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Anti-inflammatory effect of *Houttuynia cordata* injection

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Abstract

Houttuynia cordata (Saururaceae) injection (HCI) is a traditional Chinese medicine used in China. It was chosen as one of eight types of traditional Chinese medicine that play a unique role in severe acute respiratory syndrome (SARS) owing to the effect of curbing inflammation. In order to validate this plausible anti-inflammatory property, the chemical composition of HCI has been analysed by GC/MS, 22 components were identified, and the inflammation induced by carrageenan in the rat pleurisy model and by xylene in the mice ear edema model was adopted to study the anti-inflammatory activity of HCI. Injection of carrageenan into the pleural cavity elicited an acute inflammatory response characterized by protein rich fluid accumulation and leukocyte infiltration in the pleural cavity. The peak inflammatory response was obtained at 24 h when the fluid volume, protein concentration, C-reactive protein and cell infiltration were maximums. The results showed that these parameters were attenuated by HCI at any dose and touched bottom at dose of 0.54 ml/100 g, although less strong than dexamethasone. This drug was also effective in inhibiting xylene induced ear edema, and the percentage of inhibition came to 50% at dose of 80 μl/20 g. The results clearly indicate that HCI have anti-inflammatory activity.

Keywords: *Houttuynia cordata* injection; Saururaceae; Anti-inflammatory activity; Pleurisy; Ear edema

1. Introduction

Houttuynia cordata Thunb. (Saururaceae) is a traditional medicinal plant used in China for years for the treatment of cough, leucorrhea and ureteritis so on (Ji and Zhao, 2003; Zhou, 2003; Sun et al., 2004). A previous study showed that the steam distillate prepared from fresh plants of *Houttuynia cordata* Thunb. possessed direct inhibitory activity against herpes simplex virus type 1 (HSV-1), influenza virus and human immunodeficiency virus type 1 (HIV-1) without showing cytotoxicity (Hayashi et al., 1995).

Houttuynia cordata injection (HCI), a traditional Chinese medicine used in China for dozens of years, was the aqueous solution of the steam distillate from plants of *Houttuynia cordata*. At May 23, 2003, Chinese scientists unveiled a list of eight types of traditional Chinese medicine that have been shown to play a unique role in helping reduce the side-effects of western medicine and improve the immune system of patients infected with severe acute respiratory syndrome (SARS), since the Science and Technology Group of the National Headquarters for SARS Prevention and Control was set up on April 25, 2003. The eight medicines were chosen from a long list of 30 medicines that have already been used in clinical treatment in SARS-hit Beijing and Guangdong Province in South China. The list included HCI that is effective in curbing inflammation of the lungs (http://www.chinanews.com.cn, 2003), different from other traditional medicines on the list which can lessen fever, chills, headaches, muscular pain, malaise, diarrhoea, a dry cough and a lack of phlegm. But this anti-inflammatory effect of HCI was rarely reported besides few papers that showed the water extract of dried plants of *Houttuynia cordata* has this kind of activity and antiedematous action (Song, 2002). In order to test whether HCI possesses a plausible anti-inflammatory property, pleurisy induced by carrageenan and ear edema induced by xylene were thus used to study the anti-inflammatory activity of HCI in this work.

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2. Materials and methods

2.1. Drugs and material

Carrageenan was purchased from Sigma (St. Louis, MO, USA) and xylene was purchased from Hunan Normal University (Changsha, Hunan province, China). HCI (No. 0404432) was gift from Hunan Zhengqing Pharmacy Co., Ltd. (Huaihua, Hunan province, China). Dexamethasone (No. 030425-1) was obtained from Tianjin Pharmaceuticals Group Corp. (Tianjin, China).

2.2. HCI analysis

The essential oil of HCI was prepared according to the standard extracting method in Chinese Pharmacopoeia (Chinese Pharmacopoeia Committee, Publishing House of People's Health, 2000, page appendix 64). The essential oil was collected and dissolved in 2 ml hexane, and then stored in a refrigerator at 4°C before use.

GC-MS analysis was performed on a Shimadzu GC-2010 (Kyoto, Japan) gas chromatography instrument coupled to a Shimadzu QP2010 mass spectrometer (Compaq-Pro Linear data system, class5k software), equipped with a OV-1 capillary column (30 m × 0.25 mm I.D., film thickness 0.25 μm). The column was maintained at 50 °C for 6 min, and programmed to 230 °C at a rate of 10 °C/min, then held for 16 min. The temperature of the injection port and interface was set at 280 °C. Helium was used as the carrier gas with a flow rate of 0.7 ml/min. One microlitre of the samples were injected in the 1:10 split mode. The mass spectrometer was operated under electron impact (EI) mode at ionization energy of 70 eV and the scan rate was 5 scan/s. The mass spectrometer was operated with a scan mass range of 20–450 amu. The ionization source temperature was 280 °C. The analytes were identified using the NIST Mass Spectral Database. The relative responses of the individual components are expressed as percent peak area relative to total peak area.

2.3. Animals

Male Wistar rats weighing 223 ± 40 g and K.M. mice of both sexes weighing 22 ± 2 g were purchased from Animal Center, Central South University, China. All animals were kept at 23 ± 1 °C with a 12 h light/dark cycle. They had free access to water and diet and were acclimatized at least 2 week before starting the experiments. All procedures described were reviewed and approved by the University animal ethical committee.

2.4. Preparation of HCI

Ordinarily, the producing process of HCI as follow, the aerial parts of the fresh plant were picked from the field, washed and steam distilled to obtain the essential oil. The essential oil was mixed with the aqueous solution of sodium chloride solution and tween-80, a surfactant, then filtrated and sterilized, to yield HCI in the factory. The concentration of methyl nonyl ketone, a main compound in essential oil, was required to be higher than 0.8 μg/ml in the HCI, according to standard specifications of Chinese traditional medicines, the state standards for drugs issued by the ministry of public health.

2.5. Drug treatment and induction of rat pleurisy and mice ear edema

2.5.1. Pleurisy

HCI at 0.27 ml/100 g, 0.54 ml/100 g, 1.08 ml/100 g body weight was injected once intravenously everyday and lasted for 6 days. The initial concentration of methyl nonyl ketone in HCI is 1.15 μg/ml. While the reference drug dexamethasone at 0.27 ml/100 g lasted for 2 days. The initial concentration of dexamethasone is 8 mg/ml and its composition is $C_{22}H_{28}FNa_2O_8$. Pleurisy was induced by intra-thoracic injection of 0.1 ml of 2.0% carrageenan, 24 h after the last injection of tested drugs according to a previously described method (Xu et al., 2002). Injection of carrageenan was given between the seventh and eighth ribs under the right upper limbs through a gauge needle, with penetration restricted to 6 mm by inserting carefully it through a rubber disc (Peters et al., 1999; Shivkar and Kumar, 2004). Another injection of treatment drugs was given to animals 3 h after the inflammatory stimulus. Control animals received the irritant and an equal volume of sterile saline. The pleural cavity was opened and rinsed with 1 ml of cold saline containing 20 IU/ml heparin. The fluid was removed by mild suction and its volume was measured. It was evaluated for leukocyte count, protein and C-reactive protein. All parameters were compared with those of rats treated with dexamethasone and control animals.

2.5.2. Ear edema

The xylene-induced ear edema test was performed as previously described (Xu et al., 2002; Kim et al., 2004; Mujumda and Misar, 2004). HCI at 40 μl/20 g, 80 μl/20 g, 160 μl/20 g, 240 μl/20 g and 320 μl/20 g body weight was injected hypodermically near the right ear twice everyday and lasted for 3 days. A total of 20 μl of xylene was applied to the inner and outer surface of the right ear of each mouse 30 min after the last injection of tested drugs. The left ear remained untreated. Control animals received the irritant and an equal volume of sterile saline, while dexamethasone (40 μl/20 g) served as the reference. The mice were sacrificed by cervical dislocation 40 min later and the plug (9 mm in diameter) was removed with a stainless steel punch from both the treated ear and the untreated ear. The difference in weight between the two plugs was taken as a measure of oedematous response.

2.6. Measurement of total volume in pleural fluid

The thoraxes of rats were opened, the fluid was removed by mild suction and the pleural cavity was washed with 1 ml of cold saline containing 20 IU/ml heparin, and the total volume was harvested.

2.7. Leukocyte count

The 20 μl of pleural fluid was diluted with 0.38 ml diluent (distilled water containing 1.5% acetic acid and 0.01% gentian-violet) and total leukocyte count (TLC) was measured using a SB-K-25 chamber under a light microscope.

2.8. Measurement of total protein in pleural fluid

The fluid collected from the pleural cavity was centrifuged (3000 rpm for 5 min) and protein concentration in the supernatant was assayed by the Coomassie brilliant blue method because of its high sensitivity and convenience (Chial et al., 1995; Atherton et al., 1996).

2.9. Measurement of C-reactive protein (CRP) in pleural fluid

C-reactive protein is a special type of protein produced by the liver that is only present during episodes of acute inflammation. It was measured on a Hitachi 7010S automatic biochemical analyzer.

2.10. Extent of the edema and percentage of inhibition

These plugs cut from mice were weighed on an electronic balance, and the extent of the edema was expressed as the difference in the weight of plugs from inflamed and untreated ears (Kaur et al., 2004; Koo et al., 2004). The percentage of inhibition was calculated as follow:

$$\text{Percentage of inhibition} = \frac{\text{the edema extent of control} - \text{the edema extent of treated}}{\text{the edema extent of control}} \times 100\%$$

2.11. Statistical analysis

Values are expressed as mean ± S.E.M. They were further analysed using one-way analysis of variance (ANOVA) test to calculate significance of results.

3. Results and discussion

3.1. Constitutes analysis of essential oil from HCI

The essential oil from HCI was analysed and 22 components were identified. The results of the analyses showed that main compounds in HCI were β-myrcene, 1-nonanol, α-terpineol, methyl nonyl ketone, bornyl acetate, *n*-decanoic acid, caryophyllene, docosanoic acid ethyl ester (Table 1). The peak area of those components comparative to total peak area is over 80%. Methyl nonyl ketone, a main active component (Hayashi et al., 1995), is over 35%.

3.2. Effect of HCI on carrageenan induced pleurisy

Intrapleural administration of carrageenan produced acute inflammatory response. The marked exudative response was obtained which peaked at 24 h followed by a decline. For example, the volume of pleural fluid increased from 0.05 ± 0.12 ml at 0 h to 0.95 ± 0.24 ml at 24 h and decreased to 0.70 ± 0.22 ml at 48 h. While protein concentration of the exudate when measured at different time-intervals showed an increase from 2.32 ± 1.78 at 0 h to 26.18 ± 2.18 mg/ml at 24 h and decline to 18.06 ± 2.12 mg/ml at 48 h. The similar trend of the cell counts and C-reactive protein was observed.

Table 1
Composition of essential oil from HCI

Component identified	Retention time (min)	Content (%)
β-Myrcene	13.769	3.47
α-Pinene	14.726	0.63
2-Methyl-6-methylene-oct-3,7-dien-2-ol	15.835	0.50
1-Nonanol	17.224	6.21
α-Terpineol	17.810	13.24
cis-2-Methylene-3-(1-methylethenyl)-Cyclohexanol, acetate	18.776	1.10
1-Decanol	18.981	2.90
Methyl nonyl ketone	19.353	35.20
Bornyl acetate	19.380	5.52
α-Terpineol acetate	20.286	1.02
n-Decanoic acid	20.574	10.58
Acetic acid, geraniol ester	20.591	2.15
1-Decen-3-one	20.658	1.54
2-Dodecanone	20.842	0.71
Lauraldehyde	21.065	0.29
Caryophyllene	21.765	3.41
4-Tridecanone	22.294	2.46
n-Dodecanoic acid	23.137	0.53
trans-Nerolidol	23.326	0.78
Docosanoic acid ethyl ester	23.641	5.34
Caryophyllene oxide	23.932	1.88
Heptanoic acid, 3-methylbutyl ester	25.652	0.54

The anti-inflammatory effect of HCI was studied on carrageenan induced pleurisy and compared with that of dexamethasone used as a standard drug. The anti-inflammatory effect of the drugs was studied at 24 h. All dose of the anti-inflammatory drugs produced a significant decrease in the exudate volume as compared to the control (0.95 ± 0.24 ml). Although the anti-inflammation of HCI at any dose is less strong than that of dexamethasone (Table 2), HCI at three doses showed the significant difference with respect to control. The protein concentration of the exudates administrated by three doses decreased from 26.18 ± 2.18 mg/ml with administration of sterile saline to 5.47 ± 5.35, 3.35 ± 2.18, and 4.00 ± 1.67 mg/ml, respectively and the effect at dose of 0.54 ml/100 g is comparable to that of dexamethasone (Table 2). This should be the result of HCI decreasing the capillary permeability. But to suppress the inflammatory cell influx and reduce C-reactive protein concentration, HCI at dose of 0.27 ml/100 g was of no effect compared to the dose of 0.54 ml/100 g, 1.08 ml/100 g and dexamethasone (Table 2). From the data in Table 2, the anti-inflammatory effect

Table 2
Anti-inflammatory effect of HCI on carrageenan induced pleurisy ($\bar{X} \pm S$)

Treatment	Exudate volume (ml)	Protein concentration (mg/ml)	Total cells ($\times 10^6$ ml^{-1})	C-reactive protein concentration (mg/l)
Control	0.95 ± 0.24	26.18 ± 2.18	65.79 ± 32.24	1.75 ± 0.65
Dexamethasone	0.06 ± 0.03*	3.21 ± 1.68*	11.12 ± 4.76*	0.27 ± 0.22*
HCI				
0.27 ml/100 g	0.26 ± 0.07*	5.47 ± 5.35*	53.88 ± 15.65	1.81 ± 0.60
0.54 ml/100 g	0.16 ± 0.09*	3.35 ± 2.18*	29.98 ± 10.88*	0.28 ± 0.37*
1.08 ml/100 g	0.10 ± 0.05*	4.00 ± 1.67*	37.74 ± 16.35*	0.40 ± 0.35*

Each value represents mean ± S.E.M. ($n = 12$).
* $P < 0.05$ indicates significant difference with respect to control.

of HCI on carrageenan induced pleurisy at 0.54 ml/100 g or more is preferable.

3.3. Effect of HCI on xylene induced ear edema

The difference in weight between the two plugs was taken as a measure of oedematous response, and reached the maximum at 30 min followed by a decline after embrocating xylene.

Topical anti-inflammatory activity of HCI was evaluated as inhibition of the xylene-induced ear edema in mice. The xylene-induced mouse ear edema method has certain advantages for natural product testing and has a good predictive value for screening anti-inflammatory agents (Jacobs et al., 1985). The effect of HCI on xylene-induced ear edema in mice is shown in Table 3. Topical application of xylene induced cutaneous inflammation at the ears of mice and caused significant increase in ear plug weight of the right ear when compared to the left ear. When HCI was hypodermically applied at dose of 40, 80, 160, 240 and 320 μl per 20 g body weight, it provided inhibitory effect on xylene-induced ear edema formation in a dose-dependent manner (Table 3). Treatment of HCI at 80 μl/20 g gave 50% inhibition in ear plug weight (Table 3), indicating that HCI contains anti-inflammatory activity. As a positive control, dexamethasone (40 μl/20 g) gave rise to a significant inhibition of 73% in ear plug weight (Table 3). It is noted that the effect after the treatment of HCI at dose of 320 μl/20 g is of no significance. This may be attributed to extra dose bringing hypodermic damage and inflammation again. As a result, the anti-inflammatory effect of this dose decreased.

Although the anti-inflammatory effect of HCI is less strong than that of dexamethasone (Table 3), the consideration of HCI as a complicate mixture suggests that its active principle would have strong anti-inflammatory effect. The results agree with the results obtained by the carrageenan induced pleurisy test (Table 2).

Table 3
Anti-inflammatory effect of HCI on xylene induced ear edema

Treatment	Extent of the edema (mg)	Percentage of inhibition (%)
Control	19.6 ± 3.8	
Dexamethasone	5.3 ± 1.5*	73
HCI		
40 μl/20 g	13.1 ± 4.2*	33
80 μl/20 g	9.8 ± 2.8*	50
160 μl/20 g	11.0 ± 3.1*	44
240 μl/20 g	13.7 ± 4.6*	30
320 μl/20 g	15.0 ± 6.6	24

Data represent the mean of difference in ear weight (mg) ± S.E.M. ($n = 15$).
* $P < 0.05$, significant as compared to the control.

From the results obtained by these two models, HCI is effective in curbing inflammation, which was consistent with the clinical result (Song, 2002). According to the observing of Song, HCI brought down fever, cough and asthma, and eliminated the rale in capillary bronchitis. Those symptoms also appeared in SARS. So, it could suppose that the effect of curbing SARS involved in the anti-inflammatory effect. Of course, it needs validation of abound research.

4. Conclusions

The results of present study revealed anti-inflammatory activity of HCI on both mice ear edema induced by xylene and rat pleurisy induced by carrageenan model.

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