The suppression of 5-lipoxygenation of arachidonic acid in human polymorphonuclear leucocytes by the 15-lipoxygenase product (15*S*)-hydroxy-(5*Z*,8*Z*,11*Z*,13*E*)-eicosatetraenoic acid: structure–activity relationship and mechanism of action

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(15*S*)-Hydroxy-(5*Z*,8*Z*,11*Z*,13*E*)-eicosatetraenoic acid (15-HETE) suppresses in ionophore-A23187-stimulated human polymorphonuclear leucocytes (PMN) the conversion of exogenous arachidonic acid into leukotriene B₄ (LTB₄) and (5*S*)-hydroxy-(6*E*,8*Z*,11*Z*,14*Z*)-eicosatetraenoic acid (5-HETE). However, contrary to earlier suggestions, 15-HETE is not a genuine 5-lipoxygenase inhibitor under these conditions, but rather suppresses the 5-lipoxygenation of arachidonic acid by switching-over of substrate utilization, as judged from a sizeable formation of labelled (5*S*,15*S*)-dihydroxy-(6*E*,8*Z*,11*Z*,13*E*)-eicosatetraenoic acid (5,15-diHETE) from 15-[1-¹⁴C]HETE. Identical results were obtained with human recombinant 5-

lipoxygenase. In PMN the formation of 5,15-diHETE is strongly stimulated by either hydroperoxypolyenoic fatty acids or arachidonic acid, suggesting a crucial role of the hydroperoxide tone of the cell. A comparison of a selection of hydroxypolyenoic fatty acids with respect to their capability of suppressing 5-lipoxygenation of arachidonic acid revealed that 15-mono-hydroxyeicosanoids throughout exhibit the highest inhibitory potencies, whereas the other HETEs, 5,15-diHETE as well as octadecanoids, are modest or poor inhibitors. The *R* and *S* enantiomers of 15-HETE do not differ from each other, excluding a receptor-like binding of the 15-hydroxy group.

INTRODUCTION

Arachidonate 5-lipoxygenase is a pivotal enzyme in the cellular synthesis of leukotriene B₄ (LTB₄) and other proinflammatory eicosanoids [1–3]. It appears to be regulated by a number of factors such as supply of arachidonic acid [4], increase in cytosolic Ca²⁺ concentration [5,6] mediating the docking of the enzyme by a special activating protein ('FLAP') at the nuclear membranes [7], steady-state concentration of hydroperoxy fatty acids ('hydroperoxide tone') [8,9], as well as regulatory actions of eicosanoids [10,11]. An example of the latter principle is the observation by several authors that one of the products of the 15-lipoxygenase pathway of arachidonic acid metabolism, (15*S*)-hydroxy-(5*Z*,8*Z*,11*Z*,13*E*)-eicosatetraenoic acid (15-HETE), inhibits in micromolar concentrations the formation of the 5-lipoxygenase products (5*S*)-hydroxy-(6*E*,8*Z*,11*Z*,14*Z*)-eicosatetraenoic acid (5-HETE) and LTB₄ from arachidonic acid in polymorphonuclear leucocytes (PMN) [11–19]. Irrespective of the fact that the mechanism of this inhibitory action has not been studied in detail, this effect has been sometimes interpreted as a genuine inhibition of the 5-lipoxygenase [20].

In the present study we provide evidence that, at least for the reaction with exogenous arachidonic acid, 15-HETE is not a true enzyme inhibitor but rather a concurrent substrate of the 5-lipoxygenase competing with its 'natural' substrate arachidonic acid.

MATERIALS AND METHODS

Isolation of human PMN

Peripheral blood was taken from the cubital vein of healthy male

volunteers (22–50 years) who did not ingest any anti-inflammatory drug. The blood was mixed with hydroxyethyl-starch (HAES steril; 6%, w/w; Fresenius AG, Bad Homburg, Germany; 0.2 ml/ml of blood) in a silicone- and heparin-treated beaker and transferred to 10 ml tubes. After sedimentation of the cells up to two-thirds of the original volume, the leukocyte-rich plasma supernatants were underlayered with 0.5 vol. of Ficoll Paque (Pharmacia, Uppsala, Sweden) and subjected to density-gradient centrifugation as described by Böyum et al. [21]. The cells were washed with PBS. The residual erythrocytes were removed by osmotic haemolysis with ice-cold water for 30–40 s. The PMN were washed twice with PBS and resuspended in Hanks' balanced salt solution yielding a concentration of 10⁷ cells/ml and stored at 4 °C no longer than 1 h before starting the experiments. Trypan Blue-exclusion tests revealed at least 97% viable cells.

Chemicals and preparations

Human recombinant 5-lipoxygenase [22] was generously supplied by Dr. D. Riendeau (Merck-Frosst Centre for Therapeutic Research, Pointe Claire-Dorval, Québec, Canada). Its specific activity amounted to 10.2 s⁻¹ during the experiments. Racemic mixtures of 5-, 8-, 11-, 12- and 15-HETE were prepared by α -tocopherol-controlled autoxidation of arachidonic acid [23]. 9-*R/S*-HETE, 9-*R/S*-HODE and 13-*R/S*-HODE were purchased from Biomol (Hamburg, Germany). 15*S*-HETE, 5,15-diHETE, 15*S*-HEDE, 15*S*-HETriE isomers and 15*S*-HEPE were prepared from the corresponding eicosapolyenoic acids by means of soybean lipoxygenase [24] and were purified by reverse-phase

Abbreviations used: PMN, polymorphonuclear leucocytes; LTB₄, leukotriene B₄; 5-HETE, (5*S*)-hydroxy-(6*E*,8*Z*,11*Z*,14*Z*)-eicosatetraenoic acid; 15-HETE, (15*S*)-hydroxy-5*Z*,8*Z*,11*Z*,13*E*-eicosatetraenoic acid; 5,15-diHETE, (5*S*,15*S*)-dihydroxy-(6*E*,8*Z*,11*Z*,13*E*)-eicosatetraenoic acid; HETE, hydroxy-eicosatetraenoic acid; HpETE, hydroperoxyeicosatetraenoic acid; HODE, hydroxyoctadecadienoic acid; HpODE, hydroperoxyoctadecadienoic acid; HEDE, hydroxyeicosadienoic acid; HETriE, hydroxyeicosatrienoic acid; HEPE, hydroxyeicosapentaenoic acid; FLAP, 5-lipoxygenase-activating protein; PHGPx, phospholipid hydroperoxide glutathione peroxidase.

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and straight-phase HPLC (see the abbreviations footnote for definitions of HODE, diHETE, HEDE, HETriE and HEPE). The enantiomer composition of the hydroxypolyenoic fatty acids used was checked by analytical chiral-phase HPLC [25]. 15*R*-HETE and 15*S*-HETE were separated from the racemic mixture by preparative chiral-phase HPLC [25,26]. [$1\text{-}^{14}\text{C}$]15*S*-HETE was prepared from [$1\text{-}^{14}\text{C}$]arachidonic acid (Amersham International; 2.16 GBq/mmol) with soybean lipoxygenase [27].

Arachidonic acid metabolism in PMN

A 1 ml portion of the PMN suspension (10^7 cells/ml) was preincubated with hydroxypolyenoic fatty acid (dissolved in 10 μl of methanol) for 5 min at 37 °C with shaking. Thereafter 5 μl of labelled (5.6 kBq/sample) or unlabelled arachidonic acid (5–100 μM final concn.) in methanol and immediately thereafter 5 μl of ionophore A23187 (10 μM final concn.) were added and the sample was incubated for another 5 min. The reaction was stopped by the addition of 10 μl of 1 M citric acid. The lipids were twice extracted with 2 ml of ethyl acetate with vigorous shaking for 1 min. The solvent was evaporated under vacuum and the residue was dissolved in either methanol or ethyl acetate.

Radioscanning TLC

Aliquots (15 μl out of 100 μl) of the lipid extracts were applied on to precoated thin-layer plates (silica-gel 60 with concentration zone; 20 cm $\times$ 20 cm, 0.25 mm thick; Merck, Darmstadt, Germany). The chromatograms were developed at 4 °C in one of the following solvents: solvent I: n-hexane/diethyl ether/acetic acid, 40:60:1 (by vol.); solvent II: chloroform/methanol/acetic acid/water, 90:7:1:0.7 (by vol.).

After drying the plates at 100 °C, the distribution of radioactivity was scanned with a TLC Multi-Tracemaster LB 285 (Berthold, Berlin, Germany). The positions of the single fractions were determined by co-chromatography of authentic standards and correspondence with data in the literature [28]. For quantification, the peak areas related to the total radioactivity of the sample as well as to the initial concentration of [$1\text{-}^{14}\text{C}$]arachidonic acid were used.

HPLC

HPLC was performed with a Shimadzu instrument (model LC 6A) coupled to a diode array detector 1040A (Hewlett–Packard). Reverse-phase separations were carried out on a Nukleosil ODS column (250 mm $\times$ 4.6 mm; particle size 5 μm ; Macherey–Nagel KS system). Methanol/water/acetic acid (800:200:1 or 770:230:1, by vol.) were used for isocratic separations. The lipoxygenase metabolites were detected at 235 nm (HETEs), 245 nm (5,15-diHETE) and 270 nm (LTB_4 and its ω -oxidation products; hydrolysis products of LTA_4). Arachidonic acid was detected at 210 nm. The identity of the compounds was established by co-chromatography with authentic standards and by comparison of their UV spectra. The quantification of the compounds was performed by integration of the peak areas; the following molar absorption coefficients were used: 23000 $\text{M}^{-1}\cdot\text{cm}^{-1}$ (HETEs), 33000 $\text{M}^{-1}\cdot\text{cm}^{-1}$ (5,15-diHETE), 50000 $\text{M}^{-1}\cdot\text{cm}^{-1}$ (LTB_4) [29,30]. 15-HEDE (7 μg /sample) was used as internal standard.

Straight-phase HPLC separations were carried out on a ZORBAX/SIL column 250 cm $\times$ 4.6 mm, particle size 5 μm .

(du Pont). The solvent was n-hexane/isopropan-2-ol/acetic acid (200:10:1, by vol.) with a flow rate of 1 ml/min.

Assay of recombinant 5-lipoxygenase

The enzyme was preincubated in 1 ml of Hanks' solution at 14.5 °C for 5 min with the final concentrations of following additions: 0.1 mM EDTA, 0.1 mM ATP, 12 μg of phosphatidylcholine (dispersed by sonication). The reaction was started with 10 μl of 4 mM arachidonic acid in methanol and monitored spectrophotometrically at 235 nm. After 50 s the reaction largely ceased, owing to self-inactivation of the enzyme. Thereafter citric acid was added and the extraction and analysis of the lipoxygenase products were performed as described for the experiments with PMN.

RESULTS

15-HETE modifies the metabolism of [$1\text{-}^{14}\text{C}$]arachidonic acid in ionophore-A23187-stimulated human PMN

Human PMN were stimulated with ionophore A23187 and allowed to react with 5 μM [$1\text{-}^{14}\text{C}$]arachidonic acid in the presence

Figure 1 Radio-TLC plate showing the metabolism of [$1\text{-}^{14}\text{C}$]arachidonic acid in human PMN in the absence or presence of 15*S*-HETE

PMN (10^7 cells/ml) were preincubated without (a) or with 50 μM 15*S*-HETE (b) for 5 min at 37 °C and then stimulated with 10 μM ionophore A23187 and [$1\text{-}^{14}\text{C}$]arachidonic acid (5 μM , 5.6 kBq) and incubated for another 5 min. The reaction products were separated by TLC using solvent II (see the Materials and methods section).

Figure 2 Dependence of the inhibitory effect of 15*S*-HETE on the concentration of arachidonic acid

PMN (10^7 cells/ml) were preincubated without or with 30 μ M (■) or 50 μ M (▲) 15*S*-HETE for 5 min at 37 °C and then stimulated with 10 μ M ionophore A23187 and various concentrations of arachidonic acid and incubated for another 5 min. The 5-HETE formed was estimated by reverse-phase HPLC with UV detection at 235 nm.

Table 1 Inhibitory potencies of various hydroxypolyenoic fatty acids on the 5-lipoxygenation of arachidonic acid

PMN (10^7 cells/ml) were preincubated at 37 °C for 5 min with various concentrations of the hydroxypolyenoic fatty acid and then stimulated by addition of 5 μ M (final concn.) [$1\text{-}^{14}\text{C}$]arachidonic acid and 10 μ M ionophore A23187 and incubated for another 5 min. The labelled products were analysed by radioscanning TLC (see the Materials and methods section). The IC_{50} values (means $\pm$ S.D.) were estimated by fitting the dose–response curves by means of the Hill approach method [31].

Hydroxypolyenoic fatty acid	IC_{50} (μ M) for 5-HETE formation
9 <i>R</i> /5,10,12-HODE	Not inhibitory (100 μ M)
13 <i>R</i> /5,9,11-HODE	> 100*
5 <i>R</i> /5,6,8,11,14-HETE	44.9 $\pm$ 7.8
8 <i>R</i> /5,9,11,14-HETE	52.8 $\pm$ 10
9 <i>R</i> /5,7,11,14-HETE	> 50
11 <i>R</i> /5,8,12,14-HETE	51.0 $\pm$ 5.6
12 <i>R</i> /5,8,10,13-HETE	68.0 $\pm$ 10
15 <i>R</i> /5,8,11,13-HETE	11.6 $\pm$ 1.9
15 <i>S</i> /5,8,11,13-HETE	16.5
15 <i>R</i> /5,8,11,13-HETE	17.2
15 <i>S</i> /11,13-HEDE	17.6 $\pm$ 3.1
15 <i>S</i> /8,11,13-HETriE	4.6 $\pm$ 0.9
15 <i>S</i> /11,13,17-HETriE	38 $\pm$ 5
15 <i>S</i> /5,8,11,13,17-HEPE	32
5 <i>S</i> ,15 <i>S</i> /6,8,11,13-diHETE	> 100†

* 24.3 $\pm$ 15.8% inhibition at 100 μ M.

† 35% inhibition at 100 μ M.

or absence of 50 μ M 15-HETE. In agreement with previous reports in the literature, 15-HETE caused a strong suppression of the formation of 5-HETE as well as of LTB_4 and its ω -oxidation products. In contrast, the formation of labelled 15-HETE and the incorporation of arachidonic acid and/or 15-HETE into triacylglycerols and phospholipids were stimulated (Figure 1). The inhibitory effects of 15-HETE turned out to be dependent on the concentration of exogenous arachidonic acid (Figure 2); increasing the concentration of arachidonic acid counteracted the inhibition to a sizeable extent, but did not completely abolish the inhibition. In the absence of exogenous

arachidonic acid, that is, under conditions in which exclusively endogenous arachidonic acid is utilized, the inhibition of the formation of 5-HETE and LTB_4 proved to be most sensitive towards 15-HETE; the IC_{50} values were estimated to be 4.2 and 6.0 μ M respectively (results not shown), values which are in fair agreement with data from the literature [15,18,19].

Structure–activity relationship of the inhibition by hydroxypolyenoic fatty acids of the 5-lipoxygenation of arachidonic acid in PMN

A number of hydroxypolyenoic fatty acids derived from different lipoxygenation of linoleic or arachidonic acids were tested for their inhibitory effects on the 5-lipoxygenation of arachidonic acid. From their dose–response curves (not shown) the IC_{50} values were estimated (Table 1). It can be seen that the 15-monohydroxyeicosanoids are markedly more potent inhibitors than all other compounds tested. Interestingly, 13-HODE, the corresponding 15-lipoxygenase product from linoleic acid, which also exhibits some structural resemblance to 15-HETE, turned out to be a very poor inhibitor. The presence of a *cis* double bond at C_{17} lowers the inhibitory potency to some extent. Introduction of a second hydroxy group into the 15-HETE molecule (5,15-diHETE) gives rise to a strong loss of inhibitory potency.

The comparatively high inhibitory potency of 15-HEDE is particularly noteworthy, since this compound cannot serve as lipoxygenase substrate for structural reasons (absence of a *bis*-allyl methylene), in contrast with the other hydroxyeicosanoids tested.

Since the structure–activity relationship suggested a particular role of the 15-hydroxy group, we also compared the two enantiomers of 15-HETE with respect of their capability of inhibiting 5-lipoxygenation of arachidonic acid. As shown in Table 1, 15*R*-HETE and 15*S*-HETE behaved identically. This observation excludes the possibility that the 15-hydroxy group of these eicosanoids would be specifically bound to a receptor-like structure preceding the inhibitory effect.

15-HETE is substrate for the 5-lipoxygenase in human PMN

15-HETE has been reported to be converted into 5,15-diHETE in rat basophilic leukaemia cells [32] and in human alveolar macrophages [33]. Moreover, its hydroperoxy precursor 15-HpETE, has been described as substrate for the 5-lipoxygenase in human PMN [10]. For this reason we studied the metabolism of [$1\text{-}^{14}\text{C}$]15-HETE in human PMN in the presence of exogenous arachidonic acid. The reverse-phase HPLC chromatogram (Figure 3) revealed sizeable conversion into a labelled compound that was co-eluted with an authentic standard of 5,15-diHETE and possessing a UV absorption spectrum with a maximum at 244 nm reported for 5,15-diHETE [32]. The formation of 5,15-diHETE paralleled the inhibition of the formation of 5-HETE and LTB_4 occurring under these conditions. Only small amounts of labelled 5,15-diHETE were observed in the absence of exogenous arachidonic acid (not shown).

In order to find out whether 15-HETE acts as inhibitor of the 5-lipoxygenase pathway, we studied the effect of this compound on the sum of 5-lipoxygenase products. The sum of all products should be regarded as a measure of the real 5-lipoxygenase activity of the PMN under these conditions. The results (not shown) revealed that 15-HETE does not inhibit the overall 5-lipoxygenase activity in PMN; at 50 μ M 15-HETE there was even a slight enhancement of it. Therefore the suppression by 15-HETE of the formation of the 5-lipoxygenase products from exogenous arachidonic acid must not be interpreted as genuine enzyme inhibition.

Figure 3 Reverse-phase HPLC of the reaction products from [$1\text{-}^{14}\text{C}$]15-HETE and arachidonic acid

PMN (10^7 cells/ml) were preincubated with $20\text{ }\mu\text{M}$ [$1\text{-}^{14}\text{C}$]15-HETE for 5 min at $37\text{ }^\circ\text{C}$ and thereafter stimulated with $20\text{ }\mu\text{M}$ arachidonic acid and $10\text{ }\mu\text{M}$ ionophore A23187 and incubated for another 5 min. The HPLC solvent was methanol/water/acetic acid (770:230:1, by vol.). (a) UV detection at 235 nm (the insert shows the UV spectrum of 5,15-diHETE; the abscissa shows the wavelength in nm). Abbreviation: mAU, milli-absorption units. (b) Radioactivity of 1 ml fractions (Wallace 1410 β -radiation counter; Pharmacia).

Table 2 Effect of (15*S*)-hydroperoxy-(5*Z*,8*Z*,11*Z*,13*E*)-eicosatetraenoic acid (15-HpETE) on 5-lipoxygenations in human PMN

PMN (10^7 cells/ml) were preincubated at $37\text{ }^\circ\text{C}$ for 5 min with $20\text{ }\mu\text{M}$ [$1\text{-}^{14}\text{C}$]15-HETE and thereafter incubated with $5\text{ }\mu\text{M}$ arachidonic acid and $10\text{ }\mu\text{M}$ ionophore A23187 in the absence or presence of $3\text{ }\mu\text{M}$ 15-HpETE for another 5 min. The reaction products were analysed by reverse-phase HPLC with both UV detection at 235 nm (5-HETE, 15-HETE), 245 nm (5,15-diHETE) and 270 nm (LTB₄), and liquid-scintillation measurement (5,15-diHETE).

3 μM 15-HpETE present	Amount of reaction product (nmol/ 10^7 cells)				Sum of 5-lipoxygenase products
	15-HETE (residual)	5,15-diHETE	5-HETE	LTB ₄	
—	17.6	1.45	0.00	0.20	1.65
+	15.6	6.26	0.48	0.76	7.50

Since the conversion of 15-HETE into 5,15-diHETE in PMN was found to be very low in the absence of exogenous arachidonic acid, we addressed the issue as to the reason for its dependence on arachidonic acid. The 5-lipoxygenase activity of PMN has been shown to be regulated via the hydroperoxide tone [8,9], that is, hydroperoxy fatty acids are activators of lipoxygenases and must be therefore present at a certain minimal level to sustain lipoxygenase activities in the cell. This level appears to be adjusted by cellular peroxidases, in particular by the phospholipid hydroperoxide glutathione peroxidase (PHGPx) [9]. External arachidonic acid may contribute to the hydroperoxide tone via formation of 5-HpETE. Lipoxygenation of 15-HETE primarily leads to 5-hydroperoxy-15-HETE. This metabolite is apparently not capable of activating lipoxygenases during their catalytic cycle, so the lipoxygenation of 15-HETE requires the sim-

Figure 4 Stimulation of 5-lipoxygenation by (13*S*)-hydroperoxy-(9*Z*,11*E*)-octadecadienoic acid (13-HpODE)

PMN (10^7 cells/ml) were preincubated with various concentrations of [$1\text{-}^{14}\text{C}$]15-HETE and thereafter stimulated with $10\text{ }\mu\text{M}$ ionophore A23187 for another 5 min in the absence ($\square$) or presence of $3\text{ }\mu\text{M}$ 13-HpODE ($\blacksquare$). The analysis of reaction products was performed as in Table 2. The values refer to the sum of 5-HETE, LTB₄ and 5,15-diHETE.

ultaneous presence of a non-hydroxylated hydroperoxy fatty acid or a precursor of it, as demonstrated in the case of rabbit 15-lipoxygenase [34]. Starting from these considerations we added either 15-HpETE or 13-HpODE to samples containing PMN and 15-HETE. The results are shown in Table 2 and in Figure 4. In both cases there was a strong stimulation of the oxygenations of both arachidonic acid and 15-HETE. In the absence of 15-HETE, $3\text{ }\mu\text{M}$ 13-HpODE also caused a shift of the LTB₄/5-HETE ratio formed from endogenous arachidonic acid in the presence of ionophore A23187 in favour of LTB₄ (results not shown). Collectively these data indicate a crucial role of the hydroperoxide tone for the 5-lipoxygenase-mediated metabolism in PMN, in particular for that of 15-HETE.

15-HETE is also substrate for human recombinant 5-lipoxygenase

To verify the conclusions drawn from the experiments with intact cells, we studied the reaction of 15-HETE with human recombinant 5-lipoxygenase. The product analyses are compiled in Table 3 and reveal the following observations.

(i) The substrate turnovers in the samples containing arachidonic acid, 15-HETE, or both substrates together, do not differ significantly; 15-HETE is a substrate for the 5-lipoxygenase with an efficiency comparable with that of arachidonic acid.

(ii) The turnover rates of arachidonic acid and of 15-HETE if present simultaneously are not additive; that is, the substrates compete with each other for their utilization by the 5-lipoxygenase.

(iii) $20\text{ }\mu\text{M}$ 15-HETE inhibits the formation of 5-HpETE from $20\text{ }\mu\text{M}$ arachidonic acid by about 60%. This inhibition does not, however, represent a genuine enzyme inhibition, but results from a switch-over of the substrate utilization.

Thus these results are consistent with those obtained with intact PMN.

We also tried to investigate the influence of the hydroperoxide tone. However, using the optimized assay of Riendeau's laboratory [35], we failed to observe a stimulation of the 5-lipoxygenase activities by 13-HpODE. It is conceivable that the sonicated phosphatidylcholine, the addition of which was necessary to achieve optimal enzymic activity, is a possible source for activating hydroperoxides, so that the hydroperoxide tone is not

Table 3 Reaction of human recombinant 5-lipoxygenase with arachidonic acid and 15-HETE

The 5-lipoxygenase (1.7 $\mu\text{g/ml}$) was allowed to react in the assay system (see the Materials and methods section) with the substrates indicated for 50 s at 4 °C. This incubation period proved to be optimal as judged from spectrophotometric recording at 235 nm. The reaction products were analysed by reverse-phase HPLC with UV detection at 235 nm, 245 nm and 270 nm respectively.

Substrate (20 μM)	Substrate formed (nmol/ μg of 5-lipoxygenase)			
	5-HpETE	diHETEs*	5-Hydroperoxy-15-HETE	Sum
Arachidonic acid	2.10 $\pm$ 0.6	0.48 $\pm$ 0.26	—	2.58 $\pm$ 0.43†
15-HETE	—	—	1.70 $\pm$ 0.46	1.70 $\pm$ 0.46†
Arachidonic acid plus 15-HETE	0.95 $\pm$ 0.28	0.17	1.23 $\pm$ 0.4	2.35 $\pm$ 0.34†

* Hydrolysis products of leukotriene A_4 .

† Differences not significant (χ^2 test, $P < 0.05$).

limiting under these conditions, unlike in intact cells, where the hydroperoxides are continuously reduced.

DISCUSSION

The present study prompts us to re-evaluate the mode of the inhibition by 15-HETE of the 5-lipoxygenase pathway of arachidonic acid metabolism in human inflammatory cells. The lack of inhibition of total enzymic activity of the authentic pure enzyme unequivocally demonstrates that 15-HETE is not in itself a genuine 5-lipoxygenase inhibitor, as suggested in numerous reports in the literature. Using the human recombinant 5-lipoxygenase we have observed that 15-HETE actually diminishes the formation of 5-HpETE from arachidonic acid, but this effect is shown to be due to a switch-over of substrate utilization in favour of the formation of the 5-lipoxygenation product of 15-HETE.

A corresponding switch-over of substrate utilization was also observed with intact human leukocytes metabolizing exogenous arachidonic acid. The 5-lipoxygenation of 15-HETE in PMN appears to be limited by the hydroperoxide tone of the cells. For this reason it is strongly stimulated by either hydroperoxypolyenoic fatty acids (e.g. 13-HpODE or 15-HpETE) or exogenous arachidonic acid (as precursor for 5-HpETE). These compounds are expected to increase the steady-state concentration of hydroperoxypolyenoic fatty acids in the cells compensating for their continuous reduction by peroxidases, most probably by the PHGPx [9]. The apparently more pronounced dependence of the 5-lipoxygenation of 15-HETE on the hydroperoxide tone as compared with that of arachidonic acid is in line with previously obtained results on the oxygenation of 15-HETE by rabbit 15-lipoxygenase [34].

A limiting hydroperoxide tone may consequently be the reason for the low 5,15-diHETE formation from endogenous arachidonic acid in human PMN. In this case, 15-HETE exhibits its highest inhibitory potency toward the 5-lipoxygenation of arachidonic acid. Inasmuch as under these conditions a sizeable switch-over of substrate utilization was not observed, the mechanism of inhibition of the 5-lipoxygenation of arachidonic acid must therefore differ from that which obtains under conditions of utilization of exogenous arachidonic acid. With endogenous arachidonic acid as substrate for the 5-lipoxygenation in PMN, 15-HETE appears to act as 'genuine' 5-lipoxygenase inhibitor by competing with arachidonic acid and/or 5-HpETE for the substrate and/or hydroperoxy-activator binding site(s). The same mechanism may also hold true for the inhibitory effect of 15-HEDE under any conditions, since 15-HEDE cannot be an alternative lipoxygenase substrate. Collectively, our data indicate the existence of at least two different modes of the inhibitory

effect of 15-HETE on the formation of 5-HETE and LTB_4 . The action as alternative substrate for the 5-lipoxygenase proved to be the preferred way with exogenous arachidonic acid. However, under conditions in which this action is prevented, be it by a limiting hydroperoxide tone (metabolism of endogenous arachidonic acid) or be it for structural reasons (15-HEDE), the action as genuine inhibitor prevails.

Our results on the structure-activity relationship are in fair agreement with those reported for rat basophilic leukaemia (RBL-1) cells [36] and other data for various cells [12,13,15,16,19]. We showed that 15-hydroxyeicosanoids exhibit a higher inhibitory potency than other hydroxyeicosanoids provided that the molecule does not contain a second hydroxy group, whereas the number and position of double bonds appears to be of minor importance. The stereochemistry of the 15-hydroxy group, which had not been studied so far, was found in our study not to play any role at all. It is tempting to speculate that the hydroxy group must be situated only distantly enough from the carboxylic group so that the binding at the active site of the enzyme is not hindered.

The biological role of the interplay of 5- and 15-lipoxygenases in the regulation of the arachidonic acid metabolism is far from clear. The 5-lipoxygenase product 5-HETE has been reported to unmask the cryptic 15-lipoxygenase in PMN which converts arachidonic acid into 15-HETE, which, in turn, may suppress the further formation of 5-lipoxygenase products [12,13], providing a negative feedback regulation. However, 15-HETE also exerts proinflammatory actions (for reviews see [37,38]). Moreover, the formation of 5,15-diHETE from 15-HETE in PMN, in particular under conditions of an enhanced hydroperoxide tone and therefore perhaps favoured by oxidative stress, must also be considered in context with the recent findings that PMN possess a specific dehydrogenase that converts 5-hydroxy-eicosanoids into the corresponding 5-oxo derivatives [39] and that the corresponding metabolite from 5,15-diHETE, namely 5-oxo-15-HETE, exhibits a chemotactic activity which is comparable with that of LTB_4 [40]. Therefore the combined actions of 5- and 15-lipoxygenases in PMN may give rise to both proinflammatory and anti-inflammatory events.

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REFERENCES

- Samuelsson, B., Dahlén, S.-E., Lindgren, J. A., Rouzer, C. A. and Serhan, C. N. (1987) *Science* **237**, 1171–1176

- 2 Samuelsson, B. and Funk, C. D. (1989) *J. Biol. Chem.* **264**, 19469–19472
- 3 Yamamoto, S. (1991) *Free Radical Biol. Med.* **10**, 149–159
- 4 Winkler, J. D., Sung, C.-M., Hubbard, W. C. and Chilton, F. H. (1993) *Biochem. J.* **291**, 825–831
- 5 Schatz-Munding, M., Hatzelmann, A. and Ullrich, V. (1991) *Eur. J. Biochem.* **197**, 487–493
- 6 Wong, A., Cook, M. N., Foley, J. J., Sarau, H., Marshall, P. and Hwang, S. M. (1991) *Biochemistry* **30**, 9346–9354
- 7 Woods, J. W., Evans, J. F., Ethier, D., Scott, S., Vickers, P. J., Hearn, L., Heibin, J. A., Charleson S., and Singer, I. I. (1993) *J. Exp. Med.* **178**, 1935–1946
- 8 Hatzelmann, A., Schatz, M. and Ullrich, V. (1989) *Eur. J. Biochem.* **180**, 527–533
- 9 Weitzel, F. and Wendel, A. (1993) *J. Biol. Chem.* **268**, 6288–6292
- 10 McDonald, P. P., McColl, S. R., Naccache, P. H. and Borgeat, P. (1992) *Br. J. Pharmacol.* **107**, 226–232
- 11 Vanderhoek, J. Y., Bryant, R. W. and Bailey, J. M. (1980) *J. Biol. Chem.* **255**, 10064–10065
- 12 Vanderhoek, J. Y., Karmin, M. T. and Ekberg, S.-L. (1985) *J. Biol. Chem.* **260**, 15482–15487
- 13 Vanderhoek, J. Y., Wilhelmi, L. L., Ekberg, S.-L. and Karmin, M. T. (1990), *Eicosanoids* **3**, 181–185
- 14 Salari, H., Braquet, P. and Borgeat, P. (1984) *Prostaglandins Leukotrienes Med.* **13**, 53–60
- 15 Camp, R. D. R. and Fincham, N. J. (1985) *Br. J. Pharmacol.* **85**, 837–841
- 16 Chang, J., Skowronek, M. D. and Lewis, A. J. (1985) *Inflammation* **9**, 395–504
- 17 Cashman, J. R., Lambert, C. and Sigal, E. (1988) *Biochem. Biophys. Res. Commun.* **155**, 38–44
- 18 Serhan, C. N. (1989) *Biochim. Biophys. Acta* **1004**, 158–168
- 19 Iversen, L., Fogh, K., Bojesen, G. and Kragballe, K. (1991) *Agents Actions* **33**, 286–291
- 20 Kang, L.-T. and Vanderhoek, I. Y. (1995) *Biochim. Biophys. Acta* **1256**, 297–304
- 21 Böyum, A. (1968) *Scand. J. Clin. Lab. Invest.* **21**, 77–89
- 22 Denis, D., Falgoutyret, J.-P., Riendeau, D. and Abramovitz, M. (1991) *J. Biol. Chem.* **266**, 5072–5079
- 23 Peers, K. E. and Coxon, D. T. (1983) *Chem. Phys. Lipids* **32**, 49–56
- 24 Iacazio, G., Lagrand, G., Baratti, J., Bouno, G. and Triantaphylides, C. (1990) *J. Org. Chem.* **55**, 1690–1691
- 25 Kühn, H., Wiesner, R., Lankin, V. Z., Nekrasov, A., Alder, L. and Schewe, T. (1987) *Anal. Biochem.* **160**, 24–34
- 26 Kühn, H. and Wiesner, R. (1990) *J. Chromatogr.* **520**, 391–401
- 27 Crawford, C. G., van Alpen, G. H. W., Cook, H. W. and Lands, W. E. M. (1978) *Life Sci.* **23**, 1255–1262
- 28 Hurst, J. S., Flatman, S. and McDonald-Gibson, R. G. (1987) in *Prostaglandins and Related Substances: A Practical Approach* (Benedetto, C., McDonald-Gibson, R. G., Nigam, S. and Slater, T. F., eds.), pp. 53–73, IRL Press, Oxford and Washington, DC
- 29 Gibian, M. J. and Vandenberg, P. (1987) *Anal. Biochem.* **163**, 343–349
- 30 van Os, C. P. A., Rijke-Schilder, G. P. M., van Halbeck, H., Verhagen, J. and Vliegthart, J. F. G. (1981) *Biochim. Biophys. Acta* **663**, 177–193
- 31 Holzthütter, H.-G. and Colosimo, A. (1990) *Comput. Applic. Biosci.* **6**, 23–28
- 32 Wilkinson, D., Hallam, C., Helmsley, P. E., Lord, G. H. and Mitchell, P. D. (1985) *Biochem. Soc. Trans.* **13**, 180–182
- 33 Chavis, C. M., Godard, P., dePaulet, A. C. and Damon, M. (1992) *Eicosanoids* **5**, 203–211
- 34 Kühn, H., Wiesner, R., Stender, H., Schewe, T., Lankin, V. Z., Nekrasov, A. and Rapoport, S. M. (1986) *FEBS Lett.* **203**, 247–252
- 35 Percival, M., D., Denis, D., Riendeau, D. and Gresser, M. J. (1992) *Eur. J. Biochem.* **210**, 109–117
- 36 Haviv, F., Ratajczyk, I. D., DeNet, R. W., Martin, Y. C., Dyer, R. D. and Carter, G. W. (1987) *J. Med. Chem.* **30**, 254–263
- 37 Ford-Hutchinson, A. W. (1991) *Eicosanoids* **4**, 65–75
- 38 Schewe, T. and Kühn, H. (1991) *Trends Biochem. Sci.* **16**, 369–373
- 39 Powell, W. S., Gravelle, F. and Gravel, S. (1992) *J. Biol. Chem.* **267**, 19233–19241
- 40 Schwenk, U., Morita, E., Engel, R. and Schröder, J.-M. (1992) *J. Biol. Chem.* **267**, 12482–12488