Biochemical Pharmacology, Vol. 53, pp. 1215–1221, 1997.

ISSN 0006-2952/97/$17.00 + 0.00
PII S0006-2952(96)00732-0

COMMENTARY

Antipsoriatic and Proinflammatory Action of Anthralin

IMPLICATIONS FOR THE ROLE OF OXYGEN RADICALS

*Klaus Müller**

INSTITUTE OF PHARMACEUTICAL CHEMISTRY, UNIVERSITY OF MÜNSTER, D-48149 MÜNSTER, GERMANY

ABSTRACT. Anthralin is among the most effective agents for the topical treatment of psoriasis. However, this drug causes unpleasant side-effects such as inflammation and staining of the nonaffected skin surrounding a psoriatic lesion. The biochemical basis for the induction of an inflammatory response in the skin and the antipsoriatic effectiveness are uncertain, although several cellular targets of anthralin action have been identified. Because no single mechanism is operative, the view was taken that all the effects exerted by anthralin are caused by its redox activity leading to the generation of anthralin free radicals and oxygen radicals. Clear relationships between oxygen-radical production by anthralin and biological response are evident with respect to chemical lesions in cellular macromolecules such as DNA, lipid membranes, and enzymes, indicating that these species account for the antipsoriatic and proinflammatory effects elicited by anthralin. This poses new challenges for the medicinal chemist and provides impetus for identifying novel compounds having potential for an improved therapeutic index. BIOCHEM PHARMACOL 53;9:1215–1221, 1997. © 1997 Elsevier Science Inc.

KEY WORDS. anthralin; antiproliferative action; dithranol; free radicals; oxygen radicals; psoriasis; skin inflammation

Psoriasis is a common, inflammatory, and hyperproliferative skin disease, mainly characterized by abnormal keratinocyte proliferation and differentiation, accumulation of polymorphonuclear leukocytes in the skin, and T-cell activation [1]. However, the etiology of this very distressing skin disorder is unknown. The treatment for psoriasis is targeted at both the inflammatory and hyperproliferative aspect of the disease.

Anthralin (dithranol, 1,8-dihydroxy-9(10*H*)-anthracenone) is among the most effective agents for the topical treatment of psoriasis. While many therapeutics are targeted towards a single feature of the disease, all psoriatic features are resolved following topical therapy with anthralin. However, like most forms of current therapy of psoriasis, the benefit of anthralin is limited by its undesirable inflammatory effects on the skin or poor acceptability because of staining of the skin and clothing [2]. Consequently, efforts are being made to understand the molecular basis of the proinflammatory and staining properties so that anthralin may be modified structurally to remove these unpleasant effects.

Substantial evidence has been given that anthralin exhibits several parallel effects on susceptible target molecules which include certain enzymes, nucleic acids, and lipid membranes [2, 3]. Because of the chemical instability of anthralin [4], it is unlikely that the physicochemical interactions between anthralin and these biological targets provide the molecular basis for the antipsoriatic and proinflammatory effects of the drug. Also, the known metabolites, danthron and the anthralin dimer, are not effective against psoriasis. Rather, activated species that are formed during the autoxidation of anthralin may react with sensitive macromolecules and bring about changes in cellular function. In recent studies, it has been possible to elucidate the exact nature of the chemical species that account for the biochemical pharmacology of anthralin and to examine their interaction with potential biological targets. Anthralin can activate molecular oxygen by one-electron reduction to the superoxide anion radical ($O_2^{\cdot-}$), which, in turn, can be converted to more powerful species [5].

The role of oxygen radicals in human disease has become an area of intense interest [6–8]. Low concentrations of oxygen radicals are constantly formed as physiological byproducts in the human body, but they can be toxic when generated in excess. This toxicity can be of therapeutic interest, depending on the nature of the target cell. The purpose of this commentary is to summarize briefly the status of the current knowledge pointing toward the significance of oxygen-radical generation in anthralin action and induction of skin inflammation, and the application of this information to future developments of novel derivatives.

* Correspondence: Tel. 49-251-8333324; FAX 49-251-8333310.

GENERATION OF ANTHRALIN FREE RADICALS

Detection of Primary and Secondary Radicals

An ESR signal, presumed to be that of the trapped anthralin radical, was first reported by Martinmaa *et al.* [9] during autoxidation of anthralin in pyridine. Davies *et al.* [10] also reported the direct observation of an ESR signal assigned as that of the 1,8-dihydroxy-9-anthron-10-yl radical (Fig. 1). When anthralin was applied topically to the ear of a pig, the generation of a persistent free radical was observed directly in biopsy samples [11]. The ESR data observed corresponded to data reported *in vitro* for the primary anthralin radical.

Fuchs and Packer have reported that the ESR signal of anthralin-derived radicals in the skin of hairless mice does not correspond to the 1,8-dihydroxy-9-anthron-10-yl radical [12], but rather to products derived from the so-called anthralin-brown. This final oxidation product of anthralin is not well characterized chemically and is responsible for the darker shades of brown staining that occurs on the skin and clothes [2]. Low-frequency ESR studies provided direct evidence that anthralin generated secondary radicals *in vivo* in the skin of hairless mice, and it is noteworthy that the applied dose was in the therapeutic range [13].

Lack of Support for a Role of Anthralin Radical in the Mechanism of Action

However, several observations provide no support for the proposed importance of the pertinent skin radical in the processes leading to antipsoriatic activity or inflammation of the skin. First, in the study of Davies *et al.* [10], the anthralin dimer was found to be equally as good as anthralin itself as a source of the primary anthralin radical. If the anthralin free radical is the pharmacologically active species that alleviates psoriasis, then the anthralin dimer, which is not effective against psoriasis, might be expected to produce the same clinical effects. Second, radical scavengers do not influence the formation of the radical derived from anthralin in pig skin [14], whereas they inhibit anthralin inflammation of human forearm skin [15]. Thus, a link between anthralin-radical generation following topical application of anthralin and its proinflammatory property may be excluded. Third, the properties of anthralin radical have also been studied by pulse radiolysis, and it has been shown that it is a particularly stable entity that is insensitive to a vitamin E model and reacts only slowly with oxygen [16]. In further support of this, the stability of anthralin free radicals in skin [12] indicates a low reactivity of these species toward cellular target molecules and suggests that other, more reactive species derived from anthralin are responsible for mediating the biological effects of anthralin. Finally, spin-trapping experiments showed that therapeutically inactive anthrones can also form 9-anthron-10-yl radicals in aqueous buffers [17]. These observations indicate that the ability to generate these radicals is not an exclusive property of the antipsoriatic anthralin.

GENERATION OF REACTIVE OXYGEN SPECIES

During the autoxidation of anthralin anion, molecular oxygen is reduced in a non-enzymatic, univalent reduction pathway that results in the generation of several reactive intermediates. The initial species formed is $O_2^{\cdot -}$, which has been shown under physiological conditions by chemical and biochemical assays [18] and by ESR studies [19]. It is interesting to note that the direct reaction of molecular oxygen with an organic molecule occurs at significant rates only in the presence of a catalyst, such as a transition metal [20]. For a direct one-electron transfer to proceed, the reactant other than molecular oxygen must have a redox potential <-0.16 V, based on the redox potential of the $O_2/O_2^{\cdot -}$ couple (-0.16 V) [20]. With a redox potential of -0.76 V at pH 6.6 and even more strongly negative at higher pH values [21], anthralin is a potent reductant and satisfies this condition. As a consequence, $O_2^{\cdot -}$ generation from anthralin is thermodynamically favored.

Although $O_2^{\cdot -}$ is not very reactive *per se*, it is the precursor of a number of more reactive species. Under physiological conditions, it spontaneously dismutates to hydrogen peroxide (H_2O_2), which is not a radical because all outer shell electrons are paired. Unlike $O_2^{\cdot -}$, H_2O_2 easily diffuses through biological membranes. Both species can find some targets within cells at which they can do direct damage, but on the whole their reactivity is limited [22].

However, $O_2^{\cdot -}$ and H_2O_2 formed by autoxidation of anthralin give rise by an iron-catalyzed reaction (Haber–Weiss reaction) to the highly reactive hydroxyl radical ($^{\cdot}OH$). Evidence for the formation of $^{\cdot}OH$ by anthralin was given by degradation of 2-ketothiomethylbutyric acid to ethene [23], and, more specifically, by degradation of deoxyribose to malondialdehyde [24]. The results were later assessed by ESR spectroscopic studies [25].

Singlet oxygen ($^1O_2$), which is not a radical, is not produced by redox reactions but by absorption of light. Photosensitized generation of $^1O_2$ by anthralin has been determined by several indirect methods [26] and by direct detection of $^1O_2$ luminescence [27]. Molecular oxygen, which contains two unpaired electrons with parallel spin, is unable to react with biomolecules directly because the acceptance of a pair of anti-parallel electrons is restricted in the ground state and requires an electron spin inversion. By contrast, $^1O_2$ with its anti-parallel valence electron spins has no such spin restriction on reactivity. However, the role of $^1O_2$ in skin-photosensitization reactions has not yet been unequivocally assigned. The demonstration that the deprotonated anthralin interacts with $^1O_2$ with higher rates of reaction and quenching than those of important biological targets for oxidative damage suggests that anthralin anion should be preferentially oxidized [28]. On the other hand, there are other clinically interesting photosensitizers in the treatment of psoriasis that can produce $^1O_2$, such as coal tar, which contains sensitizing hydrocarbons and psoralens [e.g., psoralen + UVA (PUVA) therapy].

Figure 1 gives a summary of the pathways for the gen-

eration of primary and secondary radicals and reactive oxygen species from anthralin. The term "oxygen radicals" denotes $O_2^{\cdot-}$ and $^{\cdot}OH$, whereas "reactive oxygen species" also includes non-radical species such as H_2O_2 and $^1O_2$ [29].

CELLULAR FACTORS THAT MODULATE AND FACILITATE THE SUSCEPTIBILITY OF THE SKIN TO OXIDATIVE DAMAGE

The skin possesses a number of protective mechanisms against oxidative damage [30, 31]. The major defense mechanisms rely on enzymatically maintaining $O_2^{\cdot-}$ and H_2O_2 levels by superoxide dismutase and thioredoxin reductase or catalase and glutathione peroxidases, respectively [30–32]. Low molecular weight antioxidants such as vitamin A, α-tocopherol, ascorbic acid, and glutathione, which serve to interrupt free radical chain reactions, provide another layer of defense [31, 32].

Accordingly, this enzymatic defensive system is able to remove reactive oxygen species under normal circumstances. However, a number of factors may promote the susceptibility of human skin to oxidative damage, which is a function of the overall balance between the factors that exert prooxidant action and those that exhibit antioxidant capability. Therefore, oxidative injury of the skin can be described as a consequence of insufficient antioxidant potential or excessive local production of prooxidants that may overwhelm the protective mechanisms. Of particular interest, the activity of superoxide dismutase is decreased in psoriasis [33, 34]. Moreover, anthralin inhibits the antioxidant enzymes catalase and superoxide dismutase [35]. Furthermore, it can prime human neutrophils in inflammatory infiltrates to generate increased amounts of reactive oxidants [36].

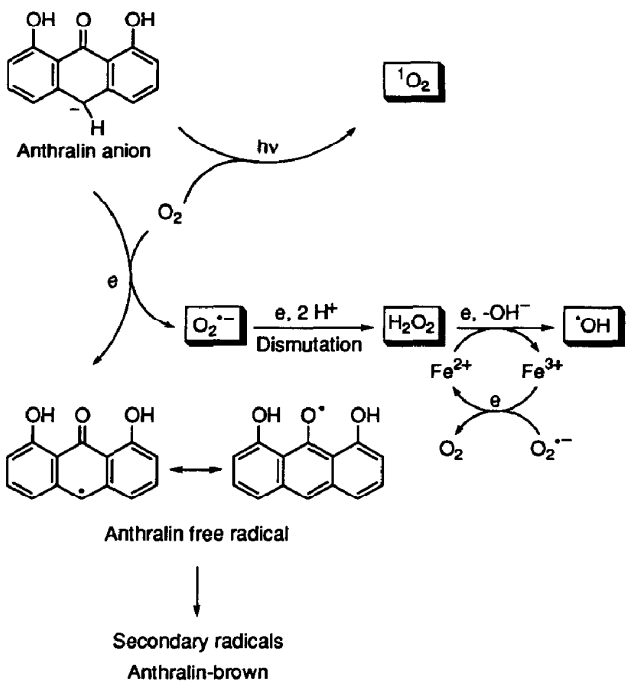

FIG. 1. Activation of anthralin to radical intermediates and concomitant generation of reactive oxygen species. Electron transfer from the anthralin anion to molecular oxygen results in the formation of $O_2^{\cdot-}$. Further steps of univalent reduction lead to H_2O_2 and, by an iron-catalyzed reaction to $^{\cdot}OH$. Energy transfer from the excited anthralin anion to molecular oxygen yields $^1O_2$.

On the other hand, the fact that psoriatic epidermis appears to be deficient in defense mechanisms against oxygen radicals, relative to healthy skin, may also increase the selective cytotoxicity of anthralin to psoriatic tissue. In addition, oxygen consumption of psoriatic lesions is about 2-fold greater than that of the uninvolved skin [37]. However, in the presence of anthralin, a significant reduction in oxygen consumption of human skin was observed, especially when exposed to UV irradiation [37], suggesting increased generation of oxygen radicals and $^1O_2$ in psoriatic skin as compared with healthy skin. In further support of this, skin exhibits a certain degree of iron turnover and is a significant site of iron excretion, which is increased in psoriasis [31]. This may further increase the concentration of highly reactive species in psoriatic tissue, as iron catalyzes the generation of $^{\cdot}OH$ by anthralin from $O_2^{\cdot-}$ and H_2O_2.

Also worth noting at this point is the fact that the intensity of anthralin inflammation in psoriatic lesions is reduced as compared with uninvolved skin [38]. This may be rationalized by the hyperactive thioredoxin reductase in psoriatic lesions compared with activity in uninvolved skin [39], because in contrast to superoxide dismutase this membrane-associated enzyme is able to remove oxygen radicals outside the cell [30].

Figure 2 summarizes the antioxidant defensive systems present within the skin and the factors that facilitate the susceptibility of the skin to anthralin-mediated oxidative damage.

OXYGEN RADICALS IN ANTHRALIN ACTION AND LESIONS THEY CAUSE IN CELLULAR MACROMOLECULES

Oxygen radicals are constantly formed as byproducts of various electron-transfer processes and may serve useful physiological functions, but they can be highly destructive if produced in excess. In the human body, there is a balance between prooxidant production and removal by antioxidants. When this balance is inclined in favor of the former, the state of oxidative stress results. Oxygen radicals have been implicated in the occurrence of cell damage in several pathological circumstances [6, 7]. Likewise, the enhancement of intracellular levels of these species has often been proposed as one of the mechanisms of cytotoxicity of a variety of drugs [40]. When rat epidermal keratinocytes were treated with anthralin, $O_2^{\cdot-}$ generation was detected both extracellularly and intracellularly and was inhibited by the addition of superoxide dismutase [41]. Evidence that anthralin-mediated cell injury is linked to oxidative stress

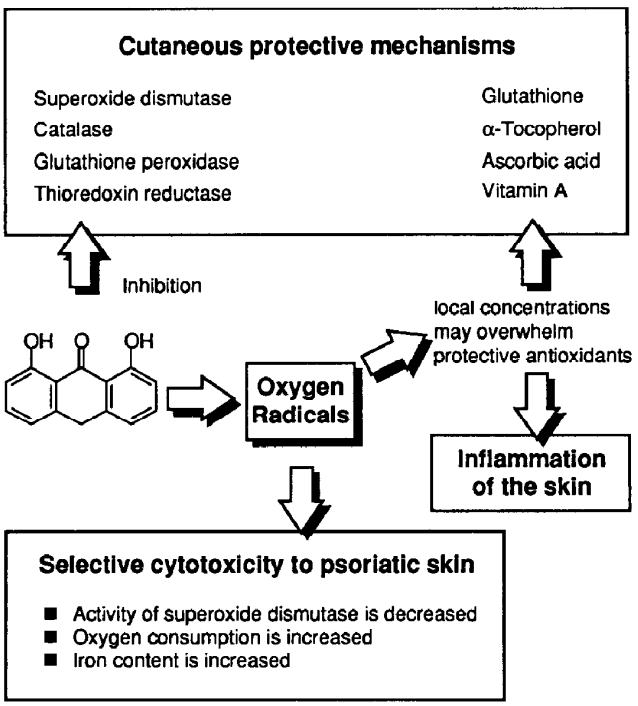

FIG. 2. Oxygen-radical defense in the skin and factors that promote selective cytotoxicity of anthralin towards psoriatic skin.

was provided by the protective effects by concurrent treatment of the cells with superoxide dismutase and catalase.

However, which target molecules are affected by anthralin, and are the same targets also affected by the active species involved? In principle, DNA, carbohydrates, lipids, and proteins have all been studied for sensitivity to oxidative modification by oxygen radicals [42], and these same cellular targets have been implicated in the mode of action of anthralin [2].

DNA

As DNA plays a central role in information transfer, it is an attractive drug target. Attention has focused on oxidative damage that can occur by direct reaction of $^{\cdot}OH$ with DNA [43]. There are two main modes of DNA attack: hydrogen abstraction from deoxyribose and addition to the π-bond of DNA bases. The former ultimately leads to sugar fragmentation, base loss, and strand fragmentation, while the latter results in damaged bases [44].

Damage to deoxyribose stimulated by anthralin was documented by the release of malondialdehyde, which was inhibited substantially by iron-binding $^{\cdot}OH$ scavengers, iron chelators, and the antioxidant enzymes superoxide dismutase and catalase, implicating the involvement of $^{\cdot}OH$ via the Fenton reaction [24]. Calf thymus DNA treated with anthralin also resulted in the modification of DNA bases, as studied by the use of the highly sensitive technique of GC–MS with selected ion monitoring [45]. Although multiple oxidative lesions are possible in DNA, 8-hydroxylation of the guanine base was one of the most abundant observed after $^{\cdot}OH$ attack by anthralin, along with 8-hydroxyadenine and formamidopyrimidine compounds [45]. These results suggest that $^{\cdot}OH$ attack on DNA sugars or bases is responsible for the induction of DNA strand breaks [46] and inhibition of DNA replication [47] by anthralin. In support of this, catalase significantly protected leukocytes from anthralin-induced DNA strand breaks, implicating H_2O_2 or $^{\cdot}OH$ as the damaging agent [48].

Lipid Membranes

Some of the primary targets of oxygen radicals are the lipids that constitute the cell membrane. Unsaturated phospholipids of biological membranes are highly vulnerable to peroxidation [49], initiated by $^{\cdot}OH$ attack at the allylic hydrogens [49]. Moreover, $^1O_2$ can accelerate lipid peroxidation by direct reaction with unsaturated fatty acyl moieties to give hydroperoxides with double bonds shifted to the allylic position [50]. This type of reaction has already been demonstrated for anthralin [26]. Furthermore, many reactive oxygen species are more soluble in a lipid environment than in aqueous systems and can readily cross biological membranes [42, 49].

Anthralin slightly increases lipid peroxidation in mouse skin after topical application [18], based on the exhalation of ethane. In addition, it was found to act as a prooxidant in peroxidation of bovine brain phospholipid liposomes [24]. When the iron-chelator desferrioxamine or chain-breaking antioxidants were included in the system, peroxidation was strongly decreased, indicating that $^{\cdot}OH$, peroxyl radicals, or alkoxyl radicals are involved. Because mitochondrial membranes are sensitive to damage resulting from the peroxidation of membrane phospholipids [51], some of the effects of anthralin on mitochondrial structure and biochemical function may be mediated by lipid peroxidation. Anthralin is an inhibitor of mitochondrial membrane functions [52], causes dramatic structural changes in mitochondria [53], and influences redox properties of energy transducing membranes [54], all of which may contribute to its antiproliferative activity.

Proteins

Proteins may be directly damaged through specific interactions of oxygen radicals with particularly susceptible amino acids crucial for protein function, resulting in global modification of many different amino acid residues and extensive fragmentation [55]. Enzyme oxidation can lead to a loss of critical sulfhydryl groups in addition to modification of amino acids such as ring-opening of histidine or tryptophan. These reactions can cause a modification of the primary, secondary, and tertiary structures of protein and may damage reactive prosthetic groups, which can result in degradation and loss of function [56].

Anthralin has been identified as a selective 12-lipoxygenase inhibitor with respect to cyclooxygenase as

well as 5-lipoxygenase [57, 58]. One might expect that anthralin reduces the active enzyme to its inactive form, according to a proposed mechanism for phenolic compounds [59]. However, we have demonstrated critical and suppressing effects on anthralin-mediated inactivation of epidermal 12-lipoxygenase by sodium benzoate, a specific 'OH scavenger, by the antioxidants 2,6-di-*tert*-butyl-4-methylphenol and β-carotene, and by the antioxidant enzymes superoxide dismutase and catalase [35]. Our results strongly suggest a role of oxygen radicals in epidermal 12-lipoxygenase inhibition either by enzyme inactivation through oxidation of amino acid residues important for enzyme activity or by reduction of the ferric iron at the active site of 12-lipoxygenase by $O_2^{\cdot-}$ to the catalytically inactive ferrous form, because ferric iron and even chelated ferric iron may be reduced by $O_2^{\cdot-}$ [60]. Inhibition of 12-lipoxygenase by oxygen radicals generated from anthralin does not necessarily exclude a specific interaction between the drug and 12-lipoxygenase, because oxygen radicals may be generated at a critical site for enzyme inactivation.

In a recent study, anthralin was described as a novel inducer of the transcription factor NF-κB [61], which can activate inflammatory genes in response to oxygen radicals [62]. The activity of the drug was related to its ability to produce oxygen radicals as messengers of NF-κB activation, supported by the observations that antioxidants inhibited, and keratinocytes overexpressing catalase significantly reduced, NF-κB activation [61].

Taken together, depending upon the proximity of anthralin to a biomolecule oxygen, radicals can give rise to DNA damage, lipid peroxidation, or modification of proteins (Fig. 3). The cumulative effects of such a cascade of oxygen radical-induced modification of cellular macromolecules may be an impairment of important cellular functions resulting in inhibition of cell growth. Furthermore, because oxygen radicals may act as signaling molecules [62],

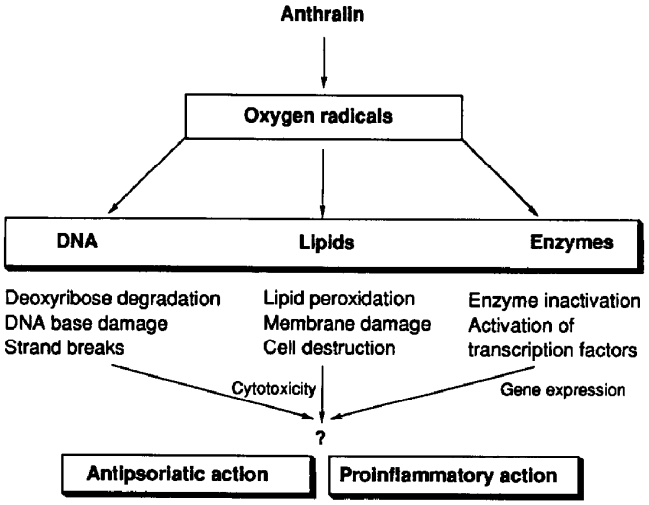

FIG. 3. Activation of anthralin with oxygen-radical generation and interactions that are currently known regarding the involvement of oxygen radicals in the mechanism of antipsoriatic and proinflammatory action of anthralin.

oxygen-radical generation by anthralin can activate transcription factors and may regulate gene expression and control cell proliferation.

OXYGEN RADICALS AND PROINFLAMMATORY ACTION OF ANTHRALIN

With the demonstration that anthralin can stimulate lipid peroxidation significantly *in vitro*, the question arises as to whether its proinflammatory action to the skin *in vivo* can be traced to a reactive-oxygen-dependent peroxidation process. The surface of the skin is especially vulnerable to damaging oxygen radicals because of its lipid-rich membranes. As a consequence, the mechanisms described above concerning the production of oxygen radicals by anthralin may provide a basis for its dose-related production of skin inflammation, aside from its potential therapeutical value.

There is now both direct and indirect evidence implicating oxygen radicals in many inflammatory skin diseases [31]. A free radical mechanism for the skin toxicity of anthralin is suggested by the ability of topically applied free radical scavengers such as α-tocopherol, retinol acetate, and butylated hydroxyanisol to inhibit anthralin inflammation of forearm skin [15]. Also, α-tocopherol effectively and persistently inhibited anthralin-induced skin irritation in the mouse ear model [63]. The role of extracellularly generated $O_2^{\cdot-}$ in anthralin-evoked ear swelling of mice is supported by the protective effect of intraperitoneally administered superoxide dismutase [64]. Similarly, intradermal injections of superoxide dismutase, the $O_2^{\cdot-}$ scavenger dihydrolipoate, and trolox, a water-soluble α-tocopherol derivative, have been reported to protect hairless mice against anthralin-induced skin inflammation [65]. Accordingly, oxygen radicals are the cause of most of the inflammatory responses associated with anthralin therapy, i.e. erythema and edema.

CONCLUSIONS AND FUTURE DIRECTIONS FOR RESEARCH

A major challenge for the medicinal chemist is the attempt to incorporate this information on anthralin action into more effective design of novel derivatives. Although oxygen radicals are responsible for the inflammation of the nonaffected psoriatic skin, the same species are central to the clinical efficacy of anthralin. Hence, it seems difficult to separate main effects from side effects. To minimize the potential of skin inflammation by anthralin, which is serious enough to prevent its use or reduce patient compliance [66], it is imperative to control and manipulate oxygen-radical generation. Strategies for overcoming these problems have been to modulate oxygen-radical generation by modifying the critical C-10 position of anthralin [67, 68]. Alternative approaches to the control of oxygen radical-formation would be to attach potential antioxidant substituents [69] or to chelate the iron that facilitates genera-

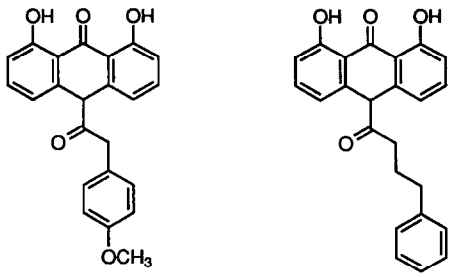

FIG. 4. Structures of novel ω-phenylacyl substituted anthralin derivatives.

tion.* As a result, several novel analogs are currently in preclinical antiinflammatory, antiproliferative, and toxicological studies. Figure 4 shows the 10-phenylbutyryl and the 10-methoxyphenylacetyl derivatives, which turned out to be the most effective representatives in other skin inflammation models and are now candidates for clinical trials.

In conclusion, anthralin evidently exerts multiple cellular effects. As a result of the detailed examination of the mechanism of action at the molecular level, the design and synthesis of antipsoriatic anthrones are being replaced on a less empirical and more rational basis. The systematic study of this important antipsoriatic agent has had the beneficial effect of stimulating the development of novel analogs that do not lead to skin inflammation.

References

1. Camisa C, *Psoriasis*. Blackwell Scientific Publishers, Oxford, 1994.
2. Kemény L, Ruzicka T and Braun-Falco O, Dithranol: A review of the mechanism of action in the treatment of psoriasis vulgaris. *Skin Pharmacol* **3:** 1–20, 1990.
3. Wiegrebe W and Müller K, Treatment of psoriasis with anthrones—Chemical principles, biochemical aspects, and approaches to the design of novel derivatives. *Skin Pharmacol* **8:** 1–24, 1995.
4. Cavey D, Caron J-C and Shroot B, Anthralin: Chemical instability and glucose-6-phosphate dehydrogenase inhibition. *J Pharm Sci* **71:** 980–983, 1982.
5. Müller K, Antipsoriatic anthrones: Aspects of oxygen radical formation, challenges and prospects. *Gen Pharmacol* (in press).
6. Halliwell B and Gutteridge JMC, Role of free radicals and catalytic metal ions in human disease: An overview. *Methods Enzymol* **186:** 1–85, 1990.
7. Gutteridge JMC, Free radicals in disease processes: A compilation of cause and consequence. *Free Radic Res Commun* **19:** 141–158, 1993.
8. 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.
9. Martinmaa J, Vanhala L and Mustakallio KK, Free radical intermediates produced by autoxidation of 1,8-dihydroxy-9-anthrone (dithranol) in pyridine. *Experientia* **34:** 872–873, 1978.
10. Davies AG, Hawari JA-A and Whitefield M, Generation and ESR spectrum of the 1,8-dihydroxy-9-anthrone-10-yl-radical. *Tetrahedron Lett* **24:** 4465–4468, 1983.
11. Shroot B and Brown C, Free radicals in skin exposed to dithranol and its derivatives. *Arzneimittelforschung* **36:** 1253–1255, 1986.
12. Fuchs J and Packer L, Investigations on anthralin free radicals in model systems and in skin of hairless mice. *J Invest Dermatol* **92:** 677–682, 1989.
13. Mäder K, Bacic G and Swartz M, *In vivo* detection of anthralin-derived free radicals in the skin of hairless mice by low-frequency electron paramagnetic resonance spectroscopy. *J Invest Dermatol* **104:** 514–517, 1995.
14. Lambelet P, Löliger J and Shroot B, Role of free radicals in the mode of action of anthralin. *Skin Pharmacol* **1:** 115–121, 1988.
15. Finnen MJ, Lawrence CM and Shuster S, Inhibition of dithranol inflammation by free-radical scavengers. *Lancet* **II:** 1129–1130, 1984.
16. Bruce JM, Dodd NFJ, Gorman AA, Hamblett I, Kerr CW, Lambert C and McNeeney SP, Anthralin-derived transients—II. Formation of the radical by spontaneous fragmentation of both singlet and triplet states of the 10,10′-dehydrodimer: Radical pair multiplicity effects. *Photochem Photobiol* **52:** 345–352, 1990.
17. Hayden PJ and Chignell CF, Detection and characterization of 9-anthron-10-yl radicals formed by antipsoriatic and tumor-promoting 9-anthrones in aqueous buffers. *Chem Res Toxicol* **6:** 231–237, 1993.
18. 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)* **320:** 59–66, 1987.
19. Bruce JM, Kerr CW and Dodd NJF, Formation of superoxide during the auto-oxidation of anthralin (1,8-dihydroxy-9-anthrone). *J Chem Soc Faraday Trans I* **83:** 85–89, 1987.
20. Miller DM, Buettner GR and Aust SD, Transition metals as catalysts of "autoxidation" reactions. *Free Radic Biol Med* **8:** 95–108, 1990.
21. 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.
22. Halliwell B, How to characterize a biological antioxidant. *Free Radic Res Commun* **9:** 1–32, 1990.
23. Müller K and Kappus H, Hydroxyl radical formation by dithranol. *Biochem Pharmacol* **37:** 4277–4280, 1988.
24. Müller K and Gürster D, Hydroxyl radical damage to DNA sugar and model membranes induced by anthralin (dithranol). *Biochem Pharmacol* **46:** 1695–1704, 1993.
25. 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.
26. Müller K, Eibler E, Mayer KK, Wiegrebe W and Klug G, Dithranol, singlet oxygen and unsaturated fatty acids. *Arch Pharm (Weinheim)* **319:** 2–9, 1986.
27. 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.
28. Müller K, Kanner RC and Foote CS, Kinetic studies on anthralin photooxidation. *Photochem Photobiol* **52:** 445–450, 1990.
29. Halliwell B, Antioxidant characterization. Methodology and mechanism. *Biochem Pharmacol* **49:** 1341–1348, 1995.
30. Schallreuter KU and Wood JM, Free radical reduction in the human epidermis. *Free Radic Biol Med* **6:** 519–532, 1989.
31. Trenam CW, Blake DR and Morris CJ, Skin inflammation:

* Müller K and Prinz H, Manuscript in preparation.

Reactive oxygen species and the role of iron. *J Invest Dermatol* **99:** 675–682, 1992.
32. Darr D and Fridovich I, Free radicals in cutaneous biology. *J Invest Dermatol* **102:** 671–675, 1994.
33. Dogan P, Soyuer Ü and Tanrikulu G, Superoxide dismutase and myeloperoxidase activity in polymorphonuclear leukocytes, and serum ceruloplasmin and copper levels, in psoriasis. *Br J Dermatol* **120:** 239–244, 1989.
34. Kobayashi T, Matsumoto M, Iizuka H and Taniguchi N, Superoxide dismutase in psoriasis, squamous cell carcinoma and basal cell epithelioma: An immunohistochemical study. *Br J Dermatol* **124:** 555–559, 1991.
35. Müller K and Gawlik I, Inactivation of mouse epidermal 12-lipoxygenase by anthralin—implications for a role of oxygen radicals. *Biochem Pharmacol* **51:** 1173–1179, 1996.
36. Anderson R, Dithranol-mediated, dose-dependent priming and activation of luminol-enhanced chemiluminescence response of human neutrophils *in vitro*. *Br J Dermatol* **121:** 1–9, 1989.
37. Raab WP, Ingram method: the precursor of photochemotherapy. *Br J Dermatol* **105** (Suppl 20): 77–81, 1981.
38. Paramsothy Y and Lawrence CM, Time course and intensity of anthralin inflammation on involved and uninvolved psoriatic skin. *Br J Dermatol* **116:** 517–519, 1987.
39. Schallreuter KU and Pittelkow MR, Anthralin inhibits elevated levels of thioredoxin reductase in psoriasis. *Arch Dermatol* **123:** 1494–1498, 1987.
40. Trush MA, Mimnaugh EG and Gram TE, Activation of pharmacological agents to radical intermediates. Implications for the role of free radicals in drug action and toxicity. *Biochem Pharmacol* **31:** 3335–3346, 1982.
41. Hsieh GC and Acosta D, Dithranol-induced cytotoxicity in primary cultures of rat epidermal keratinocytes. I. The role of reactive oxygen species. *Toxicol Appl Pharmacol* **107:** 16–26, 1991.
42. Sies H, Biochemistry of oxidative stress. *Angew Chem Int Edn Engl* **25:** 1058–1071, 1986.
43. Halliwell B and Aruoma OI, DNA damage by oxygen-derived species. Its mechanism and measurement in mammalian systems. *FEBS Lett* **281:** 9–19, 1991.
44. Breen AP and Murphy JA, Reactions of oxyl radicals with DNA. *Free Radic Biol Med* **18:** 1033–1077, 1995.
45. Müller K, Leukel P, Mayer KK and Wiegrebe W, Modification of DNA bases by anthralin and related compounds. *Biochem Pharmacol* **49:** 1607–1613, 1995.
46. Birnboim HC, DNA strand breaks in human leukocytes induced by superoxide anion, hydrogen peroxide and tumor promoters are repaired slowly compared to breaks induced by ionizing radiation. *Carcinogenesis* **7:** 1511–1517, 1986.
47. Clark JM and Hanawalt PC, Inhibition of DNA replication and repair by anthralin or danthron in cultured human cells. *J Invest Dermatol* **79:** 18–22, 1982.
48. Forrester DMT, Van Rensburg CEJ and Anderson R, Prooxidative interactions of dithranol with human phagocytes promote oxidative damage to DNA of bystander leukocyte. *Mutat Res* **247:** 39–44, 1991.
49. Halliwell B and Gutteridge JMC, *Free Radicals in Biology and Medicine*, 2nd Edn. Clarendon Press, Oxford, 1989.
50. Girotti AW, Photodynamic lipid peroxidation in biological systems. *Photochem Photobiol* **51:** 497–509, 1990.
51. Bindoli A, Lipid peroxidation in mitochondria. *Free Radic Biol Med* **5:** 247–261, 1988.
52. Fuchs J, Milbradt R and Zimmer G, Multifunctional analysis of the interaction of anthralin and its metabolites anthraquinone and anthralin dimer with the inner mitochondrial membrane. *Arch Dermatol Res* **282:** 47–55, 1990.
53. Kohen E, Kohen C, Morlière P, Santus R, Reyftmann JP, Dubertret L, Hirschberg JG and Coulomb B, A microspectrofluorometric study of the effect of anthralin, an antipsoriatic drug, on cellular structures and metabolism. *Cell Biochem Funct* **4:** 157–168, 1986.
54. Fuchs J, Nitschmann WH and Packer L, The antipsoriatic compound anthralin influences bioenergetic parameters and redox properties of energy transducing membranes. *J Invest Dermatol* **94:** 71–76, 1990.
55. Stadtman ER, Metal ion-catalyzed oxidation of proteins: Biochemical mechanism and biological consequences. *Free Radic Biol Med* **9:** 315–325, 1990.
56. Davies KJA, Delsignore ME and Lin SW, Protein damage and degradation by oxygen radicals. II. Modification of amino acids. *J Biol Chem* **262:** 9902–9907, 1987.
57. Bedord CJ, Young JM and Wagner BM, Anthralin inhibition of mouse epidermal arachidonic acid lipoxygenase *in vitro*. *J Invest Dermatol* **81:** 566–571, 1983.
58. 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.
59. 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.
60. Halliwell B and Gutteridge JMC, Role of iron in oxygen radical reactions. *Methods Enzymol* **105:** 47–56, 1984.
61. Schmidt KN, Podda M, Packer L and Baeuerle PA, Antipsoriatic drug anthralin activates transcription factor NF-κB in murine keratinocytes. *J Immunol* **156:** 4514–4519, 1996.
62. Schulze-Osthoff K, Los M and Baeuerle PA, Redox signalling by transcription factors NF-κB and AP-1 in lymphocytes. *Biochem Pharmacol* **50:** 735–741, 1995.
63. Viluksela M, Characteristics and modulation of dithranol (anthralin)-induced skin irritation in the mouse ear model. *Arch Dermatol Res* **283:** 262–268, 1991.
64. Kemény E, Csato M and Dobozy A, Pharmacological studies on dithranol-induced irritative dermatitis in mice. *Arch Dermatol Res* **281:** 362–365, 1989.
65. Fuchs J and Milbradt R, Antioxidant inhibition of skin inflammation induced by reactive oxidants: Evaluation of the redox couple dihydrolipoate/lipoate. *Skin Pharmacol* **7:** 278–284, 1994.
66. Farber EM and Nall L, Psoriasis. A review of recent advances in treatment. *Drugs* **28:** 324–346, 1984.
67. 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.
68. Müller K, Huang H-S and Wiegrebe W, Antipsoriatic anthrones with modulated redox properties. 3. 10-Thio-substituted 1,8-dihydroxy-9(10*H*)-anthracenones as inhibitors of keratinocyte growth, 5-lipoxygenase, and the formation of 12(*S*)-HETE in mouse epidermis. *J Med Chem* **39:** 3132–3138, 1996.
69. Müller K, Leukel P, Ziereis K and Gawlik I, Antipsoriatic anthrones with modulated redox properties. 2. Novel derivatives of chrysarobin and isochrysarobin—Antiproliferative activity and 5-lipoxygenase inhibition. *J Med Chem* **37:** 1660–1669, 1994.