Suppressive Effects of JCICM-6, the Extract of an Anti-arthritic Herbal Formula, on the Experimental Inflammatory and Nociceptive Models in Rodents

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JCICM-6, the extract of an anti-arthritic herbal formula composed of medicinal herbs of *Sinomenium acutum*, *Aconitum carmichaeli* Debx., *Curcuma Longa* L., *Paeonia lactiflora* Pall., and *Paeonia suffruticosa* Andr., was examined in the effectiveness and mechanism in reducing experimentally-induced inflammation and nociception using nine animal models. JCICM-6 was extracted from herbs and purified with Amberlite XAD-7HP adsorbent resin and analyzed with HPLC-fingerprint for quality consistency. In acute inflammatory models, the paw edema of rats was induced by subcutaneous injection of carrageenan or pro-inflammatory mediators, including histamine, serotonin, bradykinin, and prostaglandin E_2 (PGE_2) into the right hind paws of animals; while the ear edema of mice was induced by applying arachidonic acid or 12-*O*-tetradecanoylphorbol 13-acetate (TPA) on the ear surface. In nociceptive models, the tail-flick response induced by radiant heat stimulation was measured and the numbers of abdominal writhing episodes of mice induced by intraperitoneal injection of acetic acid were recorded. JCICM-6 orally administered in a range of dosages from 0.438 g to 1.75 g/kg significantly and dose-dependently suppressed the paw edema of rats induced by carrageenan or various pro-inflammatory mediators and the ear edema of mice induced by arachidonic acid or TPA. JCICM-6 also significantly prolonged the reaction time of rats to radiant heat stimulation and reduced the numbers of writhing episodes of mice. These results indicated that JCICM-6 possesses significant anti-inflammatory and analgesic effects, which implies that it would be a potential candidate for further investigation as a new anti-arthritic botanical drug for humans.

Key words JCICM-6; herbal formula; suppressive effect; inflammatory model; nociceptive model

In treating inflammatory and arthritic diseases, medical doctors have been commonly using nonsteroidal anti-inflammatory drugs (NSAIDs) and immunosuppressants. Previous studies have demonstrated that these drugs could inhibit synthesis or production of pro-inflammatory cytokines or mediators or their interactions. However, they sometimes also cause serious adverse effects such as gastric mucosal damage, bone marrow depression, water and salt retention (phenylbutazone, oxyphenbutazone) and even possible carcinogenesis (aminopyrine).[1] Thus, alternative agents with fewer and less severe side effects are required, and botanicals are important candidates.[2]

Chinese herbal medicines have been becoming more and more popular over the world. Actually, they are being widely used in China, Japan and South Korea for treatment of various complicated refractory diseases including arthritis. JCICM-6 is the extract of an anti-arthritic herbal formula that composes of *Sinomenium acutum* (Family Menispermaceae), *Aconitum carmichaeli* Debx. (Family Ranunculaceae), *Curcuma Longa* L. (Family Zingiberaceae), *Paeonia lactiflora* Pall. (Family Paeoniaceae), and *Paeonia suffruticosa* Andr. (Family Paeoniaceae). These five herbs are commonly prescribed together with other herbs in herbal formula to treat rheumatoid arthritis (RA) by Chinese medicine practitioners.[3] Previous chemical and pharmacological studies showed that *Sinomenium acutum* contains various bioactive alkaloids, such as sinomenine, sinoacutine, disinomenine, and magnoflorine. Of these compounds, sinomenine is predominant in the activities of anti-inflammation, analgesia, arthritis amelioration and immunosuppression.[4—6] *Curcuma Longa* L. contains curcumin, curlone and curcumenone.[7] The water extract of *Curcumae Longae* and its chemical constituent curcumin has significant anti-inflammatory and analgesic activities as well as a protective effect on gastrointestinal mucosa.[8] *Aconitum carmichaeli* Debx. contains *Aconitum* parent alkaloids of aconitine, mesaconitine and hypaconitine and their hydrolytes of benzoylaconine, benzoylmesaconine and benzoylhypaconine.[9] These three parent alkaloids are highly toxic but present in only very small amounts in the processed herbs of the *Aconitum carmichaeli* Debx.; while their hydrolytes with relatively higher contents in the processed aconite roots have significant anti-inflammatory and antinociceptive activities that are desirable for clinical treatment.[10,11] *Paeonia lactiflora* Pall. contains paeoniflorin, oxypaeoniflorin, benzoylpaeoniflorin and albilorin. Of these compounds, the major bioactive component paeoniflorin was reported to have marked anti-inflammatory, analgesic and immunomodulatory activities.[12] Our previous studies showed that sinomenine could significantly improve the bioavailability of paeoniflorin in rats when orally administered jointly and the improvement is possibly through a P-glycoprotein mechanism.[13—15] *Paeonia suffruticosa* Andr. contains paeonol, paeoniflorin, paeonoside, paeonollide and apiopaeonoside. Of these compounds, paeonol is the main bioactive component having potent anti-inflammatory and analgesic effects in carrageenan-evoked thermal hyperalgesia of rats.[16] Thus, the herbal formula comprising those five herbs with known multiple components and pharmacological

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effects may offer a comprehensive therapeutic efficacy for human arthritis *via* various mechanic pathways and cellular and molecule targets.

RA is a chronic, inflammatory, systemic autoimmune disease characterized by joint pain and swelling, pannus formation, cartilage destruction, bone erosion and joint dysfunction, in which a very complicated pathogenesis and multiple pathological changes are implicated.[17] Thus, a combinative therapy and comprehensive management for RA patients may be more appropriate to targeting on its complication of pathology. Theoretically, an ideal treatment or drug for RA should have multiple pharmacological potencies, such as anti-inflammation, analgesia, anti-arthritis and immunomodulation. The combinative therapeutics of herbal medicines may provide such multiple potencies. Our previous study has shown that QFGJS (another name of JCICM-6) had significant anti-arthritic effect in adjuvant-induced arthritis of rats.[18] Therefore, our current studies aim to examine the anti-inflammatory and analgesic effects of JCICM-6, the extract of a combination of Chinese herbal medicines, using various animal models, so as to evaluate its potential to be developed as a novel anti-arthritic botanical drug product. In this paper, the results of *in vivo* studies of JCICM-6 in nine inflammatory and nociceptive animal models are reported.

MATERIALS AND METHODS

Plant Materials *Sinomenium acutum* was purchased form Zhong-Yue Herbal Pharmaceutical Union Company in China. *Aconitum carmichaeli* DEBX. and *Curcuma Longa* L., cultivated in the Good Agricultural Practice (GAP) bases at Jiangyou Country and Shuangliu Country of Chengdu City, respectively, were purchased from the wholesale market of Chengdu City, Shichuan Province, China. *Paeonia lactiflora* PALL. and *Paeonia suffruticosa*, cultivated in the GAP bases at Bozhou City and Tongning City of Anhui Province, respectively, were purchased from the wholesale market of Bozhou City, Anhui Province, China. Prof. Lai Xiao Ping from the Guangzhou University of Traditional Chinese Medicine authenticated all herbs. The authenticated voucher specimens are kept in the School of Chinese Medicine, the Hong Kong Baptist University.

Preparation of JCICM-6 The five herbs were reduced to coarse powder by pulverization. *Sinomenium acutum*, *Aconitum carmichaeli* DEBX. and *Paeonia lactiflora* PALL. were refluxed together with 80% ethanol and concentrated to produce Extract 1. *Paeonia suffruticosa* ANDR. and *Curcuma Longa* L. were extracted firstly with supercritical CO_2 (21.7 l/h) to produce Extract 2 and Extract 3, respectively. The residues of the two herbs after CO_2 extraction were then separately refluxed with 80% ethanol and concentrated to produce Extracts 4 and 5. The mixture of Extracts 1, 4, and 5 was further purified with Amberlite XAD-7HP polymeric resin (Rohm and Haas Company, U.S.A.), and then combined with Extracts 2 and 3 to obtain JCICM-6. Three batches (20040821, 20040825, 20040901) of JCICM-6 were produced and employed in the animal studies. The chemical consistency of JCICM-6 was validated by HPLC fingerprint analysis. The average yield rate of JCICM-6 was 6.44± 0.35%, *i.e.*, a 100 g plant material that is the clinical daily dose of JCICM-6 yielded 6.44 g extract.

HPLC Fingerprint Analysis The HPLC fingerprint analysis was carried out on a Phenomenex ODS (250×4.6 mm I.D.; particle size 5 μm; Alltech Associates, Inc., U.S.A.) protected by a guard column (C_{18}, 5 μm, 7.5×4.6 mm I.D.). The separation were conducted with a mixture of acetonitrile and the buffer (containing 0.1% phosphoric acid, adjusted with triethylamine to pH 3.5±0.2) in a gradient manner. Detection was performed at a wavelength of 240 nm at room temperature. The correlation coefficient of each chromatogram to the simulative mean chromatogram was calculated.

Experimental Animals ICR mice weighing 17—23 g and SD rats weighing 150—200 g were purchased from the Laboratory Animal Services Center, the Chinese University of Hong Kong, Hong Kong. The animals were acclimated for ≥1 week under 12 h light and 12 h dark cycle at room temperature of 22±1 °C. Chow diet and water were provided *ad libitum*. Animal care and treatment procedures conformed to the Institutional Guidelines and Animal Ordinance (Department of Health, Hong Kong Special Administrative Region). Rats and mice were fasted for 48 and 24 h, respectively, before experiment.

Drugs and Reagents For all experiments, an aqueous solution of JCICM-6 was used at a concentration of 0.07 g/ml as a stock solution. The dosages of JCICM-6 employ in all experiments ranged from 0.101 to 1.75 g/kg. The highest dosage 1.75 g/kg is approximately 3.3 times of the equivalent dose of the human dosage in clinic and thus is an acceptable dosage for animal studies. All dilutions were obtained from the stock solution using a dilution vehicle that consisted of 10% peanut oil, 10% Tween ®80 and 80% distilled water. Other drugs and reagents, indomethacin, carrageenan, histamine, serotonin, prostaglandin E_2 (PGE_2), bradykinin, arachidonic acid (AA), 12-*O*-tetradecanoylphorbol 13-acetate (TPA), Tween ®80 and acetic acid, were purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.). Rotundine, an analgesic drug derived from medical plant in China, was purchased from Guangzhou Shiqiao Pharmaceutical Co., Ltd., Guangzhou, China.

Induction of Acute Inflammation in Rat Hind Paws by Carrageenan The assay was conducted as previously described by Winter.[19] Oral administration was conducted with three different doses of JCICM-6, or the reference drug or the vehicle (control, the same below), at 1 h prior to the induction of inflammation. At the induction, each rat was injected with 0.1 ml freshly prepared carrageenan (1% w/v) in physiological saline (0.9% w/v NaCl) into subplantar tissues of the right hind paw. The left hind paws without injection were used as controls. The volumes (ml) of both hind paws of each animal were measured using a plethysmometer (plethysmometer 7150, UGO Basile, Italy) at 1 h before the induction and 1, 2, 3, 4, 6 and 8 h after the induction. The increased rates in paw volume (paw edema) of the right hind paws of rats were calculated by the following equation: the increased rate (%)=(B−A)/A×100, where A and B represent the paw volumes before and at different time points after the induction, respectively. Indomethacin (10 mg/kg) administered orally was used as a reference drug.

Induction of Acute Inflammation in Rat Hind Paws by Histamine, Serotonin, Prostaglandin E_2 (PGE_2) and Bradykinin The acute inflammation in the hind paws of

rats was induced by subcutaneous injection of 0.05 ml 1% freshly prepared solutions of histamine, serotonin, bradykinin or PGE_2 (20 μg in 0.05 ml) into right hind paws of rats. The left hind paws were used as controls. Administration of JCICM-6 (0.438, 0.875, 1.75 g/kg), or of the reference drug, or of the vehicle, was conducted at 1 h prior to the induction. The volumes of the injected and control paws were measured at different time points designed from 0.5 to 6 h after injection of the phlogistic agents. Indomethacin administered orally (10 mg/kg) was used as the reference drug. The increased rates in paw volume were calculated in the same way as in carrageenan.

Induction of Acute Inflammation in Mouse Ears by AA and TPA Each mouse received 2.5 μg of TPA dissolved in 20 μl of 70% EtOH or 2% arachidonic acid dissolved in acetone. This was applied by an autopipette in 20 μl of arachidonic acid solution or TPA solution to both anterior and posterior surfaces of the right ear. The left ear, used as control, received the same volume of 70% EtOH or acetone. JCICM-6 (0.438, 0.875, 1.75 g/kg), or indomethacin (10 mg/kg), or vehicle, was orally administrated 1 h prior to the application of arachidonic acid solution. Ear thickness was measured before and after the induction from 0.5 to 8 h at different time points using a caliper. The rate of increase in mouse ear thickness was calculated by the following equation: rate of increase (%)=(B−A)/A×100; where A and B represent the ear thickness before and at different time points after the induction, respectively. The mean values of the treated animals were compared with the mean values of the control animals, using statistical methods.

Central Nociceptive Model Induced by Radiant Heat Stimulation in Rats The antinociceptive effects of JCICM-6 and the reference drug, expressed as the time required for rat tail flick after exposure to a source of radiant heat, were evaluated according to the description of D'Amour.[20] Briefly, animals were placed in a plexiglas box that allowed their tails to be free, and then the box was placed on IITC model 336 tail flick analgesia meter (IITC Inc., U.S.A.) with the tail occluding a slit over a photocell for radiant heat stimulation generated by a power lamp mounted in a reflector. The tail-flick response was elicited by applying radiant heat to the point 1/3 of length away from the tip of the tail. The apparatus was arranged so that when the operator turned on the lamp a timer was activated. When the rat felt pain and flicked its tail, light fell on the photocell such that the timer was automatically stopped. The intensity of the heat stimulus in the tail-flick test was adjusted so that the animal flicked its tail within 3 to 5 s. A 20-s cut-off time was set in order to prevent tail tissues from damage. Before the experiments, the heat stimulation latency of all animals was tested, those with response time to heat stimulation <2 s or >6 s were excluded. The tail-flick response was measured at 1, 2 and 3 h after oral administration of JCICM-6 (0.101, 0.404, 1.62 g/kg), or rotundine (100 mg/kg) as reference drug, or vehicle.

Visceral Nociceptive Model Induced by Acetic Acid Stimulation in Mice The abdominal writhing test induced by chemical stimulation of acetic acid was performed in mice as originally described by Siegmund.[21] Briefly, JCICM-6 (0.101, 0.404, 1.62 g/kg), rotundine (100 mg/kg), or vehicle was orally administrated 2 h before acetic acid injection. After intraperitoneal injection of 0.2 ml acetic acid (0.8% w/v) in physiological saline (0.9% w/v NaCl), animals were isolated for observation. The numbers of abdominal writhing syndrome/events, which consisted of the contraction of the abdominal area with extension of hind legs, were recorded during a 15 min period in each animal.

Statistical Analysis Values were expressed as the means±standard error of the means (S.E.M.). The statistical significance of the differences were assessed by ANOVA followed by *post hoc* test with LSD method. p values less than 0.05 were considered statistically significant.

RESULTS

HPLC Fingerprint of JCICM-6 Figure 1 shows the HPLC fingerprints from three batches of JCICM-6. The correlation coefficient was 0.9894±0.0056, indicating the consistence of quality among the three batches of JCICM-6.

Inhibition of Paw Edema of Rats by Treatment of JCICM-6 Figure 2 shows that JCICM-6 significantly inhibited acute paw edema evoked by carrageenan injection. The maximum phlogistic response of carrageenan was observed at 4 h after the injection in the vehicle-treated animals. Data from the JCICM-6-treated animals with the doses of 0.875 g or 1.75 g/kg at 1, 2, 3, 4, 6 and 8 h showed significant differences in the paw edema in comparison with the data of the vehicle-treated animals at the same time points, while the JCICM-6-treated animals with doses of 0.438 g/kg only showed significant differences in the paw edema at 1, 2, 3 and 4 h (Fig. 2). The reference drug, indomethacin, showed significant differences within 8 h at the dosages of 10 mg/kg. The left hind paws of rats which were used as controls showed no increase of paw volume for the entire experiment, indicating that the right hind paw edema was induced by carrageenan (data not shown). These results imply that the anti-acute inflammatory effect of JCICM-6 in rats is dose-dependent, and suggest that the effect can remain at least 9 h after oral administration.

In the case of histamine, serotonin, PGE_2 and bradykinin-induced rat paw edema, all measurements were conducted at 0.5, 1, 2, 3, 4 and 6 h after the injection of the above phlogis-

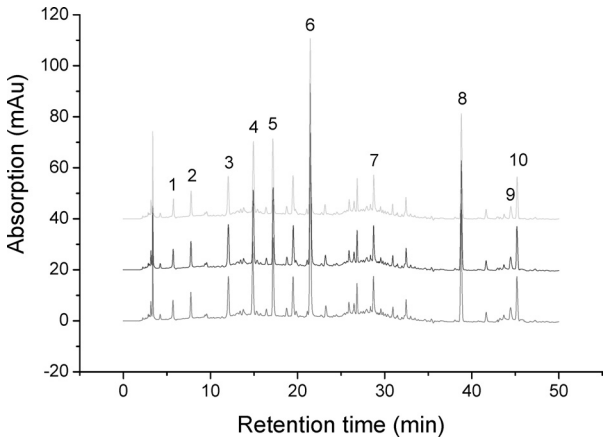

Fig. 1. HPLC Fingerprints of Three Batches (from Bottom to Top: 20040821, 20040825, 20040901) of JCICM-6

Peaks 1—10, in which peaks 3, 6, 8, and 10 were identified as sinomenine, paeoniflorin, paeonol, and curcumin, respectively, are the characteristic and representative chemical components detected from the preparation of JCICM-6.

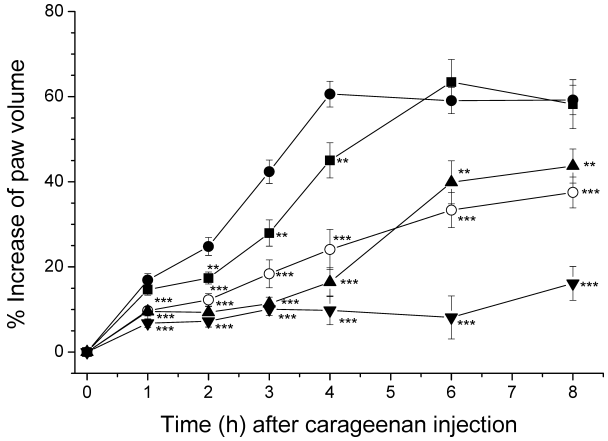

Fig. 2. Inhibition of Carrageenan-Induced Paw Edema of Rats by Treatment of JCICM-6 at Dosages of 0.438 (■), 0.875 (▲) and 1.75 (▼) g/kg, and by the Reference Drug Indomethacin at Dosage of 10 mg/kg by *p.o.* (○)

Drugs were orally administrated 1 h prior to carrageenan injection. Each point represents the mean±S.E.M. (n=10). ** p<0.01, *** p<0.001 *vs.* control animals (●) at the corresponding time point.

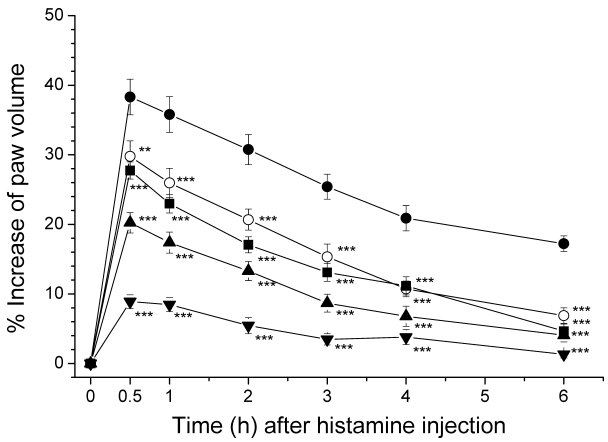

Fig. 3. Inhibition of Histamine-Induced Paw Edema of Rats by Treatment of JCICM-6 at Dosages of 0.438 (■), 0.875 (▲) and 1.75 (▼) g/kg, and by the Reference Drug Indomethacin at Dosage of 10 mg/kg by *p.o.* (●)

Drugs were orally administrated 1 h prior to histamine injection. Each point represents the mean±S.E.M. (n=10). *** p<0.001 *vs.* control animals (●) at the corresponding time point.

tic agents. Significant edema was observed in all injected paws at 0.5 h after injection. Figures 3, 4, 5 and 6 show that JCICM-6 could dose-dependently inhibit the acute inflammatory responses evoked by histamine, serotonin, PGE_2, or bradykinin over all time periods. However, the figures also show that the anti-inflammatory effect of JCICM-6, while dose-dependent, also varies according to phlogistic agents. In rat paw edema induced by histamine and bradykinin, all three doses (0.438, 0.875, 1.75 g/kg) of JCICM-6 significantly inhibited edema (Figs. 3, 6); while in the paw edema induced by serotonin and PGE_2, only higher doses (0.875, 1.75 g/kg) significantly reduced edema (Figs. 4, 5). Indomethacin (10 mg/kg) showed an anti-inflammatory effect in all animal models, although to a lesser extent than that of JCICM-6 using the relative higher doses (Figs. 3—6). Contralateral left hind paws (no pholgostic agents injected) remained constant at paw volume (data not shown).

Inhibition of Ear Edema of Mice by Treatment of JCICM-6 In Fig. 7, it can be seen that the application of

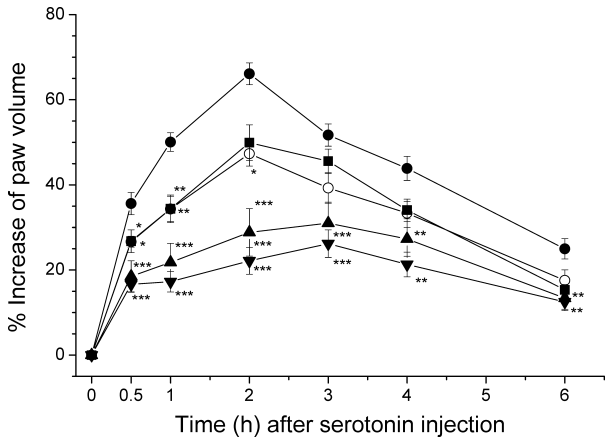

Fig. 4. Inhibition of Serotonin-Induced Paw Edema of Rats by Treatment of JCICM-6 at Dosages of 0.438 (■), 0.875 (▲) and 1.75 (▼) g/kg, and by the Reference Drug Indomethacin at Dosage of 10 mg/kg by *p.o.* (○)

Drugs were orally administrated 1 h prior to serotonin injection. Each point represents the mean±S.E.M. (n=9—10). * p<0.05, ** p<0.01, *** p<0.001 *vs.* control animals (●) at the corresponding time point.

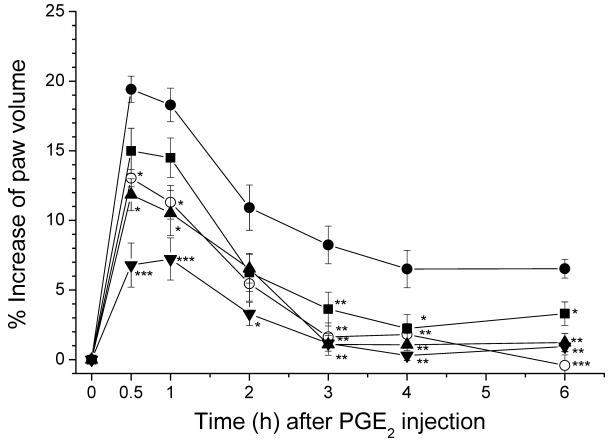

Fig. 5. Inhibition of PGE_2-Induced Paw Edema of Rats by Treatment of JCICM-6 at Dosages of 0.438 (■), 0.875 (▲) and 1.75 (▼) g/kg, and by the Reference Drug Indomethacin at Dosage of 10 mg/kg by *p.o.* (○)

Drugs were orally administrated 1 h prior to PGE_2 injection. Each point represents the mean±S.E.M. (n=9—10). * p<0.05, ** p<0.01, *** p<0.001 *vs.* control animals (●) at the corresponding time point.

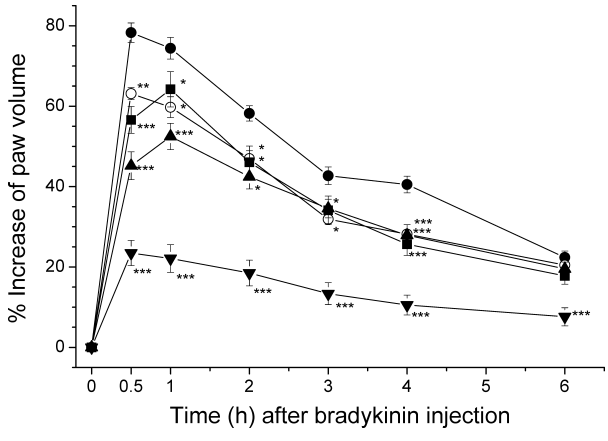

Fig. 6. Inhibition of Bradykinin-Induced Paw Edema of Rats by Treatment of JCICM-6 at Dosages of 0.438 (■), 0.875 (▲) and 1.75 (▼) g/kg, and by the Reference Drug Indomethacin at Dosage of 10 mg/kg by *p.o.* (○)

Drugs were orally administrated 1 h prior to bradykinin injection. Each point represents the mean±S.E.M. (n=10). * p<0.05, ** p<0.01, *** p<0.001 *vs.* control animals (●) at the corresponding time point.

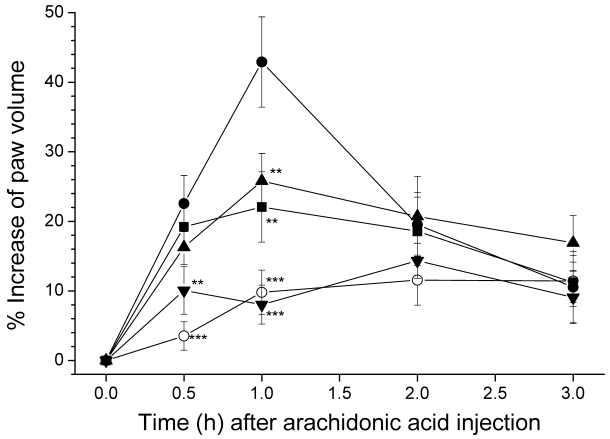

Fig. 7. Inhibition of Arachidonic Acid-Induced Ear Edema of Mice by Treatment of JCICM-6 at Dosages of 0.438 (■), 0.875 (▲) and 1.75 (▼) g/kg, and by the Reference Drug Indomethacin at Dosage of 10 mg/kg by *p.o.* (○)

Drugs were orally administered 1 h prior to the topically administration of arachidonic acid. Each point represents the mean±S.E.M. (n=10). $^{**}p<0.01$, $^{***}p<0.001$ vs. control animals (●) at the corresponding time point.

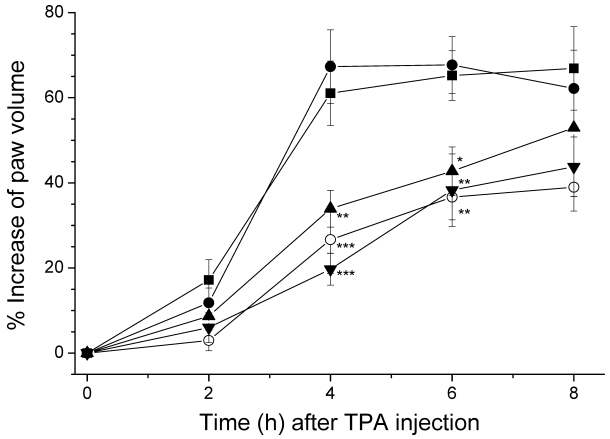

Fig. 8. Inhibition of TPA-Induced Ear Edema of Mice by Treatment of JCICM-6 at Dosages of 0.438 (■), 0.875 (▲) and 1.75 (▼) g/kg, and by the Reference Drug Indomethacin at Dosage of 10 mg/kg by *p.o.* (○)

Drugs were orally administered 1 h prior to the topically administration of TPA. Each point represents the mean±S.E.M. (n=10). $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$ *vs.* control animals (●) at the corresponding time point.

AA could effectively induce mouse ear edema with a peak at 1 h and with rapid decrease from 2 h after the induction of inflammation. Oral treatment with JCICM-6 1 h prior to the induction could significantly inhibit ear edema of mice at 0.5 and 1 h after the induction (Fig. 7). Similar result was found in the animals treated orally with indomethacin. However, at 2 and 3 h after application of AA this anti-inflammatory effect of JCICM-6 and indomethacin had almost disappeared. No ear edema was found in the contralateral left ear lobes of rats tested (data not shown).

Figure 8 shows the inflammatory pattern of TPA in the ear edema of mice as well as the inhibitory effect of JCICM-6 on this inflammatory model. It can be seen in Fig. 8 that TPA-induced ear edema appeared at a relatively later phase of the experiment in comparison with the inflammatory model evoked by AA, *i.e.*, the peak of the inflammation induced by TPA was observed at 4 h after the induction and then the ear edema lasted until the end of the experiment. The effect of

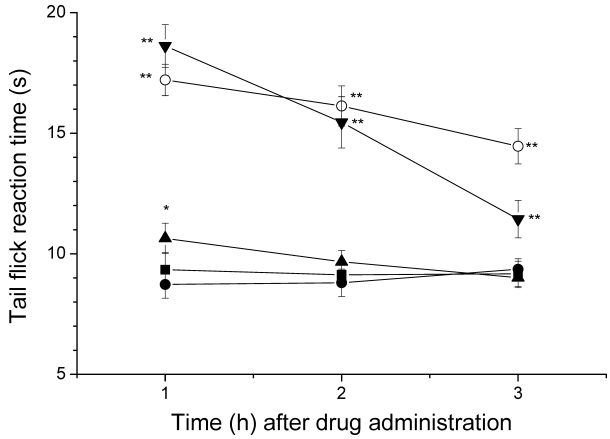

Fig. 9. Analgesic Effect of JCICM-6 at Dosages of 0.101(■), 0.404 (▲) and 1.62 (▼) g/kg and of the Reference Drug Rotundine at Dosage of 100 mg/kg (○) on Radiant Heat Stimulation-Induced Tail Flick Reaction of Rats

Drugs were orally administered and the measurements of the tail flick time were conducted at 60, 120 and 180 min after the administration. Each point represents the mean±S.E.M. (n=8—10). $^{*}p<0.05$, $^{**}p<0.01$ *vs.* control animals (●) at the corresponding time point.

JCICM-6 was also observed at later phases of the experiment, *i.e.*, at 4, 6 h even 8 h after the induction (Fig. 8). Ear edema was significantly reduced with administration of indomethacin. The left ear lobes which were used as controls showed no increase of thickness of the ear lobes for the entire experiments (data not shown).

Analgesic Effect of JCICM-6 in Nociceptive Model of Rats Both the tail flick test evoked by radiant heat simulation in rats and the writhing assay induced by peritoneal injection of acetic acid in mice were employed in the study. In Fig. 9, it is seen that the tail flick reaction time of the control animals was around 9 s at 1, 2 and 3 h after orally taking the vehicle; while JCICM-6 with the higher dose of 1.62 g/kg had significant anti-nociceptive effect, markedly prolonging tail flick reaction time. Moreover, the effective peak of JCICM-6 was seen at 60 min after oral administration and then gradually decreased (Fig. 9). Rotundine, a positive analgesic agent, prolonged the reaction time of the animals and demonstrated significant anti-nociceptive action with a slightly stronger pharmacological intensity than that of JCICM-6.

Figure 10 shows the numbers of the abdominal writhing episodes evoked by intraperitoneal injection of acetic acid in mice as well as the anti-nociceptive effect of JCICM-6. It can be seen that treatment with JCICM-6 could dose-dependently reduce the number of writhing episodes of mice in comparison with that of vehicle-treated animals; while the reference drug rotundine which showed stronger analgesic effect than JCICM-6 in this nociceptive model.

DISCUSSION

As Chinese herbal medicines have been becoming more and more popular over the world, pharmacological evidences to understand the action of these medicines and the underlying mechanisms, hence to support the proper and safe use of these medicines in clinic are indispensable. Chinese herbs are commonly prescribed as combined herbal formulae to achieve sufficient treatment in complex conditions such as

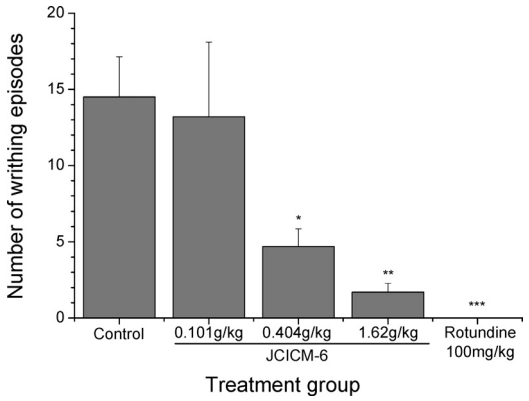

Fig. 10. Analgesic Effect of JCICM-6 at Different Dosages on Acetic Acid-Induced Writhing Response of Mice

Drugs were orally administered 2 h prior to the peritoneal injection of acetic acid. Values are the mean±S.E.M. (n=11—14). $*p<0.05$, $**p<0.01$, $***p<0.001$ *vs.* control animals.

RA through possible mechanisms known as multiple components directing to multiple targets. According the theory of Chinese medicine, not only can herbs using in proper combination exert synergistic effects to each other, but also the undesirable effects of herbs could be therefore reduced. This might be what the formula of JCICM-6 did. Traditionally, patients would cook these herbs by themselves at home. But this would make a non-standard decocting procedure as well as unstable extract that might influence the desirable therapeutic effects in clinic. Preparing Chinese herbal medicines with standardized pharmaceutical extraction processes could probably avoid this problem. In this study, JCICM-6 was prepared with an optimized standard process which mainly focused on maximizing the contents of known bioactive chemical compounds or clusters in the extract as well as preserving the major pharmacological activities of the formula. Because individual Chinese herb *per se* already contains multiple components, let alone the combination of herbs, therefore the most important issue to be considered prior to the pharmacological study of an herbal extract coming from a formula is to keep the extract chemically consistent, and thus to make the pharmacological results reliable, repeatable, and comparable. In this study, we utilized qualified plant materials from GAP bases and conducted rigorous in-process control to achieve this goal. The chemical consistency of JCICM-6 was proved by the HPLC fingerprint analysis in which the fingerprints of the three batches of JCICM-6 were almost identical. The fingerprint analysis also revealed that JCICM-6 contains the known bioactive compounds of these five Chinese herbs, including sinomenine, paeoniflorin, paeonol, and curcumin (Fig. 1). By using this extract with consistent quality, our study showed that JCICM-6 inhibited experimental inflammation and nociception in 9 animal models.

The pharmacological results of our current studies revealed that JCICM-6 elicited significant anti-inflammatory activities in carrageenan model. Carrageenan induced paw edema in rats is one of the most commonly used models of inflammation and has been accepted as a useful phlogistic tool for the investigation of new anti-inflammatory agents. Development of paw edema of rats induced by carrageenan is commonly correlated with the early exudative stage of inflammation, one of the important processes of inflammatory pathology.[22] Soon after carrageenan injection, there is sudden elevation of paw volume, correlating with the action of histamine and serotonin on vascular permeability.[23,24] At approximately 1 h after induction, inflammation begins to increase and paw edema gradually elevates to a peak during 4—6 h after induction. This second phase could be due to the liberation and over-production of bradykinin, prostaglandins and kinins in paw tissue, which accompanies leukocyte migration.[25] The inflammatory pattern in the inflamed paws of rats evoked by carrageenan in our present study is in close accordance with previous reports[23—25]; while the dose-dependent inhibition, 1—8 h after the induction of inflammation, suggests that JCICM-6 may act in both earlier and later phases of inflammation.

These results further induce us to think that the anti-inflammatory activity of JCICM-6 could be related to the impairment of pro-inflammatory mediators in the cyclooxygenase pathway, because most of the NSAIDs take their anti-inflammatory effects *via* inhibition of the production of pro-inflammatory mediators including eicosaloids. Thus, different inflammatory mediator-induced paw edema, *i.e.* histamine, serotonin, prostaglandin E_2 and bradykinin, have been studied in this experiment, so as to elucidate the anti-inflammatory effect of JCICM-6, including the underlying pharmacological mechanisms. The results here show that JCICM-6 had marked dose-dependent inhibitory effect with different pharmacological intensities on various acute inflammatory models induced by histamine, serotonin, prostaglandin E_2 or bradykinin in rats, which, moreover, suggests that the underlying anti-inflammatory mechanisms of JCICM-6 are possibly linked to the inhibition of either the synthesis, or the release, or the actions of those pro-inflammatory mediators.

Topical application of AA and TPA offers a skin inflammation model appropriate for evaluating anti-inflammatory agents. AA-induced ear edema is a good *in vivo* test useful for evaluating lipoxygenase inhibitors.[26] The present study shows that oral administration of JCICM-6 at doses of 0.438, 0.875 and 1.75 g/kg could significantly inhibit AA-induced ear inflammation, suggesting this herbal extract might act as a lipoxygenase inhibitor. In TPA-test, when JCICM-6 at doses of 0.438, 0.875 and 1.75 g/kg also produced a significant reduction in mouse ear edema. The majority of the activities of this phorbol ester appears to be involved or to be dependent on AA release and metabolism. All phospholipase A_2, or cyclooxygenase, or lipoxygenase inhibitors as well as corticoids are effective at suppressing ear edema after topical application of TPA at high doses.[26] Moreover, the topical application of TPA could induce a transient increase in prostanoid production. Using this model, orally administered inhibitors of cyclooxygenase and lipoxygenase such as phenidone and BW755C appeared to be effective at inhibiting ear edema.[27] Thus, the results of the present study suggest that JCICM-6 may have pharmacological properties similar to both lipoxygenase and cyclooxygenase inhibitors.

In arthritis, joint inflammation and pain are the most commonly co-existing symptoms. An ideal therapy for human arthritis should at least possess activities of anti-inflammation and analgesia. Thus, in the current studies, to assess the analgesic effect of JCICM-6, two nociceptive animal models were developed. According to previous reports, the tail flick test of rats evoked by radiant heat stimulation is more sensi-

tive in centrally acting analgesics whereas acetic acid-induced abdominal writhing assay in mice is commonly used for detecting both central and peripheral analgesia.[28,29] With the former test model, it was found that JCICM-6 had a significant ability to prolong the response latencies to the radiant stimulation, indicating significant increase of the nociceptive threshold in treated animals in comparison with that of non-treated animals. There are also previous reports indicating that the effectiveness of analgesic agents in tail flick pain model of rats was highly correlated with pain relief in human.[30] In Chinese and Japanese medical practice, *Sinomenium acutum* and *Aconitum carmichaeli* Debx. are usually prescribed for relieving joint and muscular pain of arthritis; both of these herbs are ingredients of JCICM-6. Thus, the anti-nociceptive effect of JCICM-6 in tail flick rat model is in good agreement with the clinical experience and therapy. In the writhing response model, acetic acid is injected into the peritoneal cavity of mice to cause nociception in abdomen due to the release of various substances that excite pain nerve endings.[31] JCICM-6 showed an ability of diminishing the numbers of the writhing episodes in a dose-dependent manner, indicating significant inhibition of the acetic acid-induced visceral nociception. The results with these two nociceptive animal models suggest that JCICM-6 might act as central and peripheral analgesic agent like rotundine (*dl*-tetrahydropalmatine). Rotundine possesses analgesic, sedative, hypnotic and antihypertensive effects. It is the active compound isolated from medical plant *Corydalis yanhusuo* and has been widely used in China as an analgesic drug in human.[32—34] While the mechanism of the analgesic effect of JCICM-6 is not readily apparent in these two models, it can, nevertheless, be speculated that this effect may be linked to processes in the prevention of sensitization of the nociceptor, down-regulation of the sensitized nociceptor and/or blockade of the nociceptor at peripheral and/or central levels.[35] Another possible mechanism may be that JCICM-6 could inhibit cyclooxygenase pathway in peripheral tissues (which has been shown in the study of anti-inflammatory activity), thus, interfering with the mechanism of transduction in primary afferent nociceptors.[36] Or JCICM-6 could be involved in AA metabolic pathway which inhibits production of prostaglandins and thromboxanes similar to acetylsalicyclic acid.[37] Moreover, JCICM-6 may block the effect or the release of endogenous substances, including PGE_2 that excites pain nerve endings and is found in writhing response test model of mice.[38]

Taken together, JCICM-6 could act in earlier and later phases of inflammation. The underlying mechanisms may be that JCICM-6 could inhibit the synthesis, release, or actions of pro-inflammatory mediators; meanwhile, JCICM-6 could also act as a lipoxygenase or cyclooxygenase inhibitors. Therefore, our up-coming studies will focus on the synthesis, formation and production of some important pro-inflammatory mediators such as cytokines, nitric oxide (NO), prostaglandins, cyclooxygenase 2 (COX-2) as well as neutrophil infiltration in the sites of inflammation impaired by treatment of the herbal extract. Particularly, the impairment of JCICM-6 on tumor necrosis factor-α (TNF-α) will be stressed, since TNF-α has an early and crucial role in the cascade of pro-inflammatory cytokine production.[39] TNF-α induces the production of interleukin-1β (IL-1β) and IL-6, thus resulting in the production of cyclooxygenase products; and it induces another cytokine, IL-8, stimulating the local production of sympathetic amines.[40,41] In contrast to the pro-inflammatory cytokines, some 'antagonist cytokines' such as IL-10 have been reported to have an inhibitory effect on the production of pro-inflammatory cytokines produced by murine Th1 lymphocytes.[42,43]

In conclusion, the anti-inflammatory and anti-nociceptive effects of JCICM-6, the extract from an anti-arthritic herbal formula, have been effectively evaluated using nine experimental animal models in rodents. The results demonstrated that JCICM-6 possesses significant properties of anti-inflammation and analgesia like reference drugs of indomethacin and rotundine, respectively, indicating JCICM-6 would be a potential candidate for further investigation as a novel anti-arthritic botanical drug.

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