

**Gosha-jinki-gan Reduces Transmitter Proteins and Sensory Receptors Associated  
with C Fiber Activation Induced by Acetic Acid in Rat Urinary Bladder**

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Short title: Effect of Gosha-jinki-gan to C fiber nerves

Word count: 2979/3000

**Abstract**

**Aims:** We determined if Gosha-jinki-gan, a traditional Chinese herbal mixture, reduced the presence of the tachykinins neurokinin A, neurokinin B, and substance P, as well as the transient receptor potential vanilloid 1 (TRPV1) and P2X3 purine receptors that are functionally associated with C fibers in the urinary bladder. **Methods:** Thirty-six female rats were fed with either a standard diet or one supplemented with 1.08% Gosha-jinki-gan. After 4 weeks, the urinary bladders were instilled with either saline or 0.1% acetic acid. After 30 minutes, the bladders were removed and expression of the tachykinins and the TRPV1 and P2X3 receptors was determined by immunohistochemistry and mRNA expression. **Results:** In rats fed with the standard diet, the tachykinins and the TRPV1 and P2X3 receptors expressed nearby or within urothelium of the acetic acid-treated rats increased compared with the saline-instilled rats. In rats pretreated with Gosha-jinki-gan, the tachykinins and the TRPV1 and P2X3 receptors in the acetic acid-treated rats also increased compared with the saline-instilled rats. However, with the instillation of acetic acid, the tachykinins and the TRPV1 and P2X3 receptors of Gosha-jinki-gan pretreated rats decreased compared with standard diet fed rats. The mRNA expression levels of neurokinin A, substance P, and the TRPV1 receptor in acetic acid-treated Gosha-jinki-gan pretreated rats were lower than that in acetic acid-treated standard diet fed rats. Gosha-jinki-gan did not destroy nerve fibers within the bladders. **Conclusions:** Gosha-jinki-gan partially reduced the tachykinins and TRPV1 and P2X3 purine receptors without destroying the nerve fibers.

(247/250 words)

**Key words: Gosha-jinki-gan; urinary bladder; C fibers; tachykinins; TRPV1 receptor; P2X3 purine receptor; rat**

## INTRODUCTION

Traditional Chinese herbal mixtures are used as complementary treatments in western medicine or as drugs to reduce side effects of therapeutic agents.<sup>1-3</sup> Gosha-jinki-gan is one of these herbal mixtures that is composed of ten natural ingredients that are produced as hot water extracts from the following sources: *Rehmannia glutinosa*, *Achyranthes* root, *Cornus officinalis*, *Dioscorea* rhizome, *Plantago* seed, *Alisma orientale*, *Porica cocos*, *Moutan* cortex, *Cinnamomum cassia*, and *Aconite* tuber.<sup>4</sup> Gosha-jinki-gan is classified with drugs that affect sensory nerves.<sup>5,6</sup> In urology clinical practices, Gosha-jinki-gan is used for treatments of patients with urinary frequency and incontinence due to storage dysfunctions and/or dysuria due to diabetes mellitus.<sup>7,8</sup> Gosha-jinki-gan decreases detrusor contractions and increases bladder capacity while not reducing voiding pressure.<sup>9,10</sup> These characteristics are similar to anti-cholinergic agents, but Gosha-jinki-gan induces few side effects such as dry mouth and residual urine due to decreased bladder compliance.<sup>11</sup> Gosha-jinki-gan studies have shown that it acts in many complicated ways.<sup>4,12-14</sup>

In this study, we focused on the response of unmyelinated C fibers within rat urinary bladders to investigate the effects of Gosha-jinki-gan. Currently these afferent nerve fibers are considered to play no role in normal micturition.<sup>15-17</sup> However, C fibers do take part in voiding reflexes when they are activated by cystitis or neurological dysfunction due to spinal cord lesions or brain diseases. The activated C fibers induce urinary frequency and/or incontinence.<sup>18-21</sup> In rats, we reported that instillation of acetic acid induces detrusor overactivity by C fiber activation in the urinary bladder.<sup>22</sup> This overactivity was mitigated by resiniferatoxin through desensitization of the C fibers. In that report, we found similar results when the rats were placed on a diet containing Gosha-jinki-gan. The tachykinins neurokinin A, neurokinin B, and substance P, which are present within sensory nerves and locally released by a number of physical and chemical stimuli,<sup>23,24</sup> have many important biological actions, including C fiber activation. Other animal studies have shown that transient receptor potential vanilloid 1 (TRPV1)<sup>18, 25</sup> and P2X3 purine receptors<sup>26</sup> also have important roles in bladder overactivity. Therefore, we sought to determine if Gosha-jinki-gan reduces these transmitter proteins and sensory receptors as one of the mechanisms to mediate acetic acid-induced C fiber activation.

## **MATERIALS AND METHODS**

### **Animals**

Female Sprague-Dawley rats (n = 36, 190-210 g, Japan SLC Inc., Shizuoka, Japan) at postnatal week 10 were used for the experiments. The animals were treated in accordance with National Institutes of Health Animal Care Guidelines and the guidelines approved by the Animal Ethics Committee of Shinshu University School of Medicine.

### **Gosha-jinki-gan Pretreatment**

We pretreated the experimental animals with Gosha-jinki-gan (TJ-107, Tsumura Co., Tokyo, Japan) according to previously described methods.<sup>22</sup> Briefly, the food purchased from the vendor (CLEA Japan, Inc., Tokyo, Japan) contained 1.08% of the herbal extract mixture Gosha-jinki-gan, the same dose as in our previous study.<sup>22</sup>

The experimental animals were randomly divided into four groups (n = 9 in each group) that were fed for 4 weeks as follows. Groups 1 and 2 were fed with a standard diet (CLEA Japan, Inc.), while Groups 3 and 4 were fed with a diet supplemented with Gosha-jinki-gan. All of the animals were maintained under a 12-hour alternating light-dark cycle, with freely available food and water. Each rat consumed about 15 g/day of the standard or Gosha-jinki-gan-supplemented diet. At 4 weeks after feeding, the animals weighed 230-250 g, and there were no significant differences within each group or among the groups.

### **Instillation of Acetic Acid into Urinary Bladder**

At the end of the 4 weeks, the rats were anesthetized with an intraperitoneal injection of ketamine hydrochloride (75 mg/kg body weight, Sankyo Eeru Medicine Co., Tokyo, Japan) and xylazine (15 mg/kg body weight, Bayer, Leverkusen, Germany). A polyethylene catheter (PE50, Nippon Becton Dickinson, Tokyo, Japan) was inserted through the urethra, and residual urine was removed. Through the catheter, the bladders of rats in Groups 1 and 3 were instilled with physiological saline (0.9% NaCl solution, Otsuka Pharmaceutical Co., Ltd., Tokyo, Japan) maintained at room temperature (27±2°C). The bladders of rats in Groups 2 and 4 were instilled with room temperature 0.1% acetic acid (Wako Co., Osaka, Japan) in saline, pH 4.0. Each bladder received 800 µl of the instilled solution. Thirty minutes after the instillation, the bladders were removed, and the animals were then sacrificed.

### **Immunohistochemistry**

The removed bladders (n = 3 in each group) were rinsed with saline, and then fixed in 4% paraformaldehyde with 4% sucrose in 0.1 M phosphate buffer for 12 hours at 4°C. The tissues were embedded with OCT compound (Sakura Co., Tokyo, Japan) in dry-ice hexane. For immunostaining, the tissue sections (5 µm) were soaked in phosphate buffered saline (PBS) for 5 minutes at 4°C. Antigen retrieval was achieved by immersion of the sections in 10 mM sodium citrate and microwaving at 100°C for 5 minutes. The specimens were coated with 1.5% normal donkey serum (Chemicon International Inc., Temecula, CA) and 1.5% non-fat milk in PBS for 1 hour at 4°C. The sections were incubated with one of the following antibodies: anti-neurokinin A (1:200, rabbit polyclonal, American Research Products, Inc., Belmont, MA), anti-neurokinin B (1:100, goat polyclonal, Santa Cruz Biotechnology Inc., Santa Cruz, CA), anti-substance P (1:100, rat monoclonal, Chemicon International Inc.), anti-TRPV1 receptor (1:1000, rabbit polyclonal, Chemicon International Inc.), or anti-P2X3 purine receptor (1:500, rabbit polyclonal, Chemicon International Inc.) for 12 hours at 4°C. Some sections were also incubated with one of the following antibodies: anti-S100 antibody (1:50, mouse monoclonal, Abcam Ltd., Cambridge, UK), a marker of nerve fibers, or anti-calcitonin gene-related peptide (CGRP) antibody (1:800, guinea pig polyclonal, Progen Biotechnik GmbH, Heidelberg, Germany), a marker of afferent nerve fibers, for 12 hours at 4°C. The sections were rinsed with PBS at 4°C, and then incubated with secondary antibodies appropriate to the primary antibodies: donkey anti-rabbit, -goat, -rat, -mouse or -guinea pig IgG conjugated with Alexa fluor 594 (1:250, Molecular Probes, Eugene, OR) for 1 hour at 4°C. Following rinsing, double staining of each section was achieved by incubation with anti-uroplakin III (UP III, 1:100, goat polyclonal, Santa Cruz Biotechnology Inc.), a marker for urothelium or anti-alpha smooth muscle actin (SMA, 1:100, mouse monoclonal, Progen) for 12 hours at 4°C. Following a rinse with PBS, they were incubated with secondary antibody consisting of donkey anti-goat or -mouse IgG conjugated with Alexa fluor 488 (1:250, Molecular Probes) for 1 hour at 4°C. Finally, cell nuclei were counterstained with 5 µg/ml 4', 6-diamidino-2-phenylindole dihydrochloride (DAPI, Molecular Probes). The slides were coated with Fluorescent Mounting Medium (Dako Cytomation, Carpinteria, CA) and observed with a Leica DAS Microscopethe (Leica Microsystems GmbH, Wetzlar, Germany).

Observers, who were not aware of the treatment status, semi-quantitatively evaluated the presence of fluorescence markers in each slide. In an area of 0.01 mm<sup>2</sup>

(100 x 100  $\mu\text{m}$ ) taken from the dome of each bladder, the presence of fluorescently tagged spots was scored as follows: +++, 31-60 spots; ++, 21-30 spots; +, 11-20 spots; -, 0-10 spots.

### **Real-Time Reverse Transcription Polymerase Chain Reaction (RT-PCR)**

Thirty minutes after instillation of saline or 0.1% acetic acid, the dome of each bladder (5 x 5 mm) was removed for estimation of mRNA expression (n = 6 in each group). Total RNA was extracted from the fragments of the bladder with the RNeasy Mini Kit (Qiagen Inc., Valencia, CA). Complementary DNA (cDNA) was synthesized from 0.2  $\mu\text{g}$  of the total RNA with the High-Capacity cDNA Archive Kit (Applied Biosystem, Foster City, CA). Real time RT-PCR of the cDNA was performed at 50°C for 2 minutes followed by 95°C for 10 minutes. These were followed by 40 cycles at 95°C for 15 seconds and 60°C for 1 minute. The following primers (Applied Biosystem) were used: Tac1 (Rn00562002\_m1) for neurokinin A, Tac2 (Rn00569785\_m1) for neurokinin B, Tac4 (Rn00597278\_m1) for substance P, Trpv1 (Rn00583117\_m1) for the TRPV1 receptor, and P2rx3 (Rn00579301\_m1) for the P2X3 purine receptor. Gene activity was expressed as the ratio to the internal standard gene beta-actin (Rn00667869\_m1).

### **Statistical Analysis**

Results were expressed as means  $\pm$  standard error of the means. Non-repeated measures Student's t-test was used to compare RNA expression levels in saline and acetic acid instilled rats within each diet condition. It was also used to compare levels of expression in saline instilled rats fed the standard and Gosha-jinki-gan diets. Statistical differences between acetic acid treated rats that were on the standard and Gosha-jinki-gan diets were determined by non-repeated measures ANOVA followed by the Scheffe's test. Differences with  $p < 0.05$  were considered significant.

## RESULTS

### Immunohistochemistry of Tachykinins and Sensory Receptors

After 4 weeks of the standard or herbal-supplemented diet and after 30 minutes instillation of either saline or 0.1% acetic acid into the urinary bladders, neurokinin A, neurokinin B, substance P, TRPV1 receptors, and P2X3 purine receptors within or nearby the urothelium of the rats in each group were visualized by immunohistochemistry (Figs. 1 and 2). The visualized tachykinins, and the TRPV1 and P2X3 receptors were semi-quantitatively assessed (Table I). In rats fed with the standard diet, the presence of the tachykinins and the TRPV1 and P2X3 receptors in the acetic acid-treated rats (Fig. 1D-F; Fig. 2C,D; Table I) increased compared with the saline-instilled rats (Fig. 1A-C; Fig. 2A,B; Table I). In rats pretreated with Gosha-jinki-gan, the presence of the tachykinins and the TRPV1 and P2X3 receptors in the acetic acid-treated rats (Fig. 1J-L; Fig. 2G,H; Table I) also increased compared with the saline-instilled rats (Fig. 1G-I; Fig. 2E,F; Table I). After instillation of saline, the presence of the tachykinins and the TRPV1 and P2X3 receptors in rats fed with standard (Fig. 1A-C and Fig. 2A,B) and Gosha-jinki-gan diets (Fig. 1G-I and Fig. 2E,F, Table I) were similar. However, in rats treated with acetic acid, the presence of the tachykinins and the TRPV1 and P2X3 receptors in the Gosha-jinki-gan pretreated rats (Fig. 1J-L; Fig. 2G,H; Table I) decreased compared with the standard diet fed rats (Fig. 1D-F; Fig. 2C,D; Table I).

### Gene Expression Levels of Tachykinins and Receptors

After the dietary and instillation periods, we estimated mRNA expression levels of the tachykinins and the TRPV1 and P2X3 receptors within the bladders (Table II). In rats fed with the standard diet, the mRNA levels of neurokinin A, neurokinin B, substance P, and TRPV1 receptor in the acetic acid-treated rats were significantly higher than in the saline-instilled rats (Table II). In contrast, the mRNA for P2X3 purine receptor was not significantly altered.

In rats pretreated with the Gosha-jinki-gan, the mRNA levels of neurokinin A, neurokinin B, and substance P in the acetic acid-treated rats were also significantly higher than in the saline-instilled rats (Table II). In contrast, the mRNA levels for TRPV1 receptor and P2X3 purine receptor were not significantly altered. In these Gosha-jinki-gan pretreated rats instilled with saline, the mRNA levels of neurokinin A and substance P were lower, and the level of neurokinin B was higher than in standard diet fed rats instilled with saline (Table II). However, the mRNA levels for TRPV1

receptor and P2X3 purine receptor were not significantly altered. In bladders instilled with acetic acid, the mRNA levels for neurokinin A, substance P, and TRPV1 receptor of Gosha-jinki-gan pretreated rats were significantly lower than that of standard diet fed rats (Table II). In contrast, the mRNA levels for neurokinin B and P2X3 purine receptor were not significantly altered.

#### **Effect of Gosha-jinki-gan on Nerve Fibers in Urinary Bladders**

Four weeks after feeding with standard and Gosha-jinki-gan diets, the nerve fibers within the urinary bladders were visualized by immunohistochemistry with S100 and CGRP antibodies. The bladders of the rats fed with the standard diet had distinct S100-positive nerve fibers under the urothelium (Fig. 2A) and CGRP-positive afferent nerve fibers within the smooth muscle layers (Fig. 2B). The bladders of the rats pretreated with the Gosha-jinki-gan also had S100-positive nerve fibers (Fig. 2C) and CGRP-positive afferent nerves (Fig. 2D). Thus, Gosha-jinki-gan did not destroy the nerve fibers present within the bladders.

## DISCUSSION

We have focused on C fibers to investigate the mechanisms by which Gosha-jinki-gan mitigates bladder overactivity. Previously we found that bladder overactivity response to the instillation of acetic acid was partially mitigated by pretreatment with Gosha-jinki-gan.<sup>22</sup> The mitigation of the bladder overactivity was confirmed in the rats desensitized with resiniferatoxin. Clinically, desensitization of C fibers is an effective treatment for bladder overactivity induced by different neurological diseases.<sup>17,27-29</sup> In this study, we found that Gosha-jinki-gan partially reduced the neurokinin A, neurokinin B, substance P, TRPV1 receptors, and P2X3 purine receptors, which are functionally associated with C fibers in the urinary bladder.

After the instillation of saline, the presence of the tachykinins and the TRPV1 and P2X3 receptors expressed within or nearby the urothelium of rats pretreated with Gosha-jinki-gan was similar to that of rats fed with standard diet. However, after the instillation of acetic acid, the presence of the tachykinins and the TRPV1 and P2X3 receptors of Gosha-jinki-gan pretreated rats decreased compared with standard diet fed rats. For the Gosha-jinki-gan pretreated rats that were instilled with acetic acid, mRNA expression levels of neurokinin A, substance P, and TRPV1 receptor were lower than that in the standard diet fed rats. In contrast, the mRNA expression levels for neurokinin B and P2X3 purine receptor of Gosha-jinki-gan pretreated rats were not significantly different from that of standard diet fed rats.

The differences between the immunohistochemical observations and quantitative analysis of mRNA expressions of neurokinin B and P2X3 purine receptor might be explained as follows. The tissues used to determine mRNA expression levels included the urothelium as well as the underlying smooth muscle layers and vascular tissues. The instillation of acetic acid directly stimulates the urothelium but not underlying tissues. In normal rat urinary bladders, the mRNA expression of neurokinin B is much lower than that of neurokinin A and substance P.<sup>24,30</sup> P2X3 purine receptors present in the urothelium have a major role in the release of ATP when the urothelium is stretched, as occurs with bladder distention. Therefore, it is possible that a longer period of acetic acid instillation would more clearly show effects of the pretreatment with Gosha-jinki-gan in the mRNA expression levels for neurokinin B and P2X3 purine receptor.

We suggest that Gosha-jinki-gan partially suppresses the immediate production of the tachykinins neurokinin A and substance P mRNA. After the instillation of saline, the rats pretreated with Gosha-jinki-gan had lower mRNA expression levels of these

tachykinins compared with the rats fed the standard diet. In contrast, the mRNA level for neurokinin B of Gosha-jinki-gan pretreated rats was higher than that of standard diet fed rats. Neurokinin A, neurokinin B, and substance P possess the highest affinity for NK2, NK3, and NK1 receptors, respectively. However, an extensive crosstalk can occur between different tachykinins and receptors.<sup>24</sup> The increased neurokinin B mRNA expression level of Gosha-jinki-gan pretreated rats suggests an increase in the transcription of the neurokinin B gene relative to the neurokinin A and substance P genes. For each of the tachykinins, the effects of Gosha-jinki-gan were found after the instillation of saline. The instillation of saline, which is a weaker stimulus than acetic acid, may stimulate C fibers because it unphysiologically produces bladder distention. Tachykinins are released by a number of physical and chemical stimuli.<sup>23,24</sup> Therefore, Gosha-jinki-gan has the potential to suppress the immediate production of tachykinin mRNAs induced by various stimuli.

TRPV1 and P2X3 purine receptors play a role in inflammatory pain and are associated with bladder overactivity through C fiber activation.<sup>18,25,26</sup> TRPV1 receptors are nonselective cationic channels. While one report indicates that they do not take part in acetic acid-stimulated bladder overactivity,<sup>31</sup> they do respond to noxious stimuli such as temperatures above 43°C and pH less than 6. Increased TRPV1 receptors have been found in patients with spinal neurogenic detrusor overactivity.<sup>32</sup> Gosha-jinki-gan reduced the presence and the mRNA expression level of TRPV1 receptors. While the mRNA expression level of P2X3 purine receptors did not change, the presence of P2X3 purine receptors expressed within or nearby the urothelium tended to be reduced by the treatment of Gosha-jinki-gan. Therefore, our study suggests that Gosha-jinki-gan has the potential to reduce these sensory receptors.

Resiniferatoxin desensitization in rats also mitigated the bladder overactivity induced by acetic acid.<sup>22</sup> However, these bladders had few CGRP-positive afferent nerve fibers compared with normal rats due to the resiniferatoxin-induced degeneration or destruction of nerve fibers.<sup>33</sup> Therefore in the current study, we determined if the changes in tachykinins and TRPV1 and P2X3 purine receptors were the result of the nerve fiber destruction by Gosha-jinki-gan. We verified by immunohistochemistry the presence of S100-positive nerve fibers under the urothelium and CGRP-positive afferent nerves within the smooth muscle layers in urinary bladders. The rats pretreated with Gosha-jinki-gan had distinct nerve fibers just as the rats fed with the standard diet. These results suggest that Gosha-jinki-gan altered the release of neurokinin A, neurokinin B, and substance P and the expression of TRPV1 receptors and P2X3 purine

receptors without destroying nerve fibers.

We have not yet determined if Gosha-jinki-gan can inhibit bladder overactivity in animal disease models such as hypertension, diabetes mellitus, urethral stricture, or a long-term chemical irritant model. However, the effects of Gosha-jinki-gan were clearly evident in the instillation of acetic acid. Therefore it is possible that Gosha-jinki-gan might mitigate bladder overactivity caused by injury and/or disease.

## CONCLUSION

In this study, we found that the traditional Chinese herbal mixture known as Gosha-jinki-gan reduced tachykinins, TRPV1 receptors, and P2X3 receptors expressed within rat bladder urothelium that was stimulated by acetic acid. Moreover, the instillation of acetic acid caused significant decreases in the mRNA expression levels of neurokinin A, substance P, and TRPV 1 receptor in Gosha-jinki-gan pretreated rats compared to standard diet fed rats. The effects of Gosha-jinki-gan were achieved without destroying the nerve fibers within the bladders. Therefore, Gosha-jinki-gan partially mediates C fiber activation through the reduction of the tachykinins, TRPV1 receptors, and P2X3 purine receptors in the urinary bladders.

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### Figure legends

**Fig. 1.** Effect of diet and bladder treatment on the presence of tachykinins. After 4 weeks of standard and Gosh-jinki-gan-supplement diet and 30 minutes of instillation of saline and 0.1% acetic acid into urinary bladders, neurokinin A (**A, D, G, and J**), neurokinin B (**B, E, H, and K**), substance P (**C, F, I, and L**) expressed within or nearby the urothelium (green) in each group were visualized. In rats fed with standard diets, the presence of tachykinins in acetic acid-treated bladders (red, arrow, **D-F**) increased compared with the saline-instilled ones (red, arrows, **A-C**). In rats pretreated with Gosha-jinki-gan, the presence of tachykinins in the acetic acid-treated bladders (red, arrows, **J-L**) also increased compared with the saline-instilled ones (red, arrow, **G-I**). After the instillation of acetic acid, the presence of tachykinins of Gosha-jinki-gan pretreated rats decreased compared with standard diet fed rats. Blue: nuclei.

**Fig. 2.** Effect of Gosha-jinki-gan on the presence of TRPV1 receptors and P2X3 purine receptors. TRPV1 receptors (**A, C, E, and G**), and P2X3 purine receptors (**B, D, F, and H**) expressed within or nearby the urothelium (green) in each group were visualized. After the instillation of saline, the presence of the both TRPV1 and P2X3 receptors of Gosha-jinki-gan pretreated rats (**E and F**) were similar to that of standard diet fed rats (**A and B**). After the instillation of acetic acid, the presence of the TRPV1 and P2X3 receptors of Gosha-jinki-gan pretreated rats (**G and H**) decreased compared with standard diet fed rats (**C and D**). Blue: nuclei.

**Fig. 3.** Effect of diet on the presence of nerve fibers within the urinary bladders. At 4 weeks after feeding, nerve fibers of the urinary bladders were visualized. (**A**) Rats fed with the standard diet had distinct S100-positive nerve fibers (red) under the urothelium (green), and (**B**) CGRP-positive afferent nerve fibers (red, arrows) within the smooth muscle layers (green). (**C**) Rats pretreated with Gosha-jinki-gan also had distinct S100-positive nerve fibers (red) under the urothelium (green), and (**D**) CGRP-positive afferent nerve fibers (red, arrows) within the smooth muscle layers (green). Blue: nuclei.

**TABLE I. Tachykinins and Receptors Expressing Nearby or Within Urothelium**

	Standard diet		Gosha-jinki-gan	
	Saline	Acetic acid	Saline	Acetic acid
Neurokinin A	-	+++	-	+
Neurokinin B	+	+++	+	++
Substance P	++	+++	+	++
TRPV1 receptor	-	+++	+	++
P2X3 purine receptor	-	++	-	+

The presence of fluorescently tagged spots 31-60; +++, 21-30; ++, 11-20; +, 0-10; -.

**TABLE II. Gene Expression Levels of Tachykinins and Receptors**

	Standard diet		Gosha-jinki-gan	
	Saline	Acetic acid	Saline	Acetic acid
Neurokinin A	87.21±0.53	173.81±3.24††	26.45±1.40§	75.11±0.46#*
Neurokinin B	2.12±0.27	7.14±2.31†	5.82±0.28§	11.12±0.42#
Substance P	66.94±4.67	145.82±36.85†	21.31±2.85§	70.80±7.37#*
TRPV1 receptor	3.98±0.18	6.97±0.34††	3.96±0.47	3.80±1.45 *
P2X3 purine receptor	1.14±0.02	2.01±0.68	0.67±0.03	1.08±0.56

†P < 0.05, ††P < 0.01, compared with the saline treated rats fed standard diet (Student's t-test).

#P < 0.01, compared with the saline treated rats pretreated with Gosha-jinki-gan (Student's t-test).

§P < 0.01, compared with the standard diet fed rats given the intravesicular instillation of saline (Student's t-test).

\*P < 0.01, compared with the standard diet fed rats given the intravesicular instillation of acetic acid (Scheffe's test).

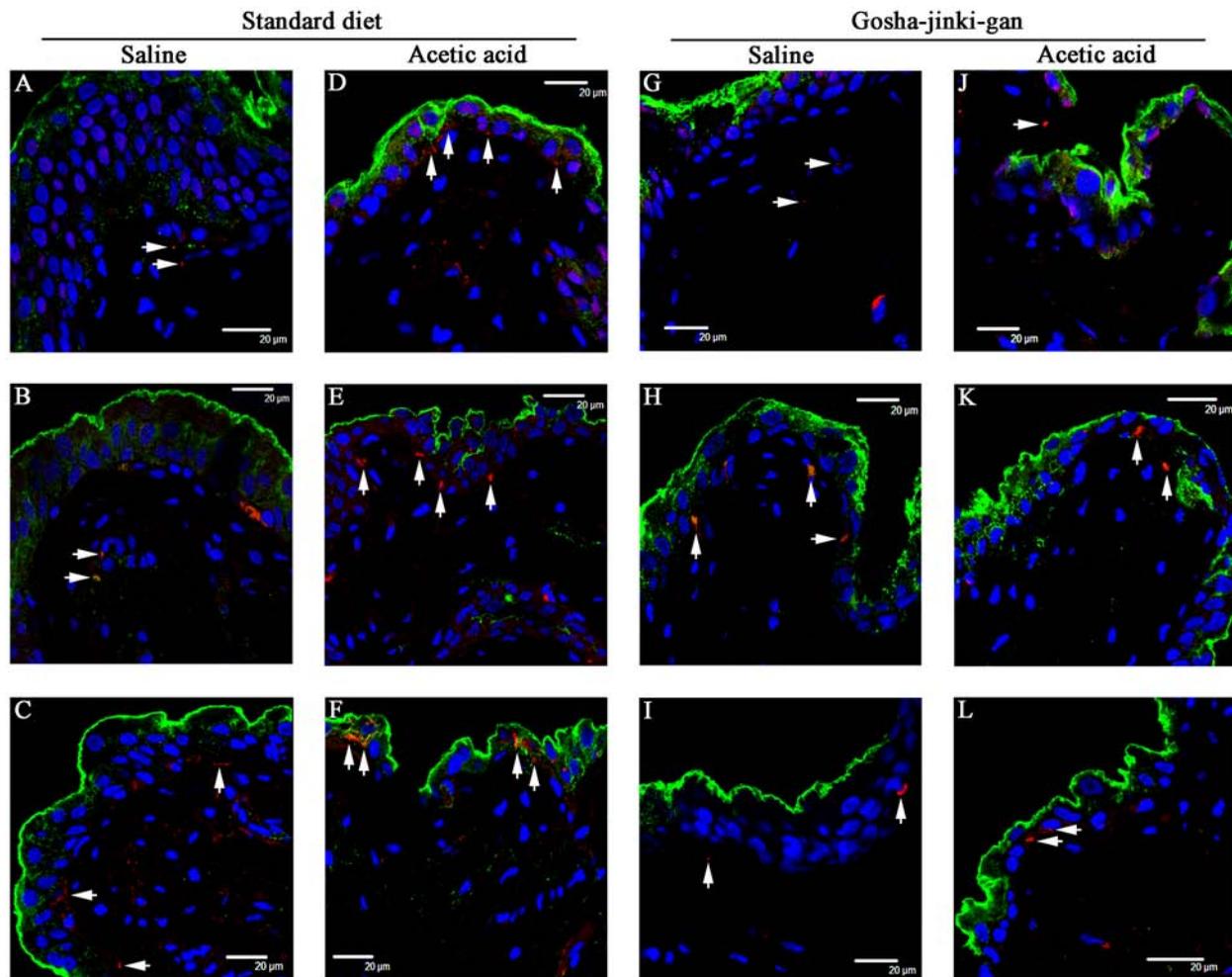


Fig. 1.



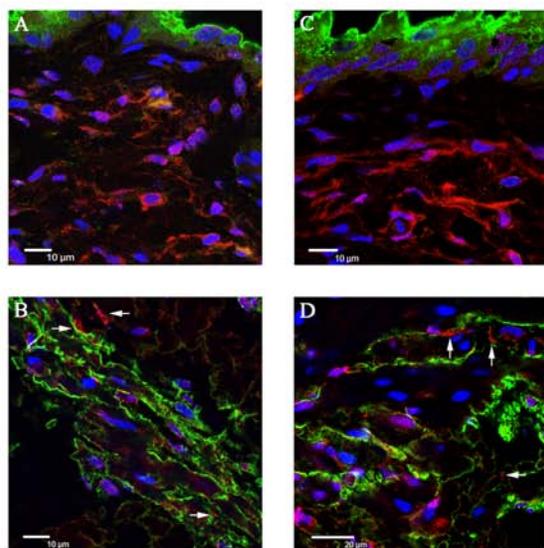


Fig. 3.