The Effects of Tripterygium Wilfordii Extract on Adjuvant Arthritis in Rats

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The Effects of *Tripterygium Wilfordii* Extract on Adjuvant Arthritis in Rats

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Abstract The effects of the extract of *Tripterygium wilfordii.* (TWE) on experimental adjuvant arthritis (AA) in the rat was studied. Lewis rats induced with AA were administered with TWE at 50 mg/kg/day for 10 weeks. The paw volume, its ratio to the body weight (paw ratio) and the radiologic changes in the feet of the rats with AA taking TWE were compared with those in the rats taking indomethacin (0.5 mg/kg/day) and no additional drugs. The rats taking TWE showed a smaller paw volume, a lower paw ratio and milder radiologic changes than the rats taking no drugs, however, the beneficial effects were weaker than those of indomethacin. We concluded that the beneficial effects of TWE on rats with AA was suggested. However, these results still need to be further confirmed by additional experiments.

Introduction

Rheumatoid arthritis (RA) is well known as a chronic inflammatory disease of the bone and joints[9]. Nonsteroidal antirheumatic drugs and corticosteroids have been used to relieve the symptoms due to inflammation and disease modifying antirheumatic drugs as well as various immunosuppressants have also been used to suppress the immunological abnormalities underlying the joint inflammation in the patients with RA[8)9)]. *Tripterygium wilfordii* (TW), a kind of vine, has been administered to RA patients in the People's Republic of China[3)6)15)16)]. It has also been recently shown that TW has immunosuppressive effects against human blood cells in vitro[7)14)] as well as autoimmunity in vivo[5)12)13)20)].

Since experimental adjuvant arthritis (AA) of the rat presents numerous similarities with human RA, we induced AA into rats[9)]. Extracts of TW (TWE) were then administered to the rats. As a result, some positive effect against the destruction of the bone and joints was suggested.

Materials and Methods

1. The induction of AA in the rat

Killed and dried tubercle bacilli were emulsified in Freund's complete adjuvant at 5.0 mg/ml. Lewis rats at 6 weeks of age were injected intradermally with 0.1 ml of the emulsion in their tail base. Thereafter, we regularly measured the body weight and paw volume of both feet by a volumeter (MK-550, Muromachi Co. Ltd, Japan) beginning from one week after the adjuvant injection.

2. Preparation of the drug-coated rats' food

Either TWE (Leigongtengpian ; Huang Shi Pharmaceutical Bureau, People's Republic of China) or indomethacin was mixed with 5% W/W of Arabian rubber. The mixture was coated on the surface of the solid food for rats. Food

containing 750 mg/Kg of TWE or 7.5 mg/Kg of indomethacin was prepared. Three groups composed of 10 rats each were then given food containing either TWE, indomethacin or drug-free food.

3. The assessment of the bone and joint changes in the AA rats

At the end of the 10-week observation period, all aninals were sacrificed and both feet were resected. The resected feet of the rats were examined by a lateral X-ray. The extent of the bone and joint changes was then determined according to the criteria indicated in Table I.

4. Statistical analysis of the data

The means and standard deviations were calculated for all variables. The statistical difference in the paw volume as well as its ratio against the body weight among each group of the rats was analyzed by the one-way analysis of variance, and then the difference at the individual observation period was analyzed by Student's t-test. The differences in the bone and joint changes on the X-ray were analyzed by the Chi-square test. P values of less than 0.05 were considered to be significant.

Results

1. Drug administration

The average administered dose of TWE for the 10-week observation period was 50 mg/kg/day, which is 4 times larger than the recommended regular dose for humans. Indomethacin was administered at 0.5 mg/kg/day which was similar to the normal dosage level for humans.

2. Changes in paw volume and its ratio to the body weight

The paw volume of the control group increased rapidly for 3 weeks and reached a peak at 4 weeks (Figure 1). Thereafter, no further change was observed until the end of 10 weeks. The paw volume of the rats administered with

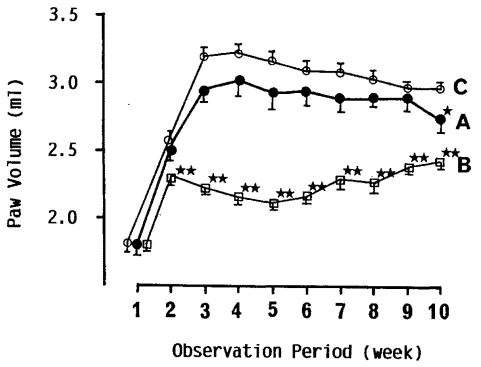

Fig. 1 Changes in the paw volume in AA in the rats.
A: TWE, B: indomethacin, C: no drug (control). Statistical difference vs the control (C) are indicated by ★ ($p<0.05$) and ★★ ($p<0.001$).

Table I Radiologic classification of the bone and joint changes in the hind paw of the adjuvant arthritis of the rat.

Grade	Tarsal bone height	Joint space narrowing	Tarsal bone destruction
I	≦	mild narrowing	moderate resorption, identifiable margin.
II	4< <8	marked narrowing, or no identifiable joint space	marked resorption, slightly identifiable margin
III	≧8	no identifiable joint space	marked resorption, no identifiable margin, ectopic mineralization

The joint space narrowing is judged in the tibiotarsal and tarsometatarsal joints. Bone destruction is judged in the tarsal bone as the presence of bone resorption, bone margin loss and ectopic mineralization around tarsal bone. An increase in the tarsal bone height due to the bone swelling is also included in this radiologic classification (tarsal bone height).

TWE showed changes similar to the control group. At 3 weeks, as well as in the following weeks, the mean paw volume of the TWE group was constantly smaller that of the control group. However, a significantly lower ratio vs the control group was obtained only at 10 weeks. On the other hand, the paw volume of the indomethacin group from 2 to 10 weeks was significantly smaller than those of the control group. In addition, a gradual decrease of the paw volume was observed as early as 3 weeks and it lasted until 5 weeks, which was then followed by a gradual increase until the end of the observation period.

Since the paw volume changes depended not only on the foot inflammation but also paralleled with the gain of total body weight of the rats, the ratio of the paw volume against that of the body weight was calculated (Figure 2). As a result, the rats of the TWE group as well as control group showed a rapid increase up to 4 weeks which was followed by a gradual decrease until 10 weeks. The indomethacin group also showed a peak at 2 weeks and then the ratio decreased until 5 weeks. The ratio at 5 weeks was similar to that at one week after the adjuvant administration, and thereafter it showed no change until 10 weeks.

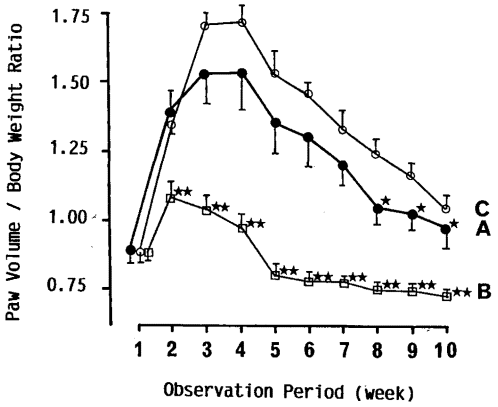

Fig. 2 Changes in the ratio of the paw volume vs body weight of AA in the rat.
A: TWE, B: indomethacin, C: no drug (control). Statistical difference vs the control (C) are indicated by ★ ($p<0.05$) and ★★ ($p<0.001$).

3. X-ray changes in the bone and joints of the foot

Bone changes in the control group were classified mainly into grade III (Table II). The changes in the indomethacin group were classified into grade I or II. The changes in the TWE group were classified into grade II and III. The χ^2-test was performed to analyze the statistical difference in the radiologic changes among the 3 groups. As a result, it was shown that the radiologic changes of the 3 groups were significantly different ($\chi^2=29.378$, $p<0.01$). The changes in both the TWE group and indomethacin group were significantly milder than those of the control group. Furthermore, the changes in the indomethacin group were milder than those of the TWE group ($\chi^2=13.286$, $p<0.01$).

Discussion

When compared with the marked effects of indomethacin to the paw volume, the effects of TWE was not striking even though the rats were administered with a dose some 4 times

Table II The distribution of the rats with adjuvant arthritis according to the radiologic classification.

Grade	I	II	III	χ^2-value vs control	Statistical significance
TWE	0	11	9	5.227	$p<0.05$
Indomethacin	3	17	0	27.048	$p<0.001$
Control	0	4	16		

The statistical significance in the grade classification was analyzed using the Chi-square test. The distribution of the rats among the 3 groups was significantly different ($\chi^2=29.378$, $p<0.01$).

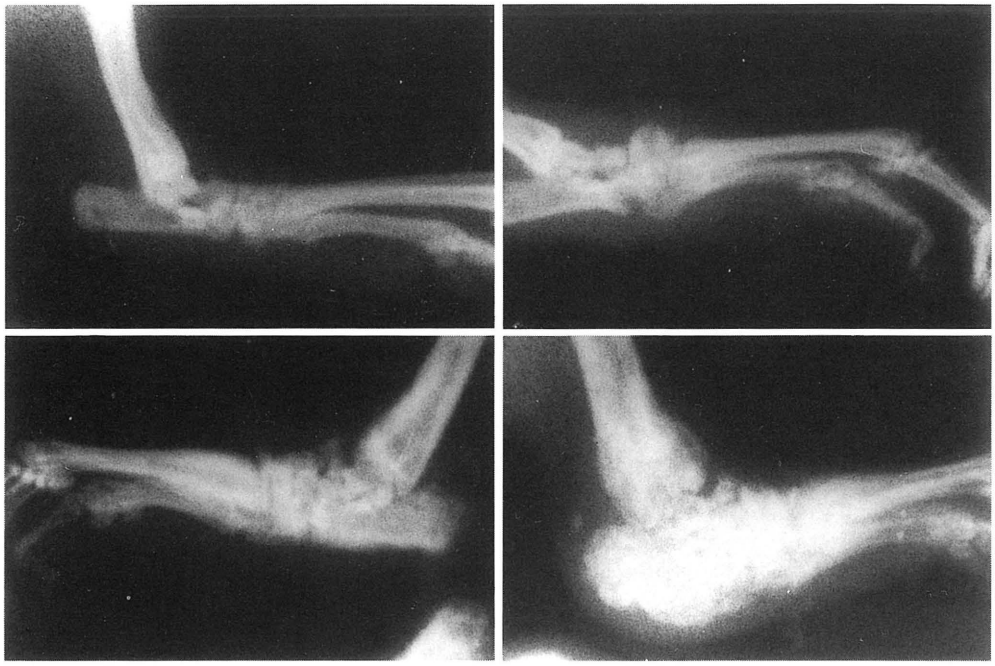

Fig. 3 Radiologic classification of the bone and joint changes of AA in the rat. Changes in AA rat's feet are judged on the tarsal bone and its joint as grade I, II or III. Normal control is shown in top left (N).

larger than that for humans. However, the mean paw volume of the rats administered with TWE was constantly smaller than that of the control group. Furthermore, the bone and joint changes in the TWE group were significantly milder than those of the control group. TWE lessened the degree of bone resorption and also inhibited pathologic mineralization in the feet. It is of interest that TWE was thus shown to have played some role against both bone and joint destruction. There have been several reports concerning the effects of TWE, in which the authors showed the immunosuppressive effects on B-cells as well as T-cells[7)14)]. However, the effects of TWE on the bone have not been reported yet.

Ectopic mineralization has been recognized as one of the characteristics of bone destruction in AA[2)9)19)]. The small number of grade III rats in the TWE group thus indicates the effectiveness of TWE. However, the bone changes in rats with TWE was so severe as to be suspicious of the drug usefulness on RA patients. In addition, ossifying periostitis leading to the ectopic mineralization, which was observed to be suppressed by both TWE and indomethacin in our experiment, is also characteristic to AA of the rat[9)]. These questions regarding the efficacy of TWE still need to be reexamined in the future on more gradually progressive joint inflammation in comparison with other immunosuppressants or immunomodulators such as cyclophosphamide or cyclosporin A[1)5)11)17)18)].

In conclusion, the administration of TWE for AA in the rat was suggested to be effective in the suppression of severe bone and joint destruction. However, these findings still need to be further reconfirmed in the future.

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(和文抄録)

ラットアジュバント関節炎に対する雷公藤の効果

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雷公藤（*Tripterygium wilfordii ; Thunder God Vine*）は藤の一種であるが，その根からの抽出物（TWE）には免疫抑制作用があることが知られている．今回，TWEの効果をヒト慢性関節リウマチの動物実験モデルであるラットアジュバント関節炎において検討した．ルイスラットにアジュバント関節炎を作成し，TWE 50 mg/kg/dayを投与した．対照には，無投薬ラットとインドメタシン0.5 mg/kg/dayを投与したラットを設けた．10週間にわたり後足趾の容積とその体重に対する比率を測定した．また，10週間の観察期間終了時点において後足趾のX線写真を撮影して骨変化を評価した．骨変化は，Tarsal bone height, Joint space narrowing, Tarsal bone destructionによりGrade I，II，IIIに分類した．その結果，TWE投与ラットの後足趾容積および体重比は無投薬対照に比し低値であったが，統計的な有意差を認めなかった（$p>0.05$）．インドメタシン投与ラットのそれは無投薬ラットに比しいずれの時期にも有意に低値を示した（$p<0.001$）．骨破壊は，TWEのGrade Iが0匹，IIが11匹，IIIが9匹に比し，無投薬では0，4，16匹でありインドメタシンでは3，17，0匹であつた．すなわち，TWEは無投薬群より骨破壊は軽度であったが（$p<0.05$），インドメタシンはTWEよりもさらに軽度であった($p<0.01$)．結論として，TWEはラットアジュバント関節炎に有効であることが示唆されたが，その効果は軽度であり，再確認が必要と思われた．