On the expression of cytosolic calcium-independent phospholipase A_2 (88 kDa) in immature and mature myeloid cells and its role in leukotriene synthesis in human granulocytes

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Abstract The human calcium-independent phospholipase A_2 ($iPLA_2$; 88 kDa) has recently been cloned (Larsson, P.K.A., Claesson, H.-E. and Kennedy, B.P. (1998) J. Biol. Chem. 272, 207–214). Here we demonstrate the expression of the human $iPLA_2$ mRNA and its splice variants in blood progenitor cells, immature leukemic cells and mature granulocytes. Chromatographical resolvable $iPLA_2$ activity was found in the cytosolic fraction of granulocytes and the activity was inhibited by the $iPLA_2$ inhibitor bromoenol lactone. This drug also inhibited leukotriene synthesis in human granulocytes, induced by low concentration of calcium ionophore A23187 (0.10–0.15 μM) or opsonized zymosan. These results suggest that $iPLA_2$ is involved in the regulation of the pool of arachidonic acid destined for leukotriene synthesis in human granulocytes.

Key words: Phospholipase A_2; Arachidonic acid; Leukotriene; Bromoenol lactone

1. Introduction

Phospholipase A_2 (PLA_2) is a family of enzymes hydrolyzing the acyl ester bond at the *sn*-2 position in phospholipids [1,2]. PLA_2 enzymes can be subdivided into extracellular or intracellular forms, based on the enzymes' localization during catalysis [2]. Intracellular PLA_2s can be further separated into two groups, calcium-dependent and calcium-independent enzymes. Calcium-dependent intracellular enzymes are represented by the well-studied 85-kDa cytosolic (c) PLA_2 [3]. Calcium-independent (i) PLA_2s, on the other hand, are a diverse group of enzymes that have a wide tissue distribution [2,4]. Different intracellular $iPLA_2$ has been purified from human myocardium [5], bovine brain [6], $P388D_1$ murine macrophages [7] and rabbit kidney [8]. Recently, much attention has been focused on an $iPLA_2$ originally purified from $P388D_1$ cells [7]. Since this $iPLA_2$ only makes up a minor fraction of the total cellular protein content and the purified enzyme is very unstable, it has been difficult to isolate and study this enzyme. However, the large scale purification of $iPLA_2$ from Chinese hamster ovary (CHO) cells as well as cDNA cloning and functional expression of the enzyme have increased our knowledge about the $iPLA_2$ protein [9]. The enzyme does not demonstrate any significant preference for fatty acids at the *sn*-2 position in phospholipids [9]. The $iPLA_2$ protein contains ankyrin repeats and a GXSXG consensus lipase motif. Ankyrin repeats are suggested to participate in protein-protein interactions and may be responsible for the $iPLA_2$ tetramer formation which is suggested to be the in vivo active form of the enzyme [9]. Recently, the full length cDNA sequence for the human form was reported as well as the expression of multiple splice variants [10]. Interestingly, two of these $iPLA_2$ splice variants would result in a protein containing just the N-terminal ankyrin repeats and not the active site. We have suggested that these forms of the $iPLA_2$ protein could function as in vivo inhibitors of the $iPLA_2$ activity because they could render the formation of a tetramer with one or several monomers that lacked the active site [10].

The murine 85-kDa $iPLA_2$ enzyme is postulated to primarily play a role in membrane phospholipid remodeling and not in the liberation of arachidonic acid [11,12]. However, the release of arachidonic acid from smooth muscle cells has been ascribed to the action of $iPLA_2$ [13]. In fact, the release of arachidonic acid from smooth muscle cells was triggered by depletion of intracellular calcium stores rather than an influx of extracellular calcium [13]. Furthermore, arachidonic acid release has been demonstrated even in the absence of increased cytosolic calcium concentrations in murine macrophages [14]. Recently a calcium-independent release of arachidonic acid in rat neutrophils through the activation of $iPLA_2$ was described [15] and this release of arachidonic acid was inhibited by the $iPLA_2$ inhibitor bromoenol lactone (BEL).

Arachidonic acid is the main precursor for important biological lipid mediators such as leukotrienes (LT) and prostaglandins [16]. The liberation of arachidonic acid, destined for leukotriene synthesis, has traditionally been ascribed to the activation of one or more calcium-dependent PLA_2s. Furthermore, $cPLA_2$ deficient mice do not produce leukotrienes or prostaglandins upon activation with calcium ionophore A23187 [17]. However, these inbred mice strains also contain a natural disruption in the $sPLA_2$ (type II) gene which makes it difficult to draw a more general conclusion about the relative role of $cPLA_2$ in eicosanoid synthesis in mice [17,18]. Results of anti-sense experiments in human monocytes indicate that there might also be species differences since the synthesis of prostaglandins, but not leukotrienes, was attenuated in cells treated with anti-sense oligonucleotides against $cPLA_2$ [19]. These studies indicate that different PLA_2 enzymes appear to be involved in the release of arachidonic acid from different membrane phospholipid pools. Thus, the released arachidonic acid can serve different functions, such as a precursor for eicosanoids or as an activator of NADPH oxidase [15,20]. A recent report demonstrated an increased activity of calcium-independent PLA_2 in HL-60 cells during differentia-

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tion to granulocytes [21]. The aim of the present study was to investigate the expression of $iPLA_2$ (88 kDa) in myeloid cells of differing maturity and the role of the enzyme in leukotriene synthesis in human granulocytes.

2. Materials and methods

2.1. Materials

HPLC solvents were purchased from Rathburn Chemicals (Walkerburn, UK). Arachidonic acid and (*E*)-6-(bromomethylene)-3-(1-naphthalenyl)-2*H*-tetrahydropyran-2-one (bromoenol lactone (BEL)) were from Biomol (Plymouth Meeting, PA, USA). Monoflow 2 scintillation liquid was obtained from National Diagnostics (Manville, NJ, USA). 1-Palmitoyl 2-[1-^{14}C]palmitoyl PC (55.5 mCi/mmol) was obtained from New England Nuclear. FPLC System and the protein purification column were from Pharmacia Biotech (Uppsala, Sweden).

2.2. Isolation of cells

Human granulocytes were isolated from concentrates of leukocytes, obtained from healthy donors at the Karolinska Hospital, by dextran sedimentation, Lymphoprep (Nycomed) centrifugation and hypotonic lysis. Cells were thereafter washed twice in phosphate buffered saline (PBS). Cellular viability was examined by trypan blue dye exclusion and was always $>95\%$. The monoclonal B cell line RAJI and the promyelocytic cell line HL-60 were cultivated in RPMI 1640 medium, supplemented with 10% fetal bovine serum, 100 μg/ml streptomycin and 100 units/ml penicillin and grown in a humidified atmosphere with 5% CO_2. The source of CD34 positive human blood progenitor cells (stem cells) was leukapheresis products harvested after treatment with granulocyte-colony stimulating factor. Cells were purified with MiniMACS Magnetic Separation System and MACS CD34 progenitor Cell Isolation Kit (QBEND/10). CD34 positive cells were analyzed with Coulter for the expression of CD34, CD38, CD33 and HLA-Dr. The source of human myeloblasts was previously Ficoll-Isopaque separated and frozen peripheral blood myeloblasts collected at diagnosis or relapse from adult patients with acute myeloid leukemia (AML), FAB (French American British) subtypes, M1, M2, M4, M5a and M5b. The results show that the purity of the progenitor cells or myeloblasts used in this study was above 98%. All patient samples were taken after informed consent from the patients. This investigation has been approved by the Ethics Committee at Karolinska Hospital.

2.3. Isolation of total cellular RNA and RT-PCR

Total cellular RNA was isolated using Trizol Reagent (Life Technologies) according to the instructions of the manufacturer. Total RNA (2 μg) was submitted to reverse transcription reaction using reverse transcriptase (USB) and oligo-dT primers. PCR was carried out using 2 μl of the reverse transcription reaction mixture. The conditions for PCR reactions were 94°C for 5 min, followed by 40 cycles of 94°C for 30 s, 60°C for 30 s, 72°C for 30 s and finally extension at 72°C for 10 min. In order to determine if the isolated cDNA was intact, PCR amplification of a β-actin fragment was performed (data not shown). Primers used for PCR were: $iPLA_2$ primer 33 (sense: 5′-CAGGGCTCTGCAGCGCCACATCAT-3′); $iPLA_2$ primer 34 (anti-sense: 5′-GGCCTTCTCGATGGCGATGAGGAG-3′).

2.4. Purification of cytosolic calcium-independent PLA_2 and $iPLA_2$ assay

Human granulocytes were resuspended in buffer A (20 mM Tris-HCl (pH 8.0), 1 mM EDTA, 1 mM EGTA, 0.5 mM dithiotreitol (DTT)) supplemented with 1 mM phenylmethanesulfonyl fluoride (PMSF) and homogenized by sonication three times for five seconds. Supernatant was obtained after centrifugation at $100\,000\times g$ for 60 min. Liquid chromatography was performed on a Pharmacia FPLC System. The $100\,000\times g$ supernatant was applied to a 6-ml anion exchange Resource-Q column, preequilibrated with buffer A. After washing, bound proteins were eluted by a 20-ml linear gradient from 0 to 1 M NaCl. Fractions of 1 ml were collected and 150-μl aliquots of these were assayed for $iPLA_2$ activity. $iPLA_2$ activity was assayed with 1-palmitoyl 2-[1-^{14}C]palmitoyl phosphatidylcholine (PC) as substrate. The phospholipids were dried under nitrogen and resuspended in 250 μl of buffer A supplemented with 1 mg/ml albumin and 10% glycerol, thus yielding a final concentration of 2 μM PC. This preparation was vortexed and sonicated in a water bath for 10 min at 4°C. The reaction was initiated by adding 150-μl aliquots of each fraction and allowed to proceed for 60 min at 37°C. Subsequently, the reaction was terminated by the addition of 2 volumes of methanol containing 0.5% acetic acid and 40 μM stearic acid, as carrier, followed by vigorous vortexing.

2.5. Incubation of intact cells and broken cell assay

Human granulocytes (10^7) were suspended in PBS (1 ml) and preincubated for 10 min at 37°C with or without BEL as indicated. Subsequently, calcium ionophore A23187 was added and the samples were incubated for 10 min at 37°C and terminated by the addition of 1 ml of methanol. For the broken cell assay, cells (10^7) were suspended in Ca^{2+}-free PBS (1 ml) supplemented with 1 mM EDTA and sonicated three times for five seconds on ice. BEL was added 10 min prior to the addition of ATP (1 mM), $CaCl_2$ (2 mM) and arachidonic acid (20 μM). The samples were thereafter incubated for 10 min at 37°C and subsequently terminated by the addition of 1 ml of methanol.

2.6. Measurement of [^{3}H]arachidonic acid release

Human granulocytes were resuspended at 2×10^6/ml in RPMI 1640 medium containing 0.25 μCi/ml [^{3}H]arachidonic acid and incubated for 60 min at 37°C. Thereafter, cells were centrifuged and washed twice to remove unincorporated arachidonic acid. Labeled cells (10^7) were resuspended in 1 ml PBS containing 1 mg/ml of fatty acid free albumin. The samples were incubated with or without BEL for 10 min at 37°C prior to the addition of 0.15 μM A23187 and thereafter incubated for another period of 10 min. The reaction was terminated with 1 volume of methanol.

2.7. Analysis of leukotrienes and free fatty acids

Methanol precipitated samples were centrifuged and the resulting supernatants were applied to octadecyl reverse-phase columns (Chromabond, C18, 100 mg, Macherey-Nagel, Düren, Germany). The extracted samples were analyzed in a reverse-phase (RP) HPLC system. The mobile phase was methanol/water/trifluoroacetic acid 70:30:0.007 (by vol.) for leukotrienes and monohydroxy acids. Elution of arachidonic acid was accomplished by using a mobile phase of methanol/water/trifluoroacetic acid 85:15:0.007 (by vol.) at a flow rate of 1.2 ml/min. Analysis was performed by comparing retention times with authentic standards. Radioactivity was detected with a β-RAM HPLC flow-through monitor system (Inus System, USA) using Monoflow 2 scintillation liquid coupled on-line to UV-spectrophotometers. Quantitative determination was performed by peak area integration.

2.8. Other procedures

Subcloning of PCR fragments into pCR2.1 was done using TOPO TA Cloning kit (Invitrogen) and the cDNA insert was sequenced using ABI PRISM Dye Terminator Cycle Sequencing Ready Reaction kit (Perkin Elmer) and an ABI 373A automated DNA sequencer. Protein content was measured with a kit (Bio-Rad) against bovine serum albumin as the standard protein. Statistical analysis was performed with Student's paired *t*-test.

3. Results and discussion

3.1. Expression of 88-kDa $iPLA_2$ and its splice variants in immature and mature myeloid cells

The selective expression of calcium-dependent $cPLA_2$ mRNA in lymphopoiesis has previously been described [22]. To determine the expression of the 88-kDa $iPLA_2$ mRNA in myelopoiesis, RT-PCR analysis was performed on total RNA isolated from various myeloid cells. In these experiments, immature myeloid cells were isolated from patients with acute myeloid leukemia (AML). The differentiation of myeloid tumor cells is blocked at an early specific stage in AML. Although these cells are malignant, they represent cell clones at various stages of differentiation, and can be used to investigate the expression of genes early in the differentiation process. Cells of different maturity, from the pluripotent blood

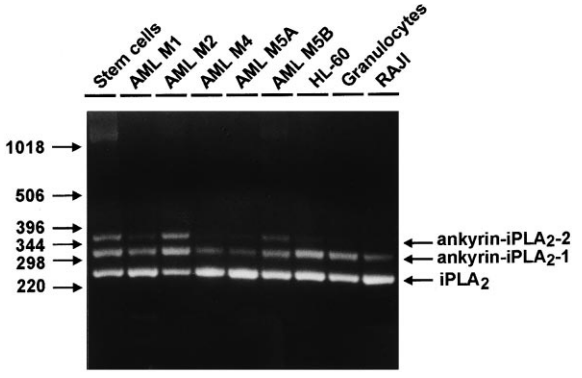

Fig. 1. RT-PCR analysis of $iPLA_2$ and its two splice variants. RT-PCR analysis of total RNA from different cells was performed as described in Section 2. A 10-μl aliquot of amplified material was analyzed on a 1.5% agarose gel, containing ethidium bromide. Arrows on the left indicate the size marker in bp. Arrows on the right indicate the position of the different splice variants of the $iPLA_2$ transcript. The source of CD34 positive human blood progenitor cells (stem cells) was leukapheresis product. The source of human myeloblasts was previously Ficoll-Isopaque separated and frozen peripheral blood myeloblasts collected at diagnosis or relapse from adult patients with acute myeloid leukemia (AML), FAB subtypes, M1, M2, M4, M5a and M5b. The purity of the blood progenitor cells or myeloblasts used in this study was more than 98%. HL-60: promyelocytic cell line; RAJI: monoclonal B cell line.

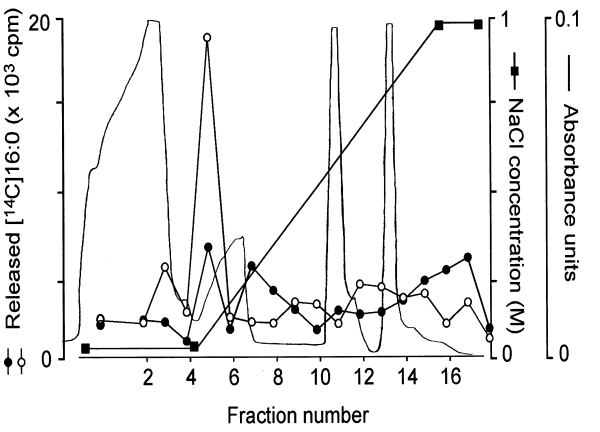

Fig. 2. Elution profile of proteins and $iPLA_2$ activity from an anion exchange column. Human granulocytes were resuspended in buffer and homogenized by sonication for three times five seconds. Supernatant was obtained after centrifugation at $100\,000 \times g$ for 60 min. The $100\,000 \times g$ supernatant was applied to a 6-ml anion exchange Resource-Q column. After washing, bound proteins were eluted by a 20-ml linear gradient from 0 to 1 M NaCl. Fractions of 1 ml were collected and 150-μl aliquots of each fraction were assayed for $iPLA_2$ by incubation for 60 min with 2 μM 1-palmitoyl [2-^{14}C]palmitoyl PC as substrate without (– ∘ –) or with (– ● –) 10 μM BEL as described in Section 2. The results are expressed as the amount of free [^{14}C]palmitic acid (cpm) in each analyzed aliquot.

progenitor cell to the terminal differentiated granulocyte, were investigated as outlined in Fig. 1. Amplification of a fragment with the expected size, 237 bp, was observed in all examined cDNAs. Interestingly, fragments of the isoforms of this $iPLA_2$, containing just the ankyrin repeats and not the active site, were easily amplified: ankyrin-$iPLA_2$-1 (isoform 1) was amplified to various extents in all reactions (289-bp fragment) whereas ankyrin-$iPLA_2$-2 (isoform 2) was amplified to a higher extent when using cDNA derived from the more immature cells (341-bp fragment) (Fig. 1). The expression of the ankyrin forms of $iPLA_2$ in myeloid cells would attenuate the $iPLA_2$ activity in immature cells since these forms may act as in vivo inhibitors. The identity of the PCR-derived fragments was verified by subcloning into pCR2.1 and subsequent sequencing using m13 forward and reverse primers (data not shown). Taken together, these results demonstrate that the $iPLA_2$ gene is constitutively expressed in the examined cells. However, the expression of alternatively spliced variants differs in the investigated cells.

3.2. *Neutrophil cytosol contains $iPLA_2$ activity*

To investigate if granulocytes possess $iPLA_2$ activity, the cytosol from granulocytes was subjected to anion-exchange chromatography using a Resource-Q column. The $100\,000 \times g$ supernatant was applied to the column and bound proteins were eluted using a linear gradient from 0 to 1 M NaCl. Aliquots of each fraction were incubated with or without BEL and incubated for 60 min under calcium-free conditions in the presence of radiolabelled phospholipids. The results outlined in Fig. 2 indicate that there is one major chromatographical resolvable $iPLA_2$ activity that is inhibited by BEL. Furthermore, the observed profile of $iPLA_2$ activity is in accordance with the elution pattern of the murine 85-kDa $iPLA_2$ enzyme, eluting with low salt wash, purified from the macrophage-like cell line P388D1 [7].

3.3. *Effects of BEL on leukotriene formation in granulocytes*

Calcium ionophore A23187 has been demonstrated to induce the release of arachidonic acid even in the absence of increased intracellular calcium concentrations [13]. This BEL-inhibitable calcium-independent release of arachidonic acid is suggested to be coupled to the depletion of intracellular calcium stores [13]. Ionophore A23187 readily penetrates the plasma membrane and can thereby mediate the passive transport of calcium ions from intracellular stores and induce calcium depletion from these sites. Since BEL inhibited the activity of $iPLA_2$ in the cytosolic fraction from neutrophils (Fig. 2), the role of BEL-sensitive enzymes in leukotriene synthesis in granulocytes was investigated. Intact granulocytes were

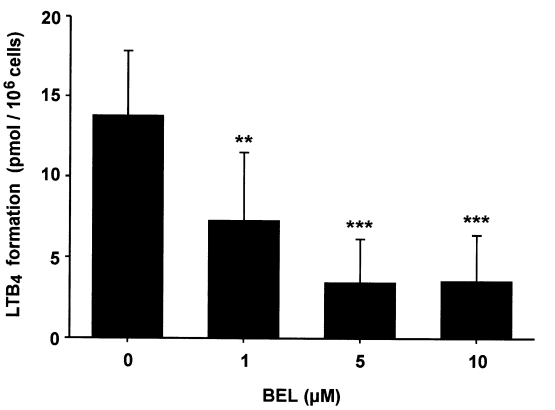

Fig. 3. Dose-response effects of BEL on ionophore A23187-induced LTB_4 formation in human granulocytes. Human granulocytes (10^7) were incubated with indicated concentrations of BEL for 10 min at 37°C and subsequently stimulated with 0.1 μM A23187 for another period of 10 min. The results are expressed as formation of LTB_4 (pmol/10^6 cells). Data show the mean ± S.D., $n = 5$ (***$P < 0.001$ vs. control and **$P < 0.01$ vs. control).

stimulated with increasing concentrations of BEL and low-dose ionophore A23187 (0.10–0.15 μM). At these concentrations of ionophore A23187, minimal amounts of the non-enzymatically products of LTA_4, i.e. 6-*trans*-LTB_4 and 6-*trans*-12-epi-LTB_4, were formed (data not shown). As depicted in Fig. 3, BEL dose-dependently inibited leukotriene synthesis, induced by 0.10 μM ionophore A23187. In another set of experiments, using 0.15 μM calcium ionophore A23187, a 69% inhibition of the formation of 5-lipoxygenase products was observed with 5 μM BEL. The total amounts of 5-lipoxygenase products, i.e. LTB_4, 6-*trans*-LTB_4, 6-*trans*-12-epi-LTB_4 and 5-hydroxyeicosatetraenoic acid (5-HETE), were 46.8 ± 6.4 and 15.2 ± 8.3 pmol/10^6 cells (data is mean ± range from two experiments performed in duplicate) in control and BEL treated cells, respectively. The inhibitory effect of BEL on LTB_4 formation at higher concentrations of A23187 (1 μM) was not as prominent as with low concentrations of ionophore A23187. The formation of 6-*trans*-LTB_4, 6-*trans*-12-epi-LTB_4 and 5-HETE was at these higher ionophore concentrations the major products and the inhibitory effect of BEL was mainly observed on the formation of these products (data not shown). Thus, the formation of 5-lipoxygenase products after stimulation with high concentrations of ionophore A23187 indicates that the cells' capacity to release arachidonic acid is not rate-limiting in LTB_4 formation but instead the activity of 5-lipoxygenase and LTA_4 hydrolase. These results indicate that there are BEL-insensitive mechanisms involved in the release of arachidonic acid destined for leukotriene synthesis. These mechanisms are likely to involve the calcium-dependent PLA_2s. Several lines of evidence suggest that calcium-dependent $cPLA_2$ plays a key role in the formation of leukotrienes A23187 [17,18]. However, the main PLA_2 activity at low concentrations of ionophore A23187 is inhibited by BEL and might therefore be ascribed to $iPLA_2$ enzymes, indicating that $iPLA_2$ is of importance for leukotriene synthesis under certain conditions.

In order to investigate the role of $iPLA_2$ on leukotriene synthesis induced by a more physiological stimulus, granulocytes were incubated with opsonized zymosan for 10 min in the presence or absence of BEL. Cells stimulated with opsonized zymosan alone produced 4.3 ± 0.5 pmol LTB_4/10^6 cells. Incubation of granulocytes with BEL (10 μM) for 10 min prior to the addition of opsonized zymosan resulted in the production of 1.7 ± 0.5 pmol LTB_4/10^6 cells (data is mean ± range from two separate experiments), thus resulting in a 60% reduction of the amounts of LTB_4.

To investigate if BEL possessed any direct inhibitory effects on 5-lipoxygenase, cellular homogenates of granulocytes were incubated with BEL. The synthesis of leukotrienes in homogenates of granulocytes was initiated by the addition of calcium, ATP and 20 μM exogenous arachidonic acid. In such experiments, BEL (10 μM) had no effect on 5-lipoxygenase activity and the levels of 5-lipoxygenase products were 380.8 ± 75.5 and 399.3 ± 77.3 5 pmol/10^6 cells (data is mean ± SD from three experiments performed in duplicate) in the absence or presence of BEL, respectively.

3.4. Effects of BEL on arachidonic acid release

BEL is reported to be about 1000-fold more specific for $iPLA_2$ in comparison to $cPLA_2$. Experiments were employed to investigate the effects of BEL on ionophore A23187-induced arachidonic acid release in human granulocytes. Cells were labelled with [^{3}H]arachidonic acid for 60 min prior to the addition of BEL or ethanol (control). Cells stimulated with 0.15 μM ionophore A23187 alone released 4230 ± 40 cpm of [^{3}H]arachidonic acid. However, preincubation of the cells with 10 μM BEL for 10 min prior to stimulation with ionophore A23187 led to decreased amounts of free [^{3}H]arachidonic acid (2370 ± 20 cpm cells) (mean ± range from one representative experiment, out of two, performed in duplicate). These results demonstrate that BEL inhibited the release of arachidonic acid in granulocytes, activated with low concentration of calcium ionophore. BEL was not cytotoxic to granulocytes since cells exposed to 10 μM BEL for 20 min at 37°C demonstrated similar viability as the control (data not shown).

3.5. Phosphatidic acid phosphohydrolase and leukotriene formation

Although BEL displays selectivity between different PLA_2 enzymes it does, however, inhibit cytosolic magnesium-dependent phosphatidic acid phosphohydrolase (PAP-1) at similar concentrations as for $iPLA_2$ [23]. Therefore, when employing BEL, caution should be taken when interpreting results. Since granulocytes contain PAP activity, and in fact 20% of the total PAP activity is cytosolic (PAP-1) [24], the potential involvement of PAP-1 in ionophore A23187-induced leukotriene synthesis in granulocytes was investigated. Propranolol, a PAP inhibitor, dose-dependently inhibits degranulation and activation of NADPH-oxidase in granulocytes at a concentration of 50–100 μM [25]. Furthermore, propranolol inhibits fMLP-induced formation of diacylglycerol (DAG), which is a possible source of arachidonic acid [26]. In order to determine whether the effect of BEL on leukotriene synthesis was mediated through inhibition of PAP, cells were preincubated with propranolol (250 μM solved in dimethylsulfoxide (DMSO)) or DMSO for 10 min and subsequently stimulated with 0.15 μM ionophore A23187 for another period of 10 min. The formation of 5-lipoxygenase products was 31.4 ± 11.0 and 31.0 ± 10.5 pmol/10^6 cells (data is mean ± range from two experiments performed in duplicate), in the absence or presence of propranolol, respectively. These results indicate that the inhibitory action of BEL on leukotriene synthesis was not due to inhibition of PAP-1. On the contrary, when using 50–100 μM propranolol, a slight increase in the formation of 5-lipoxygenase metabolites was observed (data not shown).

In a recent report it was demonstrated that different PLA_2 enzymes are involved in the release of arachidonic acid destined for eicosanoid synthesis or activation of NADPH oxidase [15]. Interestingly, the production of superoxide anions and eicosanoid synthesis was stimulus specific. Calcium ionophore A23187 induced arachidonic acid release and eicosanoid synthesis but was unable to promote superoxide production. On the other hand, polychlorinated biphenyls induced both arachidonic acid release, eicosanoid synthesis and superoxide production. The eicosanoids accounted for $< 20\%$ of the radioactive metabolites recovered from the neutrophils when stimulated with polychlorinated biphenyls. The release of arachidonic acid induced by polychlorinated biphenyls was blocked by BEL [15].

A general consensus has been that an increase of intracellular calcium is the primary event for activation of cellular calcium-dependent PLA_2s and hence the release of arachidonic acid utilized for eicosanoid synthesis. However, the

findings of this and of other recently published studies suggest that there are alternative calcium-independent mechanisms for release of arachidonic acid [13,15,27]. In conclusion, this study demonstrates the expression of the 88-kDa $iPLA_2$ mRNA and its two splice variants, ankyrin-$iPLA_2$-1 and ankyrin-$iPLA_2$-2, in immature and mature myeloid cells. Studies with BEL suggest that $iPLA_2$ is involved in leukotriene synthesis in granulocytes, upon activation with low concentration of ionophore A23187 or opsonized zymosan. Although calcium-dependent $cPLA_2$ apparently has an essential role in the release of arachidonic acid destined for leukotriene synthesis, it is likely that $iPLA_2$ is also involved in leukotriene synthesis under certain physiological conditions.

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References

[1] Dennis, E.A. (1983) in: The Enzymes, 3rd Edn. (Boyer, P.D., Ed.) pp. 307–353.
[2] Dennis, E.A. (1997) Trends Biochem. Sci. 22, 1–2.
[3] Clark, J.D., Schievella, A.R., Nalefski, E.A. and Lin, L.L. (1995) J. Lipid Med. Cell Signal 12, 83–117.
[4] Ackermann, E.J. and Dennis, E.A. (1995) Biochim. Biophys. Acta 1259, 125–136.
[5] Hazen, S.L., Hall, C.R., Ford, D.A. and Gross, R.W. (1993) J. Clin. Invest. 91, 2513–2522.
[6] Hirashima, Y., Farooqui, A.A., Mills, J.S. and Horrocks, L.A. (1992) J. Neurochem. 59, 708–714.
[7] Ackermann, E.J., Kempner, E.S. and Dennis, E.A. (1994) J. Biol. Chem. 269, 9227–9233.
[8] Portilla, D. and Dai, G. (1996) J. Biol. Chem. 271, 15451–15457.
[9] Tang, J., Kriz, R.W., Wolfman, N., Shaffer, M., Seehra, J. and Jones, S.S. (1997) J. Biol. Chem. 272, 8567–8575.
[10] Larsson, P.K.A., Claesson, H.E. and Kennedy, B.P. (1998) J. Biol. Chem. 273, 207–214.
[11] Balsinde, J., Bianco, I.D., Ackermann, E.J., Conde-Frieboes, K. and Dennis, E.A. (1995) Proc. Natl. Acad. Sci. USA 92, 8527–8531.
[12] Balsinde, J., Balboa, M.A. and Dennis, E.A. (1997) J. Biol. Chem. 272, 29317–29321.
[13] Wolf, M.J., Wang, J., Turk, J. and Gross, R.W. (1997) J. Biol. Chem. 272, 1522–1526.
[14] Lloret, S., Martinez, J. and Moreno, J.J. (1995) Arch. Biochem. Biophys. 323, 251–257.
[15] Tithof, P.K., Petersgolden, M. and Ganey, P.E. (1998) J. Immunol. 160, 953–960.
[16] Claesson, H.-E. and Dahlén, S.-E. (1998) J. Intern. Med., in press.
[17] Uozumi, N., Kume, K., Nagase, T., Nakatani, N., Ishi, S., Tashiro, F., Komagata, Y., Maki, K., Ikuta, K., Ouchi, Y., Miyazaki, J. and Shimizu, T. (1997) Nature 390, 618–622.
[18] Kennedy, B.P., Payette, P., Mudgett, J., Vadas, P., Pruzanski, V., Kwan, M., Tang, C., Rancourt, D.E. and Cromlish, W.A. (1995) J. Biol. Chem. 270, 22378–22385.
[19] Marshall, L.A., Bolognese, B., Winkler, J.D. and Roshak, A. (1997) J. Biol. Chem. 272, 759–765.
[20] Murakami, M., Nakatani, Y., Atsumi, G., Inoue, K. and Kudo, I. (1997) Crit. Rev. Immunol. 17, 225–283.
[21] Hullin-Matsuda, F., Tsujishita, Y. and Nishizuka, Y. (1997) FEBS Lett. 419, 117–120.
[22] Feltenmark, S., Runarsson, G., Larsson, P., Jakobsson, P.J., Bjorkholm, M. and Claesson, H.E. (1995) Br. J. Haematol. 90, 585–594.
[23] Balsinde, J. and Dennis, E.A. (1996) J. Biol. Chem. 271, 31937–31941.
[24] Taylor, G.S., Ladd, A., James, J., Greene, B. and English, D. (1993) Biochim. Biophys. Acta 1175, 219–224.
[25] Suchard, S.J., Nakamura, T., Abe, A., Shayman, J.A. and Boxer, L.A. (1994) J. Biol. Chem. 269, 8063–8068.
[26] Billah, M.M., Eckel, S., Mullmann, T.J., Egan, R.W. and Siegel, M.I. (1989) J. Biol. Chem. 264, 17069–17077.
[27] Gross, R.W., Rudolph, A.E., Wang, J., Sommers, C.D. and Wolf, M.J. (1995) J. Biol. Chem. 270, 14855–14858.