

# Anti-Psoriatic Drug Anthralin Activates JNK via Lipid Peroxidation: Mononuclear Cells are More Sensitive than Keratinocytes

Dominik Peus, Astrid Beyerle, Heike L. Rittner,\* Markus Pott, Alexander Meves, Cornelia Weyand,\* and Mark R. Pittelkow

Department of Dermatology, Biochemistry and Molecular Biology and \*Rheumatology, Mayo Clinic/Foundation, Rochester, Minnesota, U.S.A.

**Anthralin is a widely used, topical therapy for psoriasis. Anti-proliferative and anti-inflammatory properties of anthralin have been identified. Little is known, however, about differential sensitivities of targeted cell types and specific mechanisms of signaling pathway activation. We demonstrate that anthralin exerts potent effects on keratinocytes and mononuclear cells through strong induction of lipid peroxidation and JNK activation, a stress-induced signal transduction pathway. Lipid peroxidation was observed rapidly and half-maximal levels of lipid peroxidation were reached at a 10-fold lower concentration of anthralin for peripheral blood mononuclear cells vs normal keratinocytes. JNK activation was detected in peripheral blood mononuclear cells at a 40-fold**

**lower anthralin dose compared with keratinocytes. For both cell types, selected inhibitors of lipid peroxidation prevented JNK activation. This study demonstrates that mononuclear leukocytes are markedly more sensitive than keratinocytes to anthralin-induced lipid peroxidation and JNK activation. We identify anthralin as a novel and potent inducer of JNK activation and demonstrate that this process is mediated, at least in part, by lipid peroxidation which is among the earliest and most proximate, membrane-related responses to anthralin yet described. Key words: anthralin/c-jun-N-terminal protein kinase/keratinocytes/lipid peroxidation/reactive oxygen species. *J Invest Dermatol* 114:688–692, 2000**

**P**soriasis is a chronic relapsing, papulosquamous, inflammatory disorder of the skin. It is estimated that more than 2% of the people with European ancestry are affected by psoriasis (Stern, 1997). Lesional skin is characterized by epidermal hyperproliferation and perturbed maturation of the epidermis as well as vascular alterations. The dermal and epidermal inflammatory infiltrates are rich in CD4<sup>+</sup> and CD8<sup>+</sup> lymphocytes, respectively, and release proinflammatory cytokines that play important parts as cellular mediators in psoriasis (Bata-Csorgo *et al*, 1995; Krueger *et al*, 1995; Schon *et al*, 1997).

Anthralin (1,8-dehydroxy-9-anthrone), also known as dithranol or Cignolin was first used in psoriasis more than 80 y ago and remains a widely used topical therapy (Galewsky, 1916; Unna, 1916; Mahrle, 1997). Anthralin is relatively safe, economical, and is often used in combination with many other treatments for psoriasis. Anthralin has long been used empirically, but only more recently are its potential mechanisms of therapeutic action beginning to be characterized (Mahrle, 1997). Anthralin exhibits both anti-proliferative (Reichert *et al*, 1985) and anti-inflammatory properties in psoriasis (Schroder *et al*,

1985; ; Mrowietz *et al*, 1997), but also induces inflammation in normal skin (Finnen *et al*, 1984). In addition, modification of DNA bases (Muller, 1997) and inhibition of various enzymes (Schallreuter and Pittelkow, 1987; Kemeny *et al*, 1990) have been described. The specific pharmacologic mode of action of anthralin to these reported effects, however, is poorly understood. Rapid photo-oxidation and auto-oxidation of anthralin results in the formation of anthraquinones, anthralin dimers and, following skin application, anthralin generates reactive oxygen species (ROS) (Muller, 1997). ROS have been shown to be involved in the activation of selected mitogen-activated protein kinases (MAPK) signal transduction pathways including the c-jun-N-terminal kinase (JNK) cascade (Lo *et al*, 1996). The JNK or stress-activated protein kinase cascade (SAPK) pathway is activated by a variety of stress-related stimuli including ultraviolet radiation, heat shock, osmotic imbalance, endotoxin, and cytokines (Derijard *et al*, 1994). JNK has been shown to be required for the regulation of cellular proliferation and apoptosis (Davis, 1999). As healing of the psoriatic plaque has been associated with the disappearance of infiltrating lymphocytes along with the inhibition of keratinocyte proliferation (Christophers and Mrowietz, 1999) modulation of JNK activation may have important therapeutic implications following anthralin treatment.

In this study, we demonstrate that anthralin is a novel inducer of JNK activation and show that lipid peroxidation is among the earliest cellular responses linked to anthralin exposure. Comparing the principal cell types involved in the expression of psoriasis (Bata-Csorgo *et al*, 1995), we also find that peripheral blood mononuclear

Manuscript received November 8, 1999; accepted for publication January 7, 2000.

Reprint requests to: Dr. Dominik Peus, Department of Dermatology, Ludwig-Maximilians-University Munich, Frauenlobstr. 9–11, 80337 München, Germany. Email: D.Peus@lrz.uni-muenchen.de

Abbreviations: JNK, c-jun, N-terminal kinase; LPO, lipid peroxidation; cPA, cis-parinaric acid; NAC, N-acetylcysteine; AA6P, ascorbic acid-6-palmitate.

cells (PBMC) and lymphocytes are significantly more sensitive than keratinocytes to anthralin-induced lipid peroxidation and JNK activation.

## MATERIALS AND METHODS

**Reagents** Anthralin (Dithranol) was obtained from Biomol Research Laboratories (Plymouth Meeting, PA) and prepared in fresh DMSO (Sigma, St Louis, MO) immediately before use. *N*-acetylcysteine (NAC), ascorbic acid-6-palmitate (AA6P) and *n*-propyl gallate were purchased from Sigma. *Cis*-parinic acid (cPA) was obtained from Molecular probes (Eugene, OR) and dissolved in 97% ethanol purged with nitrogen. To this solution 1  $\mu$ g butylated hydroxytoluene per ml (Sigma) was added as antioxidant and aliquots were stored at  $-20^{\circ}\text{C}$ .

**Cell culture** Normal human keratinocytes were isolated from neonatal foreskin specimens. Primary cultures were initiated and maintained in a replicative state with complete, serum-free MCDB 153 medium. Keratinocytes from primary cultures were plated into secondary culture at  $1-10 \times 10^3$  cells per  $\text{cm}^2$ . Complete medium was supplemented with 0.1 mM calcium, 0.2% (vol/vol) bovine pituitary extract bovine pituitary extract, epidermal growth factor (10 ng per ml), insulin (5  $\mu$ g per ml), hydrocortisone ( $5 \times 10^{-7}$  M), ethanolamine ( $1 \times 10^{-4}$  M), phosphoethanolamine ( $1 \times 10^{-4}$  M), and supplemented amino acids. Cells were grown to confluence and fed with standard medium (without growth factors) for at least 48 h to achieve quiescence as previously described (Peus *et al*, 1997). PBMC from healthy blood donors were isolated by density gradient centrifugation using Ficoll-Paque (Pharmacia, Piscataway, NJ). PBMC were maintained in RPMI-1640 (Bio Whittaker, Watersville, MD) with 10% fetal bovine serum (Summit Biotechnology, Fort Collins, CO) and penicillin/streptomycin (Life Technologies, Grand Island, NJ). PBMC isolated within 3 h were used for all experiments.

**Lipid peroxidation assay** Primary human keratinocytes or PBMC were loaded with cPA (10  $\mu$ M) in complete medium or RPMI/10% fetal bovine serum, respectively, for 1 h at  $37^{\circ}\text{C}$  (Hedley and Cho, 1992). Cells were then washed and treated with anthralin (10 nM–100  $\mu$ M) followed by trypsinization, fixation with 0.4% paraformaldehyde and placement on ice. Ten thousand cells were analyzed immediately by flow cytometry using a Becton-Dickinson FACS Star Plus (San Jose, CA) with a multiline ultraviolet argon laser at 100 mW power excitation and emission wave lengths of 334–364 nm and 424 nm, respectively. The mean fluorescence for the control was consistently set at 300 arbitrary units. PBMC were gated

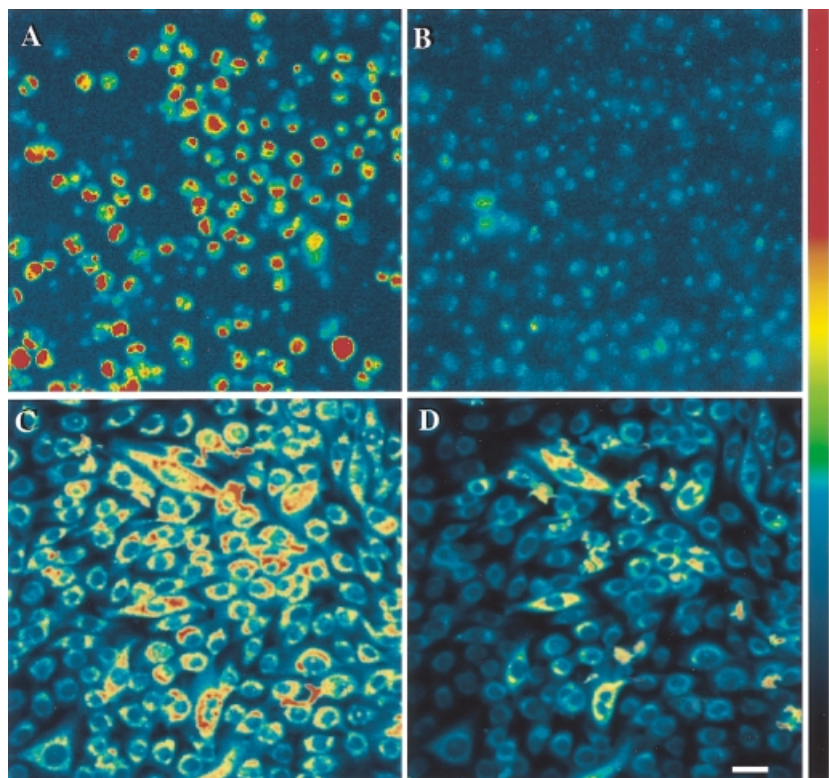
on lymphocytes for further analysis. The untreated *vs* DMSO-treated control showed no differences in lipid peroxidation for either cell type. For laser scanning confocal microscopy, cells were loaded with cPA and treated as described above. Keratinocytes were cultured on coverslips and images were obtained with an inverted laser scanning confocal microscope LSM410 (Zeiss, Jenna, Germany) equipped with a coherent ultraviolet laser, Innova 300 (Santa Clara, CA). The emitted 100 mW laser light of 364 nm for excitation is deflected by a dichroic and  $E_m$ -filter, LP395 and LP397, respectively. Images of PBMC were taken before and after anthralin treatment using a Neubauer chamber. Fluorescence intensities were converted into pseudocolors using the program Analyze (Mayo Foundation, Rochester, MN).

**JNK kinase assay** JNK activity was measured using GST-c-jun (1–223) as ligand and substrate (Hibi *et al*, 1993). Cell extracts were mixed with glutathione (GSH)-agarose beads to which GST-c-jun was bound. After incubation at  $4^{\circ}\text{C}$  for 3 h, the beads were washed extensively and incubated in kinase buffer [20 mM *N*-2-hydroxyethylpiperazine-*N'*-2ethanesulfonic acid (HEPES) (pH 7.6), 20 mM  $\text{MgCl}_2$ , 10 mM  $\beta$ -glycerophosphate, 20 mM *p*-nitrophenylphosphate, 0.5 mM  $\text{Na}_3\text{VO}_4$ , 2 mM dithiothreitol, 50  $\mu$ M adenosine triphosphate). Bound JNK was detected by the addition of 5  $\mu$ Ci of [ $\gamma$ - $^{32}\text{P}$ ]adenosine triphosphate (Dupont NEN, Boston, MA) at  $30^{\circ}\text{C}$  for 20 min. Phosphorylated proteins were separated by sodium dodecyl sulfate–polyacrylamide gel electrophoresis and analyzed by autoradiography (Kodak, New Haven, CT).

## RESULTS AND DISCUSSION

Despite decades of clinical use, the specific mechanisms of activation of intracellular signaling pathways by anthralin are not well understood. Among different types of oxidative stress, this study further examines lipid peroxidation as a prominent cellular response to anthralin and links it to stress-activated signaling pathways. For these experiments, freshly isolated PBMC and primary keratinocytes, isolated and cultured from human epidermis, were used. Epidermal keratinocytes and lymphocytes represent the two principal cell types involved in the development of psoriasis (Bata-Csorgo *et al*, 1995; Stern, 1997). To characterize the initial rapid events and damage to cellular constituents that are induced by diffusible peroxides following anthralin exposure, we measured lipid peroxidation of cellular membranes. cPA is a naturally occurring fluorescent wholly unsaturated fatty acid. It is structurally

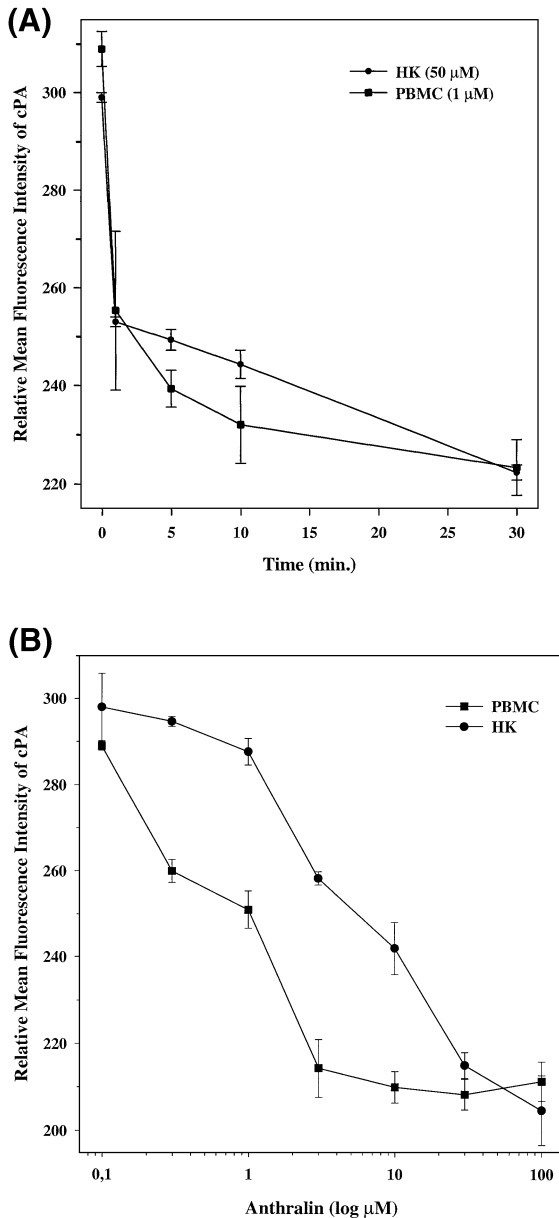
**Figure 1. Anthralin induces lipid peroxidation in keratinocytes and PBMC.** Loss of cPA fluorescence intensity indicating increased lipid peroxidation is shown using laser scanning confocal microscopy and demonstrates an increase in lipid peroxidation after treatment with anthralin of (A, B) PBMC and (C, D) keratinocytes. (A, B) Different fields of PBMC in Neubauer chamber are depicted. (C, D) Represent the same field of adherent keratinocytes. Cells were loaded with cPA for 1 h before treatment. Before and 15 min after addition of anthralin, images were obtained and fluorescence intensities were converted into pseudocolors. Scale bar: (A, B) 5  $\mu$ m; (C, D) 10  $\mu$ m.



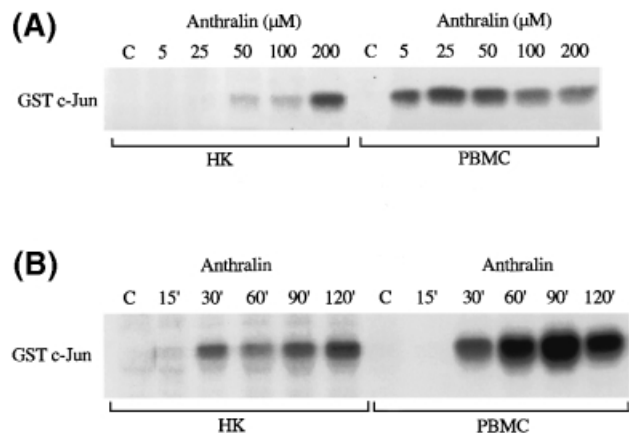
analogous to intrinsic membrane lipids and is readily incorporated into membranes (Hedley and Cho, 1992). Loss of cPA fluorescence by flow cytometry has been used as a quantitative measurement of lipid membrane damage in different cell culture systems (Kuypers *et al*, 1987; Hockenbery *et al*, 1993). We analyzed the loss of fluorescence intensity of cPA by laser-scanning confocal microscopy to quantitate rapidly the lipid peroxide-propagated chain reaction following exposure to anthralin. Fluorescence intensity of

digitized images was converted into pseudocolors as described in *Materials and Methods*. An increase in lipid peroxidation, shown by loss of cell fluorescence, was observed within 15 min of anthralin treatment of freshly isolated PBMC ( $3\text{ }\mu\text{M}$ ) (**Fig 1B**) or keratinocytes ( $100\text{ }\mu\text{M}$ ) (**Fig 1C, D**) compared with untreated control cells (**Fig 1A, C**) using optimized concentrations of anthralin for each cell type (see **Fig 2B**). The rapid onset and dramatic loss of fluorescence after anthralin exposure indicates that lipid peroxidation is a prominent early event in the cellular response to anthralin.

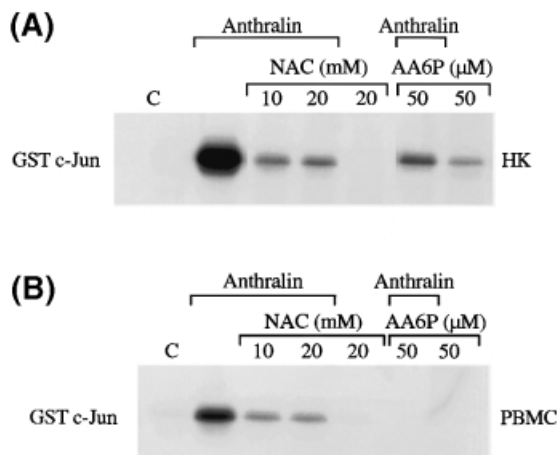
Rapid induction of lipid peroxidation by anthralin within membranes of specific cell types causing psoriasis may be linked to concentration-dependent and preferentially targeted activation of intracellular signal transduction pathways that regulate cell-cell interactions and cell viability. Moreover, therapeutic use of anthralin has been empirically refined over the decades to maximize exposure to the drug at optimized concentrations and duration of skin contact as well as to minimize toxicity to both psoriatic and normal skin. Therefore, lipid peroxidation following anthralin treatment was further analyzed in keratinocytes and PBMC. By flow cytometric gating of cPA intensities, lymphocytes could be selectively detected. Loss of cPA fluorescence was concentration- and time-dependently analyzed. Keratinocytes and PBMC were treated with optimized concentrations of anthralin,  $50\text{ }\mu\text{M}$  and  $1\text{ }\mu\text{M}$ , respectively. These specific concentrations were chosen based on the different sensitivities to lipid peroxidation of each cell type (see **Fig 2B**). Within 5 min of anthralin exposure, greater than 50% of fluorescence intensity had been lost. The onset of lipid peroxidation in both cell types is rapid and similar in profile (**Fig 2A**). Comparing concentrations of anthralin required to induce lipid peroxidation in PBMC or keratinocytes, fluorescence intensity of cPA in lymphocytes decreased in response to  $10\text{ nM}$  anthralin and showed a dramatic decline with exposure to  $0.3\text{ }\mu\text{M}$  anthralin over 30 min of treatment (**Fig 2B**). Near maximal levels of lipid peroxidation were reached at  $3\text{ }\mu\text{M}$  anthralin in lymphocytes and at  $30\text{ }\mu\text{M}$  in keratinocytes, indicating that PBMC are approximately 10 times more sensitive to anthralin-induced lipid peroxidation than keratinocytes. Monocytes exhibited a bright fluorescence pattern, presumably because of ingestion of fluorochrome, and could thus be differentiated from lymphocytes in all experiments measuring loss of cPA by flow cytometry.



**Figure 2. Effects of anthralin on lipid peroxidation in keratinocytes and PBMC.** (A) Time-dependent decrease of cPA fluorescence intensity using  $50\text{ }\mu\text{M}$  anthralin for keratinocytes or  $1\text{ }\mu\text{M}$  anthralin for PBMC (specifically gated on lymphocytes). Greater than 50% cPA fluorescence intensity had been lost in both cell types after 5 min of anthralin treatment. (B) Greater than 25% decrease in cPA fluorescence intensity was observed in PBMC after  $300\text{ nM}$  anthralin for 30 min compared with  $3\text{ }\mu\text{M}$  anthralin for keratinocytes. cPA fluorescence intensity further diminishes with higher doses of anthralin and shows nadirs at  $3\text{ }\mu\text{M}$  and  $100\text{ }\mu\text{M}$  anthralin in PBMC and keratinocytes, respectively. Fluorescence intensity of cPA was analyzed by flow cytometry using triplicates with 10,000 lymphocytes each. Fluorescence intensity of untreated and cPA-loaded cells was arbitrarily set to 300 arbitrary units. Results are representative of three independent experiments.



**Figure 3. Concentration-dependence and time course of JNK activation by anthralin.** (A) Keratinocytes and PBMC were exposed to  $5\text{--}200\text{ }\mu\text{M}$  anthralin for 90 min before cell lysates were assayed for JNK activity. JNK activity examining varying concentrations of anthralin. (B) Time course of JNK activation was analyzed using  $100\text{ }\mu\text{M}$  or  $5\text{ }\mu\text{M}$  anthralin for keratinocytes or PBMC, respectively. Cell lysates of the same numbers of cells per sample were incubated with GSH-agarose beads to which GST-c-jun (1–223) was bound for 3 h. After washing and addition of [ $\gamma\text{-}^{32}\text{P}$ ]adenosine triphosphate for 20 min followed by separation by sodium dodecyl sulfate-polyacrylamide gel electrophoresis phosphorylated c-jun was analyzed by autoradiography as described in *Materials and Methods*.



**Figure 4. Inhibition of anthralin-induced JNK activity by NAC and AA6P in keratinocytes and PBMC.** (A) Keratinocytes treated with 200  $\mu$ M anthralin for 90 min and (B) PBMC exposed to 5  $\mu$ M anthralin for 90 min were pretreated for 8 h with 10 or 20 mM NAC. Fifty micromoles per liter AA6P was added for 30 min before treatment. JNK activity was assayed as described in Fig 3.

No differences in lipid peroxidation were observed in CD4 or CD8 lymphocyte subpopulations separated by MACS column (data not shown). Furthermore, lymphocytes activated by 12-myristate 13-acetate (PMA) alone or PMA and ionomycin were more sensitive than quiescent cells to lipid peroxidation produced by anthralin (data not shown). Our data show that lipid peroxidation is induced concentration- and time-dependently by anthralin within minutes of exposure to keratinocytes or lymphocytes. To our knowledge, any more rapid effects triggering downstream signaling responses have not been described following anthralin treatment. In addition, we found that PBMC and, specifically, lymphocytes are at least 10-fold more sensitive to anthralin-induced lipid peroxidation compared with keratinocytes. These observations provide evidence that lipid peroxidation generated by anthralin is both a rapid and cell-type selective, membrane-associated event that may contribute to the therapeutic action of the drug.

Anthralin is a known inducer of ROS (Muller, 1997), and ROS are involved in the activation of JNK (Busciglio and Yankner, 1995). We therefore examined whether anthralin induces activation of JNK in keratinocytes and PBMC in a concentration- and time-dependent manner. To compare more specifically activation responses in both cell types, total JNK protein was measured using a specific JNK antibody (Santa Cruz, Biotechnology, Santa Cruz, CA) and the number of cells used for experiments were calculated accordingly. JNK activation by anthralin was concentration-dependent in both cell types and was maximally induced in keratinocytes by 200  $\mu$ M anthralin after 90 min exposure (Fig 3A). In PBMC, JNK was strongly activated at a significantly lower concentration of 5  $\mu$ M anthralin by 90 min, was maximally stimulated at 25  $\mu$ M and declined at higher anthralin concentrations. In both keratinocytes and PBMC, 100  $\mu$ M anthralin activated JNK within 30 min of exposure, and maximum activated levels were observed within 120 or 90 min, respectively (Fig 3B). Total JNK protein also was measured in PBMC and keratinocytes, and gels were equally loaded. These experiments demonstrate that anthralin activates JNK in keratinocytes and PBMC, but the threshold response in PBMC occurs at 40-fold lower concentrations of anthralin compared with keratinocytes.

Concentrations of 1% (44 mM) anthralin are commonly used for topical therapy of psoriasis. Depletion of epidermal lymphocytes has been shown to be therapeutically relevant in psoriasis (Bata-Csorgo *et al*, 1995) and, depending on the formulation, application procedure and the water content of the skin, as much as 12% of the topically applied anthralin dose has been measured in the

epidermis. Ten-fold lower concentrations of anthralin have been detected in the dermis (Schaefer *et al*, 1981). In this respect, the tissue concentration is still more than 100-fold higher than that required for JNK activation or the half-maximal induction of lipid peroxidation of PBMC, *in vitro*. These data show that the concentrations used in these experiments are well within the range used *in vivo* and underscores further the mechanistic relevance of our findings. In separate studies in keratinocytes, we have found that JNK activation shows a strong linkage with lipid peroxidation produced by selected oxidative stress-inducing agents and that lipid peroxidation is an important upstream event required for JNK activation (unpublished observation). The concentrations of anthralin that induce JNK activation correlate well with levels of lipid peroxidation for each cell type. We suggest therefore that JNK activation by anthralin for PBMC lymphocytes and keratinocytes is likely to be mediated by lipid peroxidation. To delineate better the mechanism of anthralin-induced JNK activation, cells were pretreated with the anti-oxidants, NAC or AA6P, which are known to inhibit lipid peroxidation. Pretreatment of PBMC or keratinocytes with 10–20 mM NAC or 50  $\mu$ M AA6P for 30 min potently inhibited JNK activation induced by treatment for 90 min with 5  $\mu$ M or 200  $\mu$ M anthralin, respectively (Fig 4A, B). These experiments implicate both ROS and lipid peroxides as important mediators of JNK activation induced by anthralin.

Lipid peroxidation results from the autocatalytic chain reaction of polyunsaturated fatty acids containing multiple carbon double bonds that are located within cellular membranes. Among ROS, hydroxyl radicals are considered to be the strongest initiators of lipid peroxidation (Gutteridge and Halliwell, 1989), but the precise mechanism of rapid initiation of lipid peroxidation is still unclear. A link between the rapid generation of ROS and progressive lipid peroxidation of cellular membranes has been previously identified (Hockenbery *et al*, 1993; Veis *et al*, 1993). It also has been shown that following topical application of anthralin, lipid peroxidation as well as skin irritation *in vivo* can be inhibited by anti-oxidants or inhibitors of lipid peroxidation (Finnen *et al*, 1984; Whitefield *et al*, 1985). Our findings that selected anti-oxidants and inhibitors of lipid peroxidation, such as NAC and AA6P, suppress anthralin-mediated JNK activation in keratinocytes and PBMC establish lipid peroxidation as a novel and critical upstream event in the cascade of JNK activation following anthralin treatment. This is in agreement with our finding that lipid peroxidation not only precedes JNK activation, but also that there is a close correlation between these two events following ultraviolet B irradiation and treatment with different oxidative stress inducing agents (unpublished observation).

Johnson *et al* (1996) have reported that PBMC or T lymphocytes, in comparison with keratinocytes, are more than 50-fold more sensitive to the cytotoxic effects of psoralen plus ultraviolet A radiation. It was also demonstrated that proliferating lymphoid cells are up to 100 times more sensitive than keratinocytes to the effects of methotrexate, making them the likely cellular target when treating psoriasis with methotrexate (Jeffes *et al*, 1995). As anthralin also exerts differential cytotoxic effects that preferentially target PBMC (unpublished observation), we hypothesize that lesional lymphocytes are eliminated by repeated application of anthralin to psoriatic plaques.

Protection of cells from irreversible damage and death by lipid peroxidation is mediated through various enzymatic or non-enzymatic anti-oxidant pathways. Keratinocytes may preferentially express these factors to counteract the toxic effects of lipid peroxides. In addition, the extensive and more resilient intermediate filament structure of keratinocytes and epidermis, as compared with lymphocytes and other cell types, may also play a major part in the enhanced resistance to various physical and chemical environmental stresses, including those induced by lipid peroxidation.

In summary, we identify anthralin as a novel inducer of JNK activation and characterize lipid peroxidation as the earliest response yet described that mediates activation of downstream

signaling events in PBMC and keratinocytes. Our data provide evidence that mononuclear cells are significantly more sensitive to anthralin than keratinocytes and are likely the primary target of the pharmacologic action of anthralin. Further characterization of the biochemical mechanisms of activation of signaling pathways by anthralin will lead to a better understanding of the mode of action of this drug and related analogs that are being developed for psoriasis and other skin diseases (Muller and Prinz, 1997).

We thank Dr. M. Karin (La Jolla, CA) for the gift of GST-c-Jun plasmid. The excellent technical assistance provided by R. Bortolon, M. Anderson and K. Squillace is gratefully acknowledged. We thank Lilette Peus and JoAnn Lower for secretarial assistance. D. Peus (PE 635/1-2) and H. Rittner (RI 817/1-2) were supported by grants from the Deutsche Forschungsgemeinschaft (DFG). A. Meves was supported by the German-American Fulbright Commission and the Studienstiftung des Deutschen Volkes. Additional support was provided by the Mayo Foundation.

## REFERENCES

- Bata-Csorgo Z, Hammerberg C, Voorhees JJ, Cooper KD: Intraleisional T-lymphocyte activation as a mediator of psoriatic epidermal hyperplasia. *J Invest Dermatol* 105:89S-94S, 1995
- Busciglio J, Yankner BA: Apoptosis and increased generation of reactive oxygen species in Down's syndrome neurons in vitro. *Nature* 378:776-779, 1995
- Christophers E, Mrowietz U: The inflammatory infiltrate in psoriasis. *Clin Dermatol* 13:131-135, 1995
- Davis RJ: Signal transduction by the c-Jun N-terminal kinase. *Biochem Soc Symp* 64:1-12, 1999
- Derijard B, Hibi M, Wu IH, et al: JNK1: a protein kinase stimulated by UV light and Ha-Ras that binds and phosphorylates the c-Jun activation domain. *Cell* 76:1025-1037, 1994
- Finnen MJ, Lawrence CM, Shuster S: Inhibition of dithranol inflammation by free-radical scavengers. *Lancet* ii:1129-1130, 1984
- Galewsky E: Über Cignolin, ein Ersatzpräparat des Chrysarobins. *Dermatol Wochenschr* 62:113-115, 1916
- Gutteridge JM, Halliwell B: Iron toxicity and oxygen radicals. *Baillière Clin Haematol* 2:195-256, 1989
- Hedley D, Chow S: Flow cytometric measurement of lipid peroxidation in vital cells using parinaric acid. *Cytometry* 13:686-692, 1992
- Hibi M, Lin A, Smeal T, Minden A, Karin M: Identification of an oncoprotein- and UV-responsive protein kinase that binds and potentiates the c-Jun activation domain. *Genes Development* 7:2135-2148, 1993
- Hockenbery DM, Oltvai ZN, Yin XM, Millman CL, Korsmeyer SJ: Bcl-2 functions in an antioxidant pathway to prevent apoptosis. *Cell* 75:241-251, 1993
- Jeffes EW, 3rd, McCullough JL, Pittelkow MR, et al: Methotrexate therapy of psoriasis: differential sensitivity of proliferating lymphoid and epithelial cells to the cytotoxic and growth-inhibitory effects of methotrexate. *J Invest Dermatol* 104:183-188, 1995
- Johnson R, Staiano-Coico L, Austin L, Cardinale I, Nabeya-Tsukifuji R, Krueger JG: PUVA treatment selectively induces a cell cycle block and subsequent apoptosis in human T-lymphocytes. *Photochem Photobiol* 63:566-571, 1996
- Kemeny L, Ruzicka T, Braun-Falco O: Dithranol: a review of the mechanism of action in the treatment of psoriasis vulgaris. *Skin Pharmacol* 3:1-20, 1990
- Krueger JG, Wolfe JT, Nabeya RT, et al: Successful ultraviolet B treatment of psoriasis is accompanied by a reversal of keratinocyte pathology and by selective depletion of intraepidermal T cells. *J Exp Med* 182:2057-2068, 1995
- Kuypers FA, van den Berg JJ, Schalkwijk C, Roelofsens B, Op den Kamp JA: Parinaric acid as a sensitive fluorescent probe for the determination of lipid peroxidation. *Biochim Biophys Acta* 921:266-274, 1987
- Lo YYC, Wong JMS, Cruz TF: Reactive oxygen species mediate cytokine activation of c-Jun NH2-terminal kinases. *J Biol Chem* 271:15703-15707, 1996
- Mahrle G: Dithranol. *Clin Dermatol* 15:723-737, 1997
- Mrowietz U, Jessat H, Schwarz A, Schwarz T: Anthralin (dithranol) *in vitro* inhibits human monocytes to secrete IL-6, IL-8 and TNF- $\alpha$ , but not IL-1. *Br J Dermatol* 136:542-547, 1997
- Muller K: Antipsoriatic and proinflammatory action of anthralin. Implications for the role of oxygen radicals. *Biochem Pharmacol* 53:1215-1221, 1997
- Muller K, Prinz H: Antipsoriatic anthrones with modulated redox properties. 4. Synthesis and biological activity of novel 9,10-dihydro-1,8-dihydroxy-9-oxo-2-anthracenecarboxylic and -hydroxamic acids. *J Med Chem* 40:2780-2787, 1997
- Peus D, Hamacher L, Pittelkow MR: EGF-receptor tyrosine kinase inhibition induces keratinocyte growth arrest and terminal differentiation. *J Invest Dermatol* 109:751-756, 1997
- Reichert U, Jacques Y, Grangeret M, Schmidt R: Antirespiratory and antiproliferative activity of anthralin in cultured human keratinocytes. *J Invest Dermatol* 84:130-134, 1985
- Schaefer H, Schalla W, Shroot B: Anthralin-facts, trends and unresolved problems. *Z Hautkr* 146:273-282, 1981
- Schallreuter KU, Pittelkow MR: Anthralin inhibits elevated levels of thioredoxin reductase in psoriasis. A new mode of action for this drug. *Arch Dermatol* 123:1494-1498, 1987
- Schon MP, Detmar M, Parker CM: Murine psoriasis-like disorder induced by naive CD4<sup>+</sup> T cells. *Nat Med* 3:183-188, 1997
- Schroder JM: Anthralin (1,8-dihydroxyanthrone) is a potent inhibitor of leukotriene production and LTB<sub>4</sub>-omega oxidation by human neutrophils. *J Invest Dermatol* 87:624-629, 1986
- Schroder JM, Kosfeld U, Christophers E: Multifunctional inhibition by anthralin in nonstimulated and chemotactic factor stimulated human neutrophils. *J Invest Dermatol* 85:30-34, 1985
- Stern RS: Psoriasis. *Lancet* 350:349-353, 1997
- Unna PG: Cignolin als Heilmittel der Psoriasis. *Dermatol Wochenschr* 62:116-137, 150-163, 175-183, 1916
- Veis DJ, Sorenson CM, Shutter JR, Korsmeyer SJ: Bcl-2-deficient mice demonstrate fulminant lymphoid apoptosis, polycystic kidneys, and hypopigmented hair. *Cell* 75:229-240, 1993
- Whitefield M, Leeder GM, Mosedale AR: Inhibition of dithranol inflammation by free-radical scavengers. *Lancet* i:173, 1985