Biochemical Pharmacology, Vol. 51, pp. 1173–1179, 1996.

ELSEVIER

ISSN 0006-2952/96/$15.00 + 0.00
PII S0006-2952(96)00043-3

Inactivation of Mouse Epidermal 12-Lipoxygenase by Anthralin—Implications for the Role of Oxygen Radicals

Klaus Müller and Ingo Gawlik*

INSTITUT FÜR PHARMAZIE, UNIVERSITÄT REGENSBURG, UNIVERSITÄTSSTR. 31, D-93040 REGENSBURG, GERMANY

ABSTRACT. Inactivation of 12-lipoxygenase (12-LO) in mouse epidermal homogenate by the antipsoriatic drug anthralin has been studied in detail. In view of the chemical instability of anthralin in a physiological buffer, the biological effects ascribed to the molecule itself may be related to some of its breakdown products. However, the inhibitory activity could not be attributed to the known stable oxidation product of anthralin, danthron, which did not decrease 12-LO activity. Addition of the antioxidants 2,6-di-tert-butyl-4-methylphenol (BHT) or β-carotene, or the hydroxyl radical scavenger sodium benzoate, protected against anthralin-mediated 12-LO inactivation, suggesting that pro-oxidant species derived from anthralin play a key role in the inhibitory action. Even though inhibitory effects of anthralin against catalase and superoxide dismutase (SOD) have been observed under the conditions applied in this study, these antioxidant enzymes also partially prevented the inhibition of 12-LO by anthralin when added to the incubation mixtures. Control experiments without anthralin revealed that the oxygen radical scavengers and antioxidant enzymes, themselves, did not appreciably influence epidermal 12-LO activity. A mechanism underlying the inactivation of epidermal 12-LO by anthralin is proposed, which involves active oxygen species formed during the auto-oxidation of the drug. BIOCHEM PHARMACOL 51;9:1173–1179, 1996.

KEY WORDS. anthralin; dithranol; 12-lipoxygenase inhibition; mouse skin; oxygen radicals; hydrogen peroxide

The 12-LO† pathway is the main metabolic conversion of arachidonic acid in the skin [1]. The epidermal layer, in particular, is an active site of its metabolism. Because increased production of 12-HETE has been implicated in the pathophysiology of psoriasis, 12-LO inhibition has been described as an approach for the treatment of this inflammatory skin disease [2, 3]. Moreover, the regulation of the 5-LO pathway has become an important target for therapeutic intervention [3, 4].

In recent studies of the action of anthrones upon these enzymes, the antipsoriatic drug anthralin (dithranol, 1,8-dihydroxy-9(10*H*)-anthracenone) was found to exhibit the greatest selectivity toward epidermal 12-LO [5], whereas a number of 10-substituted derivatives with modulated redox properties were more selective 5-LO inhibitors [6]. These selectivities suggested that different mechanisms determine inhibition of the two lipoxygenases, pointing to a specific enzyme interaction for the selective 5-LO inhibitors.

However, the mechanism(s) by which anthralin itself affects the production of 12-HETE is not quite clear at present. In view of the chemical instability of anthralin in aqueous solutions at pH 7.4, the biological effects ascribed to the molecule itself may, in fact, be related to some of its breakdown products [7]. In particular, activated forms of molecular oxygen produced during the auto-oxidation of anthralin [8, 9] are likely candidates. A more detailed knowledge concerning its *in vitro* mechanism of LO inactivation will be necessary for the development of improved antipsoriatic anthrones.

Many known inhibitors of lipoxygenases possess low redox potentials [10], their antioxidant potency often correlating with their inhibitory potency [11]. Furthermore, the potency of phenolic LO inhibitors may be related to their ability to directly reduce ferric iron at the active site to the inactive ferrous form, to chelate ferric iron, or a combination of both [12, 13].

In the present study, we evaluated the effects of anthralin-derived oxygen radicals on 12-LO activity, employing specific oxygen radical scavengers or antioxidant enzymes to determine which class of reactive metabolite may contribute to enzyme inactivation.

MATERIALS AND METHODS

Materials

Catalase (EC 1.11.1.6) from bovine liver, SOD (EC 1.15.1.1) from bovine erythrocytes, peroxidase (EC

* Corresponding author. Tel. +49 941-9434810; FAX +49 941-9434809.

† *Abbreviations:* BHT, 2,6-di-tert-butyl-4-methylphenol; 12-HETE, 12-hydroxyeicosatetraenoic acid; LO, lipoxygenase; SOD, superoxide dismutase.

Received 25 August 1995; accepted 18 December 1995.

1.11.1.7) from horseradish, 12(S)-HETE, and 12(R)-HETE were purchased from Sigma (Deisenhofen, Germany). Solvents for HPLC were of HPLC quality (Roth; Karlsruhe, Germany). All other reagents were of the highest grades available from Sigma (Deisenhofen, Germany), Aldrich (Steinheim, Germany), or from Merck (Darmstadt, Germany).

Epidermal 12-Lipoxygenase Assay

Preparation of the epidermal homogenate and the 12-LO assay were performed as described [5]. The epidermal homogenate (50 mg wet weight/mL) was preincubated with appropriate additions (anthralin, antioxidants, oxygen radical scavengers, or antioxidant enzymes at indicated concentrations) for 5 min at 37°C in a shaking water bath. Additions were dissolved in DMSO (final concentration of 0.4%) or PBS, pH 7.4. Control assays were conducted with inclusion of the appropriate concentration of DMSO. Calcium chloride and arachidonic acid (final concentrations 2 mM and 5 μM, respectively) were then added, and the incubation was conducted for 10 min at 37°C, unless otherwise stated. Formation of 12-HETE was terminated by the addition of 3.0 mL MeOH/CH_3CN (1 + 1) containing the antioxidant nordihydroguaiaretic acid and 1-hydroxy-8-methoxy-9,10-anthracenedione as a chromatographic marker (final concentrations 0.3 and 30 μM, respectively). The incubation mixture was kept in an ice bath for 20 min and then centrifuged at 5000 *g* for 20 min at 0°C. Pertinent controls were performed to determine the original amounts of 12-HETE already present in the homogenate. The supernatant was diluted with 5 mL of water and passed through a prewashed octadecylsilane reversed-phase cartridge (Baker) that had been washed with 5 mL of MeOH and 5 mL of water. The material was eluted with 3 mL of MeOH, diluted with 3 mL of water, and subjected to reversed-phase HPLC analysis. The isocratic elution conditions of 12-HETE were MeOH/acetonitrile/water (35 + 35 + 30, vol), plus 0.1 vol % acetic acid, pH 5.5, flow rate 1.0 mL/min (Kontron 420 pump), monitored at 232 nm with a Kontron 735 LC UV detector. Data were recorded on a MacLab data acquisition system (WissTech, Germany) and analysis was performed with the Peaks software on an Apple Macintosh computer. Integrated areas of the peaks were compared to the internal standard and to external standards of authentic samples. Molar absorption coefficients given by Borgeat and Samuelsson [14] were used for calculations; % inhibition of the formation of 12-HETE by epidermal homogenates was calculated by comparing the mean values of the test system (n = 3, SD < 10%) with control activity (n = 8, SD < 5%).

Stereochemical Analysis by Chiral Phase Chromatography

12-HETE obtained from *in vitro* incubation experiments was purified by isocratic reversed-phase HPLC, as described above. This was, in turn, analyzed by chiral phase HPLC [15] on a Chiracel OB 250× 4.6-mm column (Daicel, Tokyo, Japan). The isocratic elution conditions were hexane/2-propanol/methanol/water (99.1 + 0.68 + 0.1 + 0.03, vol), flow rate 1.0 mL/min (SP 8700 Thermo Separation Products, Darmstadt, Germany), monitored at 235 nm with a Knauer UV detector (Berlin, Germany). The retention times for 12(R)- and 12(S)-HETE were 24 and 35 min, respectively.

Catalase and SOD Assays

Catalase activity was measured following the decomposition of hydrogen peroxide at 240 nm [16]. Reaction mixtures contained 1.98 mL of PBS, 1 mL of 0.03 M hydrogen peroxide and 0.02 mL of enzyme dilution (2 U/mg protein). Incubation experiments for the determination of catalase inhibition by anthralin were performed for 30 min at 37°C in a shaking thermostat bath.

Activity of SOD was determined by measuring the inhibition of pyrogallol auto-oxidation, monitored spectrophotometrically at 420 nm [17].

RESULTS

Effects of Antioxidants, Oxygen Radical Scavengers, and Antioxidant Enzymes on Epidermal 12-LO Activity

As mentioned above, many antioxidants/free radical scavengers can inhibit lipoxygenases, and represent the largest class of inhibitors. This is not surprising because conversion of arachidonic acid into LO products is a radical-based oxidation. Accordingly, to provide evidence for a role of active oxygen species in epidermal 12-LO inhibition employing specific oxygen radical scavengers, it is important to show that inhibition of the enzyme by the scavengers, themselves, at concentrations necessary for removing active oxygen species can be excluded.

Consequently, mouse epidermal 12-LO was incubated in the presence of various antioxidants, oxygen radical scavengers, and the antioxidant enzymes catalase, SOD, and peroxidase. In these experiments, 12-HETE concentration was measured by reversed-phase HPLC. The elution pattern of the main LO product was consistent with that of an authentic sample of 12-HETE. To exclude oxidation of arachidonic acid by a nonspecific free radical mechanism that gives racemic 12-HETE, we have determined the absolute stereochemistry of the mouse epidermal 12-HETE as recently described [5]. Comparison with authentic, stereochemically pure 12-HETE enantiomeric standards demonstrated that the 12-HETE present was the 12(S)-isomer, clearly ruling out the presence of the 12(R)-isomer.

Table 1 shows the influence of antioxidants, oxygen radical scavengers, and antioxidant enzymes on the activity of 12-LO. It is interesting to note that, in the presence of the antioxidants BHT and α-tocopherol, and the hydroxyl radical scavenger sodium benzoate, the enzyme activity even increased slightly. The singlet oxygen quencher β-carotene

TABLE 1. Effects of antioxidants, oxygen radical scavengers, and antioxidant enzymes on the formation of 12-HETE by mouse epidermal 12-LO

Compound	12-HETE (ng/mL)
Control	36.5
Ascorbic acid (100 μM)	20.8
BHT (100 μM)	50.6
Catalase (167 U/mL)	47.3
β-Carotene (100 μM)	37.5
Peroxidase (6 U/mL)	17.1
Propyl gallate (100 μM)	0
Pyrogallol (100 μM)	6.3
Sodium benzoate (1 mM)	46.9
SOD (167 U/mL)	32.5
α-Tocopherol (100 μM)	45.9

Mouse epidermal homogenate (50 mg wet weight/mL) was preincubated with the additions described above for 5 min at 37°C prior to incubation with arachidonic acid (5 μM) for 10 min at 37°C. Controls were performed with inclusion of the appropriate concentration of DMSO and without additions. Indicated values are ng 12-HETE per mL incubation mixture and are the average of 3 independent experiments (SD < 10%).

did not affect the production of 12-HETE. However, the radical scavenger propyl gallate totally inhibited 12-LO at 100 μM, and pyrogallol inhibited the enzyme at the same concentration by more than 80%. Likewise, ascorbic acid strongly impaired 12-LO activity. Therefore, these antioxidants hardly serve as appropriate tools in the search for the active metabolite against 12-LO.

Addition of catalase (167 U/mL) to the incubation mixture slightly increased 12-HETE formation, and incubation of 12-LO with SOD (167 U/mL) did not appreciably influence enzyme activity. On the other hand, peroxidase decreased 12-HETE formation compared to the control mixture by more than 50% (Table 1).

Effects of Anthralin, Danthron, and Bianthrone on 12-LO Activity

Next, we studied the influence of the known oxidation products of anthralin, the anthraquinone derivative danthron, and its dimer bianthrone, on 12-LO activity in mouse epidermal homogenate. As recently described [5], incubation with anthralin itself resulted in a concentration-dependent inhibition of the enzyme and gave an IC_{50} value of 9 μM. However, the stable oxidation product, danthron, was not effective at concentrations up to 30 μM. On the other hand, the product of one-electron oxidation of anthralin and dimerization of the anthralin free radical, bianthrone, which is also further degraded to the polymeric anthralin-brown [7], inhibited epidermal 12-LO with an IC_{50} value of 13 μM.

Effects of Antioxidants and Oxygen Radical Scavengers on Epidermal 12-LO Inactivation by Anthralin

In these experiments (Fig. 1), incubations were performed with 30 μM anthralin, which decreased enzyme activity to 18% of control activity. Addition of the antioxidant BHT to the incubation mixture partially prevented the inactivation of 12-LO caused by anthralin. The specific hydroxyl radical scavenger, sodium benzoate [18], also suppressed inactivation. As compared to the concentrations of other antioxidants (100 μM) used in this study, higher concentrations (1 mM) were necessary for protection against anthralin-mediated enzyme inactivation. However, this is in agreement with the observation that sodium benzoate is less efficient in scavenging anthralin-produced hydroxyl radicals than other compounds [9]. When compared to the increased activity of 12-LO in the presence of sodium benzoate as compared to controls without additions (Table 1), anthralin-decreased activity was maintained at approximately 47%. The protective effect provided by sodium benzoate, based upon the activity that remained in the absence of antioxidants (18%), amounts to approximately 30%. The best protection was afforded by β-carotene, which did not appreciably alter 12-LO activity as compared to controls. When β-carotene (100 μM) was included in the incubation mixture, the anthralin-induced decline in 12-LO activity once more increased to approximately 58% of control activity, which yields a protection of about 40%. There is one exception in these results, however. Of all antioxidants tested, only α-tocopherol did not produce any measurable alteration compared to anthralin-treated controls.

Effects of Anthralin on Catalase and SOD Activity

It has been reported that anthralin reduces the activities of both catalase and SOD [19]. Therefore, we examined the extent to which anthralin inhibited these enzymes. It is evident from Fig. 2 that anthralin inhibits both catalase and SOD activity concentration-dependently. At concentrations between 8 and 32 μM, anthralin led to far more than a 50% decrease in catalase activity, and the activity of SOD

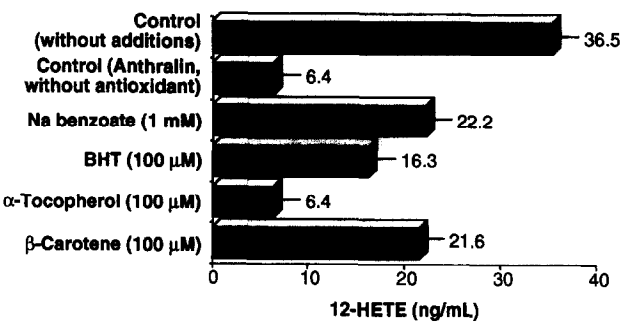

FIG. 1. Effects of antioxidants and oxygen radical scavengers on the anthralin-induced inactivation of mouse epidermal 12-LO. Mouse epidermal homogenate (50 mg wet weight/mL) was preincubated with anthralin (30 μM) and the indicated additions for 5 min at 37°C prior to incubation with arachidonic acid (5 μM) for 10 min at 37°C. Controls were performed without inclusion of anthralin, with inclusion of the appropriate concentration of DMSO, and without additions. Values are expressed as ng 12-HETE per mL incubation mixture ($n = 3$, SD < 10%).

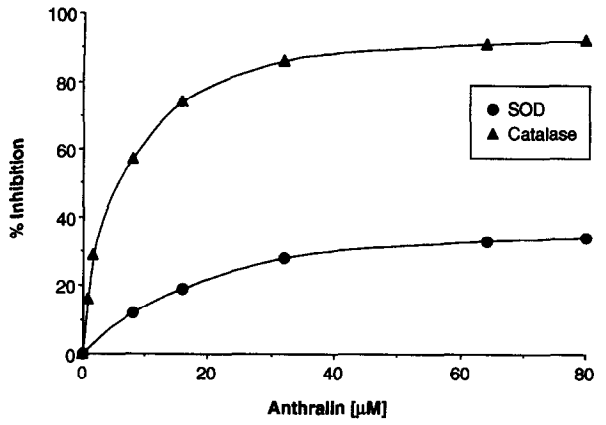

FIG. 2. Concentration-dependent effects of anthralin on the activities of catalase and SOD. Incubation mixtures contained catalase (100 U/mL) or SOD (15 U/mL) and indicated concentrations of anthralin in a final volume of 2 mL PBS, pH 7.4, for 30 min at 37°C in a shaking water bath ($n \geq 3$, SD < 5%).

declined by approximately 30%. Contrary to catalase, higher concentrations of anthralin did not result in a greater decrease in the enzyme activity of SOD. Even though the inhibitory effects of anthralin against catalase and SOD under the conditions applied in this study have to be considered, there should still be enough enzyme activity for studying the effects of these enzymes on 12-LO inactivation by anthralin.

Effects of Antioxidant Enzymes on Epidermal 12-LO Inactivation by Anthralin

In these sets of experiments, the effects of enzymes affecting the concentration of hydrogen peroxide and superoxide (i. e. catalase, peroxidase, and SOD) were examined in the anthralin-exposed 12-LO system. No significant changes in 12-HETE formation as compared to anthralin-treated controls were measured when antioxidant enzymes were added to the incubation sets, under the conditions described in the corresponding experiments with nonenzymatic antioxidants (data not shown). However, by increasing the incubation periods, pronounced changes in anthralin-decreased 12-LO activity were observed. Under these conditions, anthralin at 30 μM suppressed enzyme activity to almost negligible values. The effects of catalase, peroxidase, and SOD are depicted in Fig. 3. After incubation with any enzyme, the levels of 12-HETE increased once more. Catalase, SOD, or a combination of both enzymes enhanced 12-LO activity as compared to anthralin-treated controls to approximately 50% of the activity of untreated controls. The effect of catalase was, at least partially, due to its catalytical activity because it was diminished by heat inactivation of the enzyme. Control experiments without anthralin revealed that catalase and SOD did not appreciably influence epidermal 12-LO activity under the prolonged incubations, whereas peroxidase *per se* significantly decreased enzyme activity (data not shown). As a consequence, peroxidase only slightly affected anthralin-mediated 12-LO inactivation (Fig. 3).

DISCUSSION

In view of the instability of anthralin, inhibition of enzymes by the molecule itself is unlikely, but might be related to decomposition products formed during the auto-oxidation of the drug [7]. Indeed, coincident with the auto-oxidation process is the generation of reactive oxygen metabolites. Anthralin can activate molecular oxygen by univalent reduction to superoxide [20] which, in turn, can dismutate to produce hydrogen peroxide. The iron-dependent interaction of these two species results in the formation of the extremely reactive hydroxyl radical [21]. Hydroxyl radicals are also produced from anthralin in the presence of ferric salts [8, 9]. With the demonstration that anthralin can, indeed, significantly generate oxygen radicals, the question arises as to whether or not the biological effects of this compound can be related to this property. Although several biological targets have been shown to be damaged by oxygen radicals [22], the processes of lipid peroxidation and DNA damage are best characterized for anthralin [9, 23]. In this series of experiments, we examined the involvement of anthralin-derived oxygen radicals in the inactivation of epidermal 12-LO.

Thus far, anthralin has been identified as a selective 12-LO inhibitor with respect to 5-LO, as well as cyclooxygenase [5, 24]. However, an explanation of the mechanism of LO inhibition is still lacking. To date, the majority of evidence supporting a role of active oxygen species in LO inhibition has been derived from studies demonstrating the oxidation of the methionine residues of the enzyme by inhibitors [25, 26]. The hypothesis that oxygen radicals produced by anthralin are responsible for selective inhibition of 12-LO over 5-LO is strongly supported by observations

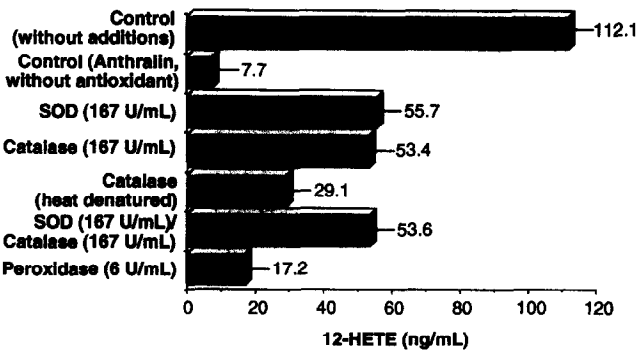

FIG. 3. Effects of incubation of catalase, peroxidase, and SOD on the anthralin-induced inactivation of mouse epidermal 12-LO. Mouse epidermal homogenate (50 mg wet weight/mL) was preincubated with anthralin (30 μM) and the indicated additions for 5 min at 37°C prior to incubation with arachidonic acid (5 μM) for 30 min at 37°C. Controls were performed without inclusion of anthralin and without additions. Values are expressed as ng 12-HETE per mL incubation mixture ($n = 3$, SD < 10%).

that 12-LO from bovine platelets is inhibited by active oxygen-producing systems, whereas the activity of 5-LO is only slightly influenced [27]. 12-LO inhibition by active oxygen species has also been observed in rabbit platelets [28]. In addition, it has been reported that LO inhibition by standard inhibitors, such as phenidone or BW755C, only occurs after oxidation of these compounds [29]. The investigators suggested that the superoxide or hydrogen peroxide may mediate LO inactivation.

In a similar fashion, 12-LO inactivation by anthralin may be related to the generation of oxygen radicals by this drug. Inhibition of 12-LO by anthralin cannot be explained by the formation of its stable oxidation product, danthron, because this compound was not able to inhibit 12-HETE production at doses up to 30 μM. Although the anthralin dimer is a comparatively potent inhibitor of 12-LO, this degradation product is only an intermediate in the autoxidation of anthralin and is further decomposed to the so-called anthralin-brown [7]. Nonetheless, this dimer has been described to generate oxygen radicals, as does anthralin [30].

The suggestion that anthralin can reduce the active enzyme to its inactive form, according to a proposed mechanism for phenolic compounds [12], might be sufficient to explain its inhibitory effect on the epidermal 12-LO (Fig. 4, pathway a). However, our data also suggest that active oxygen species may be involved (Fig. 4, pathway b), because we observed critical and suppressing effects on anthralin-mediated inactivation of 12-LO by sodium benzoate, a specific hydroxyl radical scavenger [18], by the antioxidants BHT and β-carotene, and by the antioxidant enzymes superoxide dismutase and catalase, which can remove superoxide and hydrogen peroxide. A similar protective role of these antioxidants on other oxidative systems triggered by anthralin has already been observed [8, 9]. A direct effect of the antioxidants on the anthralin molecule can be excluded

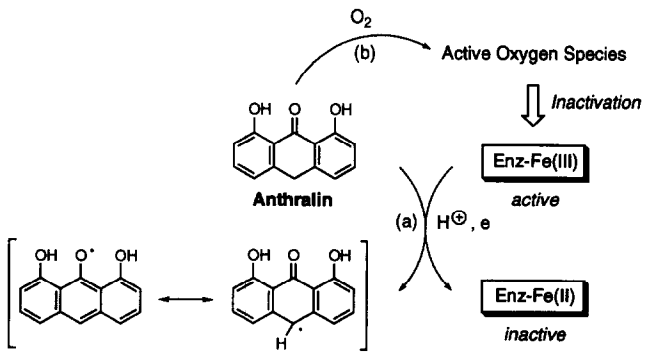

FIG. 4. Suggested mechanisms for inactivation of 12-LO by anthralin: (a) one-electron transfer from anthralin to the active ferric form of 12-LO results in an inactivated enzyme (ferrous form); (b) one-electron transfer from anthralin to oxygen results in the formation of active oxygen species ($O_2^{\cdot-}$, $^{\cdot}OH$, H_2O_2) which may inactivate the enzyme either by reduction to the ferrous form ($O_2^{\cdot-}$-mediated) or, alternatively, by oxidation of amino acid residues at the active site ($^{\cdot}OH$-mediated).

because various oxygen radical scavengers or the chain-breaking antioxidant BHT did not influence its decomposition [31]. The redox potential of the anthralin radical/anthralin couple is −0.76 V [32]. Thus, for the regeneration of anthralin from anthralin radical to proceed, which would be necessary to efficiently block its further decomposition, a redox potential <-0.76 V is required. However, most antioxidants do not satisfy this condition [33].

Catalase decomposes hydrogen peroxide to molecular oxygen and water, whereas peroxidases usually catalyze a one-electron oxidation of substrates to free radicals [34], and the product of peroxidase-catalyzed reaction is superoxide [35], which may subsequently inactivate 12-LO. This feature would account for the modest effect of peroxidase as compared to catalase and, also, for the inhibitory effect against 12-LO by the enzyme itself.

Pretreatment of mouse epidermal homogenate with the catalase, sodium benzoate, BHT, or α-tocopherol resulted in slight increases in 12-LO activity in the range of 120–130% over control, depending upon the antioxidant used, suggesting that oxygen radicals generated during arachidonic acid metabolism may inactivate the enzyme. Indeed, soybean lipoxygenase has been described to catalyze the breakdown of polyunsaturated fatty acid hydroperoxides to generate superoxide, peroxyl, and hydroxyl radicals [36, 37].

The question as to the nature of the reactive oxygen metabolite still remains open. Hydroxyl radical, superoxide, and hydrogen peroxide are all plausibly involved in the inactivation of 12-LO by anthralin, as evidenced by the effects of sodium benzoate, SOD, and catalase. Even the singlet oxygen quencher β-carotene [38] suppressed enzyme inactivation by anthralin. However, β-carotene can also function as an antioxidant in systems where singlet oxygen is not present [39]. In further support of this, reports that anthralin is only a weak sensitizer of singlet oxygen [40, 41] as well as an efficient quencher of this species [42] rather exclude singlet oxygen as an important intermediate in 12-LO inactivation.

In general, much of the biological action done by superoxide and hydrogen peroxide is thought to be due to their conversion into hydroxyl radical [43]. Thus, the susceptibility of metalloproteins to hydrogen peroxide was thought to be due to its conversion to hydroxyl radicals, site-specifically generated *via* a Fenton-type reaction [44]. If the highly reactive hydroxyl radical is generated in such a fashion at the iron-binding site of 12-LO, which is the active site of the enzyme [45], it may cause inactivation through oxidation of amino acid residues important for enzyme activity [29]. This would be expected to be irreversible if these residues are destroyed. However, it is more than conceivable that essential thiol groups are reversible (RSH to RS· or formation of disulphides). Also, in some instances, when protein radicals are formed at a specific amino acid site, they can be rapidly transferred to other sites within the protein infrastructure from methionine to cysteine *via* tryptophan and tyrosine [46]. Reactivation of 12-LO would also explain retention of activity for longer times. Alternatively,

one can envisage a mechanism such as reduction of the ferric iron at the active site of 12-LO by superoxide to the catalytically inactive ferrous form, because ferric iron and even chelated ferric iron may be reduced by superoxide [47]:

$$\text{Enzyme-Fe}^{3+}\ \text{(active)} + O_2^{\cdot -} \rightarrow \text{Enzyme-Fe}^{2+}\ \text{(inactive)} + O_2$$

Inhibition of 12-LO by oxygen radicals generated from anthralin does not necessarily exclude a specific interaction between the drug and 12-LO, because oxygen radicals may be generated at a critical site for enzyme inactivation. In this context, a plausible explanation for the lack of 12-LO protection by α-tocopherol, which efficiently inhibited anthralin-induced lipid peroxidation in model membranes [9], is to consider the inaccessibility of this molecule to the active site where oxygen radicals may be formed.

In summary, our data strongly support a role for active oxygen species as mediators of 12-LO inactivation by anthralin because protection against enzyme inactivation was accomplished by specific scavengers of these species and antioxidant enzymes.

The excellent technical assistance of K. Ziereis is gratefully acknowledged.

References

1. Ruzicka T, Eicosanoids in psoriasis. *J Dermatol Sci* **1:** 415–424, 1990.
2. Opas EE, Argenbright LW and Humes JL, An enzymatic method for distinguishing the stereoisomers of 12-hydroxyeicosatetraenoic acid in human epidermis and psoriatic scale. *Br J Dermatol* **120:** 49–58, 1989.
3. Müller K, 5-Lipoxygenase and 12-lipoxygenase: attractive targets for the development of novel antipsoriatic drugs. *Arch Pharm (Weinheim, Ger)* **327:** 3–19, 1994.
4. Musser JH and Kreft AF, 5-Lipoxygenase: properties, pharmacology, and the quinolinyl(bridged)aryl class of inhibitors. *J Med Chem* **35:** 2501–2524, 1992.
5. Müller K and Gawlik I, Novel 10-substituted antipsoriatic anthrones as inhibitors of epidermal 12-lipoxygenase and lipid peroxidation in membranes. *Biochem Pharmacol* **50:** 2077–2083, 1995.
6. Müller K, Gürster D, Piwek S and Wiegrebe W, Antipsoriatic anthrones with modulated redox properties. 1. Novel 10-substituted 1,8-dihydroxy-9(10*H*)-anthracenones as inhibitors of 5-lipoxygenase. *J Med Chem* **36:** 4099–4107, 1993.
7. Cavey D, Caron J-C and Shroot B, Anthralin: chemical instability and glucose-6-phosphate dehydrogenase inhibition. *J Pharm Sci* **71:** 980–983, 1982.
8. Müller K and Kappus H, Hydroxyl radical formation by dithranol. *Biochem Pharmacol* **37:** 4277–4280, 1988.
9. Müller K and Gürster D, Hydroxyl radical damage to DNA sugar and model membranes induced by anthralin (dithranol). *Biochem Pharmacol* **46:** 1695–1704, 1993.
10. Bruneau P, Delvare C, Edwards MP and McMillan RM, Indazolinones, a new series of redox-active 5-lipoxygenase inhibitors with built-in selectivity and oral activity. *J Med Chem* **34:** 1028–1036, 1991.
11. Thody VE, Buckle DR and Foster KA, Studies on the antioxidant activity of 5-lipoxygenase inhibitors. *Biochem Soc Trans* **15:** 416–417, 1987.
12. Kemal C, Louis-Flamberg P, Krupinski-Olsen R and Shorter AL, Reductive inactivation of soybean lipoxygenase 1 by catechols: a possible mechanism for regulation of lipoxygenase activity. *Biochemistry* **26:** 7064–7072, 1987.
13. Laughton MJ, Evans PJ, Moroney MA, Hoult JRS and Halliwell B, Inhibition of mammalian 5-lipoxygenase by flavonoids and phenolic dietary additives. *Biochem Pharmacol* **42:** 1673–1681, 1991.
14. Borgeat P and Samuelsson B, Arachidonic acid metabolism in polymorphonuclear leukocytes: effects of ionophore A23187. *Proc Natl Acad Sci USA* **76:** 2148–2152, 1979.
15. Metzger K, Angres G, Maier H and Lehmann WD, Lipoxygenase products in human saliva: patients with oral cancer compared to controls. *Free Radical Biol Med* **18:** 185–194, 1995.
16. Clairborne A, Catalase activity. In: *CRC Handbook of Methods for Oxygen Radical Research* (Ed. Greenwald, RA), pp. 283–284, CRC Press, Inc., Boca Raton, FL, 1985.
17. Marklund S and Marklund G, Involvement of the superoxide anion radical in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase. *Eur J Biochem* **47:** 469–474, 1974.
18. Winston GW and Cederbaum AI, Decarboxylation of 7-^{14}C-benzoic acid. In: *Handbook of Methods for Oxygen Radical Research* (Ed. Greenwald, RA), pp. 169–175, CRC Press, Inc., Boca Raton, FL, 1985.
19. Solanki V, Rana RS and Slaga TJ, Diminution of mouse epidermal superoxide dismutase and catalase activities by tumor promoters. *Carcinogenesis* **2:** 1141–1146, 1981.
20. Müller K, Wiegrebe W and Younes M, Formation of active oxygen species by dithranol, III. Dithranol, active oxygen species and lipid peroxidation in vivo. *Arch Pharm (Weinheim, Ger)* **320:** 59–66, 1987.
21. Halliwell B and Gutteridge JMC, Role of free radicals and catalytic metal ions in human disease: an overview. *Methods Enzymol* **186:** 1–85, 1990.
22. Halliwell B and Gutteridge JMC, *Free Radicals in Biology and Medicine* 2nd Ed. Clarendon Press, Oxford, 1989.
23. Müller K, Leukel P, Mayer KK and Wiegrebe W, Modification of DNA bases by anthralin and related compounds. *Biochem Pharmacol* **49:** 1607–1613, 1995.
24. Bedord CJ, Young JM and Wagner BM, Anthralin inhibition of mouse epidermal arachidonic acid lipoxygenase in vitro. *J Invest Dermatol* **81:** 566–571, 1983.
25. Kühn H, Holzhütter H-G, Schewe T, Hiebsch C and Rapoport SM, The mechanism of inactivation of lipoxygenase by acetylenic fatty acid. *Eur J Biochem* **139:** 577–583, 1984.
26. Galey J-B, Bombard S, Chopard C, Girerd J-J, Lederer F, Thang D-C, Nam N-H, Mansuy D and Chottard J-C, Hexanal phenylhydrazone is a mechanism-based inactivator of soybean lipoxygenase 1. *Biochemistry* **27:** 1058–1066, 1988.
27. Müller K and Ziereis K, Effects of oxygen radicals, hydrogen peroxide and water-soluble singlet oxygen carriers on 5- and 12-lipoxygenase. *Arch Pharm (Weinheim, Ger)* **326:** 819–821, 1993.
28. Sumiya T, Fujimoto Y, Nishida H, Morikawa Y, Sakuma S and Fujita T, Effects of reactive oxygen species on arachidonic acid metabolism in rabbit platelets. *Free Radical Biol Med* **15:** 101–104, 1993.
29. Cucurou C, Battioni JP, Thang DC, Nam NH and Mansuy D, Mechanisms of inactivation of lipoxygenases by phenidone and BW755C. *Biochemistry* **30:** 8964–8970, 1991.
30. Hayden PJ, Free KE and Chignell CF, Structure-activity relationships for the formation of secondary radicals and inhibition of keratinocyte proliferation by 9-anthrones. *Mol Pharmacol* **46:** 186–198, 1994.
31. Müller K, Gawlik I and Wiegrebe W, Acidity and stability of 10-substituted 1,8-dihydroxy-9(10H)-anthracenones. *Arch Pharm (Weinheim, Ger)* **328:** 359–362, 1995.

32. Halmekoski J, Vilvala R and Mustakallio KK, Polarographic studies on dithranol and its 10-acyl analogs. In: *Psoriasis* (Eds. Farber EM, Cox AJ, Nall L and Jacobs PH), pp. 381–382, Grune & Stratton, New York, 1982.
33. Buettner GR, The pecking order of free radicals and antioxidants: lipid peroxidation, α-tocopherol, and ascorbate. *Arch Biochem Biophys* **300:** 535–543, 1993.
34. O'Brien PJ, Radical formation during the peroxidase catalyzed metabolism of carcinogens and xenobiotics: the reactivity of these radicals with GSH, DNA, and unsaturated lipid. *Free Radical Biol Med* **4:** 169–183, 1988.
35. Afanas'ev IB, *Superoxide Ion: Chemistry and Biological Implications*. CRC Press, Inc., Boca Raton, FL, 1989.
36. Chamulitrat W and Mason RP, Lipid peroxyl radical intermediates in the peroxidation of polyunsaturated fatty acids by lipoxygenase. *J Biol Chem* **264:** 20968–20973, 1989.
37. Chamulitrat W, Hughes MF, Eling TE and Mason RP, Superoxide and peroxyl radical generation from the reduction of polyunsaturated fatty acid hydroperoxides by soybean lipoxygenase. *Arch Biochem Biophys* **290:** 153–159, 1991.
38. Foote CS and Denny RW, Chemistry of singlet oxygen. VII. Quenching by β-carotene. *J Am Chem Soc* **90:** 6233–6235, 1968.
39. Burton GW and Ingold KU, β-Carotene: an unusual type of lipid antioxidant. *Science* **224:** 569–573, 1984.
40. Müller K, Eibler E, Mayer KK, Wiegrebe W and Klug G, Dithranol, singlet oxygen and unsaturated fatty acids. *Arch Pharm (Weinheim, Ger)* **319:** 2–9, 1986.
41. Dabestani R, Hall RD, Sik RH and Chignell CF, Spectroscopic studies of cutaneous photosensitizing agents—XV. Anthralin and its oxidation product 1,8-dihydroxyanthraquinone. *Photochem Photobiol* **52:** 961–971, 1990.
42. Müller K, Kanner RC and Foote CS, Kinetic studies on anthralin photooxidation. *Photochem Photobiol* **52:** 445–450, 1990.
43. Halliwell B, Antioxidant characterization. Methodology and mechanism. *Biochem Pharmacol* **49:** 1341–1348, 1995.
44. Stadtman ER, Metal ion-catalyzed oxidation of proteins: biochemical mechanism and biological consequences. *Free Radical Biol Med* **9:** 315–325, 1990.
45. Kroneck PMH, Cucurou C, Ullrich V, Ueda N, Suzuki H, Yoshimoto T, Matsuda S and Yamamoto S, Porcine leukocyte 5- and 12-lipoxygenases are iron enzymes. *FEBS Lett* **287:** 105–107, 1991.
46. Rice-Evans C, Free radicals and antioxidants in normal and pathological processes. In: *Oxidative Stress, Lipoproteins and Cardiovascular Dysfunction* (Eds. Rice-Evans, C and Bruckdorfer, KR), pp. 1–32, Portland Press, London, 1995.
47. Halliwell B and Gutteridge JMC, Role of iron in oxygen radical reactions. *Methods Enzymol* **105:** 47–56, 1984.