Antioxidants attenuate anthralin-induced skin inflammation in BALB/c mice: role of specific proinflammatory cytokines

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Abstract: Anthralin is the most common therapeutic agent among a small number of pro-oxidant, 9-anthrones effective in the topical treatment of psoriasis. However, the usefulness of this drug is diminished by toxic side effects, including skin irritation and inflammation. The activities of anthralin are believed to be mediated by the generation of reactive oxygen intermediates and anthrone radicals produced in the skin. In this study, the dermal inflammatory response to anthralin was determined using a mouse ear swelling test. Maximum ear swelling induced by anthralin coincided with the elevation of cytokine mRNA expression in the skin, including interleukin-6, granulocyte-macrophage colony-stimulating factor, macrophage inflammatory protein-2, and tumor necrosis factor α at 24 h post challenge. The role of free radical generation in ear swelling and cytokine modulation were examined by systemic administration of cell permeable and impermeable antioxidants before anthralin challenge. Superoxide dismutase and α-tocopherol acetate, but not the glutathione precursor *N*-acetyl cysteine, were effective inhibitors of anthralin-induced ear swelling and cytokine elevation. Maximum inflammatory cell infiltration occurred 72–96 h post anthralin challenge and was also reduced by antioxidants. These data suggest that oxidative stress, generated at the site of anthralin treatment, alters the expression of dermal chemokines and other cytokines resulting in the recruitment of inflammatory cells. Systemic antioxidant administration may provide opportunities for therapeutic intervention against anthralin-associated toxicities. *J. Leukoc. Biol.* 64: 170–176; 1998.

Key Words: *anthralin skin lesions · free radical generation · reactive oxygen intermediates*

INTRODUCTION

Psoriasis has been treated for over 70 years with anthralin, the synthetic substitute of chrysarobin [1]. Although anthralin displays effective therapeutic properties, the use of the drug is often limited by severe inflammation of perilesional, uninvolved skin. The therapeutic and toxic effects of anthralin are thought to be mediated by reactive oxygen species (ROS), including superoxide and hydroxyl radicals [2], as well as pro-oxidant anthralin metabolites [3]. In addition to anthralin, other common proinflammatory dermatotoxicants, including ultraviolet (UV) radiation and benzene are associated with free radical generation [4]. Inflammation by exogenous agents in the skin is mediated largely by the local expression of keratinocyte-derived cytokines [5]. Among the cytokines expressed in the skin are tumor necrosis factor-alpha (TNF-α), interleukin (IL)-1, IL-6, chemotactic proteins, including macrophage inflammatory protein-2 (MIP-2), which allow for migration of polymorphonuclear leukocytes (PMNs) into the dermis, and cytokines that augment the activity of infiltrating cells, such as granulocyte-macrophage colony-stimulating factor (GM-CSF) [6]. IL-6 expression by epidermal cells correlates with the formation of psoriatic lesions and may contribute to the pathogenesis by promoting growth of epidermal cells [7]. It has been suggested that sustained free radical production in tissues may have a role in cellular signaling by the mobilization of transcription factors, such as nuclear factor-κB (NF-κB) [8], which help regulate genes controlling inflammatory cytokines and chemokines [9–11].

In previous studies we demonstrated that anthralin generates free radicals and induces the expression of inflammatory cytokines in primary human keratinocyte cultures [12]. Because the biology of intact skin cannot always be estimated by homogeneous cell cultures, it was important to determine whether similar processes occur in vivo. In vivo, anthralin-induced free radical reactions have been detected by electron spin resonance and likewise inhibited by vitamin E application in a hairless mouse model [13]. In these studies the relationship between anthralin-induced inflammation and changes in cytokine expression in mouse skin was studied. In addition, the role

Abbreviations: ROS, reactive oxygen species; TNF-α, tumor necrosis factor α; IL, interleukin; MIP-2, macrophage inflammatory protein-2; PMNs, polymorphonuclear neutrophils; GM-CSF, granulocyte-macrophage colony-stimulating factor; NF-κB, nuclear factor-κB; NAC, *N*-acetyl cysteine.

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of oxidative stress was examined by the systemic administration of antioxidants, including superoxide dismutase, *N*-acetyl cysteine (NAC) and α-tocopherol.

MATERIALS AND METHODS

Chemicals

Anthralin, croton oil, NAC, and α-tocopherol acetate (vitamin E) were obtained from Sigma Chemical Co. (St. Louis, MO). Methoxy polyethylene glycol-succinoyl-bovine superoxide dismutase (PEG-SOD) solution was a gift from Dr. Jack H. Dean of Sanofi-Winthrop, Inc. (Collegeville, PA). All other chemicals were reagent grade or better.

Animals and mouse ear swelling assay

Sixty-day-old female BALB/c mice were obtained from Charles River Laboratories (Raleigh, NC), housed three per cage, and fed ad libitum. The ear swelling assay was performed according to Gad et al. [14] as modified by Wilmer et al. [15]. Briefly, freshly prepared concentrations of anthralin were suspended in 70% ethanol/olive oil (4:1) and irritative dermatitis was elicited by applying 10 μL of anthralin suspension to the dorsal surface of both ear pinna. The ears of separate animals treated with ethanol/olive oil vehicle served as controls. To prevent oxygen radical-derived tissue injury, the hydrophilic antioxidant PEG-SOD (500 U/kg; 0.2 mL vol) and the GSH precursor NAC (200,1000 mg/kg) were dissolved in sterile water and injected intravenously 1 h before anthralin challenge. PEG-SOD equilibrates with extravascular spaces and is gradually removed over 72 h [16], whereas unmodified enzyme is completely removed from circulation within 4 h [17]. Groups of mice were administered 100 or 200 U/kg of vitamin E by gavage in a 0.2-mL vol of olive oil for 10 consecutive days before anthralin challenge on the next day. The concentrations of antioxidants tested were within the range of previously reported murine models of oxidant-mediated toxicity [18–20]. Ear thickness measurements were made using an Oditest precision caliper, model D1000 (Dyer Co., Lancaster, PA) at −1, 6, 24, 48, 72, and 96 h after anthralin challenge. Mice were killed by CO_2 inhalation and exsanguinated, ears were resected, placed in cryostat tubes for reverse transcriptase-polymerase chain reaction (RT-PCR) or histology, and frozen in liquid nitrogen. For some studies groups of mice received 10 μL of a 2.0% concentration of croton oil applied to the skin as a positive control.

RNA extraction and RT-PCR

Whole frozen pinna were minced into 2-mm squares on a clean glass slide placed on dry ice. Frozen squares were transferred to 4-mL polypropylene tubes containing 1 mL of Ultraspec™ (Biotecx Laboratories, Inc., Houston, TX) and homogenized for 45 s with a Tissumizer™ (Tekmar, Cincinnatti, OH). The total RNA was extracted according to the manufacturer's instructions and resuspended in 20 μL of distilled water. For synthesis of cDNA, 1 μg of total RNA from each sample was suspended in a 20-μL final volume of the reaction buffer (25 mM Tris-HCl, pH 8.3, 37.5 mM KCl, 10 mM dithiothreitol, 3.5 mM $MgCl_2$) containing 10 mM of each dNTP (Perkin Elmer, Foster City, CA) and 0.5 μg oligo d(T) 12–18 primer (GIBCO-BRL, Grand Island, NY). After the reaction mixture reached 42°C, 200 U Superscript® reverse transcriptase (GIBCO-BRL) was added to each tube and incubated for 30 min at 42°C. The reaction was stopped by denaturing the enzyme at 99°C for 5 min and the mixture was diluted with distilled water to a volume of 50 μL. Commercially available PCR primers for mouse TNF-α, IL-6, G3PDH, and GM-CSF were purchased from Clontech Laboratories, Inc. (Palo Alto, CA) while MIP-2 was obtained from the published sequence [21]. The primers contained the following sequences: GM-CSF (5′) primer, 5′ TGTGGTCTACAGCCTCTCAGCAC3′; GM-CSF (3′) primer, 5′ CAAAGGGGATATCAGTCAGAAAGGT3′; IL-6 (5′) primer, 5′ ATGAAGTTCCTCTCTGCAAGAGACT3′; IL-6 (3′) primer, 5′ CACTAGGTTTGCCGAGTAGATCTC3′TNF-α (5′) primer, 5′ ATGAGCACAGAAAGCATGATCCGC3′; TNF-α (3′) primer, 5′ CCAAAGTAGACCTGCCCGGACTC3′; G3PDH (5′) primer, 5′ TGAAGGTCGGTGTGAACGGATTTG3′; G3PDH (3′) primer, 5′ CATGTAGGCCATGAGGTCCACCAC3′; MIP-2 (5′) primer, 5′CAGAGCTTGAGTGTGACG3′; and MIP-2 (3′) primer, 3′TCGTACCTGATGTGCCTC3′. Amplification of cDNA was conducted as previously described [21] with the use of the Perkin-Elmer Gene Amp PCR System 9600 DNA Thermal Cycler. Optimal conditions for linearity were determined to permit semi-quantitative analysis of signal strength. Abundant messages, such as G3PDH, were amplified 25 cycles, whereas cytokine messages were amplified 30–35 cycles. When appropriate, the specificity of the PCR band was confirmed by restriction enzyme analysis of the amplified cDNA, which generated restriction fragments of the expected size (data not shown).

The PCR products were separated on a 2.0% agarose gel (UltraPure, Sigma) at 75 V for 60 min and visualized by UV illumination after staining with 0.5 μg/mL ethidium bromide. The molecular weight marker, ϕX174 DNA Hae III digest was purchased from Sigma. Gels were photographed with type 55 positive/negative film (Polaroid Corp., Cambridge, MA). Gels were scanned using the Eagle Eye II Still Video System (Stratagene Cloning Systems, Inc., La Jolla, CA). Densitometric analysis of the captured image was performed using the NIH image 1.54 analysis software and normalized for G3PDH content.

Mouse ear histology

Pinna were frozen-sectioned into 2-μm sections and placed on Probe-Plus® glass slides (Fisher Scientific, Pittsburgh, PA) for hematoxylin and eosin (H+E) staining.

Statistical analysis

The Student's *t* and Dunnett's tests were used in single and multiple comparisons of means with a common control group, respectively (Statview; Abacus Concepts, Berkeley, CA). Statistically significant differences were reported when the *P* value was less than 0.05.

RESULTS

To select a dose of anthralin that is irritating for the mouse ear, 10-μL aliquots of anthralin were applied to the dorsal surface of the ear. A nearly linear increased response in ear swelling occurred with increasing concentrations of anthralin (**Fig. 1A**). 10 mM (6 μg anthralin) concentration was selected for subsequent experiments because this was the lowest concentration that produced an easily detectable response. To characterize the time course and morphology of anthralin-induced ear swelling, ear thickness measurements and histological sections were obtained at various time points after treatment. Maximum anthralin-induced ear swelling occurred 24–48 h after anthralin challenge (Fig. 1B) and returned to normal after approximately 7 days (data not shown). The time course for ear morphology and inflammatory cell infiltration after anthralin challenge was evaluated in H+E-stained sections (**Fig. 2**). Maximal edema occurred at 24 h after challenge, which was followed by diffuse and intensive sub-epidermal PMN infiltration that peaked at 96 h.

To establish whether inflammatory cytokines are produced in skin after anthralin challenge, RT-PCR was performed from RNA isolated from samples of treated ears. In untreated ears neither IL-6, GM-CSF, nor MIP-2 mRNA transcripts were detected while TNF-α was constitutively expressed at low levels. Twenty-four hours after anthralin challenge IL-6, GM-CSF, MIP-2, and TNF-α mRNA levels were increased in anthralin as well as croton oil (positive control) ears (**Fig. 3**).

To examine the relationship between anthralin-induced oxidative stress, ear swelling, and elevated inflammatory cytokine expression, BALB/c mice received either PEG-SOD, NAC, or vitamin E before anthralin challenge. PEG-SOD and vitamin E pretreatment decreased anthralin-induced ear swell-

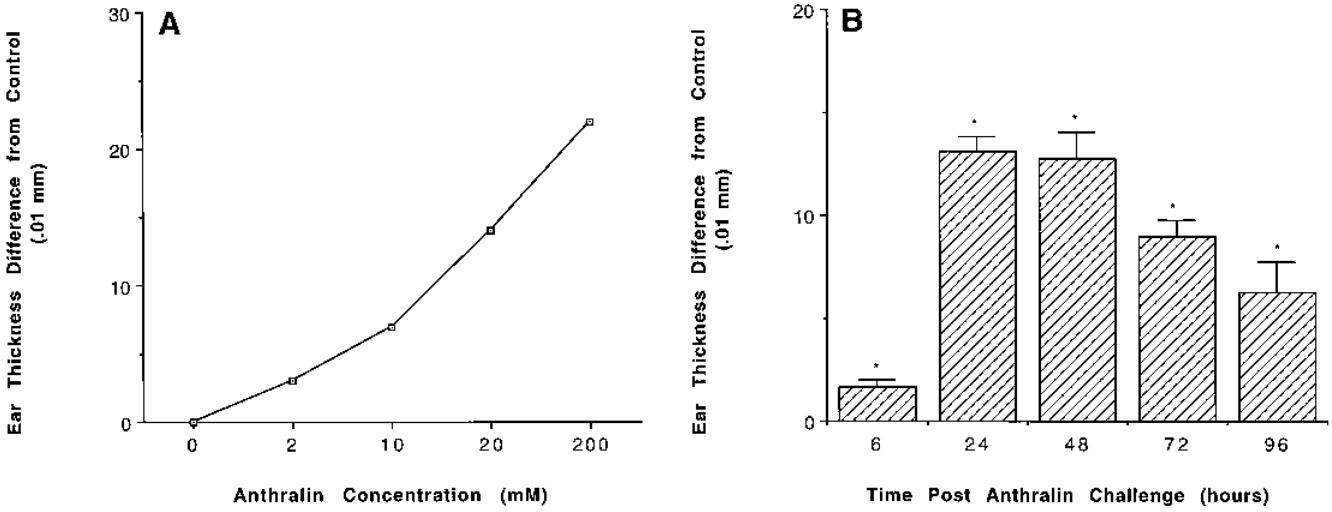

Fig. 1. Dose-response and time-course of anthralin-induced ear swelling in BALB/c mice. (A) Anthralin concentrations of 0, 2, 10, 20, and 200 mM, suspended in ethanol/olive oil vehicle (4:1), were applied in 10 µl volume to dorsum of pinna of mice and ear thickness was measured at 24 h. (B) Anthralin (10 mM, 10 µL vol) was applied to dorsum of pinna and thickness was measured at 6, 24, 48, 72, and 96 h. Results are expressed as mean ear thickness difference (0.01 mm) from vehicle-treated ears. *Significantly different from vehicle control ear thickness at $P < 0.05$, using three mice per treatment group.

ing by 40 and 75%, respectively (**Fig. 4**). On the other hand, administration of NAC before anthralin challenge had no effect on ear swelling (data not shown) and was not evaluated for its effects on cytokine gene expression. The administration of SOD, vitamin E, or vehicle alone did not affect body weight or ear swelling (data not shown). The expression of anthralin-induced cytokines were also reduced by the antioxidants and, again, showed differential effects. Anthralin-induced GM-CSF and TNF-α mRNA expression were prevented by SOD after challenge (**Fig. 5**), whereas MIP-2 and TNF-α mRNA expression were reduced by vitamin E after challenge (**Fig. 6**). The anthralin-induced expression of IL-6 mRNA was not affected

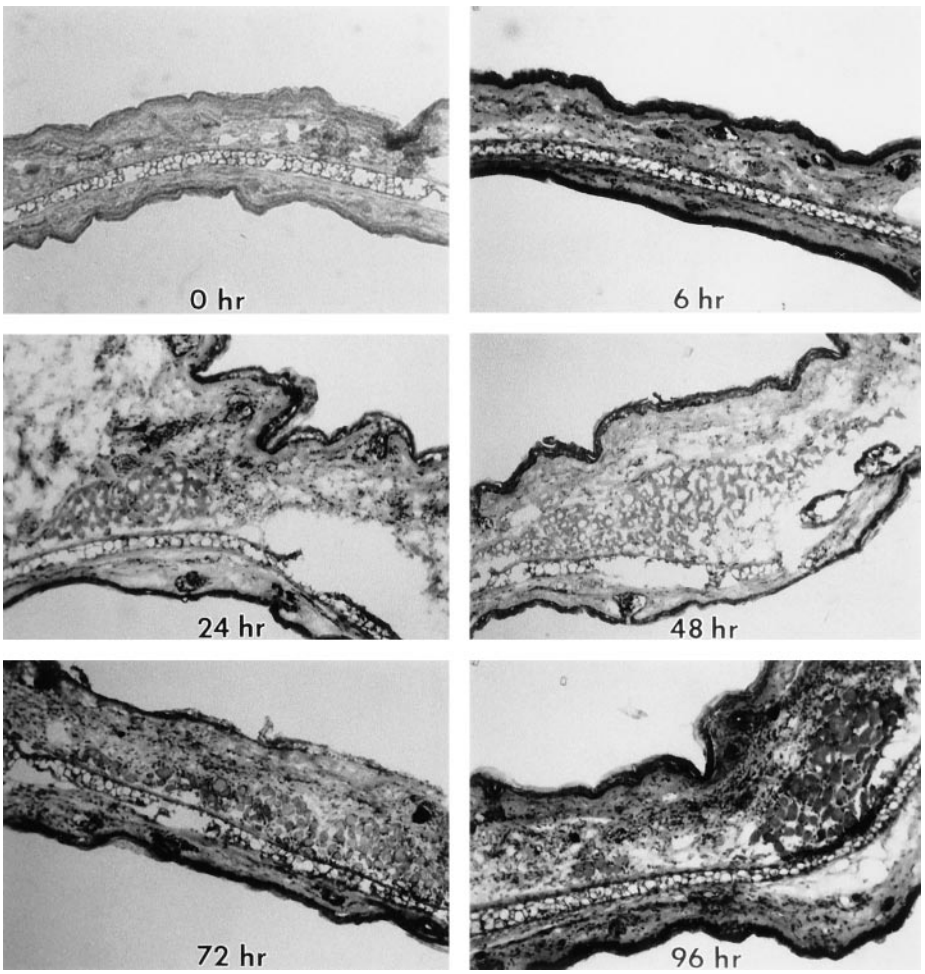

Fig. 2. Kinetics of anthralin-induced inflammatory cell infiltration in BALB/c mouse ear. Anthralin-treated BALB/c mouse ears were resected, sectioned, and stained by H+E at 0, 6, 24, 48, 72, and 96 h after a single, topical anthralin challenge. Ear thickness quantified from photo are 7, 8, 19, 16, 11, and 15 mm, respectively (naive ear = 4 mm). Magnification ×20.

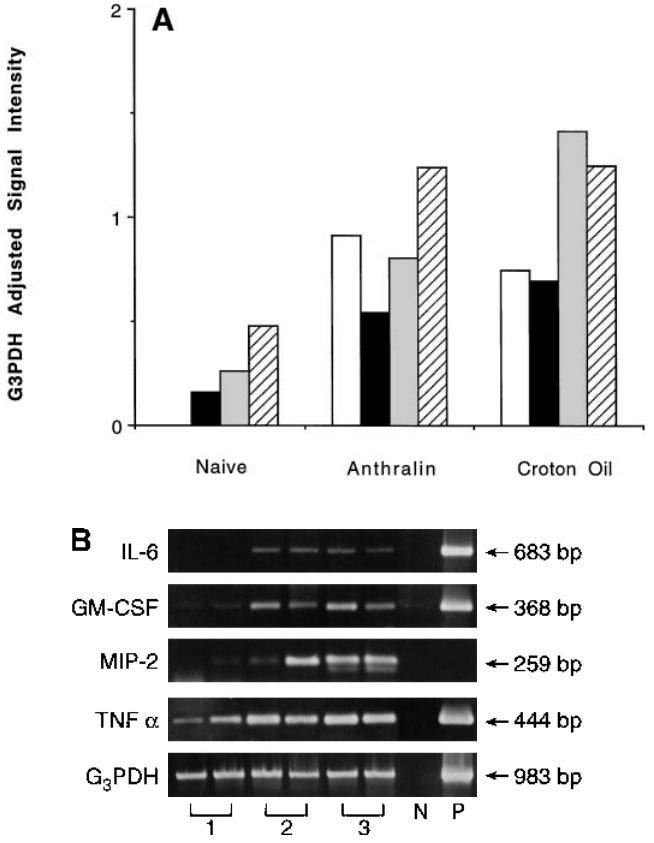

Fig. 3. RT-PCR amplification of anthralin-induced IL-6, GM-CSF, MIP-2, and TNF-α mRNAs in BALB/c mouse ears. Anthralin (10 mM) was applied in a 10 μL volume to dorsum of the pinna of each ear. Croton oil (2%) was used as a positive control. Total RNA was isolated from resected ears 24 h after challenge as described in Materials and Methods. (A) Anthralin-induced IL-6, GM-CSF, MIP-2, and TNF-α mRNA expression. Open bars, IL-6; black bars, GM-CSF; stippled bars, MIP-2; hatched bars, TNF-α. Results are expressed as mean G_3PDH adjusted signal intensity for two samples. Semi-quantitiative analysis was performed using the NIH 1.54 image analysis software. (B) PCR gel photo. 1, naive; 2, anthralin (10 mM); 3, croton oil; N, negative (water) control; P, commercial positive control.

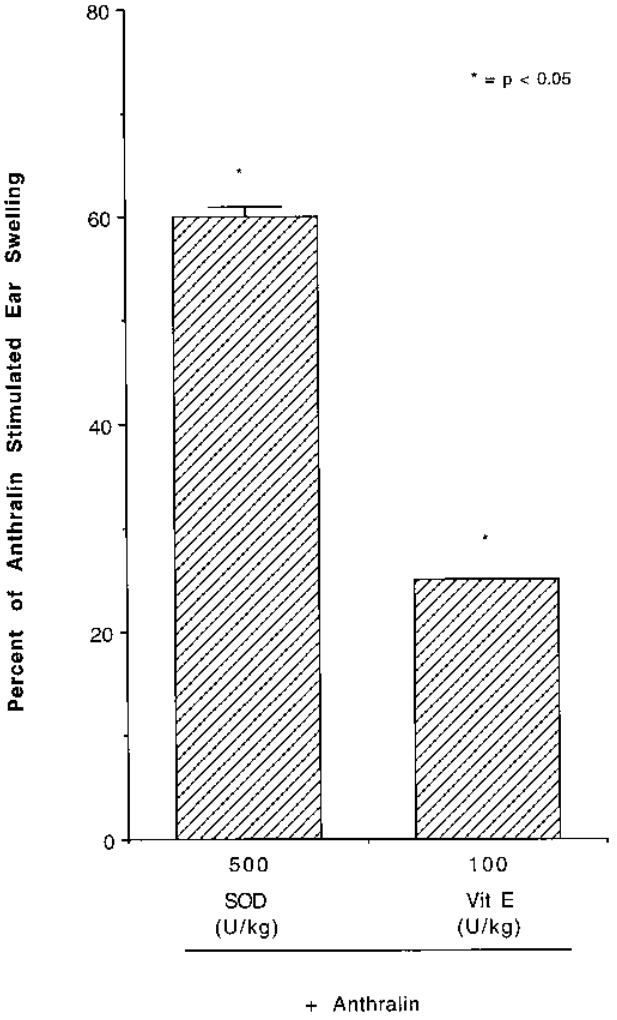

Fig. 4. Anthralin-induced ear swelling in antioxidant-treated BALB/c mice. BALB/c mice received 500 U/kg PEG-SOD (0.2 mL vol, i.v.) 1 h before anthralin challenge or 100 U/kg vitamin E (0.2 mL vol, gavage) 10 consecutive days before anthralin challenge (10 mM, 10 μL vol). Ear measurements were obtained 24 h after challenge. Results are expressed as percent of mean ± SE anthralin-induced ear thickness in the absence of antioxidants using three mice per treatment group. Data are from one of three representative experiments.

by antioxidant treatment (data not shown). For convenience, negative RT-PCR (undetectable) cytokine responses are not shown.

H+E-stained ear sections were examined to evaluate the qualitative differences in the acute inflammatory cell infiltrate of mice after anthralin and/or oxidant challenge. Similar to the ear swelling response, mice pretreated with PEG-SOD or vitamin E, before anthralin challenge, exhibited markedly reduced inflammatory cell infiltration compared with mice challenged with anthralin alone (**Fig. 7**).

DISCUSSION

Free radicals, especially those derived from oxygen, accompany inflammatory states and are suspected to mediate tissue damage [22]. Anthralin generates reactive oxygen species as well as anthralin radicals, both of which can presumably cause tissue damage [23, 24]. Using human keratinocyte cultures, we have shown previously that the oxidative stress associated with anthralin treatment is largely responsible for the expression of proinflammatory cytokines [12]. These studies confirm and extend these observations by demonstrating that application of anthralin to mouse skin evokes swelling and a significant inflammatory cytokine response that is mediated via anthralin-induced generation of ROS. Other inflammatory mediators may

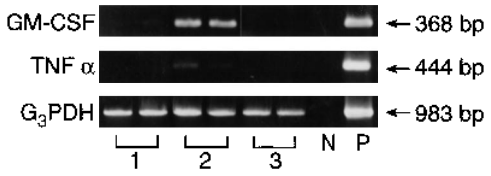

Fig. 5. Cytokine RNA RT-PCR amplification of skin samples from anthralin- and antioxidant-treated mice. BALB/c mice received 500 U/kg SOD (0.2 mL vol), i.v., 1 h before topical anthralin challenge (10 mM, 10 μL vol) on the dorsum of the ear. Total RNA was isolated from resected ears 24 h post challenge as described in Materials and Methods. Lane 1, naive; lane 2, anthralin; lane 3, anthralin + PEG-SOD; N, negative (water) control; P, commercial, positive control.

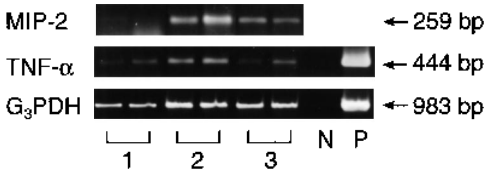

Fig. 6. Cytokine RNA RT-PCR amplification of skin samples from anthralin- and vitamin E-treated BALB/c mice. Mice received 500 U/Kg vitamin E (0.2 mL vol) by gavage 10 consecutive days before a single, topical anthralin challenge (10 mM, 10 μL vol) on the dorsum of the ear. Total RNA was isolated from resected ears 72 h after challenge as described in Materials and Methods. Lane 1, naive; lane 2, anthralin; lane 3, anthralin + vitamin E.

also be involved because in vivo studies suggest that platelet-activating factor, prostaglandins, and histamine participate in anthralin-induced, murine dermatitis [25, 26].

Anthralin is an amphiphilic molecule capable of donating an electron to membrane-associated redox components [27]. It would follow that anthralin's biological activities are associated with its ability to serve as a pro-oxidant. This was evidenced by the ability of the lipophilic antioxidant, vitamin E, to inhibit ear swelling and the expression of MIP-2 and TNF-α mRNAs following anthralin treatment. It has been reported that anthralin-induced inflammation in humans can be inhibited by topical application of tocopherol [28]. Lipid peroxidation occurs when carbon centered lipid radicals react with molecular oxygen to form the peroxyl radical (ROO·), which subsequently attacks polyunsaturated lipid molecules. Vitamin E, the major naturally occurring, lipid-soluble antioxidant in the skin [29], competes for peroxyl radicals faster than membranous, polyunsaturated fatty acids, resulting in significant antioxidant activities [30]. The inhibition of anthralin-induced MIP-2 and TNF-α by vitamin E suggests that membrane-associated factors, such as protein kinase A, are sensitive to oxidative stress and are involved in activating these cytokines. In contrast to vitamin E, the hydrophilic antioxidant SOD inhibited anthralin-induced GM-CSF and TNF-α, suggesting that an early, extracellular activity of superoxide anion is responsible for their expression. TNF-α and GM-CSF augment chemokine activity as well as regulate immune responses such as leukocyte and endothelial activation [31, 32]. Early, extracellular control of GM-CSF and TNF-α induction may provide a temporal advantage in amplifying the bioactivities of subsequently expressed chemokines and infiltrating leukocytes. Anthralin has also been reported to reduce the secretion of LPS-induced cytokine release by monocytes, including IL-6, IL-8, and TNF-α [33], although the significance of this observation is unclear.

Membrane disruption by anthralin-derived oxygen radicals can mediate cell signaling events resulting in activation of nuclear transcription factors and gene expression. The promoter region of inflammatory cytokine genes possesses binding sites for transcription factors such as AP-1, NF-κB, and NF-IL-6, which under certain conditions become activated by ROS [9–11, 34, 35]. In this respect, it has been demonstrated that anthralin induces NF-κB expression and this could be inhibited with antioxidants, including NAC [36]. The ineffectiveness of NAC to reduce swelling and cytokine expression in vivo may be due to hydrogen abstraction from thiol-containing antioxidants that generate potentially deleterious radicals [37]. Our studies demonstrate that chemical-induced expression of cytokines by ROS are not uniform, implying that expression patterns may depend on the stimulant used. Normally, phosphorylation events and subcellular localization of transcription factors regulate tissue-specific, constitutive transcription [38]. Recent studies suggest that the regulation of transcriptional

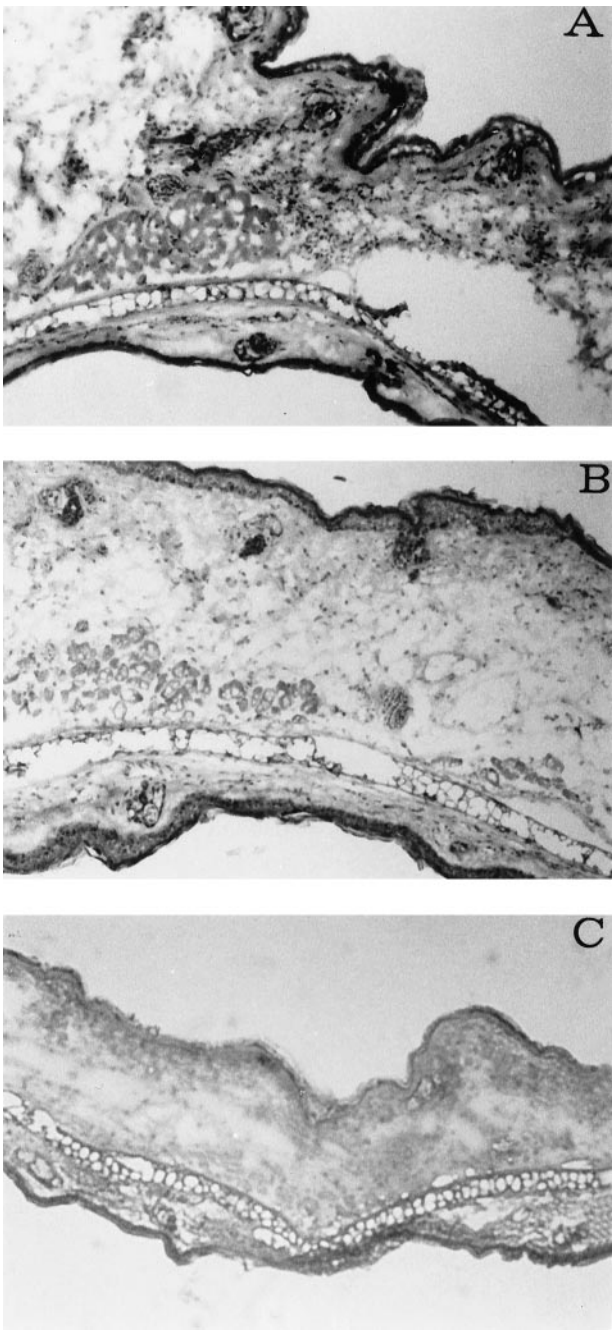

Fig. 7. Effect of PEG-SOD and vitamin E on anthralin-induced inflammatory cell infiltration of BALB/c mouse ear. Mice received 500 U/kg PEG-SOD, i.v. in 0.2-mL volumes 1 h before anthralin challenge (10 mM, 10 μL vol) or 200 U/kg vitamin E (0.2 mL vol, gavage) 10 consecutive days before anthralin challenge (10 mM, 10 μL vol) on the dorsum of ear. H+E-stained histological sections were prepared from pinna resected 24 h after challenge. (A) H+E-stained pinna sections from mice challenged with anthralin in the absence of antioxidant. (B) H+E-stained pinna sections from anthralin-challenged BALB/c mice pretreated with PEG-SOD. (C) H+E-stained ear sections from anthralin challenged BALB/c mice pretreated with vitamin E. Magnification ×20.

activation may also depend on the location of the cellular target [39] and the type of molecular damage occurring, such as protein misfolding or denaturation [40]. Along with chemokines [41], chemotaxis is controlled by other factors, including arachidonic acid products [42] and components of the complement cascade [43].

It is interesting that anthralin-induced PMN infiltration is reduced by SOD pretreatment in the absence of MIP-2 modulation. This suggests that other neutrophil chemoattractants or chemokines are important for oxidative stress-induced PMN recruitment. We also observed that anthralin-induced neutrophil infiltration peaks at 72–96 h rather than at 24–48 h, as observed with croton oil and other irritants [44]. Anthralin-induced DNA damage of leukocytes [45] may account for the delayed PMN accumulation. However, anthralin also activates the oxidative metabolism of phagocytes [46], which in turn can induce cytokine gene expression.

In summary, it was observed that anthralin is a potent mediator of irritancy and inflammatory cytokine expression in mouse skin. Furthermore, this response was induced largely by lipid and intracellular oxidants generated on topical application. Currently, with the exception of superoxide dismutase, the clinical use of antioxidants is rare in dermatology [47]. Supplemental antioxidant treatment that provides strategic targeting to affected cellular sites may represent potential advantages in protection from oxidative damage. Rationally designed antioxidant preparations based on tissue-specific antioxidant expression at the cellular site of radical interactions may be necessary to improve efficacy.

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REFERENCES

1. Ashton, R. E., Andre, P., Lowe, N. J., Whitfield, M. (1983) Anthralin: historical and current perspectives. *J. Am. Acad. Dermatol.* **9**, 173–192.
2. Hayden, P. J., Free, K. E., Chignell, C. F. (1994) Structure-activity relationships for the formation of secondary radicals and inhibition of keratinocyte proliferation by 9-anthrones. *Mol. Pharmacol.* **46**, 186–198.
3. Kemeny, L., Ruzicka, T., Braun-Falco, O. (1990) Dithranol: a review of the mechanism of action in the treatment of psoriasis vulgaris. *Skin Pharmacol.* **3**, 1–20.
4. Gutteridge, J. M. C. (1993) Free radicals in disease processes: a compilation of cause and consequence. *Free Rad. Res. Commun.* **19**, 141–158.
5. Lisby, S., Muller, K. M., Jongeneel, V., Saurat, J., Hauser, C. (1995) Nickel and skin irritants up-regulate tumor necrosis factor-α mRNA in keratinocytes by different but potentially synergistic mechanisms. *Int. Immunol.* **7**, 343–352.
6. Sauder, D. N., Pastore, S. (1994) Cytokines in contact dermatitis. *Am. J. Contact Dermatitis* **4**, 215–224.
7. Ohta, Y., Katayama, T., Funato, T., Yokozeki, H., Nishiyama, S., Hirano, T., Kishimoto, T., Nishioka, K. (1991) In situ expression of messenger RNA of interleukin-1 and interleukin-6 in psoriasis: Interleukin-6 involved in formation of psoriatic lesions. *Arch. Dermatol. Res.* **283**, 351–356.
8. Schreck, R., Baeuerle, P. A. (1991) A role for oxygen radicals as second messengers. *Trends Cell Biol.* **1**, 39–42.
9. Schreck, R., Alberman, K., Baeuerle, P. A. (1992) Nuclear factor κB: an oxidative stress-responsive transcription factor of eukaryotic cells (a review). *Free Rad. Res. Commun.* **17**, 221–237.
10. Simeonova, P. P., Luster, M. I. (1995) Iron and reactive oxygen species in the asbestos-induced tumor necrosis factor-α (TNF-α) response from rat alveolar macrophages. *Am. J. Respir. Cell Mol. Biol.* **12**, 676–683.
11. Simeonova, P. P., Luster, M. I. (1996) Asbestos induction of nuclear transcription factors and interleukin-8 gene regulation. *Am. J. Respir. Cell Mol. Biol.* **15**, 787–795.
12. Lange, R. W., Hayden, P. J., Chignell, C. F., Luster, M. I. (1998) Anthralin stimulates keratinocyte-derived proinflammatory cytokines via generation of reactive oxygen species. *Inflamm. Res.* **47,** 174–181.
13. Mader, K., Bacic, G., Schwartz, H. M. (1995) 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.
14. Gad, S. C., Dunn, B. J., Dobbs, D. W., Reilly, C., Walsh, R. D. (1986) Development and validation of an alternative dermal sensitization test: the Mouse Ear Swelling Test (MEST). *Toxicol. Appl. Pharmacol.* **84**, 93–114.
15. Wilmer, J. L., Burleson, F. G., Kayama, F., Kanno, J., Luster, M. I. (1994) Cytokine induction in human epidermal keratinocytes exposed to contact irritants and its relation to chemically-induced inflammation. *J. Invest. Dermatol.* **102**, 915–922.
16. Fuchs, J., Milbradt, R. (1994) Antioxidant inhibition of skin inflammation induced by reactive oxidants: evaluation of the redox couple dihydrolipoate/lipoate. *Skin Pharmacol.* **7**, 278–284.
17. Pyatak, P. S., Abuchowski, A., Davis, F. F. (1980) Preparation of a polyethylene glycol: superoxide dismutase adduct, and an examination of its blood circulating life and anti-inflammatory activity. *Res. Commun. Chem. Pathol. Pharmacol.* **29**, 113–127.
18. Senaldi, G., Pointairre, P., Piquet, P-F., Grau, G. E. (1994) Protective effect of *N*-acetylcysteine in hapten-induced irritant and contact hypersensitivty reactions. *J. Invest. Dermatol.* **102**, 934–937.
19. Fuchs, J., Milbrandt, R. (1994) Antioxidant inhibition of skin inflammation induced by reactive oxidants: Evaluation of the redox couple dihydrolipoate/lipoate. *Skin Pharmacol.* **7**, 278–284.
20. Iqbal, Z. M. (1984) In vivo nitrosation of amines in mice by inhaled nitrogen dioxide and inhibition of biosynthesis of *N*-nitrosamines. *IARC Sci. Pub.* **57**, 291–300.
21. Kayama, F., Yoshida, T., Elwell, M. R., Luster, M. I. (1995) Role of tumor necrosis factor-α in cadmium-induced hepatotoxicity. *Toxicol. Appl. Pharmacol.* **131**, 224–234.
22. Freeman, B. A., Crapo, J. D. (1982) Biology of disease free radicals and tissue injury. *Lab. Invest.* **47**, 412–426.
23. Fuchs, J., Packer, L. (1989) Investigations of anthralin free radicals in model systems and in skin of hairless mice. *J. Invest. Dermatol.* **92**, 677–682.
24. Motten, A. G., Sik, R. H., Chignell, C. F., Hayden, P. J. (1994) An EPR study of free radicals formed by antipsoriatic and tumor-promoting 9-anthrones in nonpolar solutions. *Chem. Res. Toxicol.* **7**, 877–881.
25. Kemeny, L., Csato, M., Dobozy, A. (1989) Pharmacological studies on dithranol-induced irritative dermatitis in mice. *Arch. Dermatol. Res.* **281**, 362–365.
26. Viluksela, M. (1991) Characteristics and modulation of dithranol anthralin-induced skin irritation in the mouse ear model. *Arch. Dermatol. Res.* **283**, 262–268.
27. Fuchs, J., Nitschmann, W. H., Packer, L. (1990) The antipsoriatic compound anthralin influences bioenergetic parameters and redox properties of energy transducing membranes. *J. Invest. Dermatol.* **94**, 71–76.
28. Finnen, M. J., Lawrence, C. M., Shuster, S. (1984) Inhibition of dithranol inflammation by free radical scavengers. *Lancet* **8412**, 1129–1130.
29. Nachbar, F., Korting, H. C. (1995) The role of vitamin E in normal and damaged skin. *J. Mol. Med.* **73**, 7–17.
30. Burton, G. W., Traber, M. G. (1990) Vitamin E: antioxidant activity, biokinetics, and bioavailability. *Annu. Rev. Nutr.* **10**, 357–382.
31. Jaatela, M. (1991) Biology of disease: biologic activities and mechanisms of action of tumor necrosis factor-α/cachectin. *Lab. Invest.* **64**, 724–742.
32. Gasson, J. C. (1991) Molecular physiology of granulocyte-macrophage colony-stimulating factor. *Blood* **77**, 1131–1145.
33. Mrowietz, U., Jessat, H., Schwarz, A., Schwarz, T. (1997) Anthralin (dithranol) in vivo inhibits human monocytes to secrete IL-6, IL-8 and TNF-α, but not IL-1. *Br. J. Dermatol.* **136**, 542–547.
34. Kishimoto, T., Taga, T., Akira, S. (1994) Cytokine signal transduction. *Cell* **76**, 253–262.
35. Burleson, F. G., Simeonova, P. P., Germolec, D. R., Luster, M. I. (1996) Dermatotoxic chemicals stimulate *c-jun* and *c-fos* transcription and AP-1 DNA binding in human keratinocytes. *Res. Commun. Mol. Pathol. Pharmacol.* **93**, 131–148.
36. Schmidt, K. N., Podda, M., Packer, L., Baeuerle, P. A. (1996) Antipsoriatic drug anthralin activates transcription factor NF-κB in murine keratinocytes. *J. Immunol.* **156**, 4514–4519.

37. Aruoma, O., Halliwell, B., Hoey, B. M., Butler, J. (1989) The antioxidant action of *N*-acetylcysteine: Its reaction with hydrogen peroxide, hydroxyl radical, superoxide, and hypochlorous acid. *Free Rad. Biol. Med.* **6**, 593–597.
38. Karin, M., Hunter, T. (1995) Transcriptional control by protein phosphorylation: Signal transmission from the cell surface to the nucleus. *Curr. Biol.* **5**, 747–757.
39. Vile, G. F., Tanew-Ilitschew, A., Tyrrell, R. M. (1995) Activation of NF-κB in human skin fibroblasts by the oxidative stress generated by UVA radiation. *Photochem. Photobiol.* **62**, 463–468.
40. Tacchini, L., Pogliaghi, G., Radice, L., Anzon, E., Bernelli-Zazzera, A. (1995) Differential activation of heat-shock and oxidation-specific stress genes in chemically-induced oxidative stress. *Biochem. J.* **309**, 453–459.
41. Oppenheim, J. J., Zachariae, C. O. C., Mukaida, N., Matsushima, K. (1991) Properties of the novel pro-inflammatory supergene ''intercrine'' cytokine family. *Annu. Rev. Immunol.* **9**, 617–648.
42. Ford-Hutchinson, A. W., Bray, M. A., Doig, M. V., Shipley, M. E., Smith, M. J. H. (1980) Leukotriene B, a potent chemokinetic and aggregating substance released from polymorphonuclear leukocytes. *Nature* **286**, 264–265.
43. Fernandez, H. N., Henson, P. M., Otani, A., Hugli, T. E. (1978) Chemotactic response to human C3a and C5a anaphylatoxins I. Evaluation of C3a and C5a leukotaxis in vitro and under simulated in vivo conditions. *J. Immunol.* **120**, 109–115.
44. Anderson, C. (1988) Dermal cell infiltrates: allergic, toxic, irritant, and type I reactions. *Acta. Dermatol. Venereol.* **68** (Suppl. 135), 24–27.
45. Forrester, D. M. T., Van Rensburg, C. E. J., Anderson, R. (1991) Pro-oxidative interactions of dithranol with human phagocytes promote oxidative damage to DNA of bystander leucocytes. *Mutat. Res.* **247**, 39–44.
46. Anderson, R. (1989) Dithranol-mediated, dose-dependent priming and activation of luminol-enhanced chemiluminescence responses of human neutrophils in vitro. *Br. J. Dermatol.* **121**, 1–9.
47. Trenam, C. W., Blake, D. R., Morris, C. J. (1992) Skin inflammation: reactive oxygen species and the role of iron. *J. Invest. Dermatol.* **99**, 675–682.