Anthralin (1,8-Dihydroxyanthrone) Is a Potent Inhibitor of Leukotriene Production and LTB₄-ω Oxidation by Human Neutrophils*

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The effect of anthralin and its oxidation products danthrone and anthralin-dimer on the production of 5-lipoxygenase products (5-HETE, leukotriene B₄, ω-oxidized LTB₄) by Ca-ionophore A 23187-stimulated human neutrophils has been studied in vitro. Anthralin exhibited dose-dependent inhibitory activity showing 50% inhibition at 7 μM with 10⁷ neutrophils. Inhibitory effects strongly depended upon cell densities and maximal inhibition occurred at low cell concentrations, whereas inhibitory rates of anthralin were low at high cell densities.

Inhibition of leukotriene production persisted after washing of anthralin-treated neutrophils. Also, with increasing amounts of arachidonic acid as substrate only slight changes of inhibitory activity were detected, indicating a noncompetitive way of action. In addition to the inhibition of leukotriene-production, the formation of ω-OH-LTB₄ from LTB₄ as well as ω-COOH-LTB₄ from ω-OH-LTB₄ was inhibited with IC₅₀ (half maximum inhibition concentration) near 4.4 μM and 2.2 μM, respectively. In contrast to anthralin, both metabolites—danthrone as well as anthralin-dimer—did not show any effect on leukotriene production and ω-oxidation even at high concentrations (up to 70 μM and 44 μM, respectively). J Invest Dermatol 87:624-629, 1986

Anthralin is a widely used topical antipsoriatic remedy [1]. Despite numerous clinical and experimental investigations the way that anthralin affects clearing of psoriatic lesions is still not fully understood. In the past, it has been shown that anthralin inhibits glycolytic enzymes [2,3]. Other studies have shown that under certain circumstances anthralin may have antiproliferative as well as antirespiratory activity [4-7]. Recent work in our laboratory revealed that anthralin inhibits various functional responses of human neutrophils [8]. Because clinical trials to successfully apply anthralin to inflamatory skin diseases other than psoriasis have mostly failed, it appears reasonable to suppose that the way anthralin works in skin may be related to specific features of the psoriatic tissue changes.

In psoriasis, elevated levels of free arachidonic acid as well as 12-HETE have recently been detected [9]. In addition, 5-lipoxygenase products were found in the diseased epidermis [10] and LTB₄-like material was demonstrated in scales [11] from patients with psoriasis.

Although the origin of these potent inflammatory mediators is speculative at the moment, theoretically 12-lipoxygenase products could originate from epidermal cells whereas 5-lipoxygenase products could mainly derive from infiltrate cells, possibly neutrophils.

In a recent study, Bedord et al [12] have shown that anthralin effectively reduces the production of 12-HETE from mouse epidermal homogenates without affecting synthesis of cyclooxygenase products. In the present study the effects of anthralin as well as of its metabolites danthrone and dimeric anthralin on the release of 5-lipoxygenase products (leukotrienes and 5-HETE) from Ca-ionophore-stimulated human neutrophils were investigated. Our results suggest that part of the antipsoriatic activity of anthralin may be related to inhibition of leukotriene production in infiltrating neutrophils.

MATERIALS AND METHODS

Calcium ionophore A 23187, arachidonic acid (99% pure), prostaglandin B₂ (PGB₂), and 1,8-dihydroxy-dianthron (danthron) were purchased from Sigma Chemie, Munich, F.R.G. Standard LTB₄ was a generous gift of Dr. J. Rokach, Merck Frosst, Canada. Standard 5-HETE was a generous gift of Dr. Bartmann, Hoechst AG, Frankfurt. Standard ω-OH-LTB₄ as well as ω-COOH-LTB₄ and 5,6 DiHETEs were from Paesel GmbH, Frankfurt; standard 6-trans-LTB₄ and 12-epi-6-trans-LTB₄ were a generous gift of Dr. G. Loschen, Chemie Grünenthal GmbH, Stolberg (F.R.G.) or have been isolated from Ca-ionophore-stimulated human neutrophils by published methods [13]. LTB₄, ω-OH-LTB₄, and 5-
HETE were further characterized by inherent chemotactic activity and UV spectra. 1,8-Dihydroxyanthrone (anthranil, dithanol) was a gift from Bayer AG, Leverkusen, F.R.G., and was free of impurities as assessed by high-performance liquid chromatography (HPLC) analysis. It was dissolved in acetone (10 mg/ml) and diluted in phosphate-buffered saline (PBS), pH 7.2, containing 0.1% (w/v) bovine serum albumin (Sigma) just before experiments were performed.

Dimeric anthranil (1,8,1',8'-tetrahydroxydianthrone) was synthesized according to the method of Aturhoff and Schrefl [14]. Final purification was assessed by preparative thin-layer chromatography. Identity and purity have been proved by melting point, IR- and 1H-nuclear magnetic resonance spectra. Dimeric anthranil has been dissolved in acetone (5 mg/ml) and diluted as described for anthranil.

**Cell Preparation** Polymorphonuclear leukocytes (PMN) from healthy donors were isolated as previously described [15] using a slight modification of the method of Henson [16]. Because of variable amounts of contaminating mononuclear cells as well as platelets, crude PMN preparations (containing >90% PMN) were further purified by Ficol-Hypaque centrifugation. The sediment was washed twice in Ca++- and Mg++-free PBS, containing 1% (w/v) bovine serum albumin (BSA), pH 7.2, and stored at 4°C until experiments started. Using this technique final cell preparations contained more than 98% PMN (>93% neutrophils) with a viability greater than 97% as assessed by trypan blue exclusion. The main contaminants of these preparations were eosinophils (2–7%). Platelet contamination did not exceed 2% of the granulocytes.

**Incubation Procedures** Polymorphonuclear leukocytes (10⁷ cells/assay) were preincubated with anthera-derivatives in PBS, pH 7.2, containing 128 mM NaCl, 8.1 mM Na₂HPO₄, 1.5 mM KH₂PO₄, 2.7 mM KCl, 0.1% (v/v) glucose, and 0.1% (w/v) BSA, for 15 min at 37°C, followed by a 5-min incubation with 10 μM arachidonic acid (prepared by evaporation of methanolic stock solution and subsequent solvent with 50 μl 0.1 M NaHCO₃ and PBS containing 0.1% BSA). Thereafter CaCl₂ (0.6 mM) as well as Ca⁺⁺-ionophore A-23187 (10 μM) were added. Incubation was carried out in a shaker bath for 10 min and then stopped by cooling in an ice bath. After centrifugation supernatants were collected and 100 ng PGB₂ as well as the radical scavenger 4-hydroxy-2,2,6,6-tetramethylpiperidinoxyl free radical (2 μg) [17] were added and stored under argon at −70°C until they were analyzed by reversed-phase (RP)-HPLC.

In experiments, where LTB₁ metabolism was investigated, human PMN (10⁷ cells/ml) were incubated with 200 ng LTB₁ for 30 min at 37°C in the presence or absence of anthranil. Preincubation with anthranil as well as termination of enzymatic reaction was performed as described.

**Reversed-Phase High-Performance Liquid Chromatography** For extraction of leukotrienes octadeyleic (C18)-RP cartridges (Sep Pak, Waters) were used, which were first washed with methanol and water, respectively, according to the manufacturer’s instructions.

After applying the cell supernatant (pH 7.2) to the RP-18-cartridge (3x) and washing with water, adsorbed leukotrienes were eluted with methanol (usually 3 ml). Recovery for all leukotrienes as well as 5-HETE and the internal standard PGB₂ was found to be better than 80%.

Methanolic solutions of leukotrienes were evaporated under argon and the residue solubilized in 400 μl of methanol/0.12% ammonium acetate-buffer, pH 5.4 (50:50).

High-performance liquid chromatography was performed with a 4.6 x 250 mm Altex Ultrasound ODS 5-μm column, attached to a Spectra Physics SP 8700 pump and a Kratos Spectroflow 783 UV detector.

The solvent system was a gradient of methanol (A) and 0.12% ammonium acetate, pH 5.4 (B). The column was eluted with the following protocol: time 0 min: 50% A, 50% B; time 6 min: 60% A, 40% B; time 12 min: 60% A, 40% B; time 28 min: 70% A, 30% B; time 50 min: 70% A; 30% B; time 52 min: 100% A, time 65 min: 100% A. A flow rate of 1.0 ml/min at 50°C was maintained and the effluent was monitored at 270 nm (leukotrienes) or 237 nm (monohydroxy acids).

Peak areas were integrated with a Spectra Physics SP 4270 computing integrator to quantify leukotrienes by relation to external standards of authentic samples. The recovery of the international standard PGB₂ was used to correct for losses during sample preparation. Molar absorption coefficients (M⁻¹ cm⁻¹) of 28,650 for PGB₂ and 50,000 for the leukotrienes at 270 nm and 29,500 for the monohydroxy acids at 237 nm [17] were used.

**Statistics** The Wilcoxon rank-sum test for paired and unpaired samples was used to assess statistical significance.

**RESULTS**

**Arachidonate Metabolism by Ionophore-Stimulated Neutrophils** When neutrophils were incubated with arachidonic acid (10 μM) as well as Ca-ionophore (10 μM) for 10 min at 37°C followed by termination of the reaction by cooling in an ice bath, a typical HPLC profile was obtained (Fig 1).

The gradient system used in our HPLC analyses allowed simultaneous assessment of ω-oxidized leukotrienes, leukotrienes, and HETES. As shown in Fig 1, typical elution times were 6.3 min for ω-COOH LTB₁, 9.5 min for ω-OH-LTB₁, 22.3 min for trans-LTB₂, 23.8 min for epi-trans-LTB₂, 25.5 min for LTB₄, and 43.0 min for 5-HETE.

In this system we are unable to distinguish between LTB₁ and the interaction products 5(S),12(S)-diHETE; however further analysis of the LTB₁ peak by a different HPLC solvent system [tetrhydrofurans:methanol:ammonium acetate (0.1%, w/v, pH 5.4) (25:30:45)] demonstrated negligible (5%) contamination of 5,12-diHETE. This is evidence that by our isolation and purification procedure platelet contamination of the PMN preparations is nearly absent.

**Effects of Anthranil Pretreatment on Leukotriene Production** Pretreatment of neutrophils with anthranil (30 μM) and subsequent activation of PMN-5-lipoxygenase by Ca-ionophore

![Figure 1](https://example.com/figure1.png)
Figure 2. Dose-dependent inhibition of ionophore-stimulated LT production of human PMN (10^7 cells/3 ml) by anthralin. Mean amounts of LTB₄ (●-●), total LTB₄ (= sum of LTB₄, ω-OH-LTB₄, and ω-COOH-LTB₄) (△-△), ω-OH-LTB₄ (●-●), and ω-COOH-LTB₄ (●-●) as well as 5-HETE (●-●) of 6 experiments were expressed in percent of the vehicle control, corrected for recovery by recovery of the PGB₂ control. Typical amounts isolated from stimulated neutrophils were 83.2 ng LTB₄, 112.6 ng ω-OH-LTB₄, and 30.4 ng ω-COOH-LTB₄ (= 100% control). Note increased amounts (104.2 ng) of LTB₄ at low (2-5 μM) anthralin concentrations and lower IC₅₀ for production of ω-COOH-LTB₄ and ω-OH-LTB₄. Significantly enhanced amounts of LTB₄ are indicated by the asterisk (p < 0.05). Bars, SD.

in the presence of arachidonic acid results in nearly complete absence of all 5-lipoxynase-products of LTB₄, trans-LTB₄, epitrans-LTB₄, and 5-HETE, as well as both ω-oxidation products of LTB₄, ω-OH-LTB₄ and ω-COOH-LTB₄ (Figs 1, 2).

To exclude a direct chemical reaction of anthralin with these arachidonic acid metabolites, supernatants of ionophore-stimulated neutrophils (containing all 5-lipoxynase products mentioned above) were incubated with anthralin (20 μM) for 20 min. After HPLC analysis no significant loss of these 5-lipoxynase products could be detected, indicating absence of direct effects of anthralin on the production of leukotrienes (data not shown).

A dose-response study revealed an inhibition of the 5-lipoxynase-product formation within a narrow concentration range of 1-10 μM anthralin (Fig 2). Comparison of the half maximum inhibition concentration (IC₅₀) of different 5-lipoxynase products revealed similar IC₅₀ for trans-LTB₄, epi-trans-LTB₄ (data not shown), and 5-HETE. Leukotriene B₄ alone shows significantly increased yields at low anthralin concentrations (Fig 2), whereas dose responses of ω-OH-LTB₄ and ω-COOH-LTB₄ in comparison with 5-HETE are shifted to lower anthralin concentrations.

Total LT₄ (the sum of LT₄ plus ω-OH-LTB₄ plus ω-COOH-LTB₄) produced by PMN resulted in the same IC₅₀ as that seen for 5-HETE (Fig 2).

Effect of Anthralin on ω-Oxidation of LTB₄ Anthralin pre-treatment of human neutrophils resulted not only in diminished formation of LTB₄ and 5-HETE, but also in decreased amounts of ω-OH-LTB₄ and ω-COOH-LTB₄ (Fig 2). Since this finding could be the result of decreased LTB₄ synthesis as well as decreased ω-oxidation, neutrophils were incubated with LTB₄ in the presence or absence of different concentrations of anthralin. Typical HPLC chromatograms of these experiments are shown in Fig 3. This figure reveals that anthralin inhibits formation of ω-COOH-LTB₄ as well as ω-OH-LTB₄. Dose-response studies shown in Fig 4 make it possible to calculate the IC₅₀ for formation of ω-OH-LTB₄ by inhibition of total ω-OH-LTB₄, which is the sum of ω-OH-LTB₄ plus ω-COOH-LTB₄.

It is noteworthy that IC₅₀ for the formation of ω-COOH-LTB₄ is lower (2.2 μM) compared with the formation of ω-OH-LTB₄ (4.4 μM).

Figure 3. Reversed phase HPLC chromatograms of LTB₄ metabolites synthesized by human neutrophils (10⁷ cells/3 ml) in the absence (control) and presence of 1.4 or 7.1 μM anthralin. Peak identification: 1, ω-COOH-LTB₄; 2, ω-OH-LTB₄; 3, internal standard PGB₂; 4, LTB₄. Note increased amounts of ω-OH-LTB₄ (73.2 ng compared with 46.2 ng in the control) as well as decreased amounts of ω-COOH-LTB₄ (68.4 ng compared with 88.2 ng in the control) after treatment with 1.4 μM anthralin. One typical experiment is shown.

Figure 4. Dose-dependent inhibition of LTB₄ metabolism to ω-OH-LTB₄ and ω-COOH-OTB₄. Typical yields of leukotrienes after treatment of PMN with ω-OH-LTB₄ were 54.2 ng ω-OH-LTB₄ and 74.6 ng ω-COOH-LTB₄ (= 100% control). Neutrophils (10⁷ cells/3 ml) were incubated for 30 min at 37°C with 200 ng LTB₄ in the presence or absence of anthralin. Mean amounts of ω-OH-LTB₄ (●-●), total LT₄ (sum of ω-OH-LTB₄ and ω-COOH-LTB₄) (●-●), and ω-COOH-LTB₄ (●-●) of 6 experiments were expressed in percent of the vehicle control (0.1% acetonitrile) corrected for recovery by recovery of the internal standard PGB₂. Note increased yields of ω-OH-LTB₄ at low (1 μM) anthralin concentrations and shifted dose responses of ω-COOH-LTB₄ and total ω-OH-LTB₄. Significantly enhanced amounts of ω-OH-LTB₄ are indicated by the asterisk (p < 0.05). The 0 value is expressed on abscissa. Bars, SD.
Table I.  Effect of Washing on Anthralin (74 μM)-Dependent Inhibition of Leukotriene Production by Human Polymorphonuclear Leukocytes (PMN) (3 × 10³/3 ml)

<table>
<thead>
<tr>
<th>Product</th>
<th>% Control</th>
<th>% Control</th>
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<tbody>
<tr>
<td>ω-OH-LTB₄</td>
<td>54.8 ± 12.7</td>
<td>21.2 ± 6.1</td>
</tr>
<tr>
<td>trans-LTB₄</td>
<td>24.4 ± 8.3</td>
<td>14.4 ± 3.4</td>
</tr>
<tr>
<td>epi-trans-LTB₄</td>
<td>26.3 ± 10.1</td>
<td>16.1 ± 4.9</td>
</tr>
<tr>
<td>LTB₄</td>
<td>78.9 ± 16.3</td>
<td>28.9 ± 5.8</td>
</tr>
<tr>
<td>5-HETE</td>
<td>39.1 ± 7.2</td>
<td>18.5 ± 4.4</td>
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</table>

*PMN were washed 2 times with PBS. ^PMN were not washed. Data are the result of 3 experiments ± SD.

Effect of Washing of Anthralin-Treated Cells on Leukotriene Production

In an additional series of experiments neutrophils were thoroughly washed after preincubation with anthralin. As shown in Table I, leukotriene production is inhibited after washing of the cells, however with increased IC₅₀.

Effect of Anthralin at Different Cell Densities

In these experiments different neutrophil densities (2 × 10⁶ to 30 × 10⁶ PMN/ml) were preincubated at constant anthralin concentrations (7, 29, and 74 μM) for a constant length of time (20 min) at 37°C. As shown in Fig 5, the inhibitory activity of anthralin on PMN lipoxygenase pathways not only depends upon the anthralin concentration but also upon the cell density used. When 4 × 10⁷ PMN per assay were used, an IC₅₀ near 74 μM was found; however with 10⁶ PMN the IC₅₀ decreased to 7 μM. Using as low as 3 × 10⁶ PMN per assay, an IC₅₀ of 0.9 μM was observed (data not shown).

Effect of Danthron and Dimeric Anthralin on Leukotriene Production

Because anthralin easily decomposes to both metabolites (danthron and dimeric anthralin), we studied the influence of these components on leukotriene synthesis. With both compounds no significant inhibitory effect was seen at concentrations up to 70 μM for danthron or up to 44 μM for dimeric anthralin (Table II).

Effect of Different Substrate Concentrations on Anthralin-Dependent Inhibition of Leukotriene Production

The effect of increasing arachidonic acid concentrations on LTB₄ as well as 5-HETE production at different anthralin concentrations is shown in Table III. Only when large amounts of arachidonic acid were used did leukotriene production increase.

Table II.  Influence of Danthron and Dimeric Anthralin on Leukotriene and 5-HETE Production by Polymorphonuclear Leukocytes (PMN) (10⁷/ml)

<table>
<thead>
<tr>
<th>Product</th>
<th>% Control</th>
<th>% Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>ω-OH-LTB₄</td>
<td>93 ± 11</td>
<td>96 ± 12</td>
</tr>
<tr>
<td>trans-LTB₄</td>
<td>95 ± 9</td>
<td>91 ± 8</td>
</tr>
<tr>
<td>epi-trans-LTB₄</td>
<td>98 ± 5</td>
<td>99 ± 13</td>
</tr>
<tr>
<td>LTB₄</td>
<td>104 ± 12</td>
<td>96 ± 16</td>
</tr>
<tr>
<td>5-HETE</td>
<td>103 ± 9</td>
<td>103 ± 15</td>
</tr>
</tbody>
</table>

*PMN were preincubated with 70 μM danthron. ^PMN were preincubated with 44 μM dimeric anthralin. Data show results of 3 experiments ± SD.

DISCUSSION

Human neutrophils contain enzyme systems capable of generating leukotrienes and 5-HETE from exogenous arachidonic acid [13]. The main products released by neutrophils after stimulation with Ca-ionophore A-23187 are potent chemotaxins like LTB₄ and its metabolite ω-OH-LTB₄, [19] as well as 2 nonchemotactic isomers of LTB₄, 6-trans-LTB₄ and 12-epi-6-trans-LTB₄, and in addition 5-HETE [20].

In the present study we investigated the influence of anthralin on the production of several 5-lipoxygenase products of Ca-ionophore-stimulated human neutrophils.

The results demonstrate that the clinically active antispasmodic drug anthralin—in contrast to its metabolites danthron and dimeric anthralin—is a potent inhibitor of leukotriene and 5-HETE production by human neutrophils (Figs 1, 2; Table II). The IC₅₀ for 5-HETE and leukotriene production is shown to be near 7.5 μM when 10⁷ PMN were used. This is nearly 5-fold lower than the IC₅₀ found for 12-HETE production of crude epidermal homogenates [12].

Because earlier investigations [17, 21] as well as additional spectroscopic and biologic studies carried out in our laboratory revealed that it is possible to characterize most of the 5-lipoxygenase products released from neutrophils by RP-HPLC techniques, our studies were performed without the use of radioactive labeled arachidonic acid.

Using this procedure, no interference in retention time was seen with the drugs used in this study.

The mechanism(s) by which anthralin affects the production of leukotrienes, is not clear at the present time. However some of the findings reported here as well as previously published observations [8] may help to identify particular aspects of the in vivo action of anthralin. It is noteworthy that the IC₅₀ for the production of 5-HETE as well as total LTB₄ and both nonchemotactic isomers of LTB₄, which are formed nonenzymatically by hydrolysis of LT₄α, is nearly identical. Therefore it is reasonable

Table III.  Effect of Substrate Concentration on the Inhibition of Ca-Ionophore-Stimulated 5-HETE and Total LTB₄ Production by Anthralin-Treated Neutrophils (10³/3 ml)^

<table>
<thead>
<tr>
<th>Percent Inhibition</th>
<th>Arachidonic Acid (μM)</th>
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<tr>
<td></td>
<td>1.5</td>
</tr>
<tr>
<td>5-HETE production</td>
<td>20</td>
</tr>
<tr>
<td>production</td>
<td>40</td>
</tr>
<tr>
<td>LTB₄ production</td>
<td>20</td>
</tr>
<tr>
<td>production</td>
<td>40</td>
</tr>
</tbody>
</table>

^Results of 6 experiments ± SD are shown.

*By means the sum of LTB₄, ω-OH-LTB₄, and ω-COOH-LTB₄ produced by human neutrophils.

*Significantly lower (p < 0.05) than control.

Figure 5. Effect of anthralin at different cell densities. Neutrophils were preincubated with 7, 29, and 74 μM anthralin at different cell densities and stimulated thereafter with 10 μM arachidonic acid. Data (mean amounts of total formed LTB₄) are expressed in percent of the vehicle (0.1% acetone) control. The results of 4 experiments ± SD (bars) are shown.
to suggest that anthralin inhibits the 5-lipoxygenase by interference with the formation of the intermediate 5-HPTE, which is a precursor of LTBA. Thus anthralin may possibly function as a radical trapping antioxidant.

A priori, one could expect such a phenolic agent to be a relatively nonspecific inhibitor of all lipoxygenases, since these enzymatic reactions proceed via radical mechanisms. It should be noted, however, that the standard antioxidant nordihydroguaiaretic acid shows different inhibitory activities in platelet 12-lipoxygenase and PMN-5-lipoxygenase (unpublished). Therefore it is possible that differential sensitivity of these lipoxygenases may exist toward antioxidants. Alternatively it may be possible that anthralin inhibits the activation of 5-lipoxygenase via enzyme activation by Ca$^{2+}$. 5-Lipoxygenase requires Ca$^{2+}$ for activation in vitro; however, in contrast to this, 12-lipoxygenase does not [22].

An unexpected result is the potent inhibitory effect of anthralin on the catalysis of LTB$_4$ to $\omega$-OH-LTB$_4$ and $\omega$-COOH-LTB$_4$. This LTB$_4$ oxidation seems to be of higher sensitivity to anthralin treatment as compared with the synthesis of LTB$_4$. The profound inhibitory effect of anthralin seen in the $\omega$-oxidation of LTB$_4$ leads to a decrease of $\omega$-oxidation products of anthralin at concentrations which only slightly affect the synthesis of LTB$_4$. As a consequence of this inhibitory activity on the catalysis of LTB$_4$ accumulates (Fig 4).

It is interesting to note that high concentrations of arachidonic acid (>20 $\mu$M) also lead to a decreased synthesis of $\omega$-COOH-LTB$_4$ and partly $\omega$-OH-LTB$_4$—as first mentioned by Salari et al [23].

To further evaluate the inhibitory role of anthralin on LTB$_4$ catalysis, we performed separate inhibition experiments with anthralin using LTB$_4$ and $\omega$-OH-LTB$_4$, respectively, as substrates. In this system the IC$_{50}$ for the formation of $\omega$-COOH-LTB$_4$ (Figs 3, 4) was lowest compared to formation of $\omega$-OH-LTB$_4$.

Inhibition of LTB$_4$-$\omega$-oxidation is possibly linked with inhibition of LTB$_4$-20-hydroxylase, which has recently been isolated and characterized in human neutrophils [24,25]. The synthesizing capacity of this enzyme results from a cytochrome P-450 oxygenation system [26]. To our knowledge—apart from carbon monoxide [27] and long-chain terminal acetylenic fatty acids [28]—anthralin is the first agent that is known to inhibit $\omega$-oxidation of LTB$_4$ in vitro. Looking at the conditions which are present in vivo, inhibition of LTB$_4$ catalysis by human neutrophil-LTB$_4$-20-hydroxylase, however, may not play a significant role because anthralin concentrations measured in epidermis of psoriasis after treatment are reported to be 10- to 100-fold higher than IC$_{50}$ of $\omega$-oxidation [29,30]. Under these conditions production of leukotrienes appears to be totally blocked (Fig 2). In view of these observations it is interesting to speculate that anthralin may also inhibit other P-450 systems, which are seen in various tissues, and are able to catalyze $\omega$-oxidation of prostaglandins and long-chain fatty acids [31–33], and could result in accumulation of these compounds.

The anthralin-induced inhibition of leukotriene synthesis was reduced in neutrophils preincubated with anthralin followed by washing (Table I). In addition to this the inhibitory effects of anthralin are only slightly influenced by increased substrate concentrations (Table III). This indicates that inhibition of the leukotriene generating system mainly works in the noncompetitive way.

It should be noted that anthralin-treated neutrophils—even after washing—show an intense brownish color, which appears to depend on a chemical reaction of anthralin with cellular (membrane) components as has been shown earlier [8]. This, however, did not result in cytotoxicity, since in all experiments performed with anthralin, cytotoxic effects were not responsible for the effects described here. This was evidenced by the absence of any release of the cytoplasmatic enzyme lactate dehydrogenase [8].

As an important observation we could show that the inhibitory effect of anthralin depends not only on the concentration of anthralin used for preincubation but also on the cell density. This is in accordance with the hydrophobicity of anthralin. At lowest cell densities inhibition of leukotriene synthesis was strongest (Fig 5), which gives additional evidence for the irreversible type of inhibition by anthralin. With these results we are able to calculate the critical amount of anthralin necessary to produce half maximum leukotriene synthesis per neutrophil. This is 1 pg anthralin per cell.

Taken together, the important part of the mode of action of anthralin seems to be related to cutaneous inflammation by inhibition of cellular LTBA production together with effects on the other cellular functions in psoriasis. This may help to explain the puzzling fact that therapeutic efficacy of anthralin is for the most part restricted to this skin disease.

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