A systematic comparison between collagen-induced arthritis and pristane-induced arthritis in Dark Agouti rats

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Abstract

Objective Both collagen-induced arthritis (CIA) and pristane-induced arthritis (PIA) are commonly used rat models of rheumatoid arthritis (RA). The aim of this study was systematically to compare the differences between CIA and PIA in Dark Agouti

(DA) rats.

The CIA was induced by immunising DA rats intradermally with collagen type II (CII) and PIA was induced by injecting subcutaneously with pristane. The arthritis was evaluated macroscopically and microscopically. Nitric oxide (NO) level of plasma was determined by Griess reaction method. Plasma autoimmune antibodies, including CII specific IgG antibody (anti-CII IgG), cyclic citrullinated peptide specific IgG antibody (anti-CCP IgG), IgM and IgG rheumatoid factors (IgM RF and IgG RF), were detected by the enzyme-linked immunosorbent assay.

Results

The onset of PIA rats was earlier than that of CIA rats. The involved sites of PIA rats were mostly wrist/ankle and metacarpophalangeal/metatarsophalangeal (MCP/MTP) joints while those of CIA rats were primarily distal interphalangeal (DIP) joints. NO level of plasma was increased in PIA rats, as anti-CII IgG, anti-CCP IgG, IgM RF and IgG RF levels of plasma were increased in CIA rats. The kidney hyaline casts were more frequent in CIA rats than in control rats, with 9/12 in PIA group, 8/8 in CIA, and 4/8 in control, respectively.

Conclusion

PIA mainly affected wrist/ankle joints and MCP/MTP joints, had more severe inflammation and hardly involved other organs; while CIA mostly influenced DIP joints, had more autoimmune antibodies in plasma, and always showed hyaline casts in kidney. These findings will be useful to select the animal model of RA.

Key words

Collagen-induced arthritis, pristane-induced arthritis, animal model, rheumatoid arthritis

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Introduction

Rheumatoid arthritis (RA) is a chronic inflammatory disease which is characterised by symmetrical polyarticular synovitis with cartilage and bone damage (1, 2). Most RA patients are seropositive for autoimmune antibodies. Rheumatoid factors (RF) have been adopted as diagnostic and prognostic indicators of RA, and antibodies to cyclic citrullinated proteins (ACPA) are highly specific markers for RA (3, 4). Nitric oxide (NO) is known as a signal molecule and plays an important role in bone resorption in the inflamed joints (5, 6). The immunological strategies of inhibiting the synthesis or action of NO have formed the basis of new therapies for RA (7-9).

Many rat models are used to mimic RA, including collagen type II induced arthritis (CIA) (10), pristane-induced arthritis (PIA), adjuvant induced arthritis, oil-induced arthritis, avridine-induced arthritis, collagen type XI induced arthritis and cartilage oligomeric matrix protein-induced arthritis (11-13). Different models have distinct symptoms and pathogenic mechanisms, and each model reflects only one or several aspects of RA (14, 15).

CIA is one of the most commonly used models of RA, and it can be easily induced by intradermal injection of autologous or heterologous collagen typeII (CII) with incomplete Freund's adjuvant in susceptible rats and mice (16-19). CII is a normal constituent of the articular cartilage and can trigger an immune response in vivo against the cognate protein when injected into animals (20). Another commonly used model of RA is PIA, which is induced by a single subcutaneous injection of pristane (2, 6, 10, 14-tetramethylpentadecane). Pristane, a small alkane molecule, can not form a stable complex with a MHC classIImolecule itself (21). It has been shown that PIA is a T-cell-dependent disease (22). PIA and CIA share most of the arthritis susceptibility loci in rats and both have some similarities with RA (11). However, the involvements of other organs in CIA and PIA have not been studied; neither has the comparison study performed between these two models in the same strain of rats.

Here we compared clinical manifestations, plasma indicators, pathology of joints and other organs between CIA and PIA in DA rats. Our results indicated that CIA and PIA showed significant differences in clinical and plasma features.

Materials and methods Animals

DA rats (originating from Zentralinstitut Fur Versuchstierzucht, Hannover, Germany) were bred in the animal house under a specific pathogen-free condition and with 12h light/dark cycles. The rats were housed in polystyrene cages: 4 rats per cage with standard rodent chow and water ad libitum (23). Forty rats at age of 8 to 12 weeks were randomly divided into 3 groups matched by sex and age, among which 16 rats were used for the CIA group, 16 rats for the PIA group and 8 for the control group. Each group was divided into acute (sacrificed at 26 days after induction) and chronic (sacrificed at 70 days after induction) models. The experiment had been approved by the Institutional Animal Ethics Committee of the University.

Preparation of CII

CII was prepared from the DA rat xiphoid process cartilage by pepsin-digested method (24). Briefly, homogenised rat xiphoid process cartilage was digested with pepsin and further extracted with the buffer 1.0M NaCl/50 mM Tris/pH 7.5. A 0.9M NaCl was utilised to precipitate CII. The collagen was lyophilised, weighed, and then it was dissolved and stored in 0.1 M acetic acid until used. The purity of CII was detected by Coomassie Blue staining after SDS-PAGE analysis.

Induction of arthritis

In the CIA model, rats were immunised by a single intradermal injection of 150µl emulsion containing 150µg CII dissolved in 75µL 0.1mol/L acetic acid and 75µL incomplete Freund's adjuvant (sigma-aldrich, USA). In the PIA model, rats were subcutaneously injected with 150µL pristane (Acros Organics, Belgium) at the base of the tail. Control rats were subcutaneously injected with 150µL phosphate buffered saline.

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Clinical evaluation of arthritis

A macroscopic scoring system was used to monitor arthritis development in all 4 limbs. Briefly, 1 point is given for each swollen or red metacarpophalangeal/ metatarsophalangeal (MCP/MTP) joint; 1 point is also given for swollen or red interphalangeal (IP) joints of each toe, 5 points at most for each wrist/ankle joint (1, slight redness; 2, inside, outside or central of the joint swelling; 3, moderately swelling/erythema; 4, severe but not entire joint swelling; 5, entire joint swelling/erythema). The maximum score for each paw is 15. The rats were observed 3 times per week after arthritis induction.

Analysis of symmetry

Symmetry was indicated by the rate of paired affected joints. Thirty pairs of joints, including wrist/ankle, MCP/ MTP and IP joints, were observed in each rat. The symmetry rate was calculated according to the following formula:

Symmetry rate (%)=number of symmetric damaged joint pairs/(number of affected joints-number of symmetric damaged joint pairs)×100%

Delayed type hypersensitivity (DTH)

Twenty-four hours before being euthanised, all the rats were injected intradermally with 20μ L of 1 mg/mL CII dissolved in 0.05mol/L acetic acid in the left ear, and 20μ L of 0.05mol/L acetic acid was injected in the right ear as a control. After the euthanasia of the rats, all of the ears were separated, perforated at the same position and weighed. The difference of weight between each pair of ears represented the magnitude of DTH.

Collection of plasma samples

Rats were anesthetised by intraperitoneal administration of 2% pentobarbital sodium (0.15mL/100g body weight) at 26 or 70 days after induction. Blood samples were collected into heparin sodium anticoagulation tubes (Jiangxi Sophisticated Medical Equipment Co., LTD, China) by puncturing abdominal aorta, then separated by centrifuge at 3,000rpm for 20min and stored at -80°C.

NO level of plasma

One hundred microlitres of blood plasma was mixed with 80µL of 375mM ZnSO₄ and 120µL of 275mM NaOH, and then centrifuged at 13,000rpm for 20min. Supernatant was obtained and added with 400mg of Cu (copper) plated Cd (cadmium), then shaken for 2.5h at room temperature after adding 100µL of 0.2M glycine buffer. The 100µL of supernatant was added into 96 well plates, and then reacted with 100µL of Griess reagent (0.1% N (1-Naphthyl) ethyl-enediamine dihydrocholride: 1% sulfanilamide) to develop color. The absorbance was measured at 545nm wavelengths with a microplate reader (Thermo Electron Corporation, Finland). The NO concentration was obtained from calculating OD values against a standard curve from gradient concentrations of sodium nitrite.

Autoimmune antibody levels of plasma

The anti-CII IgG antibodies (anti-CII IgG) were detected by enzyme-linked immunosorbent assay (ELISA). Briefly, flat-bottomed 96-well microtiter plates (Corning Costar CO., LTD, USA) were coated overnight at 4°C with 50µL of 10µg/mL rat CII in carbonate buffer solution, then washed, blocked with 2% BSA and incubated for 1h at 37°C. Plates were washed and plasma was added at a dilution of 1:100. Plates were incubated for 1 h at 37°C and washed, and horseradish peroxidase-conjugated rabbit anti-rat IgG antibody (Beijing Biosynthesis Biotechnology Co., Ltd, China) at a dilution of 1:10,000 was added and incubated for 1h in 37°C. Then 100µL of 0.1mg/ml tetramethyl benzidine was added to each well and plates were incubated for 30min at room temperature, then 1M H₂SO₄ was added as stop buffer. Plates were read at 450nm by micro plate reader (Thermo Electron Corporation, Finland). Other antibodies were assayed by the similar method. For RF detection, 50µL of 0.1µg/mL rabbit IgG (Beijing Biosynthesis Biotechnology CO., LTD, China) was used as the coating antigen, and horseradish peroxidase conjugated rabbit anti-rat IgG antibody or anti-rat IgM antibodies (Beijing Biosynthesis Biotechnology

Co., Ltd, China) was used as detecting antibodies at a dilution of 1:10,000. For anti-CCP antibody detection, 50μ L of 1μ g/mL CCP (The sequence of CCP: HQ (CHQESTXGRSRGRC) GRSGS Cys3-Cys16, X=Citrulline. Shanghai Science Peptide Biological Technology CO., LTD, China) was coated on plates and plasma with a dilution of 1:200 was added.

Pathological evaluation of arthritis

Left hind paws of rats were removed and fixed, then decalcified in 12.5% EDTA (ethylenediamine tetraacetic acid) solution for 4 weeks, during which the solution was changed every 2 days. The decalcified samples were subsequently embedded in paraffin and cut into 6 µm tissue sections, which were then stained with haematoxylin and eosin (H.E). A pathological scoring system was adopted to evaluate the severity of arthritis. Synovitis was evaluated by numbers of synovial lining cell layers, area of pannus coating articular surface, infiltration of synovial inflammation cells and formation of new vessels; and each index was given from 0 to 3 points. Articular destructions were assessed by cartilage erosion, bone erosion, synarthrophysis and joint structure; and each was given from 0 to 3 points. Repair was indicated by the formation of new cartilage and bone and each was given from 0 to 3 points also.

Pathological evaluation of other organs

Brain, lung, kidney, liver, spleen, thymus, thyroid gland, cornea, ovary/testis, cardiac muscle, inguinal lymph nodes and popliteral lymph nodes (ILN and PLN) were harvested and weighed immediately after rat euthanisation. All the tissues/organs were fixed in 4% paraformaldehyde and stained with H.E. Then pathological changes of these tissues/organs were evaluated by two pathologists under the blind fashion.

Statistical analysis

Comparisons of macroscopic and microscopic scores, symmetry rate, NO and antibodies were analysed by the Mann-Whitney test. The comparison of onset joint rate was analysed by the



Fig. 1. Clinical comparisons between CIA and PIA in DA rats. (**A**) Cumulative arthritis incidence of CIA (n=8) and PIA (n=8) models. CIA was induced by a single subcutaneous injection of 150 μ g CII and PIA was induced by 150 μ L pristane instead. (**B**) Onset joint rate of CIA and PIA models. Each paw was divided into four regions: wrist/ankle joint, MCP/MTP joints, PIP and DIP joints. (**C**) Symmetry was indicated by rate of paired affected joints. (**D**) Clinical arthritis scores of total or separate joints arthritis. Onset joint rate comparison was analysed by Fisher's exact test. Differences of symmetry rate and arthritis severity between PIA and CIA were analysed by Mann-Whitney test. (C) and (D) Data are shown as mean± SEM with 13 rats in CIA group and 16 rats in PIA group. *Indicates *p*<0.05 comparing two groups.

Fisher's exact test. Organ/body weight ratios were analysed by independentsamples *t*-test. Quantitative data were expressed as mean±SEM. A *p*-value less than 0.05 was considered as statistically significant.

Results

Clinical comparisons between CIA and PIA models

In DA rats, PIA was more susceptible with an incidence of 100% (16/16) compared with 81.25% (13/16) of CIA. The prevalence rate of PIA was fluctuating during the chronic phase, whereas that of CIA was permanent (Fig. 1A). The onset of PIA rats was earlier than that of CIA (p<0.05), with 14.2±1.7 and 18.6±1.5 days (mean±SD), respectively. PIA reached the maximum score earlier than CIA (p<0.05), with 21.4±4.9 and 28.5±10.4 days. However, there was no difference of maximum score earlier was no difference of maximum score earlier was not difference earlier was not difference earlier was not e

mum arthritis scores between the two models, with 16.5 ± 6.9 and 20.4 ± 14.6 for PIA and CIA, respectively.

The onset joints of PIA were predominantly wrist/ankle joints and metacarpelphalangeal/metatarcelphalangeal (MCP/MTP) joints, whereas those of CIA were distal interphalangeal (DIP) joints, with significant difference (Fig. 1B). The symmetry of CIA was high in the early phase and decreased during the chronic phase while that of PIA showed an opposite trend (Fig. 1C). PIA had an acute process with two disease peaks and a chronic fluctuating process, while CIA was a chronic and permanent arthritis model. Similar to the onset sites, PIA mainly influenced wrist/ankle joints and MCP/MTP joints, while CIA predominantly influenced DIP joints during the disease course (Fig. 1D).

We also analysed the difference be-

tween female and male rats from both models and found that there was no significant difference in incidence and disease severity (data not shown).

Comparisons of DTH, plasma NO and autoimmune antibodies between CIA and PIA

The DTH of acute CIA rats was significantly severer than that of control and acute PIA rats (Fig. 2A). Plasma NO level was increased in acute and chronic PIA rats (Fig. 2B). The plasma anti-CII IgG level was significantly higher in acute and chronic CIA rats (Fig. 2C), and the anti-CCP IgG level was higher in chronic CIA rats than in chronic PIA rats (Fig. 2D). As expected, the plasma IgM RF level was higher in acute CIA rats than in chronic CIA rats, while the IgG RF level had dominantly increased in chronic CIA rats (Fig. 2E and Fig. 2F). The antibodies in PIA rats did not

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Fig. 2. Comparisions of DTH, plasma NO and autoimmune antibodies between CIA and PIA. (A) All the rats were injected intradermally with collagen CII dissolved in 0.05mol/L acetic acid in the left ear, and injected with 0.05mol/L acetic acid as control in the right ear. The difference of weight between both ears represented the magnitude of DTH. (B) Nitric oxide level of plasma was assayed by Griess reaction. (C), (D), (E) and (F) Autoimmune antibody levels of plasma were detected by ELISA, arbitrary unit was used with setting control group 1.0. Mann-Whitney test was used for analysis. *Indicates p<0.05 comparing with control group or between two groups.

DTH: delayed type hypersensitivity; CII: collagen type II; CCP: cyclic citrullinated peptide; RF: rheumatoid factor; aCIA: acute collagen-induced arthritis; aPIA: acute pristane-induced arthritis; cCIA: chronic collagen-induced arthritis; cPIA: chronic pristane-induced arthritis.

significantly vary with the control although IgM RF had an increasing trend in acute phase of PIA.

Pathological analysis of ankle joints

Left ankle joints were examined from control, PIA and CIA rats. There was no difference in the total pathological score of each ankle joint between these two arthritis models. Then the pathological changes of the ankle joints were divided into three parts and independently analysed for synovitis, destruction and repair. The synovitis score of acute CIA rats was higher than chronic CIA rats, and the result of PIA rats was similar to CIA though no statistical significance was found. The destruction and repair of the ankle joints were mainly shown in chronic models with no difference between CIA and PIA (Fig. 3).

Pathological analysis of other organs in CIA and PIA

The organ/body weight ratio was analysed. ILN and PLN in CIA and PIA rats, especially in acute PIA rats, were significantly larger than in control rats. The organ/body weight ratio of spleen and brain was increased in acute CIA rats; the heart was bigger in chronic CIA rats than in acute CIA rats; the lung was enlarged in chronic PIA rats compared to the control rats; the liver was increased in both chronic CIA and PIA rats compared to the control rats (Fig. 4A).

Then the pathological changes of the brain, lung, kidney, liver, spleen, thymus, thyroid gland, cornea, ovary/testis, cardiac muscle, ILN and PLN were analysed. The number of rats with kidney hyaline casts was 9/12 in PIA group, 8/8 in CIA, and 4/8 in control, respectively, with significantly increasing in CIA than control (p<0.05) (Fig. 4B). There was no specific pathological change found in other organs in both arthritis models.

Discussion

Our research implicated that CIA and PIA had many different features. PIA mainly affected wrist/ankle joints and MCP/MTP joints, had more severe inflammation and hardly involved other organs; while CIA mostly influenced DIP joints, had more autoimmune antibodies in plasma, and always showed hyaline casts in kidney.

It has been shown that the incidence of PIA was higher than CIA despite the difference in rat strains, which was consistent with our results (11, 25). This was caused by the difference of induction substances. The previous findings have shown that CII needs to be a triple helical structure rather than denatured CII to induce arthritis (20). CII can be denatured by heating, shaking and ultraviolet radiation (26). So the incidence of CIA hardly reaches 100% even by increasing either the quantity or times of CII for immunisation (27, 28). Pristane is an isoprenoid alkane and can easily enter the local tissue profited from its small molecule (29). Therefore, arthritis can technically be easily induced by pristane in rats.

Rheumatoid arthritis is a symmetric disease, and the common definition of symmetry requires that at least 50% of the involved joints should be symmetric pairs (30). Symmetry is more evident in proximal IP (PIP) joints than in MCP and wrist joints in RA patients (31). Our results showed that symmetry of CIA was higher in the acute phase, while that of PIA was increased with the disease development, which is similar with RA(2). The symmetry of affected joints is mediated by precipitating of circulating immune complexes equally well in both sides of the body (32). On the other hand, in RA patients, the MCP/MTP, PIP/thumb IP and wrist/ankle joints are more commonly involved than elbow/ knee and shoulder/hip joints. The DIP joints are involved only in the presence of a coexisting MCP or PIP involvement (33). In CIA, our results showed that the onset joints were frequently DIP joints but not PIP joints, MCP/ MTP or wrist/ankle joints. The previous study indicated that the inflammation of DIP joints was more serious than that of PIP joints (34). In our observation, even without other inflamed joints, DIP joints were still involved. In PIA, the onset joints were frequently wrist/ankle joints and MCP/MTP joints rather than



Fig. 3. Pathological analysis of ankle joints. (A) Different pathological changes of arthritis models (CIA was used for example). (B) Pathological scores of different arthritis models. Mann-Whitney test was used for analysis. *Indicates p<0.05 comparing between two groups.



Fig. 4. Pathological analysis of other organs in CIA and PIA. (A) Organ/body weight ratios of different groups. (B) Pathological changes of kidney. Independent-Samples *t*-test was used for analysis. *Indicates p<0.05 comparing between two groups.

IP joints, and the mainly injured joints were MCP/MTP joints and wrist/ankle joints instead of IP joints. It was previously shown that the inflammatory severity of PIA was less in the chronic phase (after >56d) than in the first acute phase of the disease, but there were more pronounced deformities of peripheral joints in the chronic phase (21). In brief, PIA seems to be more similar to RA in clinical features. The plasma level of NO is higher, and the activity of inducible nitric oxide synthase (iNOS) is increased in RA patients in comparison to the control (8, 9). NO reflects an immune-activated state with up-regulated activity of iNOS by inflammatory mediators (35). Our results showed that the plasma level of NO was increases in PIA but not in CIA rats. Previous studies have shown that pristane can induce lymphoid neogenesis and promote secretion of proinflammatory cytokines, and PIA is a T-cell-mediated MHC class IIrestricted arthritis model (8, 29, 36). In summary, PIA has more severe inflammation than CIA.

The RF is one of the diagnostic criteria for rheumatoid arthritis. The ACPA is extremely specific for RA patients and is present early in the disease (37). In recent years, the combined usage of these two types of autoimmune antibodies has increased the diagnostic sensitivity and specificity (38). In our results, the anti-CII IgG was increased in both acute CIA rats and chronic CIA rats, the IgM RF was increased in the acute model of CIA rats, the IgG RF and anti-CCP IgG were increased in the chronic model of CIA rats. Interestingly, we also found that IgM RF had an increasing trend in acute phase of PIA, which was consistent with the previous finding (39). CIA was characterised by the generation of antibodies toward self antigens, and the IgM RF was increased in both rats and mice CIA models (40-43). These autoimmune antibodies can form immune complexes and deposits onto the joints of CIA rats or mice, and the serum from CIA rats can transfer arthritis to naive recipients (42). Therefore, CIA was a humoural immune mediated arthritis model.

RA, as a systemic disease, can also affect extra-articular tissues/organs to lead to pathological conditions, such as rheumatoid nodules, vasculitis and myocarditis (2). It has been reported that PIA is mostly a joint-specific arthritis model whereas CIA affects both joints and extra-articular cartilage (25). In this study, we examined histological changes of extra-articular organs and did not observe the specific pathological abnormalities in both CIA and PIA. However, CIA rats had more kidney hyaline casts than that of control rats. Hyaline casts consist of Tamm-Horsfall protein and can be observed in association with proteinuria of renal or extrarenal origin. This may be caused by the presence of excessive serum protein in the tubular lumen (44). In CIA, the kidney hyaline casts may be due to the increase of autoimmune circulating antibodies.

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Taken together, PIA compared with CIA frequently affected PIP, MCP/ MTP and wrist/ankle joints rather than DIP joints. PIA showed higher incidence with earlier onset, and the symmetry of PIA was increased with the disease development. PIA was a more joint-restricted disease hardly influencing other organs.

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