Effects of Picrorrhiza Rhizoma on Acute Inflammation in Mice

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(Received May 21, 2008; Accepted June 25, 2008)

Abstract – The effects of Picrorrhiza Rhizoma (PR) aqueous extracts were observed on xylene-induced acute inflammation. The xylene was topically applied 60 min after administration of 500, 250 and 125 mg/kg of PR extracts, and all animals were sacrificed 2 hrs after xylene application. The changes on ear weights, histopathological analyses of ear were evaluated as compared with indomethacin and dexamethasone 15 mg/kg treated groups - well known anti-inflammatory agents. Xylene application resulted in marked increases in induced ear weights as compared with intact control ear. Severe vasodilation, edematous changes of ear skin and increase in the thickness of the ear tissues as acute inflammation were detected in xylene-treated control ears at histopathological observation. However, these xylene-induced acute inflammatory changes were dose-dependently decreased by oral treatment of PR extracts. Therefore, it is concluded that PR extracts has favorable anti-inflammatory effects on xylene-applied acute ear inflamed mice.

Keywords: Picrorrhiza Rhizoma, extracts, acute inflammation, xylene, histology, mouse

INTRODUCTION

Although inflammation has been regarded as an essential protective process preserving the integrity of organisms against various irritations like Physical damage, chemical substances, micro-organisms and other agents, the inflammatory response to several insults frequently leads to erroneous damage to normal tissues (Habashy *et al.*, 2005).

A traditional Korean herbal medicine, Picrorrhiza Rhizoma (PR) is a dried root and stem of *Picrorrhiza kurroa*, and has been used as hepatoprotective agents such as jaundice. Until now, the nitric oxide scavenging activity (Jagetia and Baliga, 2004), cardioprotective effect (Senthil Kumar *et al.*, 2001), anti-cancer effect (Jeena *et al.*, 1999; Joy *et al.*, 2000), anti-diabetic activity (Joy and Kuttan, 1999), anti-viral effect (Mehoratra *et al.*, 1990), hypolipidemic and hepatoprotective effects (Lee *et al.*, 2006ab; Lee *et al.*, 2008) of PR extracts have been evaluated. However, there are no experimental reports on the anti-

inflammatory activities PR extracts as we know. Traditionally, water decoction of PR extracts has used as Korean medicine, the effects of aqueous PR extracts on the xylene-induced acute inflammation were investigated in the present study.

MATERIALS AND METHODS

Animals and husbandry

Sixty-three male ICR mice (6-week-old upon receipt; SLC, Japan) were used after acclimatization for 7 days. Animals were housed five or four per polycarbonate cage in a temperature (20-25°C) and humidity (40-45%) controlled room with a 12 hrs:12 hrs light:dark cycle. Feed (Samyang, Korea) and water were supplied *ad libitum*. All animals were fasted overnight before dosing and sacrifice (about 18 h with *ad libitum* access to water).

Experimental design

The PR was purchased from Cho-Heung Pharmaceutical Ind. Co. (Daegu, Korea) after confirming the morphology under microscopy. The prepared PR (103 g) was boiled in 2 l of distilled water for 2 hrs and filtrated. The fil-

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trate was decompressed using a rotary vacuum evaporator (Lab. Camp, Korea) and lyophilized in a programmable freeze dryer (IIShin Lab., Korea). Total acquired PR extracts was 26.4 g (yield 25.65%). Powders of PR extracts were stored in a desiccator to protect from light and moisture. Indomethacin (Fluka, Switzerland) and dexamethasone (Sigma, USA) were used as reference drugs in this study. The animals were distributed into seven groups with 9 mice per group: intact control, xylene-treated control, 500, 250, and 125 mg/kg PR extracts dosing groups, 15 mg/kg indomethacin and dexamethasone dosing groups. PR extracts was orally administered once, and indomethacin and dexamethasone were administered intraperitoneally once. PR extracts was dissolved in distilled water, and indomethacin and dexamethasone were dissolved or suspended in saline.

Induction of acute inflammation

1 hr after oral administration with test substances, 0.03 ml of xylene (Merck, Germany) was topically applied to the anterior surface of the right ear. The left ear was considered a control.

Ear weight measurement

Two hours after topical application of xylene, all animals were sacrificed, and both ears were removed. Circular sections were taken using a cork borer with a 7-mm diameter and weighed as previously described (Kou *et al.*, 2005). The absolute wet-weight of the induced ear was measured and then, the relative weights (% of body weights) of the ears were calculated to reduce the differ-

ences from individual body weights. In addition, the differences between the intact ear and induced ear were also calculated as follows:

$$\begin{aligned} \text{Relative weight (\%)} &= \\ &(\text{absolute ear weight/body weight at sacrifice}) \times 100 \\ \text{Differences in weights (g)} &= \\ &\text{induced ear weight} - \text{intact ear weight} \end{aligned}$$

Histology and histomorphometry

Induced ears were trimmed crosswise to include ear cartilage, and fixed in 10% neutral buffered formalin, then embedded in paraffin, sectioned (3~4 μm) and stained with Hematoxylin & Eosin stain. The histological profiles of the ears were observed as compared with intact or xylene-treated control. The thickness of the induced ear was calculated using automated image analysis (DMI-300; DMI, Korea). The thicknesses from epidermis to dermis of induced ear skin (anterior skin thickness; μm) and anterior to posterior ear (full ear thickness, μm) were detected as histomorphometry on crossly trimmed ear specimens in regular corresponding regions in all induced ear samples.

Statistical analyses

Multiple comparison tests of the different dose groups were carried out. The variance homogeneity was examined using the Levene test. If the Levene test indicated no significant deviations from the variance homogeneity, the data obtained was analyzed using a one way ANOVA test followed by a least-significant difference multi-com-

Table I. Changes in ear weights in intact or xylene-applied mice

Group	Absolute weight (mg)			Relative Weight (%)		
	Intact ear	Induced ear	Difference	Intact ear	Induced ear	Difference
Controls						
Intact	117.78 $\pm$ 3.99	121.67 $\pm$ 5.55	3.89 $\pm$ 3.14	0.42 $\pm$ 0.01	0.43 $\pm$ 0.02	0.01 $\pm$ 0.01
Xylene	114.56 $\pm$ 9.19	154.78 $\pm$ 6.65*	40.22 $\pm$ 11.39*	0.41 $\pm$ 0.04	0.56 $\pm$ 0.02*	0.14 $\pm$ 0.04*
References						
Dexamethasone	115.22 $\pm$ 12.91	124.89 $\pm$ 12.44 [#]	9.67 $\pm$ 10.76 [#]	0.42 $\pm$ 0.04	0.45 $\pm$ 0.04 [#]	0.04 $\pm$ 0.04 [#]
Indomethacin	114.33 $\pm$ 6.60	132.67 $\pm$ 9.41 ^{**} , [#]	18.33 $\pm$ 10.48 [*] , [#]	0.41 $\pm$ 0.02	0.48 $\pm$ 0.04 [*] , [#]	0.07 $\pm$ 0.04 [*] , [#]
PR extracts						
500 mg/kg	115.11 $\pm$ 4.96	130.89 $\pm$ 10.13 ^{**} , [#]	15.78 $\pm$ 12.12 ^{**} , [#]	0.41 $\pm$ 0.01	0.47 $\pm$ 0.04 ^{**} , [#]	0.06 $\pm$ 0.04 [*] , [#]
250 mg/kg	114.22 $\pm$ 4.47	135.44 $\pm$ 6.17 [*] , [#]	21.22 $\pm$ 7.22 [*] , [#]	0.41 $\pm$ 0.02	0.49 $\pm$ 0.03 [*] , [#]	0.08 $\pm$ 0.03 [*] , [#]
125 mg/kg	115.33 $\pm$ 3.64	141.78 $\pm$ 10.89 [*] , [#]	26.44 $\pm$ 13.21 [*] , ^{##}	0.41 $\pm$ 0.02	0.51 $\pm$ 0.05 [*] , [#]	0.10 $\pm$ 0.05 [*] , ^{##}

Mean $\pm$ S.D. of nine mice; Relative weight (%)=(absolute ear weight/body weight at sacrifice) $\times$ 100; Differences=induced ear weight-intact ear weight; * P <0.01 and ** P <0.05 compared with. intact control; [#] P <0.01 and ^{##} P <0.05 compared with xylene control by MW test.

parison test to determine which pairs of group comparisons were significantly different. A non-parametric comparison test, Kruskal-Wallis H test was performed in the case of significant deviations from the variance homogeneity was observed at Levene test. When a significant difference was observed in the Kruskal-Wallis H test, the Mann-Whitney U-Wilcoxon Rank Sum W test was used to determine the specific pairs of group comparisons that were significantly different. The SPSS statistical software (Release 6.1.3, SPSS Inc., USA) was used and a P value <0.05 was considered significant.

RESULTS

Changes in ear weights

Significant ($P<0.01$) increases in the weights of the induced ear were detected in xylene-treated control as compared with intact control and, accordingly, the differences between intact and induced ear were also significantly ($P<0.01$) increased regardless of absolute and relative weights. However, ear weights and the differences between intact and induced ear of all administered groups were significantly ($P<0.01$ or $P<0.05$) decreased as compared with xylene-treated control, respectively (Table I).

Histological analyses of induced ear

Severe vasodilation and edematous changes of ear skin, as acute inflammation, was detected in xylene-treated control at histopathological observations. However, these histological indicators of acute inflammation were markedly and dose-dependently decreased in all three different dosages of PR extracts administered groups as compared with xylene-treated control (Fig. 1). Histomorphometrically, both anterior skin and full ear thicknesses were significantly ($P<0.01$) increased in xylene-treated control as compared with intact control, but these increases in ear thickness induced by xylene applications were also significantly ($P<0.01$) and dose-dependently inhibited by treatment of all three different dosages of PR extracts like two references (Table II).

Table II. Changes on the histomorphometry analyses of induced ear in intact or xylene-applied mice

Group	Full ear thickness (μm)	Anterior skin thickness (μm)
Controls		
Intact	766.77 $\pm$ 127.71	324.39 $\pm$ 94.26
Xylene	1916.07 $\pm$ 147.05*	1495.32 $\pm$ 166.73*
References		
Dexamethasone	763.84 $\pm$ 103.99#	328.82 $\pm$ 71.95#
Indomethacin	1059.19 $\pm$ 221.11*,#	739.26 $\pm$ 185.30*,#
PR extracts		
500 mg/kg	1007.32 $\pm$ 136.44*,#	690.09 $\pm$ 151.01*,#
250 mg/kg	1341.77 $\pm$ 222.92*,#	1081.21 $\pm$ 239.93*,#
125 mg/kg	1641.45 $\pm$ 176.29*,#	1166.73 $\pm$ 163.32*,#

Mean $\pm$ S.D. of nine mice; Anterior skin thickness=thicknesses from epidermis to dermis of induced ear skin; Full ear thickness=thicknesses from anterior to posterior ear of induced ear, * $P<0.01$ compared with intact control; # $P<0.01$ and ## $P<0.05$ compared with xylene control.

Fig. 1. Histological profiles of induced ear detected in intact control (A, B), xylene control (C, D), dexamethasone (E, F), indomethacin (G, H), 500 (I, J), 250 (K, L) and 125 (M, N) of PR extracts-administered groups.

Note the classic histological profile of acute inflammation as severe vasodilation and edematous changes of skin were detected in xylene control. However, these inflammation indicators were dramatically decreased in all dosing groups tested. All Hematoxylin & Eosin staining, Scale bars=160 μm .

DISCUSSION

It is generally known that acute inflammation was easily induced by topical application of xylene on the ear skin, and the anti-inflammatory effect of a drug has been evaluated based on the changes of ear weight and histopathology (Hosseinzadeh *et al.*, 2003; Kou *et al.*, 2005). In the present study, the effects of PR extracts on xylene-induced acute inflammation were observed as compared with indomethacin (cyclooxygenase inhibitors) and dexamethasone (glucocorticoid), frequently used as references as development of the new anti-inflammatory drugs (Hosseinzadeh and Younsei 2002; Ramprasath *et al.*, 2006).

Marked increases of ear weights (Al-Majed *et al.*, 2003; Ojewole 2005), and severe vasodilation and edematous changes of ear skin at histopathological observation (De La Puerta *et al.*, 1996; Kou *et al.*, 2003; Rotelli *et al.*, 2003; Kim *et al.*, 2007) were detected as signs of acute inflammation after topical application of xylene in the present study. However, ear weight increases and histopathological changes were dose-dependently decreased after pretreatment with three different dosages of PR extracts in the present study. This inhibition was considered as direct evidence that the PR extracts used in this study has favorable effects on reducing the acute inflammatory response. These are considered as direct evidence that PR extracts inhibited the acute inflammatory response. Nitric oxide plays an important role in inflammation, and nitric oxide synthase inhibitors can reverse several inflammatory symptoms (Amin *et al.*, 1995). Therefore, previously reported antioxidative effects of PR extracts (Jagetia and Baliga, 2004) were considered one of the major mechanisms of the anti-inflammatory effect of PR extracts detected in the present study. Based on the results, we therefore, conclude that the PR extracts has favorable effect on the reduction of the acute inflammatory response induced by xylene application in mice.

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