitive neurons distributed in gastrointestinal tract act as devices of molecular integration and introduced such information as thermal, mechanical, chemical, and ischemic stimuli to the central nerve system [12, 32].  $VR_1$  was expressed in these capsaicin-sensitive neurons and other histiocytes such as gastric epithelial cells, and  $VR_1$  was involved in the cellular protection of the gastric mucosa [33].

CGRP, a neuropeptide consisting of 37 amino acids that is secreted by capsaicin-sensitive neurons and other

histiocytes, has been confirmed to have a protective effect on myocardial and endothelial cells subjected to I–R [34–36], particularly exhibiting a critical effect on reducing stress-induced gastric mucosal injury. Interestingly, the levels of CGRP in gastric tissue were affected by estrogen and isoflavone in ovariectomized rats [37]. Our study also showed that upregulation of CGRP was related to genistein postconditioning and that the effect was prevented by capsazepine and excess capsaicin. RT-PCR and Western blotting showed that expression of CGRP in DRG and gastric mucosa was upregulated in  $Geni_{500}$ , instead of capsazepine and capsaicin. The upregulated expression of CGRP suggested that the effect of genistein postconditioning was related to activation of  $VR_1$  and was mainly mediated by CGRP. Furthermore, CGRP8-37 was administered prior to genistein postconditioning to further explore CGRP mediation of the protective effect against I–R-induced gastric mucosal injury. As the results showed, gastric mucosal injury index of CGRP8-37 remarkably increased in comparison with  $Geni_{500}$ .

The antiapoptotic effect of the PI3K–Akt pathway is well established [38]. Many studies have demonstrated that the PI3k–Akt pathway is involved in the repair of brain and heart injury induced by ischemia–reperfusion [39, 40]. LY294002 specifically inhibits the class I phosphatidylinositol-3-kinase (PI3K)/Akt pathway [40]. In this study, LY294002 reversed the protective effects of genistein. According to Fig. 8, compared with genistein, the gastric mucosal injury index increased and expression of p-Akt decreased. These results suggest that the PI3k–Akt pathway participates in the protective effect of genistein postconditioning.

Based on all the results of our present study, genistein postconditioning was confirmed to have protective effects on I–R-induced gastric injury, in spite of reports that genistein exerts pharmacological postconditioning with potency similar to  $17\beta$ -estradiol by activating the estrogen receptor [31]. Our present findings suggest that the capsaicin receptor ( $VR_1$ ) is activated in genistein postconditioning. Capsaicin-sensitive neurons and gastric epithelial cells further released bioactive peptide CGRP to reduce I–R-induced gastric mucosal injury. In addition, the PI3k–Akt pathway was involved in the protective effects of genistein postconditioning, consistent with results in other tissues subjected to I–R injury.

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**Conflict of interest statement** There is no conflict of interest.

## References

- Souzeau E, Belanger S, Picard S, et al. Dietary isoflavones during pregnancy and lactation provide cardio protection to offspring rats in adulthood. *Am J Physiol Heart Circ Physiol*. 2005;289:H715–H721.
- Walker HA, Dean TS, Sanders TA, et al. The phytoestrogen genistein produces acute nitric oxide-dependent dilation of human forearm vasculature with similar potency to 17beta-estradiol. *Circulation*. 2001;103:258–262.
- Rishi RK. Phytoestrogens in health and illness. *Indian J Pharmacol*. 2002;34:311–320.
- Jiang F, Jones GT, Husband AJ. Cardiovascular protective effects of synthetic isoflavone derivatives in apolipoprotein E-deficient mice. *J Vasc Res*. 2003;40:276–284.
- Ji ES, Yue H, Wu YM. Effects of phytoestrogen genistein on myocardial ischaemia-reperfusion injury and apoptosis in rabbits. *Acta Pharmacol Sin*. 2004;25:306–312.
- Amira S, Rotondo A, Mulè F. Relaxant effects of flavonoids on the mouse isolated stomach: structure-activity relationships. *Eur J Pharmacol*. 2008;599:126–130.
- Takekawa S, Matsui T, Arakawa Y. The protective effect of the soybean polyphenol genistein against stress-induced gastric mucosal lesions in rats, and its hormonal mechanisms. *J Nutr Sci Vitaminol*. 2006;52:274–280.
- Purcell AL, Carew TJ. Modulation of excitability in Aplysia tail sensory neurons by tyrosine kinases. *J Neurophysiol*. 2001;85:2398–2411.
- Linford NJ, Dorsa DM. 17beta-Estradiol and the phytoestrogen genistein attenuate neuronal apoptosis induced by the endoplasmic reticulum calcium-ATPase inhibitor thapsigargin. *Steroids*. 2002;67:1029–1040.
- Ward SM, Bayguinov J, Won KJ, et al. Distribution of the vanilloid receptor (VR1) in the gastrointestinal tract. *J Comp Neurol*. 2003;465:121–135.
- Patterson LM, Zheng H, Ward SM, et al. Vanilloid receptor (VR1) expression in vagal afferent neurons innervating the gastrointestinal tract. *Cell Tissue Res*. 2003;311:277–287.
- Sasamra T, Kuraishi Y. Peripheral and central actions of capsaicin and VR1 receptor. *Jpn J Pharmacol*. 1999;80:275–280.
- Faussone-Pellegrini MS, Taddei A, Bizzoco E, et al. Distribution of the vanilloid (capsaicin) receptor type 1 in the human stomach. *Histochem Cell Biol*. 2005;124:61–68.
- Kang JY, Teng CH, Chen FC, et al. Effect of capsaicin and cimetidine on the healing of acetic acid induced gastric ulceration in the rat. *Gut*. 1996;38:832–836.
- Holzer P. Efferent-like roles of afferent neurons in the gut: Blood flow regulation and tissue protection. *Auton Neurosci*. 2006;125:70–75.
- Kwiecien S, Brzozowski T, Konturek PC, et al. The role of reactive oxygen species and capsaicin-sensitive sensory nerves in the pathomechanisms of gastric ulcers induced by stress. *J Physiol Pharmacol*. 2003;54:423–437.
- Osaki M, Kase S, Adachi K, et al. Inhibition of the PI3K-Akt signaling pathway enhances the sensitivity of Fas-mediated apoptosis in human gastric carcinoma cell line, MKN-45. *J Cancer Res Clin Oncol*. 2004;130:8–14.
- Plastaras JP, Dorsey JF, Carroll K, et al. Role of PI3K/Akt signaling in TRAIL- and radiation-induced gastrointestinal apoptosis. *Cancer Biol Ther*. 2008;7:2047–2053.
- Wada K, Kamisaki Y, Kitano M, et al. A new gastric ulcer model induced by ischaemia-reperfusion in the rat: role of leukocytes on ulceration in rat stomach. *Life Sci*. 59:PL295–PL301.
- Guth PH, Aures D, Poulsen G. Topical aspirin plus HCl gastric lesions in the rat. Cytoprotective effect of prostaglandin, cimetidine and probanthine. *Gastroenterology*. 1979;76:88–93.
- Towbin H, Staehelin T, Gordon J, et al. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. *Proc Natl Acad Sci USA*. 1979;76:4350–4354.
- Farber JL, Chien KR, Mittnacht S Jr. Myocardial ischaemia: the pathogenesis of irreversible cell injury in ischaemia. *Am J Pathol*. 1981;102:271–281.
- Cheung JY, Bonventre JV, Malis CD, et al. Calcium and ischemic injury. *N Engl J Med*. 1986;314:1670–1676.
- Piper HM, Meuter K, Schafer C, et al. Cellular mechanisms of ischaemia reperfusion injury. *Ann Thorac Surg*. 2003;75:S644–S668.
- Zhao ZQ, Corvera JS, Halkos ME, et al. Inhibition of myocardial injury by ischemic postconditioning during reperfusion: comparison with ischemic preconditioning. *Am J Physiol Heart Circ Physiol*. 2003;285:H579–H588.
- Kin H, Zhao ZQ, Sun HY, et al. Postconditioning attenuates myocardial ischaemia-reperfusion injury by inhibiting events in the early minutes of reperfusion. *Cardiovasc Res*. 2004;62(1):74–85.
- Sun K, Liu ZS, Sun Q. Role of mitochondria in cell apoptosis during hepatic ischaemia-reperfusion injury and protective effect of ischemic postconditioning. *World J Gastroenterol*. 2004;10:1934–1938.
- Akiyama T, Ishida J, Nakagawa S, et al. Genistein, a specific inhibitor of tyrosine-specific protein kinases. *J Biochem*. 1987;262:5592–5595.
- Deodato B, Altavilla D, Squadrito G. Cardioprotection by the phytoestrogen genistein in experimental myocardial ischaemia-reperfusion injury. *Br J Pharmacol*. 1999;128:1683–1690.
- Tissier R, Waintraub X, Couvreur N. Pharmacological postconditioning with the phytoestrogen genistein. *J Molecul Cellul Cardiol*. 2007;42:79–87.
- Cesare P, Moriondo A, Vellani V. Ion channels gated by heat. *Proc Natl Acad Sci*. 1999;96:7658–7663.
- Katoa S, Aihara E, Nakamura A, et al. Expression of vanilloid receptors in rat gastric epithelial cells: role in cellular protection. *Biochem Pharmacol*. 2003;66:1115–1121.
- Ren YS, Ma TG, Wang HB, et al. Protective effects of calcitonin gene-related peptide (CGRP) on myocardial cell injury and magnesium contents following severe hypoxia and simulated reperfusion. *Med Sci Res*. 1993;21:177–178.
- Li YJ, Peng CF, Li NS, et al. Calcitonin gene-related peptide protects against endothelial cell damage due to oxidized low-density lipoprotein. *Med Sci Res*. 1993;23:253–254.
- Li YJ, Peng J. The cardioprotection of calcitonin gene-related peptide mediated preconditioning. *Eur J Pharmacol*. 2002;442:173–177.
- Shimozawa N, Okajima K, Harada N, et al. Estrogen and isoflavone attenuate stress-induced gastric mucosal injury by inhibiting decreases in gastric tissue levels of CGRP in ovariectomized rats. *Am J Physiol Gastrointest Liver Physiol*. 2007;292:G615–G619.
- Franke TF, Hornik CP, Segev L, et al. Sugimoto C: PI3K/Akt and apoptosis: size matters. *Oncogene*. 2003;22:8983–8998.
- Shibata M, Yamawki T, Sasaki T. Upregulation of Akt phosphorylation at the early stage of middle cerebral artery occlusion in mice. *Brain Res*. 2002;942:1–10.
- Matsui T, Tao J, del Monte F, et al. Akt activation preserves cardiac function and prevents injury after transient cardiac ischaemia in vivo. *Circulation*. 2001;104:330–335.
- Xing C, Zhu B, Liu H, et al. Class I phosphatidylinositol 3-kinase inhibitor LY294002 activates autophagy and induces apoptosis through p53 pathway in gastric cancer cell line SGC7901. *Acta Biochim Biophys Sin*. 2008;40:194–201.