

Cyclooxygenase-catalyzed formation of 9-hydroxylinoleic acid by guinea pig alveolar macrophages under non-stimulated conditions

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Data are presented on the formation of a linoleic acid oxygenation product under basal conditions by guinea pig alveolar macrophages (AM). Under non-stimulated conditions 20×10^6 AM released about 1 nmol 9-hydroxylinoleic acid (9-HODE) during a 15 min incubation. Furthermore, AM released more than 2 nmol 12-hydroxy-5,8,10-heptadecatrienoic acid (HHT), a byproduct of the formation of thromboxane A_2 . 9-HODE and HHT were the most prominent products formed under the incubation conditions used. These products were formed via a cyclooxygenase-catalyzed reaction, since their formation was inhibited by $1 \mu\text{M}$ indomethacin. Thus, under basal conditions AM possess substantial cyclooxygenase activity. The biological significance of 9-HODE and related substances is discussed.

Alveolar macrophage 9-Hydroxylinoleic acid Cyclooxygenase Enzyme activity Indomethacin

1. INTRODUCTION

Phagocytic cells represent an important defense mechanism in the lungs against inhaled foreign particles. In particular alveolar macrophages (AM) play an important role in the initial events of bacterial clearance [1,2]. Macrophages release various fatty acid metabolites, including prostaglandins and leukotrienes, upon stimulation [3]. These fatty acid-derived mediators are crucial in the full development of the inflammatory response. In recent years emphasis has been placed on the production and the biological significance of arachidonic acid-derived mediators, including prostaglandins and leukotrienes. Macrophage membrane phospholipids, however, also contain linoleic acid which can be liberated by phospholipase activity upon cell stimulation [4]. Little information is available on the production of linoleic acid-derived mediators from inflammatory cells, and their possible role in the inflammatory

process. We are the first to describe the release of 9-hydroxylinoleic acid by AM under basal, i.e. non-stimulated, conditions from endogenous linoleic acid. The biological significance of this linoleate metabolite will be discussed.

2. MATERIALS AND METHODS

2.1. Isolation and incubation of macrophages

Alveolar macrophages were obtained from male Dunkin Hartley guinea pigs (400–600 g) through lung lavage in situ as described [5]. The macrophages were incubated at 20×10^6 cells/ml in Krebs-bicarbonate solution for 15 min at 37°C . In one incubation $1 \mu\text{M}$ indomethacin was added at the start of the incubation period. After the incubation period the incubates were centrifuged at $10000 \times g$ for 15 min. The resulting supernatant was further subjected to extraction and fractionation procedures as described below.

2.2. Extraction and analysis of fatty acid metabolites

Supernatants of incubates were applied to disposable reverse-phase extraction columns (J.T. Baker Chemicals, Deventer, The Netherlands), after which lipid-soluble material was eluted from the column with methanol. The methanol eluate was subsequently fractionated by silica gel chromatography according to [6], yielding fractions containing monohydroxy acids, dihydroxy acids, and prostaglandins, respectively. These fractions were initially analyzed by reverse-phase high-performance liquid chromatography (RP-HPLC) using a system to separate various leukotrienes and monohydroxy acids (system I) [7]. The HPLC apparatus consisted of a Pye Unicam PU4002 pump, PU4031 oven, and a PU4021 photodiode array detector. Chromatographic parameters and analyses of results were controlled by a PU4850 microcomputer. The analysis was performed at

30°C on a Nucleosil 5C18 column (250 × 4.6 mm, Chrompack, Middelburg, The Netherlands) and the elution was carried out with tetrahydrofuran-methanol-water-acetic acid (25:30:45:0.1) (pH 5.5 with ammonia). The aqueous phase contained 0.1% EDTA. The flow rate was maintained at 0.9 ml/min. The eluate was monitored in chromascan mode, in which a UV spectrum is taken every 1 s, resulting in a 3-dimensional plot of time and wavelength vs absorption. For identification purposes, a second RP-HPLC system was used, which separates monohydroxy acids (system II) [6], i.e. tetrahydrofuran-acetonitrile-water-acetic acid (22:40:38:0.05) at a flow rate of 1.0 ml/min.

3. RESULTS

RP-HPLC analysis of the incubation fluid of non-stimulated guinea pig AM revealed three fatty acid metabolites which were identified as

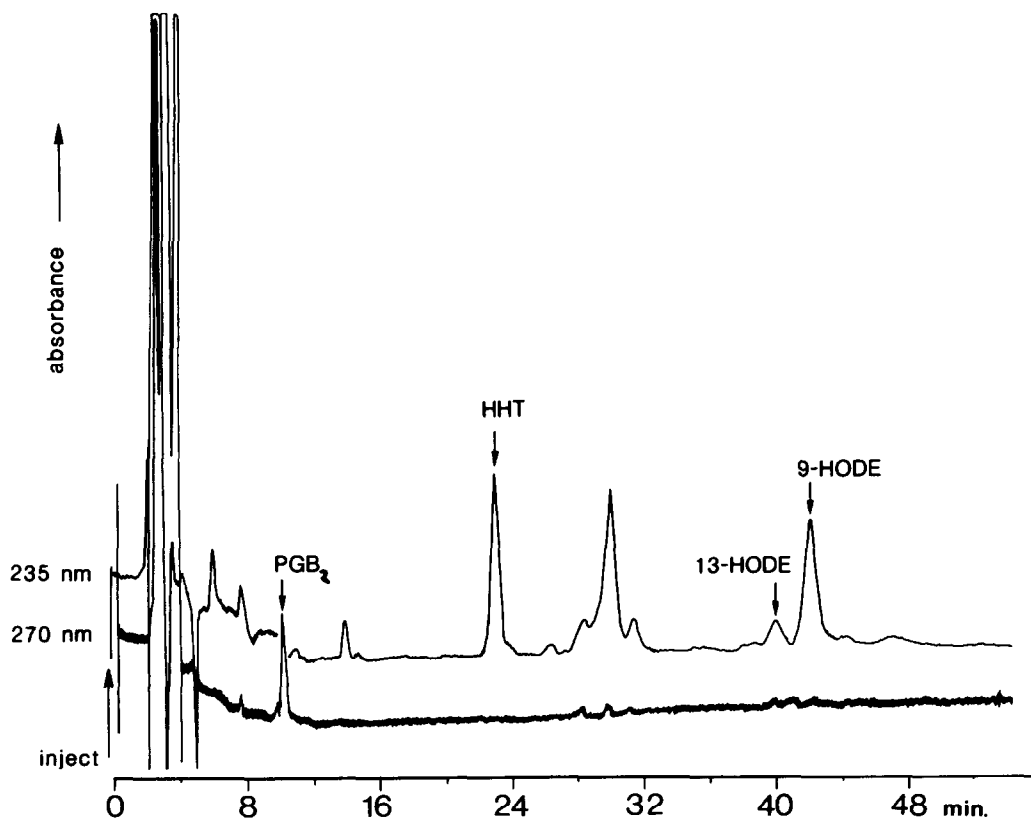


Fig.1. RP-HPLC analysis of an incubate of non-stimulated guinea pig AM (system I). This chromatogram is representative of the 4 analyses carried out. Monohydroxy acids were monitored at 235 nm. Synthetic prostaglandin B₂ was used as an internal standard to correct for losses during the extraction procedures.

12-hydroxy-5,8,10-heptadecatrienoic acid (HHT), 13-hydroxy-9,11-octadecadienoic acid (13-HODE, 13-hydroxylinoleic acid), and 9-hydroxy-10,12-octadecadienoic acid (9-HODE, 9-hydroxylinoleic acid), respectively (see fig.1). The three metabolites displayed maximal UV absorption at 230 nm, indicative of monohydroxy acids. The HHT, 13-HODE and 9-HODE peaks coeluted with their respective authentic standards in silica gel fractionation (all compounds only present in the monohydroxy acids fraction), in RP-HPLC system I (retention time: 23, 41, 43 min, respectively), and in RP-HPLC system II (retention time: 7, 11, 12 min, respectively). Formation of HHT and 9-HODE was totally inhibited by 1 μ M indomethacin, whereas 13-HODE formation was not. Eluting between the HHT and 13-HODE peaks, several peaks were seen which have not been identified as yet. In an additional experiment the identity of HHT and 9-HODE was further checked by labeling the macrophages with [3 H]arachidonic acid for 45 min prior to the incubations. About 60% of the added label was taken up by the cells. After collection of the HPLC eluate the HHT peak appeared to contain radioactivity, whereas the 9-HODE peak did not. It was calculated that about 1 nmol 9-HODE per 20×10^6 cells was formed in the 15 min incubation period, whereas more than 2 nmol HHT were formed (mean data from 4 experiments). In one experiment we observed that adhering macrophages, devoid of contaminating lymphocytes, also synthesized 9-HODE and HHT. We cannot exclude, however, that lymphocytes also produce these mediators.

4. DISCUSSION

Here, we have shown that under basal conditions guinea pig AM possess considerable cyclooxygenase activity, as judged by the amount of HHT formed during a 15 min incubation. HHT is considered to be formed concomitant with thromboxane A_2 (TxA_2) formation, which is catalyzed by cyclooxygenase and thromboxane synthase enzymes [8,9]. Indeed, in additional experiments we also found TxB_2 , a stable breakdown product of TxA_2 , in incubates of AM (not shown). We also observed the formation of a linoleic acid metabolite, 9-HODE, by non-stimulated guinea pig AM. To our best knowledge no data are

available in the literature on the release of 9-HODE by inflammatory cells. Linoleic acid has been shown to be converted to monohydroxy metabolites by microsomal fractions from sheep seminal vesicles [10], homogenates of VX₂ carcinoma tissue [11], and by blood vessels [12]. The production of these metabolites could be inhibited by indomethacin, suggesting that they are formed through a cyclooxygenase-catalyzed reaction. In rabbit peritoneal tissue, however, only 9-HODE formation was clearly dependent on cyclooxygenase activity, whereas 13-HODE was formed through lipoxygenase-like activity [13]. In porcine leukocytes, 13-HODE was formed via a lipoxygenase-catalyzed reaction, since it could only be inhibited by the lipoxygenase inhibitor 5,8,11,14-eicosatetraynoic acid (ETYA) and not by indomethacin [6]. Our present results are in agreement with these findings, in that 9-HODE formation by guinea pig AM could be inhibited by indomethacin. We also found small amounts of 13-HODE, which was not affected by indomethacin. It cannot be excluded that 13-HODE was formed by a lipoxygenase enzyme, although we could not detect the formation of any lipoxygenase products from arachidonic acid in our incubations. It is equally possible that 13-HODE was formed non-enzymatically, since oxidation of linoleic acid in aqueous buffers favours the formation of 13 positional isomers of the hydroxy acid [14]. The fact that only limited information is available on the formation of linoleic acid metabolites by inflammatory cells can be explained by the experimental conditions usually employed in such studies. Most studies focus on the production of arachidonic acid-derived mediators, and exogenous arachidonic acid is added, or a stimulus is used which liberates arachidonic acid from membrane lipids. It has been shown, however, that arachidonic acid exerts a strong inhibition on the metabolism of linoleic acid [12].

The biological significance of 9-HODE formation is not known as yet. Structurally related monohydroxy derivatives of arachidonic acid (HETEs) exert a modulatory role on inflammatory processes, e.g. by interfering with the metabolism of arachidonic acid [15]. For example, 15-HETE inhibits 5- and 12-lipoxygenase, whereas 5- and 12-HETE inhibit 15-lipoxygenase. 13-HODE also has inhibitory properties towards 5-lipoxygenase

activity, albeit less than 15-HETE [16]. HETEs have also been reported to stimulate chemotaxis and chemokinesis of neutrophils and eosinophils and to induce neutrophil aggregation and degranulation [17–19]. Whether 9-HODE has similar properties remains to be determined. Most interestingly, recent support for a role for monohydroxy acids in inflammatory processes has been obtained with patients with familial Mediterranean fever [20]. In these patients, who suffer from recurrent episodes of fever, serositis and arthritis, high levels of monohydroxy acids, including 9-HODE and 13-HODE, were found circulating in the blood.

HETEs and HODEs are formed from their respective precursor fatty acids through a hydroperoxide intermediate. These hydroperoxides may also have a modulatory function in the formation of fatty acid-derived mediators, either by providing the cyclooxygenase and lipoxygenase enzymes with the required peroxide tone [21], or by influencing phospholipase activity [22]. Other metabolites may be formed from linoleic acid as well, including epoxyhydroxy and trihydroxy derivatives [6,12]. Little is known about the biological significance of these products. In our incubations with AM we have not been able to detect any of these metabolites. Another interesting class of linoleic acid metabolites is produced by neutrophils and can also be found in rat lung lavage fluid after pure oxygen breathing [23,24], an experimental model to study inflammatory processes in the lungs. These epoxide derivatives, named leukotoxins, show a typical uncoupling effect on mitochondrial respiration and relax rat stomach smooth muscle.

In conclusion, it is evident that besides the extensively studied arachidonic acid-derived prostaglandins and leukotrienes, linoleic acid-derived products may also be synthesized by inflammatory cells, and further research is necessary to acknowledge fully their possible role in inflammatory processes.

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