Pergamon

Biochemical Pharmacology, Vol. 50, No. 3, pp. 347–353, 1995.

0006-2952/95 $9.50 + 0.00

0006-2952(95)00148-4

EFFECTS OF AN ANTI-INFLAMMATORY PEPTIDE (ANTIFLAMMIN 2) ON CELL INFLUX, EICOSANOID BIOSYNTHESIS AND OEDEMA FORMATION BY ARACHIDONIC ACID AND TETRADECANOYL PHORBOL DERMAL APPLICATION

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(*Received* 21 *November* 1994; *accepted* 15 *March* 1995)

Abstract—Antiflammins are synthetic peptides with sequence homology to proteins inhibitory for phospholipase A_2 (EC 3.1.1.4). The effect of antiflammin 2 on murine arachidonate or 12-*O*-tetradecanoylphorbol 13-acetate (TPA)-induced ear oedema has been studied. Topical application of arachidonic acid (AA) produced a short-lived oedema response with rapid onset associated with marked increases in prostaglandin E_2 levels. TPA produced a longer-lasting oedema associated with marked influx of neutrophils and mononuclear cells as well as predominant formation of leukotriene B_4 (LTB_4). Topical pretreatment with indomethacin or dexamethasone reduced plasma leakage, oedema and prostaglandin E_2 biosynthesis in AA-induced oedema, whereas antiflammin 2 had no effect. However, topical pretreatment with antiflammin 2 dose-dependently reduced plasma leakage, cell influx, oedema and LTB_4 levels in response to TPA. These results indicate that the anti-inflammatory effect of antiflammins can be attributed to AA mobilization and/or 5 lipoxygenase inhibition but can be dissociated from an effect on arachidonic acid metabolism by the cyclooxygenase pathway.

Key words: antiflammins; anti-inflammatory peptides; phospholipase A_2; prostaglandin E_2; leukotriene B_4; inflammation

Glucocorticoids are among the most effective drugs for the treatment of inflammatory diseases. The anti-inflammatory activity of glucocorticoids is the result of several modulating actions on cells involved in the inflammatory process [1]. Inhibition of biosynthesis and release of eicosanoids is an important mechanism of steroid action. It has been proposed that this effect could be the result of the synthesis and/or release of proteins with anti-PLA_2† activity such as lipocortins [2, 3] demonstrated by Ahluwalia *et al.* [4] to increase in the skin following application of a glucocorticoid. On the other hand, Levin *et al.* [5] reported that uteroglobin, a progesterone-induced protein, also inhibited PLA_2 (EC 3.1.1.4) activity. Recently, several reviews on lipocortin 1, uteroglobin and lipocortin-derived peptides have attempted to assess the role of these peptides in mediating glucocorticoid-induced effects on inflammation [6–8].

Two basic synthetic nonapeptides with amino acid sequences derived from regions of high similarity in uteroglobin and lipocortin I have been described as inhibitors of porcine pancreatic PLA_2 [9, 10]. The nonapeptide MQMKKVLDS, called P1 or AF-1, is equivalent to the nine amino acid C-terminal portion of α-helix 3 in uteroglobin, whereas nonapeptide P2 or AF-2, HDMNKVLDL, corresponds to the 246–254 sequence of lipocortin I. These peptides, named "antiflammins", also showed an anti-inflammatory effect in carrageenan-induced rat paw oedema [9, 10]. The ability of antiflammins to inhibit pancreatic and *Naja naja* PLA_2 *in vitro* as well as their anti-inflammatory activity *in vivo* has been questioned by several authors [11–15]. However, we demonstrated that these peptides were able to inhibit acute inflammatory processes induced by carrageenan or phorbol esters when administered locally or parenterally [16, 17]. In contrast, antiflammins had no effect on the inflammation induced by exogenous PLA_2 administration [18]. These results indicate that the antiflammins may have a direct inhibitory effect on PLA_2 activation but not on enzyme or enzyme–substrate interaction.

The purpose of this paper was to study the effect of antiflammins on cells and inflammatory mediators involved in the development of ear oedema induced by AA or TPA.

MATERIALS AND METHODS

Animals. Male Swiss Webster mice (Charles River, Barcelona, Spain) were used. The animals were housed with a 12 hr lighting schedule (06:00–18:00 hr

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† Abbreviations: PLA_2, phospholipase A_2; TPA, 12-*O*-tetra-decanoylphorbol 13-acetate; AA, arachidonic acid; LTB_4, leukotriene B_4; PGE_2, prostaglandin E_2; AF-2, antiflammin 2; HTAB, hexadecyltrimethylammonium bromide; MPO, myeloperoxidase; NAG, *N*-acetyl-β-D-glucosaminidase; 6-keto-$PGF_{1\alpha}$, 6-keto-prostaglandin $F_{1\alpha}$; RIA, radioimmunoassay; COX, cyclooxygenase; Dex, dexamethasone; Ind, indomethacin; DMF, dimethylformamide

on) with access to food and water *ad libitum*. Housing and experimental procedures were conducted according to standard conditions.

Materials. AA, DHTAB, Ind, *o*-dianisidine 2HCl and TPA were purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.). Human polymorphonuclear leukocyte MPO, NAG from beef kidney and *p*-nitrophenyl-2-acetamido-β-D-glucopyranoside were obtained from Calbiochem (San Diego, CA, U.S.A.). AF-2 was purchased from Bachem Feinchemikalien AG (Switzerland) with a purity by HPLC > 98%. It was stored under argon in sealed glass vials and desiccated at −20° until use. Prior to opening the flask it was warmed to room temperature to avoid water condensation, and then dissolved before each assay in Tris-HCl 10 mM pH 8.0 buffer. Peptide was never stored in solution. All other reagents and chemicals used in the experiments were of analytical grade.

Experimental protocols. To minimize the possibility of any variability due to a diurnal cycle, all the experiments were conducted between 08:00 and 17:00 hr. All drugs were administered topically in a volume of 20 μL/ear 5 min before AA or TPA application. An exception was dexamethasone, which was administered 2 hr before AA application. Indomethacin and dexamethasone were dissolved in acetone; this solvent did not have any action on inflammatory processes.

AA and TPA inflammation models. Groups of mice received arachidonic acid (2 mg) or TPA (10 μg) on the right ear, dissolved in acetone at concentrations of 100 mg and 500 μg/mL, respectively. The phlogogenic agents were applied in 10 μL each to the inner and outer surface of the ear. The left ear received acetone, delivered in the same manner. Treatments were given topically 5 min after AA or TPA application except for dexamethasone, which was administered topically 2 hr before inflammation was induced. Mice were killed by CO_2 inhalation and a 7 mm diameter section of the right and left auditory pinna, measured from the apex, were cut and the samples weighed and used for MPO and NAG measurements as described below. The measurements were taken from 0 to 3 hr after AA-induced inflammation and from 0 to 24 hr after TPA application.

Vascular permeability. To determine vascular permeability in response to arachidonic acid or phorbol ester, mice were intravenously injected into tail veins with Evans blue (300 mg/kg) 30 min before topical application of arachidonic acid or TPA. Mice were killed and ears removed, and the Evans blue was extracted with DMF (2 mL/ear; Istral polytron, speed 10, 15 sec) and centrifuged (32,000 *g*, 10 min). Absorbance readings were measured spectrophotometrically at 590 nm in quartz cells and the amount (μg) of Evans blue calculated using a standard curve. Evans blue removed from ears was proportional to the vascular permeability increase and oedema formation.

Measurement of myeloperoxidase and N-acetyl-β-D-glucosaminidase activities. Myeloperoxidase, a haemoprotein located in azurophil granules of neutrophils where it plays a role in bacterial killing, has been used as an enzyme marker of neutrophil infiltration into inflamed tissues, as proposed by Bradley *et al.* [19]. In a similar form, *N*-acetyl-β-D-glucosaminidase levels have been used as an indicator of mononuclear cell infiltration [20].

Ear samples were homogenized in a Ystral polytron in 3 mL of 50 mM potassium phosphate buffer (pH 6.0) and centrifuged (30,000 *g*, 20 min), and supernatants were discarded. Tissue pellets were reextracted into potassium phosphate buffer (50 mM, pH 6.0) with 0.5% HTAB, freeze–thawed three times, and centrifuged to collect supernatants used in MPO and NAG assays.

MPO assays were performed according to procedures described by Bradley *et al.* [19] adapted to a 96-well plate format. Briefly, 10 μl of test samples or MPO standards were added to the wells. The reaction was initiated by the addition of 200 μL of assay buffer containing 0.167 mg/mL *o*-dianisidine and 0.0005% hydrogen peroxide (pH 6.0). The rate of change of absorbance at 405 nm was monitored by a Titertek Uniskan reader.

For NAG assays, 25 μL samples were transferred to a 96-well plate followed by 25 μL of 2.24 mM solution of *p*-nitrophenyl-2-acetamide-β-D-glucopyranoside and 100 μL of citrate buffer (50 mM, pH 4.5). Following a 60 min incubation at 37°, the reaction was stopped by the addition of 100 μL of glycine buffer (200 mM, pH 10.4), and absorbance read at 405 nm. Human neutrophil MPO and NAG from beef kidney were used as the reference standard.

Extraction of eicosanoid metabolites. Mouse ears were homogenized with 1 mL of methanol containing 1% 1 M HCl. Then, 2 mL of distilled water was added to each tube, and the tubes were kept on ice for 30 min. The resulting insoluble proteins were removed by centrifugation (30,000 *g*, 20 min). Arachidonic acid metabolites were measured as we previously described [21]. The eicosanoids were extracted with ethyl acetate (6 mL) and after the aqueous phase was discarded, the organic phase was evaporated in a stream of nitrogen. PGE_2, 6-keto-$PGF_{1\alpha}$ and LTB_4 present in the extracts were measured using the specific protocols described by RIA kits from New England Nuclear (Boston, MA, U.S.A.). The overall recoveries for the extraction procedure were established by including tritiated eicosanoids and were 85% for LTB_4 and 80% for both PGE_2 and 6-keto-$PGF_{1\alpha}$. The lower limit of detection of prostaglandins and leukotrienes by RIA was 7 pg/mL. The antibody for LTB_4 cross-reacted with LTC_4 and LTD_4 (36%). The antibodies for 6-keto-$PGF_{1\alpha}$ and PGE_2 minimally cross-reacted with other E-series prostaglandins (<1%).

Statistical analysis. Data are expressed as the mean ± SEM. Because experiments were performed on tissue pairs, control ear and ear administered with phlogogenic agent, the determinations were fit simultaneously to pairs of ears in order to obtain parameter estimates free of animal-to-animal variation. The percentage of inhibition is defined by the following equation:

$$\% \text{ inhibition} = (Cr - Tr)/Cr \times 100$$

where Cr and Tr refer to values in AA (or TPA)

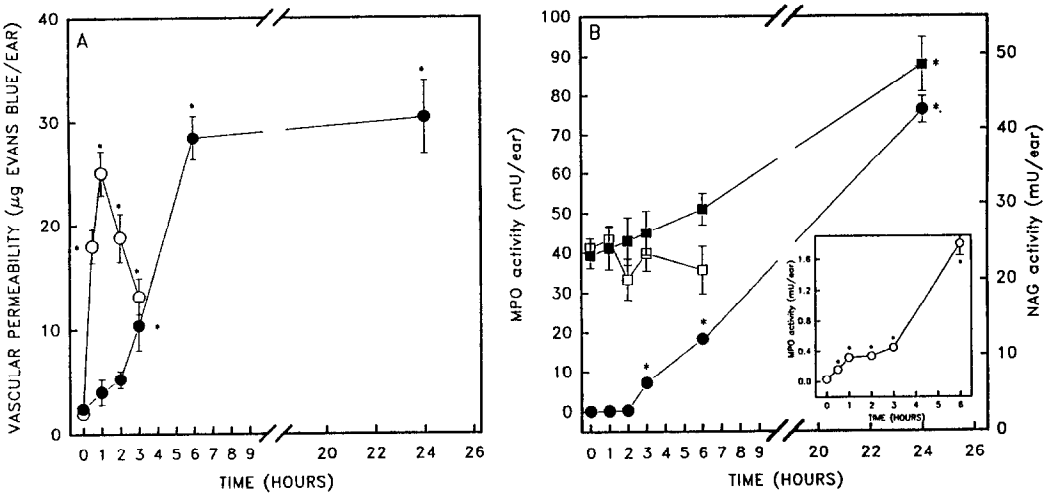

Fig. 1. Time course of increased vascular permeability (A) induced by topical application of AA (○, 2 mg/ear) or TPA (●, 10 μg/ear). Results expressed as the increase of Evans blue extravasated are shown as mean ± SEM of six to eight determinations. Time course of neutrophil or monocyte/macrophage influx (B) induced by topical application of AA (○ □, 2 mg/ear) or TPA (● ■, 10 μg/ear). Results expressed as MPO activity (circles) or NAG activity (squares), respectively are shown as mean ± SEM of six to eight determinations. * Significantly different from control values ($P < 0.05$).

drug-non-treated groups and AA (or TPA) drug-treated groups, respectively.

Statistical significance was assessed by one-tailed Student's *t*-test for unpaired samples, with $P < 0.05$ regarded as significant.

RESULTS

Topical application of AA or TPA produced dose-dependent increases in mouse ear weight (data not shown). Consistent with earlier reports [22], the AA-induced oedema response was rapid in onset with a short duration. In contrast, TPA produced a longer-lasting response with a delayed onset. Topical application of AA transiently increased vascular permeability measured by Evans blue extravasation (Fig. 1A). Vascular permeability and oedema had a similar time course. Similarly, TPA-induced changes in vascular permeability paralleled ear oedema.

The accumulation of neutrophils and monocytes/macrophages is a characteristic feature of dermal inflammatory responses. Topical application of arachidonic acid (2 mg/ear) resulted in time-dependent increases in mouse ear MPO levels that lagged behind the oedema response (Fig. 1B). MPO constitutes nearly 5% of neutrophil protein content [23] and the utility of its measurement to correlate neutrophil influx is well established [19]. Topical TPA application (10 μg/ear) resulted in a gradual increase in MPO levels, which persisted up to 24 hr (Fig. 1B). Influx of monocytes/macrophages typically occurs later than neutrophil influx. Levels of NAG were used as an indicator of the presence of monocyte/macrophage cells. AA application did not increase NAG levels over control levels during the period studied (Fig. 1B). Phorbol ester, in contrast, increased NAG levels nearly two-fold at 6 hr, when MPO levels and ear oedema were also significantly raised.

In order to define the nature of the mediators of inflammation in these experimental models, the levels of PGE_2, LTB_4 and 6-keto-$PGF_{1\alpha}$ were measured in ear samples following topical application of AA or TPA. Following AA application, levels of PGE_2 markedly increased for 30 min, and decreased thereafter. There were also smaller increases in levels of LTB_4 (Fig. 2). Following the topical application of TPA (10 μg/ear), the only measurable changes in levels of metabolites from the cyclo-oxygenase pathway were seen in 6-keto-$PGF_{1\alpha}$. Levels of LTB_4 were increased slowly (Fig. 2).

The effects of AF-2, Ind and Dex on oedema response, MPO and NAG levels, PGE_2 and LTB_4 levels, and vascular permeability were examined both in TPA and arachidonate models. Ind (ED_{50} 170 μg/ear or 470 nmols/ear), Dex (ED_{50} 6.5 μg/ear or 16 nmols/ear) and AF-2 (ED_{50} 24 μg/ear or 21 nmols/ear) administered topically attenuated the TPA-induced inflammatory response (Fig. 2). Despite substantial reductions in oedema response, indomethacin had only a slight effect on MPO levels. However, the treatment with dexamethasone or AF-2 significantly inhibited MPO and NAG levels (Table 1). Peak changes in vascular permeability were seen 6 hr after application of TPA. Topical treatments with Dex or AF-2 were more efficacious than Ind in attenuating inflammatory response as determined by oedema (Fig. 2) and by vascular permeability changes (Fig. 3).

Topical application of indomethacin and dexamethasone significantly attenuated the AA-induced oedema response in a dose-dependent fashion (Fig. 2). Moreover, AF-2, at doses that did not attenuate the AA-induced oedema response, did not affect

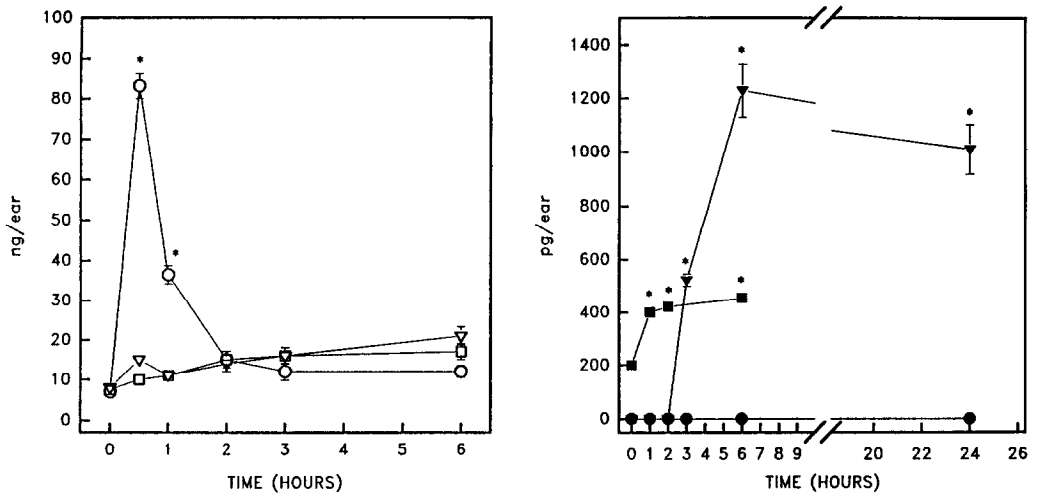

Fig. 2. Time course of PGE_2 levels (circles), LTB_4 (triangles) or 6-keto-$PGF_{1\alpha}$ (squares) levels in AA (open symbols) and TPA (closed symbols) induced ear oedema. Results are expressed as mean ± SEM of six to eight animals. * Significantly different from control values ($P < 0.05$).

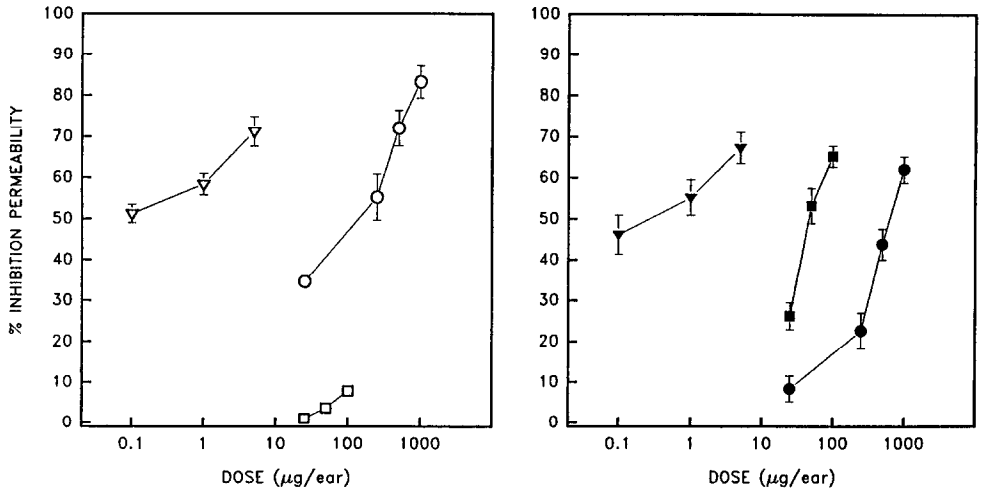

Fig. 3. Inhibition of increased vascular permeability induced by AA (open symbols) or TPA (closed symbols) upon topical administration of Ind (circles), Dex (triangles) or AF-2 (squares) 5 min before phlogogens except for Dex, which was administered 1 hr before AA. The effects of drugs were determined 1 hr or 6 hr after AA or TPA application, respectively. Results expressed as the inhibitory effect on Evans blue extravasation are shown as mean ± SEM of six to eight mice. * Significantly different from the non-treated group ($P < 0.05$).

the influx of neutrophils as measured by increases in MPO (Table 2). However, Dex inhibited the AA-induced increases in neutrophil influx in a dose-independent manner (Table 2). Furthermore, Ind and Dex attenuated vascular permeability changes in the arachidonate model. In contrast, AF-2 was ineffective (Table 2). Taken together, these results strongly support the hypothesis that the lack of effect of AF-2 on PGE_2 biosynthesis is related to the lack of anti-inflammatory action of the nonapeptide when inflammation was induced with AA.

DISCUSSION

Acute inflammatory reactions are characterized by changes in vascular permeability and vasodilatation, resulting in oedema and cell influx.

In the present study, both AA and TPA were able to induce vascular permeability increase, oedema, and cellular influx, inferred from MPO or NAG measurement, with markedly different time course profiles and mediators.

AA elicited an inflammatory response charac-

Table 1. Effect of Ind, Dex and AF-2 on neutrophil and mononuclear cell influx and 6-keto-$PGF_{1\alpha}$/LTB_4 levels in TPA-induced ear

Treatment (μg/ear)	MPO levels (mU/ear)	NAG levels (mU/ear)	6-keto-$PGF_{1\alpha}$ (pg/ear)	LTB_4 levels (pg/ear)
Non-treated	0.02	23.1 ± 1.1	198 ± 22	0.30
TPA	75.1 ± 3.2	47.5 ± 2.9	467 ± 23	1252 ± 163
TPA + Ind (1000)	56.3 ± 2.1*	36.6 ± 2.7	212 ± 13*	663 ± 153*
TPA + Ind (500)	60.0 ± 3.2	39.0 ± 2.8	256 ± 21*	857 ± 193
TPA + Ind (250)	64.7 ± 2.7	41.3 ± 3.2	302 ± 28*	1173 ± 201
TPA + Ind (25)	67.0 ± 2.9	45.1 ± 3.1	376 ± 32	ND
TPA + Dex (5)	24.7 ± 2.1*	20.2 ± 2.5*	218 ± 16*	263 ± 107*
TPA + Dex (1)	31.5 ± 2.0*	24.8 ± 2.7*	225 ± 23*	303 ± 112*
TPA + Dex (0.1)	41.2 ± 2.6*	28.2 ± 2.8*	267 ± 28*	563 ± 126*
TPA + AF-2 (100)	38.2 ± 2.5*	27.1 ± 2.6*	254 ± 26*	523 ± 128*
TPA + AF-2 (50)	42.7 ± 3.1*	30.9 ± 2.9*	ND	697 ± 131*
TPA + AF-2 (25)	48.2 ± 3.2*	39.1 ± 3.1	381 ± 31	893 ± 161

Cell influx and 6-keto-$PGF_{1\alpha}$/LTB_4 levels were tested in TPA-induced ear oedema as described in the Methods Section. Ind and Dex were dissolved in acetone whereas AF-2 was dissolved in Tris-HCl 10 mM pH 8.0 buffer. Drugs were administered topically in a volume of 20 μL/ear 5 min before TPA application. TPA effect was determined 6 hr after eliciting the inflammation. Results are mean ± SEM of six to eight mice.

* Significantly different ($P < 0.05$ or less) from non-treated group. ND, not determined.

Table 2. Effect of indomethacin, Dex and AF-2 on neutrophil influx and PGE_2/LTB_4 levels in AA-induced ear oedema

Treatment (μg/ear)	MPO levels (mU/ear)	PGE_2 levels (ng/ear)	LTB_4 levels (ng/ear)
Non-treated	0.03	0	0
AA	1.70 ± 0.21	85.3 ± 3.6	17.1 ± 2.0
AA + Ind (1000)	0.82 ± 0.13*	6.2 ± 1.1*	6.8 ± 1.1*
AA + Ind (500)	0.85 ± 0.11*	19.0 ± 1.3*	9.9 ± 0.9
AA + Ind (250)	1.01 ± 0.15	33.5 ± 1.9*	14.2 ± 1.1
AA + Ind (25)	1.39 ± 0.21	49.6 ± 2.2	16.3 ± 1.2
AA + Dex (5)	0.36 ± 0.12*	9.7 ± 2.1*	5.2 ± 0.8*
AA + Dex (1)	0.43 ± 0.13*	17.3 ± 1.8*	7.6 ± 1.2*
AA + Dex (0.1)	0.51 ± 0.11*	21.6 ± 2.0*	9.3 ± 1.1
AA + AF-2 (100)	1.68 ± 0.21	80.1 ± 3.2	18.1 ± 2.2
AA + AF-2 (50)	1.73 ± 0.13	79.2 ± 3.1	17.6 ± 1.3
AA + AF-2 (25)	1.72 ± 0.20	83.2 ± 2.8	15.2 ± 1.2

Cell influx and PGE_2/LTB_4 levels were tested in AA-induced ear oedema as described in the Methods section. Ind and Dex were dissolved in acetone whereas AF-2 was dissolved in Tris-HCl 10 mM pH 8.0 buffer. Drugs were administered topically in a volume of 20 μL/ear 5 min before AA application, except for Dex, which was administered 120 min before AA application. Drug effects were determined 30 min after eliciting the inflammation. Results are mean ± SEM of six to eight mice.

* Significantly different ($P < 0.05$ or less) from non-treated group.

terized by rapid onset of oedema and vascular permeability and marked prostaglandin biosynthesis, minimal cellular influx, and short duration of action. In contrast, the phorbol ester (TPA) elicited an inflammatory response characterized by a delayed time of onset, longer-lasting inflammation associated with marked cellular influx and moderate eicosanoid biosynthesis. TPA, but not AA, increased NAG levels, suggesting an increase in monocyte/macrophage cell influx. Interestingly, unlike arachidonate, TPA-elicited changes in vascular permeability paralleled oedema and MPO changes and were longer lasting. The AA and TPA models differed considerably in their mediator biosynthetic

profile, with the former dominated by prostanoids and the latter by LTB_4 as recently reported by Rao *et al.* [24]. At the inflammatory site where there is damaged endothelium, vasodilators may potentiate plasma leakage and hence oedema formation by increasing blood flow [25]. In the present study, plasma leakage in AA-induced oedema appeared to be dependent on prostaglandins, whereas plasma leakage in the TPA-induced oedema could be dependent on LTB_4 released from either neutrophils or monocytes. These results are consistent with earlier reports of dermal inflammation in guinea pig [26, 27].

Two nonapeptides with amino acid sequences contained in the 36 kDa proteins uteroglobin and lipocortin I were described by Miele *et al.* [9] as potent inhibitors of PLA_2 with striking anti-inflammatory activity *in vivo*. These authors suggested that the oligopeptides AF-1 and AF-2 are able to mimic the biological effect of these proteins because they may represent an active site, or part of an active site, of the proteins. Our previous results using AA or TPA-induced ear inflammation models [16, 17] are in line with Miele *et al.*'s hypothesis [9].

It is evident that the rate-limiting steps of these experimental models of acute inflammation are represented by the coordinated activation of PLA_2, arachidonate metabolism and cell influx. The question of whether antiflammins inhibit PLA_2 activity and arachidonate cascade is still controversial. Our purpose was to study the effect of antiflammins on cells and inflammatory mediators involved in the development of these inflammatory models.

The data presented here demonstrate that AF-2 does not affect ear oedema induced by arachidonate or by TPA in the same fashion. Thus, the inhibitory effect of the nonapeptide was dose-dependent on TPA-induced oedema, whereas we observed a lack of significant action on AA-induced oedema. However, the response to AA-induced oedema was substantially reduced by the COX inhibitor, indomethacin, indicating that this inflammation is mediated by prostaglandin-like substances, as shown here. Additionally, the response to PGE_2 biosynthesis in an AA-induced oedema model was found to be sensitive to Ind and Dex, whereas AF-2 had no action, strongly suggesting that the anti-inflammatory effects of AF-2 were not brought about through reduction of cyclooxygenase activity, as proposed recently by Perretti *et al.* [28]. These results are apparently contradictory to the data reported by Calderano *et al.* [29], who observed that AF-2 inhibits COX activity in rabbit distal colonic mucosa *in vitro*. We should bear in mind that COX exists in at least two isoforms with similar molecular structures. COX-1 is expressed constitutively and was the first to be characterized, whereas COX-2 is induced in cells exposed to proinflammatory agents, and the two isoforms can be differentially inhibited.

The anti-inflammatory activity of AF-2 in the murine model of phorbol ester-induced inflammation is consistent with previous results [16]. Furthermore, we now report that AF-2 treatment was able to inhibit neutrophil and mononuclear accumulation induced by TPA. Vasanthakumar *et al.* [30] demonstrated that uteroglobin inhibited human and rabbit phagocyte chemotaxis. Recently, Camussi *et al.* [31] demonstrated that antiflammins inhibit neutrophil chemotaxis *in vitro*, leading them to suggest that antiflammins could inhibit synthesis of inflammatory mediators such as leukotrienes derived from AA. In addition, they proposed that LTB_4 is a potent chemotactic agent that mediates changes in vascular permeability. The raised levels of LTB_4 observed in this experimental model further support an important role of leukotrienes in cell influx as well as phorbol ester-induced oedema. Besides, the results of the present study clearly show that AF-2 reduced LTB_4 levels, and this effect may be correlated with its inhibitory action on cell influx, vascular permeability increase or oedema induced by TPA. Recently, we observed that antiflammins may be able to reduce mast cell degranulation and oedema induced by *Naja naja* PLA_2 [17]. We can not exclude the possibility that mast cell degranulation induced by LTB_4 may play a role in TPA-induced ear oedema that could be affected by antiflammins.

In summary, the results of the present study suggest a role for both COX and lipoxygenase products in acute inflammatory responses elicited by AA or TPA. Interestingly, the anti-inflammatory effects of AF-2 in the TPA model suggest that this nonapeptide could affect AA mobilization and/or AA metabolism by 5-lipoxygenase. Furthermore, our results provide evidence that the actions of AF-2 do not involve inhibition of AA metabolism by COX under our experimental conditions. However, an alternative mechanism to anti-inflammatory effect of antiflammins may involve the antichemotactic effects of fragments of lipocortins or uteroglobin such as antiflammins. Further studies to examine the mechanism of anti-inflammatory action of antiflammins in cells involved in inflammatory processes are necessary.

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