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Suppression of adjuvant arthritis by hesperidin in rats and its mechanisms

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Abstract

The citrus flavonoid hesperidin has been reported to possess a wide range of pharmacological properties. We have investigated the preventive and therapeutic effects of hesperidin on the development of adjuvant arthritis (AA), a rat model of rheumatoid arthritis (RA). Freund's complete adjuvant was used to induce AA in rats. Secondary paw swelling, polyarthritis index and histopathological assessment of ankle joints were used to evaluate the effects of hesperidin on AA rats. Concanavalin-A-induced T-lymphocyte proliferation and interleukin (IL)-2 production by splenocytes were measured using the MTT assay. Levels of IL-1, IL-6 and tumour necrosis factor (TNF)-α secreted by peritoneal macrophages (PM) were measured by RIA. Intragastric administration of hesperidin significantly attenuated secondary paw swelling and reduced the polyarthritis index of AA rats in a dose-dependent manner. In addition, hesperidin clearly ameliorated the pathological changes in AA rats. Hesperidin also restored the suppression of T-lymphocyte proliferation and IL-2 production, and downregulated production of IL-1, IL-6 and TNF-α by PM in AA rats. Our results suggest that hesperidin improves AA by downregulating the function of over-active macrophages and by up-regulating the activities of dysfunctional T lymphocytes. Hesperidin may therefore have therapeutic value for the clinical treatment of RA. Further research is required to clarify the detailed mechanisms of the protective effects of hesperidin on AA.

Introduction

Rheumatoid arthritis (RA) is a chronic autoimmune disease characterized by swelling joints, inflammation of the synovium and degeneration of cartilage. The chronic nature of this disease results in progressive joint destruction, which leads to severe locomotive disability and deterioration in quality of life (Verhoeven et al 1998). The prevalence of RA worldwide varies from 0.5% to 2% (Koch 1998). Investigations on the pathogenesis have revealed the primary involvement of cellular immune responses, such as an increase in production of interleukin (IL)-1 by macrophages, a decrease in T-lymphocyte proliferation and IL-2 production, and decreased function of Ts cells (Firestein et al 1987; Miossec et al 1987). Adjuvant arthritis (AA) in rats is an experimental model that shares some features with human RA, such as swelling of the extremities, cartilage degradation, loss of joint function and lymphocyte infiltration into diseased joints (Cicala et al 2000). The similarities in joint pathology between AA and RA are useful in screening new drugs for the treatment of RA.

Cytokines play an important role in the inflammation cascade, and changes in these proteins can be monitored to determine how a given treatment alters disease progression. Some of the important pro-inflammatory cytokines involved in RA such as IL-1, IL-6 and tumour necrosis factor (TNF)-α are thought to play key roles in the destruction of cartilaginous and bony tissues in joints affected by RA (Feldmann et al 1996).

Although a few drugs are effective in the treatment of RA, such as non-steroidal anti-inflammatory drugs, steroids and immunosuppressants, their side effects and toxicities leave a need for new and more powerful natural agents. Natural products have been used efficiently for several centuries with no obvious toxicities or side effects. In the past few

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decades, the anti-rheumatic effects of various natural products have been reported (Kumar et al 2003; Zhang et al 2004; Jung et al 2005).

The flavonoid hesperidin is a flavanone glycoside composed of the flavanone hesperetin and the disaccharide rutinose, and is a plentiful and inexpensive by-product of citrus cultivation. Deficiency of this substance in the diet has been linked with abnormal capillary leakiness, as well as pain in the extremities, which causes aches, weakness and nighttime leg cramps. No signs of toxicity have been observed with the normal intake of hesperidin or related compounds (Erlund et al 2001; Garg et al 2001). Hesperidin has been found to possess a wide range of pharmacological properties, such as antioxidant (Tirkey et al 2005), anti-inflammatory (Emim et al 1994; Guardia et al 2001), hypolipidaemic (Monforte et al 1995) and anticarcinogenic (Tanaka et al 1997) actions. Hesperidin has also been reported to have anti-rheumatic effects (Rotelli et al 2003; Kawaguchi et al 2006). However, the mechanisms of action of hesperidin in AA rats remain unknown and further work is therefore necessary. In our study, we explored the effects of hesperidin on AA and clarified its immunological mechanisms.

The Chinese herbal remedy *Tripterygium wilfordii Hook f.* (TWHF) has been known for thousands of years as a therapeutic agent for RA. Glucoside of *T. wilfordii* (TPT), extracted from the root of TWHF, displays both immunosuppressive and anti-inflammatory activities (Asano et al 1998). In this study, TPT was used as a positive control.

Materials and Methods

Chemicals

Hesperidin (purity 98.8%) is a yellow needle-crystal powder, kindly provided by Dr Wen-ming Cheng (College of Pharmacy, Anhui Medical University, China) who purchased it from the National Institute for the Control of Pharmaceutical and Biological Products (Peking, China) (batch number 0721-200406). TPT was purchased from Fudan Fuhua Pharmaceutical Factory (Shanghai, China). TPT and hesperidin were both made into solutions of the required concentration using 0.5% carboxymethylcellulose sodium (CMC-Na) solution and administered intragastrically.

3-[4,5-Dimethyl-2-thiazolyl]-2,5-diphenyl-2H-tetrazolium bromide (MTT), lipopolysaccharide (LPS), and concanavalin A (ConA) were purchased from Sigma Chemical Co. (St Louis, MO, USA). Bacillus Calmette–Guerin (BCG) was purchased from Shanghai Biological Products Factory (Shanghai, China). RPMI-1640 medium was purchased from Gibco Co. (CA, USA). The RPMI-1640 medium was supplemented with 25 mM Hepes, 1 mM sodium pyruvate, 2 mM L-glutamine, 50 μM 2-mercaptoethanol, 100 kU L^{-1} penicillin sodium, 100 mg L^{-1} streptomycin and 10% fetal calf serum (FCS), and the pH adjusted to 7.2. Commercial RIA kits for IL-1β, IL-6 and TNF-α were purchased from Beijing North Institute of Biological Technology (Peking, China).

Animals

Male Sprague–Dawley (SD) rats weighing 160–180 g and male BALB/c mice weighing 20–22 g were obtained from the Animal Department of Anhui Medical University and maintained in a temperature- (23 ± 2 °C) and humidity- (55–60%) controlled room with a 12 h light–dark cycle (lights on from 06:00 to 18:00). Animals were housed in plastic cages with free access to food and water. All animals received care in compliance with the guideline of the Animal Care and Use of Laboratory Animals as set by the Association of Laboratory Animal Sciences and the Center for Laboratory Animal Sciences at Anhui Medical University. Ethical approval for this study was obtained from the Ethics Committee of Anhui Medical University (licence number 2006DZ1109).

Induction of AA in rats

Sixty male SD rats with no remarkable differences in health status or paw volume were selected from 70 rats after observation for 1 week. The rats were randomly divided into six groups of 10 rats: a normal control group, an AA model group, hesperidin (40, 80 and 160 mg kg^{-1}) groups and a TPT (40 mg kg^{-1}) group. Freund's complete adjuvant (FCA) containing 10 mg heat-inactivated BCG in 1 mL paraffin oil was given by intradermal injection into the left hind paw of all rats except the normal control group.

Assessment of AA in rats

The volume of the right hind paw volume was measured using a digiti pedis volume meter (YLS-7A, Academy of Medical Science of Shandong Province, China) before immunization with FCA (baseline, day 0) and on days 12, 16, 20, 24 and 28 after immunization. Paw swelling at each time point was defined as the increase in paw volume since immunization, given in mL. Polyarthritis severity was graded on a scale of 0–4 (Sasai et al 1999) where 0 = no swelling; 1 = isolated phalanx joint involvement; 2 = involvement of the phalanx joint and digits; 3 = involvement of the entire region down to the ankle; 4 = involvement of the entire paw, including the ankle. The secondary inflammation index (polyarthritis score) was the total score for the three secondary paws, and had a maximum score of 12.

Drug administration

Hesperidin and TPT solutions were prepared by dissolving the corresponding drug in 0.5% CMC-Na solution. Rats in the AA+ hesperidin and AA + TPT groups were given different doses of hesperidin or TPT intragastrically in a volume of 10 mL kg^{-1}, once a day from day 12 to day 21 after AA induction. Each rat in the normal control group and the AA model group was given equivalent volumes of 0.5% CMC-Na solution.

Measurement of concanavalin-A-induced T-Lymphocyte proliferation

Animals were killed on day 28 after immunization. The spleen was dissected in sterile conditions and a splenic

lymphocyte suspension was prepared according to the method of Mosmann (1983). Cells were suspended in RPMI-1640 medium containing 10% FCS at a concentration of 1×10^{10} cells L^{-1}. The cell suspension (100 μL) and ConA (100 μL; final concentration 5 mg L^{-1}) were seeded into 96-well culture plates simultaneously, in triplicate. The cultures were incubated at 37°C in an atmosphere of 5% CO_2 for 48 h. Six hours before the end of this period, 10 μL MTT (5 g L^{-1}) was added to each well. The cultures were centrifuged (10 min at 2000 rpm) and the supernatants discarded. After adding 120 μL isopropanol containing HCl 0.04 M to each well, the plates were oscillated for 30 s to dissolve the formazan crystals. The absorbance was measured at 570 nm using a plate reader. The results were the mean of triplicate measurements.

ConA-induced IL-2 production by splenocytes

Spleen cells from AA rats (final concentration 5×10^9 cells L^{-1}) were cultured with ConA (final concentration 3 mg L^{-1}) in 24-well plates for 48 h at 37°C in 5% CO_2 humidified air. The plates were then centrifuged (10 min at 2000 rpm) and the supernatants collected for assay of IL-2 activity. Biological activity of IL-2 was determined using the activated-splenocyte proliferation assay described by Chen & Wei (2002). Briefly, spleen cells from BALB/c mice were suspended at a concentration of 2×10^9 cells L^{-1} and incubated with ConA (final concentration 3 mg L^{-1}) at 37°C, 5% CO_2 for 48 h to activate the splenocytes. The cell suspension was adjusted to 1×10^9 cells L^{-1} with RPMI-1640 medium. A sample of suspension (100 μL) and IL-2 supernatants (100 μL) were added to each well of a 96-well culture plate and incubated at 37°C, 5% CO_2 for 48 h. Six hours before the end, 10 μL MTT (5 g L^{-1}) was added to each well. The remainder of the procedure is as described above for ConA-induced T-lymphocyte proliferation.

Induction of IL-1, IL-6 and TNF-α from peritoneal macrophages (PM)

Peritoneal exudated cells were collected by i.p. injection of D-Hank's solution (twice, 5 mL each time). After centrifuging (10 min at 2000 rpm), the cells were washed three times with RPMI-1640 medium. The cell suspension was adjusted to 2×10^9 cells L^{-1} in RPMI-1640 medium containing 10% FCS, and 1 mL added to each well of 24-well culture plates. After 2 h incubation at 37°C in an atmosphere of 5% CO_2, non-adherent cells were removed by washing with RPMI-1640 medium. LPS (1 mL with a final concentration of 6 mg L^{-1}) was added to each well to induce generation of IL-1, IL-6 and TNF-α by PM. The plates were then incubated at 37°C in an atmosphere of 5% CO_2 for 24 h. After incubation, the supernatants were collected by centrifugation (10 min at 2000 rpm) and stored at −20°C for subsequent measurement of IL-1, IL-6 and TNF-α levels using commercially available RIA kits.

Histological examination and assessment of arthritis damage

Animals were killed on day 28 of AA induction and the hind paws were fixed with 10% formalin solution, dehydrated in graded alcohols and embedded in paraffin. Paraffin sections (3 μm) of the tissues were prepared and stained with haematoxylin and eosin (H&E). Sections stained with H&E were evaluated under a light microscope (Olympus LX70, Olympus, Tokyo, Japan) and representative photos were taken. Bone destruction, vascular proliferation, synovial hyperplasia and inflammatory cell infiltration were assessed. The severity of lesions was classified into four grades: no detectable changes (0), mild (1), moderate (2) and severe (3) (Tastekin et al 2007). Pathological evaluation was performed randomly by a pathologist trained in rat joint pathology who was blind to the treatment of the samples.

Statistical analysis

Results are expressed as mean ± s.d. Data were analysed by one-way analysis of variance followed by the Student–Newman–Keuls test. A P value below 0.05 was considered significant.

Results

Effect of hesperidin on secondary inflammation in AA rats

Inflammatory polyarthritis was induced in all immunized rats. Intragastric hesperidin and TPT were given from day 12 to day 21 after immunization with FCA. Paw swelling and polyarthritis index were measured every 4 days from day 12 to day 28. Our results showed that the swelling peaked on day 24. Compared with the AA control group, administration of hesperidin (80 and 160 mg kg^{-1}) and TPT (40 mg kg^{-1}) significantly suppressed secondary paw swelling (Table 1) and reduced the polyarthritis index of AA rats on days 20, 24 and 28 (Table 2).

Effect of hesperidin on ConA-induced T-lymphocyte proliferation

ConA-induced T-lymphocyte proliferation was measured using the MTT assay on day 28 after AA induction. In contrast to the normal control group, T-lymphocyte proliferation was markedly decreased in AA control rats. Hesperidin (40, 80 and 160 mg kg^{-1}) enhanced ConA-induced T-lymphocyte proliferation compared with the AA control group (Figure 1). However, TPT, which is an immunosuppresssant, aggravated the reduction of T-lymphocyte proliferation.

Effect of hesperidin on ConA-induced IL-2 production by spleen cells

The ConA-induced IL-2 production by spleen cells was measured on day 28 of AA induction. As shown in Figure 1, IL-2 production was reduced significantly in AA rats. Hesperidin (80 and 160 mg kg^{-1}) markedly recovered the IL-2 production compared with the AA control group. TPT did not show any effect.

Table 1 Effect of hesperidin on paw swelling (change in volume in mL) in rats with adjuvant arthritis (AA)

	Day				
	12	16	20	24	28
Normal	0.095 ± 0.085	0.112 ± 0.044	0.149 ± 0.052	0.208 ± 0.086	0.228 ± 0.078
AA	0.458 ± 0.167††	0.550 ± 0.158††	0.729 ± 0.258††	0.906 ± 0.301††	0.734 ± 0.255††
Hesperidin 40 mg kg^{-1}	0.440 ± 0.147	0.516 ± 0.148	0.653 ± 0.189	0.810 ± 0.260	0.650 ± 0.172
Hesperidin 80 mg kg^{-1}	0.405 ± 0.168	0.482 ± 0.109	0.564 ± 0.165*	0.729 ± 0.240*	0.559 ± 0.169*
Hesperidin 160 mg kg^{-1}	0.430 ± 0.171	0.477 ± 0.216	0.539 ± 0.182*	0.638 ± 0.247**	0.421 ± 0.205**
TPT 40 mg kg^{-1}	0.394 ± 0.166	0.452 ± 0.168	0.457 ± 0.144**	0.563 ± 0.198**	0.475 ± 0.192**

Data are mean ± s.d., n = 10 per group. TPT was the positive control. ††$P < 0.01$ vs normal control group; *$P < 0.05$, **$P < 0.01$ vs AA group.

Table 2 Effect of hesperidin on polyarthritis index in rats with adjuvant arthritis (AA)

	Day				
	12	16	20	24	28
Normal	0.55 ± 0.87	0.74 ± 1.06	0.91 ± 1.12	0.97 ± 1.22	0.96 ± 1.42
AA	5.80 ± 2.05††	6.93 ± 2.56††	8.15 ± 2.41††	9.23 ± 2.56††	7.52 ± 2.27††
Hesperidin 40 mg kg^{-1}	5.42 ± 1.98	6.56 ± 2.55	7.27 ± 2.02	7.90 ± 2.51	6.16 ± 1.78
Hesperidin 80 mg kg^{-1}	5.71 ± 1.52	5.64 ± 2.06	6.24 ± 2.21*	6.85 ± 2.73*	4.95 ± 1.52**
Hesperidin 160 mg kg^{-1}	5.25 ± 1.78	5.78 ± 1.97	5.52 ± 1.84**	5.49 ± 1.82**	3.54 ± 1.37**
TPT 40 mg kg^{-1}	5.59 ± 1.29	5.87 ± 2.14	5.07 ± 1.69**	4.93 ± 1.48**	3.93 ± 1.23**

Data are mean ± s.d., n = 10 per group. TPT was the positive control. ††$P < 0.01$ vs normal control group; *$P < 0.05$, **$P < 0.01$ vs AA group.

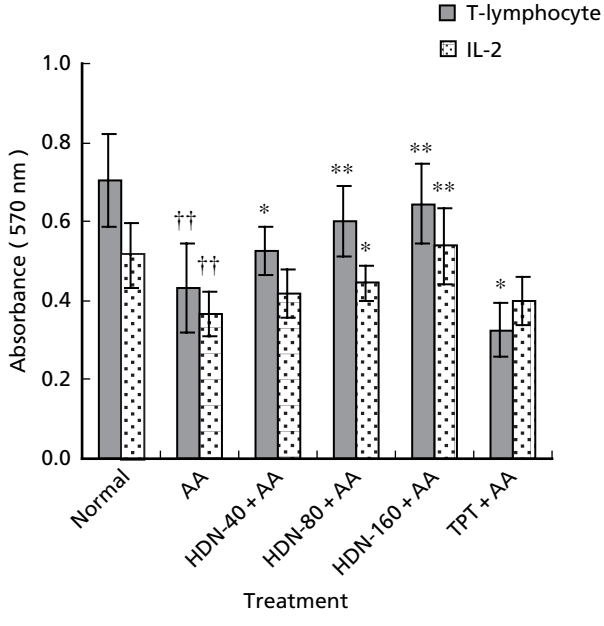

Figure 1 Effects of hesperidin (HDN-40/80/160 mg kg^{-1}) on concanavalin-A-induced T-lymphocyte proliferation and interleukin-2 (IL-2) production by splenocytes from rats with adjuvant arthritis (AA). Data are mean ± s.d., n = 10 per group. ††$P < 0.01$ vs normal control group; *$P < 0.05$, **$P < 0.01$ vs AA group. TPT (40 mg kg^{-1}) was the positive control.

Effects of hesperidin on pro-inflammatory cytokines produced by PM

The secretion of IL-1, IL-6 and TNF-α from PM was measured on day 28 of AA induction. As shown in Table 3, LPS-induced IL-1, IL-6 and TNF-α production by PM of AA control rats was significantly higher than that of normal rats. Hesperidin (40, 80 and 160 mg kg^{-1}) and TPT (40 mg kg^{-1}) effectively inhibited the production of IL-1 and IL-6. Hesperidin (160 mg kg^{-1}) and TPT (40 mg kg^{-1}) markedly decreased the production of TNF-α. These results show that hesperidin significantly reduced the production of pro-inflammatory cytokines by PM, as with TPT.

Histopathological evaluation

Photomicrographs of sections stained with H&E illustrated the disease severity and effect of hesperidin on joint histology (Figure 2). No inflammation or tissue destruction was seen in sections from normal rats (Figure 2A). In contrast, AA rats showed pannus formation, extensive inflammation (Figure 2B), synovial hyperplasia and bone destruction (Figure 2C). Hesperidin-treated (80 mg kg^{-1}) AA rats exhibited moderate synovial hyperplasia and mild cartilage degradation (Figure 2D). Hesperidin (160 mg kg^{-1}) and TPT (40 mg kg^{-1}) ameliorated joint destruction, although slight synovial hyperplasia remained (Figures 2E and F). Histological analysis showed that hesperidin markedly inhibited bone

Table 3 Effects of hesperidin on production of interleukin (IL)-1, IL-6 and tumour necrosis factor (TNF)-α by peritoneal macrophages in rats with adjuvant arthritis (AA)

	IL-1 (ng L^{-1})	**IL-6 (ng L^{-1})**	**TNF-α (ng L^{-1})**
Normal	58.34 ± 16.41	56.27 ± 11.72	352.63 ± 103.63
AA	123.38 ± 26.90††	116.79 ± 23.63††	510.20 ± 104.13††
Hesperidin 40 mg kg^{-1}	98.75 ± 25.68*	72.64 ± 15.51**	457.88 ± 97.81
Hesperidin 80 mg kg^{-1}	80.50 ± 16.54**	61.07 ± 12.48**	425.83 ± 85.23
Hesperidin 160 mg kg^{-1}	63.13 ± 14.04**	40.61 ± 9.75**	402.13 ± 72.17*
TPT 40 mg kg^{-1}	60.75 ± 12.63**	43.95 ± 10.03**	408.75 ± 119.01*

Data are mean ± s.d., n = 10 per group. TPT was the positive control. $^{\dagger\dagger}P < 0.01$ vs normal control group; $^{*}P < 0.05$, $^{**}P < 0.01$ vs AA group.

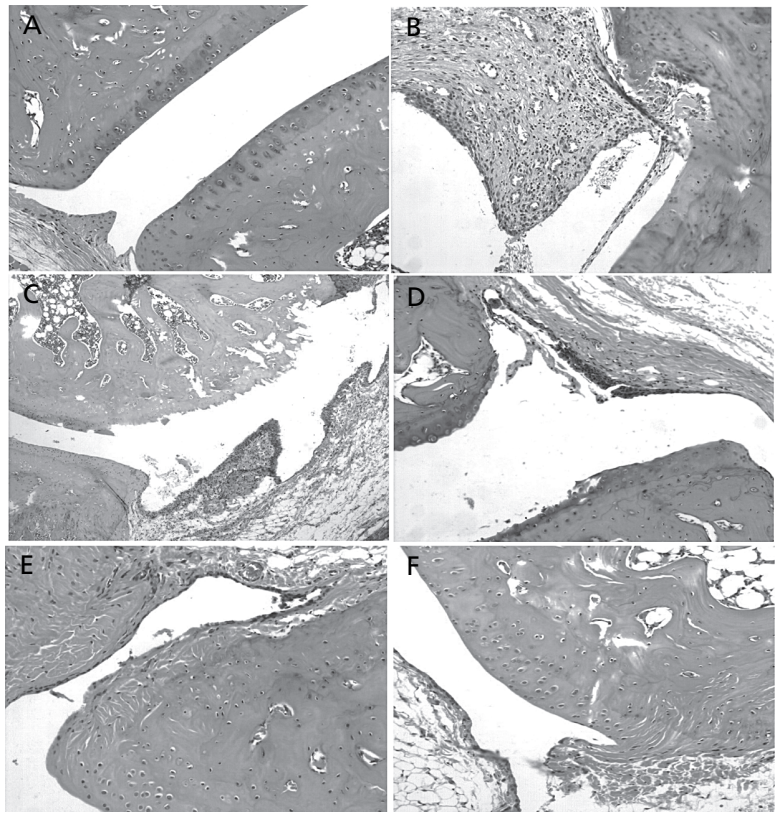

Figure 2 Histopathological examinations under a light microscope of ankle joints stained with haematoxylin and eosin (magnification ×100): (A) this section from a normal rat shows normal histological features with no inflammation or tissue destruction; (B) extensive inflammation and vascular proliferation were found in rats with adjuvant arthritis (AA); (C) AA rats showed obvious bone destruction, synovial hyperplasia or inflammatory cell infiltration; (D) AA rats treated with hesperidin (80 mg kg^{-1}) showed moderate synovial hyperplasia and mild cartilage degradation; (E) this section from an AA rat treated with hesperidin (160 mg kg^{-1}) shows amelioration of pathological changes. (F) AA rats treated with TPT (40 mg kg^{-1}) showed histopathological improvements similar to those seen with hesperidin 160 mg kg^{-1}.

destruction, synovial hyperplasia and inflammatory cells infiltration in a dose-dependent manner (Figures 3A, C and D). In addition, hesperidin significantly attenuated vascular proliferation at a dose of 160 mg kg^{-1} (Figure 3B). The same results were also observed in the TPT group.

Discussion

RA is an autoimmune disease characterized by synoviocyte proliferation, thickening of the synovial lining, various types of cell invasion, and pannus formation. AA in rats has similar

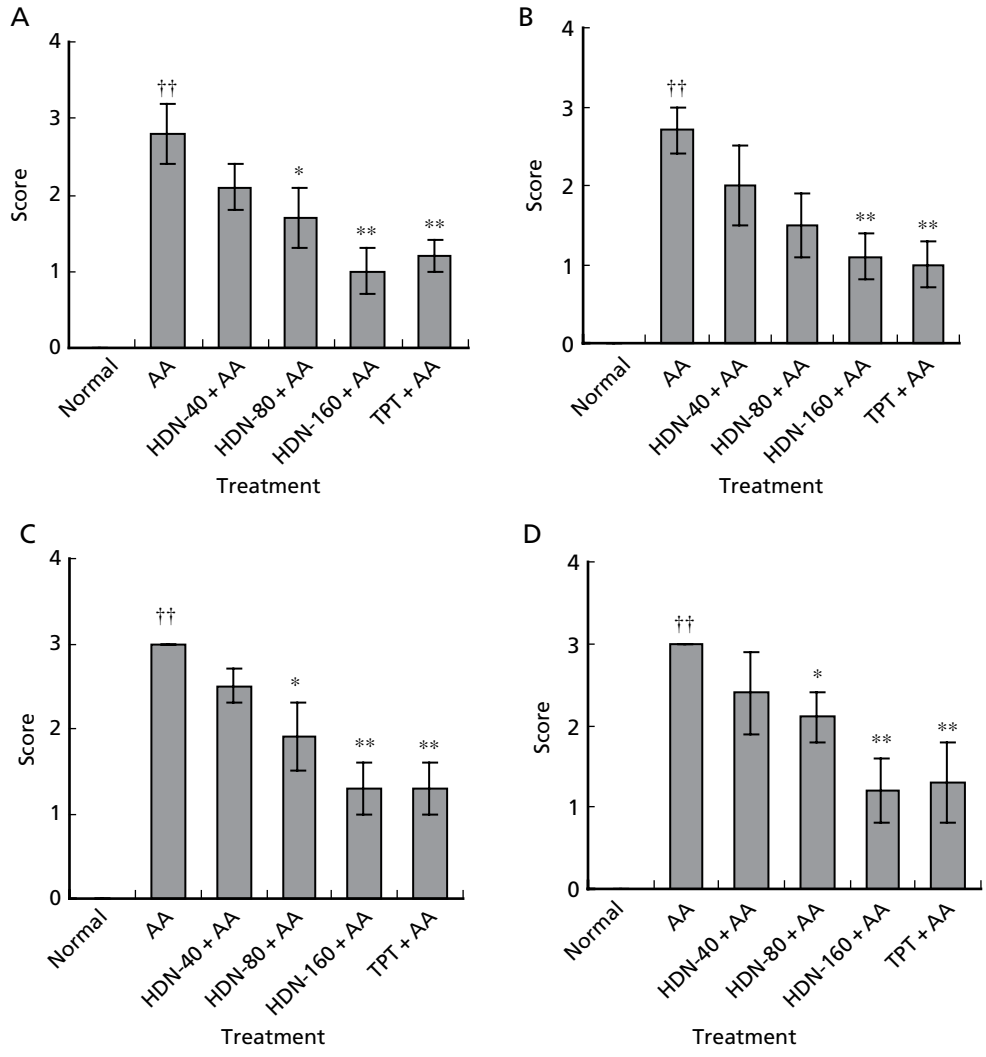

Figure 3 Inhibitory effects of hesperidin (HDN-40/80/160 mg kg^{-1}) on histological scores in ankle joints of rats with adjuvant arthritis (AA): (A) bone destruction; (B) vascular proliferation; (C) synovial hyperplasia; (D) inflammatory cell infiltration. Data are mean ± s.d., n = 10 per group. $^{\dagger\dagger}P < 0.01$ vs normal control group; $^{*}P < 0.05$, $^{**}P < 0.01$ vs AA group. TPT (40 mg kg^{-1}) was the positive control.

characteristics to RA in terms of histology and immunology and is widely used as a model for polyarthritis such as RA. In the AA model, arthritis develops within 2 weeks and is typified by decreased body weight, paw oedema, arthrodynia, deformity and cartilage degradation.

In the present study, we examined the therapeutic effects of hesperidin on AA rats and its immunological mechanisms. Our results showed that hesperidin remarkably inhibited joint swelling and reduced the polyarthritis index in AA rats. These results suggest that hesperidin reduces secondary inflammation in AA rats and may therefore be effective in chronic autoimmune diseases such as RA. The main pathological changes of AA include synovitis, pannus formation and inflammatory cell infiltration, which lead to cartilage erosion and articular destruction. Histological examination revealed that hesperidin significantly ameliorated these pathological changes in AA rats.

The dysfunction of T lymphocytes and the abnormal activation of mononuclear macrophages are important in the pathogenesis of arthritis. ConA-induced T-lymphocyte proliferation and IL-2 production were impaired in AA rats, suggesting functional abnormity of lymphocytes (Franch et al 1994; Jiang & Xu 2003). Macrophages play an important role in RA: the rheumatoid synovium is intensively infiltrated by macrophages and their numbers correlate with clinical scores and articular destruction in RA (Mulherin et al 1996). Once activated in-vivo by autoantibodies or by antigen-specific T-cell-derived lymphokines, macrophages are the major source of IL-1, IL-6 and TNF-α during immune responses (St Clair et al 1996; Ma & Pope 2005). IL-1 is a polypeptide produced mainly by macrophages, which has multiple biological functions. It is thought to be an essential mediator of inflammation. Many investigators have reported the importance of IL-1 in RA (Jeong et al 2004; Fleischmann et al 2004) and

have shown that IL-1 production correlates not only with the degree of inflammation but also with the degree of joint destruction. It is therefore believed that an inhibitor of IL-1 generation could be a useful therapeutic agent in the treatment of RA. IL-6 and TNF-α are also pro-inflammatory cytokines that play important parts in the aetiology of RA (Camussi & Lupia 1998; Nishimoto 2006). Both IL-1 and TNF-α promote synovitis by direct effects on joint tissues and induction of other pro-inflammatory cytokines. Selective inhibition of IL-1 and TNF-α pathways could represent an important new approach to the treatment of RA (Dayer et al 2001; Taylor 2001). The abnormal production of IL-1, IL-6 and TNF-α may be involved in the pathogenesis of autoimmune diseases such as RA (Deleuran et al 1992; Li et al 2002), and it is possible that these cytokines mediate the increase in paw volume as well as the decrease in bone density (Takagi et al 1997).

In order to understand the mechanisms of the therapeutic effects of hesperidin, we explored its modulatory activities on the cellular immune response in AA rats. In AA control rats, T-lymphocyte proliferation and IL-2 production by splenocytes were decreased, and the production of IL-1, IL-6 and TNF-α by over-activated macrophages was increased. It is clear that such disorders in immune response make AA harder to cure using currently available immunosuppressants. In fact, although TPT inhibited inflammation in AA rats, it did not restore the ability of T lymphocytes to proliferate, or the production of IL-2. Hesperidin significantly inhibited the production of IL-1, IL-6 and TNF-α. Interestingly, hesperidin enhanced T-lymphocyte proliferation and IL-2 production at the same time. From the above results, we deduced that hesperidin mediates its inhibitory activities on AA through down-regulating the function of over-activated macrophages and by up-regulating the function of dysfunctional T lymphocytes. These properties of hesperidin are quite different from immunosuppressants such as TPT and may be advantageous in the treatment of chronic RA with an underlying immune system disorder.

Conclusion

Our study shows that hesperidin has a remarkable protective action against FCA-induced AA in rats. Hesperidin can efficiently ameliorate disease severity, as assessed by paw swelling, polyarthritis score and histological evaluation. The inhibitory effects of hesperidin on AA are associated with its ability to inhibit the production of pro-inflammatory cytokines secreted from activated macrophages. In addition, hesperidin can markedly up-regulate the suppressed T-lymphocyte proliferation and IL-2 production by splenocytes. The results suggest that hesperidin has significant anti-rheumatic activity and its mechanism is related, at least in part, to its immunomodulatory properties. Further work is needed to clarify the mechanisms of action of hesperidin at the molecular level.

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