Novel synthetic retinoic acid inhibits rat collagen arthritis and differentially affects serum immunoglobulin subclass levels

Kenji Kuwabara[a], Koichi Shudo[b], Yozo Hori[a,*]

[a]*Discovery Research Laboratories II, Shionogi and Co., 3-1-1 Futaba-cho, Toyonaka, Osaka 561, Japan*
[b]*Faculty of Pharmaceutical Sciences, University of Tokyo, Bunkyo-ku, Tokyo 113, Japan*

Received 24 October 1995; revised version received 30 November 1995

Abstract Retinoids affect many biological processes such as cell proliferation, differentiation and morphogenesis, but their effects on arthritic patients and animal models of arthritis are controversial. We tested the effect of a novel synthetic retinoic acid, Am-80 (4-[(5,6,7,8-tetrahydro-5,5,8,8,-tetramethyl-2-naphthalenyl) carbamoyl] benzoic acid), on type-II collagen (CII)-induced arthritis (CIA) in rats. Am-80 markedly suppressed the incidence of arthritis, hindpaw swelling and bone destruction. In contrast, 13-*cis*-retinoic acid (13-*cis*-RA) hardly inhibited these CIA symptoms. Moreover, Am-80, but not 13-*cis*-RA, strongly reduced the serum level of anti-CII antibody and differentially affected the levels of immunoglobulin (Ig) subclasses in vivo: IgG1 and IgG2a levels were decreased, while IgA level was increased without any change in the IgM level. These findings indicate that Am-80 may be one of the lead retinoic acids of a new class of anti-inflammatory agents.

Key words: Synthetic retinoid; Am-80; Collagen-induced arthritis; Serum immunoglobulin

1. Introduction

Retinoids, natural and synthetic analogues of vitamin A, have been shown to play important roles in cell proliferation, differentiation and morphogenesis [1]. They also have a modulating function on a wide variety of inflammatory and immune structural cells [2]. Based on these properties, retinoids have been used to try to control incurable proliferative inflammatory diseases, such as psoriasis and rheumatoid arthritis.

While trials with dermatological diseases have been successful [3], the effects of retinoids on both arthritic patients and animal models of arthritis have been inconsistent. Etretinate improved the severity of psoriatic arthritis [4], while arthritic symptoms were observed during etretinate or isotretinoin treatment for acne [5,6]. In animal arthritic models, 13-*cis*-RA was found to inhibit rat adjuvant arthritis [7], but enhanced rat CIA without affecting anti-CII antibody level and delayed-type hypersensitivity.

Am-80 (4-[(5,6,7,8-tetrahydro-5,5,8,8,-tetramethyl-2-naphthalenyl) carbamoyl]benzoic acid) is a novel synthetic retinoic acid having potent binding activity to retinoic acid receptor (RAR)α and β but not to RARγ [9,10]. Although many retinoids have already been tested in human arthritiic patients as well as in animal models of arthritis, there is no report on the anti-arthritic effect of retinoids which lack RARγ-binding activity. Since type-γ receptors are highly expressed in cartilage cell lineage as well as in differentiating skin and early embryos [11,12], this type of retinoids may display different patterns of effects on arthritic models in comparison to the retinoids reported thus far.

Here, we report Am-80 as the first retinoic acid which strongly inhibits rat CIA concomitant with a profound decrease of the anti-CII Ab level and differential regulation of Ig subclass levels in vivo.

2. Materials and methods

2.1. Collagen arthritis in the rat

Female Lewis rats (7–8 weeks old; Charles River Japan, Kanagawa, Japan) were used. Collagen arthritis was induced according to the method described by Trentham et al. [13]. Emulsion was prepared by adding 1.6 mg/ml of bovine CII (Cosmo Bio, Tokyo, Japan) and 0.4 mg/ml of adjuvant peptide solution (Peptide Institute, Osaka, Japan) to an equal volume of Freund's incomplete adjuvant (DIFCO, MI) and stirring with homogenizer for 15 min, 4°C at 10,000 rpm. On day 0, each animal received 0.8 mg of collagen in 1 ml of emulsion intradermaly. Am-80 (synthesized at Shionogi) and 13-*cis*-RA (Sigma, MO) were suspended in water containing 0.5% arabic gum and were administered orally once daily from day 1 to day 23.

2.2. Arthritis assessment

The volumes of two hindpaws were measured with a plethysmometer and the mean volume for both was calculated during the course of arthritis from day 1 to day 24. On day 24, animals were bled and the sera were stored at −20°C. Thymus and spleen weights were measured. Radiographs of hindpaws were taken on day 24 using an X-ray unit (40 kV, 3 mA, 40 s: Japan Softex, Tokyo, Japan) and radiographic scoring (radiographic index) was assessed by the extent of joint space narrowing, bone destruction and periosteal new bone formation. Scores were assigned as integers from 0 to 2 per joint (0 = normal, 2 = maximum joint destruction) and were determined by blind investigation. The radiographic index represents the sum of the left and right joint scores from each rat, with maximum possible scores of four per rat. Ankle joints were collected on day 24, decalcified and stained with hematoxylin and eosin for histological examination.

2.3. Measurement of serum anticollagen antibody and immunoglobulin levels

The serum level of anti-CII antibody was measured in serum diluted to 1:2000 by ELISA system (K-72; Cosmo Bio, Tokyo, Japan). The serum levels of IgA, IgM, IgG and IgG subclass were quantified by single radial immunodiffusion method [14,15] using commercially available kits (Radial Immunodiffusion Kits, Serotec, Oxford, UK).

2.4. Statistical analysis

For the radiographic index, the mean values were compared between the groups by Wilcoxon *U*-test. For the other data, the mean values were compared by Dunnett's *t*-test.

*Corresponding author. Fax: (81) (6) 332 6385.

Abbreviations: CII, type-II collagen; CIA, collagen-induced arthritis; Ig, immunoglobulin; IL, interleukin; 13-*cis*-RA, 13-*cis*-retinoic acid; *trans*-RA, all-*trans*-retinoic acid; RAR, retinoic acid receptor; TGF-β, transforming growth factor β.

SSDI 0014-5793(95)01440-3

3. Results

3.1. Effects on severity of arthritis

The hindpaw volume of the vehicle-treated group increased gradually and reached maximum on day 18. Am-80 significantly inhibited the incidence of arthritis and hindpaw swelling in a dose-dependent manner at doses from 0.3 to 3 mg/kg. On day 24, 1 mg/kg and 3 mg/kg inhibited paw swelling by 62.4% and 91.2% in comparison to the vehicle-treated group, respectively (Fig. 1A, Table 1). By contrast, 13-*cis*-RA had no inhibitory effect on the incidence of arthritis and only 17.8% inhibition of hindpaw swelling was noted with 100 mg/kg at day 24 (Fig. 1B). The body weight of the vehicle-treated group decreased along with the increase of paw swelling. Am-80, but not 13-*cis*-RA, led to significant recovery of this body weight loss on day 24. Decrease of thymus weight and increase of spleen weight were observed in the vehicle-reated group in comparison to the normal group. Am-80 led to thymus weight recovery in a dose-dependent manner, but further potentiated the spleen weight increase. In contrast, 13-*cis*-RA did not affect thymus weight, but increased spleen weight (Table 1).

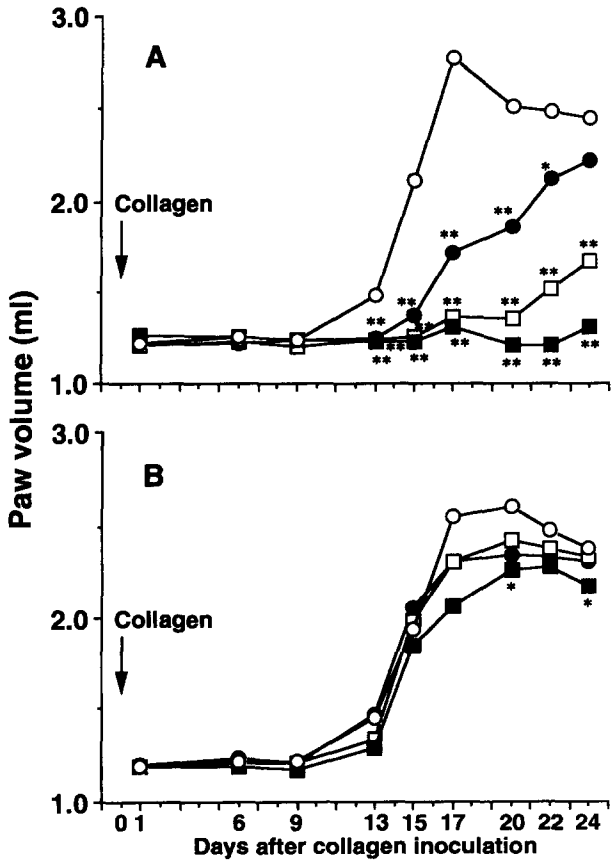

Fig. 1. Effect of Am-80 (A) and 13-*cis*-RA (B) on paw volume changes in rat collagen arthritis. (A) Am-80, (○) vehicle, (●) 0.3, (□) 1, (■) 3 mg/kg/day. (B) 13-*cis*-RA, (○) vehicle, (●) 10, (□) 30, (■) 100 mg/kg/day. Collagen arthritis was induced by intradermal injection of 0.8 mg/kg of bovine type-II collagen on day 0. Hindpaw volume was measured by water displacement method with a plethysmometer and the mean volume for both hindpaws was calculated. Compounds were administered orally once daily from day 1 to day 23. Each value represents the mean of 8 animals. $^{*}P < 0.05$ and $^{**}P < 0.01$ vs. vehicle control (Dunnett's *t*-test).

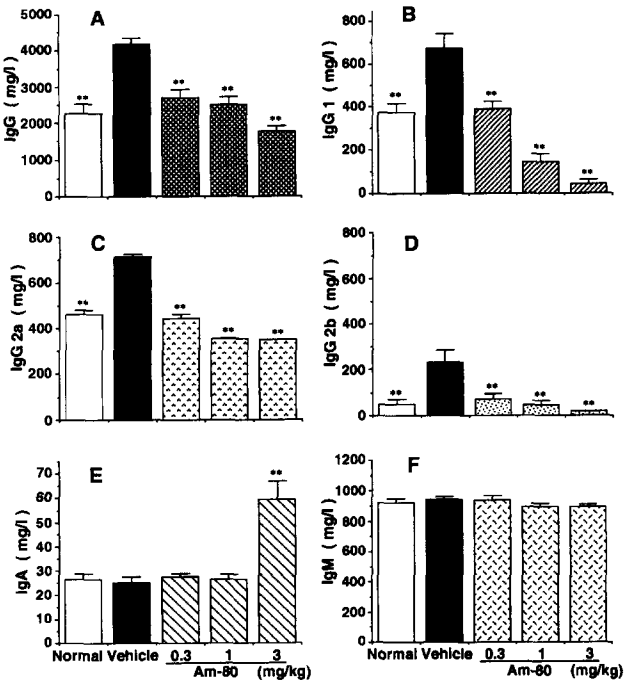

Fig. 2. Differential modulation by Am-80 of sera levels of IgG (A), IgG1 (B), IgG2a (C), IgG2b (D), IgA (E) and IgM (F) in rat collagen arthritis. Arthritis was induced as described in Fig. 1 and on day 24 blood was sampled and quantified with radial immunodiffusion kits. Vertical lines denote ± 1 S.E. ($n = 8$ in all cases except $n = 7$ for normal rats). $^{**}P < 0.01$ vs. vehicle control (Dunnett's *t*-test).

On radiographic scoring, vehicle-treated rats showed destruction of knee and ankle joints, with the severity of ankle joint destruction being greater than that of the knee joint. Am-80 reduced the bone destruction with complete inhibition at 3 mg/kg. 13-*cis*-RA did not inhibit bone damage and even led to significant enhancement of knee joint damage when given at 10 mg/kg (Table 1).

Histological analysis also revealed that Am-80 at 3 mg/kg offered complete protection against the following pathological changes observed in the vehicle-treated group: narrowing of joint space, pannus formation, mononuclear cells and polymorphonuclear leukocyte infiltration, articular cartilage and bone erosion. 13-*cis*-RA did not inhibit these changes even at 100 mg/kg (data not shown).

3.2. Effects on serum anticollagen antibody and immunoglobulin levels

Am-80, but not 13-*cis*-RA, strongly inhibited the increase of CII antibody and the level of the 3 mg/kg treated group decreased to a nearly normal level (Table 1). To determine the effect of Am-80 on Ig subclasses, the total serum levels of IgA, IgM, IgG and IgG subclasses were quantified. As shown in Fig. 2, the lower doses of Am-80 had almost no effect on IgA and IgM levels, but 3 mg/kg enhanced IgA levels to above those of normal rats. On the other hand, a dose of 0.3 mg/kg Am-80 was enough to inhibit the increase of total IgG, IgG1, IgG2a and IgG2b levels in arthritic rats to those of normal rats and the IgG1 level was further decreased to a level much lower than that of normal rats when they were treated with 1 and 3 mg/kg Am-80.

Table 1
Effect of Am-80 and 13-*cis*-RA on collagen arthritis in the rat[a]

Dose (mg/kg/day p.o.)		Incidence of arthritis	Paw volume (ml)	Inhibition (%)	Body weight (g)	Thymus (mg/100 g body weight)	Spleen (mg/100 g body weight)	Radiographic index: Knee joint	Radiographic index: Ankle joint	Antibody to CII[b]
Normal		0/7[†]	1.24 ± 0.01**		196 ± 3**	216 ± 3**	233 ± 9*	0 ± 0	0 ± 0[¶]	0.05 ± 0.01**
Vehicle		9/9	2.45 ± 0.05		153 ± 1	96 ± 9	270 ± 10	0.63 ± 0.32	2.63 ± 0.18	0.41 ± 0.06
Am-80	0.3	8/8	2.22 ± 0.13	18.4	164 ± 3*	129 ± 11*	365 ± 9**	0.50 ± 0.27	1.38 ± 0.26[‖]	0.14 ± 0.02**
	1	8/8	1.67 ± 0.07**	62.4	170 ± 3**	172 ± 12**	346 ± 7**	0 ± 0	0.75 ± 0.25[‖]	0.09 ± 0.01**
	3	2/8[†]	1.31 ± 0.02**	91.2	172 ± 4**	146 ± 8**	355 ± 9**	0 ± 0	0 ± 0[¶]	0.06 ± 0.00**
Vehicle		8/8	2.38 ± 0.05		160 ± 1	101 ± 11	295 ± 8	0.13 ± 0.13	3.00 ± 0.27	0.51 ± 0.07
13-*cis*-RA	10	8/8	2.31 ± 0.06	5.9	160 ± 3	99 ± 12	312 ± 9	1.25 ± 0.16[§]	3.13 ± 0.13	0.39 ± 0.09
	30	8/8	2.33 ± 0.03	4.2	158 ± 1	112 ± 8	332 ± 9**	0.50 ± 0.27	3.13 ± 0.13	0.39 ± 0.05
	100	8/8	2.17 ± 0.02*	17.8	153 ± 2*	108 ± 6	332 ± 9*	0.63 ± 0.26	3.00 ± 0.27	0.43 ± 0.08

[a] Collagen arthritis was induced by intradermal injection of 0.8 mg of bovine type-II collagen into the back skin of rat on day 0. Compounds were administered orally from day 1 to day 23. Parameters were measured on day 24. Mean ± S.E.
[b] OD at 450 nm of a 1:2000 dilution.
[†] $P < 0.01$ vs. vehicle control (Fisher's exact probability test).
[§] $P < 0.05$, [‖] $P < 0.01$ and [¶] $P < 0.001$ vs. vehicle control (Wilcoxon *U*-test).
*$P < 0.05$ and **$P < 0.01$ vs. vehicle control (Dunnett's *t*-test).

4. Discussion

CIA in rats is an animal model for rheumatoid arthritis, requiring T cells [16], anti-CII antibody [17] and complement system [18] for its induction and persistence. Recent clinical reports on the presence of anti-CII antibody particularly in early rheumatoid arthritis further warrant its importance for studying the pathogenesis of rheumatoid arthritis [19]. The present study is the first report on the inhibition of rat CIA by retinoids. Am-80 strongly suppressed the incidence of arthritis, hindpaw swelling and bone damage in this model. Although several retinoids including natural and synthetic ones have been shown to ameliorate rat adjuvant-induced [7] or streptococcal cell wall-induced arthritis [20], they aggravated rat CIA [8,21]. Indeed, 13-*cis*-RA significantly increased knee joint damage in the present experiments.

It is well-established that in rat CIA CII antibody binds to the collagen of the articular cartilage, activates the complement system and initiates tissue damage [18]. These observations suggest that Am-80 can inhibit CIA by reducing CII antibody production and this was supported by the lack of suppression of both CII antibody level and joint damage with 13-*cis*-RA treatment. More interestingly, Am-80 not only suppressed CII antibody level but also differentially modulated Ig subclass levels. Since IgG2a is the most potent activator of the classical complement cascade and Fc receptor bearing macrophages [22], the present findings further support the inhibitory mechanism of Am-80 and the pathogenic role of IgG2a in rat CIA [23].

Although the mechanisms by which Am-80 suppressed CIA and CII Ab production are probably very complex as in the case of many retinoids, the following direct or indirect effects could be involved. (1) Direct inhibition of growth of B cell precursors as reported for all-trans retinoic acid (*trans*-RA) and 9-*cis*-RA [24]. (2) Differential modulation of Ig subclasses through the stimulated synthesis of transforming growth factor β (TGF-β). The present results are consistent with the recent in vitro findings that *trans*-RA selectively enhanced IgA production but inhibited IgG1 production from LPS-stimulated murine splenocytes partly through the synthesis of TGF-β [25], because retinoids are strong inducers of TGF-β and this growth factor is well-known as an IgA class-switching factor [26]. In fact, an increase of TGF-β immunostain in rat testicular tissue was observed after oral treatment for one month with 2 mg/kg/day of Am-80, while a dose of 0.4 mg/kg/day was insufficient to increase the TGF-β immunostain (unpubl. data). This might also explain the lack of increase of IgA levels by Am-80 with doses less than 2 mg/kg/day in the present experiments. (3) Since IgA downregulates the release of inflammatory cytokines, such as tumor necrosis factor and interleukin (IL)-6 from monocytes [27], the increased levels of IgA provoked by Am-80 might be responsible for the strong inhibition of CIA via downregulation of these arthritogenic cytokines. (4) Inhibition of synthesis of collagenases that degrade basement membrane and extracellular matrix, by suppressing collagenase gene expression [28]. Moreover, retinoids have been shown to modulate IL-1, IL-2, IL-6, interferon-γ and tumor necrosis factor production from various types of cells [29–33], therefore, the direct in vitro effect of Am-80 on the production of these cytokines also remains to be studied.

Although the development of retinoids as anti-inflammatory drugs for rheumatology has been hampered by their wide range of toxicity [3] and Am-80 actually shares some of these toxicities [34], the strong inhibition by Am-80 of rat CIA indicates that retinoids which lack RARγ-binding activity may be the lead retinoic acids as a new class of anti-inflammatory agents for incurable proliferative diseases, such as rheumatoid arthritis and psoriasis. Furthermore, the delineation of changes of RARs expression in lymphoid systems and articular tissue during the course of rat CIA as well as their modification by Am-80 should offer new insights into the possible involvement of RARs in the pathogenesis of rheumatoid arthritis.

References

[1] Gudas, L.J., Sporn, M.B. and Roberts, A.B. (1994) in The Retinoids: Biology, Chemistry and Medicine. 2nd Ed. (Sporn, M.B., Roberts, A.B. and Goodman, D.S., Eds.), pp. 443–520. Raven, New York, NY.
[2] Ross, A.C. and Hämmerling, U.G. (1994) ibid., pp. 521–543.
[3] Peck, G.L. and DiGiovanna, J.J. (1994) ibid., pp. 631–658.

[4] Hopkins, R., Bird, H.A., Jones, H., Hill, J., Surrel, K.E., Astbury, C., Miller, A. and Wright, V. (1985) Ann. Rheum. Dis. 44, 189–193.
[5] Matsuoka, L.Y., Wortsman, J. and Pepper, J.J. (1984) Arch. Intern. Med. 144, 1870–1871.
[6] Kaplan, G. and Haettich, B. (1991) Baillieres Clin. Rheumatol. 5, 77–97.
[7] Brinckerhoff, C.E., Coffey, J.W. and Sullivan, A.C. (1983) Science 221, 756–758.
[8] Trentham, D.E. and Brinckerhoff, C.E. (1982) J. Immunol. 129, 2668–2672.
[9] Kagechika, H., Kawachi, E., Hashimoto, Y., Himi, T. and Shudo, K. (1988) J. Med. Chem. 31, 2182–2192.
[10] Hashimoto, Y. (1991) Cell Struct. Funct. 16, 113–123.
[11] Dolle, P., Ruberte, E., Kastner, P., Petkovich, M., Stoner, C.M., Gudas, L.J. and Chambon, P. (1989) Nature (London) 342, 702–705.
[12] Kastner, P., Krust, A., Mendelsohn, C., Garnier, J.M., Zelent, A., Leroy, P., Staub, A. and Chambon, P. (1990) Proc. Natl. Acad. Sci. USA 87, 2700–2704.
[13] Trentham, D.E., Townes, S.A. and Kang, A.H. (1977) J. Exp. Med. 146, 857–868.
[14] Mancini, G., Carbonara, A. and Heremans, F.J. (1965) Immunochem. 2, 235–254.
[15] Fahey, J.L. and McKelvey, F.J. (1965) J. Immunol. 94, 84–90.
[16] Kerwar, S.S., Englert, E.M., McReynolds, A.R., Landes, J.M., Lloyd, M.J., Oronsky, L.A. and Wilson, J.F. (1983) Arthritis Rheum. 26, 1120–1131.
[17] Stuart, J.M., Cremer, B.R., Townes, S.A. and Kang, H.A. (1982) J. Exp. Med. 155, 1–16.
[18] Morgan, K., Clague, B.R., Shaw, J.M., Firth, A.S., Towose, M.T. and Holt, P.J.M. (1981) Arthritis Rheum. 24, 1356–1362.
[19] Fujii, K., Tsuji, M., Kitamura, A. and Murota, K. (1992) Int. Orthoped. 16, 272–276.
[20] Haraoui, B., Wilder, R.L., Allen, J.B., Sporn, M.B., Helfgott, R.K. and Brinkerhoff, C.E. (1985) Int. J. Immunopharmacol. 7, 903–916.
[21] Bradsaw, D., Cashin, H.C. and Kennedy, J.A. (1984) in Retinoids: New Trends in Research and Therapy (Saurat, Ed.), pp. 204–218, Karger, Basel, Switzerland.
[22] Ezekowitz, R.A.B., Bampton, M. and Gorden, S. (1983). J. Exp. Med. 157, 807–812.
[23] Firth, S.A., Morgan, K., Evans, B.H. and Holt, P.J.M. (1984) Immunol. Lett. 7, 243–247.
[24] Fahlman, C., Jacobsen, S.E., Smeland, E.B., Lømo, J., Næss, C.E., Funderud, S. and Blomhoff, H.K. (1995) J. Immunol. 155, 58–65.
[25] Tokuyama, H. and Tokuyama, Y. (1993) Cell. Immunol. 150, 353–363.
[26] Coffman, R., Lebman, D. and Schrader, B. (1989) J. Exp. Med. 170, 1039–1044.
[27] Wolf, H.M., Fischer, M.B., Pühringer, H., Samstag, A., Vogel, E. and Eibl, M.M. (1994) Blood 83, 1278–1288.
[28] Pan, L. and Brinckerhoff, C.E. (1994) Ann. N.Y. Acad. Sci. 732, 335–347.
[29] Dillehay, D.L., Walia, A.S. and Lamon, E.W. (1988) J. Leukocyte Biol. 44, 353–360.
[30] Felli, M.P., Vacca, A., Meco, D., Screpatini, I., Farina, A.R., Maroder, M., Martinotti, S., Petrangeli, E., Frati, L. and Gulino, A. (1991) Mol. Cell. Biol. 11, 4771–4778.
[31] Zitnik, R.J., Kotloff, R.M., Latifpour, J., Zheng, T., Whiting, N.L., Schwalb, J. and Elias, J.A. (1994) J. Immunol. 152, 1419–1427.
[32] Mehta, K., McQueen, T., Tucker, S., Pandita, R. and Aggarwal, B.B. (1994) J. Leukocyte Biol. 55, 336–342.
[33] Abb, J., Abb, H. and Deinhardt, F. (1982) Immunopharmacology 4, 303–310.
[34] Von Schroeder, H.P., Hashimoto, Y. and Heersche, J.N.M. (1994) Teratology 50, 54–62.