Am J Transl Res 2016;8(9):3678-3688
www.ajtr.org /ISSN:1943-8141/AJTR0027760

Original Article

Effect of paeoniflorin on the calcium ion concentration in salivary gland cells using confocal laser scanning microscopy

Xian Qian[1], Xiaolu Shi[1], Hongyi Wang[2]

[1]Department of Rheumatism, The Affiliated Hospital of Nanjing University of Chinese Medicine, Nanjing 210029, China; [2]Department of Pharmacology, Nanjing Medical University, Nanjing 210029, China

Received October 13, 2015; Accepted July 9, 2016; Epub September 15, 2016; Published September 30, 2016

Abstract: Objective: To investigate the effects of paeoniflorin, the main monomer component of Jinxueyuan granules, on the Ca^{2+} concentrations in salivary gland cells to further explore the salivation-promoting mechanism and effective monomer components of Jinxueyuan granules. Methods: The salivary gland cells of suckling rats were cultured in vitro and loaded with a Fluo-3AM fluorescent probe, and changes in the intracellular Ca^{2+} concentrations were observed using a confocal laser scanning microscope. Results: No significant changes in the intracellular Ca^{2+} concentrations were demonstrated ($P>0.05$) in the paeoniflorin-free Hank's media treatment group or in the higher-dose paeoniflorin (10^{-2} mol/L) Hank's media treatment group; however, a significant increase in the intracellular Ca^{2+} concentration in the lower-dose paeoniflorin (10^{-4} mol/L) treatment group was observed ($P=0.001$). Further study showed that treatment with the calcium channel blocker verapamil hydrochloride or with Ca^{2+}-free D-Hank's media did not block the paeoniflorin-induced (10^{-4} mol/L) increase in intracellular Ca^{2+} ($P<0.05$). Conclusion: Paeoniflorin promotes the release of endogenous calcium to upregulate the intracellular Ca^{2+} concentration. Further studies should be performed to investigate the association between paeoniflorin and the Ca^{2+} concentration in salivary gland cells and to elucidate the corresponding functional pathways.

Keywords: Paeoniflorin, Sjögren's syndrome (SS), salivary gland cell, calcium ion, confocal laser scanning microscope

Introduction

Xerostomia is a symptom associated with multiple diseases and is often observed in Sjögren's syndrome (SS) [1]. SS, which is characterized by systemic chronic inflammation, is an autoimmune disease with a high prevalence in women that ranks second after that of rheumatoid arthritis [2, 3]. Exocrine glands, especially salivary glands, are inflamed in SS, and the clinical manifestations include dry mouth and dry eyes, which lead to rampant caries and blindness in severe cases. The pathogenesis and pathological processes of SS are unclear, and the treatment strategy is usually limited to symptom relief. The application of sialogogues, such as pilocarpine and cevimeline, can stimulate secretion from salivary and lacrimal glands. However, side effects such as sweating, facial hot flush and a higher frequency of urination decrease the value of these treatment strategies.

Chinese herbal medicines have been widely applied in the treatment of SS-like xerostomia. Clinical studies report that Jinxueyuan granules, the main component of which is peony, relieve dry mouth, dry eyes and systemic dryness with good drug compliance and without obvious toxicity and side effects, and an 85% clinical curative effect can be achieved [4, 5]. Our earlier animal experiments showed that this medication promoted salivary gland secretion in mouse and rat models of SS, and it prevented the observed pathological changes. Furthermore, some studies have demonstrated that this drug increased the intracellular Ca^{2+} concentrations in rat salivary gland cells in a dose-dependent manner [6-10]. Furthermore, in some reports, peony stimulated salivary

secretion [11, 12], although the underlying mechanism is unclear.

Calcium ions are essential intracellular components of signal transduction. The triggering of receptors, such as acetylcholine receptors, on acinic cell membranes elicits the G-protein-mediated transduction of salivation-associated signals, which then activate phospholipase C to catalyze the hydrolysis of phosphatidylinositol 4,5-bisphosphate into diacylglycerol (DAG) and inositol triphosphate 3 (IP3). IP3 is a water-soluble substance that diffuses from the cell membrane into the cytoplasm and binds to IP3 receptors (IP3Rs) on the endoplasmic reticulum membrane. This binding initiates the rapid release of stored Ca^{2+} into the cytoplasm, which increases the free Ca^{2+} concentration and triggers Ca^{2+}-dependent salivary secretion [13, 14]. Therefore, the abundance of Ca^{2+} in the cytoplasm allows for sustained salivary secretion in acinic cells.

Paeoniflorin is the main pharmacological component of peonies [15]. The potential association between paeoniflorin and the intracellular calcium ion concentrations in salivary gland cells is not known, and no published correlation studies could be found. The present study analyzed the changes in the intracellular calcium ion concentrations in salivary gland cells due to paeoniflorin treatment using in vitro cell culture and confocal laser scanning microscopy (CLSM). This study provides experimental evidence to aid in the screening of effective Chinese herbal medicines and compounds that trigger saliva production.

Materials and methods

Isolation and culture of salivary gland cells

One female adult rat (80-100 g) and six 8-day-old suckling mice (three males and three females) supplied by the Institute of Laboratory Animal Science, Chinese Academy of Medical Sciences (certificate no. Scxk (SU) 2008-0004) were used as donor animals for submandibular gland cells in this study. The animals were soaked with 75% alcohol for 5-10 min for sterilization and anesthesia. The salivary gland was then isolated and transferred into a culture dish on a clean bench under aseptic conditions. The envelope, fat and connective tissue were removed with ophthalmic scissors, and the glands were washed 3 times with Hank's media, placed into another culture dish and then cut into chylous effusions at a block size of 0.1-0.3 mm. The tissue was washed again 3 times with Hank's media and digested twice (once for 20 min) at 37°C with 4 ml of 0.125% trypsin+0.1% collagenase.

The cell suspension was collected and centrifuged twice at 800 rpm (2×5 min), the cell precipitate was harvested, and an F12/DMEM medium (6 ml) containing 10% FBS was used to resuspend the cells. The suspension was then transferred into a 50-ml culture flask and cultured at a constant temperature for 1 h. After shaking, the cell suspension was observed under an inverted microscope (Olympus CKX41), and 4 ml of each suspension was seeded in type I collagen (25 ml)-coated plastic culture flasks at a concentration of 4×10^5 cells/ml. The cells were cultured at 37°C in 5% CO_2, and the culture medium was changed every 3 days. The cells reached confluence after 9 days of culturing. Subculturing was performed using the trypsin-EDTA method. Briefly, the culture supernatant was discarded, and the cells were washed twice with 2 ml of F12/DMEM, digested with 1 ml of 0.25% trypsin, and observed under an inverted phase-contrast microscope. The digestion was terminated using 1.5 ml of F12/DMEM with 10% FBS, and the cells were detached from the walls of the flask by repeated disruption. The cell suspension was transferred into 10-ml centrifuge tubes and centrifuged at 800 rpm for 5 min. The cell pellets then were harvested, resuspended in a culture medium, and seeded into type I collagen-coated plastic culture flasks at a concentration of 4×10^5 cells/ml.

Immunocytochemical staining

Rat salivary gland cells were digested with trypsin at passage 2 and seeded onto cover slips in a 24-well culture plate with 0.5 ml of cell suspension (1×10^5/ml) per well. The cells were cultured at 37°C in 5% CO_2 for 72 h until they reached 95-100% confluence on each cover slip. The cells on the cover slip were removed from the wells, washed 3 times (3×3 min) with PBS at 37°C, fixed with 4% paraformaldehyde for 20 min, washed 3 times (once for 3 min), treated with 0.2% TritonX-100 for 5 min, washed again with PBS, treated with 3% H_2O_2

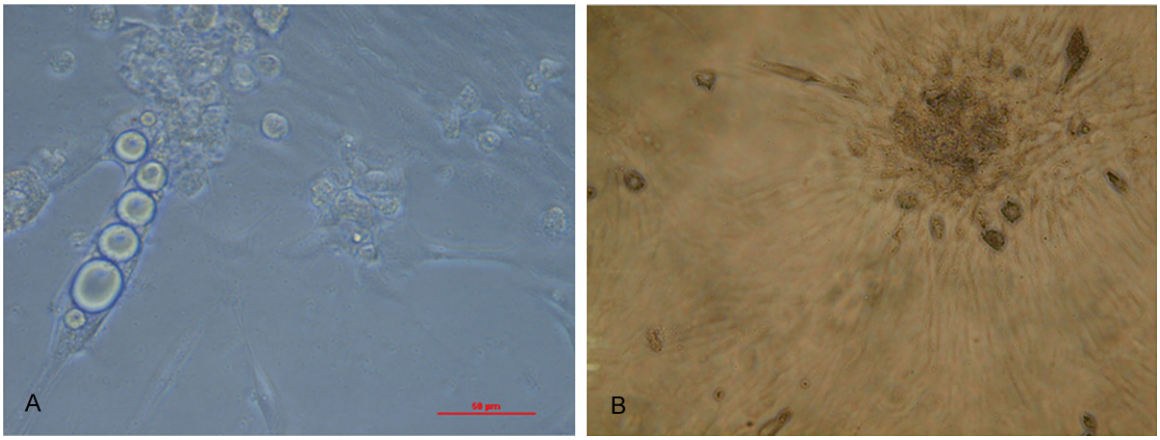

Figure 1. Cell growth and staining were observed using inverted phase-contrast microscopy. A. SD rat salivary gland cells. The cells grew as aggregates and proliferated actively under good conditions (×400). B. CK8 immunocytes in SD rat salivary glands. The reaction products were evenly distributed as keratin-positive claybank particles in the cytoplasm. The culture was identified as salivary gland cells.

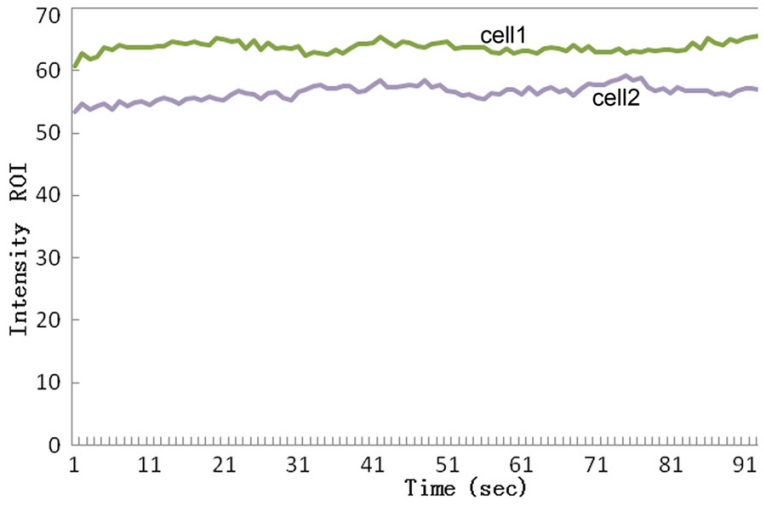

Figure 2. Changes in the intracellular calcium ion levels in suckling rat salivary gland cells treated with drug-free Hank's media.

for 5-10 min to eliminate endogenous peroxidase activity and washed. The cells were incubated in a blocking buffer containing normal goat serum at room temperature for 10-15 min and incubated with rabbit Anti-CK8 (bs-1106R, Bioss, Beijing China) at a concentration of 1:300 at 37°C for 2-3 h.

Goat anti-rabbit IgG labeled with biotin (SP-9001, ZSGB-Bio, China) was added for 15 min at room temperature. The cells were washed, incubated with an HRP-streptavidin (S-A/HRP) solution at room temperature for 15 min and stained with DAB (ZLI-9032, ZSGB-Bio, China) for 2-10 min. Following observation under an inverted microscope and washing, counterstaining was performed with hematoxylin for 5-10 min. Gradient concentrations of 80%, 95% and 100% ethanol were used for dehydration. At each step, 3-4 washes were performed (3-5 min per wash), and an alcohol clearing wash was performed twice with xylene (once for 10 min). The samples were sealed with neutral gum, the slips were placed into an electrothermal dry box at a constant temperature (37°C) for 24 h, and the cell staining was examined.

Salivary gland cell group and confocal laser scanning microscope observations

Suckling rat salivary gland cells at passage 3 were washed 2-3 times with Hank's media and incubated in a mixture including 20 μl of KHBS-diluted calcium ion fluorescent probe (Fluo-3AM, F1242, molecular weight: 1129.86, Invitrogen, USA) at 20 μmol/L and 4 μl of non-ionic polyhydric alcohol surfactant containing 20% F127 at 37°C in 5% CO_2 for 30 min. The cells were washed twice with Ca^{2+}-free, Mg^{2+}-free D-Hank's media and twice with insulin- or glucose-free KHBS. The following groups of

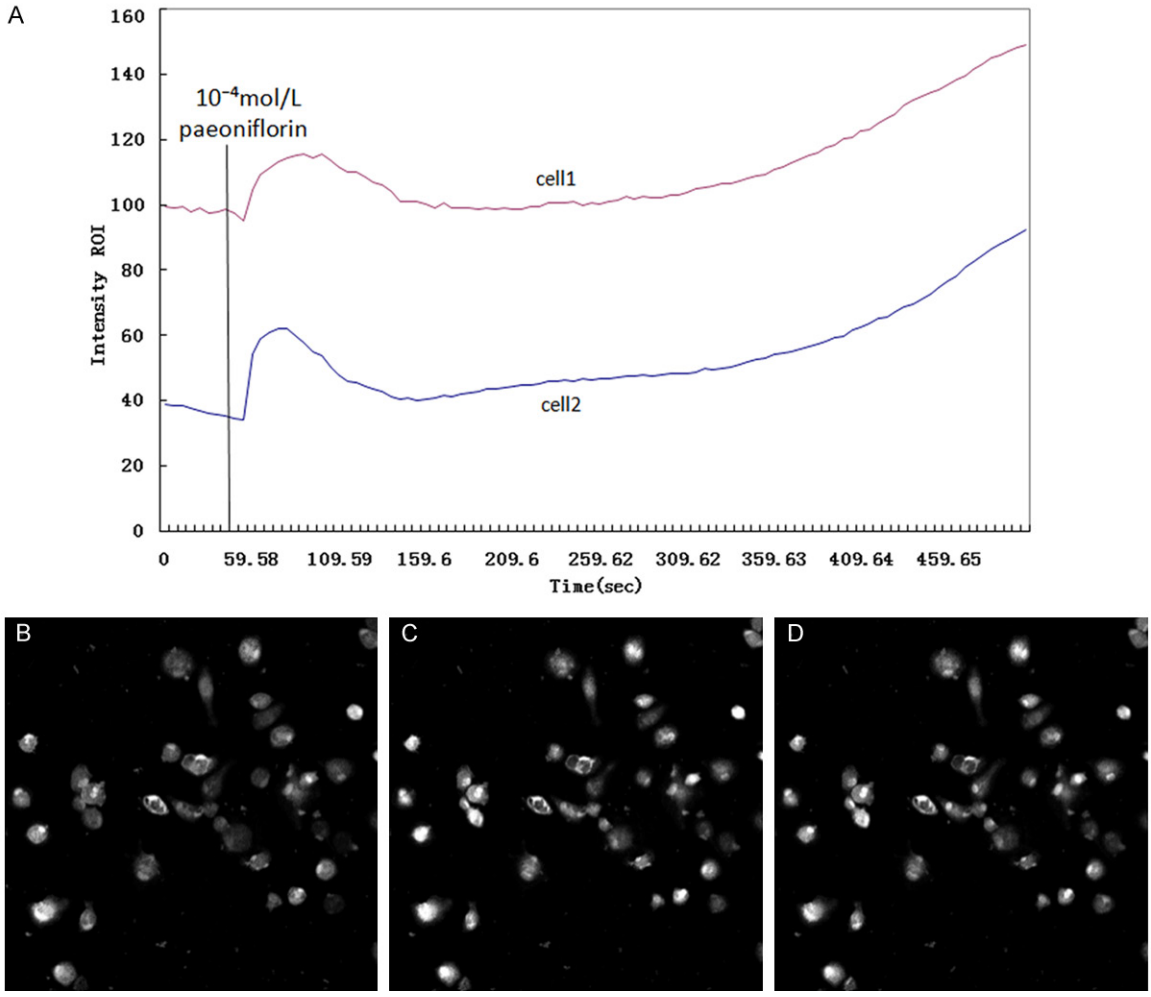

Figure 3. The line graph shows the effects of paeoniflorin (10^{-4} mol/L) on the intracellular Ca^{2+} level in suckling rat salivary gland cells (A), while the photos below show the changes in cell brightness, indicating the changes in the intracellular Ca^{2+} levels before (B) and after treatment of a suckling rat salivary gland with paeoniflorin in Hank's media (10^{-4} mol/L) for 20 s (C) and 60 s (D).

salivary gland cells were used to examine Fluo-3AM loading: drug-free Hank's media (blank control); Hank's media+paeoniflorin (20110-818, Shangfang Union, Beijing, China; dose: 20 mg, purity: 98.23%, molecular weight: 480.47) at working concentrations of 10^{-2} mol/L and 10^{-4} mol/L; Hank's media+verapamil hydrochloride (central pharmacy of Jiangsu Provincial Hospital of Traditional Chinese Medicine; formula: 2 ml: 5 mg) at a working concentration of 0.005 mol/L; paeoniflorin at working concentrations of 10^{-2} mol/L and 10^{-4} mol/L; and D-Hank's media+paeoniflorin at a working concentration of 10^{-4} mol/L.

The cover slip was observed using a confocal laser scanning microscope (Carl Zeiss LSM710, resolution 1024×1024, Argon laser, Ex/Em: 488 nm/BP5052550, pinhole: 1.0, Germany, 2009). The appropriate salivary gland cells were located in the visual field and scanned every 5 s of each interval. Changes in fluorescence intensity, which indicated changes in the calcium ion concentration post-drug treatment, were recorded.

Statistical analysis

Semi-quantitative analysis of the relative fluorescence intensity was performed using Zeiss LSM710 Image Examiner software. Statistical analysis was performed using SPSS 19.0 statistical software. All differences were considered statistically significant at $P<0.05$.

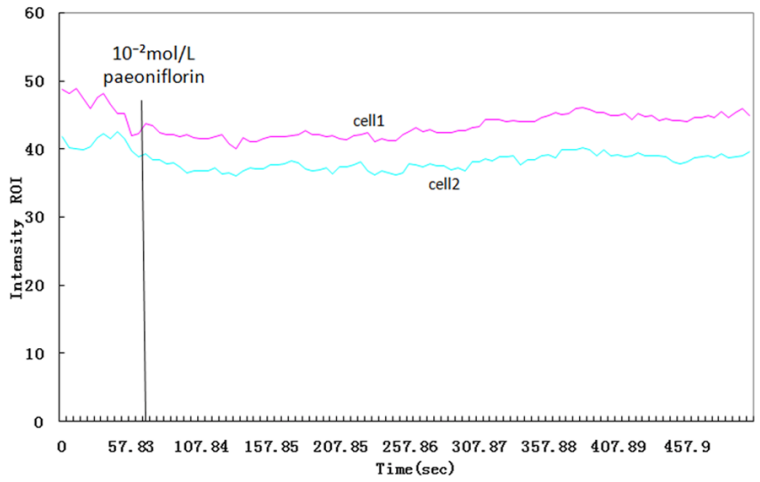

Figure 4. The effects of 10^{-2} mol/L paeoniflorin on the intracellular Ca^{2+} level in suckling rat salivary gland cells.

The effects of paeoniflorin in Hank's media on the intracellular calcium ion levels in salivary gland cells

No significant changes in calcium ion levels were detected in salivary gland cells at passage 3 after treatment with Hank's media containing Ca^{2+}. The level of intracellular Ca^{2+} increased significantly at 15 s (P=0.001) (**Figure 3**) and continued to increase 300 s after treatment with 10^{-4} mol/L paeoniflorin. The intracellular Ca^{2+} concentration exhibited slight fluctuations after 10^{-2} mol/L paeoniflorin treatment (P>0.05) (**Figure 4**).

Results

Salivary gland cell culture and staining observations

Adherent growth of the cell aggregates was observed using an inverted phase-contrast microscope. The cells presented round, oval and pebble-like shapes with rich cytoplasm containing secretory granules. Karyokinesis and vacuoles were found in cells that were actively proliferating under good growth conditions (**Figure 1A**). CK8 immunocytes in SD rat salivary gland cells were observed using an inverted microscope, and the reaction products were evenly distributed as buffy or sepia keratin-positive particles in the cytoplasm. The nuclei presented a negative reaction. Salivary gland cells were identified using these methods (**Figure 1B**). By using CK8 antibody immunocytochemical staining, positive cellular morphology and characteristic epithelial cell staining were observed, suggesting that the cultured cells were submandibular gland cells.

Changes in intracellular calcium ion levels in salivary gland cells treated with drug-free Hank's media

No significant changes in the Ca^{2+} level (fluorescence intensity) were detected in the cells treated with drug-free Hank's media (P>0.05). The calcium levels primarily remained at a constant level (**Figure 2**).

The effects of paeoniflorin and verapamil hydrochloride in Hank's media on the intracellular calcium ions in salivary gland cells

Verapamil hydrochloride (0.005 mol/L) and paeoniflorin (10^{-4} mol/L) were added to the Ca^{2+}-containing Hank's media, which was then applied to salivary gland cells at passage 3. The intracellular Ca^{2+} levels increased significantly (P<0.05), and this increase was maintained for approximately 10 s. The Ca^{2+} levels decreased gradually until another increase occurred at 100 s (**Figure 5**). When the concentration of paeoniflorin was changed to 10^{-2} mol/L, the intracellular Ca^{2+} gradually increased (P<0.05) (**Figure 6**).

The effects of paeoniflorin in D-Hank's media on the intracellular calcium ion concentration in salivary gland cells

Intracellular Ca^{2+} levels constantly increased after treatment with paeoniflorin (10^{-4} mol/L) in Ca^{2+}-free D-Hank's media (**Figure 7**).

Discussion

SS is a chronic inflammatory autoimmune disease that affects exocrine glands, particularly the salivary and lacrimal glands. SS is characterized by clinical manifestations such as dry mouth, dry eyes, rampant caries, and blindness as well as injury to other organs. Sialagogue has been applied to relieve dry mouth because this drug stimulates the salivary glands.

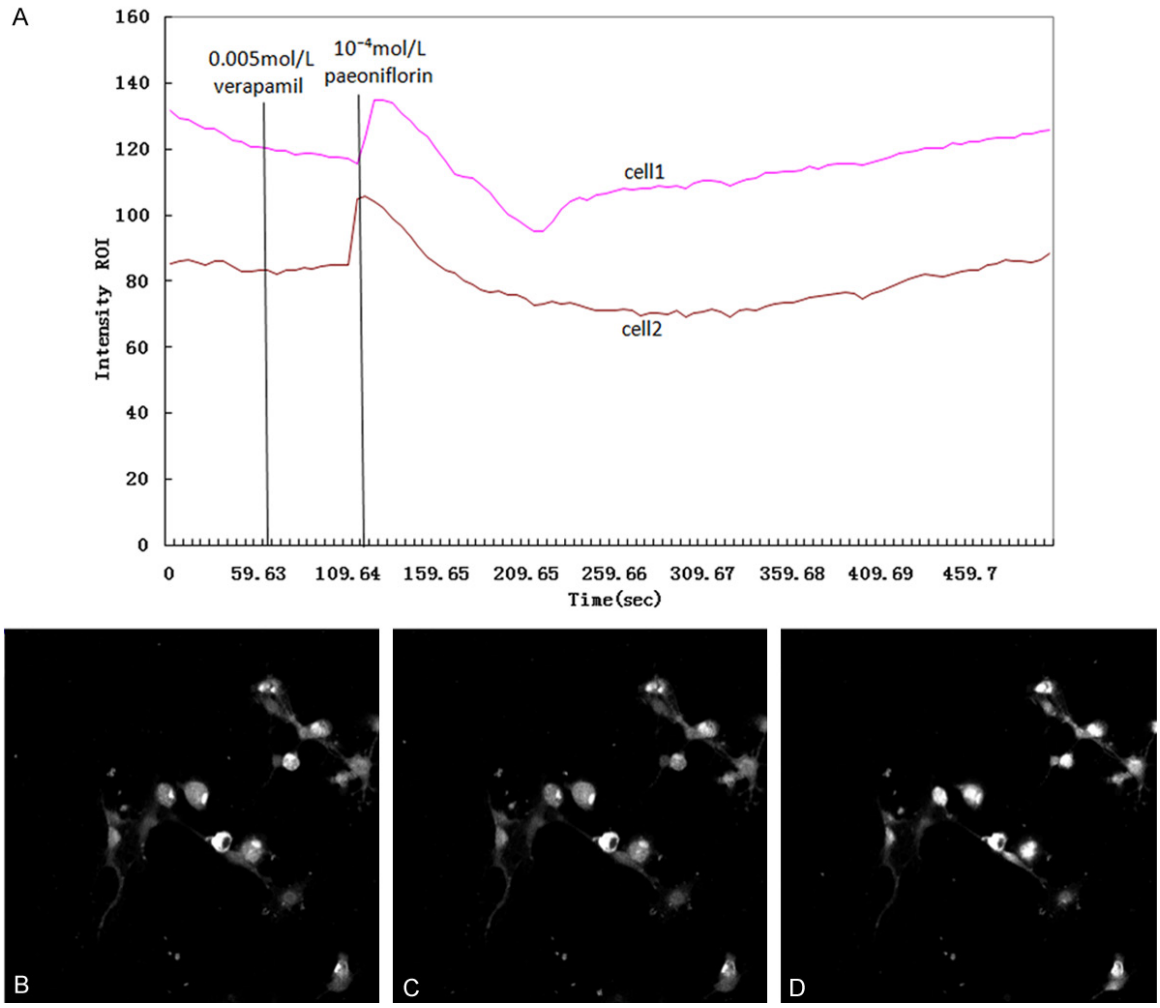

Figure 5. The line graph shows the effects of paeoniflorin (10^{-4} mol/L) in verapamil hydrochloride-containing Hank's media on the intracellular calcium ions in the salivary gland cells of suckling rats (A), while the photos below show the changes in cell brightness, indicating changes in the intracellular Ca^{2+} levels before (B) and 30 s after verapamil hydrochloride treatment of a suckling rat salivary gland (C) as well as 10 s after treatment with paeoniflorin (10^{-4} mol/L) (D) in Hank's media.

However, the side effects of sialagogue, including sweating, facial hot flush and a higher frequency of urination, often cause poor drug compliance. Glucocorticoids and other immunosuppressants, such as cyclophosphamide, could be used to control or suspend the immune response-mediated injury of tissues and organs; however, the toxicity and side effects of these drugs decrease their clinical value. Therefore, it is critical to understand the pathogenesis of SS and to develop improved medical treatments. Clinical feedback has indicated that these treatments have sufficient efficacy; however, no clear functional mechanism has been identified. Previous studies were focused at the organ level; therefore, associated cellular or molecular biological investigations are lacking. Only two research articles from CNKI had been indexed by the end of 2013, and these articles described the protection of salivary gland cells and secretion stimulation by Chinese herbal compounds [16, 17]. The only associated study indexed in PubMed showed that a *Dendrobium officinale* extract upregulated the labial gland AQP5 to stimulate salivary secretion in SS [18]. However, the mechanisms by which Chinese herbal medicines and compounds facilitate salivary gland secretion are not known. For example, the observed multi-target and multi-level inhibition of salivary gland injury, the regulation of aquaporin expression and the changes in intracellular calcium ion distribution require further investigation.

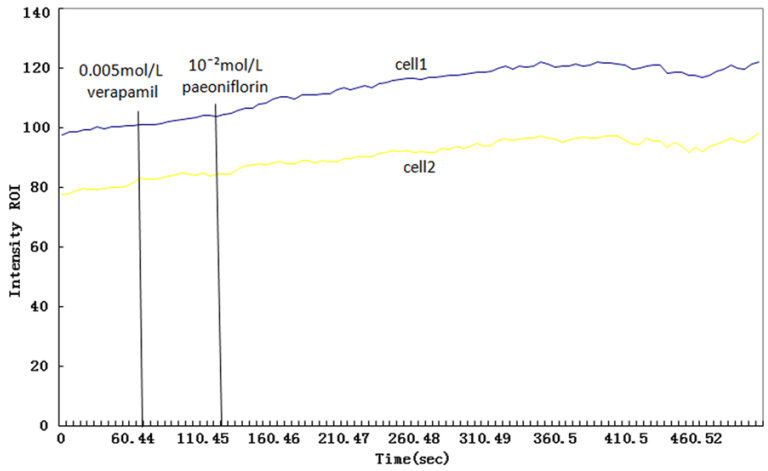

Figure 6. The effects of paeoniflorin (10^{-2} mol/L) in verapamil hydrochloride-containing Hank's media on the intracellular calcium ions in the salivary gland cells of suckling rats.

Developments in immunology and molecular biology have improved our understanding of the pathogenesis and drug treatment of SS, and recent studies have especially focused specifically on salivary gland protection and secretion facilitation. Our reference analysis revealed that studies on the secretory mechanisms of salivary glands suggest roles for AQP5 (a transmembrane aquaporin) [19-22], the M3 receptor (a type III muscarinic acetylcholine receptor) and α-1 adrenergic receptors [23-25] in salivary gland protection and secretion facilitation. Fewer studies have reported correlations between salivary secretion function and intracellular calcium ion concentrations in salivary gland cells. Previous studies revealed that calcium ions have primary intracellular roles in the regulation of salivation, lacrimal secretion and pancreatic protein secretion [26-33], indicating the significance of facilitating salivation to relieve dry mouth symptoms in SS using Chinese medicines that can regulate calcium ion concentrations. Currently, there are no published studies in this field.

In our previous work, a Jinxueyuan compound significantly facilitated salivation in an SS rat model and increased intracellular calcium ion concentrations. The peony, the main pharmacological component in Jinxueyuan, is also a common saliva-producing compound in Chinese herbal medicine. Few studies examining the effects of paeoniflorin on external secretion have been performed [15, 34], although many of its pharmacological functions have been discovered. A search of PubMed and CNKI using the keyword combination of "paeoniflorin" with "salivary glands", "salivary secretion", and "mechanism of action" revealed only 2 reports, published in early 2014, that associated peonies and salivation. Ping et al. tested the effects of *Polygonatum odoratum*, *Radix ophiopogonis*, *Asparagus cochinchinensis*, smoked plum, peony and *Salvia miltiorrhiza* on salivary gland perfusion in vitro and found that all of these herbs, except for *Polygonatum odoratum,* stimulated salivation [11]. Kuixin et al. found that peonies and peach kernel improved salivation at different levels. However, neither of these studies explored the mechanisms underlying these observed effects [12].

Numerous studies have shown that Ca^{2+} plays quite an important role in salivation. By reviewing the literature [31, 35-37], we have found that there are two mechanisms underlying acinous cell membrane receptor activation-induced salivation, one of which is the activation of phosphatidic acid C and the movement of Ca^{2+}, which is known as Ca^{2+}-dependent salivation. It is believed that the intracellular Ca^{2+} pool is composed of two fractions: the predominant stored Ca^{2+} pool and the low concentration of free Ca^{2+} in the cytoplasm. Ca^{2+} pumps located on the membranes of cells can pump Ca^{2+} from the cytoplasm to the outside of the cell or into Ca^{2+} pools, thus maintaining large concentration differences between the free Ca^{2+} in the cytoplasm and outside the cell as well as the stored Ca^{2+} pools.

Academics currently generally believe that, for saliva secretion, a stimulus first activates phosphatidic acid C, causing the release of inositol triphosphate (IP3, one channel activation signal and the messenger for Ca^{2+} movement), which leads to the influx of extracellular Ca^{2+} or release of the intracellular Ca^{2+} pool into the cytoplasm. Once in the cytoplasm, Ca^{2+} will bind to calmodulin to activate protein kinase C, resulting in the phosphorylation of proteins as

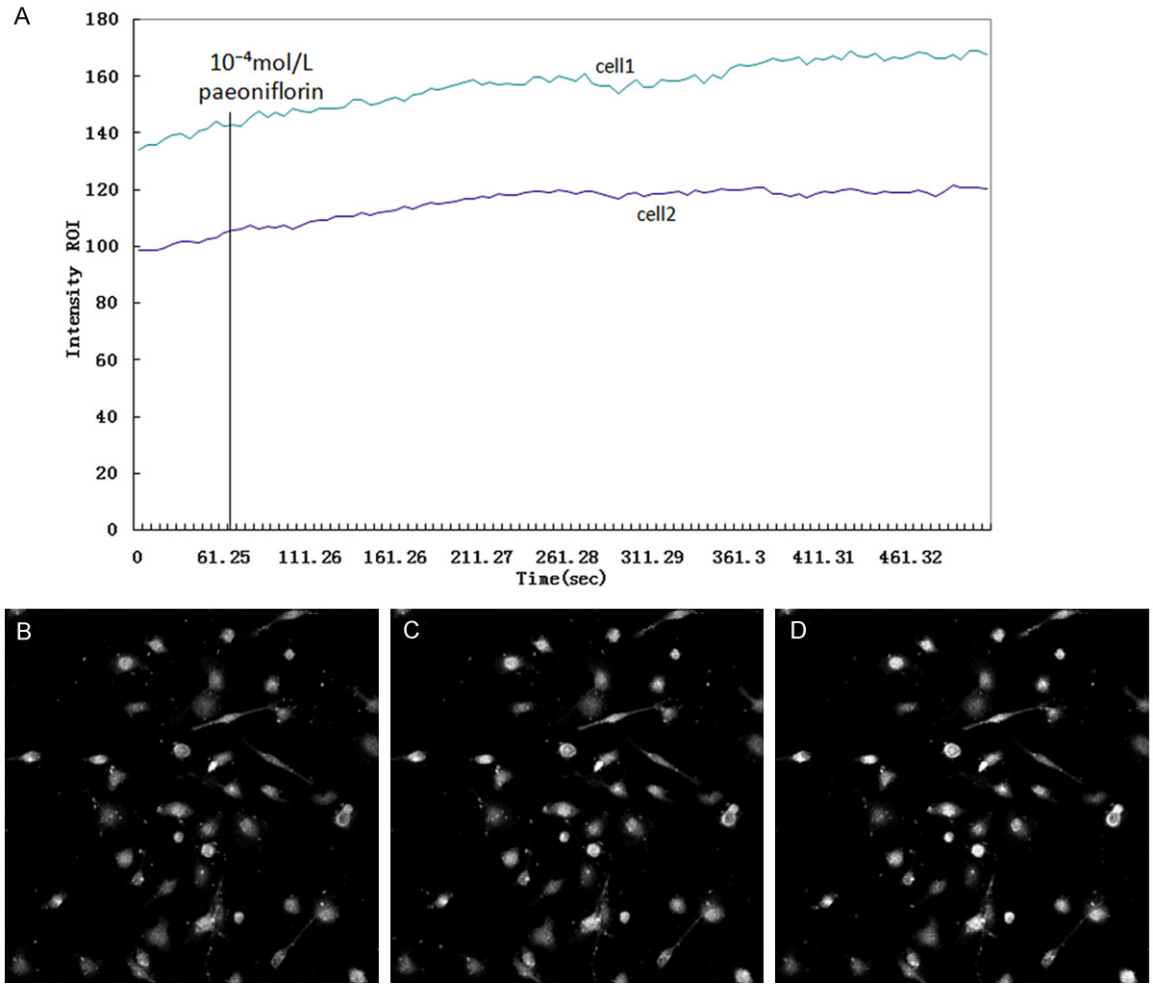

Figure 7. The line graph shows the effects of paeoniflorin (10^{-4} mol/L) in D-Hank's media on the intracellular Ca^{2+} concentration in the salivary gland cells of suckling rats (A), while the photos below show the cell brightness changes, which indicate changes in the intracellular Ca^{2+} levels before (B) and after treatment of suckling rat salivary gland cells with paeoniflorin in D-Hank's media (10^{-4} mol/L) for 100 s (C) or 400 s (D).

well as salivation. In this study, after using D-Hank's solution lacking external calcium and blocking Ca^{2+} influx with verapamil, paeoniflorin was still able to stimulate an increase in intracellular Ca^{2+} (**Figures 5**-**7**). After repeated experiments, we now believe that paeoniflorin may have stimulated the influx of external Ca^{2+} and the release of the intracellular Ca^{2+} pool, and this strong stimulus maintained the intracellular Ca^{2+} at a stable, high level, which further promoted the continuous secretion of saliva.

Slingh et al. showed that neurotransmitters stimulate saliva secretion from the salivary glands, and this secretion is regulated by the concentration of intracellular Ca^{2+} [33]. High concentrations of agonist produced a dual-phase increase in cytoplasmic Ca^{2+}, an initial, rapid increase in the $[Ca^{2+}]_i$ followed by a lower-concentration but longer-lasting increase in the $[Ca^{2+}]_i$. This finding is in accordance with the trend of the Ca^{2+} concentration curve after the paeoniflorin stimulus (**Figures 3** and **5**), but it cannot explain the lack of an apparent increase in the initial Ca^{2+} concentration in submandibular gland cells stimulated with high concentrations of paeoniflorin (**Figures 4** and **6**). However, previous studies using the whole-cell patch-clamp technique have demonstrated that glycoside monomers can regulate the calcium current density by regulating voltage-gated calcium channels, thus coordinating the intracellular and extracellular calcium concentrations [38,

39]. We believe that a similar mechanism might underlie the action of paeoniflorin on the Ca^{2+} concentration; therefore, future studies will apply new techniques to develop a deeper understanding of this process.

This study found that paeoniflorin increased the intracellular calcium ion levels in salivary gland cells, with a peak increase that occurred within seconds and was maintained at a constant level for 20-30 s post-treatment with a lower drug concentration. These dynamic changes are consistent with the characteristics of Ca^{2+}-dependent salivation. Furthermore, a higher concentration of paeoniflorin did not significantly increase the intracellular calcium ion levels, which indicated the presence of dose-dependent effects on intracellular calcium ions even during treatment with the same drug monomer. Verapamil hydrochloride, a calcium channel blocker, and paeoniflorin in Ca^{2+}-free D-Hank's media increased the intracellular calcium ion levels and maintained this increase at higher levels in salivary gland cells. This result indicated a potential association between endogenous calcium release and the paeoniflorin-initiated increase of intracellular calcium ions.

This study has several limitations. Further work on the dose-effect relationship between paeoniflorin and intracellular calcium ion levels in salivary gland cells should be performed. The effect of this relationship on exogenous calcium influx and endogenous calcium release should also be investigated.

Paeoniflorin increases the intracellular calcium ion levels in salivary gland cells, which provides a pharmacological mechanism for the Jinxueyuan granule-initiated increase in the intracellular calcium ion concentration in these cells. These results suggest a potential mechanism of Chinese herbaceous peony-stimulated salivation via an increase in intracellular calcium ion levels in salivary gland cells.

Acknowledgements

This study was supported by the Natural Science Foundation of Jiangsu Province (BK-2011870) and the National Natural Science Funds (81273714). We thank the Department of Pharmacology, Nanjing Medical University, for offering laboratory space and experimental reagents, and we are grateful to the researchers for their helpful technical support.

Disclosure of conflict of interest

None.

Address correspondence to: Xian Qian, Department of Rheumatism, The Affiliated Hospital of Nanjing University of Chinese Medicine, No. 155 Hanzhong Road, Nanjing 210029, China. Tel: +8613851444-984; E-mail: moneyfirst1@163.com

References

[1] Fox RI, Konttinen Y and Fisher A. Use of muscarinic agonists in the treatment of Sjögren's syndrome. Clin Immunol 2001; 101: 249-263.

[2] Fox RI, Stern M and Michelson P. Update in Sjögren syndrome. Curr Opin Rheumatol 2000; 12: 391-398.

[3] Delaleu N, Jonsson MV and Jonsson R. Disease mechanisms of Sjögren's syndrome. Drug Discovery Today: Disease Mechanisms 2004; 1: 329-336.

[4] Qian X, Ma YZ, Zhang MJ, Ji W, Lu SJ and Lu Y. Clinical observation of the treatment of Sicca Syndrome with Jinxueyuan. Chinese Journal of Integrative Medicine 2004; 24: 368-369.

[5] Qian X, Ma YZ, Zhang MJ, Ji W, Lu SJ and Lu Y. Clinical study on the treatment of Sicca Syndrome with Jinxueyuan. Chinese Journal of Clinical Medicine 2003; 16: 975-977.

[6] Zhu XX, Qian X, Ni WP, Wu XT, Ding Y and Su M. Intervention study on the treatment of Sicca Syndrome with Jinxueyuan granule. Chinese Archives of Traditional Chinese Medicine 2009; 27: 972-974.

[7] Zhu XX, Qian X, Ni WP, Wu XT, Ding Y and Su M. Experimental study on Jinxueyuan granule using rat model of Sicca Syndrome. Chinese Archives of Traditional Chinese Medicine 2008; 26: 2607-2609.

[8] Xu Q, Qian X and Wang HY. The preliminary discussion on the treatment application of Jinxueyuan granule in rat models of Sicca Syndrome. Pharmacology and Clinics of Chinese Materia Medica 2011; 27: 86-88.

[9] Qian X, Wang JR, Wang HY and Xu Chao. Study of AQP5 expression up-regulation in Salivary gland of SS rats by Jinxueyuan Granula. China Journal of Traditional Chinese Medicine and Pharmacy 2011; 26: 2375-2377.

[10] Wang HY, Xu C, Li QP and Qian X. The observation on treatment activity of Jinxueyuan granule in rat model of Sicca Syndrome. Lishizhen Medicine and Materia Medica Research 2011; 22: 1469-1471.

[11] Hu P, Cunshang ZL, Zhang QD, Ding W, Guo J and Wei MX. The function of perfusion into salivary gland in vitro with Chinese herbal medicine replenishing the vital essence and removing heat and invigorate the circulation of blood in rat. Chinese Journal of Microcirculation 2010; 20: 22-25.
[12] Wei KX, Ding W, H Ping, Wei F, Cunshang ZL, Huang Q and Zhang QD. The study on the effects of Salvia, a Chinese herbal medicine activating blood and dissolving stasis, on salivation. Mcademic Conference of Microcirculation: China Society of Integrated Traditional Chinese and Western Medicine; 2009. p. 26.
[13] Evans R and Turner R. New insights into the upregulation and function of the salivary Na+-K+-2Cl-cotransporter. Eur J Morphol 1998; 36: 142-146.
[14] Kaplan MD, Taylor SE and Ambudkar IS. G-protein- and capacitatively regulated Ca2+ entry pathways are activated by muscarinic receptor stimulation in a human submandibular ductal cell line. Pflugers Arch 1994; 428: 439-445.
[15] Sun LR, Cao X, Hou FQ, Zhu XH and Gao TM. Research advance in paeoniflorin. China Journal of Chinese Materia Medica 2008; 33: 2028-2032.
[16] Zhang YL, Qin LP, Wang KL and Li B. Protective effect of traditional Chinese medicine hu-qi extractum on radiation-induced salivary glands in Wistar rats. The 3rd International Oncology Academic Conference of Chinese Traditional Medicine and Integrated Chinese and Western Medicine & The 12 the China Oncology Academic Conference of Integrated Chinese and Western Medicine; 2010. p. 120-125.
[17] Zhou DH, Zhang QD, Wei KX and Xu Y. The effects of Dandiqiongyu granule on rat of Sicca Syndrome. Journal of Nanjing University of Traditional Chinese Medicine 2005; 25: 266-267.
[18] Xiao L, Ng TB, Feng YB, Yao T, Wong JH, Yao RM, Li L, Mo FZ, Xiao Y, Shaw PC, Li ZM, Sze SC and Zhang KY. Dendrobium candidum extract increases the expression of aquaporin-5 in labial glands from patients with Sjogren's syndrome. Phytomedicine 2011; 18: 194-198.
[19] Kozono D, Yasui M, King LS and Agre P. Aquaporin water channels: atomic structure molecular dynamics meet clinical medicine. J Clin Invest 2002; 109: 1395-1399.
[20] Agre P, King LS, Yasui M, Guggino WB, Ottersen OP, Fujiyoshi Y, Engel A and Nielsen S. Aquaporin water channels--from atomic structure to clinical medicine. J Physiol 2002; 542: 3-16.
[21] Nejsum LN, Kwon TH, Jensen UB, Fumagalli O, Frokiaer J, Krane CM, Menon AG, King LS, Agre PC and Nielsen S. Functional requirement of aquaporin-5 in plasma membranes of sweat glands. Proc Natl Acad Sci U S A 2002; 99: 511-516.
[22] Takagi K, Yamaguchi K, Sakurai T, Asari T, Hashimoto K and Terakawa S. Secretion of saliva in X-irradiated rat submandibular glands. Radiat Res 2003; 159: 351-360.
[23] Baum BJ. Principles of saliva secretion. Ann N Y Acad Sci 1993; 694: 17-23.
[24] Yamamoto H, Sims NE, Macauley SP, Nguyen KH, Nakagawa Y and Humphreys-Beher MG. Alterations in the secretory response of non-obese diabetic (NOD) mice to muscarinic receptor stimulation. Clin Immunol Immunopathol 1996; 78: 245-255.
[25] Bacman SR, Berra A, Sterin-Borda L and Borda ES. Human primary Sjogren's syndrome autoantibodies as mediators of nitric oxide release coupled to lacrimal gland muscarinic acetylcholine receptors. Curr Eye Res 1998; 17: 1135-1142.
[26] Ambudkar IS. Regulation of calcium in salivary gland secretion. Crit Rev Oral Biol Med 2000; 11: 4-25.
[27] Melvin JE, Yule D, Shuttleworth T and Begenisich T. Regulation of fluid and electrolyte secretion in salivary gland acinar cells. Annu Rev Physiol 2005; 67: 445-469.
[28] Mikoshiba K, Hisatsune C, Futatsugi A, Mizutani A, Nakamura T and Miyachi K. The role of Ca2+ signaling in cell function with special reference to exocrine secretion. Cornea 2008; 27 Suppl 1: S3-8.
[29] Shan Z. Calcium ion and salivary secretion. Beijing Journal of Stomatology 2004; 12: 110-113.
[30] Foskett JK. [Ca2+]i modulation of Cl- content controls cell volume in single salivary acinar cells during fluid secretion. Am J Physiol 1990; 259: C998-1004.
[31] Kopach O, Kruglikov I, Pivneva T, Voitenko N, Verkhratsky A and Fedirko N. Mitochondria adjust Ca(2+) signaling regime to a pattern of stimulation in salivary acinar cells. Biochim Biophys Acta 2011; 1813: 1740-1748.
[32] Lee MG, Xu X, Zeng W, Diaz J, Kuo TH, Wuytack F, Racymaekers L and Muallem S. Polarized expression of Ca2+ pumps in pancreatic and salivary gland cells. Role in initiation and propagation of [Ca2+]i waves. J Biol Chem 1997; 272: 15771-15776.
[33] Singh BB, Zheng C, Liu X, Lockwich T, Liao D, Zhu MX, Birnbaumer L and Ambudkar IS. Trp1-dependent enhancement of salivary gland fluid secretion: role of store-operated calcium entry. FASEB J 2001; 15: 1652-1654.
[34] Hu N, Xu HY, Chen ZW and Xing GH. The pharmacological research advance of paeoniflorin. Journal of Qiqihar University of Medicine 2007; 28: 1093-1095.

[35] Ambudkar IS. Dissection of calcium signaling events in exocrine secretion. Neurochem Res 2011; 36: 1212-1221.
[36] Almassy J, Won JH, Begenisich TB and Yule DI. Apical Ca2+-activated potassium channels in mouse parotid acinar cells. J Gen Physiol 2012; 139: 121-133.
[37] Zhang W, Wang XR, Xu YZ and Ouyang J. Study on mechanism of saliva secretion (II). Journal of Comprehensive Stomatology 2000; 16: 38.
[38] Tao J, Wang H, Chen J, Xu H and Li S. Effects of saponin monomer 13 of dwarf lilyturf tuber on L-type calcium currents in adult rat ventricular myocytes. Am J Chin Med 2005; 33: 797-806.
[39] Tao J, Wang H, Zhou H and Li S. The saponin monomer of dwarf lilyturf tuber, DT-13, reduces L-type calcium currents during hypoxia in adult rat ventricular myocytes. Life Sci 2005; 77: 3021-3030.