Arch Dermatol Res (2010) 302:221–227
DOI 10.1007/s00403-009-1017-8

SHORT COMMUNICATION

Platelet activating factor stimulates arachidonic acid release in differentiated keratinocytes via arachidonyl non-selective phospholipase A_2

Katarina Mariann Jørgensen · Hanne Solvang Felberg · Rolf K. Berge · Astrid Lægreid · Berit Johansen

Received: 3 November 2009 / Revised: 10 December 2009 / Accepted: 14 December 2009 / Published online: 30 December 2009
© The Author(s) 2009. This article is published with open access at Springerlink.com

Abstract Platelet activating factor (PAF, 1-*O*-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine) is known to be present in excess in psoriatic skin, but its exact role is uncertain. In the present study we demonstrate for the first time the role of group VI PLA_2 in PAF-induced arachidonic acid release in highly differentiated human keratinocytes. The group IVα PLA_2 also participates in the release, while secretory PLA_2s play a minor role. Two anti-inflammatory synthetic fatty acids, tetradecylthioacetic acid and tetradecylselenoacetic acid, are shown to interfere with signalling events upstream of group IVα PLA_2 activation. In summary, our major novel finding is the involvement of the arachidonyl non-selective group VI PLA_2 in PAF-induced inflammatory responses.

Keywords Phospholipase A_2 · Platelet activating factor · Arachidonic acid · Tetradecylthioacetic acid · Tetradecylselenoacetic acid

Abbreviations

AA	Arachidonic acid
$AACOCF_3$	Arachidonyl trifluoromethyl ketone
BEL	Bromoenol lactone
BSA	Bovine serum albumin
FCS	Fetal calf serum
MAFP	Methyl arachidonyl fluorophosphonate
OA	Oleic acid
PA	Palmitic acid
$PACOCF_3$	Palmitoyl trifluoromethyl ketone
PAF	Platelet activating factor
PAF-R	Platelet activating factor receptor
PLA_2	Phospholipase A_2
$cPLA_2$	Cytosolic phospholipase A_2
$iPLA_2$	Ca^{2+}-independent phospholipase A_2
$sPLA_2$	Secretory phospholipase A_2
PPAR	Peroxisome proliferator-activated receptor
TSA	Tetradecylselenoacetic acid
TTA	Tetradecylthioacetic acid

K. M. Jørgensen · H. S. Felberg · B. Johansen (✉)
Department of Biology, Norwegian University of Science and Technology, NTNU, Trondheim, Norway
e-mail: Berit.Johansen@bio.ntnu.no; bejo@nt.ntnu.no

A. Lægreid
Department of Cancer Research and Molecular Medicine, Norwegian University of Science and Technology, NTNU, Trondheim, Norway

R. K. Berge
Division of Clinical Biochemistry, Haukeland Hospital, Bergen, Norway

Introduction

The lipid mediator platelet activating factor (PAF, 1-*O*-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine) is found in excess in psoriatic scale and plasma [15] as well as in other inflammatory skin conditions [34]. Bayerl et al. [6] also reveal increased expression of PAF receptors in psoriatic skin.

Phospholipase A_2 enzymes are central mediators of inflammatory responses, including psoriasis [30]. The PLA_2 superfamily of enzymes is often divided into three broader categories: (1) extracellular secretory PLA_2s ($sPLA_2$), (2) cytosolic calcium-dependent PLA_2s ($cPLA_2$) and (3) cytosolic calcium-independent PLA_2s ($iPLA_2$). Recent evidence suggests that all classes of PLA_2 enzymes

may participate in agonist-induced arachidonic acid (AA) release [30, 36]. In HaCaT keratinocytes, we previously detected expression of $sPLA_2$ isoenzymes group IIa, IId, V and X, as well as the ubiquitous group IVα ($cPLA_2$) and VI ($iPLA_2$) [4]. PAF is known to activate $cPLA_2$ in several cell types [14], but its role in activating other PLA_2 isoenzymes is less well characterized.

The two synthetic fatty acids tetradecylthioacetic acid (TTA) and tetradecylselenoacetic acid (TSA) [22, 33] have previously been reported to have anti-inflammatory properties [35]. To further investigate PLA_2-involvement in inflammatory skin conditions this paper examines and compares the activation and participation of PLA_2 isoenzymes in PAF and calcium ionophore (A23187)-induced AA-release in keratinocytes, and if TTA and TSA interfere with this.

Results and discussion

Keratinocytes in the outer layer of the epidermis are differentiated, as opposed to proliferating keratinocytes attached to basal laminae. In order to obtain a cell state resembling mature skin, HaCaT keratinocytes [7] used in this study (kindly provided by Prof. N. Fusenig, Heidelberg, Germany) were differentiated in culture for 3 days prior to experimentation (protocol as described in refs. [3, 4]). The differentiation state of the cells was documented by examining the expression of three markers of suprabasal keratinocytes: Keratin 10 [8], S100A7 (psoriasin) [9] and $sPLA_2$ IIA [2]. They all show an upregulation of more than fivefold compared to HaCaT undifferentiated state (Fig. 1a). [RNA was isolated using a Qiagen RNeasy Mini kit according to kit protocol, and RT^2-PCR performed using the MX3000 system from Stratagene (primer sequences shown in Table 1)]. Other studies of HaCaT cells also demonstrate that upregulation of these genes are associated with a highly differentiated state [16, 29], thus the data suggest that the cultivation protocol used, indeed, differentiates the keratinocytes.

Previous studies of PAF-stimulated AA-release in keratinocytes have used actively proliferating cells [10, 27]. In order to establish the pro-inflammatory potential of PAF in differentiated keratinocytes, HaCaT cells were stimulated with PAF-16 (purchased from Calbiochem), and the subsequent release of $[^3H]$AA and $[^{14}C]$ oleic acid (OA) (NEN, Perkin Elmer) was measured. Changes in the levels of these free fatty acids may indicate the involvement of AA-selective or non-selective phospholipases, respectively [30]. (Protocol as described previously [3, 4], except that bovine serum albumin was excluded due to evidence that it may inhibit PAF activity [13].) At 60 min exposure and 20 μM concentration, PAF typically induces an AA induction of about twofold in a dose-dependent manner (Fig. 1b). The fold induction of OA is nearly as large as for AA, which is especially notable, since OA release is typically a minor fraction of the fold induction of AA-release [4, 25], and only rarely equal to it [36]. We may conclude that PAF has a pro-inflammatory effect in fully differentiated HaCaT, by induction of AA-release. Furthermore, AA-non-selective phospholipase A_2s, as indicated by the observed OA release, contribute substantially to this effect.

The physiological relevance of the PAF concentration used deserves to be further commented upon. HaCaT cells

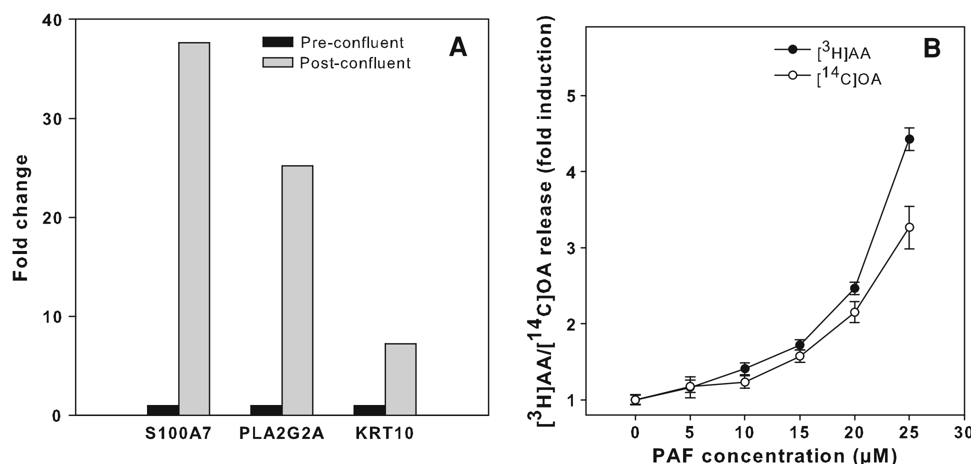

Fig. 1 PAF induces AA and OA release in differentiated keratinocytes. **a** Initial cell state. Fold induction of differentiation specific marker genes in proliferating vs post-confluent HaCaT cells. ($N = 2$, result shown from one representative experiment). **b** Dose–response relationship for PAF stimulation of HaCaT cells. The response is measured as fold induction of $[^3H]$ arachidonic acid and $[^{14}C]$ oleic acids compared to the unstimulated control. PAF exposure time was 60 min. (All dose–response data in cell culture have been statistically validated using one way ANOVA at the 95% confidence level, and the results shown are representative of at least three consecutive experiments, using at least three parallel samples in each experiment)

Table 1 Primers for keratinocyte differentiation markers

Entrez ID	Symbol	Size	Forward primer	Reverse primer
3858	KRT10	106	TGCACACAGTAGCGACCTTC	TGAGACGTAATGTACAAGCTCTGG
6278	S100A7	148	CTCCCAGCAAGGACAGAAAC	CTGCTGACGATGATGAAGGA
5320	PLA2G2A	167	GAGCTAGGCCAGTCCATCTG	TAACTGAGTGCGGCTTCCTT

were 100% viable at this PAF concentration as determined by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenoltetrazolium bromide (MTT) dye (Sigma) uptake assay [21] (data not shown). This study uses natural PAF, a readily degradable lipid—it has a half life of 0.5–2 s in serum [24]. HaCaT cells express both $sPLA_2$ GX [4] and PAF-AH II [19], which are both known to degrade PAF [12, 19]. Additionally, recent developments in new quantitative methods for PAF suggest that the PAF concentration in serum in healthy humans may be as high as 0.2–0.3 µM [26], which is at least 100 times higher than previously thought. Thus, the PAF concentration used in this study is non-toxic and physiologically plausible.

Group IVα is the only truly AA-selective PLA_2 isoenzyme [30]. In order to examine activation of group IVα PLA_2 in PAF-mediated AA-release, HaCaT cells were treated with PAF in the presence or absence of the group IVα inhibitor methyl arachidonyl fluorophosphonate (MAFP) [11] (obtained from Cayman Chemicals), followed by lysis and analysis by in vitro group IVα PLA_2 enzyme activity assay (for method description see [3, 18]). [All inhibitors used, their vehicles and the calcium ionophore do not affect viability in the concentrations applied in this study, as verified by MTT assays (not shown).] PAF-induced activation of group IVα PLA_2 enzyme in HaCaT cells, and this response is reduced by 50% by MAFP (Fig. 2a). This in vitro assay is highly specific and the result thus strongly indicates that group IVα PLA_2 participates in PAF-induced AA-release [18].

In order to determine the importance of the contribution of group IVα PLA_2 in the total PAF-response, PAF-mediated [^{3}H]AA and [^{14}C]OA release in cells treated with PAF in the presence or absence of MAFP [11] was determined. We found that MAFP inhibited both [^{3}H]AA and [^{14}C]OA release in a dose-dependent manner by a maximum of 51 and 28.5% respectively (see Fig. 2b). Since MAFP is also known to have some effect on group VI PLA_2 [25, 28], we cannot exclude the involvement of PLA_2s of this type in the response. However, our previous studies show that it is possible to attenuate AA-release in HaCaT cells completely in the IL-1β pathway using only 10 µM of MAFP [4]. The partial attenuation of PAF-mediated AA-release

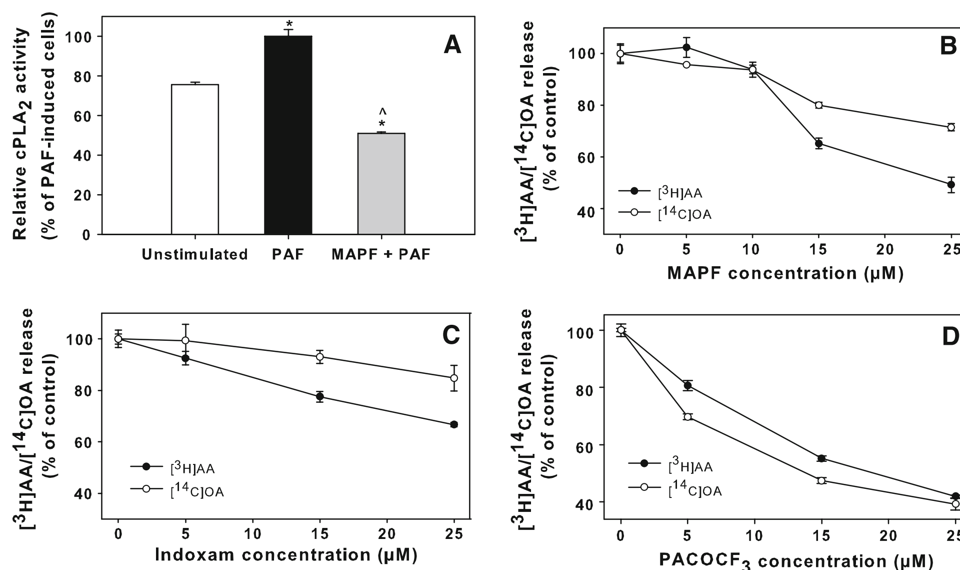

Fig. 2 Different PLA_2 inhibitors reduce PAF-induced AA and OA release. **a** In vitro $cPLA_2$ activity assay on lysate from HaCaT cells pre-treated with 25 µM MAFP (90 min) and stimulated by 20 µM PAF (60 min) prior to lysis. [95% significance indicated by *asterisk* (compared to unstimulated control) and Δ (compared to PAF-stimulated sample) as determined by Student's *t* test]. Results shown are representative of at least three consecutive experiments, using at least three parallel samples in each experiment. Release assay of [^{3}H] arachidonic acid and [^{14}C] oleic acids compared to the unstimulated control (in %). Stimulus is 20 µM PAF for 60 min. **b** Dose–response inhibition by MAFP (pretreatment time 90 min.), **c** inhibition by indoxam (pretreatment time 90 min), **d** dose–response inhibition by $PACOCF_3$ (pretreatment time 15 min). Statistical testing and number of experiments as for Fig. 1b

achieved with MAFP therefore suggests a significant contribution to AA-release by AA-nonspecific enzymes in addition to the contribution by group IVα PLA_2. However, our in vitro enzyme assay clearly shows that group IVα PLA_2 is activated by PAF, thus our conclusion is that group IVα PLA_2 participates in PAF-mediated AA-release together with AA-nonspecific PLA_2 subtypes.

To our knowledge, there are no previous reports of PAF-induced $sPLA_2$ activation in the literature. Secretory PLA_2 enzymes would be candidate enzymes for the OA release observed; we then examined the role of $sPLA_2$ subtypes in PAF-mediated AA-release. The $sPLA_2$-selective inhibitor indoxam [31] (a generous gift from Shionigi Ltd, Japan), gives a dose-dependent inhibition with a maximum of 33% of PAF-induced AA-release and 15% of OA release at 25 μM (Fig. 2c). Similar results were obtained using SB203347 (a kind gift from Lisa Marshall, SmithKline Beecham, PA, USA), another $sPLA_2$ inhibitor [20, 31] (results not shown). Interestingly, Fig. 2c thus shows that the inhibition found with indoxam predominantly affects AA-release. In several cell types, $sPLA_2$ isoenzymes IIa, IId and V, but not X, have been shown to be more strongly arachidonyl-selective when operating intracellularly, a mechanism which involves $sPLA_2$ isoenzyme selective caveolin-mediated endocytosis [23]. Our data thus suggest that one or several of these three $sPLA_2$s (IIa, IId or V) may participate in PAF-mediated intracellular AA-release in differentiated HaCaT cells.

Lastly, we examined the possible role of group VI PLA_2 in the PAF-induced OA response. We found that the PLA_2 subtype VI-specific inhibitor palmitoyl trifluoromethyl ketone ($PACOCF_3$) [1] (from Calbiochem) dose-dependently reduced the PAF-induced AA-release by 58% and OA release by 61% at a 25-μM concentration (Fig. 2d). These results were confirmed with application of bromoenol lactone (BEL, from Cayman Chemicals) [1], another group VI inhibitor, which produced comparable levels of maximum inhibition (data not shown). Although $PACOCF_3$ is also known to inhibit group IV PLA_2 [11], BEL [1] is not known to do so. As $sPLA_2$ inhibitors were shown to preferentially inhibit AA-release, the incomplete attenuation achieved with the group IVα/VI inhibitor MAFP, and the more successful inhibition with $PACOCF_3$, the conclusion supported is that group VI PLA_2 most likely plays a major role in PAF-mediated AA-release. The group VI PLA_2 enzyme is probably at least as important as group IVα PLA_2, judging by its ability to contribute to the high OA release. The participation as well as the notable significance of group VI PLA_2 in the PAF-mediated response is a novel finding.

Taken together, our data suggest the participation of both calcium-dependent and -independent cytosolic PLA_2 subtypes IVα and VI, as well as of secretory PLA_2 subtypes, in the PAF-induced response in differentiated HaCaT keratinocytes.

The palmitic acid (PA)-derived lipids TTA and TSA [22, 33] are already shown to exhibit anti-inflammatory

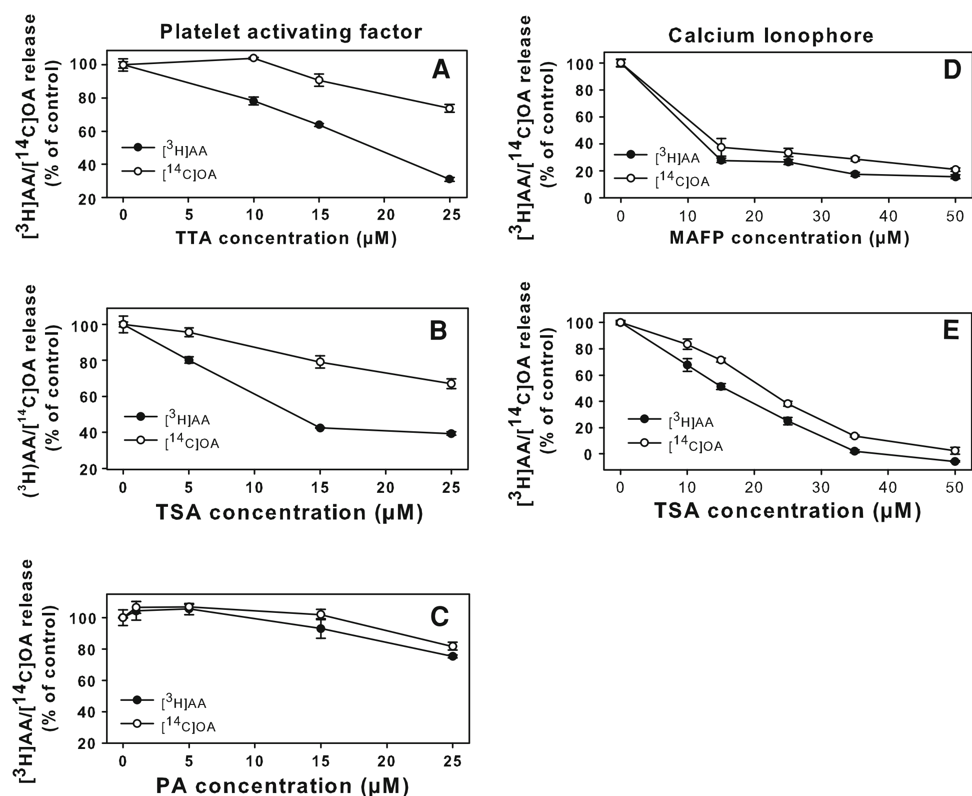

Fig. 3 Tetradecylthioacetic acid and tetradecylselenoacetic acid inhibit arachidonic acid release. The response is measured as % release of $[^3H]$ arachidonic acid and $[^{14}C]$ oleic acids compared to the unstimulated control. Pretreatment time for all inhibitors is 90 min. Effects on the PAF-induced response (*left*) (20 μM PAF, 60 min). Dose–response inhibition by **a** TTA and **b** TSA. **c** Dose–response result for Palmitic acid. PA acts as a control, since TTA and TSA are PA derivatives. Effects on the calcium ionophore (A23187)-induced response (*right*) (1 μM A23187, 60 min). **d** Dose–response inhibition by MAFP, **e** dose–response inhibition by TSA. Statistical testing and number of experiments as for Fig. 1b

properties [35]. Most published studies of TTA and TSA show their roles as PPAR ligands [35], however, their potency as anti-inflammatory and anti-apoptotic agents are not fully explained by this mechanism. It would therefore be interesting to test whether their anti-inflammatory properties include inhibition of AA-release. TTA (Fig. 3a) and TSA (Fig. 3b) show a similar overall trend with maximum AA inhibition of 60–70% of the PAF-induced AA-release at 25 μM concentration. Further experiments have therefore been carried out with only one of these two inhibitors. TTA and TSA are both derivatives of PA [22], which was used as a control (Fig. 3c). The data suggests that the inhibitory effect is specific to TTA and TSA, and not shared by their common precursor, PA.

We also tested these inhibitors in the calcium ionophore (A23187) (Sigma) response, to compare with the PAF response. Calcium ionophore acts by releasing calcium from both the mitochondrion and the extracellular matrix [28], and can induce activation of calcium-dependent enzymes such as group IVα PLA_2 [17]. Twenty-five micromolar of the group IVα PLA_2 inhibitor MAFP [11] was found to strongly inhibit calcium ionophore induced AA-release (73.5%; Fig. 3d, right). In cells pre-treated with 35 μM of TSA, a near-complete attenuation of A23187-induced AA-release, 98%, was found (Fig. 3e).

Thus, TTA and TSA give a strong arachidonyl-selective inhibition in both the PAF and calcium ionophore responses, suggesting that they affect the group IVα PLA_2-regulated pathway. The data suggest a prominent role for AA-specific PLA_2 subtypes in the response to calcium ionophore, consistent with our previous studies [32].

We then wished to determine whether TSA actually acts as inhibitor of the group IVα PLA_2 enzyme. The effect of TSA on a recombinant group IVα PLA_2 substrate was tested using the in vitro enzyme activity assay [18], and compared to the effects of $AACOCF_3$ and $PACOCF_3$. $AACOCF_3$ (Cayman Chemicals) is a potent, reversible group IVα PLA_2 inhibitor, with an effect similar to that of MAFP [11]. Forty nanomolar $AACOCF_3$ gives a 70% reduction in group IVα PLA_2 activity (Fig. 4a). A similar concentration of the group VI-selective inhibitor $PACOCF_3$ gives a 55% reduction in group IVα PLA_2 activity, which is consistent with previous studies showing that $PACOCF_3$ is a poorer inhibitor of group IVα PLA_2 than $AACOCF_3$ is [11]. TSA shows only a 20% reduction in group IVα PLA_2 activity, suggesting that it has a poor direct effect on group IVα PLA_2. Thus, the observed strong reduction of cellular AA-release by TSA must mainly be due to a signalling mechanism leading to the activation of group IVα PLA_2, rather than on the enzyme itself.

Since $PACOCF_3$ and TTA are both PA derivates, we lastly wanted to examine whether these inhibitors act through similar or different mechanisms. TTA and $PACOCF_3$ were applied to cells both separately and together, and AA-release was induced with PAF. Both TTA and $PACOCF_3$ inhibited AA-release by approx 30%, respectively, while both together inhibited AA-release by almost 60% (Fig. 4b). The effect of TTA and $PACOCF_3$ combined is clearly additive. This suggests that these inhibitors work on parallel, separate mechanisms, where TTA acts on signalling involving group IVα PLA_2, while $PACOCF_3$ works on the group VI PLA_2 enzyme.

Measuring cellular AA and OA release in the presence of different PLA_2 inhibitors is an established method within the PLA_2 research community [18, 30]. We have used two structurally and mechanistically different inhibitors against each class of PLA_2 in our experiments. Both of the $cPLA_2$ (MAFP, $AACOCF_3$) [11] and $iPLA_2$ inhibitors ($PACOCF_3$, BEL) [1, 5] used here are very well

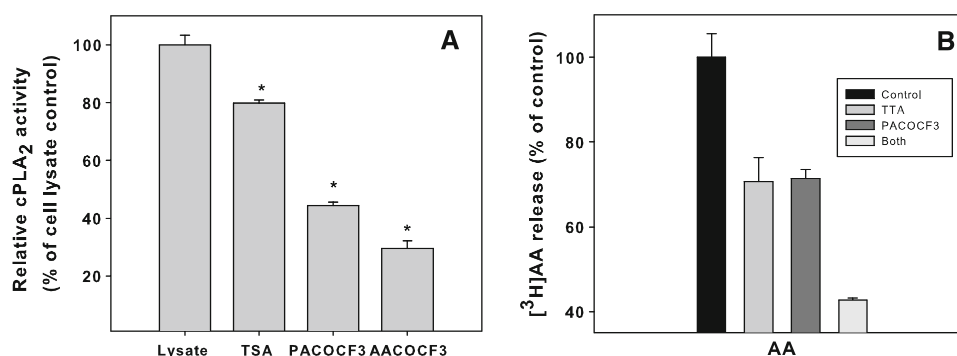

Fig. 4 Tetradecylthioacetic acid and tetradecylselenoacetic acid point of action in AA-release is upstream of group IVα PLA_2. **a** In vitro activity assay applying inhibitors directly to lysate from group IVα PLA_2 overexpressing insect cells (95% significance compared to control indicated by *asterisk* as determined by Student's *t* test). **b** Simultaneous application of TTA and $PACOCF_3$ is additive. The response is measured as % release of [^{3}H] arachidonic acid compared to the unstimulated control. Stimulus is 20 μM PAF for 60 min. Pretreatment time for TTA is 90 min, $PACOCF_3$ was added 15 min, before PAF stimulation. Results shown are representative of at least three consecutive experiments, using at least three parallel samples in each experiment

established. The two latter inhibitors display similar inhibitory properties, and knowing that BEL has high selectivity for $iPLA_2$ over $cPLA_2$, our conclusions are strongly supported. The selectivity of the $sPLA_2$ inhibitor indoxam has been thoroughly characterized [31, 37], and as a result has become a preferred inhibitor for the secretory enzyme. The in vitro assay utilizes knowledge on substrate specificity, enzyme structure and calcium requirements to positively identify $cPLA_2$: The calcium requirement for $sPLA_2$ is 100-fold lower than for $cPLA_2$, the assay buffer contains dithiothreitol which reduces the disulfide bridges stabilizing the $sPLA_2$ structure, while $iPLA_2$ activity requires ATP [18]. The total evidence obtained with cell culture as well as in vitro assays compared with known information on isoenzyme substrate specificity and inhibitor selectivity against the same isoenzyme thus adds up to a meaningful overall interpretation. In summary, PAF induces arachidonic and oleic acid release in differentiated HaCaT keratinocytes. The main participating PLA_2 isoenzyme is group VI, with contribution from group IVα PLA_2 and to some extent the $sPLA_2$ subtypes. The important role of group VI PLA_2 in PAF-mediated AA-release is a novel finding, and may therefore represent a novel intervention point in inflammatory dermatoses.

Acknowledgments This work was funded by the NTNU thematic priority area for Medical Technology (K.M. Jørgensen). We thank Randi Sommerfeldt for technical assistance. Prof. Rolf K Berge wishes to acknowledge the support of Nordic Centre Mitohealth and the EU-project Athero Remo.

Open Access This article is distributed under the terms of the Creative Commons Attribution Noncommercial License which permits any noncommercial use, distribution, and reproduction in any medium, provided the original author(s) and source are credited.

References

1. Ackermann EJ, Conde-Frieboes K, Dennis EA (1995) Inhibition of macrophage Ca(2 +)-independent phospholipase A2 by bromoenol lactone and trifluoromethyl ketones. J Biol Chem 270(1):445–450
2. Andersen S, Sjursen W, Laegreid A, Volden G, Johansen B (1994) Elevated expression of human nonpancreatic phospholipase A2 in psoriatic tissue. Inflammation 18(1):1–12
3. Anthonsen MW, Andersen S, Solhaug A, Johansen B (2001) Atypical lambda/iota PKC conveys 5-lipoxygenase/leukotriene B4-mediated cross-talk between phospholipase A2 s regulating NF-kappa B activation in response to tumor necrosis factor-alpha and interleukin-1beta. J Biol Chem 276(38):35344–35351
4. Anthonsen MW, Solhaug A, Johansen B (2001) Functional coupling between secretory and cytosolic phospholipase A2 modulates tumor necrosis factor-alpha- and interleukin-1beta-induced NF-kappa B activation. J Biol Chem 276(32):30527–30536
5. Balsinde J, Dennis EA (1996) Bromoenol lactone inhibits magnesium-dependent phosphatidate phosphohydrolase and blocks triacylglycerol biosynthesis in mouse P388D1 macrophages. J Biol Chem 271(50):31937–31941
6. Bayerl C, Brandt H, Niemczyk M, Muller-Decker K, Gretz N (2003) PAF-receptor in inflammatory versus non inflammatory human epidermis, cell cultures and embryonal cells. Inflamm Res 52(7):283–286
7. Boukamp P, Petrussevska RT, Breitkreutz D, Hornung J, Markham A, Fusenig NE (1988) Normal keratinization in a spontaneously immortalized aneuploid human keratinocyte cell line. J Cell Biol 106(3):761–771
8. Candi E, Schmidt R, Melino G (2005) The cornified envelope: a model of cell death in the skin. Nat Rev Mol Cell Biol 6(4):328–340
9. Eckert RL, Lee KC (2006) S100A7 (Psoriasin): a story of mice and men. J Invest Dermatol 126(7):1442–1444
10. Fisher GJ, Talwar HS, Ryder NS, Voorhees JJ (1989) Differential activation of human skin cells by platelet activating factor: stimulation of phosphoinositide turnover and arachidonic acid mobilization in keratinocytes but not in fibroblasts. Biochem Biophys Res Commun 163(3):1344–1350
11. Ghomashchi F, Loo R, Balsinde J, Bartoli F, Apitz-Castro R, Clark JD, Dennis EA, Gelb MH (1999) Trifluoromethyl ketones and methyl fluorophosphonates as inhibitors of group IV and VI phospholipases A(2): structure–function studies with vesicle, micelle, and membrane assays. Biochim Biophys Acta 1420(1–2):45–56
12. Gora S, Lambeau G, Bollinger JG, Gelb M, Ninio E, Karabina SA (2006) The proinflammatory mediator platelet activating factor is an effective substrate for human group X secreted phospholipase A2. Biochim Biophys Acta 1761(9):1093–1099
13. Grigoriadis G, Stewart AG (1992) Albumin inhibits platelet-activating factor (PAF)-induced responses in platelets and macrophages: implications for the biologically active form of PAF. Br J Pharmacol 107(1):73–77
14. Ishii S, Shimizu T (2000) Platelet-activating factor (PAF) receptor and genetically engineered PAF receptor mutant mice. Prog Lipid Res 39(1):41–82
15. Izaki S, Yamamoto T, Goto Y, Ishimaru S, Yudate F, Kitamura K, Matsuzaki M (1996) Platelet-activating factor and arachidonic acid metabolites in psoriatic inflammation. Br J Dermatol 134(6):1060–1064
16. Lemaitre G, Lamartine J, Pitaval A, Vaigot P, Garin J, Bouet S, Petat C, Soularue P, Gidrol X, Martin MT, Waksman G (2004) Expression profiling of genes and proteins in HaCaT keratinocytes: proliferating versus differentiated state. J Cell Biochem 93(5):1048–1062
17. Lin LL, Lin AY, Knopf JL (1992) Cytosolic phospholipase A2 is coupled to hormonally regulated release of arachidonic acid. Proc Natl Acad Sci USA 89(13):6147–6151
18. Lucas KK, Dennis EA (2005) Distinguishing phospholipase A2 types in biological samples by employing group-specific assays in the presence of inhibitors. Prostaglandins Other Lipid Mediat 77(1–4):235–248
19. Marques M, Pei Y, Southall MD, Johnston JM, Arai H, Aoki J, Inoue T, Seltmann H, Zouboulis CC, Travers JB (2002) Identification of platelet-activating factor acetylhydrolase II in human skin. J Invest Dermatol 119(4):913–919
20. Marshall LA, Hall RH, Winkler JD, Badger A, Bolognese B, Roshak A, Flamberg PL, Sung CM, Chabot-Fletcher M, Adams JL et al (1995) SB 203347, an inhibitor of 14 kDa phospholipase A2, alters human neutrophil arachidonic acid release and metabolism and prolongs survival in murine endotoxin shock. J Pharmacol Exp Ther 274(3):1254–1262
21. Mosmann T (1983) Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J Immunol Methods 65(1–2):55–63

22. Muna ZA, Bolann BJ, Chen X, Songstad J, Berge RK (2000) Tetradecylthioacetic acid and tetradecylselenoacetic acid inhibit lipid peroxidation and interact with superoxide radical. Free Radic Biol Med 28(7):1068–1078
23. Murakami M, Koduri RS, Enomoto A, Shimbara S, Seki M, Yoshihara K, Singer A, Valentin E, Ghomashchi F, Lambeau G, Gelb MH, Kudo I (2001) Distinct arachidonate-releasing functions of mammalian secreted phospholipase A2 s in human embryonic kidney 293 and rat mastocytoma RBL-2H3 cells through heparan sulfate shuttling and external plasma membrane mechanisms. J Biol Chem 276(13):10083–10096
24. O'Flaherty JT, Surles JR, Redman J, Jacobson D, Piantadosi C, Wykle RL (1986) Binding and metabolism of platelet-activating factor by human neutrophils. J Clin Invest 78(2):381–388
25. Oestvang J, Anthonsen MW, Johansen B (2003) Role of secretory and cytosolic phospholipase A(2) enzymes in lysophosphatidylcholine-stimulated monocyte arachidonic acid release. FEBS Lett 555(2):257–262
26. Owen JS, Wykle RL, Samuel MP, Thomas MJ (2005) An improved assay for platelet-activating factor using HPLC-tandem mass spectrometry. J Lipid Res 46(2):373–382
27. Pei Y, Barber LA, Murphy RC, Johnson CA, Kelley SW, Dy LC, Fertel RH, Nguyen TM, Williams DA, Travers JB (1998) Activation of the epidermal platelet-activating factor receptor results in cytokine and cyclooxygenase-2 biosynthesis. J Immunol 161(4):1954–1961
28. Pressman BC (1976) Biological applications of ionophores. Annu Rev Biochem 45:501–530
29. Ryle CM, Breitkreutz D, Stark HJ, Leigh IM, Steinert PM, Roop D, Fusenig NE (1989) Density-dependent modulation of synthesis of keratins 1 and 10 in the human keratinocyte line HACAT and in ras-transfected tumorigenic clones. Differentiation 40(1):42–54
30. Schaloske RH, Dennis EA (2006) The phospholipase A2 superfamily and its group numbering system. Biochim Biophys Acta 1761(11):1246–1259
31. Singer AG, Ghomashchi F, Le Calvez C, Bollinger J, Bezzine S, Rouault M, Sadilek M, Nguyen E, Lazdunski M, Lambeau G, Gelb MH (2002) Interfacial kinetic and binding properties of the complete set of human and mouse groups I, II, V, X, and XII secreted phospholipases A2. J Biol Chem 277(50):48535–48549
32. Sjursen W, Brekke OL, Johansen B (2000) Secretory and cytosolic phospholipase A(2)regulate the long-term cytokine-induced eicosanoid production in human keratinocytes. Cytokine 12(8):1189–1194
33. Spydevold O, Bremer J (1989) Induction of peroxisomal beta-oxidation in 7800 C1 Morris hepatoma cells in steady state by fatty acids and fatty acid analogues. Biochim Biophys Acta 1003(1):72–79
34. Travers JB, Murphy RC, Johnson CA, Pei Y, Morin SM, Clay KL, Barber LA, Hood AF, Morelli JG, Williams DA (1998) Identification and pharmacological characterization of platelet-activating factor and related 1-palmitoyl species in human inflammatory blistering diseases. Prostaglandins Other Lipid Mediat 56(5–6):305–324
35. Westergaard M, Henningsen J, Svendsen ML, Johansen C, Jensen UB, Schroder HD, Kratchmarova I, Berge RK, Iversen L, Bolund L, Kragballe K, Kristiansen K (2001) Modulation of keratinocyte gene expression and differentiation by PPAR-selective ligands and tetradecylthioacetic acid. J Invest Dermatol 116(5):702–712
36. Yellaturu CR, Rao GN (2003) A requirement for calcium-independent phospholipase A2 in thrombin-induced arachidonic acid release and growth in vascular smooth muscle cells. J Biol Chem 278(44):43831–43837
37. Yokota Y, Hanasaki K, Ono T, Nakazato H, Kobayashi T, Arita H (1999) Suppression of murine endotoxic shock by sPLA2 inhibitor, indoxam, through group IIA sPLA2-independent mechanisms. Biochim Biophys Acta 1438(2):213–222