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# Effects of puerarin on the inflammatory role of burn-related procedural pain mediated by $P2X_7$ receptors

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ABSTRACT

*Background:* Burn injury can induce an inflammatory response in the blood and wound of patients. Procedural activities in burn patients are particularly problematic in burn care due to their high intensity and frequency; hence, procedural pain evoked by burn dressing changes is a common severe issue. Previous studies demonstrated that purinergic signalling is one of the major pathways involved in the initiation, progression and down-regulation of the inflammatory response. Adenosine 5′-triphosphate (ATP) contributes to inflammation, and increased extracellular ATP levels amplify inflammation *in vivo* via the $P2X_7$ receptor. In the present study, the effect of puerarin, an active ingredient extracted from Chinese herbal medicine Ge Gen, on pain relief of burn patients during dressing change and the mechanism related to the regulation of the purinergic signalling pathway were investigated.

*Methods:* Burn patients were randomly divided into the normal saline group (NS-treated burn patients) and the puerarin-treated group (PUE-treated burn patients), and healthy volunteers were recruited as a control group. The visual Analogue Scale (VAS) scores, heart rate (HR) and respiratory rate (RR) of NS- and PUE-treated burn patients were observed. In addition, interleukin (IL)-1 and IL-4 levels in blood samples, as well as expression of $P2X_7$ receptor messenger RNA (mRNA) and protein in peripheral blood mononuclear cells (PBMCs) were determined.

*Results:* The IL-1 levels in the PUE-treated burn patients at post-dressing changes were significantly decreased in comparison with those in NS-treated burn patients; in contrast, the IL-4 levels in PUE-treated burn patients were increased. The expression levels of $P2X_7$ protein and mRNA in PBMCs of PUE-treated burn patients were significantly decreased in comparison with those in NS-treated burn patients.

*Conclusions:* The inflammation and associated pain involved in dressing changes of burn patients were relieved by puerarin treatment. The effects were correlated with the decreased expression level of $P2X_7$ receptor mRNA and protein in PBMCs of burn patients.



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Pain is a major issue for burn patients. Burn pain includes procedural pain, background pain and breakthrough pain [1]. Despite increasing emphasis on pain management, burn wound-care-related pain continues to be documented as an ongoing issue. Changing of burn dressings is a common clinical practice in burn treatment. Apart from being one of the most difficult forms of acute pain to treat, procedural burn pain also occurs at relatively high frequency, intensity and variability. Wound healing is a complex process involving a series of overlapping phases, including haemostasis, inflammation, proliferation and resolution [2]. In some cases, robust inflammation, such as that associated with burn injury, may be detrimental to wound closure [3].

Inflammation is a complex response generated by an interacting network of stimulatory and inhibitory signals. Belonging to a superfamily of inflammation-related gene-products, interleukin (IL)-1 is a potent pro-inflammatory cytokine that causes the accumulation of arachidonic acid metabolites, up-regulates inducible nitric oxide (NO) synthase and sustains NO production [4]. In recent years, there is increasing awareness that adenosine 5′-triphosphate (ATP) is a candidate damage signal locally released at the inception of inflammation [5]. The mechanism by which IL-1 activates pro-inflammatory responses in target cells is fairly known. ATP-induced IL-1 processing and secretion is triggered by the $P2X_7$ receptor [6]. IL-4 is an anti-inflammatory cytokine. It has marked inhibitory effects on the expression and release of the pro-inflammatory cytokines [7]. IL-4 is a highly pleiotropic cytokine that is able to influence Th cell differentiation [8].

Extracellular purines and pyrimidines are important molecules. They produce biological action through the specific cell surface purine and pyrimidine receptors [9]. Two families of purine receptors were recognised in 1978, including P1 receptors sensitive to adenosine and P2 receptors sensitive to ATP and adenosine diphosphate (ADP). Later, P2 receptors were divided into ionotropic P2X receptors and metabotropic P2Y receptors on the basis of mechanism of action, pharmacology and molecular cloning. Cloning of these receptors in the early 1990s was a turning point in the acceptance of the purinergic signalling hypothesis. Currently, there are four subtypes of P1 receptors, seven subunits that make several subtypes of the P2X ion-channel receptor and eight sub-types of G protein-coupled receptors in the literature [10]. The levels of nucleotides in the extracellular space are normally low and are regulated by ectonucleotidases. However, in the event of localised trauma, high concentrations of nucleotides capable of activating the P2 receptors were observed [11]. The nucleotide receptor $P2X_7$ is a potential therapeutic target and a biomarker for many inflammatory diseases, and expressed in several cell types, including monocytes, macrophages, osteoblasts, osteoclasts, astrocytes and microglia [12,13]. The $P2X_7$ receptor is a ligand-gated cation channel activated by extracellular ATP, in a form of a reversible plasma-membrane pore permeable to hydrophilic solutes of molecular mass up to 900 Da [14,15]. $P2X_7$ activation also leads to the release and maturation of both IL-1β and IL-18 from lipopolysaccharide (LPS)-activated monocytes and macrophages [16], as well as release of tumour necrosis factor-α (TNF-α) from rat brain microglia [17].

Puerarin ($C_{21}H_{20}O_9$) is a major isoflavonoid derived from the Chinese medical herb Radix puerariae (kudzu root). In China, puerarin had been used for the treatment of cardiovascular diseases [18,19] and cerebral ischaemia [20]. It has been reported that puerarin-induced neuroprotection is related to the control of inflammation [18–21]. In our previous studies, puerarin has been found to antagonise the nociceptive or pain transmission mediated by $P2X_3$ and/or $P2X_{2/3}$ receptors in primary afferent neurons [22–24]. The purpose of this study is to further investigate the effects of puerarin on inflammatory responses mediated by the $P2X_7$ receptor in peripheral blood mononuclear cells (PBMCs) and associated pain relief of burn patients.

## 1. Materials and methods

### 1.1. *Sample collection*

Thirty-two burn patients and 10 healthy controls were enrolled at the Burn Center of the First Affiliated Hospital of Nanchang University, China, from 2008 to 2010. Following local ethics committee approval, written informed consent was obtained from them. Inclusion criteria of burn patients include age between 25 and 50 years, weighing from 50 to 80 kg, 2nd- and 3rd-degree burns with total body surface area (TBSA) between 10% and 50% and 3rd-degree burns with TBSA less than 15%, and no pre-medication. The nature of the burns was flame and scald. The patients had been through the shock stage. The time interval was 48–72 h between injury and entry into the study. In addition, their visual Analogue Scale (VAS) score in burn dressing changes was $\geqq 5$. Burn patients were excluded if they were confused, had injury which precluded vital signs on an electrocardiography machine, pregnancy and lactation or had a history of substance abuse. Healthy controls include volunteers aged between 20 and 40 years with no history of substance abuse. Burn patients were randomly divided into the normal saline group (NS-treated burn patients, $n = 15$) and the puerarin-treated group (PUE-treated burn patients, $n = 17$) by random allocation with dice method. The caregivers and investigators were blinded to the treatments. The NS group received no analgesics during wound care. The NS-treated burn patients were applied with 100 ml 0.9% sodium chloride injection. The PUE-treated burn patients were applied with 100 ml puerarin glucose injection (Yangtze River Pharmaceutical Group, SFDA Licence No. H20020450, including puerarin 200 mg). Both administrations by intravenous drip within 30–40 min and at 30–40 drops per min lasted for 3 days before dressing changes after the burn shock. Other routine clinic manner and practice patterns were not changed during the study period. No treatment was given in healthy controls (Table 1).

### 1.2. *Visual Analogue Scale*

VAS was used as the instrument for measuring the pain scale level. It is a universal, reliable method for assessing pain. A range of 0–10 was used. The scores were grouped as follows: no pain (0–1), mild pain (2–4), moderate pain (5–7) and severe pain (8–10). The pain was evaluated 10 min prior to start, after the