Volume 291, number 2, 249–252 FEBS 10296 October 1991

ADONIS 001457939101 10063

PDGF suppresses the activation of group II phospholipase A_2 gene expression by interleukin 1 and forskolin in mesangial cells

Heiko Muhl[1], Thomas Geiger[1], Werner Pignat[1], Fritz Markl[1], Henk van den Bosch[2], Klaus Vosbeck[1] and Josef Pfeilschifter[1]

[1]*Research Department, Pharmaceuticals Division, Ciba-Geigy Ltd, CH-4002 Basel, Switzerland* and [2]*Centre for Biomembranes and Lipid Enzymology, Padualaan 8, 3584 CH Utrecht, The Netherlands*

Received 14 Augustus 1991

Treatment of rat mesangial cells with interleukin 1β (IL-1β) and forskolin greatly enhanced the expression of group II phospholipase A_2 (PLA_2) mRNA, with subsequent increased synthesis and secretion of PLA_2, as detected by PLA_2 activity measurements and immunoprecipitation of culture media of [^{35}S]methionine-labelled mesangial cells PDGF–BB dose-dependently suppressed the IL-1β- and forskolin-induced elevation of PLA_2 mRNA, as well as PLA_2 synthesis and secretion In contrast, PDGF–AA had no inhibitory effect The tyrosine kinase inhibitor genistein dose-dependently antagonized the inhibitory effect of PDGF-BB on IL-1β-stimulated PLA_2 secretion, thus suggesting that tyrosine phosphorylation may be required for PDGF-BB inhibition of PLA_2 gene expression in mesangial cells

Phospholipase A_2, Interleukin 1, Phospholipase A_2 mRNA, PDGF, Genistein, Mesangial cell

1 INTRODUCTION

Arachidonic acid release from the sn-2 position of phospholipids by PLA_2 activation is thought to be the rate-limiting step of eicosanoid synthesis in many cells [1,2] But PLA_2 not only plays an important intracellular role, it is also secreted from a variety of cells during inflammatory processes and may thereby contribute to the pathological alterations observed [3] PLA_2s comprise a diverse group of enzymes that can be divided into a class of high mol. wt. PLA_2s of 60–110 kDa and a class of low mol wt PLA_2s of 14 kDa Members of both classes from human and rat sources have recently been cloned [4–8]. The low mol wt PLA_2s are further divided into group I (the pancreatic type of PLA_2) and group II PLA_2 [9] Group II PLA_2 is synthesized and secreted from many tissues and cell types and is believed to play a role in the initiation and propagation of inflammatory processes [3] In addition, potent pro-inflammatory cytokines, such as IL-1 or tumour necrosis factor α (TNFα) have been found to increase group II PLA_2 gene expression in rabbit chondrocytes [10,11], rat vascular smooth muscle cells [12] and HepG2 cells [13]

Glomerular mesangial cells are being increasingly recognized as important target and effector cells in the pathogenesis of acute and chronic forms of renal diseases [14] Recently we have shown that stimulation of mesangial cells with IL-1α, IL-1β, TNFα or forskolin increases the synthesis and secretion of prostaglandin E_2 and group II PLA_2 [15–17]. Extracellular PLA_2 secreted by mesangial cells may participate in inducing and maintaining inflammatory reaction within the glomerulus. The latter suggestion gains support from our recent observations, demonstrating that anti-inflammatory steroids and transforming growth factor β_2 potentially antagonize IL-1β- and TNFα-induced PLA_2 synthesis [18–20]

Platelet-derived growth factor (PDGF) is a dimeric protein composed of two closely related but not identical chains, denoted A and B, which are linked by disulphide bonds All three possible dimeric molecules of PDGF have been isolated from natural sources. We report here that PDGF-BB is another potent inhibitor of IL-1β and forskolin-induced PLA_2 synthesis acting at the level of PLA_2 gene expression

Abbreviations PLA_2, phospholipase A_2, IL-1β, interleukin 1β, PDGF, platelet derived growth factor

Correspondence address J Pfeilschifter, Ciba-Geigy Ltd, R-1056 P 23, CH-4002 Basel, Switzerland Fax (41) (61) 6973968

2. MATERIALS AND METHODS

2 1 *Cell cultures and incubation*

Rat glomerular mesangial cells were cultivated as described previously [21] In a second step, single cells were cloned by limiting dilution using 96-microwell plates Clones with apparent mesangial cell morphology were characterized [22] and used for further processing The cells were grown in RPMI 1640 supplemented with 10% fetal calf serum, penicillin (100 U/ml), streptomycin (100 μl/ml) and bovine insulin at 0 66 U/ml For the experiments passages 12-41 of mesangial cells were used Confluent mesangial cells cultured in 16 mm diameter wells or 60 mm diameter dishes (for PLA_2 immunoprecipitation), were washed twice with PBS and incubated with 1 ml of RPMI 1640, containing 0 1 mg/ml of fatty acid-free bovine serum albumin (Sigma)

with or without agents for the indicated time periods Thereafter, the medium was withdrawn and centrifuged for 10 min at 1000 rpm in an IEC Centra-7R lab centrifuge The supernatant was removed and frozen in liquid nitrogen and stored until assayed for protein content and PLA_2 activity Protein was determined by the method of Bradford [23] with bovine serum albumin as the standard 80 μCi of [^{35}S]methionine were added to 2 ml of methionine-free culture medium for the labelling of newly synthesized proteins The incubation time was 6 h

2 2 *Phospholipase A_2 assay*

PLA_2 activity was determined using [1-^{14}S]oleate-labelled *E coli* as substrate as described [24] Assay mixtures (1 0 ml) contained 100 mM Tris-HCl (pH 7 4), 1 0 mM $CaCl_2$, 5 nM *E coli* phospholipid (3000–5000 cpm) and the enzyme to be tested at a dilution producing approximately 5% substrate hydrolysis Reactions were stopped after 1 h and the liberated [^{14}C]oleate was extracted by a modified Dole extraction procedure [24]

2 3 *Immunoprecipitation*

For immunoprecipitation, 1 ml of culture supernatant was added to 6 ml of buffer A (20 mM Tris-HCl, pH 7 6, 140 mM NaCl, 5 mM EDTA, 1% (v/v) Triton X–100) containing 1 mM phenylmethylsulfonyl fluoride After addition of 10 μl of antibodies [25 26] and incubation at 4°C overnight, the antigen–antibody complexes were bound to 10 mg (dry weight) of a mixture of protein A-Sepharose and protein G-Sepharose, washed four times with buffer A and twice with 50 mM sodium phosphate buffer, pH 7 5 Elution was routinely achieved by incubation with 0 1 M Tris-HCl, pH 6 8 5% mercaptoethanol, 5% sodium dodecyl sulfate, and 10% glycerol at 95°C for 3 min The eluted proteins were analyzed by SDS-PAGE using a 10–15% polyacrylamide step gel [27] and subjected to fluorography [28]

2 4 *Northern blot analysis*

Confluent mesangial cells were harvested from 60 mm diameter culture dishes with a rubber policeman Total RNA was extracted from the cells using the guanidinium thiocyanate/phenol/chloroform method [29] The RNA was precipitated with isopropyl alcohol, the pellets were washed with 75% ethanol, and samples of 15 μg RNA were separated on 1% agarose gels containing 6 6% formaldehyde prior to transfer to Gene Screen membranes (New England Nuclear) [30] After baking the filters at 80°C for 2 h and prehybridization for 6 h, the filters were hybridized with a 39-mer antisense oligonucleotide for rat group II PLA_2 (nucleotide 58–96, [6]), that had been labelled with T4-polynucleotide kinase and [γ-^{32}P]ATP [30] To correct for variations in RNA amount a parallel filter was hybridized with a genomic clone for rat 28 S ribosomal RNA [31], labelled with the random primer technique described by Feinberg and Vogelstein [32] After hybridization, the membrane was washed 3 times in 4 × SSPE, 0 1% sodium dodecyl sulfate at 50°C for 20 min (20 × SSPE 3 6 M sodium chloride, 0 2 M sodium phosphate, 0 02 M EDTA pH 7 4) The signal was detected by autoradiography

2 5 *Chemicals*

[1-^{14}C]Oleic acid and nylon membranes (Gene Screen) were purchased from DuPont de Nemours International (Regensdorf, Switzerland), adenosine 5'-[γ-^{32}P]triphosphate (specific activity >110 TBq/mM) and [^{35}S]methionine (specific activity >37 TBq/mM) were purchased from Amersham International (UK), forskolin was from Calbiochem (Lucerne, Switzerland), recombinant human IL-1β was prepared by the Biotechnology Department of Ciba-Geigy Ltd (Basel, Switzerland), recombinant human PDGF-AA and -BB were kindly donated by Dr M Pech from F Hoffmann-La Roche Ltd (Basel, Switzerland), prestained markers were from Bio-Rad Laboratories (Glattbrugg, Switzerland), T4-polynucleotide kinase and all cell culture nutrients were from Boehringer-Mannheim (Germany), and all other chemicals used were from either Merck (Darmstadt, Germany) or Fluka (Buchs, Switzerland)

3 RESULTS

As already shown previously, mesangial cells secrete low amounts of PLA_2 into the culture medium, and this secretion of PLA_2 is markedly stimulated by IL-1β (Table I) Addition of PDGF-BB (100 ng/ml) significantly inhibited the basal release of PLA_2 from mesangial cells Furthermore, PDGF-BB dose-dependently attenuated the stimulatory effect of IL-1β on PLA_2 secretion In contrast, PDGF-AA at 100 ng/ml had no effect on both basal and cytokine-induced PLA_2 release

To study the biosynthesis of PLA_2, mesangial cells were stimulated with IL-1β and forskolin (10 μM) for 22 h and [^{35}S]methionine was added for the last 6 h of the stimulation periods. A combination of IL-1β and forskolin has been shown to synergistically stimulate PLA_2 secretion from mesangial cells [16,17] PLA_2 was immunoprecipitated from the medium with a monoclonal group II PLA_2 antibody [25,26] and subjected to SDS-PAGE As shown in Fig 1, no 14 kDa group II PLA_2 could be detected in the culture supernatants of unstimulated cells Addition of IL-1β plus forskolin markedly stimulated the synthesis of PLA_2, an effect that was dose-dependently antagonized by PDGF-BB (Fig 1)

Northern blot analysis using an oligonucleotide probe for group II PLA_2 revealed that the amount of group II PLA_2 mRNA was massively increased by IL-1β plus forskolin as compared to the levels found in control cells (Fig 2). Addition of PDGF-BB potently antagonized IL-1β plus forskolin-induced PLA_2 mRNA accumulation as shown in Fig 2

In order to get some hints on the mechanisms of PDGF-BB inhibition of cytokine-induced PLA_2 secre-

Table I

Effects of PDGF-homodimers on IL-1β-stimulated PLA_2 secretion from mesangial cells

Addition	PLA_2 secretion (cpm/100 μl)
Control	321 ± 22
PDGF-BB (100 ng/ml)	201 ± 19*
PDGF-AA (100 ng/ml)	288 ± 22
IL-1β (1 nM)	5695 ± 746*
IL-1β (1 nM) + PDGF-BB (1 ng/ml)	5695 ± 385
IL-1β (1 nM) + PDGF-BB (10 ng/ml)	4571 ± 195
IL-1β (1 nM) + PDGF-BB (50 ng/ml)	1382 ± 95**
IL-1β (1 nM) + PDGF-BB (100 ng/ml)	744 ± 27**
IL-1β (1 nM) + PDGF-AA (100 ng/ml)	6435 ± 200

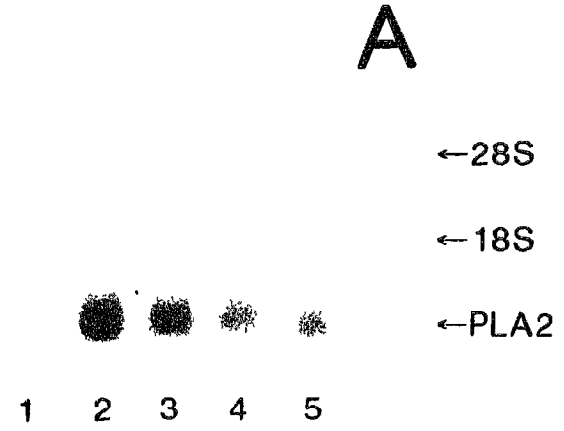
Confluent mesangial cells were incubated with the indicated concentrations of IL-1β, PDGF-AA or PDGF-BB for 24 h Thereafter, the medium was withdrawn and PLA_2 activity determined as described in section 2 Results are means ± SE (n=4)

*P<0 05 versus control

**P<0 05 versus IL-1β (Student's t-test)

Fig 1 Dose-dependent inhibition by PDGF-BB of the synthesis of PLA_2 in stimulated mesangial cells Mesangial cells were incubated with vehicle (1), IL-1β (10 nM) plus forskolin (10 μM) alone (2) or in combination with PDGF-BB 10 ng/ml (3), 30 ng/ml (4), 100 ng/ml (5), or 300 ng/ml (6) for 16 h and then labelled for a further 6 h with [^{35}S]methionine The culture supernatants were analyzed for the presence of group II PLA_2 with immunoprecipitation as described in section 2

tion, we investigated the effect of genistein, a tyrosine kinase inhibitor, that is known to be active against the PDGF receptor tyrosine kinase [33] The data in Table II show that genistein completely reversed the inhibitory action of PDGF-BB on IL-1β-stimulated PLA_2 secretion from mesangial cells



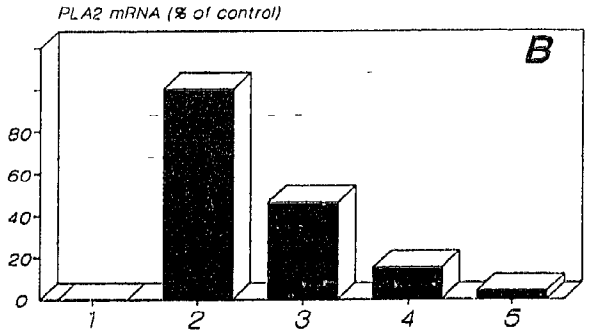


Fig 2 Effect of PDGF-BB on group II PLA_2 mRNA levels in stimulated mesangial cells (A) Mesangial cells were incubated with vehicle (1), IL-1β (10 nM) plus forskolin (10 μM) alone (2) or in combination with PDGF-BB 10 ng/ml (3), 100 ng/ml (4), or 300 ng/ml (5) for 21 h Cellular RNA (15 μg) was analyzed by Northern blotting as described in section 2 (B) For quantitation, autoradiograms of the filters were scanned densitometrically and expressed as a percentage of RNA found in maximally stimulated cells

4. DISCUSSION

Previously we have shown that pro-inflammatory cytokines, such as IL-1α, IL-1β, and TNFα as well as cAMP elevating agents, stimulate the synthesis and secretion of group II PLA_2 from mesangial cells [15–19] Addition of actinomycin D or cycloheximide completely suppressed the basal, as well as the cytokine- and forskolin-stimulated release of PLA_2 [15,16] These results, together with a lag period of several hours before the onset of PLA_2 secretion, indicated that RNA synthesis and protein synthesis are necessary for the observed enzyme secretion [15–17] In the present study, we demonstrate that IL-1β and forskolin indeed stimulate group II PLA_2 gene expression in mesangial cells (Fig. 2) Increased levels of group II mRNA could be detected within 4 h after addition of IL-1β plus forskolin, and maximum levels were obtained between 22 h and 40 h after onset of stimulation (H Muhl, T Geiger and J Pfeilschifter, unpublished observations) An increased synthesis and secretion of PLA_2 after stimulation with IL-1, TNF or cAMP have also been reported for vascular smooth muscle cells [12,34] and were found to be due to augmented froup II PLA_2 gene expression [12]

The main finding of the present report is the potent inhibition of IL-1β- and forskolin-induced PLA_2 gene expression by PDGF-BB (Fig 2) This inhibitory action of PDGF-BB was also evident from the marked reduction of PLA_2 protein (Fig 1) and PLA_2 activity (Table I) in the culture supernatant of stimulated mesangial cells The tyrosine kinase inhibitor genistein reversed the inhibitory effect of PDGF-BB on IL-1β and forskolin stimulated PLA_2 secretion, thus suggesting that tyrosine phosphorylation may be required for PDGF-BB inhibition of PLA_2 gene expression in mesangial cells

Table II

Effects of genistein on PDGF-BB inhibition of IL-1β-stimulated PLA_2 secretion from mesangial cells

Addition	PLA_2 secretion (cpm/100 μl)
Control	537 ± 57
IL-1β	9331 ± 882
IL-1β + PDGF-BB	1439 ± 87*
IL-1β + PDGF-BB + Genistein (1 μM)	1355 ± 89
IL-1β + PDGF-BB + Genistein (10 μM)	2472 ± 122**
IL-1β + PDGF-BB + Genistein (50 μM)	10826 ± 402**

Confluent mesangial cells were incubated with IL-1β (1 nM), PDGF-BB (100 ng/ml) or vehicle together with the indicated concentrations of genistein for 24 h Thereafter, the medium was withdrawn and PLA_2 activity determined as described in Section 2 Results are means ± SE (n=4)

*P<0 05 versus IL-1β

**P<0 05 versus IL-1β + PDGF-BB (Student's t-test)

In contrast, PDGF-AA had no significant effect on PLA_2 induction in mesangial cells This may be explained by the observation that mesangial cells exclusively bind the BB homodimer of PDGF and respond only to the BB isoform in terms of phosphoinositide turnover, prostaglandin and DNA synthesis [35], thus suggesting that mesengial cells possess only the β-type of PDGF receptor Abboud and colleagues [36,37] have reported that mesangial cells express both PDGF A-chain and B-chain mRNAs, release a PDGF-like factor and proliferate in response to PDGF, thus suggesting a possible autocrine function for PDGF in glomerular pathophysiology. In addition, an up-regulation of PDGF receptors has been reported for inflamed kidney as compared to normal kidney [38] Inhibition of cytokine-induced PLA_2 secretion by PDGF may represent an attenuating mechanism for the acute inflammatory process Extracellular PLA_2 is vasoactive and pro-inflammatory when administered by different routes (for review see [3]). PDGF may act in line with TGFβ [20] and glucocorticoids [18,19] to protect the kidney from damage resulting from cytokine-stimulated mediator release and subsequent inflammatory reactions In a manner reminiscent of TGFβ [20], PDGF-BB inhibits cytokine-induced PLA_2 expression and secretion (this paper), but potentiates cytokine-stimulated prostaglandin synthesis [39] TGFβ has been reported to modulate the expression of PDGF receptor α- and β-subunits in fibroblasts and smooth muscle cells [40,41]. Further experiments are required to investigate whether this also applies to our cell culture model Thus, mesangial cells may provide a model system to study the cross-talk between both cytokines and polypeptide growth factors

REFERENCES

[1] Van den Bosch, H (1980) Biochim Biophys Acta 604 191–246
[2] Irvine, R F (1982) Biochem J 204 3–16
[3] Pruzanski, W and Vadas, P (1991) Immunol Today 12, 143–146
[4] Kramer, R M , Hession, C , Johansen, B , Hayes, G , McGray, P , Chow, E P , Tizard, R and Pepinsky, R B (1989) J Biol Chem 264, 5768–5775
[5] Seilhamer, J J , Pruzanski, W , Vadas, P , Plant, S , Miller, J A , Kloss, J and Johnson, L K (1989) J Biol Chem 264, 5335–5338
[6] Ishizaki, J , Ohara, O , Nakamura, F , Tamaki, M , Ono, T , Kanda, A , Yoshida, N , Teraoka, H , Tojo, H and Okamoto, M (1989) Biochem Biophys Res Commun 162, 1030–1036
[7] Komada, M , Kudo, I and Inoue, K (1990) Biochem Biophys Res Commun 168, 1059–1065
[8] Clark, J , Lin, L L , Kriz, R W , Ramesha, C S , Sultzman, L A , Lin, A Y , Milona, N and Knopf, J L (1991) Cell 65, 1043–1051
[9] Heinrikson, R L , Krueger, E T and Keim, P S (1977) J Biol Chem 252, 4913–4921
[10] Lyons-Giordano, B , Davis, G L , Galbraith, W , Pratta, M and Arner, E (1989) Biochem Biophys Res Commun 164, 488–495
[11] Kerr, J , Stevens, T , Davis, G , McLaughlin, J and Harris, R (1989) Biochem Biophys Res Commun 165, 1079–1084
[12] Nakano, T , Ohara, O , Teraoka, H and Arita, H (1990) FEBS Lett 261, 171–174
[13] Crowl, R M , Stoller, T J , Conroy, R R and Stouer, C R (1991) J Biol Chem 266, 2647–2651
[14] Hawkins, N J , Wakefield, D and Charlesworth, J A (1990) Pathology 22, 24–32
[15] Pfeilschifter, J , Pignat, W , Vosbeck, K and Marki, F (1989) Biochem Biophys Res Commun 159, 385–394
[16] Pfeilschifter, J , Leighton, J , Pignat, W , Märki, F and Vosbeck, K (1991) Biochem J 273, 199–204
[17] Schalkwijk, C , Pfeilschifter, J , Marki, F and Van den Bosch, H (1991) Biochem Biophys Res Commun 174, 268–275
[18] Pfeilschifter, J , Pignat, W , Vosbeck, K , Marki, F and Wiesenberg, I (1989) Biochem Soc Transact 17, 916–917
[19] Schalkwijk, C , Vervoordeldonk, M , Pfeilschifter, J , Marki, F and Van den Bosch, H (1991) Biochem Biophys Res Commun (in press)
[20] Pfeilschifter, J , Pignat, W , Leighton, J , Marki, F , Vosbeck, K and Alkan, S (1990) Biochem J 270, 269–271
[21] Pfeilschifter, J , Kurtz, A and Bauer, C (1984) Biochem J 223, 855–859
[22] Pfeilschifter, J and Vosbeck, K (1991) Biochem Biophys Res Commun 175, 372–379
[23] Bradford, M M (1976) Anal Biochem 72, 248–254
[24] Marki, F and Franson, R (1986) Biochim Biophys Acta 879, 149–156
[25] Aarsman, A J , De Jong, J G N Arnoldussen, E , Neys F W , Van Wassenaar, P D and Van den Bosch, H (1989) J Biol Chem 264, 10008–10014
[26] De Jong, J G N , Amesz, H , Aarsman, A J , Lentig, H B M and Van den Bosch, H (1987) Eur J Biochem 164, 129–135
[27] Laemmli, U K (1970) Nature 227, 680–685
[28] Bonner, W M and Lasky, R A (1974) Eur J Biochem 46, 83–88
[29] Chomczynski, P and Sacchi, N (1987) Anal Biochem 162, 156–159
[30] Sambrook, J , Fritsch, J and Maniatis, T (1989) Molecular Cloning, a Laboratory Manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY
[31] Geiger, T Andus, T , Klapproth, J , Northoff, H and Heinrich, P C (1988) J Biol Chem 263, 7141–7146
[32] Feinberg, A P and Vogelstein, B (1983) Anal Biochem 132, 6–13
[33] Akiyama, T , Ishida, J , Nakagawa, S , Ogawara, H , Watanabe, S , Itoh, N , Shibuya, M and Fukami, Y (1987) J Biol Chem 262, 5592–5595
[34] Pfeilschifter J , Pignat, W , Märki, F and Wiesenberg, I (1989) Eur J Biochem 181, 237–242
[35] Pfeilschifter, J and Hosang, M (1991) Cell Signalling (in press)
[36] Abboud, H E , Poptic, E and DiCorleto P (1987) J Clin Invest 80, 675–683
[37] Shultz, P J , DiCorleto, P E , Silver, B J and Abboud, H E (1988) Am J Physiol 255, F674–F684
[38] Fellstrom, B , Klareskog, L , Heldin, C H , Larsson, E , Rönnstrand, L , Terracio, L , Tufveson, G , Wahlberg, J and Rubin, K (1989) Kidney Int 36, 1099–1102
[39] Floege, J , Topley, N , Wessel, K , Kaever V , Radeke, H , Hoppe, J , Kshimoto, T and Resch, K (1990) Kidney Int 37, 859–869
[40] Gronwald, R G K , Seifert, R A and Bowen-Pope, D F (1989) J Biol Chem 264, 8120–8125
[41] Majack, R A , Majesky, M W and Goodman, L V (1990) J Cell Biol 111, 239–247