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Short communication

Suppression of rat adjuvant arthritis by some acyclic nucleotide analogs

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Abstract

The antiarthritic potential of two different acyclic nucleotide analogs, i.e. 9-(2-phosphonomethoxyethyl)adenine (PMEA), its bis(pivaloyloxymethyl)ester (Bis-POM-PMEA), and 1-(*S*)-(3-hydroxy-2-phosphonomethoxyethyl) cytosine (HPMPC) was investigated in the rat model of mycobacterial adjuvant-induced arthritis. With dependence on the dose, timing and route of administration, as well as on the genetic constitution of the arthritis-prone animals, PMEA was able to delay the onset, and substantially reduce or nearly completely inhibit the development of arthritic paw swelling. HPMPC was less active in this model. As compared with PMEA, its prodrug, Bis-POM-PMEA, expressed much more pronounced beneficial effects after both oral and i.p. administration.

Keywords: Acyclic nucleotide analog; PMEA (9-(2-phosphonomethoxyethyl)adenine); HPMPC (1-(*S*)-(3-hydroxy-2-phosphonomethoxyethyl)cytosine); Antiarthritic effect; Adjuvant arthritis

1. Introduction

9-(2-Phosphonomethoxyethyl)adenine (PMEA) is a potent inhibitor of retroviruses (including human immunodeficiency virus) and DNA viruses (Balzarini et al., 1989; Naesens et al., 1994) and has thus become an attractive candidate for treatment of the acquired immunodeficiency syndrome (Walker et al., 1993). Its antiproliferative properties have also been observed in other biological systems (Otová et al., 1993; Křen et al., 1993; Zídek et al., unpublished results).

Inasmuch as many antiproliferative agents are efficient modulators of immune-mediated disease conditions (Pullar and Veale, 1993), we have addressed the question of whether PMEA also exhibits such activity. In the present study we investigated the effect of PMEA and its orally available prodrug, bis(pivaloyloxymethyl) 9-(2-phosphonylmethoxyethyl)adenine (Bis-POM-PMEA), on the development of adjuvant-induced arthritis in the rat. This disease is a model of chronic inflammation with presumed autoimmune origin. Due to its similarity, albeit not absolute, to human rheumatoid arthritis and Reiter's syndrome, arthritis in rats has become the most widely used model for testing antiinflammatory/antirheumatic properties of agents (Billingham, 1983). For comparison, we have included in this study another phosphonate-based antiviral, 1-(*S*)-(3-hydroxy-2-phosphonomethoxyethyl)cytosine (HPMPC).

The results of this panel study showed that PMEA and Bis-POM-PMEA substantially reduce the development of arthritic swelling of paws in adjuvant-treated rats.

2. Materials and methods

2.1. Materials

Female rats of the inbred strains Lewis (LEW/Crl/CrlBR) and Brown Norway (BN/CrlBR), were purchased from Charles River (Sulzfeld, Germany). Their weight ranged between 165–180 g at the beginning of the experiments. The rats were housed in groups of five animals per cage, and were allowed standard pelleted diet and water ad libitum. The test substances, i.e. 9-(2-phosphonomethoxyethyl)adenine (PMEA), Na-salt, and 1-(*S*)-(3-hydroxy-2-phosphono-

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methoxyethyl)cytosine (HPMPC) were synthesized according to the procedures described elsewhere (Holý et al., 1989a,b). Bis-POM-PMEA, i.e. the bis(pivaloyloxymethyl)ester of PMEA, was kindly donated by Gilead Sciences (Foster City, CA, USA). Fresh solutions were prepared in sterile saline and applied i.p., s.c. (in the nape of the neck), or orally by gavage (p.o.) at the dose of 50 mg/kg (unless stated otherwise). Control animals were given sterile saline.

2.2. Induction of arthritis and its evaluation

Adjuvant arthritis was induced by single intraplantar injection of 0.5 mg of *Mycobacteria tuberculosis*, strain H37RA (Difco Lab., Detroit, MI, USA), finely ground in 0.1 ml of paraffin oil. The volume of the uninjected paw was recorded every other day after adjuvant administration using a plethysmometer (Ugo Basile 7150). Changes in paw volume (swelling) were expressed as differences from preapplication values to the nearest 0.01 ml. The overall paw volume changes were evaluated by means of individual areas under the curve (AUC). Each group was composed of five animals. The experiments were run in four consecutive blocks A, B, C, D (Table 1) within a period of 7 months.

2.3. Statistical analysis

Analysis of variance and Dunnett's test were employed to determine statistical significances of differences among the group means.

3. Results

The introductory screening experiment (Table 1, block A) indicated that repeated administration of PMEA (given i.p. on days 0–10) was able to reduce substantially the development of arthritic swelling of paws in Lewis rats injected with mycobacterial adjuvant. Doses of 100 mg/kg or 50 mg/kg were found to be highly and about equally effective (Table 1, groups

Table 1
Effects of acyclic nucleotide analogs, PMEA, Bis-POM-PMEA, and HPMPC, on development of adjuvant-induced arthritis

Dose (mg/kg)	Route	Timing (days)	Rats	Group/ block	Paw swelling (ml × 100) on days					AUC (%)
					14	16	19	22	29	
Control										
–	–	–	LEW	1/A	45 ± 16	69 ± 20	86 ± 19	107 ± 21	85 ± 17	100 ± 23
–	–	–	LEW	2/B	46 ± 5	60 ± 6	103 ± 5	133 ± 14	ND	100 ± 10
–	–	–	LEW	3/C	58 ± 8	88 ± 11	109 ± 12	110 ± 7	79 ± 11	100 ± 10
–	–	–	LEW	4/D	47 ± 10	ND	96 ± 19	113 ± 17	ND	100 ± 14
–	–	–	BN	5/D	45 ± 2	72 ± 5	85 ± 6	83 ± 6	58 ± 6	100 ± 8
PMEA										
100	i.p.	0–10	LEW	6/A	0 ± 1	8 ± 4	26 ± 6	38 ± 4	17 ± 4	25 ± 3 [b]
50	i.p.	0–10	LEW	7/A	1 ± 4	19 ± 5	29 ± 10	31 ± 6	19 ± 4	27 ± 2 [b]
50	i.p.	0–10	LEW	8/B	16 ± 3	27 ± 5	37 ± 3	50 ± 6	ND	34 ± 4 [b]
50	i.p.	0–10	LEW	9/C	7 ± 2	31 ± 6	38 ± 4	42 ± 6	33 ± 3	35 ± 5 [b]
50	i.p.	0–10	LEW	10/D	3 ± 4	ND	42 ± 7	58 ± 4	ND	33 ± 4 [b]
50	i.p.	0–17	LEW	11/B	12 ± 9	33 ± 13	34 ± 12	47 ± 12	ND	27 ± 6 [b]
10	i.p.	0–10	LEW	12/A	14 ± 12	28 ± 13	47 ± 16	44 ± 17	32 ± 9	46 ± 14 [a]
10	i.p.	0–10	LEW	13/C	31 ± 9	42 ± 11	65 ± 12	70 ± 9	52 ± 15	59 ± 12
50	s.c.	0–10	LEW	14/D	5 ± 6	ND	35 ± 8	55 ± 12	ND	32 ± 7 [b]
50	p.o.	0–10	LEW	15/C	48 ± 4	62 ± 5	75 ± 13	71 ± 13	60 ± 16	74 ± 12
50	p.o.	0–10	LEW	16/D	33 ± 10	ND	61 ± 20	70 ± 20	ND	62 ± 19
50	i.p.	0–10	BN	17/D	2 ± 1	8 ± 1	11 ± 2	13 ± 2	13 ± 2	14 ± 1 [c]
Bis-POM-PMEA										
50	i.p.	0–10	LEW	18/C	6 ± 4	7 ± 7	10 ± 7	20 ± 7	14 ± 6	11 ± 6 [c]
50	p.o.	0–10	LEW	19/C	6 ± 3	21 ± 3	45 ± 8	67 ± 8	49 ± 10	44 ± 6 [b]
HPMPC										
50	i.p.	0–10	LEW	20/A	28 ± 7	21 ± 4	33 ± 6	44 ± 11	32 ± 10	44 ± 8 [a]
25	i.p.	0–10	LEW	21/A	7 ± 6	53 ± 9	73 ± 14	89 ± 16	91 ± 15	79 ± 12
10	i.p.	0–10	LEW	22/A	30 ± 5	54 ± 8	67 ± 9	79 ± 13	86 ± 19	80 ± 15

Swelling of uninjected hind paw of Lewis (LEW) or Brown Norway (BN) rats was determined at various time intervals following intraplantar injection (day 0) of 0.1 ml of paraffin oil containing 0.5 mg of *M. tuberculosis*. Administration of test agents (i.p., s.c., or by gavage – p.o.) started on day 0 and usually continued till day 10. Control animals were given sterile saline. The experiments, run in 4 blocks A, B, C, D, were performed during a period of 7 months. Paw volumes (in ml to the nearest 0.01 ml) were determined plethysmometrically, and changes against day 0 were recorded. Data are the means ± S.E.M. from 5 animals in each group. The overall effect of drugs was evaluated statistically by calculating areas under the curve (AUC), reflecting both the severity and onset of paw swelling. AUC of the individual block controls was taken as 100%. [a] $P < 0.05$, [b] $P < 0.01$, [c] $P < 0.001$ compared to the corresponding adjuvant control. ND = not determined.

6/A and 7/A, respectively), while the 10 mg/kg dose had only a mild (statistically significant) suppressive effect (group 12/A). It can be noticed that not only was the severity of adjuvant arthritis reduced, but, also its onset was considerably delayed. This was a typical, common feature for all other effective dose regimens (each experimental group to be compared with corresponding controls, i.e. groups 1/A, 2/B, 3/C, 4/D, 5/D, Table 1). A combined measure of these two disease parameters was expressed and statistically evaluated by calculating the area under the curve (Table 1). The other test nucleotide analog, HPMPC (Table 1, groups 20/A, 21/A, 22/A) proved to be less effective than PMEA. Therefore, further experiments were designed to better describe the antiarthritic potential of PMEA. No differences were found between i.p. and s.c. PMEA injections (groups 10/D and 14/D, respectively), while the same dose (50 mg/kg) given orally had only a negligible suppressive effect (group 16/D). Prolongation of PMEA treatment from 10 to a total of 17 daily i.p. injections did not further enhance its beneficial influence (group 11/B). It can be noticed that the antiarthritic effects of PMEA are very well reproducible. The dose of 50 mg/kg proved to uniformly reduce paw swelling throughout all four experimental blocks (groups 7/A, 8/B, 9/C, 10/D, Table 1). Similarly, comparable effects were found in the two groups administered 10 mg/kg i.p. (12/A, 13/C) or 50 mg/kg orally (15/C, 16/D). Other time-dosing schedules for PMEA (50 mg/kg) i.p. administration (not shown in Table 1), i.e. when given on days 0–6, 7–10, 0, 5, 10, 11–15, −3–0, or singly on day 0, or 7, had only a marginal, not statistically significant reducing effect.

Bis-POM-PMEA showed significantly enhanced antiarthritic activity when compared with its parent compound PMEA ($P < 0.01$). Its i.p. effectiveness was almost completely inhibitory (group 18/C) and oral administration (group 19/C) was nearly twice as effective as the oral administration of PMEA (group 15/C).

The expression of antiarthritic potential of PMEA is obviously dependent on the genetic constitution of animals. Rats of the Brown Norway strain remained practically free of the disease when treated i.p. with this compound (group 17/D).

4. Discussion

These observations demonstrate that the acyclic analog of adenine, i.e. 9-(2-phosphonomethoxyethyl)-adenine (PMEA) and to a lesser extent the acyclic analog of cytosine, 1-(*S*)-(3-hydroxy-2-phosphonomethoxyethyl)cytosine (HPMPC) are effective to reduce the development of adjuvant-induced arthritis in the Lewis and the Brown Norway rats. The effect has been found to depend largely on the dose and timing of PMEA. It was most prominent (reduction by about 60–70%) after 50–100 mg/kg (although 10 mg/kg was still effective) given daily either i.p. or s.c. during the period of the first 10 days following adjuvant injection. Some other time-dosing i.p. schedules (50 mg/kg) as well as oral administration were only marginally effective. It was interesting to observe that the antiarthritic potential of PMEA was much better expressed in the Brown Norway (in fact, practically complete inhibition of arthritis was achieved) than in the Lewis rats. The lack of PMEA activity upon oral administration is consistent with its negligible oral bioavailability. The Bis-POM-PMEA derivative which was developed to overcome this obstacle (Starrett et al., 1994) proved to be more effective than the parent molecule of PMEA. Bis-POM-PMEA injected i.p. almost completely inhibited the development of arthritis, and even the suppression after its oral administration was of remarkable magnitude, paw swelling being reduced by about 50%.

Due to the systemic character and complexity of the disease and with respect to its rather poorly understood etiologic and pathogenetic background, elucidation of mechanisms of antiarthritic actions of drugs is generally considered to be an extremely difficult task. It is assumed that the pathogenesis of various immunopathies including rheumatoid arthritis is largely influenced by impaired suppressor cell activity (Gilman et al., 1987). Some experimental data seem to indicate a possible involvement of suppressor cells in the antiarthritic effects of PMEA: it has been found to enhance the proportion of killer/suppressor cells (Otová et al., 1993) and prevent acute graft-versus-host disease in the rat (Křen et al., 1993).

Otherwise, relevant information about the pharmacological and immunopharmacological profile of PMEA or related compounds has hitherto remained very limited. Calio et al. (1994) reported that PMEA is an effective inducer of interferons alpha and beta. However, any antiarthritic effectiveness of these cytokines is obviously a controversial issue (Aoyagi et al., 1994; Kiely and Bruckner, 1994). No data are as yet available on the possible interference of PMEA or Bis-POM-PMEA with biosynthesis of, for example, prostaglandins or nitric oxide which are known to be closely associated with arthritic immunopathies (Stefanovic-Racic et al., 1993).

Further studies are under way in our laboratory to analyse more closely the mechanism of the antiarthritogenic activity of PMEA. It will be also important to evaluate its therapeutic potency and efficacy, possible advantages or disadvantages, notably in comparison with the most frequently used immune-active drugs in rheumatoid arthritis, such as azathioprine, methotrexate, chlorambucil and cyclophosphamide (Pullar and Veale, 1993).

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