Glycoconj J (2012) 29:305–313
DOI 10.1007/s10719-012-9407-0

Therapeutic effects of lactosyl derivative Gu-4 in a collagen-induced arthritis rat model

Jie Fan · Huiting Zhou · Shihui Wang · Hailian Wang · Yushun Zhang · Yingtao Guo · Qing Li · Zhongjun Li · Zhihui Zhao

Received: 9 April 2012 / Revised: 25 May 2012 / Accepted: 28 May 2012 / Published online: 12 June 2012

Abstract Rheumatoid arthritis (RA) is an inflammatory disorder that is characterized by persistent recurrence of joint inflammation leading to cartilage and bone destruction. The present anti-arthritis therapies failed to achieve satisfactory remission in all patients; therefore, it is still necessary to develop novel approaches to fulfill the demand in clinic. Here, we reported the therapeutic effects of lactosyl derivative Gu-4, a synthetic compound that was previously identified as a selective inhibitor against leukocyte integrin CD11b, in a bovine type II collagen induced arthritis (CIA) rat model. First, prophylactic administration of Gu-4 (1.2728 mg/kg) to rats by intraperitoneal injection every 2 days from the first day of collagen immunization significantly decreased the incidence of CIA, diminished the mean paw volume increase, and reduced the number of swollen paws. Second, administration of Gu-4 (1.2728 mg/kg) to rats at early-onset stage of CIA prevented the progression of the pathological process of RA, accelerated the remission of paw edema, and declined the arthritis score; after 5 weeks treatment, X-ray and histological examinations were carried out, the ankle joint of hind limb of Gu-4 treated CIA rats exhibited slighter bone erosion and much less inflammatory cell infiltration compared to those of saline treated animals; furthermore, Gu-4 remarkably attenuated the production of rheumatoid factor (RF) in the serum of CIA rats as determined by ELISA. Moreover, we performed *in vitro* lymphocyte proliferation assay and found that Gu-4 significantly inhibited the proliferation of splenic lymphocytes isolated from CIA rats in a dose-dependent manner. Our results suggest that Gu-4 can effectively ameliorate CIA and might be an alternative option for the treatment of RA.

Keywords Collagen-induced arthritis · Gu-4 · Rheumatoid arthritis · Rheumatoid factor · Lymphocyte proliferation

Rheumatoid arthritis (RA) is a chronic, systemic inflammatory disorder that may affect many tissues and organs, but principally attacks synovial joints [1]. The affected joints display hyperplasia of the synovia with increased synovial fluid volume, large cellular infiltrates in the synovial and periarticular regions, complement deposition, high levels of proinflammatory cytokine expression, and eventual erosion and remodeling of cartilage and bone of the joint [2]. Although the aetiology of RA is not fully understood, autoimmunity plays a pivotal role in both its chronicity and progression [3].

Our current knowledge about the fundamental mechanisms underpinning RA comes from extensive studies in rodent models [4, 5]. In collagen-induced arthritis (CIA) in mice, for example, the autoimmune reaction mediated by immune complex formation shares many similarities with human RA, indicating that anti-collagen antibody deposition in the joints triggers the complete range of arthritic symptoms [6–8].

Jie Fan, Huiting Zhou and Qing Li contributed equally to this paper.

J. Fan · H. Zhou · S. Wang · H. Wang · Y. Zhang · Y. Guo · Z. Zhao (✉)
Jiangsu Province Key Laboratory for Molecular and Medical Biotechnology, College of Life Science,
Nanjing Normal University,
Nanjing 210046, China
e-mail: zhaozhihui_1964@yahoo.com.cn

Q. Li · Z. Li (✉)
State Key Laboratory of Natural and Biomimetic Drugs,
Department of Chemical Biology,
School of Pharmaceutical Sciences, Peking University,
Beijing 100083, China
e-mail: zjli@bjmu.edu.cn

At the site that immune complex deposits, the disturbance of tissue homeostasis initiated by immune complexes is recognized either by professional tissue-resident sentinel cells, such as macrophages and mast cells, or by stromal cells, these sentinel cells release a series of proinflammatory mediators and neutrophil-active chemoattractants [9–12]. Although the exact chemoattractant responsible for the recruitment of neutrophils into peripheral tissues is unclear, neutrophils are considered to be the first cells that infiltrate into the inflamed joint of RA. Moreover, the recruited neutrophils are more active transcriptionally and translationally than their counterparts in the blood, and they release many mediators such as chemokines, cytokines and lipid mediators to orchestrate further inflammatory reactions. Many mediators released by neutrophils themselves are neutrophil chemoattractants [13]. Therefore, neutrophils might recruit other neutrophils. In immune-complex induced arthritis, the recruitment of neutrophils by neutrophils into the joint is apparently crucial for the development of arthritis [14].

During the journey of extravasation, neutrophils engage in a sequence of physical interactions with endothelial cells, referred to as the leukocyte adhesion cascade. In recent years, the leukocyte adhesion cascade has been thought to consist of several distinct steps [15]: slow rolling, adhesion strengthening, intraluminal crawling, activation, and arrest, followed by diapedesis, each requiring distinct molecular mechanisms. The first step, rolling, is mediated predominantly by selectins, while the β2-integrins lymphocyte function-associated antigen (LFA)-1 (αLβ2-integrin, CD11a/CD18) and macrophage-1 antigen (Mac-1) (αMβ2-integrin, CD11b/CD18) mediate adhesion and the subsequent intraluminal crawling of the neutrophils. Transmigration of neutrophils *via* the paracellular route is mediated by integrins, junction adhesion molecules, and other adhesion molecules, and is thought to be the predominant route for neutrophil diapedesis [15, 16]. As the above sequential cascade must be run through entirely, it provides multiple points for intervening therapeutically to attenuate neutrophil recruitment into tissues, and thereby attenuating the development of full-blown arthritis.

In addition to neutrophils, a body of evidence suggests that B cells and T cells are essential to RA. It has been shown that B cells might play multiple roles in the development and maintenance of RA, including the secretion of pathogenic antibodies and cytokines (both inflammatory and inhibitory), and/or the activation of T cells [17–19].

In recent years, patients with RA have benefited a lot from newly developed biological agents, especially enbrel (inhibitors of tumour necrosis factor), rituximab (anti-CD20), abatacept (cytotoxic T-lymphocyte antigen 4 immunoglobulin), and tocilizumab (anti-interleukin 6 receptor) [20]. However, even with the treatment of these drugs, the frequency and degree of responses of RA patients are restricted, which points to multiplicity and redundancy of events leading to rheumatoid arthritis and indicates the need to search for further therapies and treatment principles to increase response rates and to achieve high frequencies of remission or even cure in rheumatoid arthritis.

Previously, we have reported that Gu-4, a lactose derivative (N-[2-(1,3-dilactosyl)-propanyl]-2-amino-pentandiamide), exerts a therapeutic effect on severe burn rats by targeting CD11b, the α subunit of αMβ2-integrin [21]. Further investigations revealed that Gu-4 blocked the adhesion and transendothelial migration of granulocytes by inhibiting the affinity and avidity modulation of CD11b [22]. In this study, we assessed the effect of Gu-4 on a CIA model of rat and found that Gu-4 greatly attenuated the joint damages of the animals.

Materials and methods

Animals

Female Wistar rats (160–180 g body weight) were obtained from Shanghai Experimental Animal Center. Rats were housed in groups under standard conditions with a 12 h light–dark cycle and freely accessed to water and food. Laboratory animal handling and experimental procedures were performed in accordance with the requirements of Provisions and General Recommendation of Chinese Experimental Animals Administration Legislation, which were approved by Science and Technology Department of Jiangsu Province.

Preparation of Gu-4

Gu-4 was prepared in our laboratory, and the structure of which was confirmed by nuclear magnetic resonance (NMR), mass-spectrometry (MS), and by elemental analysis [23]. For more detailed information about the structure of Gu-4, please see ref. [21].

Induction of collagen-induced arthritis

Rats were immunized with 100 μg of Bovine Type II Collagen (Chondrex, Inc., USA) emulsified in 100 μl of Incomplete Freund's Adjuvant (IFA) (Chondrex, Inc., USA). Injections were given subcutaneously at 1–2 cm above the base of the tail. After 7 days, two more injections of Type II Collagen in IFA were administered at two points in the back region to booster the first immunization [5]. At both time points, rats injected subcutaneously the same amount of 0.05 M acetic acid were assigned to normal control group.

Prophylactic Gu-4 treatment

50 rats were randomly allocated into five groups as described for each experiment ($n=10$ per group). (i) Normal control group. The animals were subjected to control immunization and administered with saline (5 ml/kg, intraperitoneally (*i.p.*)). (ii) Saline group. The animals were subjected to collagen immunization and administered with saline (5 ml/kg, *i.p.*). (iii) Lactose group. The animals were subjected to collagen immunization and administered with lactose (Sigma, 800 nmol/kg, *i.p.*). (iv) Gu-4 group. The animals were subjected to collagen immunization and administered with Gu-4 (1.2728 mg/kg, *i.p.*). (v) Enbrel group. The animals were subjected to collagen immunization and administered with Enbrel (Shanghai CP Guojian Pharmaceutical Co., Ltd., 0.083 mg/kg, subcutaneously (*s.c.*)). All drugs were dissolved in sterile saline before use. Animals began to receive one dose of injection immediately after the first inoculation, and this was repeated every 2 days for 3 weeks. In this experimental setting, the biological activity of Gu-4 on arthritis was assessed by evaluating the following aspects, the incidence of CIA, the change of paw volume, and the number of swollen paws.

Gu-4 therapy after onset of RA

To examine the therapeutic effects of Gu-4 on rats with ascertained clinical signs, collagen immunized animals that have been diagnosed with early-onset arthritis (arthritis score≥1) were selected. And those rats with similar arthritis score were allocated to different groups, namely, saline group, lactose group, Gu-4 group, and Enbrel group ($n=10$ per group). The corresponding treatments (similar to prophylactic Gu-4 treatment) were initiated after the completion of group assignment and continued for 6 weeks. In this experimental setting, the therapeutic effects of Gu-4 on arthritis were assessed by evaluating the following aspects, namely, arthritis score, paw volume change, the number of swollen paws, histology, X-ray examination, serum rheumatoid factor (RF) measurement and lymphocyte proliferation assay.

Clinical assessment of arthritis

The severity of arthritis in all rats was assessed by three independent observers. (i) Arthritis score: for each paw, swelling was evaluated by using a well-established score system [24]: 0, no swelling; 1, mild, but definite redness and swelling of ankle or wrist joints; 2, moderate redness and swelling of ankle or wrist joints; 3, severe redness and swelling of the entire paw including all digits; 4, maximally inflamed limb with involvement of multiple joints. The sum of the paw swelling score for each paw was defined as total arthritis score (0–4 per paw). In a given group, arthritis score was defined as the mean of total arthritis score of all rats within the group. (ii) Incidence rate: for a given group at an indicated time point, incidence rate was defined as the percentage of rats with onset diagnosis of arthritis to total rats. The establishment of the onset diagnosis of arthritis was based on the presence of swelling paw (at least one paw) with an arthritis score≥1. (iii) Number of swollen paws: this index was defined as the sum of paws with an arthritis score≥1 within a given group.

Measurement of paw volume

Paw volume was measured by means of a volume displacement method using a Plethysmometer (YLS-7B, YiYan Science & Technology Development Co., Ltd., Jinan, China). The repeated measurements were conducted by the same person. Paw volume was measured immediately after the first inoculation of collagen and at later indicated time points. Paw edema for a given group was expressed as mean paw volume or as mean increase in paw volume relative to the basal values.

X-ray examination

For rats subjected to different treatments after onset of RA, the X-ray examination was carried out using a medical diagnostic X-ray machine (Beijing Wandong Medical Equipment Co., Ltd., HF50-R32, Beijing, China). The rats were anesthetized with pentobarbital sodium (15 mg/kg, *i.p.*) (Sinopharm Chemical Reagent Co., Ltd., Shanghai, China) and were faced down. The X-ray parameters were 43.0 kv and 1.60 mAS.

Detection of serum RF by ELISA

Withdrawal of 100 μL blood from the orbital veniplex of rat was carried out under ether anaesthesia at indicated time point. Leave the sample for 1 h at room temperature, and then aspirate the serum carefully. Centrifuge the serum at 4000 rpm for 20 min at 4 °C, remove the supernatant to a clean tube and stored at −70 °C. The level of RF in serum aliquots were measured by using Rat RF Elisa Kit (Lot 201110, R&D Systems, Minneapolis, MN). Samples and standards were prepared following the manufacturer's instructions and analyzed on a fluorescence plate reader (Bio-Tek SynergyII).

Histological examination

In the experimental setting of Gu-4 therapy after onset of RA, rats were sacrificed at the fifth week after the initiation of treatment with Gu-4. The hind limbs of rats were fixed in

10 % formalin and decalcified by immersing in bone decalcifier solution (Decal Chemical, Congers, NY) for 2 days with gentle rocking and daily replacement of the solution, then embedded in paraffin. Tissue sections (4 μm) were stained with hematoxylin and eosin (H&E) and observed under light microscope. The extent of inflammation, bone erosion and cartilage damage were evaluated by two investigators.

Lymphocyte proliferation assay

Spleens were removed aseptically from normal rats and CIA rats in model group when they were sacrificed and cell suspension was prepared from the spleen of each rat. Spleen cells were cultured in a 96-well plate (5×10^5 cells per well) containing RPMI1640 (GIBCO) supplemented with 15 % (v/v) fetal bovine serum for 48 h at 37 °C in a humidified 5 % CO_2 incubator. Lymphocyte proliferation was determined by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay [25].

Statistical analysis

All data were presented as mean ± S.D. One-way ANOVA was used for the statistical analysis by Statistic Package for Social Science Version 13.0 (SPSS Inc. Chicago, IL, USA) software. p values less than 0.05 were considered significant.

Results

Prophylactic Gu-4 treatment prevented the development of CIA

To determine the anti-arthritic effect of Gu-4, the mean increase of paw volume was determined at 1, 2, 3, 4, 5 and 6 weeks, repectively, after the first immunization of bovine type II collagen, the incidence rate of RA and the number of swollen paws were assessed at 7 d after the booster injection of bovine type II collagen. In saline and lactose treated group, at 2 weeks after injection, collagen elicited apparent paw edema in rats, and the swelling became more severe over the following 2 weeks. The administration of Gu-4 significantly reduced the occurrence of paw swelling and attenuated the development of collagen-induced arthritis (Fig. 1). Notably, Gu-4 showed comparable therapeutic effects as Enbrel (Fig. 1c).

Gu-4 inhibited the progress of joint inflammation and bone destruction in CIA rats

In order to recapitulate the process of rheumatoid arthritis in clinical practice, we further observed the therapeutic effects of Gu-4 on rats after the onset of CIA. During 5 weeks treatment, mean paw volume, arthritis score and the number of swollen paw were dynamically assessed. At the beginning of the treatment, as shown in Fig. 2, the three measured indexes in rats of all groups were similar, significant differences among different groups appeared as the treatments continued. For animals in saline treated group, the mean paw volume of which reached a maximum at about 11 d after the initiation of treatment and declined gradually thereafter, however, the arthritis score and the number of swollen paws in this group kept at high level during the whole process of treatment. The change patterns of the three measured indexes in lactose treated rats were similar to those in saline group, and there were no significant differences between these two groups at most of the check points. For rats in Gu-4 treated group, as compared to those in saline treated group, the progress of edema in rat paws was significantly inhibited, and the arthritis score and the number of swollen paws were markedly decreased.

At the end of 5 weeks treatments, X-ray and histological examinations were carried out to further assess the therapeutic effects of Gu-4 on CIA rats. For saline and lactose treated CIA rats, as shown in Fig. 3, the joint space was narrow and the joint structure was broken, and there were apparent synovial hyperplasia and inflammatory cell infiltration in the affected joints. For Gu-4 and Enbrel treated groups, the destruction level and inflammation degree were obviously slighter than those in saline treated CIA rats.

Gu-4 treatment significantly decreased the level of RF in serum of CIA rats

In rheumatoid arthritis, the serum level of RF is usually high. The presence of RF is believed to be associated with the response to anti-arthritis therapy [26]. We therefore measured the serum level of RF in rats that received 5 weeks treatment after the onset of CIA by ELISA. As shown in Fig. 4, serum RF level in saline or lactose treated CIA rats was significantly increased compared to that in normal control rats. Gu-4 treatment successfully attenuated the increase of serum RF in CIA rats, predicting a good therapeutic effect of Gu-4 and an ideal outcome, as evidenced by X-ray and histological examinations.

Gu-4 inhibited the proliferation of splenic lymphocytes

In order to determine whether the proliferation of spleen lymphocytes would be affected by Gu-4 treatment, spleen cells separated from both the normal and the CIA rats were cultured *in vitro* for 48 h. In comparison, splenic lymphocytes isolated from CIA rats exhibited much higher viability than those from normal rats as determined by MTT assay. Importantly, Gu-4 treatment significantly inhibited the

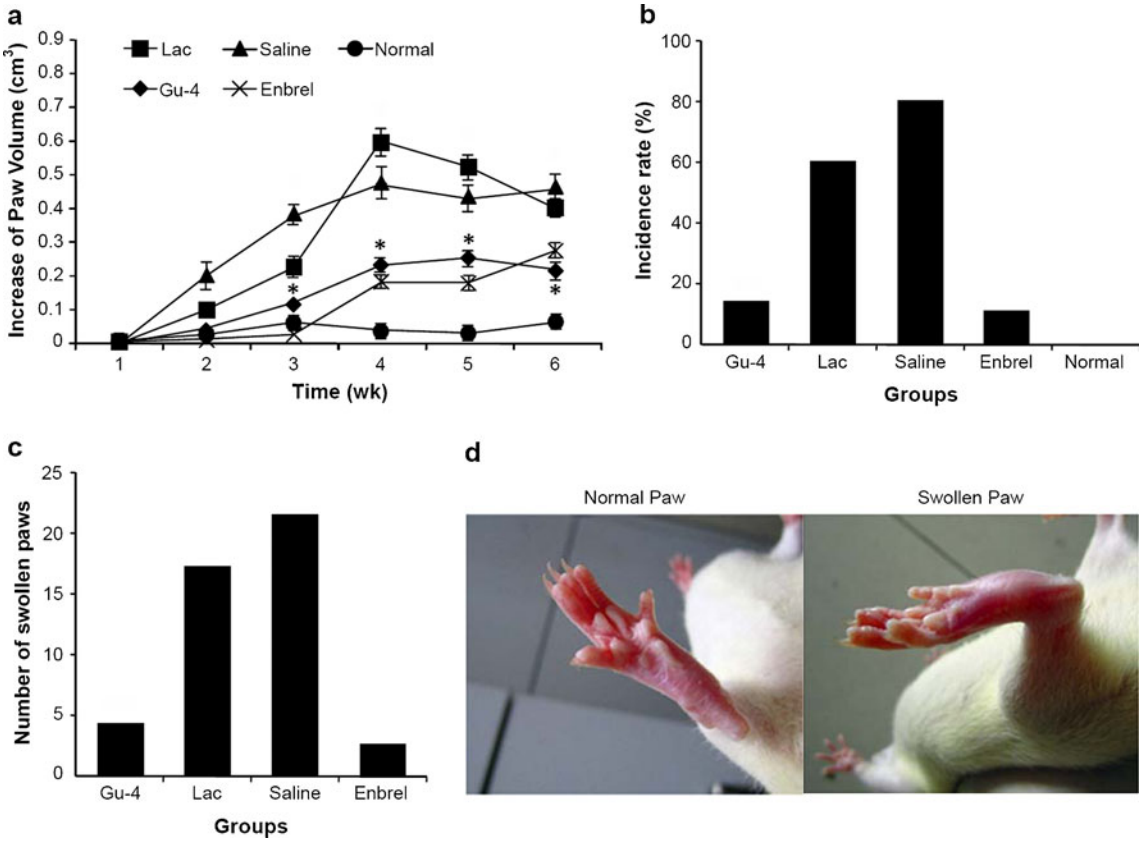

Fig. 1 Prophylactic Gu-4 treatment prevented the onset of CIA in rats. Wistar rats were treated with saline, Gu-4 (1.2728 mg/kg), lactose (Lac, 0.27384 mg/kg) or Enbrel (0.083 mg/kg) every 2 days after collagen immunization. Mean paw volume increase was dynamically measured for 6 weeks (**a**); the incidence rate of arthritis (**b**) and the number of swollen paws (**c**) were determined at 2 week after the first collagen immunization. Photographs in (**d**) illustrated a representative of swollen paws. Data in (**d**) were analyzed using one-way ANOVA and results are mean ± S.D. from 10 rats/group, * $p<0.05$, Gu-4 group compared to the saline group

viability of spleen cells isolated from CIA rats with a Gu-4 dose-dependent manner (Fig. 5), the most prominent inhitory effect appeared when the dose of Gu-4 is 40 nmol/ml. However, higher doses of Gu-4 (80 and 160 nmol/ml) achieved not stronger but slightly lower inhibitory effects as compared to that of 40 nmol/ml ($p>0.05$), further investigation is required to clarify the mechanism of this phenomenon. In addition, Enbrel also exhibited significant inhibitory effects on splenic lymphocytes isolated from CIA rats with a dose of 10 ng/ml (data not shown).

Discussion

Rheumatoid arthritis (RA) is an inflammatory autoimmune disorder that afflicts almost 1 % population of the world [27, 28]. If not adequately treated, progressive destruction of cartilage and bone can lead to substantial loss of functioning and mobility of the patients. Although many newly developed anti-arthritis drugs have greatly increased the treatment options for RA, more effective therapies are still needed to achieve complete remission of the disease [29, 30].

Neutrophils are prominent participants in the joint inflammation of human RA patients. Some previous studies have demonstrated that neutrophils play a crucial role in the initiation and progression of RA [12, 14]. The extravasation of leukocytes toward the synovium is important for the establishment of a chronic inflammatory process in RA. This multi-step process involves interactions with endothelial cells through cell adhesion molecules and complex cytokine and chemokine pathways. The heavily infiltrated neutrophils in synovial tissue not only produce pro-inflammatory cytokines but also release large amounts of destructive enzymes, such as metalloproteases, contributing to joint erosions [31]. Interestingly, it had been demonstrated that neutrophil depletion in animal models of arthritis prevented joint inflammation [32]. Thus, neutrophils constitute the major driving force of RA, and approaches to interfere with the migration and the activation of neutrophils could be alternative strategies for RA therapy.

In this study, we investigated the potential anti-arthritis role of Gu-4, a synthetic lactose derivative that we have previously identified as an antagonist against leukocyte integrin CD11b [21, 22]. Since our previous *in vivo* and *in*

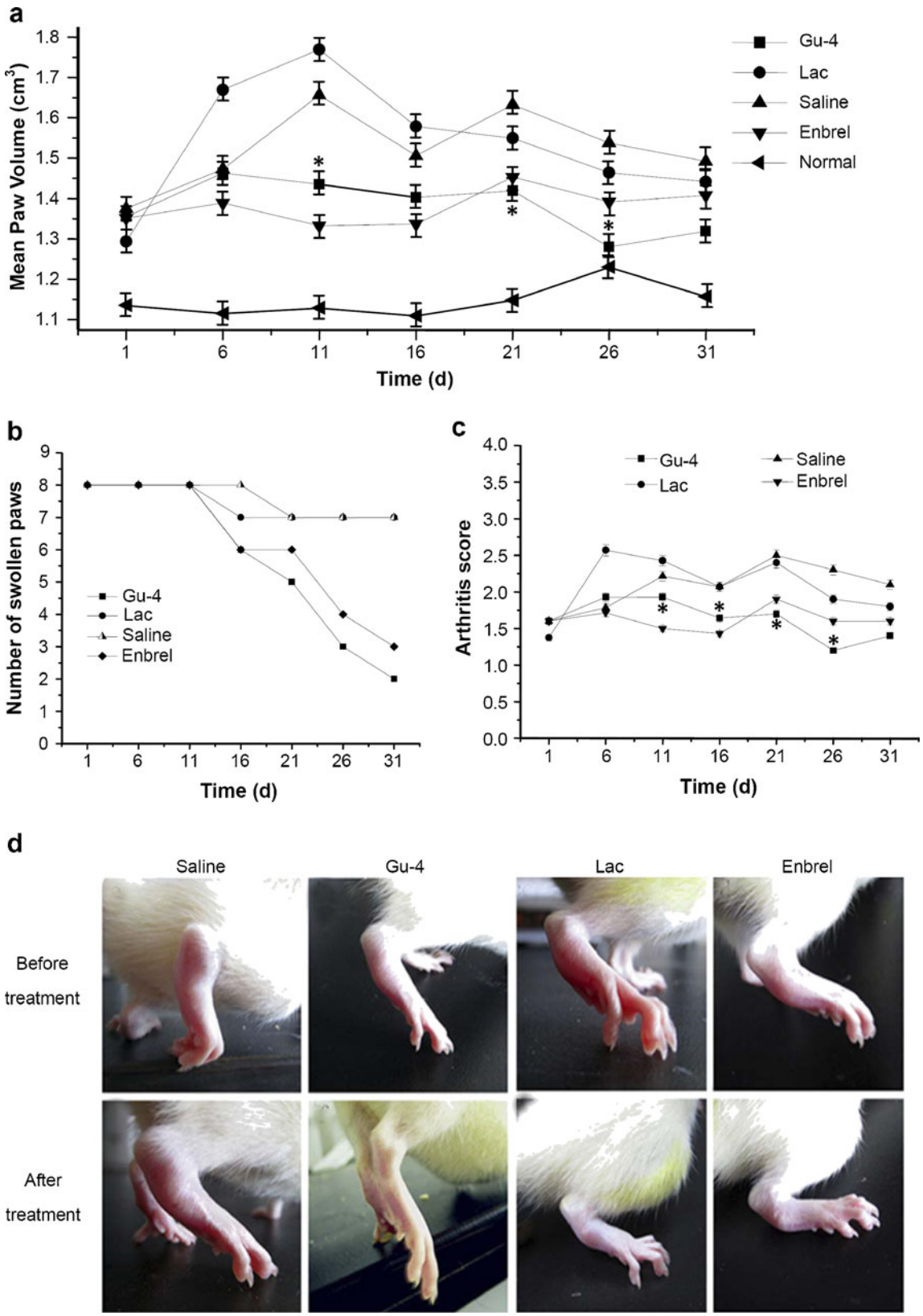

Fig. 2 Gu-4 treatment slowed down the progression of CIA in rats. Wistar rats that have been diagnosed with early-onset of CIA were treated with saline, Gu-4 (1.2728 mg/kg), lactose (Lac, 0.27384 mg/kg) or Enbrel (0.083 mg/kg) every 2 days (n=10 per group). Mean paw volume (**a**), the number of swollen paws (**b**) and arthritis score (**c**) of rats were determined at 1, 6, 11, 16, 21, 26 and 31 days respectively, after the beginning of treatment. Representative photographs in (**d**) illustrate paws of CIA rats just prior to treatment and after 5 weeks of treatment. Data in (**a**) and (**c**) are mean ± S.D. and were analyzed using one-way ANOVA. * $p<0.05$, Gu-4 group compared to the saline treated group

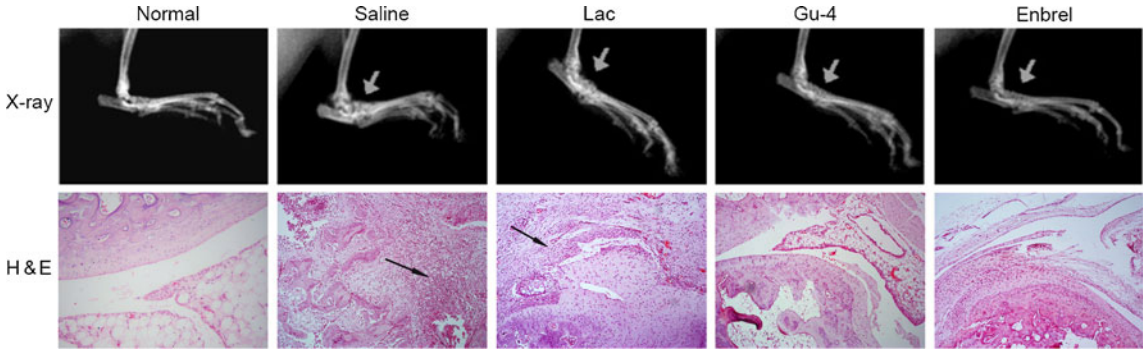

Fig. 3 Gu-4 treatment ameliorated synovial inflammation and bone destruction in CIA rats. After 5 weeks treatment of Gu-4, lactose, Enbrel or saline, the hind limbs of CIA rats from all groups were examined by x-ray radiograph and H&E staining of the ankle joint. Upper row: representative radiograph from each group of 10 rats. Lower row: representative H&E histology staining photograph from each group of 10 rats, original magnification, ×100

vitro studies have demonstrated that Gu-4 has the ability to inhibit the adhesion and migration of granulocytes, especially polymorphonuclear leukocytes, we therefore hypothesized that RA might be an indication for Gu-4. Here, prophylactic treatment with Gu-4 effectively prevented the occurrence of collagen induced arthritis in rats as evaluated by paw volume increase, RA incidence rate and the number of swollen paws, suggesting the potential value of Gu-4 as a preventing agent for RA. Moreover, administration of Gu-4 also exhibited remarkable positive effects on the onset of CIA, and the significantly reduced inflammatory cell ingress in synovial tissue after the Gu-4 treatment indicated that inhibition of leukocyte adhesion may be one of the underlying mechanisms for Gu-4 anti-arthritis activity.

In addition to neutrophils, several lines of evidence indicate that lymphocytes, *i.e.*, T cells and B cells, play essential roles in the pathogenesis of RA. T cells are one of the major cells in inflamed synovial membranes in RA [33]. $CD4^+$ T cells are not only involved in bone destruction but also in the production of inflammatory cytokines such as TNFα and IL-1. The most persuading evidence for the role of B cells in RA came from an experimental study on animal models and clinical trials, for B cell-deficient mice do not develop type II collagen induced arthritis [34], and selective B cell depletion using rituximab (an anti-CD20 monoclonal antibody) significantly improved the clinical course of RA patients [35]. In addition, activation of B cells plays a crucial role in the synthesis of rheumatoid factor (RF), a kind of humoral component often occurring in a large proportion of RA patients as well as in experimental models [36]. It is speculated that the presence and persistence of RF implies the survival and proliferation of autoreactive B cell clones in RA patients under a continuous stimulation. RF thus represents a very valuable tool in both diagnostic and prognostic terms [26]. In the present study, we showed that Gu-4 significantly inhibited the proliferation of splenic

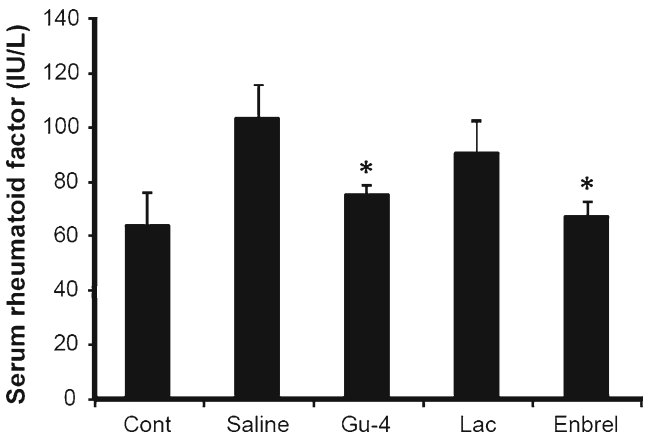

Fig. 4 Gu-4 treatment reduced serum RF level in CIA rats. Blood samples were taken from CIA rats, which had been treated with Gu-4, lactose, Enbrel or saline for 5 weeks, and serum RF level was determined by ELISA. Gu-4 treatment significantly diminished the increase of serum RF levels in CIA rats. Results are mean ± S.D. of 5 animals. * $p<0.05$ compared to saline treated group

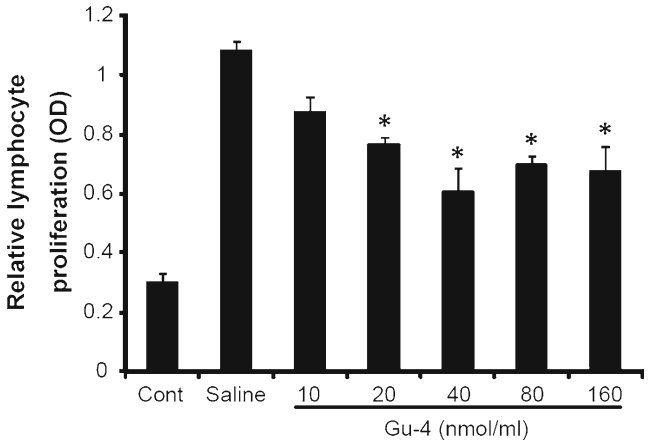

Fig. 5 Gu-4 inhibited the proliferation of lymphocytes from spleen of CIA rats. Lymphocytes were separated from spleens of CIA rats. Proliferation of lymphocytes subjected to treatments with different concentrations of Gu-4 was determined by MTT assay. Data are reported as mean ± S.D. and were analyzed using one-way ANOVA. * $p<0.05$ compared to the saline treated group

lymphocytes isolated from CIA rats, which implies that the procedures involved in the activation of lymphocytes were interfered by Gu-4. Accordingly, a relatively low level of serum RF was observed in Gu-4 treated CIA rats as examined by ELISA. This corroborated the results of *in vitro* lymphocyte proliferation assay.

In conclusion, we made a preliminary exploration on CIA rats to assess the potential therapeutic effect of lactosyl derivative Gu-4 our results demonstrated that Gu-4 could effectively prevent the occurrence of CIA, and at the early-onset stage of CIA therapy with Gu-4 can also improve the outcome of CIA rats. In addition, we also showed that Gu-4 treatment reduced the level of serum RF in CIA animals, which may contribute to the improved outcome of CIA. Further investigations on the underlying mechanisms of Gu-4 action may provide more rationale for future clinical trials.

Acknowledgments This work was financially supported by the Natural Science Foundation of China (Grant No. 2008104GZ40166), the State New Drug Innovation Plan (Grant No. 2009ZX09103-044), and a project from the Priority Academic Program Development of Jiangsu Higher Education Institutions (PAPD). The authors thank Dr. Lei Lan for her help with the revision of this manuscript.

References

1. Sweeney, S.E., Firestein, G.S.: Rheumatoid arthritis: regulation of synovial inflammation. Int. J. Biochem. Cell Biol. **36**, 372–378 (2004)
2. Brennan, F.M., Maini, R.N., Feldmann, M.: Role of pro-inflammatory cytokines in rheumatoid arthritis. Springer Semin. Immunopathol. **20**, 133–147 (1998)
3. Raptopoulou, A., Sidiropoulos, P., Katsouraki, M., Boumpas, D.T.: Anti-citrulline antibodies in the diagnosis and prognosis of rheumatoid arthritis: evolving concepts. Crit. Rev. Clin. Lab. Sci. **44**, 339–363 (2007)
4. Joe, B., Griffiths, M.M., Remmers, E.F., Wilder, R.L.: Animal models of rheumatoid arthritis and related inflammation. Curr. Rheumatol. Rep. **1**, 139–148 (1999)
5. Asquith, D.L., Miller, A.M., McInnes, I.B., Liew, F.Y.: Animal models of rheumatoid arthritis. Eur. J. Immunol. **39**, 2040–2044 (2009)
6. Myers, L.K., Rosloniec, E.F., Cremer, M.A., Kang, A.H.: Collagen-induced arthritis, an animal model of autoimmunity. Life Sci. **61**, 1861–1878 (1997)
7. Terato, K., Hasty, K.A., Reife, R.A., Cremer, M.A., Kang, A.H., *et al.*: Induction of arthritis with monoclonal antibodies to collagen. J. Immunol. **148**, 2103–2108 (1992)
8. Kleinau, S., Martinsson, P., Heyman, B.: Induction and suppression of collagen-induced arthritis is dependent on distinct Fcγ receptors. J. Exp. Med. **191**, 1611 (2000)
9. Fonseca, J.E., Cortez-Dias, N., Francisco, A., Sobral, M., Canhao, H., *et al.*: Inflammatory cell infiltrate and RANKL/OPG expression in rheumatoid synovium: comparison with other inflammatory arthropathies and correlation with outcome. Clin. Exp. Rheumatol. **23**, 185–192 (2005)
10. Kim, H.J., Krenn, V., Steinhauser, G., Berek, C.: Plasma cell development in synovial germinal centers in patients with rheumatoid and reactive arthritis. J. Immunol. **162**, 3053–3062 (1999)
11. Pillinger, M.H., Abramson, S.B.: The neutrophil in rheumatoid arthritis. Rheum. Dis. Clin. North Am. **21**, 691–714 (1995)
12. Wipke, B.T., Allen, P.M.: Essential role of neutrophils in the initiation and progression of a murine model of rheumatoid arthritis. J. Immunol. **167**, 1601–1608 (2001)
13. McDonald, B., Pittman, K., Menezes, G.B., Hirota, S.A., Slaba, I., *et al.*: Intravascular danger signals guide neutrophils to sites of sterile inflammation. Sci. STKE **330**, 362 (2010)
14. Sadik, C.D., Kim, N.D., Luster, A.D.: Neutrophils cascading their way to inflammation. Trends Immunol. **32**, 452–460 (2011)
15. Ley, K., Laudanna, C., Cybulsky, M.I., Nourshargh, S.: Getting to the site of inflammation: the leukocyte adhesion cascade updated. Nat. Rev. Immunol. **7**, 678–689 (2007)
16. Williams, M.R., Azcutia, V., Newton, G., Alcaide, P., Luscinskas, F.W.: Emerging mechanisms of neutrophil recruitment across endothelium. Trends Immunol. **32**, 461–469 (2011)
17. Kong, Y.Y., Feige, U., Sarosi, I., Bolon, B., Tafuri, A., *et al.*: Activated T cells regulate bone loss and joint destruction in adjuvant arthritis through osteoprotegerin ligand. Nature **402**, 304–309 (1999)
18. De Vita, S., Zaja, F., Sacco, S., De Candia, A., Fanin, R., *et al.*: Efficacy of selective B cell blockade in the treatment of rheumatoid arthritis: evidence for a pathogenetic role of B cells. Arthritis Rheum. **46**, 2029–2033 (2002)
19. Nakken, B., Munthe, L.A., Konttinen, Y.T., Sandberg, A.K., Szekanecz, Z., *et al.*: B-cells and their targeting in rheumatoid arthritis–current concepts and future perspectives. Autoimmun. Rev. **11**, 28–34 (2011)
20. Tanaka, Y.: Recent progress in clinical trials for rheumatic diseases. Nihon Rinsho **67**, 619–625 (2009)
21. Zhao, Z., Li, Q., Hu, J., Li, Z., Liu, J., *et al.*: Lactosyl derivatives function in a rat model of severe burn shock by acting as antagonists against CD11b of integrin on leukocytes. Glycoconj. J. **26**, 173–188 (2009)
22. Yan, T., Li, Q., Zhou, H., Zhao, Y., Yu, S., *et al.*: Gu-4 suppresses affinity and avidity modulation of CD11b and improves the outcome of mice with endotoxemia and sepsis. PLoS One **7**, e30110 (2012)
23. Li, H., Li, Q., Cai, M.S., Li, Z.J.: Synthesis of galactosyl and lactosyl derivatives as potential anti-metastasis compounds. Carbohydr. Res. **328**, 611–615 (2000)
24. Butz, D.E., Li, G., Huebner, S.M., Cook, M.E.: A mechanistic approach to understanding conjugated linoleic acid's role in inflammation using murine models of rheumatoid arthritis. Am. J. Physiol. Regul. Integr. Comp. Physiol. **293**, R669–R676 (2007)
25. Tomita, T., Kakiuchi, Y., Tsao, P.S.: THR0921, a novel peroxisome proliferator-activated receptor gamma agonist, reduces the severity of collagen-induced arthritis. Arthritis Res. Ther. **8**, R7 (2006)
26. Nell, V.P., Machold, K.P., Stamm, T.A., Eberl, G., Heinzl, H., *et al.*: Autoantibody profiling as early diagnostic and prognostic tool for rheumatoid arthritis. Ann. Rheum. Dis. **64**, 1731–1736 (2005)
27. Gabriel, S.E.: The epidemiology of rheumatoid arthritis. Rheum. Dis. Clin. North Am. **27**, 269–281 (2001)
28. Majithia, V., Geraci, S.A.: Rheumatoid arthritis: diagnosis and management. Am. J. Med. **120**, 936–939 (2007)
29. Smolen, J.S., Steiner, G.: Therapeutic strategies for rheumatoid arthritis. Nat. Rev. Drug Discov. **2**, 473–488 (2003)
30. Firth, J.: Rheumatoid arthritis: diagnosis and multidisciplinary management. Br. J. Nurs. **20**, 1179–1180, 1182, 1184–1175 (2011)
31. Cascao, R., Rosario, H.S., Souto-Carneiro, M.M., Fonseca, J.E.: Neutrophils in rheumatoid arthritis: more than simple final effectors. Autoimmun. Rev. **9**, 531–535 (2010)
32. Cascao, R., Rosario, H.S., Fonseca, J.E.: Neutrophils: warriors and commanders in immune mediated inflammatory diseases. Acta Reumatol. Port. **34**, 313–326 (2009)

33. Ponchel, F., Morgan, A.W., Bingham, S.J., Quinn, M., Buch, M., *et al.*: Dysregulated lymphocyte proliferation and differentiation in patients with rheumatoid arthritis. Blood **100**, 4550–4556 (2002)
34. Svensson, L., Jirholt, J., Holmdahl, R., Jansson, L.: B cell-deficient mice do not develop type II collagen-induced arthritis (CIA). Clin. Exp. Immunol. **111**, 521–526 (1998)
35. Edwards, J.C., Szczepanski, L., Szechinski, J., Filipowicz-Sosnowska, A., Emery, P., *et al.*: Efficacy of B-cell-targeted therapy with rituximab in patients with rheumatoid arthritis. N. Engl. J. Med. **350**, 2572–2581 (2004)
36. Wipke, B.T., Wang, Z., Nagengast, W., Reichert, D.E., Allen, P.M.: Staging the initiation of autoantibody-induced arthritis: a critical role for immune complexes. J. Immunol. **172**, 7694–7702 (2004)