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European Journal of Pharmacology 376 (1999) 91–100

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Chemokines, nitric oxide and antiarthritic effects of 9-(2-phosphonomethoxyethyl)adenine (Adefovir)

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Received 28 December 1998; received in revised form 3 May 1999; accepted 5 May 1999

Abstract

Antiarthritic effects of two acyclic nucleoside phosphonates, 9-(2-phosphonomethoxyethyl)adenine (PMEA; Adefovir) and 9-(2-phosphonomethoxypropyl)adenine (PMPA), as well as their more bioavailable prodrugs, bis(pivaloyloxymethyl)ester of PMEA [bis(POM)-PMEA; Adefovir Dipivoxil] and bis(isopropyloxycarbonyloxymethyl)ester of PMPA [bis(POC)-PMPA], were investigated in a model of adjuvant-induced arthritis in Lewis rats. The drugs were injected subcutaneously at doses of 5–50 mg/kg. PMEA and its prodrug inhibited by $>80\%$ arthritic paw swelling, splenomegaly and fibroadhesive perisplenitis. Both prophylactic and therapeutic dosing regimens were effective. Neither PMPA nor bis(POC)-PMPA suppressed development of arthritic lesions. Substantially reduced nitrite + nitrate levels were detected in serum and urine of PMEA-treated animals as compared to those of untreated diseased controls. Also, complete suppression of the disease-associated, greatly enhanced systemic levels of the chemokine, RANTES (regulated upon activation, normal T cell expressed and secreted), was observed in rats injected with PMEA. Additional in vitro studies showed that PMEA does not change, PMPA enhances, and both prodrugs inhibit the immune-activated NO production. Under the same conditions PMEA inhibits, while PMPA slightly stimulates, secretion of RANTES. Collectively, these data suggest that the in vivo-inhibited production of nitric oxide (NO) is a consequence rather than a mechanism of antiarthritic action of PMEA. Possible mechanisms for the anti-RANTES activity of PMEA remains to be firmly established.

Keywords: PMEA (9-(2-phosphonomethoxyethyl)adenine); PMPA (9-(2-phosphonomethoxypropyl)adenine); Arthritis; Nitric oxide (NO); RANTES (regulated upon activation, normal T cell expressed and secreted)

1. Introduction

Rheumatoid arthritis is considered to be a disease of autoimmune etiology, though the network of factors determining and modifying its development and progression is only incompletely understood. This type of chronic inflammation apparently results from a multitude of different pathways (Van Arman, 1976; Kiely, 1998), and this situation is necessarily reflected in a rather poor efficacy of current therapeutic modalities. The search for new drugs is thus a permanent challenge for pharmacological research. The hallmarks of the human disease are cartilage destruction, chronic synovitis and bone resorption, which are under the control of a plethora of cytokines (Arend and Dayer, 1990), stimulating a number of locally and/or systemically produced proinflammatory mediators. The animal immunological model of progressive joint swelling, destruction and bone erosion, adjuvant-induced arthritis in rats, has been shown to share certain clinical and immunological features with human arthritis (Pearson, 1956; Weichman, 1989). The model is thus widely used, with a relatively high degree of validity, for testing antiinflammatory and antirheumatic properties of drugs (Billingham, 1983; Borah et al., 1995).

Our previous experiments demonstrated prominent antiarthritic properties of an acyclic nucleoside phosphonate 9-(2-phosphonomethoxyethyl)adenine (PMEA; Adefovir) and its more bioavailable prodrug, bis(POM)-PMEA (Adefovir Dipivoxil), i.e. bis(pivaloyloxymethyl) ester of PMEA (Zídek et al., 1995b). PMEA represents a prototype compound in a new series of outstanding antivirals, effective against some DNA viruses and retroviruses, including

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0014-2999/99/$ - see front matter
PII: S0014-2999(99)00343-X

human immunodeficiency virus (HIV) (De Clercq, 1991; Naesens et al., 1995; Barditch-Crovo et al., 1997). The postulated mechanism involves transformation of the phosphonate residue by intracellular kinases to the corresponding mono- and diphosphoryl derivatives which are assumed to suppress the viral DNA synthesis de novo mediated by inhibition of replicative DNA-polymerases. Besides, some acyclic nucleoside phosphonates are potent immunomodulators, interfering with the production of cytokines and nitric oxide (NO) (Zídek et al., 1997b).

Recent data have indicated a significant role of NO (Stefanovic-Racic et al., 1994; Stichtenoth et al., 1994; Odeh, 1997) and chemoattractive cytokines, namely the ''regulated upon activation, normal T cell expressed and secreted'' (RANTES) (Barnes et al., 1998; Volin et al., 1998) in the pathogenesis of arthritic processes, both in rheumatic patients and in rats with adjuvant-induced arthritis. The aim of the present study was, therefore, to investigate whether these factors could be targets for the antiinflammatory action of PMEA. Included in the study was another member of the group of acyclic nucleoside phosphonates, the *R*-enantiomer of 9-(2-phosphonomethoxypropyl)adenine (PMPA), that has been shown to completely prevent the development of acquired immunodeficiency syndrome (AIDS) in a simian model of the disease (Tsai et al., 1995; Balzarini et al., 1996; Bischofberger et al., 1996). As with PMEA, the orally more bioavailable prodrug of PMPA, i.e. its bis(isopropyloxycarbonyloxymethyl)ester [bis(POC)-PMPA] (Naesens et al., 1997), was tested as well.

In summary, the present data identify the PMEA and its prodrug bis(POM)-PMEA as outstanding inhibitors of the development of adjuvant-induced arthritis in rats. The antiarthritic effect has been found to be associated with greatly suppressed systemic levels of NO and a chemokine, RANTES, production. Both PMPA and bis(POC)-PMPA were ineffective in this experimental system.

2. Materials and methods

2.1. Animals, induction of arthritis and its evaluation

Female rats of the inbred strain, Lewis (LEW/Crl/CrlBR), weighing 170–185 g, were purchased from Charles River (Sulzfeld, Germany). They were housed in groups of four to six animals per cage, and were allowed standard pelleted diet and water ad libitum. Adjuvant arthritis was induced by single intraplantar injection of 0.5 mg of *Mycobacterium tuberculosis*, strain H37RA (Difco Labs, Detroit, MI, USA), finely ground in 0.1 ml of paraffin oil. The day of injection was taken as day 0. The volume of hind paws was recorded using a plethysmometer (Ugo Basile 7150, Varese, Italy). The overall severity of edematous inflammation developing during the whole observation period (20–22 days) was evaluated on the basis of individual areas under the curve (AUC), calculated from the differences from the preapplication paw volume.

2.2. Acyclic nucleoside phosphonates

PMEA (Adefovir), PMPA, and their more bioavailable prodrugs bis(POM)-PMEA (Adefovir Dipivoxil) and bis(POC)-PMPA, respectively, were kindly donated by Gilead Sciences (Foster City, CA, USA). Their structure is shown in Fig. 1. Fresh solutions were prepared in sterile saline, and applied s.c. (in the nape of the neck) daily, either on days 0–9 or 10–19, with respect to the induction of adjuvant arthritis. Controls were injected with saline.

2.3. Cell cultures

Peritoneal cells were harvested at the time intervals indicated after the injection of mycobacterial adjuvant, washed three times, resuspended in RPMI-1640 medium (SEVAC, Prague, CZ), and placed in 96-well flat-bottom

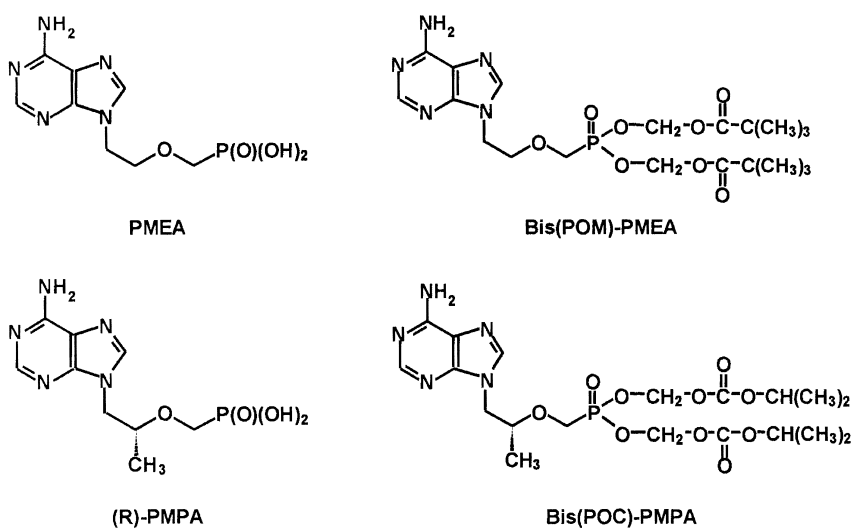

Fig. 1. Structure of acyclic nucleoside phosphonates tested.

microplates (Costar, Cambridge, MA, USA) in a density of 2×10^5 cells/well in a final 100 μl. Adherent peritoneal cells (macrophages) were obtained after a standard adherence procedure (2 h at 37°C, 5% CO_2), when the medium was replaced with complete RPMI-1640 containing 10% heat-inactivated fetal bovine serum, 50 μg/ml gentamicin, 2 mM L-glutamine, and 5×10^{-5} M 2-mercaptoethanol (all Sigma, St. Louis, MO, USA).

Single-cell suspensions of lymphocytes were prepared from spleens of rats after lysis of erythrocytes, using a red blood cell lysing buffer (Sigma). The cells were cultured (3×10^5 cells/well in final 100 μl) in the medium described above.

The cultures were maintained at 37°C and 5% CO_2, in a humidified Heraeus incubator for the intervals shown below.

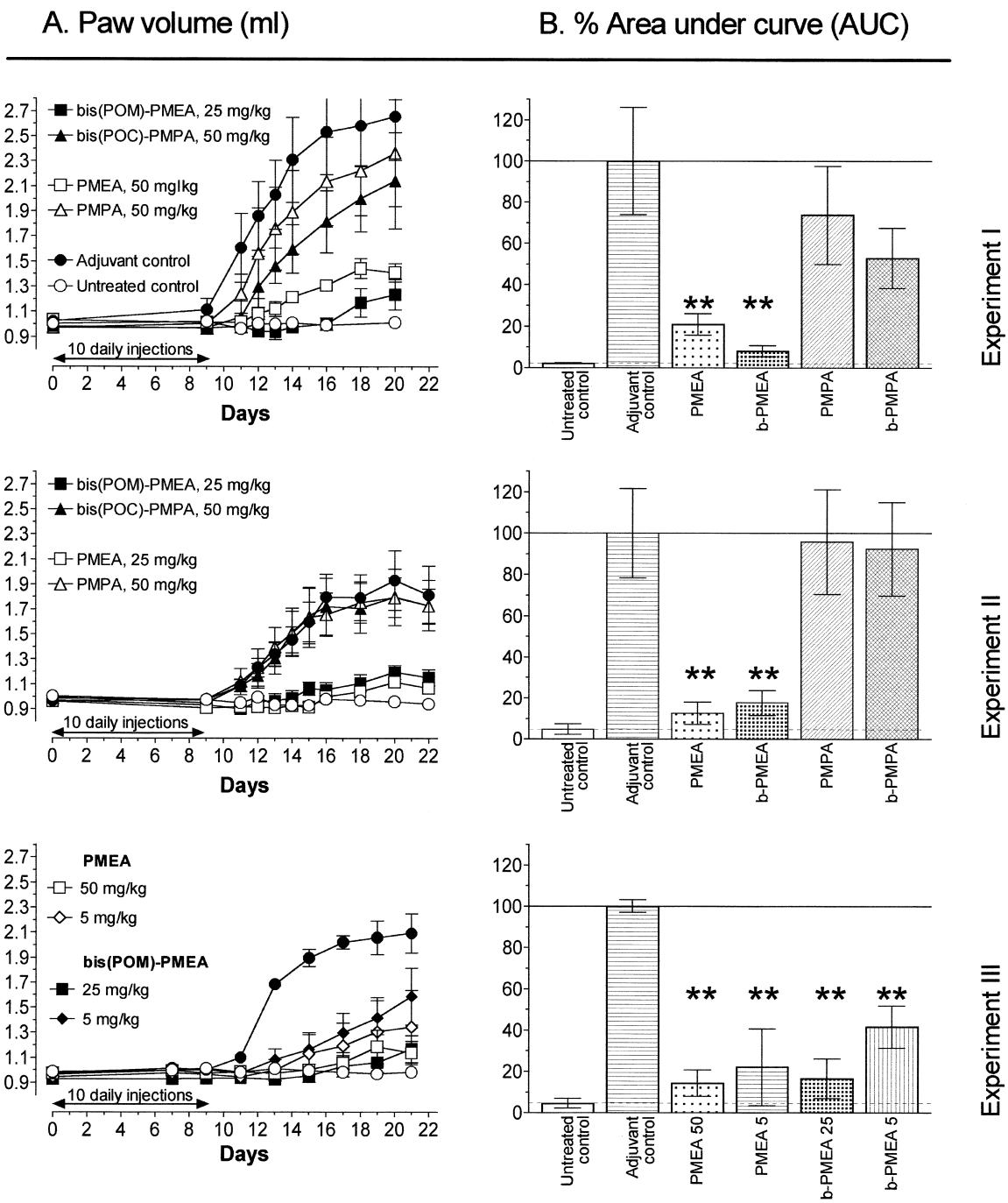

Fig. 2. Effect of s.c. applied acyclic nucleoside phosphonates PMEA, PMPA, and their ester prodrugs bis(POM)-PMEA and bis(POC)-PMPA, respectively, on development of adjuvant-induced arthritis in Lewis rats. The swelling of adjuvant-uninjected right hind paw (A) was measured plethysmometrically, and the overall severity of paw edema was evaluated by calculating the area under the curve (B). Each point or bar is a mean ± S.E.M. ($n = 5$–6 rats). ** Statistically significant at $P < 0.01$.

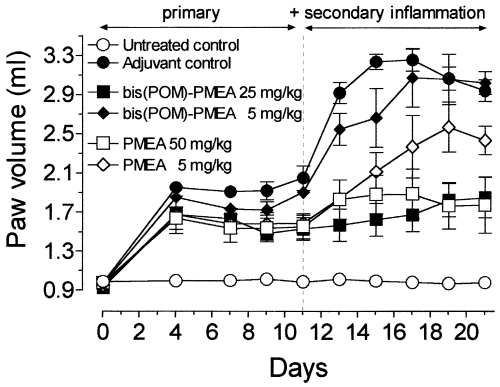

Fig. 3. Swelling of the ipsilateral, i.e. mycobacterial adjuvant-injected left hind paw of rats. The test drugs were applied s.c. on days 0–9. Data are means ± S.E.M. ($n = 6$ rats).

2.4. NO assay

The concentration of nitrites in supernatants of macrophages was assayed after their 24-h cultivation. Urine was collected for 22 h in metabolism cages (Tecniplast Gazzada, Buguggiate, Italy). During this time and for the preceding 12 h, the rats were allowed to drink 7% glucose in distilled water in order to minimize external sources of nitrate contamination. The concentration of nitrites + nitrates in urine (μmol/h) and in serum (μM) was determined after the reduction of nitrates to nitrites, using the enzyme, nitrate reductase, from *Aspergillus* sp. (Boehringer Mannheim, Mannheim, Germany), following the method of Schmidt et al. (1992). The samples were incubated for 10 min with Griess reagent (1% sulfanilamide/0.1% naphtylethylendiamine/2.5% H_3PO_4), and absorbance at 540 nm was recorded using a microplate reader, Rainbow Thermo (Tecan, Grödig, Austria). A nitrite calibration curve was used to convert absorbance into μM nitrite.

2.5. RANTES assay

The chemokine was determined in plasma, and in supernatants of macrophages or splenocytes cultured alone or in the presence of lipopolysaccharide (LPS; 5 μg/ml) for 16 h, as described above. A human ELISA kit (R&D Systems, Minneapolis, MN, USA) which is cross-reactive with rat RANTES (Barnes et al., 1998) was employed for this purpose. Instead of human standard, rat RANTES (Endogen, Woburn, MA, USA) was used to make the calibration curve. Absorbances were read at 450 nm using a microplate reader Rainbow Thermo (Tecan).

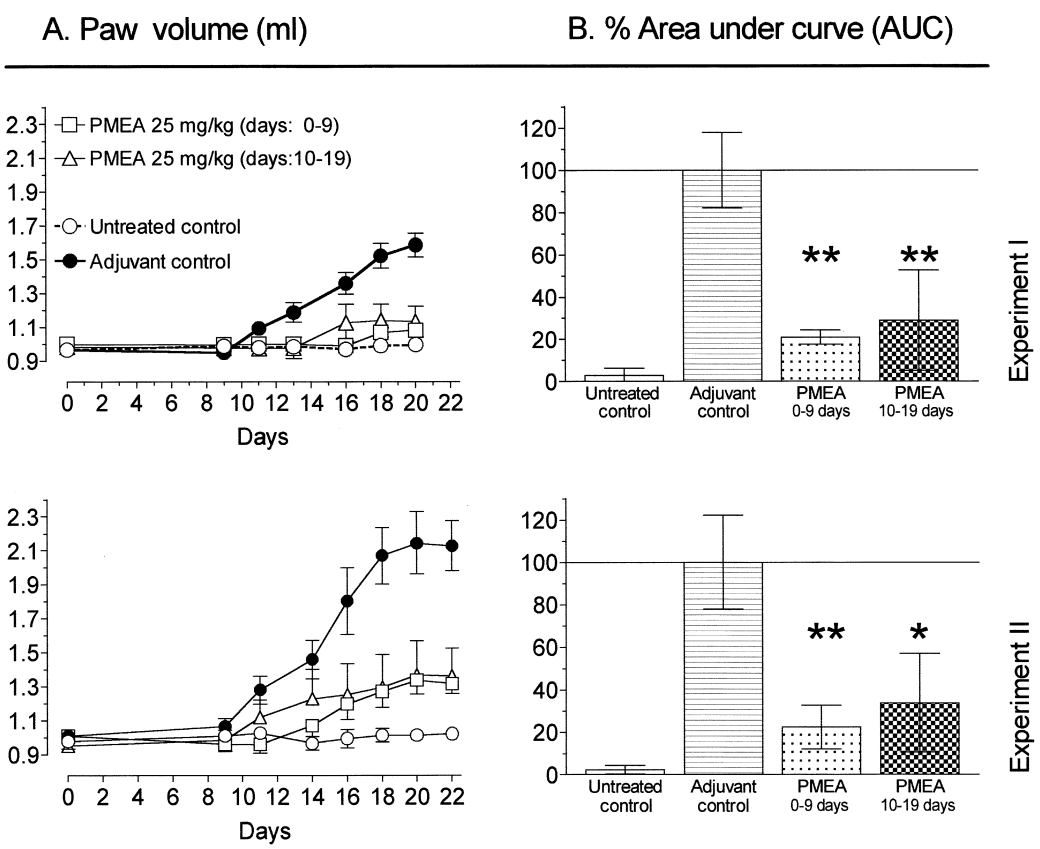

Fig. 4. Suppression of arthritic rat paw edema (A) by PMEA injected s.c. either prophylactically on days 0–9, or therapeutically on days 10–19. The efficacy of dosing regimens was evaluated from the area under the curve (B). The data are means ± S.E.M. observed in two independent experiments ($n = 6$ rats in Experiment I, and $n = 5$ rats in Experiment II). Statistically significant at $^{*}P < 0.05$ or $^{**}P < 0.01$, respectively.

2.6. Statistical analysis

The data were evaluated by analysis of variance and subsequent Dunnett's test, using the Prism program (GraphPad Software, San Diego, CA, USA).

3. Results

3.1. Swelling of paws

Several independent experiments were performed 2–3 months apart. Although there were some differences between experiments for the magnitude of the paw volume increase produced by the mycobacterial adjuvant in the controls, almost identical antiinflammatory effects of PMEA and bis(POM)-PMEA were observed throughout (Fig. 2A). The overall severity of paw swelling was statistically evaluated by calculating areas under curve (AUC) (Fig. 2B). No substantial difference between the effects of PMEA (50 mg/kg s.c.) versus those of bis(POM)-PMEA (25 mg/kg s.c.) was detected; both drugs reduced the arthritic swelling by about 80% ($P < 0.01$). The lower dose applied, 5 mg/kg s.c. (Fig. 2, Experiment III), also exerted a statistically significant effect ($P < 0.01$). Not only the swelling of the contralateral, i.e. adjuvant-uninjected paw, but also the swelling of the ipsilateral, adjuvant-injected paw, was reduced by PMEA and bis(POM)-PMEA (Fig. 3). The mild suppression was discernible even during the phase of primary inflammation, shortly after adjuvant administration [$P < 0.05$, except for bis(POM)-PMEA ($P > 0.05$)], while it was more prominent during the subsequent phase of secondary, i.e. arthritic paw swelling on days 11–21 ($P < 0.01$). A lower dose (5 mg/kg) was less effective ($P < 0.05$ for PMEA) or ineffective [$P > 0.05$ for bis(POM)-PMEA], however. Statistically significant ($P < 0.01$) antiarthritic effects also were seen in the two additional experiments, where daily administration of PMEA (25 mg/kg s.c.) started on day 10 (Fig. 4) instead of day 0 as in the experiments above. Beneficial outcomes of this therapeutic dosing regimen were indistinguishable from those achieved with prophylactic administration of PMEA ($P > 0.60$).

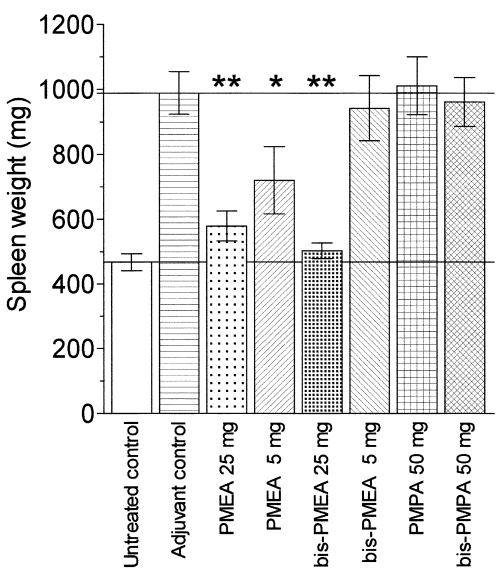

Fig. 5. Development of splenomegaly in arthritic rats was inhibited by PMEA and bis(POM)-PMEA. The agents were applied s.c. on days 0–9, and the spleens were weighed on day 21. The bars represent means ± S.E.M. ($n = 6$ rats). Statistically significant at $^{*}P < 0.05$ or $^{**}P < 0.01$, respectively.

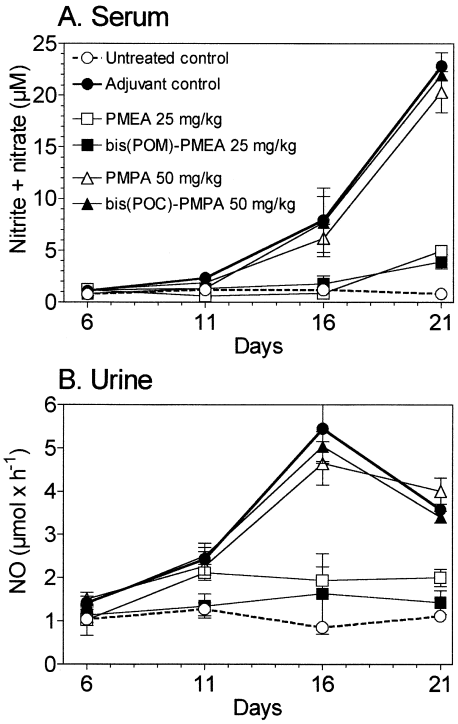

Fig. 6. Formation of end-products (nitrites + nitrates) of nitric oxide synthesis as detected in serum (A) or urine (B) of mycobacterial adjuvant-injected rats, either controls or treated s.c. on days 0–9 with acyclic nucleoside phosphonates. Each point, representing successive days of arthritis progression, is the mean ± S.E.M. ($n = 4$ rats).

PMPA and bis(POC)-PMPA were found to be virtually devoid of antiinflammatory activity in this experimental system (Fig. 2, Experiments I–II).

3.2. Splenomegaly and fibroadhesive splenitis

An approximately two-fold increase in spleen weight was observed in arthritic animals (Fig. 5). The enlargement was significantly suppressed in rats treated with PMEA or bis(POM)-PMEA ($P < 0.01$), approaching the values typical for control, adjuvant-untreated animals. The effect of a 5-mg/kg dose was less significant for PMEA ($P < 0.5$) or non-significant for bis(POM)-PMEA ($P > 0.05$). Neither PMPA nor bis(POC)-PMPA was able to inhibit the development of splenomegaly.

Gross inflammatory pathology, manifested as fibroadhesive splenitis, was associated with arthritic splenomegaly. It was either undetectable or was expressed as a mild form of infrequent focal capsules on the spleen surface in PMEA- and bis(POM)-PMEA-injected animals. Again, PMPA or bis(POC)-PMPA did not reduce this symptom.

3.3. Systemic changes in NO production

Beginning day 11, an elevated concentration of nitrite + nitrate in serum (Fig. 6A) and urine (Fig. 6B) of control arthritic rats became apparent, and increased further towards day 21 in serum (28-fold increase) or day 16 in

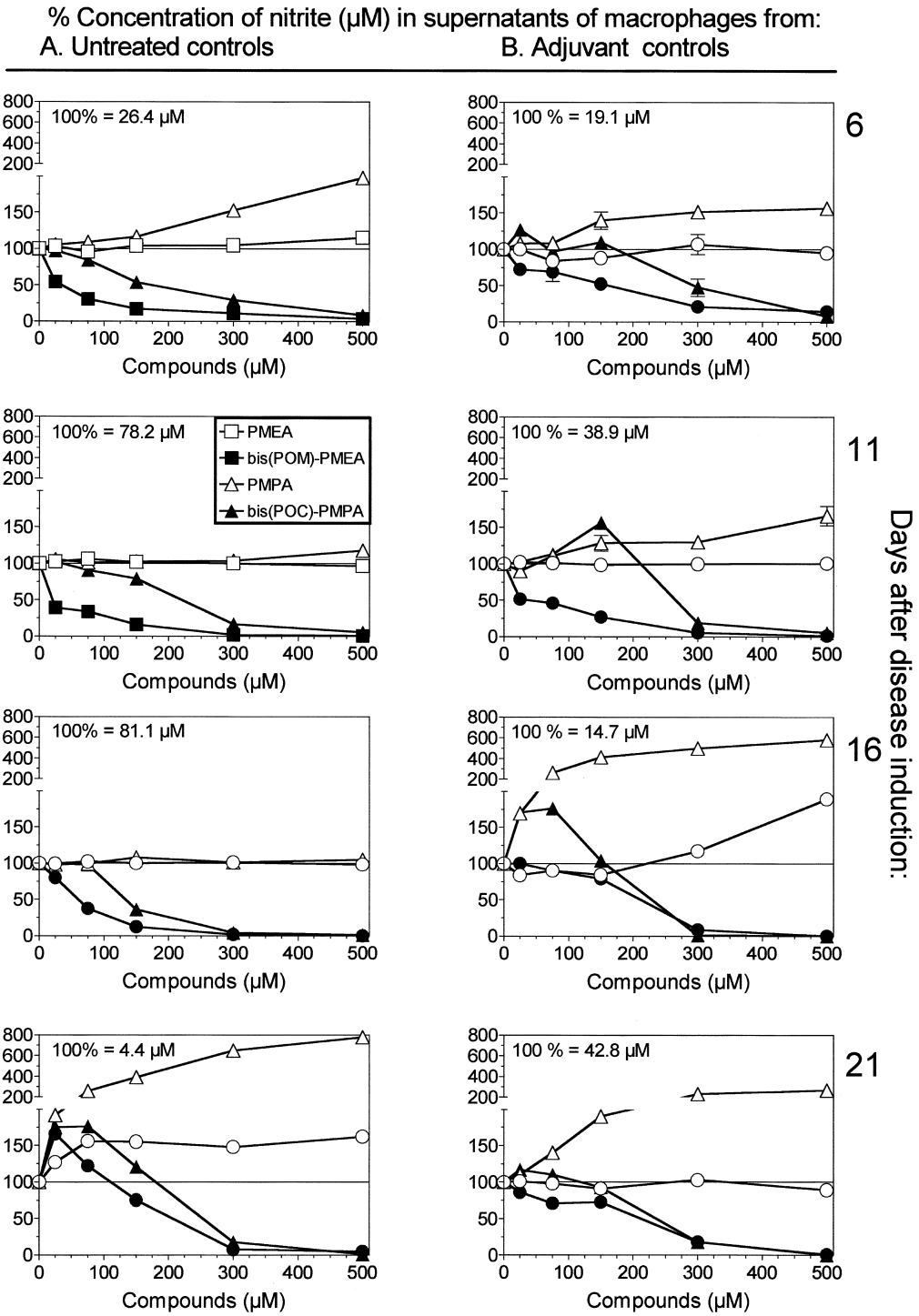

Fig. 7. Interference of acyclic nucleoside phosphonates with in vitro production of nitric oxide by peritoneal macrophages harvested from control untreated (A) or control arthritic (B) rats ($n = 4$) on various days following the injection of mycobacterial adjuvant. Concentration of nitrite in macrophage supernatants was determined after 24 h of cell cultivation. Each point is the mean ± S.E.M.

urine (6-fold increase). However, only a mild or no NO increase was observed in animals treated with PMEA or bis(POM)-PMEA, while administration of PMPA or bis(POC)-PMPA failed to suppress the formation of NO.

3.4. Interference of test compounds with in vitro production of NO by macrophages

Peritoneal macrophages were harvested from untreated or arthritic controls ($n = 4$, each group) on days 6, 11, 16, and 21 following injection of mycobacterial adjuvant, and were cultured in the absence or presence of varying concentrations of test compounds for 24 h, when the supernatant concentration of nitrite was determined (Fig. 7). It should be noticed that except day 21, the NO production by macrophages obtained from adjuvant-treated controls was lower than that from untreated controls; it ranged from 4 μM (day 21) to 81 μM (day 16). Irrespective of the macrophage origin (i.e. untreated versus adjuvant controls) or magnitude of their spontaneous NO activity, the NO production was inhibited by bis(POM)-PMEA and bis(POC)-PMPA. PMEA virtually did not interfere with NO formation, whereas PMPA augmented it, especially when the background NO production was relatively low (see untreated controls on day 21, and arthritic controls on days 16 and 21). The effects were dose-dependent.

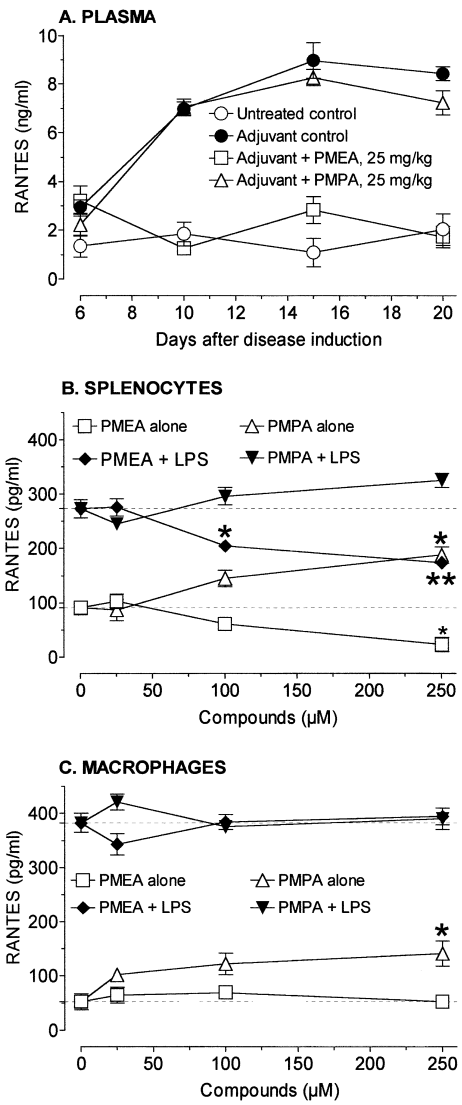

Fig. 8. Effect of PMEA and PMPA on production of RANTES, assayed by ELISA. The chemokine was measured either in the plasma of arthritic rats (A) which were either untreated or s.c. injected with test drugs on days 0–9 ($n = 4$) or in supernatants of splenocytes (B) or peritoneal macrophages (C) obtained from control untreated animals ($n = 3$). The cells were cultured in triplicate for 16 h in the absence or presence of LPS (5 μg/ml). Each point is a mean ± S.E.M. Statistically significant at $^{*}P < 0.05$ or $^{**}P < 0.01$, respectively.

3.5. RANTES production

The concentration of RANTES in plasma of control diseased rats was substantially increased during the whole observation period of 20 days ($P < 0.01$). It was completely inhibited in rats treated with PMEA on days 0–9, whereas PMPA was ineffective in this respect (Fig. 8A).

Additional in vitro studies showed that production of RANTES by splenocytes (Fig. 8B) or macrophages (Fig. 8C) can be stimulated by LPS. Its secretion could be dose dependently reduced by PMEA in splenocyte ($P < 0.01$), but not in macrophage cultures. On the contrary, PMPA augmented the control as well as the LPS-induced RANTES synthesis in both cell types ($P < 0.05$).

4. Discussion

The present data demonstrate the potential of acyclic nucleoside phosphonate PMEA and bis(POM)-PMEA to inhibit the development of adjuvant-induced arthritis in rats. When evaluated from the swelling of the arthritic, i.e. adjuvant-uninjected paw, the suppressive effect was > 80%. The progression of primary inflammation into the secondary arthritic phase at the site of the ipsilateral, i.e. adjuvant-injected hind paw also was significantly attenuated, though the primary swelling (days 0–11) was only marginally affected. In agreement with data of others (Engelhardt et al., 1995; Tanahashi et al., 1998), about two-fold increase in spleen weight, considered to be an expression of immunological abnormalities, developed in arthritic rats. Our findings extend the description of arthritic spleen pathology by the observation of severe fibroadhesive perisplenitis. The splenomegaly was greatly reduced, and perisplenitis was either clinically undiscernible or only manifested as focal capsules on the spleen surface in PMEA- and bis(POM)-PMEA-treated rats. The well reproducible antiarthritic activity was found to be dose-dependent, a dose of 5 mg/kg s.c. being still significantly effective to inhibit secondary paw swelling. It is important

that PMEA exhibits an antiinflammatory potential, not only during the inductive phase of arthritis, but also during the phase of established disease, i.e. when its administration is started at the time of first discernible signs of paw arthritic lesions (on day 10). An approximately equipotent efficacy of PMEA and bis(POM)-PMEA applied at doses of 50 and 25 mg/kg, respectively, was recorded. The prodrug is rapidly and completely hydrolyzed to PMEA (Naesens et al., 1996), and in comparison to the parent structure, it has a many-fold increased cellular uptake (Srinivas et al., 1993) and about two-fold higher oral bioavailability in rats (Starett et al., 1994). With respect to the about twice higher molecular weight of bis(POM)-PMEA than of PMEA, it can be presumed that the antiarthritic efficacy of the prodrug is approximately four-fold higher than that of the parent compound. In addition, oral administration of bis(POM)-PMEA has also been found to inhibit the development of arthritis in rats (Zídek et al., 1995b). An effective dose of 5 mg/kg of PMEA is close to that reported to exhibit anti-HIV activity (Barditch-Crovo et al., 1997). Interestingly, another well recognized antiviral acyclic nucleoside analogue, PMPA, has not been found to possess an antiarthritic potential. The difference between the effects of PMEA and PMPA suggests that the mechanism(s) of the antiarthritic action differs from the mechanism of antiviral activities of these compounds.

One of the major events determining severity and progression of arthritis is a redistribution of antigen-primed immunocompetent cells in the body, their enhanced affinity and migration to the joint synovium. Although the involvement of distinct cell types is controversial, abundant experimental evidence suggests that major lesions of adjuvant arthritis can be passively transferred by both synovial macrophages (Ramos-Ruiz et al., 1992) and T cells from various lymphatic organs, especially when the cells are ex vivo stimulated with concanavalin A (Taurog et al., 1983). The adoptive transfer experiments showed that recruitment of mature host T lymphocytes is crucial for arthritis induction. It has been suggested that these cells activate host macrophages and direct their preferential migration to the joints (Van de Langerijt et al., 1994). As a result, arthritic joint synovium, that is otherwise devoid of cells in the healthy state, becomes rich in all macrophages, T cells and plasma cells (Odeh, 1997), and both locally proliferating and infiltrating cells contribute to a substantially thickened synovial lining (Van den Berg and Van Lent, 1996). The major stimulus for migration of lymphocytes and macrophages is the presence of the C–C family of chemoattractant cytokines (chemokines), such as macrophage inflammatory protein-1α (MIP-1α) and RANTES (Schall et al., 1993; Bacon et al., 1995; Taub et al., 1996). Specifically, RANTES is an important chemoattractant for monocytes in joints of rheumatic patients (Volin et al., 1998). The accumulation of cells in the synovium may be a source of a number of proinflammatory molecules such as cytokines and reactive oxygen and nitrogen species, arachidonic acid metabolites, histamine, serotonin, bradykinin, etc., that either initiate or turn the pathology into a chronic form.

In order to seek for a plausible explanation of antiarthritogenic potential of PMEA, we investigated its possible interference with some of these factors. In accordance with reports of others (Stefanovic-Racic et al., 1994; Stichtenoth et al., 1994; Ueki et al., 1996), we found enhanced levels of NO breakdown products, nitrites + nitrates, in plasma and urine of arthritic rats, but their concentration was substantially suppressed in rats treated with PMEA or bis(POM)-PMEA. There are numerous data showing that as with human rheumatoid arthritis (Farrell et al., 1992), the development of rat arthritis can be suppressed by inhibitors of NO production (Stefanovic-Racic et al., 1994; Connor et al., 1995). However, despite the deep decrease in systemic NO levels in PMEA-treated animals, the antiinflammatory effect of PMEA can hardly be considered to depend on such a mechanism, since therapeutic intervention with the test drugs is diametrically opposed to their ability to interfere with the production of NO by macrophages obtained from either control or arthritic animals. Thus, PMEA does not change NO production, PMPA enhances it, whereas both prodrugs, i.e. bis(POM)-PMEA and bis(POC)-PMPA, inhibit NO formation, no matter at which stage of the disease development the cells are assayed. These findings argue for the view that suppressed systemic levels of NO are to be taken as a consequence of therapeutic effectiveness, rather than as an indicator of the mechanism of the antiarthritogenic action of PMEA. This conclusion may be supported by the fact that attenuation of arthritis severity by these agents is much more profound than that which can be achieved with NO inhibitors (Tanaka et al., 1998). The data contribute to and support the view that high-output NO production is a non-specific subsidiary consequence of arthritis progression, rather than its etiological principle (Tanaka et al., 1996; Gilkeson et al., 1997).

We have already described and analysed a similar interference of PMEA, bis(POM)-PMEA and PMPA with NO production in an in vitro model of murine and rat macrophages (Zídek et al., 1997a, 1999). The NO inhibitory effect of bis(POC)-PMPA is a novel finding, the mechanism of action of which remains to be elucidated.

Incidentally, one special aspect of NO production by rat macrophages should be pointed out. Macrophages from various animal species contain inducible NO synthase which is not expressed constitutively (Förstermann et al., 1995). Paradoxically, cultured peritoneal macrophages from healthy rats may produce NO spontaneously, without an apparent requirement for an exogenous activation signal (Zídek et al., 1995a). We have encountered the same phenomenon in this (viz. Fig. 7) as well as in other experiments (unpublished data) where the concentration of nitrite in supernatants of peritoneal macrophages obtained from conventional, specific-pathogen free, and even

germ-free rats ranged from 0 to 100 μM for individual animals. Cultured rat astrocytes (Stewart et al., 1997) and certain cells in normal rat kidney (Nathan and Xie, 1994) also express inducible NO synthase spontaneously. It should be realized that this peculiar activity might have a serious impact upon the analysis of a role of NO in rat adjuvant arthritis, since its neglect could easily lead to either negative or false positive interpretations.

All standard disease-modifying antirheumatic drugs, such as methotrexate, sulfasalazine, cyclosporin A, glucocorticoids, gold salts, chloroquine and hydroxychloroquine, inhibit production of tumor necrosis factor-α (TNF-α) (Danning and Boumpas, 1998), which may contribute to their antiarthritic effects. PMEA, however, does not share this activity, but rather enhances the secretion of TNF-α (Zídek et al., 1999). On the other hand, it prevents the enhanced systemic levels of RANTES arising in rats with arthritis. This chemokine, produced by human rheumatoid synovial fibroblasts (Rathanaswami et al., 1993) and synovial T cells (Robinson et al., 1995), as well as by other cell types including epithelial cells (Wang et al., 1996) and macrophages (Devergne et al., 1994), is a powerful chemoattractant for monocytes and mainly $CD4^+$ T lymphocytes (Bacon et al., 1995). It has recently been shown that its local and systemic levels are elevated in arthritic animals, and antiRANTES antibodies are able to reduce disease severity (Barnes et al., 1998). The present experiments also showed that the in vitro stimulated synthesis of RANTES by lymphocytes (although not by macrophages) can be suppressed by PMEA but not by PMPA, which is devoid of antiarthritic potential. Therefore, in contrast to interference with NO production, the suppressive influence on RANTES is in parallel with the antiinflammatory intervention of these drugs. In any case, a possible participation of this effect in the intrinsic mechanism(s) of antiarthritic activity of PMEA remains to be firmly established. Involvement of other immunobiological activities of PMEA cannot be excluded. Interestingly, PMEA possesses a more pronounced cytostatic activity, especially directed against T lymphocytes, than does PMPA (Holý et al., 1996).

Although no definitive conclusions have been reached regarding the causal relationship between arthritis and HIV infection, it has been suggested that a common, i.e. immunosuppressive antiarthritic therapy, should be avoided because of potentially detrimental effects in patients with AIDS (Rosenberg et al., 1989; Munoz-Fernandez et al., 1991). As the main therapeutic indication for PMEA is AIDS, this drug could become a promising candidate for the treatment of rheumatic disorders in these patients.

Acknowledgements

This work was supported by grant No. 307/97/0069 from the Grant Agency of the Czech Republic.

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