Free Radical Research, October 2009; 43(10): 951–956

informa
healthcare

Pitfalls in the use of arachidonic acid oxidation products to assign lipoxygenase activity in cancer cells

MARZIEH NIKNAMI[1], QIHAN DONG[1], & PAUL K. WITTING[2,3]

[1]*Cancer Biology Group, Department of Endocrinology and Sydney Cancer Centre, Central Clinical School, The University of Sydney, NSW 2006, Australia,* [2]*Vascular Biology Group, ANZAC Research Institute, Concord Hospital, Sydney, NSW 2139, Australia, and* [3]*Redox Biology Group, Discipline of Pathology, Bosch Institute, Faculty of Medicine, The University of Sydney, NSW 2006, Australia*

(Received 28 April 2009; revised 24 June 2009)

Abstract
Arachidonic acid (AA) reaction with cyclooxygenase (COX) and lipoxygenases (LOX) yield eicosanoids that can mediate prostate cancer proliferation and enhance both tumour vascularization and metastasis. Increasingly measurement of eicosanoids with liquid chromatography is employed to implicate LOX activity in different biological systems and in particular link LOX activity to the progression of cancer in experimental models. This study demonstrates that simply identifying patterns of eicosanoid regio-isomerism is insufficient to designate LOX activity in prostate cancer cells and the analysis *must* include complete stereochemical assignment of the various isomers in order to validate the assignment of LOX activity.

Keywords: *Eicosanoid, arachidonic acid, lipid oxidation, stereochemistry, regiochemistry*

Introduction

Eicosanoids and related compounds prostaglandins such as prostacyclin, leukotrienes and thromboxanes are metabolites of arachidonic acid that are produced by many different cells types. Although their primary physiological actions are generally related to inflammation and haemostasis, by nature they are vasoactive agents that can modulate cardiovascular function, particularly in relation to the maintenance of blood pressure and vascular tone [1]. Their effects are highly localized and collectively they can act as paracrine hormones being released locally by cells to act on nearby cells.

The synthesis and biological action of eicosanoids has increasingly become a focus for therapeutic development in the setting of cancer [2,3]. Recent evidence has demonstrated that eicosanoids are potent cell cycle regulators with specific 15-LOX isoforms and their products associated with anti-tumour activity, while the 5- and 12-LOX enzymes and their respective eicosanoid products increase during tumour development in some cancers [3,4]. Consistent with this idea, pharmacologic and natural inhibitors of 5-LOX are effective tumour suppressors in some experimental models of cancer [5,6].

It is also well established that eicosanoid synthesis from labelled AA is greater in malignant than benign prostate cancer tissue, suggesting an increased AA flux through the COX and LOX pathways [7]. Monitoring hydroxyeicosatetraenoic acid (HETE) as an index of LOX activity has been used in studies of cancer [8–11] and other inflammatory disorders [12–14] as the primary means to assign LOX activity.

LOX enzymes generate regio-selective hydroperoxyeicosatetraenoic acid products (H(P)ETE) that are reduced to the corresponding HETE. The chemical structures of the 5-, 12- and 15-H(P)ETE and the corresponding HETE products are shown in

Correspondence: Dr Paul Witting, Redox Biology Group, Discipline of Pathology, Bosch Institute, Faculty of Medicine, The University of Sydney, NSW 2006, Australia. Tel: 61-2-9114-0524. Fax: 61-2-9351-3429. Email: pwitting@med.usyd.edu.au

ISSN 1071-5762 print/ISSN 1029-2470 online
DOI: 10.1080/10715760903145013

Scheme 1 with their respective stereogenic centres highlighted.

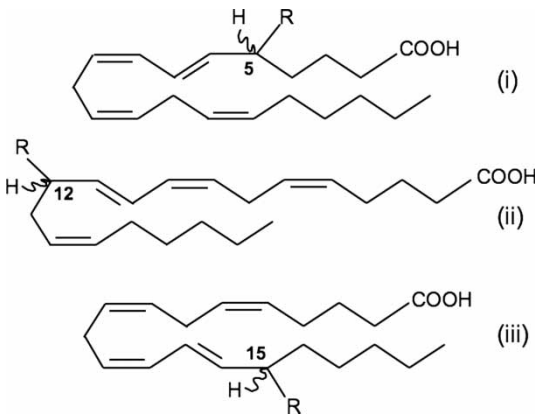
Each AA oxidation product is characterized by the site of modification on the fatty acid backbone. For example, 5-, 12- or 15-HETE products are generated by the corresponding 5-, 12- or 15-LOX. Each regio-specific product contains a mixture of *R*- and *S*-HETE stereo-isomers (termed racemic when the *S*/*R* ratio = 1). Irrespective of regio-outcome, LOX activity yields primarily the *S*-HETE in enantiomeric excess and this affords a *S*/*R* ratio of HETE >1 [15,16]. This combined regio- and stereo-specificity is confounded by the production of peroxyl radicals that 'leak' from the LOX active site [17] or through the autoxidation of AA. Free radicals stimulate AA oxidation by a radical-chain mechanism yielding similar regio-isomeric distributions as LOX. The key to differentiating non-enzymatic (non-stereo-selective) from enzymatic (stereo-selective) oxidation is determination of the *S*/*R*-HETE ratio since free radical-mediated oxidation affords the *S*- and *R*-HETE in equal yield and affords a *S*/*R* ratio of ~ 1.

In this study we demonstrate that the defining parameter for the unambiguous assignment of LOX activity in cultured prostate cancer cells is the identification of the ratio of *S*/*R*-HETE products and not the assessment of the regio-isomeric distribution.

Materials and methods

Cells

Two prostate cancer cell lines (LNCaP and PC3) were used as models of prostate cancer. These cell lines differ by isolation site and androgen sensitivity. LNCaP (#CRL-1740) and PC3 (#CRL-1435) cell lines were purchased from American Type Cell Collection (ATCC) (Rockville, MD). LNCaP cells were isolated from a supraclavicular lymph node metastasis of human prostate cancer. This cell line is androgen sensitive and is used as the model for androgen-dependent prostate cancer [18]. Cultured PC3 cells represent an androgen insensitive cell line that was derived from bone metastasis.



Scheme 1. Chemical structures of LOX-mediated AA oxidation products with the stereogenic centres shown at the 5, 12 or 15-regioisomer. Structures with R = -OOH correspond to the (i) 5-, (ii) 12- and (iii) 15-H(P)ETE products while structures with R = -OH correspond to the (i) 5-, (ii) 12- and (iii) 15-HETE products. Note, these AA oxidation products are distinct from the isoprostane series of AA oxidation products that contain a fused pentacyclic ring.

Cell culture

Prostate cancer cells were routinely cultured in RPMI-1640 media (containing 11 mM glucose) supplemented with 10% v/v Foetal Calf Serum (FCS), Penicillin (50 U/ml) and Streptomycin (100 g/ml). Cells were cultured in sterile (75 cm^3) tissue culture flasks (Asahi Technoglass, Tokyo, Japan) at 37°C with 5% $CO_{2(g)}$ in a humidified atmosphere to $\sim$80% confluence before use in further experiments. Where required, cells were harvested by washing the monolayers in warm phosphate buffered saline (PBS) containing 33% trypsin (0.05% v/v)-EDTA for 15 min at 37°C and then neutralizing the trypsin by addition of an equal volume of warm FCS. Cells from 8–10 culture flasks were combined to yield a single pooled cell pellet for use in AA oxidation studies. Total protein (BCA assay, Sigma Australia) was then measured in these pooled samples prior to their use in AA oxidation studies. The values obtained were as follows: PC3 cells protein = 8.7 ± 0.1 mg/mL; LNCaP cells = 9.5 ± 0.2 mg/mL. The high cell densities were required to generate significant amounts of AA oxidation products suitable for HPLC analysis.

Cell studies

Cultured LNCaP and PC3 prostate cancer cell lines were initially treated with 1 and 1.5 mM Ibuprofen, respectively—DMSO was employed as control. Ibuprofen was employed as a non-specific COX inhibitor to minimize the contribution of COX to AA metabolism in these cell preparations. Next, the cell samples were incubated at 37°C for 72 h, harvested, cell density was adjusted to yield final cell pellets $\sim$10 mg total cell protein/mL and then isolated cell samples were incubated with 0.1 mM purified AA at 37°C for 1 h. The ratio of added AA to total cell protein in these reactions were determined to be $\sim$3400 and 3800 pg/μg protein for LNCaP and PC3 cells, respectively. These levels of AA are comparable to total AA determined in the brain of rodents (9632 pg/μg protein [19]), albeit that this concentration of lipid is a measure of total tissue AA and not necessarily a measure of the pool of free AA available for enzymic oxidation.

Note, commercial AA contains oxidation products that must be removed with reversed-phase chromatography and analytical purity verified before use—

refer below for chromatographic method. Samples containing phosphate buffered saline (PBS; pH 7.4; NaCl 150 mM) and purified AA served as the vehicle control. Total lipids were extracted into chloroform/methanol/water (5:1:1 v/v/v), centrifuged ($3600 \times g$, 15 min), the chloroform phase dried and the residue reconstituted with methanol/water (9:1 v/v) for use in the chromatographic separations below.

In some experiments cultured LNCaP cells were pre-treated with the selective inhibitor of 5-LOX, MK886 (Sigma, Australia), prior to exposure to AA and assessment of the S-to-R ratio of HETE products. Under these conditions cells were pre-incubated at 37°C for 48 h with the inhibitor present at a final concentration of 18 μM. This dose was selected as cytotoxicity studies showed that this concentration corresponded approximately to the $\sim IC_{50}$ value for this inhibitor (data not shown).

Autoxidation of purified AA

Stock solutions of AA were purified by HPLC (see below) and diluted to a final concentration of 100 mM. Samples designated for autoxidation were placed into plastic screw cap tubes and allowed to stand at 37°C with constant exposure to ambient light. After 72 h, the samples were extracted for assessment of HETE products as outlined below.

Liquid chromatography

Native and oxidized AA in the cell samples were separated by reversed-phase HPLC using a LC18-DB column (25×0.46 cm, particle size 5 μm) eluted with methanol/water/ethanoic acid (90:10:0.1, v/v/v) at 1 mL/min. Unresolved mixtures of (*R*/*S*)-H(P)ETEs eluted between 4–7 min and authentic AA at 15–18 min, as determined by comparison with appropriate standards (Cayman, Australia). The eluting (*R*/*S*)-H(P)ETEs were collected, dried and resuspended in methanol then treated with 5 mM sodium borohydrate to generate the corresponding HETEs. The mixture was mixed vigorously, centrifuged ($3600 \times g$, 15 min) and extracted into chloroform/methanol/water (5:1:1 v/v/v). The chloroform layer was removed, the aqueous phase re-extracted with chloroform and the combined extracts dried and the residue resuspended in hexane.

Enantiomeric mixtures of 5(*R*/*S*)-, 8(*R*/*S*)-, 11(*R*/*S*)-, 12(*R*/*S*)- and 15(*R*/*S*)-HETE were separated by normal-phase chromatography using a LC-Si column (25×0.46 cm, particle size 5 μm) eluted with hexane/isopropylalcohol/acetic acid (120:2:0.1 v/v/v) at 1 mL/min. The 12(*R*/*S*)-, 15(*R*/*S*)- 11(*R*/*S*)-, 8(*R*/*S*)- and 5(*R*/*S*)-HETE eluted between 7–8, 9–11, 14–16, 20–25 and 35–45 min, respectively. The mixtures of 5(*R*/*S*)-, 8(*R*/*S*)-, 11(*R*/*S*)-, 12(*R*/*S*)- and 15(*R*/*S*)-HETE were collected, dried and resuspended in hexane then resolved into *R* or *S* stereo-isomers with a Chiralcel chiral column (Diacel Industries) eluted with hexane/isopropylalcohol/acetic acid (115:5:0.1v/v/v) at 1 mL/min and monitoring products at 238 nm.

Results

Cell-derived eicosanoids were initially separated from native AA to decrease the possibility of adventitial oxidation after isolation from the cell fraction (Figure 1A). Any ongoing oxidation in the absence of cell proteins is likely to involve radical-mediated processes and this may affect the final stereoisomeric distribution of HETE products. The regio-distribution of this mixture was determined with normal-phase chromatography [15,16]. As anticipated the regio-distribution of HETE products isolated from mixing AA with (Figure 1B) or without (Figure 1C) cancer cells were similar. These data confirmed that same regio-isomeric distribution of products can be obtained through enzymic or free radical-mediated AA modification. Next, the isolated 12 and 15 regio-isomers (corresponding to the major AA oxidation products) were separated into the *S*- and *R*-HETE products with chiral chromatography.

Separation of the various stereo-isomers in this way allowed a comprehensive quantification of the ratio of enantiomers for these major products (Table I). Typically, AA oxidation stimulated by either cancer cell type yielded predominantly 15(*S*)-HETE (Figure 2). The *S*/*R* ratio of HETEs obtained from mixing cells with AA is > 1, indicative of LOX enzymic activity. Whereas, in the absence of cells the *S*/*R* ratio of ~ 1 for the recovered HETE oxidation products is typical of non-stereo-selective AA oxidation that occurs as a result of free radical-mediated oxidation (Table I).

In separate studies, we investigated the effect of the 5-LOX inhibitor MK886 on the stereoisomer distribution of 5-HETE products obtained from LNCaP cells (Table II). Consistent with enzymic oxidation of added AA by 5-LOX, the *S*/*R* ratio of 5-HETE products was > 1 in the absence of MK886. Pre-treatment with MK886 significantly inhibited the yield of racemic 5-HETE (relative yield 0.35 ± 0.13, representing a decrease of 65%; $p < 0.05$; $n = 3$) consistent with a majority of the accumulated 5-HETE products resulting from enzymic oxidation of AA. However, some significant residual oxidation remained after inhibition of 5-LOX and the isolated products yielded 5-HETE with an *S*/*R* ratio ~ 1. This latter observation indicated that inhibition of 5-LOX did not completely ablate AA oxidation and that non-enzymic pathways continued to accumulate (*S* and *R*) 5-HETE products in near equal proportions.

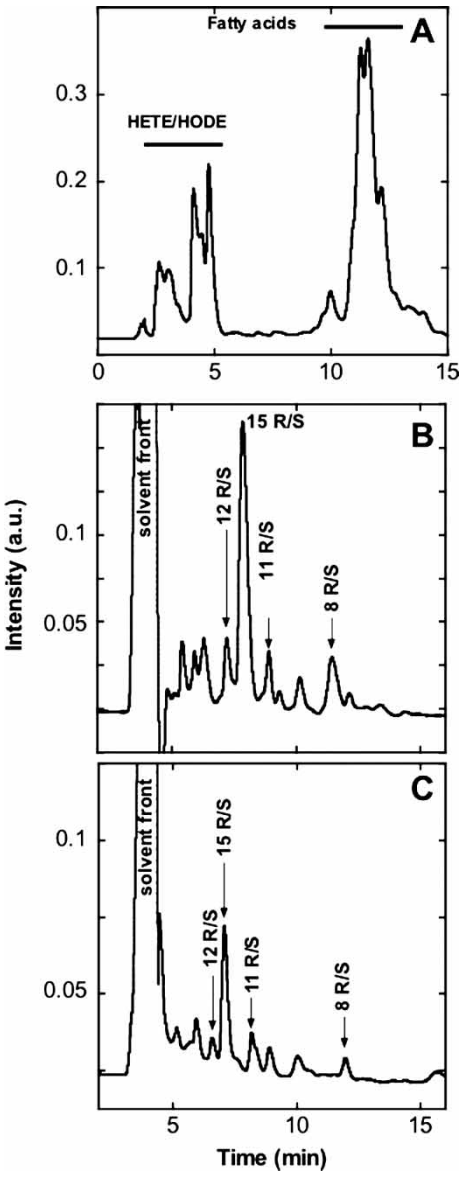


Figure 1. Purified AA (1mM) free of detectable oxidation products was exposed to cultured LNCaP cells. (A) Oxidation products were isolated from excess AA (see Methods). (B) The isolated products were separated into HETE regio-isomers using normal-phase chromatography. (C) AA autoxidation yields a similar pattern of HETE regio-isomers to that in the presence of cells. Data are representative of that obtained from at least 3 independent experiments using different cell preparations.

Table I. Stereo-selective AA oxidation by prostate cancer cells.[a]

Cell-type	12-HETE (ratio *S*/*R*)	15-HETE (ratio *S*/*R*)
LNCaP	2.8±0.4 (2.6–3.2)⋆	1.8±0.8 (1.2–2.1)⋆
PC3	2.9±0.4 (2.6–3.2)⋆	2.1±0.5 (1.7–2.7)⋆
Cell free	1.0±0.1 (0.9–1.0)	1.0±0.0 (0.99–1.1)

[a]*S*/*R* ratios were determined by chiral separation as described in the methods. The *S*/*R* ratio represents a quotient of the corresponding peak areas from the resolution of the 12- and 15-HETE stereo-isomers by chiral HPLC. Data represent mean ± SD, $n = 3$–4 experiments (data range in brackets).

⋆ Significantly different to the cell free system; $p < 0.05$.

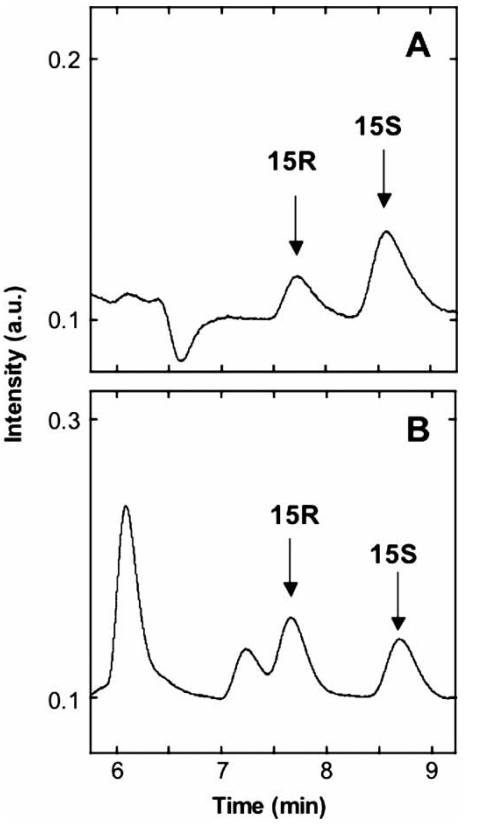


Figure 2. Isolated HETE regio-isomers from cell (A) or cell free studies (B) were separated into *R* or *S* stereo-isomers with chiral chromatography (confirmed by spiking with authentic 12/15-*S*-HETE). Peak area was used to assess the *S*-to-*R* ratio as a surrogate for LOX activity. Data are representative of that obtained from at least 3 independent experiments using different cell preparations.

Discussion

We wished to monitor LOX activity in cultured prostate cancer cells. In reviewing the available data and methodologies, we have identified that in many cases researchers in various fields including cancer have commonly utilized determinations of regio-isomer distribution combined with pharmacologic inhibition to imply changes in LOX activity [8,9,11,20–23]. This practice can lead to the incorrect interpretation of the data since (i) free radical-mediated AA oxidation leads to the same regio-isomer distribution and (ii) it is not clear whether LOX inhibitors may also act as chain breaking antioxidants or exhibit other LOX-independent activities. For example, mitochondria are direct targets of the 5-LOX inhibitor MK886. Where added to PC3 with prostate cancer cells at a final concentration of 10 μM, MK886-mediated changes to the mitochondrial transition pore, inhibited respiration and increased cell death [24]. By contrast, addition of 1 μM MK886 had no major effect on PC3 cell survival [24]. Interestingly, the free radical spin trap *N*-tertbutylnitroxide (NTBN) promotes apoptosis in lung and pancreatic cancer cells by the same mechanism as the 5-LOX inhibitor MK866, but acts distinctly from the pro-apoptotic 5-LOX inhibitor

Table II. AA oxidation mediated by LNCaP cells in the presence or absence of the 5-LOX inhibitor Mk886.[a]

Reaction conditions	5-HETE (ratio *S*/*R*)
LNCaP – 18 μM Mk886	1.7 ± 0.4 (1.3–2.0)
LNCaP + 18 μM Mk886	0.94 ± 0.3 (0.91–0.97)

[a]*S*/*R* ratios were determined by chiral separation as described in the methods. The *S*/*R* ratio represents a quotient of the corresponding peak areas from the resolution of the 5-HETE stereo-isomers by chiral HPLC. Data represent mean ± SD (data range in brackets).

SC41661A [25,26]. These data are suggestive that inhibiting radical-mediated oxidation of AA can yield a similar anti-tumourigenic effect as blockade of the 5-LOX pathway and further emphasizes the importance of appropriate validation for implicating LOX enzyme activity in cancer studies.

Reversed-phase chromatography readily separates regio-isomers of (^{3}H)-(*R*/*S*)HETE in malignant prostate cancer cells exposed to (^{3}H)-labelled AA. Assignment of LOX activity is made by comparing product retention times with the corresponding (^{3}H)-regio-isomer (racemic mixture) or the pure (*S*) or (*R*) HETEs. Note, racemic (*R*/*S*)HETE elutes with identical retention time as the pure HETEs. Although monitoring (^{3}H)-labelled HETEs is useful for assigning HETE regio-isomers, monitoring regio-isomer distribution is not sufficient to assign LOX activity as free radical-mediated oxidation of AA yields the identical HETE products. This complication has been identified in other areas of medical research such as atherosclerosis where the production of HETE stereoisomers has been employed to ascertain the contribution of enzymic vs non-enzymic lipid oxidation to lipoprotein modification *in vitro* [27] and in atherosclerotic lesions formed in the vessel wall [28,29].

In addition to the combined use of reversed- and chiral-phase liquid chromatography for the isolation of HETE enantiomers, the development of techniques that couple liquid chromatography with mass spectrometry represents a significant advance in the study of LOX activity in biological samples [30]. The ability to now reproducibly detect and quantify a wide array of HETE stereo-isomers in cells and intact human tissues [31] is an important step in the rigorous identification of roles for LOX and their eicosanoid products in cancer progression. Another alternate method for assessing HETE products is use of a commercial non-radiolabelled ELISA approach, although we are unaware of any study that compares and validates this method with HPLC- or mass spectrometry-based techniques.

As a final caveat to this study, it is important to point out that at least some of the *R*-HETE products of AA oxidation have biological activity in different tissues, so that while the *S*-HETE enantiomers may predominate in tissues where LOX activity is enhanced, formation of *R*-HETE products can lead to altered function in some biological pathways [32,33]. Moreover, enzymes forming *R*-configuration products are found in both the animal and plant kingdoms: R-LOX are prevalent in aquatic invertebrates with their *R*-HETE products playing a role in reproductive function. Also, a 12(*R*)-LOX has been found recently in humans and mice and 12(*R*)-HETE appears to be involved in the pathophysiology of psoriasis and other proliferative skin diseases [34].

In conclusion, the assignment of LOX activity based on monitoring HETE regio-isomers alone may be misleading. The widespread assessment of AA oxidation through determining HETE regio-isomer as a surrogate for LOX activity continues to be misinterpreted in many fields, but in particular cancer research. Therefore, it is imperative to fully assign HETE stereo-isomerism and, thereby, LOX activity in cancer cells or tissue. Continued inappropriate use of HETE regio-distributions [35] or other non-specific determination of lipid oxidation [36] as surrogate measures of LOX activity will only confound future studies.

Acknowledgements

Grant funds for this research were awarded to PKW by the Australian Research Council Discovery (Grant: DP0878559).

***Declaration of interest*:** The authors have no conflict of interest to declare. The authors alone are responsible for generating the data and preparing the manuscript.

References

[1] Stocker R, Keaney Jr JF. Role of oxidative modifications in atherosclerosis. Physiol Rev 2004;84:1381–1478.
[2] Marnett LJ, Honn KV. Overview of articles on eicosanoids and cancer. Cancer Metas Rev 1994;13:237–239.
[3] Pidgeon GP, Lysaght J, Krishnamoorthy S, Reynolds JV, O'Byrne K, Nie D, Honn KV. Lipoxygenase metabolism: roles in tumor progression and survival. Cancer Metas Rev 2007;26:503–524.
[4] Patel MI, Kurek C, Dong Q. The arachidonic acid pathway and its role in prostate cancer development and progression. J Urol 2008;179:1668–1675.
[5] Pidgeon GP, Kandouz M, Meram A, Honn KV. Mechanisms controlling cell cycle arrest and induction of apoptosis after 12-lipoxygenase inhibition in prostate cancer cells. Cancer Res 2002;62:2717–2721.
[6] Tong WG, Ding XZ, Adrian TE. The mechanisms of lipoxygenase inhibitor-induced apoptosis in human breast cancer cells. Biochem Biophys Res Commun 2002;296:942–948.
[7] Faas FH, Dang AQ, Pollard M, Hong XM, Fan K, Luckert PH, Schutz M. Increased phospholipid fatty acid remodeling in human and rat prostatic adenocarcinoma tissues. J Urol 1996;156:243–248.
[8] Hong J, Bose M, Ju J, Ryu JH, Chen X, Sang S, Lee MJ, Yang CS. Modulation of arachidonic acid metabolism by curcumin

and related beta-diketone derivatives: effects on cytosolic phospholipase A(2), cyclooxygenases and 5-lipoxygenase. Carcinogenesis 2004;25:1671–1679.

[9] Nony PA, Kennett SB, Glasgow WC, Olden K, Roberts JD. 15S-Lipoxygenase -2 mediates arachidonic acid-stimulated adhesion of human breast carcinoma cells through the activation of TAK1, MKK6, and p38 MAPK. J Biol Chem 2005;280:31413–31419.

[10] Shappell SB, Boeglin WE, Olson SJ, Kasper S, Brash AR. 15-Lipoxygenase-2 (15-LOX-2) is expressed in benign prostatic epithelium and reduced in prostate adenocarcinoma. Am J Pathol 1999;155:235–245.

[11] Shappell SB, Gupta RA, Manning S, Whitehead R, Boeglin WE, Schneider C, Case T, Price J, Jack GS, Wheeler TM, Matusik RJ, Brash AR, Dubois RN. 15 s-Hydroxyeicosatetraenoic acid activates peroxisome proliferator-activated receptor gamma and inhibits proliferation in PC3 prostate carcinoma cells. Cancer Res 2001;61:497–503.

[12] Kaduce TL, Fang X, Harmon SD, Oltman CL, Dellsperger KC, Teesch LM, Gopal VR, Falck JR, Campbell WB, Weintraub NL, Spector AA. 20-hydroxyeicosatetraenoic acid (20-HETE) metabolism in coronary endothelial cells. J Biol Chem 2004;279:2648–2656.

[13] Pommery J, Pommery N, Henichart JP. Modification of eicosanoid profile in human blood treated by dual COX/LOX inhibitors. Prost Leuko Essent Fatty Acids 2005;73:411–417.

[14] Upston JM, Witting PK, Brown AJ, Stocker R, Keaney JF Jr. Effect of vitamin E on aortic lipid oxidation and intimal proliferation after arterial injury in cholesterol-fed rabbits. Free Radic Biol Med 2001;31:1245–1253.

[15] Rayner BS, Stocker R, Lay PA, Witting PK. Regio- and stereo-chemical oxidation of linoleic acid by human myoglobin and hydrogen peroxide: Tyr(103) affects rate and product distribution. Biochem J 2004;381:365–372.

[16] Upston JM, Neuzil J, Witting PK, Alleva R, Stocker R. Oxidation of free fatty acids in low density lipoprotein by 15-lipoxygenase stimulates nonenzymic, alpha-tocopherol-mediated peroxidation of cholesteryl esters. J Biol Chem 1997;272:30067–30074.

[17] Chamulitrat W, Mason RP. Lipid peroxyl radical intermediates in the peroxidation of polyunsaturated fatty acids by lipoxygenase. Direct electron spin resonance investigations. J Biol Chem 1989;264:20968–20973.

[18] Sved P, Scott KF, McLeod D, King N, Singh J, Tsatralis T, Nikolov B, Boulas J, Nallan L, Gelb MH, Sajinovic M, Russell P, Dong Q. Oncogenic action of secreted phospholipase A2 in prostate cancer. Cancer Res 2004;64:6934–6940.

[19] Yue H, Jansen SA, Strauss KI, Borenstein MR, Barbe MF, Rossi LJ, Murphy E. A liquid chromatography/mass spectrometric method for simultaneous analysis of arachidonic acid and its endogenous eicosanoid metabolites prostaglandins, dihydroxyeicosatrienoic acids, hydroxyeicosatetraenoic acids, and epoxyeicosatrienoic acids in rat brain tissue. J Pharm Biomed Anal 2007;43:1122–1134.

[20] Berg C, Hammarström S, Herbertsson H, Lindström E, Svensson AC, Söderström M, Tengvall P, Bengtsson T. Platelet-induced growth of human fibroblasts is associated with an increased expression of 5-lipoxygenase. Thromb Haemost 2006;96:652–659.

[21] Espada CE, Berra MA, Martinez MJ, Eynard AR, Pasqualini ME. Effect of Chia oil (Salvia Hispanica) rich in ϖ-3 fatty acids on the eicosanoid release, apoptosis and T-lymphocyte tumor infiltration in a murine mammary gland adenocarcinoma. Prost Leuko Essent Fatty Acids 2007;77:21–28.

[22] Ma KF, Yang HY, Chen Z, Qi LY, Zhu DY, Lou YJ. Enhanced expressions and activations of leukotriene C4 synthesis enzymes in D-galactosamine/lipopolysaccharide-induced rat fulminant hepatic failure model. World J Gastroenterol 2008;14:2748–2756.

[23] Shappell SB, Boeglin WE, Olson SJ, Kasper S, Brash AR. 15-lipoxygenase-2 (15-LOX-2) is expressed in benign prostatic epithelium and reduced in prostate adenocarcinoma. Am J Pathol 1999;155:235–245.

[24] Gugliucci A, Ranzato L, Scorrano L, Colonna R, Petronilli V, Cusan C, Prato M, Mancini M, Pagano F, Bernardi P. Mitochondria are direct targets of the lipoxygenase inhibitor MK886. A strategy for cell killing by combined treatment with MK886 and cyclooxygenase inhibitors. J Biol Chem 2002l;277:31789–31795.

[25] Anderson KM, Seed T, Alrefai W, Ou D, Harris JE. NTBN, a free radical spin trap induces programmed cell death in human pancreatic cancer (PANC-1) cells. Anticancer Res 1998;18:3213–3222.

[26] Ou D, Bonomi P, Jao W, Jadko S, Harris JE, Anderson KM. The mode of cell death in H-358 lung cancer cells cultured with inhibitors of 5-lipoxygenase or the free radical spin trap, NTBN. Cancer Let 2001;166:223–231.

[27] Heydeck D, Upston JM, Viita H, Ylâ-Herttuala S, Stocker R. Oxidation of LDL by rabbit and human 15-lipoxygenase: prevalence of nonenzymatic reactions. J Lipid Res 2001;42:1082–1088.

[28] Kuhn H, Chaitidis P, Roffeis J, Walther M. Arachidonic acid metabolites in the cardiovascular system: the role of lipoxygenase isoforms in atherogenesis with particular emphasis on vascular remodeling. J Cardiovasc Pharm 2007;50:609–620.

[29] Kuhn H, Heydeck D, Hugou I, Gniwotta C. In vivo action of 15-lipoxygenase in early stages of human atherogenesis. J Clin Invest 1997;99:888–893.

[30] Deems R, Buczynski MW, Bowers-Gentry R, Harkewicz R, Dennis EA. Detection and quantitation of eicosanoids via high performance liquid chromatography-electrospray ionization-mass spectrometry. Methods Enzymol 2007;432:59–82.

[31] Blewett AJ, Varma D, Gilles T, Libonati JR, Jansen SA. Development and validation of a high-performance liquid chromatography-electrospray mass spectrometry method for the simultaneous determination of 23 eicosanoids. J Pharm Biomed Anal 2008;46:653–662.

[32] Chiaro CR, Patel RD, Perdew GH. 12(R)-Hydroxy-5(Z),8(Z),10(E),14(Z)-eicosatetraenoic acid [12(R)-HETE], an arachidonic acid derivative, is an activator of the aryl hydrocarbon receptor. Mol Pharmacol 2008;74:1649–1656.

[33] Ciccoli R, Sahi S, Singh S, Prakash H, Zafiriou MP, Ishdorj G, Kock JL, Nigam S. Oxygenation by COX-2 (cyclooxygenase-2) of 3-HETE (3-hydroxy-eicosatetraenoic acid), a fungal mimetic of arachidonic acid, produces a cascade of novel bioactive 3-hydroxyeicosanoids. Biochem J 2005;390:737–747.

[34] Schneider C, Brash AR. Lipoxygenase-catalyzed formation of R-configuration hydroperoxides. Prostaglandins Other Lipid Mediat 2002;68–69:291–301.

[35] Frohberg P, Drutkowski G, Wobst I. Monitoring eicosanoid biosynthesis via lipoxygenase and cyclooxygenase pathways in human whole blood by single HPLC run. J Pharm Biomed Anal 2006;41:1317–1324.

[36] Somvanshi RK, Singh AK, Saxena M, Mishra B, Dey S. Development of novel peptide inhibitor of Lipoxygenase based on biochemical and BIAcore evidences. Biochim Biophys Acta 2008;1784:1812–1817.

This paper was first published online on iFirst on 12 August 2009.