Journal of Integrative Agriculture
Advance Online Publication 2014 Doi：10.1016/S2095-3119(14)60824-8

Influence of Gallic Acid on Porcine Neutrophils Phosphodiesterase 4, IL-6, TNF-α and Rat Arthritis Model[1]

JIANG Dai-xun, ZHANG Mei-hua, ZHANG Qian, CHEN Yi-shan, MA Wen-jing, WU Wei-peng, MU Xiang and CHEN Wu

Beijing Key Laboratory of Traditional Chinese Veterinary Medicine, Beijing University of Agriculture, Beijing 102206, P.R.China

ABSTRACT

Our previous studies showed the anti-inflammatory effects of Paeonia lactiflora roots extract may be mediated, at least in part, through its gallic acid content, and this effect may be regulated in part by an inhibition on cAMP-phosphodiesterase (PDE). To explore the anti-inflammatory effect and mechanism, we further studied the influence of gallic acid on neutrophils PDE4 activity and expression, TNF-α and IL-6 content and rat arthritis model. PDE4 activity and gene express was calculated respectively by substrate cAMP change examined with HPLC and the method of real-time RT-PCR. The concentration of IL-6 and TNF- α in supernatant were assayed by ELISA method. Model of rat arthritis was caused by complete Freund's adjuvant. Results showed that gallic acid had a dose-dependent restraint on PDE4 activity of neutrophils in vitro, promoted significantly PDE4A expression (P<0.01), and had no influence on expressions of PDE4B and 4D. However, PDE4C expression was not detected. Gallic acid could promote IL-6 release (p<0.05), and inhibit TNF-α release of neutrophils (P<0.05). The experiment in vivo showed that gallic acid had obvious restraint on local inflammation of animal model (P<0.05). Therefore, the anti-inflammatory effect of gallic acid may be mediated in part through an inhibition on PDE4 activity and further an increase of IL-6 and a decrease of TNF- α of neutrophils, and this effect seemed to have no relationship with PDE4 expression.

Key words: gallic acid, phosphodiesterase 4, inflammation, IL-6, TNF-α, neutrophils

Correspondence CHEN Wu, Tel/Fax: +86-10-80799434, E-mail: tcvmchenwu@hotmail.com

 Doi：10.1016/S2095-3119(14)60824-8

1. Introduction

Gallic acid is a type of polyphenolic compounds distributed widely in nature, and mainly exists in plants of the families of Cornaceae, Punicaceae, Paeoniaceae and so on. Commonly used herbs in clinic such as Fructus Corni, Pericarpium Granati, Fructus Chebulae, Raidix Paeoniae Alba (*Paeonia lactiflora* roots) and Galla Chinensis contain gallic acid. In recent years, researchers have conducted further studies on gallic acid, a common active ingredient in many herbs, and have found that it has many beneficial effects, including anti-inflammation (Hsiang *et al.* 2013), antianaphylaxis (Kim *et al.* 2006), anti-tumor (Verma *et al.* 2013), antiradiation (Gandhi and Nair 2005) and control of insulin level (Latha and Daisy 2011), of which the molecular mechanism has relationship with improvement of antioxidant enzymes activity, increase of glutathione content, intervention of cytochrome P450, inhibition of the transcription factor NF- κB and so on (Tung *et al.* 2009; Choi *et al.* 2009). However, the mechanism of gallic acid on phosphodiesterase has not been extensively studied.

Our preliminary studies show that gallic acid is one of the main effective ingredients in *Paeonia lactiflora* roots, and that its anti-inflammatory effects may be mediated, at least in part, through its gallic acid content. This effect may be regulated in part by an inhibition on cAMP-PDE in neutrophils (Jiang *et al.* 2011). Neutrophils play an important role in nonspecific immunity. The increase of cAMP content in neutrophils can inhibit the respiratory burst and degranulation, and thereby inhibit the inflammatory response (Luo and Chen 2000). PDE4, specifically hydrolyzing cAMP, is the main PDE family in neutrophils (Jiang *et al.* 2007). Due to the decisive role on cAMP signal in inflammatory cells such as neutrophils, PDE4 is recognized as a new anti-inflammatory target (Lugnier 2006). Therefore, each big pharmaceutical company in the world devotes significant research into PDE4 inhibitor with the hope that the inhibitor can be used to treat various inflammatory diseases. An example is roflumilast, being sold as a new PDE4 inhibitor for use in the auxiliary treatment of chronic obstructive pulmonary disease, however, the clinical dose must be limited to mitigate side effects such as nausea and emesis (Yan *et al.* 2013). Our studies focused on the influence of gallic acid on activity and expression of PDE4, release of IL-6 and TNF-α in neutrophil, and the anti-inflammatory role on animal model. Our results suggest a new theory for the preparation and clinical application of gallic acid.

 Doi：10.1016/S2095-3119(14)60824-8

2. Materials and methods

2.1. Chemicals

Quantikine ELISA porcine IL-6 and TNF-αkits were purchased from R&D Systems, RevertAidTM First Strand cDNA Synthesis Kit was from Fermentas. fMLP, cAMP, rolipram and HEPES were from Sigma, Trizol Reagent was from Invitrogen, RQ1 RNase-Free DNase was from Promega, TransStartTM Green qPCR SuperMix (ROX I) was from Beijing Transgen Biotech CO., Ltd. Dextran T-500 from Phamarcia, RPMI1640 from Gibco, lymphocyte separating medium from the Tianjin Haoyang Biological Product Technology Co., Ltd, China, HPLC grade methanol was from Fisher Scientific. Gallic acid was from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). All other reagents were all analytical pure.

2.2. Experimental animal

Sprague-Dawley rats (150 g each, 55 d of age) and China experimental minipig (20 kg, one year of age) were used. The animals were housed in an air-conditioned room (20–23◦C) with controlled lighting (from 7:00 a.m. to 6:00 p.m.). The animals were fed on pelleted food, and tap water was available ad libitum. All animals were monitored and maintained in accordance with the ethical recommendations of the Universities Federation for Animal Welfare. The animals were acclimatized to the laboratory for at least 3 d before testing.

2.3. Isolation of neutrophils

Blood was drawn in heparin from the jugular vein of the China experimental minipig, which was anesthetized with an ear vein injection of thiopental sodium (15 mg kg^{-1}). Highly purified neutrophils were obtained by Chen's method (Chen *et al.* 2003).

2.4 Measurement of PDE4 activity

Neutrophils were rinsed twice with RPMI1640, and the concentration was adjusted to 5×10^8 cells mL^{-1}. RPMI1640 and neutrophils suspension (5.0×10^7 cells) was added into each well of a 24-well cell culture plate. GA was gallic acid (0.1, 0.25, 0.5, 1, 2.5, 10 mmol L^{-1}, dissolved in

 Doi：10.1016/S2095-3119(14)60824-8

RPMI1640, pH 7.4). Plates were incubated for 10 min at 37◦C in a 5% CO_2 incubator. FMLP, an inflammatory factor, was added into each well to a final concentration of 1 nmol L^{-1} with the exception of the blank control. Following incubation for 30 min at 37◦C in 5% CO_2, the contents of each well were removed into centrifuge tubes and centrifuged for 5 min at 1000×g. The supernatant was discarded, and the cells were rinsed three times with Ca^{2+}- and Mg^{2+}-PBS and adjusted to 1×10^8 cells mL^{-1} with Ca^{2+}- and Mg^{2+}-PBS. Cells were homogenized with a vitric homogenizer in an ice water bath and subsequently used to assay PDE4 activity.

The cAMP-PDE activity was detected using the method described in our previous report (Jiang *et al.* 2006). To identify the cAMP-PDE4 activity, a specific inhibitor, rolipram, was added to the incubation mixture at a final concentration of 10 μmol L^{-1} (Levallet *et al.* 2008). The PDE activity measured in the presence of rolipram was subtracted from the total activity to quantify the PDE4 activity.

2.5. Measurement of PDE4 gene expression

According to the inhibition of different concentrations of gallic acid on PDE4 activity in cultured neutrophils, we determined 1 mmol L^{-1} gallic acid, due to its proper inhibition ratio of approximately 20% on PDE4, as the effective concentration to study the influence on neutrophils PDE4 expression *in vitro* at various times.

The assay procedures of gallic acid on PDE4 expression were identical to that of gallic acid on PDE4 activity except for positive control rolipram (1 μmol L^{-1}) and the last cultured time of 15 min, 1, 3 and 6 h. After the centrifugation, RNA of the cells were extracted to further determine the expression of PDE4A, 4B, 4C and 4D, and the supernatant was used to assay the concentration of TNF-α and IL-6.

RNA of neutrophils was isolated with Trizol reagent according to the manufacturer's instructions. Five micrograms of RNA were used for cDNA synthesis. The gene expression level was determined by real-time RT-PCR using a TransStartTM Green qPCR SuperMix (ROX I). β-actin was used as a reference gene. Primers 5'-GGATGCAGAAGGAGATCACG-3' and 5'-ACTCCTGCTTGCTGATCCAC-3' were used for β-actin. Primers 5'-GCGAGCAGACTTAGCAGATAG-3' and 5'-GTCCTCAAGGCAGGCAGA-3' were used for PDE4A. Primers 5'-TTGTATCGGCAATGGACAGA-3' and

 Doi：10.1016/S2095-3119(14)60824-8

5’-CAGAAGCCGTGTGCTTATCA-3’ were used for PDE4B. Primers 5’-GAGAACCATCACCTGGCTGT -3’ and 5’-GCCAGGAGGTTCATGTGTTT-3’ were used for PDE4C. Primers 5’-TGATGCACAGCTCCAGTT-3’ and 5’-AGGCCGGTTACCAGACAGC-3’ were used for PDE4D. The RT-PCR conditions were as follows: 95℃ for 2 min, followed by 40 cycles of 95℃ for 20 s, 60℃ for 20 s and 72℃ for 30 s. The expression level was normalized to the β-actin control, and relative expression values were determined against PDE 4A, 4B, 4C, 4D control at 15 min respectively using the comparative Ct method.

2.6. Measurement of TNF-α and IL-6

The concentrations of TNF-α and IL-6 in the supernatant were assayed according to the manufacturer’s instructions of TNF-α and IL-6 ELISA kits.

2.7. In vivo anti-inflammatory activity of gallic acid on rat arthritis model

50 male Sprague-Dawley rats were randomly divided into five groups. The control group was intraperitoneally given physiological saline, the experimental groups were given gallic acid (1, 5, 10 μg g^{-1} body weight), and the positive control group was given rolipram (2.75 μg g^{-1} body weight). After 30 min, 0.05 mL complete Freund’s adjuvant was injected under the plantar fascia of each rat’s right rear foot to cause local arthritis. The thickness of the tissue at the reaction site was measured with a micrometer before injection of Freund’s adjuvant, and at 3, 6, 15 and 24 h after injection. The degree of swelling was calculated as the difference in thickness at the different measurement times.

2.8. Statistical analysis

The experimental results were expressed as the mean±standard error of the mean (SEM) and the statistical significance was evaluated by using the student’s t-test. P-values of less than 0.05 implied significance.

3. Results

3.1. Influence of gallic acid on PDE4 activity

 Doi：10.1016/S2095-3119(14)60824-8

PDE4 activity of control group was significantly raised from that of blank control group ($P<$ 0.01; Fig. 1), demonstrating that 1 nmol L^{-1} of fMLP had obviously promotion on PDE4 activity. Gallic acid demonstrated a dose-dependent inhibitory effect on PDE4 activity. At concentrations of 0.1, 0.25, 1, 2.5, and 10 mmol L^{-1}, PDE4 activity of neutrophils was inhibited approximately 1.65%, 8.77%, 22.58%, 7.89% and 66.33% compared with the control.

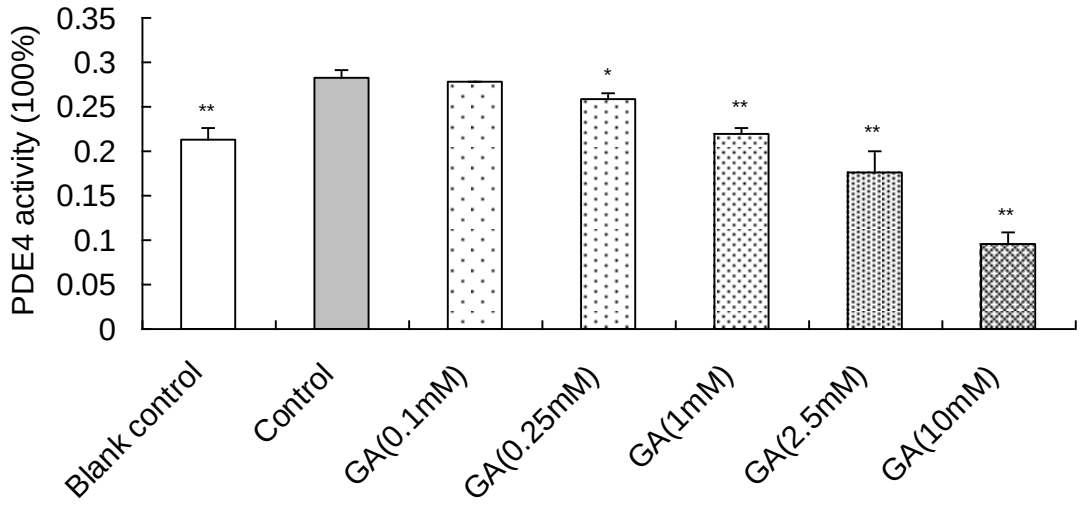


Fig. 1 Influence of gallic acid (GA) on PDE4 activity of neutrophils *in vitro*. Activity was shown as the degradation percentage of substrate cAMP. Blank control had no stimulus of fMLP. Control =negative control (with same volume of PBS instead of GA). Data are expressed as the means ±SEM (n=3). *$P < 0.05$; ** $P < 0.01$ compared with control.

3.2. Influence of gallic acid on PDE4A expression

The relative gene expression of PDE4A in each group had no significant change in 15 min and 1 h (Fig. 2). However, the expression of PDE4A in gallic acid and rolipram groups were significantly increased compared with that of the control group at 3 and 6 h, respectively ($P<0.01$), showing that gallic acid and rolipram can promote PDE4A expression.

 Doi：10.1016/S2095-3119(14)60824-8

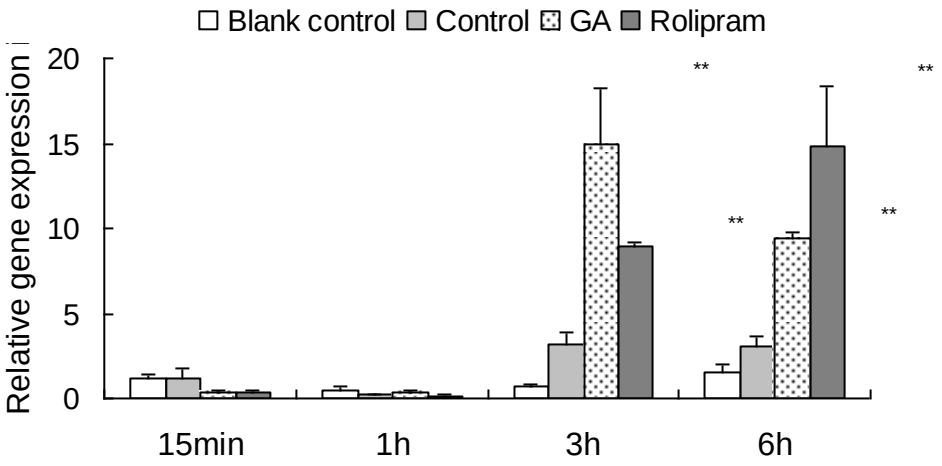
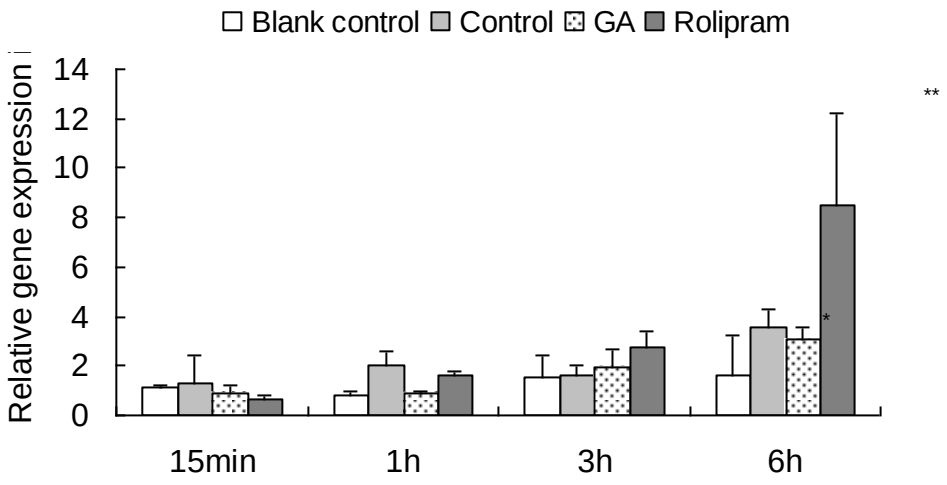


Fig. 2 Influence of gallic acid (GA) on PDE4A expression of neutrophils *in vitro*. Blank control had no stimulus of fMLP. Control=negative control (with same volume of PBS instead of GA). Data are expressed as the means±SEM (n=3). ***P* < 0.01 compared with control.

3.3. Influence of gallic acid on PDE4B expression

The relative gene expression of PDE4B in each group had no significant change in 15 min, 1 h and 3 h (Fig. 3). Compared with that of blank control group, the expression of PDE4B of control group was significantly raised at 6 h (P<0.05), showing that fMLP could promote PDE4B expression. There was no manifest change of PDE4B expression in gallic acid group, however, the expression of PDE4B was significantly increased in rolipram group at 6 h (P<0.01), showing that rolipram can promote PDE4B expression.



 Doi：10.1016/S2095-3119(14)60824-8

Fig. 3 Influence of gallic acid (GA) on PDE4B expression of neutrophils *in vitro*. Blank control had no stimulus of fMLP. Control=negative control (with same volume of PBS instead of GA). Data are expressed as the means±SEM (n=3). *P < 0.05; **P < 0.01 compared with control.

3.4. Influence of gallic acid on PDE4D expression

The relative gene expression of PDE4D in each group had no significant change in 15 min and 1 h (Fig. 4). Compared with that of blank control group, the expression of PDE4D of control group was significantly raised at 6 h (P<0.05), showing that fMLP can PDE4D expression. There was no obvious change of PDE4D expression in gallic acid group, however, the expression of PDE4D was significantly increased in rolipram group at 3 and 6 h compared with that of control group (P<0.05), showing that rolipram can promote PDE4B expression.

In addition, the relative gene expression of PDE4C in neutrophils was not detected at the different time measurements in this study, and this may have a relationship to the low expression of PDE4C in neutrophil.

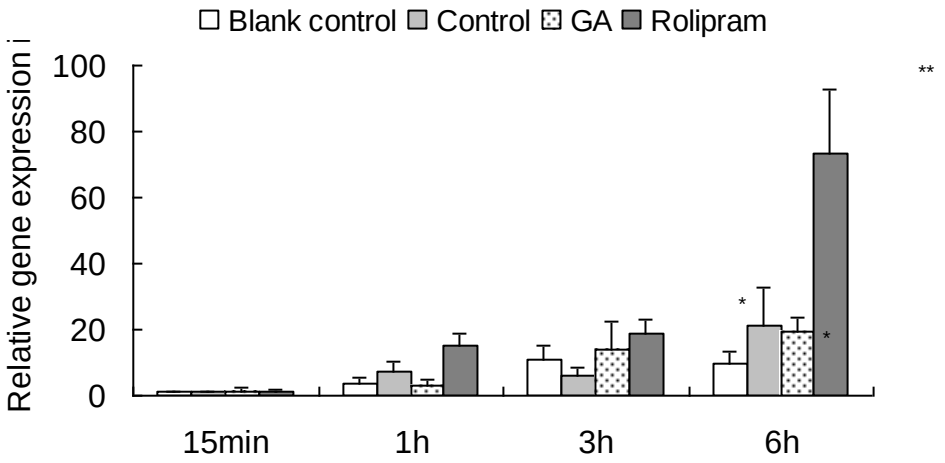


Fig. 4 Influence of gallic acid (GA) on PDE4D expression of neutrophils *in vitro*. Blank control had no stimulus of fMLP. Control=negative control (with same volume of PBS instead of GA). Data are expressed as the means±SEM (n=3). *P < 0.05; **P < 0.01 compared with control.

3.5. Influence of gallic acid on IL-6 concentration

 Doi：10.1016/S2095-3119(14)60824-8

The IL-6 concentration in each group had no significant change in 15 min (Fig. 5). Compared with that of blank control group, IL-6 concentration of control group was significantly raised at 3 and 6 h (P<0.05), showing that fMLP can promote the production of IL-6. IL-6 concentration of gallic acid group was significantly raised at 3 and 6 h compared with that of control group (P<0.05), showing that gallic acid can promote IL-6 production. The IL-6 concentration was significantly increased in rolipram group at 1 h (P<0.01).

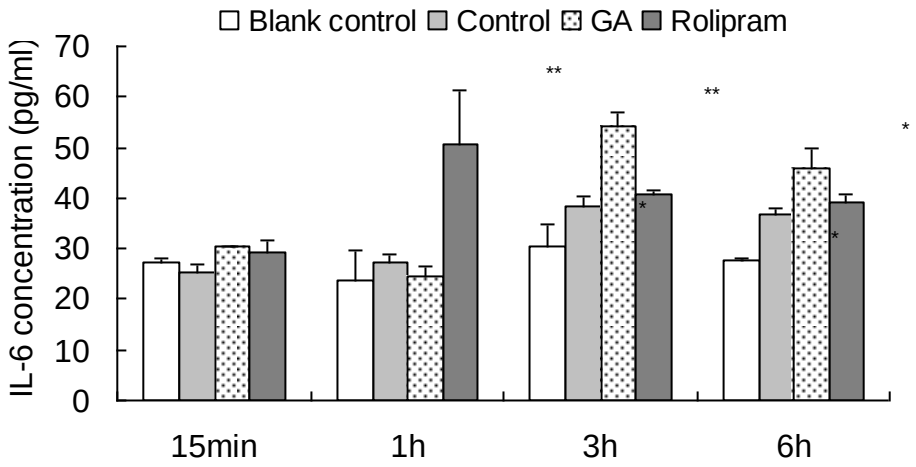


Fig. 5 Influence of gallic acid (GA) on IL-6 concentration in neutrophils supernatant *in vitro*. Blank control had no stimulus of fMLP. Control=negative control (with same volume of PBS instead of GA). Data are expressed as the means±SEM (n=3). *P < 0.05; **P < 0.01 compared with control.

3.6. Influence of gallic acid on TNF-α concentration

There was no significant change of TNF-α concentration between blank control group and control group (Fig. 6). Compared with that of control group, TNF-α concentration of gallic acid group was significantly reduced at 15 min, 3 h and 6 h (P<0.05), showing that gallic acid can inhibit TNF-α production. The TNF-α concentration was significantly lowered in rolipram group only at 1 h (P<0.05).

 Doi：10.1016/S2095-3119(14)60824-8

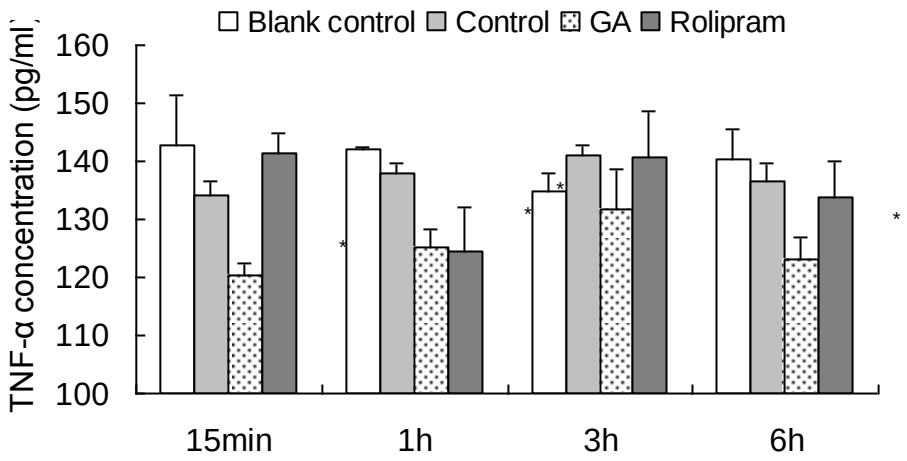


Fig. 6 Influence of gallic acid (GA) on TNF-α concentration in neutrophils supernatant *in vitro*. Blank control had no stimulus of fMLP. Control=negative control (with same volume of PBS instead of GA). Data are expressed as the means±SEM (n=3). **P* < 0.05 compared with control.

3.7. Influence of gallic acid on rat arthritis model

Gallic acid administered at 1, 5, 10 μg g^{-1} body weight significantly inhibited experimentally induced, localized arthritis inflammation at 3, 6 and 15 h (*P*<0.05; Fig. 7). Relative inhibition decreased with time between 3 and 24 h. At 1 μg g^{-1} body weight, swelling was inhibited by 4.38%, 4.42 and 3.64% at 3, 6 and 15 h, respectively. At 5 μg/g body weight, swelling was inhibited by 8.58, 5.86 and 3.74% at 3, 6 and 15 h, respectively. At 10 μg g^{-1} body weight, swelling was inhibited by 8.70, 6.06 and 3.86% at 3, 6 and 15 h, respectively. Rolipram at 2.75 μg g^{-1} of body weight inhibited swelling by 7.29, 10.05 and 6.60% at 3, 6 and 15 h, respectively.

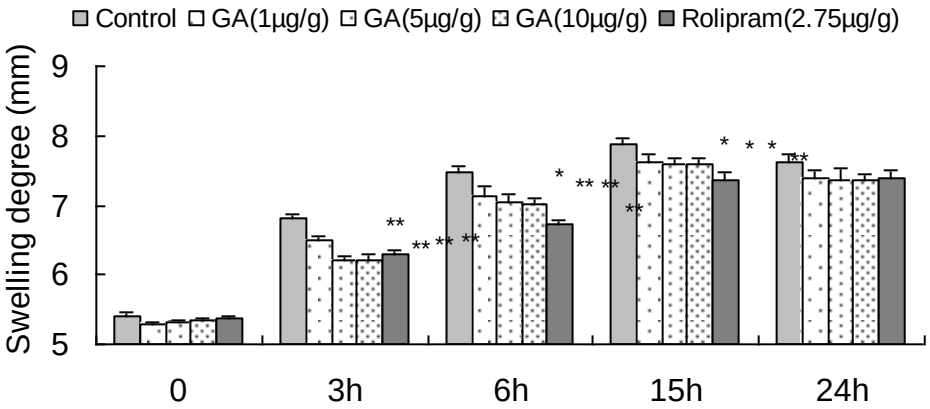


Fig. 7 Influence of gallic acid (GA) on rat arthritis model *in vivo*. Control=negative control (with

 Doi：10.1016/S2095-3119(14)60824-8

same volume of physiologic saline instead of GA). Data are expressed as the means±SEM (n=10). *P<0.05; **P<0.01 compared with control.

4. Discussion

Herbs are recognized to be relatively safe with little side effects. Developing herb-derived drugs or leading compounds has become the hope to protect human health. A large number of studies have shown that herbs can increase cAMP level through inhibiting PDE activity (Jiang *et al.* 2006). Gallic acid is a selective cAMP-PDE inhibitor sieved from *Paeonia lactiflora roots* (Jiang *et al.* 2011), and our studies show that it has a dose-dependent manner and has a high selectivity on PDE4 activity. The inhibition ratio of 1 mmol L^{-1} gallic acid on PDE4 activity in cultured neutrophils *in vitro* was 22.58%.

PDE4 gene expression can be influenced by agents such as inflammatory factor, PDE4 inhibitors. Lipopolysaccharide can induce PDE4B expression of human monocytes, but has no effect on PDE4B expression of neutrophils (Wang *et al.* 1999). Rolipram can decrease PDE4A expression but does not change PDE4B expression in hippocampus, and does not change PDE4A expression and increased PDE4B expression in cerebral cortex (Dlaboga *et al.* 2006). From the results, fMLP could significantly contribute to the expression of PDE4B (6 h), 4D (6 h), with no effect on PDE4A expression. Rolipram increased the expression of PDE4A (3 h, 6 h), 4B (6 h), 4D (3 h, 6 h). These actions indicate that PDE4 may have a feedback regulation on the influence of fMLP and rolipram. Gallic acid (3 h, 6 h) significantly contributed only to PDE4A expression, but had no effect on the expression of PDE4B, 4D. Because PDE4B accounted for the major activity of PDE4 in neutrophils (Wang *et al.* 1999), gallic acid perhaps did not exist in feedback regulation of PDE4 compared with that of rolipram. The significant promotion of PDE4A expression requires further study.

TNF- α is a multifunctional proinflammatory cytokine, having the actions of activation and induction of respiratory burst and degranulation, release of oxygen free radicals, enhancement of neutrophil phagocytosis, increase of adhesion factor expression in neutrophils. Studies show that rolipram, a PDE4 selective inhibitor, can significantly inhibit TNF-α release of white blood cells (Marx *et al.* 2002; Kambayashi *et al.* 1995), which accords with our results that rolipram

 Doi：10.1016/S2095-3119(14)60824-8

significantly inhibited TNF-α release (1 h). Similarly, gallic acid also had a significant inhibition on TNF-α release of neutrophil (except 3 h), and showed a stronger inhibition than that of rolipram. It appears, therefore, that gallic acid's reduction of neutrophils TNF-α content relates to its inhibition on PDE4 activity.

IL-6 can induce inflammatory cells to play a proinflammatory role (Suwa *et al.* 2000; Ottonello *et al.* 2002), but recent studies show an anti-inflammatory effect of IL-6 on animal model of acute lung injury and Duchenne Muscular Dystrophy (Bhargava *et al.* 2013; Kostek *et al.* 2012), including the promotion of neutrophils apoptosis (Ganeshan *et al.* 2013). Results showed that gallic acid had a significant promotion on IL-6 release of neutrophil with the extension of time. Taking the anti-inflammatory effect of gallic acid in animal models of inflammation into account, increase of IL-6 in neutrophils, induced by gallic acid, likely serves an anti-inflammatory function and may promoted the apoptosis of neutrophils.

As a selective PDE4 inhibitor from *Paeonia lactiflora* roots, gallic acid *in vivo* could obviously reduce a local inflammatory response, caused by inflammatory reagent in this study, with one time medication in short time, of which the effect was consistent with that of *Paeonia lactiflora* roots extract (Jiang *et al.* 2011), and that further prove gallic acid is the main anti-inflammatory ingredient in *Paeonia lactiflora* roots. This effect may be mediated through the reduction of PDE4 activity in neutrophils, and possibly also in other inflammatory cells.

In addition, gallic acid could be promising agent in the field of cancer chemoprevention. The inhibitory effect of gallic acid on cancer cell growth is mediated via the modulation of genes which encodes for cell cycle, metastasis, angiogenesis and apoptosis. Gallic acid inhibits activation of nuclear factor-κB and Akt signaling pathways along with the activity of cycloxygenase, ribonucleotide reductase and so on to prevent the processes of carcinogenesis (Verma *et al.* 2013). Research shows that cAMP can inhibit the proliferation, promote the differentiation, induce the apoptosis, and reverse the multidrug resistance of cancer cells (He and Dong 2002). Gallic acid can increase cAMP content by inhibiting PDE4 activity, mediating cancer chemoprevention of gallic acid, at least in part, through an inhibition on PDE4.

5. Conclusion

The anti-inflammatory effects of gallic acid may be mediated in part through an inhibition on

PDE4 activity and further an increase of IL-6 and a decrease of TNF- α of neutrophils, and this effect seemed to have no relationship with PDE4 expression.

Acknowledgements

We gratefully acknowledge the financial support of the Beijing Natural Science Foundation of China (No. 6112007), the Funding Project for Academic Human Resources Development in Institutions of Higher Learning under the Jurisdiction of Beijing Municipality (PHR201107134), and 2012 Scientific Research Quality Raising Funds of Beijing University of Agriculture (PXM2012_014207_000010/ PXM2012_014207_000013).

References

Bhargava R, Janssen W, Altmann C, Andrés-Hernando A, Okamura K, Vandivier R W, Ahuja N, Faubel S. 2013. Intratracheal IL-6 protects against lung inflammation in direct, but not indirect, causes of acute lung injury in mice. *Plos One*, **8**, e61405.

Chen W, Jiang D X, Yang L, Hu Y, Zhang C X, Yu T Q, Lu Q. 2003. Extraction and activity detection of cAMP-phosphodiesterase from porcine neutrophils. *Journal of Beijing Agricultural College*, **18**, 241–244.

Choi K C, Lee Y H, Jung M G, Kwon S H, Kim M J, Jun W J, Lee J, Lee J M, Yoon H. G. 2009. Gallic acid suppresses lipopolysaccharide-induced nuclear factor-kappaB signaling by preventing RelA acetylation in A549 lung cancer cells. *Molecular Cancer Research*, **7**, 2011-2021.

Dlaboga D, Hajjhussein H, O'Donnell J M. 2006. Regulation of phosphodiesterase-4 (PDE4) expression in mouse brain by repeated antidepressant treatment: comparison with rolipram. *Brain Research*, **1096**, 104-112.

Gandhi N M, Nair C K. 2005. Protection of DNA and membrane from gamma radiation induced damage by gallic acid. *Molecular and Cellular Biochemistry*, **278**, 111-117.

Ganeshan K, Johnston L K, Bryce P J. 2013. TGF-β1 limits the onset of innate lung inflammation by promoting mast cell-derived IL-6. *Journal of Immunology*, **190**, 5731-5738.

He Y, Dong X. 2002. Signal system after cAMP receptor and differentiation of induction of tumor

cells. Journal of Gannan Medical College, **22**, 400-403.

Hsiang C Y, Hseu Y C, Chang Y C, Kumar K J, Ho T Y, Yang H L. 2013. Toona sinensis and its major bioactive compound gallic acid inhibit LPS-induced inflammation in nuclear factor-κB transgenic mice as evaluated by *in vivo* bioluminescence imaging. Food Chemistry, **136**, 426-434.

Jiang D X, Chen W, Gu J N, Xu J Q, Yu T Q, Lu P, Mu X. 2007. Study on activity and gene expression of phosphodiesterase in porcine neutrophils. *Scientia Agricultura Sinica*, **40**, 1265-1270.

Jiang D X, Chen W, Xu J Q. 2006. Progress on regulation effect of traditional Chinese medicine on the level of cyclic nucleotide. *Journal of Traditional Chinese Veterinary Medicine*, **5**, 23-26.

Jiang D X, Chen W, Yu T Q, Lu P, Yang L, Mu X. 2006. Preliminary study of herbs on the influence of phosphodiesterase 4 activity. *Journal of Beijing Agricultural College*, **21**, 7-9.

Jiang D X, Chen Y S, Hou X T, Xu J F, Mu X, Chen W. 2011. Influence of *Paeonia lactiflora* roots extract on cAMP-phosphodiesterase activity and related anti-inflammatory action. *Journal of Ethnopharmacology*, **137**, 914-920.

Kambayashi T, Jacob C O, Zhou D, Mazurek N, Fong M, Strassmann G. 1995. Cyclic nucleotide phosphodiesterase type IV participates in the regulation of IL-10 and in the subsequent inhibition of TNF-alpha and IL-6 release by endotoxin-stimulated macrophages. *Journal of Immunology*, **155**, 4909-4916.

Kim S H, Jun C D, Suk K, Choi B J, Lim H, Park S, Lee S H, Shin H Y, Kim D K, Shin T Y. 2006. Gallic acid inhibits histamine release and proinflammatory cytokine production in mast cells. *Toxicological Sciences*, **91**, 123-131.

Kostek M C, Nagaraju K, Pistilli E, Sali A, Lai S H, Gordon B, Chen Y W. 2012. IL-6 signaling blockade increases inflammation but does not affect muscle function in the mdx mouse. *BMC Musculoskeletal Disordors*, **13**, 106.

Latha R C, Daisy P. 2011. Insulin-secretagogue, antihyperlipidemic and other protective effects of gallic acid isolated from *Terminalia bellerica* Roxb. in streptozotocin-induced diabetic rats. *Chemico-biological Interactions*, **189**, 112-118.

Levallet G, Levallet J, Bonnamy P J. 2008. FSH-induced phosphoprotein phosphatase 2A-mediated deactivation of particulate phosphodiesterase-4 activities is abolished after alteration in proteoglycan synthesis in immature rat Sertoli cells. *Journal of Endocrinology*, **197**, 45-54.

Lugnier C. 2006. Cyclic nucleotide phosphodiesterase (PDE) superfamily: A new target for the development of specific therapeutic agents. *Pharmacology & Therapeutics*, **109**, 366-398.

Luo F R, Chen H S. 2000. Neutrophils' signal transduction pathway of respiratory burst and degranulation. *Foreign Medical Sciences ·Section of Pathophysiology and Clinical Medicine*, **20**, 219-222.

Marx D, Tassabehji M, Heer S, Hüttenbrink K B, Szelenyi I. 2002. Modulation of TNF and GM-CSF release from dispersed human nasal polyp cells and human whole blood by inhibitors of different PDE isoenzymes and glucocorticoids. *Pulmonary Pharmacology & Therapeutics*, **15**, 7-15.

Ottonello L, Frumento G, Arduino N, Bertolotto M, Dapino P, Mancini M, Dallegri F. 2002. Differential regulation of spontaneous and immune complex-induced neutrophil apoptosis by proinflammatory cytokines. Role of oxidants, Bax and caspase-3. *Journal of Leukocyte Biology*, **72**, 125-132.

Suwa T, Hogg J C, English D, Van Eeden S F. 2000. Interleukin-6 induces demargination of intravascular neutrophils and shortens their transit in marrow. *American Journal of Physiology-heart and Circulatory Physiology*, **279**, H2954-H2960.

Tung Y T, Wu J H, Huang C C, Peng H C, Chen Y L, Yang S C, Chang S T. 2009. Protective effect of Acacia confusa bark extract and its active compound gallic acid against carbon tetrachloride-induced chronic liver injury in rats. *Food and Chemical Toxicology*, **47**, 1385-1392.

Verma S, Singh A, Mishra A. 2013. Gallic acid: molecular rival of cancer. *Environmental Toxicology and Pharmacology*, **35**, 473-485.

Wang P, Wu P, Ohleth K M, Egan R W, Billah M M. 1999. Phosphodiesterase 4B2 is the predominant phosphodiesterase species and undergoes differential regulation of gene expression in human monocytes and neutrophils. *Molecular Pharmacology*, **56**, 170-174.

Yan J H, Gu W J, Pan L. 2014. Efficacy and safety of roflumilast in patients with stable chronic obstructive pulmonary disease: A meta-analysis. *Pulmonary Pharmacology & Therapeutics*, **27**, 83-89.