Inhibitory Effects of Shale Oils (Ichthyols) on the Secretion of Chemotactic Leukotrienes From Human Leukocytes and on Leukocyte Migration

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Ichthyols are sulfated shale oils with well-known anti-inflammatory effects in dermatologic diseases. Their possible mechanisms of action were studied by measuring chemotactic factor release from peripheral human leukocytes in vitro. Ichthyols caused no release of such factors by themselves but inhibited ionophore-induced release. After elution of the cell supernatants on reverse-phase high-pressure liquid chromatography, followed by analysis of the fractions in the chemotaxis assay and the radioimmunoassay, Ichthyols caused a reduction of lipids at marker positions for leukotriene B₄ (LTB₄) and 20-COOH-LTB₄. The inhibition was also evident in the LTB₄ radioimmunoassay, was dose- and time-dependent, and occurred in noncytotoxic concentrations of the agents. Ichthyols also inhibit the chemotactic response of neutrophils toward LTB₄ and the unstimulated migration of cells. These inhibitory effects of Ichthyols on secretion of chemotactic arachidonate metabolites from leukocytes and on cell migration provide a plausible explanation for their anti-inflammatory activity. J Invest Dermatol 87:694–697, 1986

Ichthyols are water-soluble ammonium bituminosulfonates [12] that have been used in dermatology as anti-inflammatory and antipruriginous agents since 1882 [13]. They have been shown to be effective in treating eczema of diverse causes, crysipelas, acne vulgaris, rosacea, and arthritis [13–15]. Ichthyols are available as dark or as light mixtures. The dark Ichthyol is obtained during treatment of shale oil extracts with sulfuric acid at high temperatures, and the light Ichthyol results from prolonged sulfation of the extracts at lower temperatures, yielding low-molecular-weight compounds (<800) and a thinner solution [16; information of the manufacturer].

In the present investigation, we have studied the effect of the Ichthyols on the generation of chemotactic factors (CFs), particularly of LTB₄ and its biologically active metabolites, using both bioassay and analytical procedures. The modulation of neutrophil chemotaxis was studied also for control. The in vitro data provide a possible explanation for the anti-inflammatory properties of the Ichthyols, as observed in vivo.

MATERIALS AND METHODS

Preparation of Cells Blood was obtained by venipuncture from healthy volunteers and separated by Ficoll/Hypaque, to obtain a fraction containing lymphocytes, monocytes, and basophils (LMB) and a fraction containing polymorphonuclear neutrophils (PMN) (>95% pure) and red blood cells. The latter were removed by an additional step of dextran sedimentation (see [17] for details).

Stimulation of Cells Release of mediators was studied by incubating 1 × 10⁷ cells in 1 ml Hanks’ buffer (Gibco, Glasgow, Scotland) with the calcium ionophore A 23187 (Eli Lilly, St. Louis, Missouri) at 1 × 10⁻⁷ M, 37°C for 15 min, or for the time indicated (see [17] for details). The 2 Ichthyols, prepared as described above and kindly provided by the Ichthyol-Gesellschaft, Hamburg, F.R.G., were preincubated for 10 min with the cells alone prior to stimulation with the ionophore. In some experiments, the time of exposure of the cells to the Ichthyols was

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Table I. Inhibition of Ionophore-Stimulated Release of Chemotactic Leukotrienes from Polymorphonuclear Neutrophils (PMN) and from Lymphocytes, Monocytes, and Basophils (LMB) by Different Concentrations of Light Ichthyols

<table>
<thead>
<tr>
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<th>Percent Inhibition</th>
<th>CF Release</th>
<th>LTB4 Release (RIA)</th>
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<tr>
<td>PMN + Ichthyol at:</td>
<td></td>
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<td></td>
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<tr>
<td>1.0 mg/ml</td>
<td>100 ± 2</td>
<td>100 ± 0</td>
<td></td>
</tr>
<tr>
<td>0.1 mg/ml</td>
<td>77 ± 12</td>
<td>0 ± 0</td>
<td></td>
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<tr>
<td>0.01 mg/ml</td>
<td>55 ± 15</td>
<td>0 ± 0</td>
<td></td>
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<tr>
<td>LMB + Ichthyol at:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.0 mg/ml</td>
<td>100 ± 0</td>
<td>55 ± 12</td>
<td></td>
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<tr>
<td>0.1 mg/ml</td>
<td>100 ± 0</td>
<td>36 ± 8</td>
<td></td>
</tr>
<tr>
<td>0.01 mg/ml</td>
<td>90 ± 6</td>
<td>28 ± 9</td>
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Results are expressed as mean ± 1 SD (n = 5). Control release with the ionophore was 5 × 10⁻⁴ M LTB4 for PMN and 1 × 10⁻⁴ M LTB4 for LMB. Note the greater inhibition of the release of chemotactic activity than of LTB4, as measured in the RIA, which may be due to a direct effect of the diluted (1:10) Ichthyols in the test solution in the chemotactic assay. The viability of the cells after incubation was >98%.

Table II. Effect of Dark Ichthyol on Human Polymorphonuclear Neutrophil Migration

<table>
<thead>
<tr>
<th>Ichthyol (mg/ml)</th>
<th>Above</th>
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<tbody>
<tr>
<td></td>
<td>1.0</td>
<td>0.75</td>
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<tr>
<td>1.0</td>
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<tr>
<td>0.75</td>
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<td>0.5</td>
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<tr>
<td>Below</td>
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<tr>
<td>0.25</td>
<td>0</td>
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<tr>
<td>0.1</td>
<td>0</td>
<td>0</td>
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<tr>
<td>0.01</td>
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<tr>
<td>C</td>
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</tr>
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</table>

Representative checkerboard analysis of the effect of dark Ichthyol and buffer alone (C) on human PMN migration, with Ichthyol placed above and/or below the filter in the Boyden chamber. Results are expressed as mean (5 determinations) number of cells that have migrated through filters in 5 HFP. There is a dose-dependent inhibition of chemokinetic (vertical column) and of chemokinetic (horizontal and diagonal columns) migration of the cells. Similar results were obtained with light Ichthyol and in 2 other experiments with different donor leukocytes.
dent manner, and this held for both types of Ichthyols in 2 other experiments (not shown).

The chemotactic migration of PMN toward LTB4 was also reduced in a dose-dependent manner by both agents (Fig 1). Ichthyol dark was less inhibitory in 2 of 3 experiments and equally inhibitory as Ichthyol light in a third experiment. Studies of the effect of Ichthyols on LMB chemotaxis were not done since normal LMB exhibit no significant random mobility and are not chemotactic toward LTB4 [22].

The kinetics of inhibition of CF release were studied next by preincubating cells with the Ichthyols (0.1 mg/ml) for varying time periods, followed by stimulation with the ionophore for 15 min (Fig 2). Both agents needed about 10 min of contact with cells before a significant reduction of release was noted. The final concentration of Ichthyols in the cell supernatants, when studied in the chemotaxis assay, was 0.01 mg/ml and contributed therefore only little to the time-dependent inhibition of CF release, as shown in Fig 2.

A reduction of the amount of LTB4 and its metabolites in the cell supernatants was confirmed by HPLC analysis. Contact of the cells with Ichthyols at 1 mg/ml for only 5 min, followed by a 15-min ionophore stimulation, caused no reduction of the HPLC peaks or of LTB4 in the unfractinated cell supernatants and in the HPLC fractions, as measured by RIA (not shown). Inhibition was, however, clearly evident after a 15-min preincubation (Fig 3). Both peaks at the LTB4 position were reduced, and so was the peak at the 20-COOH-LTB4 position in the presence of Ichthyol light. Ichthyol dark itself caused a broad peak at fraction 2 of the HPLC. This peak extended into the following fractions and caused even an increase in the size of the PGB2 standard peak. Analysis of the fractions showed a complete inhibition of the CF activity at marker positions for LTB4 and 20-COOH-LTB4 and of the LTB4-RIA measurements (fractions 8 and 3, respectively) by both Ichthyols (Fig 3). The size of the peak of synthetic LTB4 was not inhibited by preincubation with either of the Ichthyols.

**DISCUSSION**

The data presented here show 2 basic properties of the Ichthyols which provide a basis for the anti-inflammatory effects of the agents in vivo: (1) There is an inhibition of unstimulated and LTB4-induced in vitro neutrophil migration, and (2) the amount of CFs in stimulated leukocyte supernatants is reduced. LTB4 is one of the CFs that is identified in these supernatants and that is affected by the Ichthyols.

The inhibition of neutrophil migration occurs at noncytotoxic doses and affects the cells in a nongradient setting (Table II). Stimulation of cell migration, as reported recently by another group [23], was never observed by us with cells from 5 different human donors. Reasons for this divergence of results might be related to the time of incubation of the chemotaxis chambers, the concentrations of albumin in the buffer, or the method of evaluation of cell migration. In agreement with the latter authors who observed inhibition of chemotaxis toward formyl peptides, ECF, and activated serum [23], we found a dose-dependent inhibition of LTB4-induced chemotaxis of PMN by the Ichthyols (Fig 1).

The findings are relevant in that Ichthyols would thus suppress the biologic effects of CFs which are present in inflamed tissue. In accordance with our observations on the inhibitory rather than stimulatory activity of Ichthyols on normal PMN, these agents cause no inflammatory changes when applied to normal skin [13].

The lack of in vivo inflammatory effects of the Ichthyols fit also with the inability of these agents to induce CF secretion from PMN or LMB, as reported here. Furthermore, both Ichthyols inhibit ionophore-induced CF secretion (Table I, Fig 2). LTB4 and its metabolites are known to be the main chemotactic products during ionophore-induced stimulation of neutrophils [24,25]. LTB4, and less so its two 20-C oxidation products, have been shown to be biologically active in decreasing order of magnitude in the chemotaxis assay, as employed here [26]. Our data, showing reduced values of the LTB4-RIA and lower peaks and biologic activities at the LTB4-marker positions on HPLC, give support, although no final proof, to the notion that reduction of LTB4,

![Figure 2](image1.png)

**Figure 2.** Time-dependent inhibition of CF release from PMN by Ichthyol dark or light at 0.1 mg/ml. Cells were preincubated with buffer alone (100%) or with the Ichthyols for the time indicated and were then stimulated for CF release by the calcium ionophore A23187 for 15 min. Viability after incubation was always >98%, Bars, SE.

![Figure 3](image2.png)

**Figure 3.** Reverse-phase HPLC elution curve of ionophore-stimulated PMN supernatants alone (left) or after preincubation with cells with dark (right) or light (center) Ichthyols. Chemotactic activity (cells/5 HPF) and LTB4-RIA (ng/ml) measurements are shown in the insets. The positions of LTB4 markers and of the internal PGB2 standard are shown by arrows.
secretion by cells does indeed occur. The need for a prolonged incubation before a reduction of ionophore-stimulated LTB₄ and CFs are measured, suggests that Ichthyol affects the secretory process of the cell in addition to the biologic effect of its secreted products.

Ichthyols have been shown in the past to be reducing agents [13], to lower total lipids in the epidermis [15], and to inhibit the activity of esterases that act on short-chain fatty acids [27]. These findings are in line with the present data which show that Ichthyols reduce arachidonate-dependent, lipoxigenase-driven LTB₄ production. The biologic effects of Ichthyols, as reported until now in the literature, and their inhibitory effects on unstimulated and CF-induced cell migration and on ionophore-induced LTB₄ secretion, as observed here, provide a seemingly plausible basis for the anti-inflammatory properties of the agents in vivo.

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REFERENCES


