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ORIGINAL ARTICLE

Puerarin alleviates burn-related procedural pain mediated by $P2X_3$ receptors

Xin Li · Jun Zhang · Yun Gao · Yang Yang · Changshui Xu · Guilin Li · Guanghua Guo · Shuangmei Liu · Jinyan Xie · Shangdong Liang

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Abstract Pain is a major problem after burns. Procedural pain evoked by burn dressing changes is common in patients, and its management is a critical part of treatment in acute burn injuries. Burn pain is very likely the most difficult form of acute pain to treat. ATP contributes to inflammation, and ATP is implicated in peripheral pain signaling via actions upon $P2X_3$ receptors. Puerarin is extracted from a traditional Chinese medicine and may act on $P2X_3$ receptor mechanisms. The Visual Analogue Scale (VAS) has been shown to be a sensitive indicator of pain intensity and treatment effects. Peripheral blood mononuclear cells (PBMCs) are involved in nociception or pain after burn injury. Burn patients were randomly divided into normal saline (NS) group (salt solution is saline) and puerarin-treated group and pain (Visual Analogue Scale scores) and inflammation (PBMCs) measured. Burn pain produces a stress response, so blood glucose, insulin, and cortisol levels in burn patients were determined. Furthermore, the expression of $P2X_3$ protein and mRNA in PBMCs was detected. The VAS scores in the puerarin-treated group were lower than those in NS group. The blood glucose, insulin, and cortisol levels in the puerarin-treated group at post-dressing changes were significantly decreased in comparison with those in NS group. The expression levels of $P2X_3$ protein and mRNA in PBMCs of burn patients in NS group were significantly increased in comparison with those in the puerarin-treated group. Puerarin can antagonize inflammatory factors (such as ATP) and decrease the upregulated expressions of $P2X_3$ protein and mRNA in PBMCs after burns to decrease VAS. Thus, puerarin had an analgesic effect on procedural pain in dressing changes of burn patients related to $P2X_3$ receptors.

Keywords $P2X_3$ receptors · Burn · Puerarin

Introduction

Burn injuries are frequent and disabling problems in most areas of the world [14]. Pain is a major problem after burns [49] and is one of the patients' most serious and persisting complaints. Adequate pain control is therefore of utmost importance. Burn pain includes procedural pain, background pain, and breakthrough pain [23]. There are two main features of burn pain, one is its long-lasting course which frequently exceeds healing time, and the other is the repetition of highly nociceptive procedures which can lead to severe psychological disturbances if pain control is inappropriate [28]. Procedural pain evoked by burn dressing changes is more common and painful in patients, and its management is a critical part of treatment in acute burn injuries. Burn pain is very likely the most difficult form of acute pain to treat [41]. Management of burn pain is still a challenge in clinical settings, although several treatment options have been suggested [61]. It is necessary to

Xin Li, Jun Zhang, Yun Gao, and Yang Yang are jointly first authors.

X. Li · J. Zhang · Y. Gao · C. Xu · G. Li · S. Liu · J. Xie · S. Liang (✉)
Department of Physiology,
Medical College of Nanchang University,
Nanchang, Jiangxi 330006, People's Republic of China
e-mail: liangsd@hotmail.com

Y. Yang
Department of First Clinical Medicine (2 class-2007 grade),
Medical College of Nanchang University,
Nanchang, Jiangxi 330006, People's Republic of China

G. Guo
First Affiliated Hospital, Medical College of Nanchang University,
Nanchang, Jiangxi 330006, People's Republic of China

combine antinociceptive treatment with antineuropathic treatment for the management of burn pain [45]. Our objective is to explore a safe, effective, and feasible analgesic drug and a new mechanism of pain formation.

P2 purinoceptors responding to ATP are divided into ionotropic receptors (P2X, seven types; $P2X_{1-7}$) coupled to ion channels and metabotropic receptors (P2Y, eight types; $P2Y_{1,2,4,6,11,12,13,14}$) coupled to intracellular second-messenger systems through heterotrimeric G-proteins [6]. P2 receptors expressed in blood cells regulate responses such as cell proliferation, differentiation, chemotaxis, cytokine release, and immune and inflammatory responses [8, 11]. ATP acts on P2X receptors on nociceptive sensory neurons and participate in the transmission of pain signals from the periphery to the spinal cord. ATP can activate peripheral nociceptors and produces intense pain when skin is inflamed [10, 21, 22]. ATP is implicated in peripheral pain signaling via actions upon $P2X_3$ receptors [4, 7]. $P2X_3$ knock-down animals show noticeable reductions in chronic inflammation-induced thermal and mechanical hyperalgesia, and spinal nerve ligation-induced mechanical allodynia [37]. A role for the $P2X_3$ receptors in peripheral pain mechanisms, such as chronic inflammatory pain and some features of neuropathic pain has been suggested.

Puerarin ($C_{21}H_{20}O_9$) is an isoflavone isolated from a traditional Chinese medicine Ge-gen (Radix Puerariae) [19]. It seems to be a vasodilator similar to papaverine. Puerarin is widely used in clinical treatment of myocardial infarction [19], cerebral ischemia [15], and diabetes [52] in China. Puerarin protects the heart from arrhythmias induced by $BaCl_2$ and aconitine in rats [19]. Puerarin given intravenously to patients is reported to relieve angina, lower blood pressure, and slow heart rate [15, 19, 52]. Puerarin can significantly increase blood supply of cardiac patients, improve microcirculation, and protect ischemic myocardium. Puerarin reduces blood sugar and cholesterol [52]. It has been reported that puerarin possesses protective effects, at least in part, which are related to its ability to increase superoxide dismutase activity, decrease lipid peroxidation, and enhance fibrinolysis [19]. Puerarin is a scavenger of oxygen-free radicals and is capable of prophylactically antagonizing against the oxidative injury by H_2O_2 and superoxide anion [60]. Recent studies demonstrate that puerarin may inhibit oxidative stress induced by acute alcoholism [59], reducing the production of PGE2, TNF, and IL-6 [42] and act as an anti-inflammatory agent by blocking NF–kappa B signaling, so it may possibly be developed as a useful agent for the chemoprevention of atherosclerosis [55]. In preclinical studies, after burn injury in the rats, puerarin could reduce the nociceptive transmission of burn injury pain mediated by $P2X_3$ receptors and alleviate hyperalgesia induced by $P2X_3$ receptor activation [54]. The purpose of this study is to investigate the relationship between burn pain and the expression of $P2X_3$ receptors on peripheral blood mononuclear cells (PBMCs) and the effects of puerarin on burn pain in humans.

Methods

Patients

The study was conducted in the Burn Center of the First Affiliated Hospital of Nanchang University, China. Following local ethics committee approval, written informed consent was obtained from 40 patients of either sex, aged between 25 to 50 years with second- and third-degree burns of 10–55% of total body surface area (TBSA) and less than 19% of TBSA to third degree. Patients weighting 50 to 80 kg were enrolled. They were admitted to hospital within 24 h after burns and received no medication pre-hospital. The time of patients' each dressing change was no less than 8 min and Visual Analogue Scale (VAS) score was ≧5.

Patients who were confused, who had a history of substance abuse, who had injury too severe to allow normal use of intravenously drip and multi-function ECG monitor, or who had compromised airway, or pregnancy and lactation were excluded from the study. Other patients excluded were those who required inotropic support or mechanical ventilatory support, those with arterial oxygen saturation (SpO_2) of <90% on room air, those with significant renal or hepatic insufficiency, and those with a known hypersensitivity to puerarin.

Patient groups

Patients were randomly divided into normal saline (NS) group (A group) and puerarin-treated group (B group). "A" group was applied with 100 ml 0.9% sodium chloride injection and "B" group with 100 ml puerarin glucose injection (Yangtze River Pharmaceutical Group, SFDA Licence Number H20020450, including puerarin 200 mg). Both administrations by intravenously drip (30–40 drops/min, within 30–40 min) lasted for 3 days before dressing changes after the burn shock. Other routine clinic treatment was the same. There was no difference in sex, age, weight, and burned body surface (% body surface area burned = BSA) between two groups before the study ($p>0.05$; Table 1).

Visual Analogue Scale

The VAS is one of the most commonly used pain assessment instruments and is regarded as the 'gold standard' in research and clinical practice [36, 38, 56].

Table 1 Basic comparison of patients in each group

	A group	B group
Sex (female/male)	6/12	8/14
Age, years	39.69±12.57	42.29±17.53
Weight, kg	60.53±8.77	63.59±9.52
BSA, %	27.63±11.04	29.38±10.55

A group: normal saline (NS)-treated group; B group: puerarin (PUE)-treated group. There was no difference in sex, age, weight, and burned body surface (% body surface area burned = BSA) between two groups before the study ($p>0.05$)

The VAS consists of a 100-mm horizontal or vertical straight line with anchors indicating, for example, 'no pain' and 'worst pain imaginable'. The pain experience is recorded by marking the appropriate point on the line [38, 39]. Patients considered suitable for the study were instructed in the use of VAS for assessment of pain intensity. After administration, VAS was applied at pre-, mid-, and post-dressing changes. Six time points respectively were at 10 min pre-dressing changes, 1 min and 8 min mid-dressing, 10 min, 30 min, and 60 min post-dressing changes.

The determination of blood glucose, insulin, and cortisol

Peripheral blood samples ($n=40$) were collected from patients at three time points, when pre-treated on the first day and post-treated on the second and third day after dressing changes. Eligible participants included ten healthy volunteers who did not undergo any treatment. Blood in ten healthy volunteers was collected and transported in an identical manner compared with that in patients. Approximately 6–8 ml peripheral blood was collected in vacuum blood collection tubes at each time point. About 2 ml peripheral blood was transported to the clinical laboratory of the first affiliated hospital of Nanchang University for the analysis of blood glucose, insulin, and cortisol and others preserved on ice sent to physiology laboratory. Reference values of blood glucose, insulin, and cortisol were 3.8~6.1 mmol/L, 5~10 mIU/L, and 4~20 μg/dl, respectively.

Isolation of PBMCs

Approximately 4–6 ml blood samples at each time point were diluted (V ratio1:1) with 0.1 M phosphate-buffered saline (PBS) and subjected to density gradient centrifugation using Ficoll (Beijing Broad-Wright Science and Technology, LTS1077) at 2,200 rpm for 25 min. PBMCs were isolated and washed twice in sterile 0.1 M PBS solution at 1,500 rpm for 5 min. After washing, $2–3\times10^6$ cells were collected in two centrifuge tubes with each one about 1×10^6 cells. In one tube, 4% paraformaldehyde (0.5 ml) was added and chilled on 4°C for 1 day, and then used for immunohistochemistry. Other cells were used to extract the total RNA.

Immunohistochemistry

Isolated PBMCs were fixed and embedded in OCT (tissue-freezing medium, Sakura Finetek USA, Inc.). Preparations were cut into 8 μm thick on the cryostat. Sections were first rinsed for 3×5 min in 0.1 M PBS and then blocked for endogenous peroxidase activity with 0.3% hydrogen peroxide for 10 min at room temperature, then next with 10% goat serum for 30 min at room temperature to block non-specific binding, and followed by an incubation overnight at 4°C with a rabbit anti-$P2X_3$ (1:2,000 diluted in PBS; CHEMICON International, Inc., USA). On the second day, after 3×5 min rinse in PBS, the sections were incubated with biotinylated goat anti-rabbit secondary antibody (Beijing Zhongshan Goldenbridge Biotechnology Co. Ltd, SP-9001 kit) for 40 min at 37°C. Finally, streptavidin–horseradish peroxidase (Beijing Zhongshan Goldenbridge Biotechnology Co. Ltd, SP-9001 kit) was added, and slides were incubated for 20 min at 37°C. Diaminobenzidine was used as the chromogen, and color development was stopped by gently dipping slides into distilled water. Image scanning analysis system (HMIV-2000, Wuhan) was used to analyze the changes in stain values (integrated optical density) of $P2X_3$ in isolated cells. Background was determined by averaging the optical density of ten random areas.

Reverse transcription polymerase chain reaction (RT-PCR)

The total RNA was extracted from the isolated PBMCs using TRIZOL reagent according to the manufacturer's instructions (TIANGEN Co.). TRIZOL (1 ml) was added to 1×10^6 cells to extract the total RNA. It was reverse-transcribed into complementary DNA (cDNA) with Revert Aid First Strand cDNA Synthesis Kit (Fermentas Co.). cDNA was amplified in 50 μl of polymerase chain reaction (PCR) mixture (TIANGEN Co.). The samples were amplified for 35 cycles. Each cycle consisted of denaturation at 94°C for 45 s, annealing at 48°C for 45 s, and extension at 72°C for 1 min. The primers used were: human $P2X_3$ receptors (Gen Bank TM accession number NM_002559): sense 5-CTCCTTCCCAACCTGACA-3 and antisense 5-CTCATTCACCTCCTCAAACT-3. β-actin message was used to normalize the cDNA amount to be used. The electrophoresis of PCR products was made on 1.5% agarose gel and visualized by ethidium bromide staining.

Statistical analysis

Statistical analyses of the data were performed by SPSS 11.5. All results were expressed as mean±SEM. Statistical significance was determined by one-factor analysis of variance followed by the Fisher post hoc test for multiple comparisons. Student's *t* test was used for other data analysis. $p<0.05$ was considered significant.

Results

Effects of puerarin on Visual Analogue Scale

Analysis of VAS responses was given by each patient at six time points after administration. There was no statistical significance between NS-treated group (A group, $n=18$) and puerarin (PUE)-treated group (B group, $n=22$) before dressing changes ($p>0.05$). During the dressing change procedure, mean VAS scores of A group at mid- and post-dressing changes were higher than those at pre-dressing changes ($p<0.05$) from the first day to third day. The lower values in B group were presented in comparison with those in A group at 8 min mid-dressing and every time point at post-dressing from the first day to third day after the burn shock ($p<0.05$). Especially at 8 min mid-dressing on the third day, mean VAS scores in B group was decreased by 32%, from 4.67±0.80 to 3.17±0.60. At 30 and 60 min post-dressing, mean VAS scores in B group came back to the basic and even lower than those at pre-dressing (Table 2).

Effects of puerarin on blood glucose, insulin,, and cortisol

Ten healthy volunteers were used as a control group (C group). The values of blood glucose, insulin, and cortisol in burn patients of NS-treated group (A group, $n=18$) from the first day to third day were not different ($p>0.05$), but were significantly higher than those in C group ($p<0.05$). The values of blood glucose and insulin in burn patients of PUE-treated group (B group, $n=22$) on the second and third days were lower than those on the first day ($p<0.05$) but were also significantly decreased in comparison with those in burn patients of A group at post-dressing on the second day and third day ($p<0.01$; Table 3).

Effects of puerarin on the expression of $P2X_3$ immunoreactivity in PBMCs

$P2X_3$ receptor immunoreactivity in the PBMCs was detected using immunohistochemistry. The stain values (integrated optical density) of $P2X_3$ expression in C group (healthy volunteers) from the first to third day were not different ($p>0.05$). The stain value of $P2X_3$ expression in C group was 62.4±6.33. The stain values of $P2X_3$ expression from the first to third day in burn patients of A group (NS-treated group) were 105.9±6.48, 111.8±9.18, and 110.6±4.48, respectively. And those in burn patients of B group (PUE-treated group) were 110.1±10.56, 102.0±6.25, and 81.6±8.41, respectively.

The expression values of $P2X_3$ immunoreactivity from the first to third days in A and B group were significantly higher than those in C group ($p<0.01$), and no difference was found between A and B group before dressing changes ($p>0.05$). The integrated optical density of $P2X_3$ expression in B group on the second day was lower than that on the first day ($p<0.05$) but also lower than that in A group on the second day ($p<0.01$) and third day ($p<0.05$). The stain values of $P2X_3$ expression in B group on the second day and third day were sharply decreased in comparison with those in A group ($p<0.01$) but still higher than those in C group ($p<0.01$; Fig. 1).

Effects of puerarin on the expression of $P2X_3$ mRNA in PBMCs

The expression level of $P2X_3$ mRNA in PBMCs was detected by RT-PCR. The gray scale values of $P2X_3$/β-actin

Table 2 Effects of puerarin on Visual Analogue Scale

Group	Day	10 min pre-dressing	1 min mid	8 min mid	10 min post	30 min post	60 min post
A group ($n=18$)	1	1.75±0.48$^{\#}$	4.50±1.04	5.00±1.08$^{\#}$	3.50±1.19$^{\#}$	2.75±1.11$^{\#}$	2.50±1.19$^{\#}$
	2	1.50±0.28$^{\#}$	5.00±1.08	5.50±1.56$^{\#}$	3.50±0.96$^{\#}$	1.75±0.47$^{\#}$	1.75±0.48$^{\#}$
	3	1.75±0.25$^{\#}$	4.00±0.81	4.50±1.26$^{\#}$	3.00±1.0$^{\#}$	2.00±0.70$^{\#}$	1.50±0.29$^{\#}$
B group ($n=22$)	1	1.83±0.31	5.83±0.91	4.67±0.80*	2.50±0.43*	1.67±0.21*	1.17±0.17*
	2	1.67±0.21	5.17±1.05	5.00±0.68*	3.17±0.60*	2.00±0.45*	1.33±0.21*
	3	1.50±0.22	4.50±0.76	3.17±0.60*	1.67±0.21*	1.33±0.21*	1.17±0.17*

A group: normal saline (NS)-treated group; B group: puerarin (PUE)-treated group. The VAS scores of A group at mid- and post-dressing changes were higher than those at pre-dressing changes $^{\#}$($p<0.05$) from the first day to third day. The lower values in B group were presented in comparison with those in A group at 8 min mid-dressing and every time point at post-dressing from the first day to third day after the burn shock *($p<0.05$)

Table 3 Effects of puerarin on blood glucose, insulin, and cortisol

	Healthy	NS d1	NS d2	NS d3	PUE d1	PUE d2	PUE d3
Blood glucose, mmol/L	5.18±0.52	7.83±1.27$^{\#}$	7.47±2.11$^{\#}$	7.43±2.48$^{\#}$	7.55±1.27	5.72±1.13*$^{\#\#}$	5.60±1.14*$^{\#\#}$
Insulin, mIU/L	7.42±1.40	21.77±4.90$^{\#}$	23.23±7.72$^{\#}$	21.00±9.90$^{\#}$	24.75±6.08	20.00±11.34*$^{\#\#}$	13.67±1.04*$^{\#\#}$
Cortisol, μg/dl	11.45±2.68	18.80±3.76$^{\#}$	21.48±3.71$^{\#}$	18.00±3.46$^{\#}$	11.28±2.12	14.03±2.74*$^{\#\#}$	10.1±4.84*$^{\#\#}$

A group: normal saline (NS)-treated group; B group: puerarin (PUE)-treated group; C group: healthy volunteers (control). The values of blood glucose, insulin, and cortisol of A group from the first day to third day were significantly higher than those in C group $^{\#}$ ($p<0.05$), and the values of B group on the second and third days were lower than those on the first day *($p<0.05$), but were also significantly decreased in comparison with those in A group at post-dressing on the second day and third days $^{\#\#}$ ($p<0.01$)

in C group (healthy volunteers) from the first to third day were not different ($p>0.05$). The expression value of $P2X_3$ mRNA in C group was 0.5935±0.0080. The stain values of $P2X_3$ mRNA expression from the first to third days in burn patients of A group (NS-treated group) were 1.1001±0.1476, 1.0143±0.0221, and 1.0923±0.0197, respectively. The expression values of $P2X_3$ mRNA from the first to third days in burn patients of B group (PUE-treated group) were 1.1715±0.0308, 0.9848±0.0145, and 0.7188±0.0086, respectively.

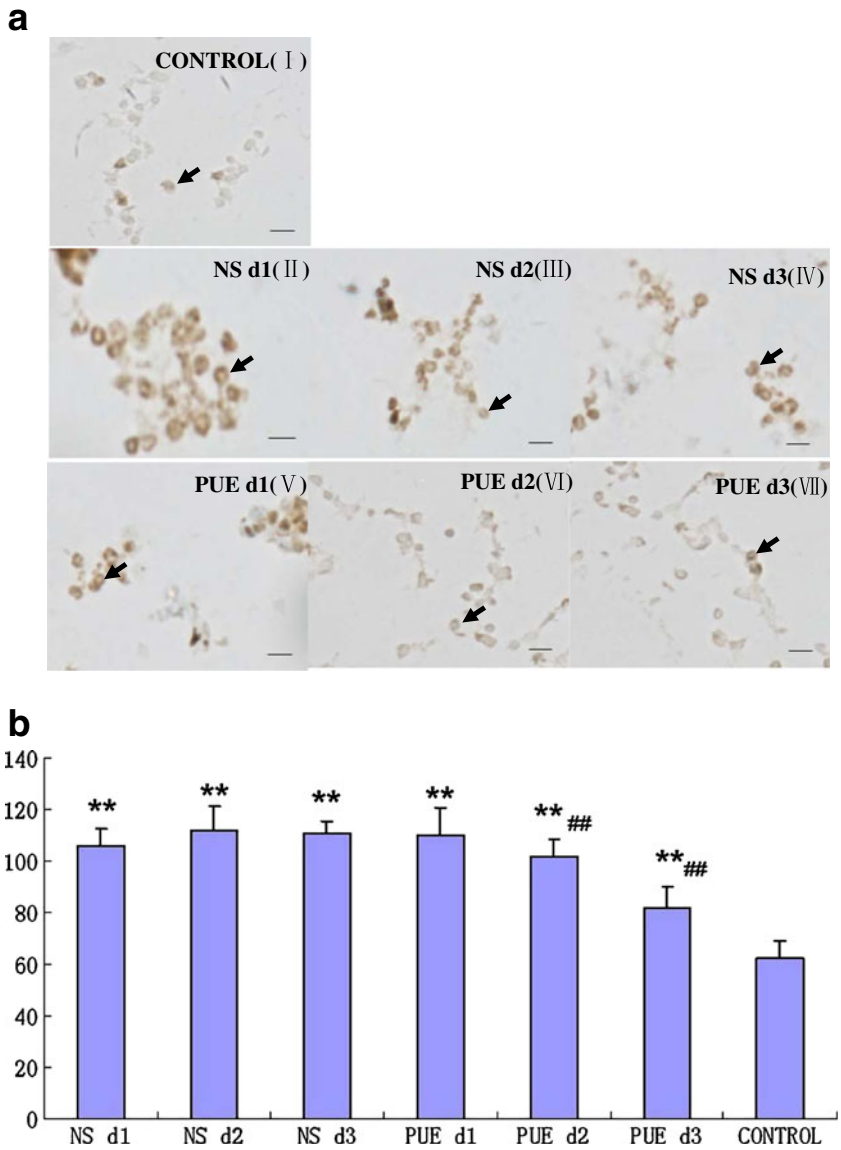

Fig. 1 Effect of puerarin on the expression of $P2X_3$ receptors in PBMCs by immunohistochemistry. **a** *I*: healthy volunteers (control); *II*: NS-treated group on the first day; *III*: NS-treated group on the second day; *IV*: NS-treated group on the third day; *V*: PUE-treated group on the first day; *VI*: PUE-treated group on the second day; *VII*: PUE-treated on the third day. (*Arrows* indicate the immunostaining of PBMCs; scale bars, 20 μm). **b** **$p<0.01$, compared with Healthy group(control); $^{\#\#}p<0.01$, compared with NS-treated group in histogram

The expression values of $P2X_3$ mRNA from the first to third days in A and B groups were significantly higher than those in the C group ($p<0.01$). The expression levels of $P2X_3$ mRNA in B group on the second and third days were not only significantly lower than those on the first day ($p<0.01$) but also lower than those in A group from the first to third days ($p<0.01$; Fig. 2).

Discussion

Acute pain following burn is due to the stimulation of skin nociceptors. Burns will immediately prompt an intense inflammatory response and the release of chemical mediators that sensitize the active nociceptors at the site of injury. This will lead to the wound being sensitive to mechanical stimuli such as touch, rubbing, or debridement, as well as chemical stimuli such as antiseptics or other topical applications, which is called primary hyperalgesia [41, 43]. Our results show that the VAS pain scores in the puerarin-treated group were lower compared with those in the saline group at 8 min mid-dressing and at each time point post-dressing from the first to third days after the burn shock. Especially at 8 min mid-dressing on the third day, the VAS scores in the puerarin-treated group were significantly decreased. The VAS has been shown to be a sensitive indicator of pain intensity and treatment effects [36, 56], and the degree of pain relief is significantly correlated with a decrease in VAS [36, 56]. It appears that the treatment with puerarin can relieve the procedural pain involved in dressing changes of burn patients.

Pain accompanies burns and involves emotion and physical stress and dysregulation of the hypothalamic–pituitary–adrenocortical axis and immune system [14, 40]. This rapid response is brought about by sudden increases in sympathetic nervous system activity and endogenous stress hormone levels [14, 50]. It has been reported that the secretions of plasma cortisol, adrenocorticotropic hormone, and aldosterone are significantly increased immediately after severe burns, and the extent of this is associated with intensity of stress [14, 40]. Furthermore, the levels of blood glucose, insulin, and cortisol in burn patients are enhanced. In patients with large areas of burn, particularly third-degree burns, lasting hyperglycemia and hypermetabolism promote secretion from Islets of Langerhans to accelerate glycogenolysis, which elevate blood glucose [14, 25, 40, 51]. Increased levels of cortisols are further associated with enhanced hepatic gluconeogenesis and a reduced insulin-mediated glucose uptake into skeletal muscle and adipose tissue [20, 44]. These phenomena may lead to elevated blood glucose levels in association with normal or elevated serum insulin concentrations [53, 57]. The blood glucose,

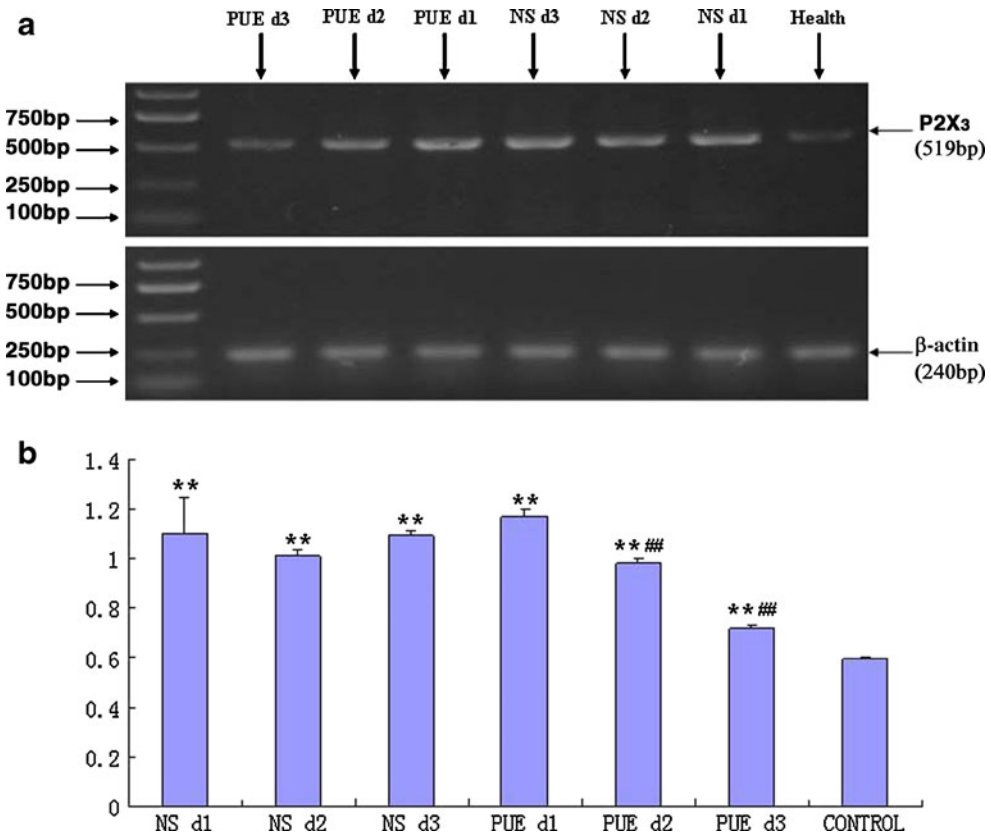

Fig. 2 Effects of puerarin on the expression of $P2X_3$ mRNA in PBMCs were detected by RT-PCR. **a** The expression of $P2X_3$ receptors mRNA in NS and PUE group was higher than that in the healthy control group ($p<0.01$). The expression of $P2X_3$ mRNA in group PUE d2 and PUE d3 was significantly lower than that in PUE d1 ($p<0.01$) and significantly lower than that in NS group($p<0.01$), although still higher than that in the healthy control group ($p<0.01$). **b** $^{**}p<0.01$, compared with Healthy group (control); $^{\#\#}p<0.01$, compared with NS-treated group in histogram

insulin, and cortisol levels of burn patients in the puerarin-treated group at post-dressing change times were not only lower than those at pre-dressing but were also significantly decreased in comparison with those in the saline group at post-dressing change times. After treatment with puerarin, the decrease in VAS correlated with the changes of blood glucose, insulin, and cortisol levels. The levels of blood glucose, insulin, and cortisol in burn patients were enhanced after stress response-induced by burn pain. The pain relief in dressing changes of burn patients followed the reduction of blood glucose, insulin, and cortisol levels. These observations further indicate that puerarin has an analgesic effect on procedural pain in burn dressing changes.

It has been reported that puerarin could decrease the sensitization of $P2X_3$ receptors involved in hyperalgesia after burn injury in the rats [54]. Does the mechanism of puerarin inhibiting procedural pain in burn dressing changes of patients also affect $P2X_3$ receptors?

ATP is involved in peripheral pain signaling by acting upon $P2X_3$ receptors [3–5, 7, 24], and $P2X_3$ receptors are crucial in mediating both acute pain and chronic pain [7, 12, 16–18, 29–34, 46–48, 58]. The $P2X_{2/3}$ knockouts display significant reduction in pain reception in response to ATP [9]. Neurons expressing $P2X_3$ receptors are dramatically more responsive to ATP with even small increases in temperature [13]. $P2X_3$ expression in sensory neurons was increased after burn injury in the rats [18, 54], and $P2X_3$ receptors are relevant with burn injury pain [18, 54]. PBMCs are a key link in inflammatory response [35]. There are strong immunoreactivities for β-endorphin, EM-1, and EM-2 in leukocytes of inflamed subcutaneous paw tissue at 4 days after intraplantar injection of complete Freund's adjuvant [27]. These leukocytes had morphological appearances consistent with mononuclear cells and granulocytes. The pain of tissue damage is conveyed by the release of cytosolic ATP onto receptors expressed by nociceptive sensory neurons (nociceptors) [1, 2, 26]. ATP can also be released by macrophages in response to cytokines, osmotic stress, and mechanical stimulation. Puerarin may decrease the release of ATP at the site of burn injury and inhibit the intense inflammatory response to relieve the burn-related procedural pain evoked by dressing changes.

We investigated whether $P2X_3$ receptors are involved in puerarin actions. Our results show that the expression of $P2X_3$ protein and mRNA in PBMCs of burn patients is significantly increased. $P2X_3$ receptors on PBMCs appear to be involved in nociception or pain after burn injury. The expression of $P2X_3$ protein and mRNA in PBMCs of burn patients was significantly decreased in the puerarin-treated group at post-dressing times. The effect of puerarin on $P2X_3$ receptor expression of PBMCs in humans is in agreement with previous studies that show downregulation of the expression of $P2X_3$ receptors following in burn rats [18, 54]. The effect mechanisms of puerarin on burn pain may include antagonism of inflammatory factors (such as ATP) by downregulated expression of $P2X_3$ receptors in PBMCs of burn patients.

Conclusions

In summary, $P2X_3$ receptors are involved in procedural pain in burn dressing changes. Puerarin can decrease the stress response to burn pain, antagonize the inflammatory response mediated by ATP, and decreases the upregulated expression of $P2X_3$ protein and mRNA in PBMCs after burns to decrease VAS. These actions may mediate the analgesic effect of puerarin on procedural pain in dressing changes of burn patients related to $P2X_3$ receptors.

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