

Recovery process of arthritis induced by 6-Sulfanilamidoindazole (6SAI) in rats

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Summary. 6-Sulfanilamidoindazole (6SAI) is known to induce not only an acute arthritis but also serositis and arteritis which resemble those induced by some vasodilators in rats. In this study, the recovery process of ankle lesions was examined histopathologically for up to 12 weeks of recovery period in rats bearing arthritis induced by administration of 6SAI (500mg/kg) for 2 weeks. At 2 weeks of 6SAI-treatment, exudative synovitis and exudative/edematous peri-arthritis with marked formation of granulation tissues and periosteal reactive bone formation were noted in the ankles, but no remarkable neutrophil infiltration was detected in those lesions. The ankle swelling induced by 6SAI diminished by 4 weeks of recovery period, and the elevated plasma fibrinogen levels were normalized by 2 weeks of recovery period. Although fibrosis and newly-formed periosteal bone were still observed after 2 weeks of recovery period, no inflammatory lesion was detected at that point. At 4 or 12 weeks of recovery periods, the ankles showed an almost normal appearance. These results indicate that 6SAI-induced arthritis is reversible in nature and does not develop into chronic phase.

Key words: Rat, 6-Sulfanilamidoindazole, Arthritis, Histopathology, Recovery process

Introduction

6-Sulfanilamidoindazole (6SAI) is known to induce an acute, self-limiting arthritis and peri-arthritis in rats (Mielens and Rozitis, 1964; Miller et al., 1970). Although 6SAI-induced arthritis has been used for assessing anti-inflammatory agents, the arthritis in this model was not suppressed by immunosuppressive agents (Sigg et al., 1967), protease inhibitor (Hirschelmann et al., 1980), or superoxide dismutase (Hirschelmann and Schade, 1986). Thus, this arthritis model is thought to be a special type different from other immunologically-

induced arthritis such as adjuvant arthritis and type-II collagen-induced arthritis in rats. However, the pathogenesis of this arthritis model is still obscure.

Our previous work on the pathology of 6SAI-treated rats revealed that this agent induced not only ankle inflammation but also serositis and arteritis, and the arterial changes were similar to those induced by several vasodilators (Ohmachi et al., 1998). The articular changes induced by 6SAI were characterized by exudative synovitis and periarticular inflammation without bone/cartilage destruction in a 4-week treatment study, and this indicates that this model is also different from so-called rodent rheumatoid arthritis models (Ohmachi et al., 1998). As mentioned above, 6SAI-induced arthritis is said to be an acute self-limiting one. However, whether the arthritis could recover or not has not yet been fully examined.

In the present study, to clarify this point, we examined the recovery process of the ankle lesions pathologically for up to 12 weeks of recovery period in rats treated with 6SAI for 2 weeks.

Materials and methods

Chemicals

6SAI was obtained from Aldrich Japan, Inc (Tokyo). 6SAI was suspended in 1.0% carboxymethylcellulose before daily use.

Animals

Nine-week-old male Crj:CD(SD) IGS rats (Charles River Japan Inc., Tsukuba) were used. They were kept in an animal room controlled at a temperature of 23±1 °C and a relative humidity of 55±5% with a 12-hour light-dark cycle, and fed standard commercial laboratory diet (CE-2, Oriental Yeast Co. Ltd, Tokyo) and tap water *ad libitum*.

Treatments

The animals were assigned to two groups and

administered with 6SAI orally at the dose level of 0 (control, n=12) and 500 mg/kg/day (n=30) for 2 weeks, respectively. The animals were observed daily and body weights were recorded twice a week. The condition of the ankle joint was grossly evaluated by "Arthritis score" as previously described (0 = no changes; 1 = slight swelling of a part of the ankle; 2 = swelling of the whole ankle; 3 = involvement of metatarsal joints; 4 = involvement of toes; 5 = severe swelling and redness of the entire paw) (Ohmachi et al., 1998).

The nineteen animals which gave the arthritis score over '3' during the 6SAI-treatment period were selected at 2 weeks after the first dosing (2W). Four to six rats from these selected animals and 3 animals of the control group were killed by exsanguination under ether anesthesia at 0, 2, 4, and 12 weeks of recovery period (R2W, R4W and R12W), respectively.

Hematology and blood biochemistry

Blood samples obtained from each animal were used for hematological and serum biochemical analyses.

Histopathology

The amputated ankles were fixed in 10% neutral-buffered formalin. The ankles of each animal were decalcified with 10% formic acid. Paraffin sections at 5 μ m were stained with hematoxylin and eosin (HE). For evaluating the proliferative activity of various cells in

articular lesions, immunohistochemical staining for proliferating cell nuclear antigen (PCNA) was carried out on paraffin sections by the avidin-biotin-peroxidase complex method using Simple stain kit (Nichirei, Tokyo). Mouse anti-PCNA antibody (clone PC 10; Dako, USA) was used as the primary antibody. The sections were visualized by DAB reaction and then counterstained with hematoxylin.

Results

Clinical findings

In the 6SAI-treated group, body weight significantly decreased at the end of the treatment period. Ankle swelling and redness developed almost bilaterally in the 6SAI-treated rats from 3 days (3D) after the first dosing. The degree of the changes peaked at 10 or 11D, and then began to diminish (Fig. 1). The gross changes in ankles mostly disappeared by R4W.

Hematological and blood biochemical findings

In the 6SAI-treated rats, fibrinogen values which are known to be closely related to the intensity of ankle inflammation in this model (Ohmachi et al., 1998) markedly increased at 2W, but the values returned to normal levels at R2W (Table 1). Mild anemia was noted at 2W, R2W and R4W. Serum albumin levels significantly decreased at the end of dosing period,

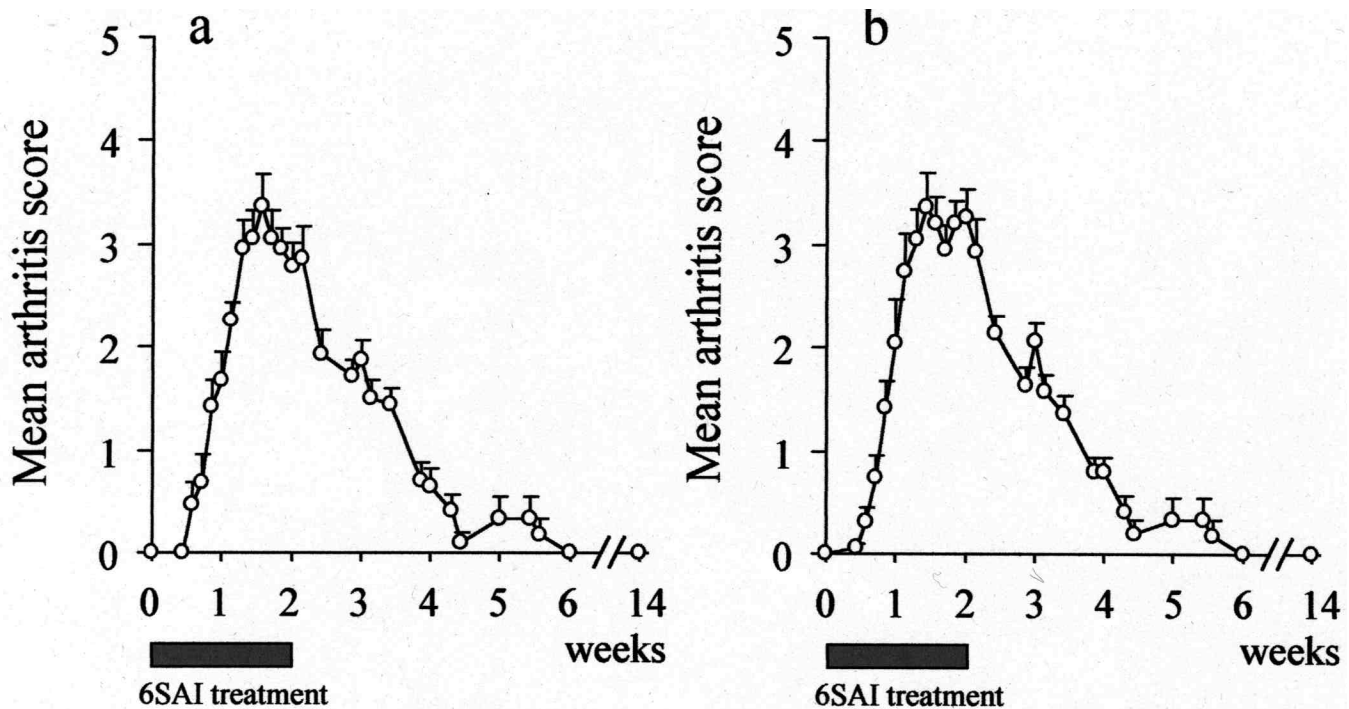


Fig. 1. Changes in mean arthritis score of right (a) and left (b) hindpaws of rats treated orally with 6SAI for 2 weeks and followed by 0 to 12 weeks recovery period. Each point represents mean \pm SE.

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although total protein levels did not show significant changes. There were no abnormal changes in the control group.

Histopathological findings

At 2W, moderate to severe exudative synovitis and

exudative/edematous periartthritis were observed in the 6SAI-treated rats. The changes were characterized by hypertrophy and desquamation of synovial lining cells, infiltration of numerous mononuclear cells and a small number of polymorphonuclear leukocytes in the synovium with focal hemorrhage and fibrin deposition, and fibrin exudation into the synovial lumen (Fig. 2a-c).

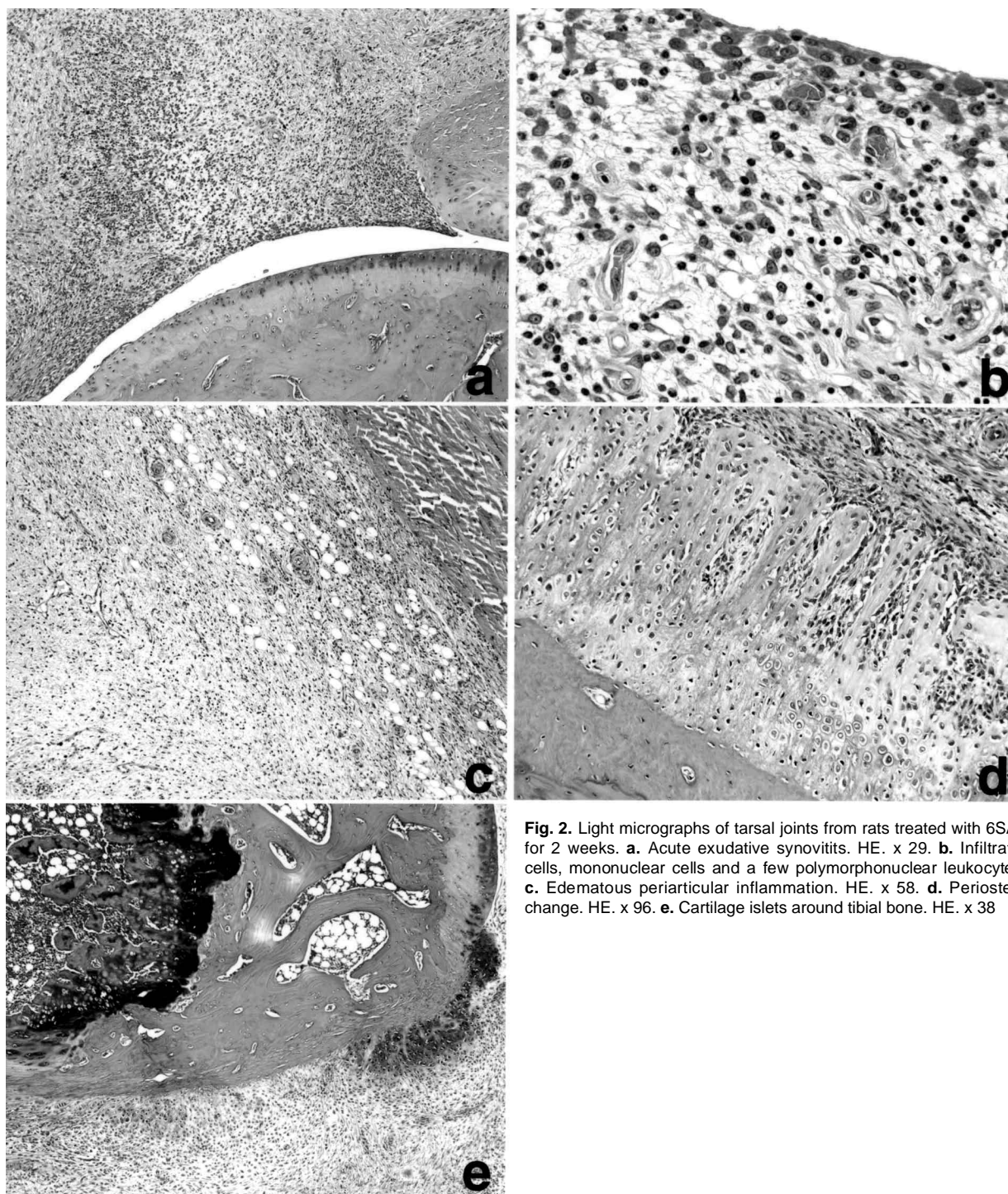


Fig. 2. Light micrographs of tarsal joints from rats treated with 6SAI (500 mg/kg) for 2 weeks. **a.** Acute exudative synovitis. HE. x 29. **b.** Infiltration of stellate cells, mononuclear cells and a few polymorphonuclear leukocytes. HE. x 290. **c.** Edematous periarticular inflammation. HE. x 58. **d.** Periosteal osteogenic change. HE. x 96. **e.** Cartilage islets around tibial bone. HE. x 38

The mononuclear cells infiltrated in the lesions were mainly composed of large stellate cells (macrophage- and fibroblast-like cells) and monocyte-like round cells,

and also included a small number of lymphocyte-like small round cells. In the lesions, marked proliferation of stellate cells and neovascularization were observed. In

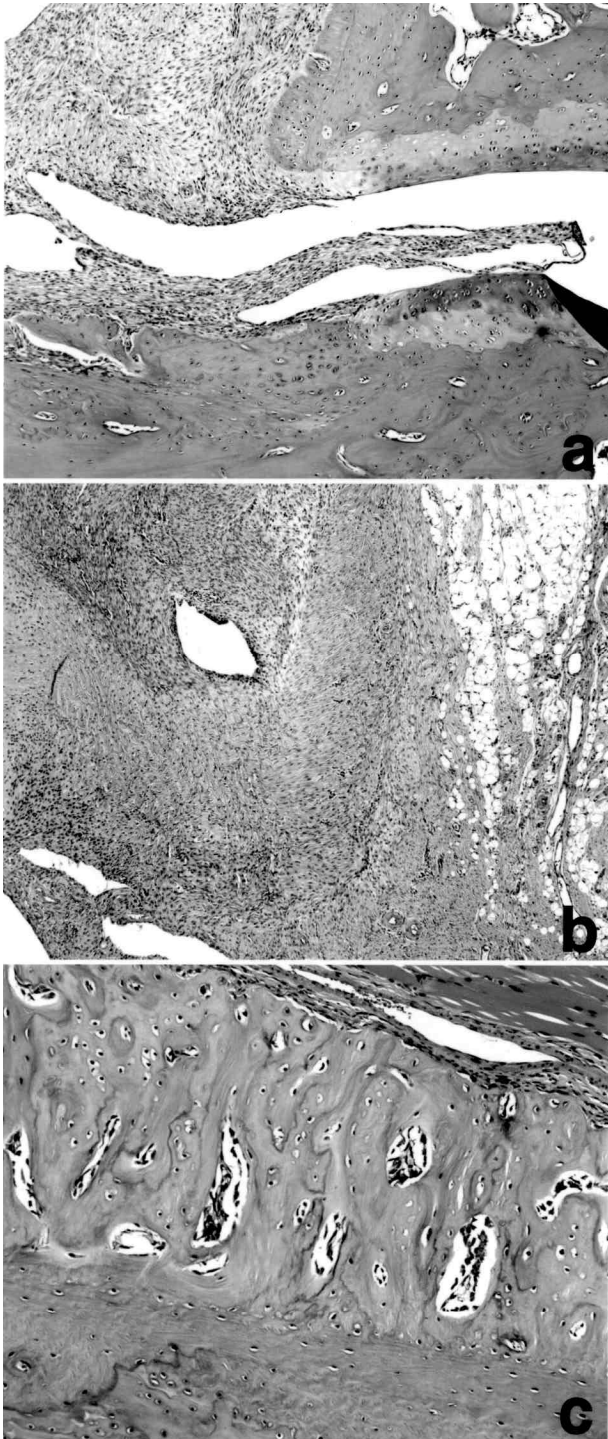


Fig. 3. Light micrographs of tarsal joints from rats treated with 6SAI for 2 weeks and followed by 2 weeks of recovery period. **a.** Fibrosis of synovium. HE. x 58. **b.** Fibrosis of periarticular connective tissues. HE. x 38. **c.** Periosteal new bone formation. HE. x 96

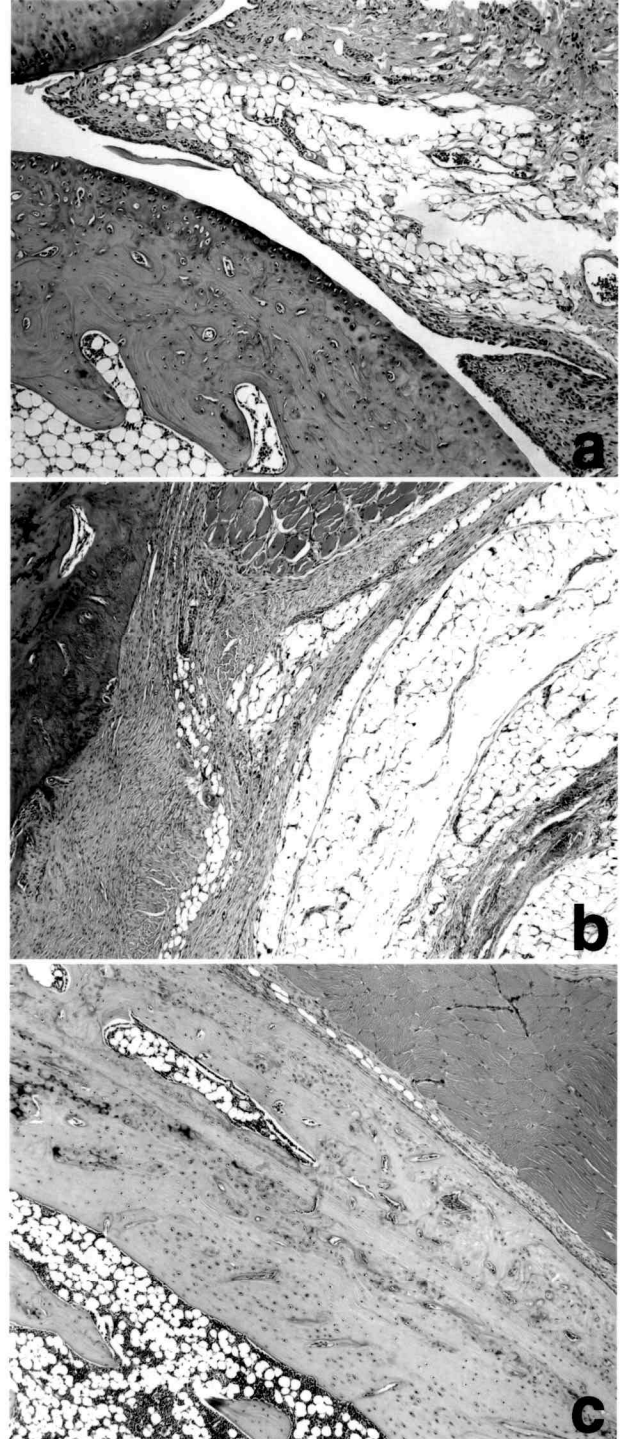


Fig. 4. Light micrographs of tarsal joints from rats treated with 6SAI for 2 weeks and followed by 4 weeks of recovery period. **a.** Focal fibrosis in synovium. HE. x 58. **b.** Focal fibrosis around bone and muscle. HE. x 38. **c.** Newly-formed bone is barely noted. HE. x 38

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addition, arteritis of small-sized arteries was found in the inflamed periarticular tissues. At the distal end of the tibia/fibura, tarsal, metatarsal and calcaneal bones showing marked periosteal inflammation, periosteal osteogenic and chondrogenic changes were noted (Fig.

2d,e). The osteogenic layer of the periosteum was thickened, being difficult to be distinguished from surrounding fibroplastic inflamed tissues. In such portion, proliferation of osteoblasts with matrix production around the newly-formed blood vessels was

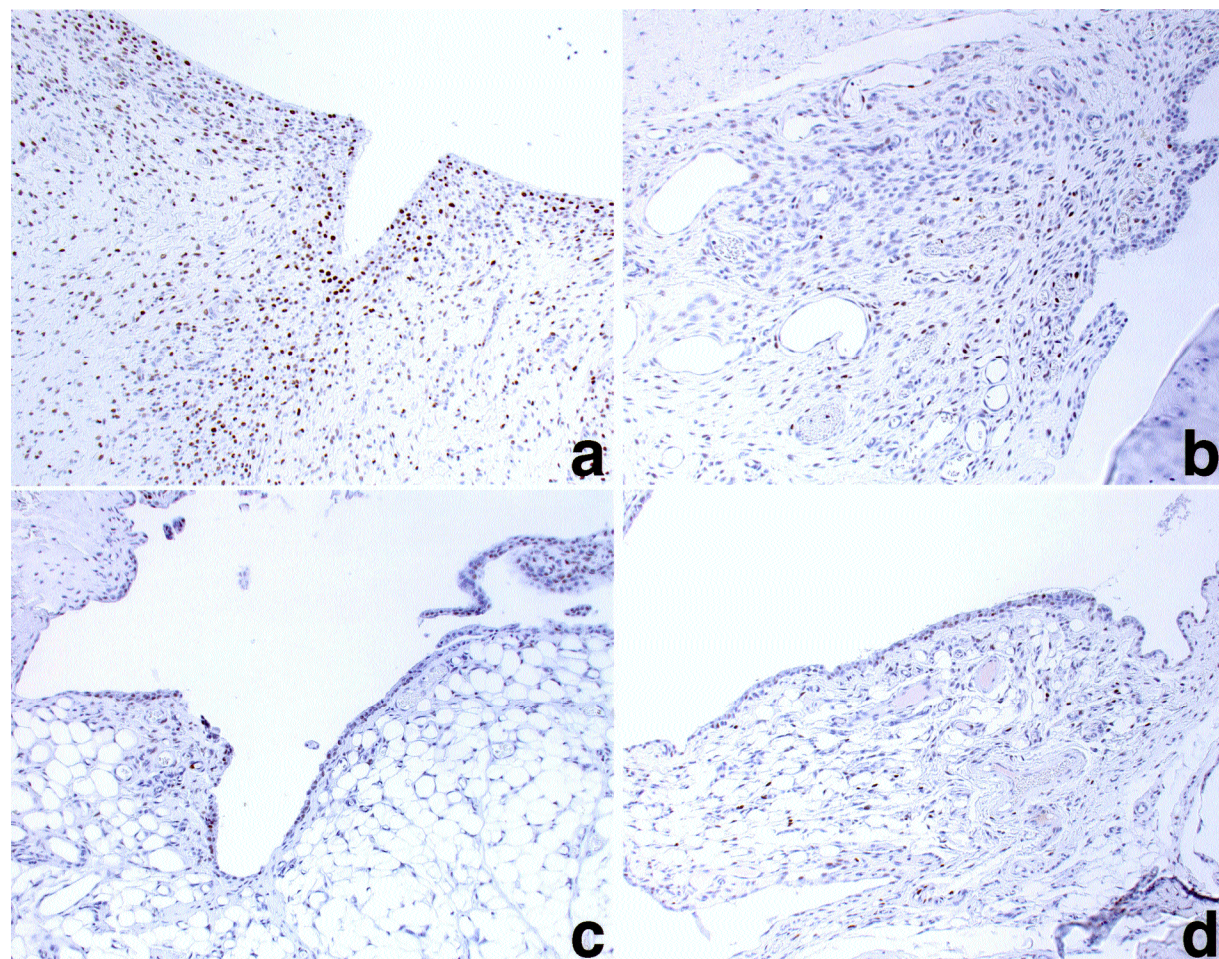


Fig. 5. PCNA immunoreactivity in synovium. **a.** Numerous PCNA-positive cells are noted in a rat treated with 6SAI for 2 weeks. x 97. **b.** Several spindle cells in the synovium are PCNA-positive in a rat treated with 6SAI for 2 weeks and followed by 2 weeks of recovery period. x 97. **c.** A few sinovial lining cells are PCNA-positive in a rat treated with 6SAI for 2 weeks and followed by 12 weeks of recovery period. x 97. **d.** A few sinovial lining cells are PCNA-positive in a rat of control group. x 97

Table 1. Hematology in rats treated with 6SAI.

GROUP	PERIOD ^a	RBC (10 ⁴ /μl)	Hb (g/dl)	Ht (%)	WBC (10 ² /μl)	Bas (%)	Eos (%)	Neu (%)	Lym (%)	Mon (%)	PLT (10 ⁴ /μl)	FIBRINOGEN (mg/dl)	TP	ALB
Control	2W	780±2 ^b	15.9±0.1	43.8±0.6	125±17	0.0±0.0	0.8±0.8	9.8±2.9	89.0±3.3	0.3±0.2	114.5±7.6	204±4	5.67±0.20	2.25±0.04
	R2W	823±16	16.2±0.3	45.2±0.8	89±9	0.0±0.0	1.3±0.7	11.3±2.6	86.5±3.1	0.8±0.3	117.4±6.9	204±9	6.04±0.25	2.24±0.05
	R4W	837±10	15.9±0.4	45.3±1.2	86±9	0.2±0.2	0.3±0.2	6.8±1.4	92.5±1.5	0.2±0.2	115.7±4.1	229±5	6.13±0.06	2.27±0.03
	R10W	973±31	15.8±0.3	44.6±1.1	99±22	0.0±0.0	0.2±0.2	11.3±1.2	87.2±0.8	1.3±0.3	118.1±6.8	216±10	6.37±0.16	2.30±0.02
6SAI	2W	762±24	15.1±0.5	40.8±1.3	124±11	0.3±0.2	0.0±0.1	11.1±3.0	86.8±3.1	1.8±0.5	107.1±7.3	587±92*	5.84±0.06	1.88±0.06**
	R2W	703±23*	14.5±0.3**	41.3±1.0*	121±21	0.0±0.0	0.1±0.1	10.1±3.6	89.3±3.6	0.6±0.1	161.9±26.3	197±4	5.66±0.10	2.23±0.03
	R4W	762±13**	15.3±0.2	44.3±0.7	94±7	0.3±0.2	0.6±0.2	10.5±0.5	88.3±0.7	0.4±0.2	118.0±4.2	219±7	5.91±0.13	2.19±0.01*
	R10W	848±10	15.6±0.2	44.0±0.7	86±10	0.2±0.1	1.0±0.3	9.3±1.3	89.1±1.3	0.5±0.2	110.0±2.7	219±7	6.29±0.12	2.27±0.05

^a: See materials and methods. ^b: mean±SE; *: p<0.05; **:p<0.01; Significantly different from controls.

seen as a trabecular pattern generally arranged perpendicular to the mature bone cortex and parallel to each other, like an endosteum (Fig. 2d). The irregular islands of cartilage were sometimes observed in the granulation tissues adjacent to the epiphyseal cartilage or near the articular cartilage (Fig. 2e).

At R2W, though the focal inflammatory cell infiltration was still observed in the synovium of the tarsal joints, marked fibrosis in the synovium and periarticular tissues was the main feature of the affected ankles (Fig. 3a,b). The newly-formed tibia, tarsal or calcaneal bones were already ossificated and the related osteogenic layers of the periosteum became thinner (Fig. 3c). The cavity in the newly-formed bone became narrower. In some cases, bone marrow formation was seen in the reactive bones and sometimes communicated with the preexisting marrow in the old bones through large-sized blood vessels. At the surface of the reactive bones, a few osteoclasts were sometimes noted. On the other hand, reactive cartilage formation which was seen around the inflamed bones at 2W disappeared.

At R4 and R12W, except for mild fibrous thickening of peritenon, the ankles showed an almost normal appearance (Fig. 4a,b), and the bone cortex and the periosteum in the ankles showed almost normal appearance at R4 and 12W (Fig. 4c).

PCNA immunoreactivity was frequently observed in the inflamed synovium and periarticular connective tissues, and also in the thickened osteogenic layers around the bones adjacent to the inflamed tissue at 2W (Fig. 5a). In these inflamed tissues, numerous stellate cells and monocyte-like round cells were positive for PCNA, and synovial lining cells were sometimes PCNA-positive. On the other hand, lymphocyte-like small round cells were almost negative for PCNA staining. In the synovium from the control rats, a few synovial cells were positive for PCNA (Fig. 5d). Although PCNA immunoreactivity was still observed in some stellate cells in the fibrotic lesions in the synovium, periarticular connective tissues and periosteum of newly-formed bones at R2W (Fig. 5b), PCNA-staining pattern in the synovium, periarticular tissues and bones was similar with that in the normal tissues at R4 and 12W (Fig. 5c,d).

Discussion

The recovery process of 6SAI-induced arthritis was examined.

The kinetics of ankle swelling during the treatment period in this study was quite similar to that observed in our previous study on rats treated with 6SAI for up to 4 weeks (Ohmachi et al., 1998), in which the ankle swelling peaked at about 11 days after the first dosing, and the mean maximum arthritis score was about 3.5 at that point.

Throughout the study, the recurrence of inflammation never occurred, and 6SAI-induced arthritis did not progress into the chronic phase. After the last dosage, the lesions recovered rapidly and were

completely cured after the 12 weeks of recovery periods. Both the results of hematological and serum biochemical examinations also showed that the animals did not develop inflammation during the recovery period.

In several experimental arthritis, such as type-II collagen-induced arthritis (Abe et al., 1995), *Lactobacillus casei* cell wall extract-induced arthritis (Lehman et al., 1983), adjuvant arthritis (Abe et al., 1995), muramyl dipeptide-induced arthritis (Sugawara et al., 1995) and Streptococcal cell wall-induced arthritis (Cromartie et al., 1977), the arthritides always progressed from the acute phase into the chronic phase. In these models, inflammation in the acute phase was characterized by marked infiltration of neutrophils and lymphocytes, and inflammation in the chronic phase was characterized by continuing lymphocyte infiltration and bone/cartilage destruction with proliferating granulation tissues, i.e. pannus. In other types of arthritis models, such as pristane-induced arthritis and spontaneous arthritis in MRL lpr/lpr mice, acute- and chronic-type inflammations progressed simultaneously (Abe et al., 1995). Judging from the similarities in the pathological features at the active phase, it seems that intra-articular inflammation is usually accompanied by marked neutrophil infiltration and tends to progress into chronic inflammation including bone/cartilage destruction in the above-mentioned arthritis models.

On the other hand, the typical histopathological features of 6SAI-induced arthritis were confirmed to be an exudative/edematous synovitis and periartthritis, and they never developed into the chronic or active phase. The major pathological difference between 6SAI-induced ankle inflammation and other arthritis models was that the latter was accompanied with marked neutrophil infiltration while the former was not.

Neutrophil is known to play an important role in an acute inflammation and tissue damage through releasing superoxide and lysosomal enzymes, and synthesizing lipoxygenase products. The severities of arthritis and cartilage destruction are reported to be suppressed by inhibiting inflammatory or tissue-damaging effects of neutrophils using leukotriene B4 receptor antagonist (Kuwabara et al., 2000). In neutropenic mice, joint inflammation was virtually absent, and loss of cartilage proteoglycan was abolished in cationic immune-complex-induced arthritis (von Lent et al., 1994). Hashida et al. (1996) reported that a factor derived from polymorphonuclear leukocytes enhances the synthesis of prostaglandin (PG) in cultured rat synovial cells and the production of IL-1-induced synovial cell collagenase and PGE2 in rats. Prostaglandins are also well-known mediators of cellular and vascular events in inflammatory reactions. Neutrophils may activate matrix metalloproteinase (MMP)-1 and MMP-3, and inflammation and cartilage destruction in adjuvant-induced arthritis in rats are suppressed by a MMP-inhibitor (Hamada et al., 2000). These findings strongly suggest that the absence or suppression of neutrophils at the inflamed joint tissues may reduce the severities of

articular inflammation and cartilage destruction in joints.

Although the reason why only a few neutrophils infiltrated in the synovial lesions of 6SAI-induced arthritis is not clear at the present, it is supposed that the type of inflammation caused by 6SAI may be important. Similar findings had been obtained from our recent studies on the early development of 6SAI-induced arthritis (Ohmachi et al., 2001a). Namely, mononuclear cell infiltration was noted in the joint synovium, peritenon and periarticular connective tissues within a few days after the first dosage of 6SAI. The lesion became edematous with infiltration of mononuclear cells and a small number of polymorphonuclear leukocytes, and thereafter, the lesion was replaced by granulation tissues. 6SAI induces not only arthritis but also arteritis and serositis in various organs and tissues. The histopathological features of the arteritis induced by 6SAI was quite similar to those induced by vasodilating agents (Ohmachi et al., 1998). In addition, 6SAI-treated animals showed almost no apparent changes in the white blood cell count including lymphocyte and neutrophil ratio and serum albumin/globulin ratio in this study, and this indicates that the systemic immunological alteration might not be a major factor of the development of 6SAI-induced arthritis. In addition, 6SAI enhanced the ankle inflammation in lipopolysaccharide (LPS)-treated rats, and histopathologically, 6SAI caused marked edema with fibrin exudation and leukocyte infiltration (Ohmachi et al., 2001b). So, 6SAI-induced arthritis is supposed to be an inflammation based on the 6SAI-induced local vascular damage. In several hypertension models the cardiovascular lesions usually lacks in apparent neutrophil infiltration. For example, long term administration of N⁻-nitro-L-arginine methyl ester, an inhibitor of nitric oxide synthesis, causes hypertension and produces arteritis characterized by infiltration of monocytes and appearance of myofibroblasts in the blood vessels (Kato et al., 1998). The recovery process of the 6SAI-induced arthritis is thought to be similar to vascular remodeling in artery in several hypertension models.

In the present study, we conducted PCNA staining to examine the proliferating activity of inflammatory cells. At 2W, many stellate cells were PCNA-positive, but PCNA-positive cells were scarcely observed in the lesions during recovery period. These results suggested the PCNA-positive cells mainly played a part in the wound healing, and did not contribute to the development of inflammation in this model.

By the way, marked periosteal-reactive bone formation was constantly observed in the treated animals. It seemed to diminish during the 4th and 12th week of recovery periods, and the affected bones showed normal appearance after several weeks of recovery. Since the osteogenic lesions in this model were not modified by destructive changes, such as pannus invasion or bone destruction, the 6SAI-induced arthritis model is supposed to be a model for examining pathophysiology of inflammatory reactive osteogenesis and its recovery.

In conclusion, this study confirmed that 6SAI-induced arthritis is an exudative synovitis and exudative/edematous periarticular inflammation and never develops into chronic arthritis, and the lack of prominent neutrophil infiltration in the lesion was supposed to have a close relation with its self-limiting character.

Acknowledgements. We thank Mr S. Kurabe, Ms T. Yahagi, Mrs M. Takahashi, Mrs N. Shimazu, and Mrs K. Takahashi, for their kindful technical assistance.

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Accepted December 26, 2001