Glucocorticoid in Inflammatory Proliferative Skin Disease Reduces Arachidonic and Hydroxyeicosatetraenoic Acids

Abstract. *Psoriasis is a prototype of several common, glucocorticoid responsive, inflammatory proliferative skin diseases. Within 28 hours, glucocorticoid reduced the increased concentration of free arachidonic acid in diseased tissue. This reduction was observed prior to visible improvement of disease and may be an important molecular mechanism for the therapeutic efficacy of glucocorticoids in psoriasis and similar inflammatory diseases.*

Lesional epidermis of the skin disease psoriasis is characterized by glycogen accumulation, increased cell proliferation, and reduced terminal differentiation (*1*). The increased ratio of guanosine 3′,5′-monophosphate (cyclic GMP) to adenosine 3′,5′-monophosphate (cyclic AMP) in the lesions may in part account for these characteristics (*2*). We previously reported mean increases of arachidonic acid and 12L-hydroxy-5,8,10,14-eicosatetraenoic acid (HETE) concentrations in lesional epithelium of psoriasis of 26- and 82-fold, respectively (*3*). The increase in free arachidonic acid may be important in the initiation or maintenance of the cyclic nucleotide imbalance since arachidonic acid and other free fatty acids stimulate the guanylate cyclase of platelets (*4*), fibroblasts (*5*), and fat cells (*6*) and can inhibit adenylate cyclase of fat cells (*7*).

Psoriasis is prototypic of several common glucocorticoid-responsive, inflammatory proliferative skin diseases (*8*). Anti-inflammatory glucocorticoids can prevent the release of free arachidonic acid in certain tissues (*9*) and cells (*10*). We now report that glucocorticoid applied to lesions of psoriasis normalized the free arachidonic acid content and reduced HETE concentrations, and suggest that this may be an important component of the mechanisms responsible for the therapeutic effect of glucocorticoids in this disease.

A new, highly effective topical glucocorticoid known to clear psoriasis (*11*), diflorasone diacetate 0.05 percent in cream vehicle (*12*), or cream vehicle (*13*) alone (control), was applied under occlusive plastic film (Saran Wrap) to four separate lesions (*14*). Neither physicians nor patients knew which lesions received which treatment. The area of each lesion was approximately 25 cm². Thus glucocorticoid or control application was randomly assigned to four separate lesions of similar severity in each of 12 psoriasis patients such that two of these lesions received glucocorticoid and two received control applications.

Each of the four sites received one application that was in place for 24 hours continuously. Cross contamination among the four sites was prevented by the plastic-film dressings and a distance of 15 cm or more between sites. The patients were 18 years of age or older (seven males and five females) and had provided written informed consent. Pregnant women were excluded. Individuals using oral or topical drugs for 1 week prior to the study were excluded. Twenty-four hours after application of the creams the plastic film was removed, and the lesions were gently cleansed and air-dried for 4 hours. Twenty-eight hours after application, two of the four lesions, one of which had received glucocorticoid and the other a control application (*15*), were cleansed with hexachlorophene and anesthetized by local injection of 1 percent lidocaine hydrochloride without epinephrine. One biopsy (1 by 1 to 2 by 4 cm) was taken from each of the two sites as previously described (*16*) by means of a Castroviejo keratome with a mean 0.375-mm shim setting for removal of the full thickness of the lesion (*17*). A small portion of the tissue was processed for histological examination of frozen sections so that the correct depth could be verified. The remainder was frozen in liquid nitrogen within 5 to 8 seconds of removal from patients.

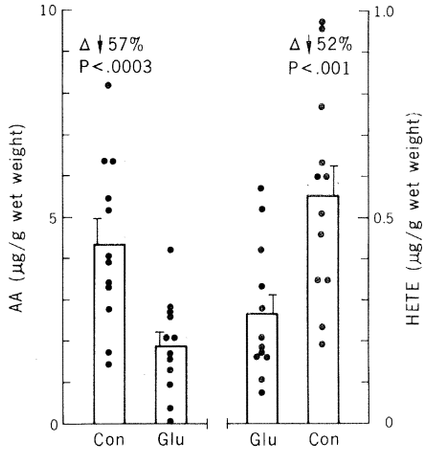

Fig. 1. Arachidonic acid (*AA*) and 12L-hydroxy-5,8,10,14-eicosatetraenoic acid (*HETE*) concentrations in 12 control (*Con*) and 12 glucocorticoid-treated (*Glu*) specimens of involved epidermis of psoriasis. The bars represent mean values and the vertical lines show one standard error of the mean ($N = 12$). Δ ↓ indicates the percentage decrease in glucocorticoid-treated versus control. Glucocorticoid treatment reduced the arachidonic acid content by 57 percent ($P < .0003$) and the HETE content by 52 percent ($P < .001$). Data were analyzed by means of Student's *t*-test for paired data, 11 degrees of freedom, and a one-tailed hypothesis.

The other two lesions were similarly treated (one glucocorticoid and one control) but no biopsies were taken. These unbiopsied (in vivo) lesions were observed at 3 days, but hormone and control applications were discontinued 2 days prior to observation. Tissues taken at biopsy were ground to powders (70 to 360 mg) at −78°C and suspended in a mixture of chloroform and methanol containing deuterium-labeled arachidonic acid (8 μg) and HETE (1.7 μg) as carriers. The carrier compounds were also labeled with ^3H or ^{14}C to aid subsequent purification by silicic acid chromatography and (after methylation) thin-layer chromatography. After conversion of HETE to the trimethylsilyl ether derivative, both compounds were subjected to combined gas-liquid chromatography and mass spectrometry. The degree of isotope dilution was determined by recording two ions representing deuterium-labeled and unlabeled species, respectively, during analyses [see (*3*)]. The biochemical data were analyzed statistically as summarized in Fig. 1.

The mean arachidonic acid concentration was 4.3 ± 0.6 (± standard error; $N = 12$) μg/g (wet weight) in control lesions and 1.9 ± 0.3 μg/g in glucocorticoid-treated lesions (57 percent decrease, $P < .0003$), whereas the corresponding HETE levels were 0.55 ± 0.07 and 0.27 ± 0.05 μg/g, respectively (52 percent decrease, $P < .001$). Similar results were obtained when values were expressed as nanograms per milligram of lesional protein [AA: 23.5 ± 3.3 ($N = 12$) and 9.8 ± 1.9, 58 percent reduction ($P < .0005$); HETE: 3.05 ± 0.47 and 1.53 ± 0.28, 50 percent reduction ($P < .0005$)] or as micrograms per milligram of lesional DNA [AA: 1.03 ± 0.18 ($N = 8$) and 0.516 ± 0.091, 50 percent reduction ($P < .008$); HETE: 0.125 ± 0.019 and 0.070 ± 0.014, 44 percent reduction ($P < .008$)]. These data show that glucocorticoid depresses the concentrations of arachidonic acid and HETE in lesional tissue. This may be brought about by inhibition of arachidonic acid release from lesional membrane phospholipids as indicated in Fig. 2, possibly by induction of phospholipase A_2 inhibitors, inhibition of phospholipase A_2

synthesis, increased degradation of phospholipase A_2, or to stimulation of re-esterification of arachidonic acid into membrane phospholipids or combinations of these.

Arachidonic acid and HETE concentrations in control lesions were lower than those we previously reported for untreated lesions (*3*) and than concentrations found in untreated lesions during the course of the present investigation. This may indicate that a component of the cream vehicle or the occlusive plastic dressings per se, or both (*13*), inhibit arachidonic acid release in psoriasis epidermis. It is interesting that the cream vehicle alone (*18*) and the occlusive plastic dressings alone (*19*) have therapeutic effects in psoriasis. The fact that we observed statistically significant depressions of arachidonic acid and HETE in lesions treated with glucocorticoid plus cream vehicle in comparison to those treated with cream vehicle alone indicates that the glucocorticoid has a high potency in normalizing the concentrations of arachidonic acid and HETE in psoriasis [the mean concentrations in glucocorticoid plus cream–treated lesions were reduced by 94 percent (HETE) and 95 percent (AA) in comparison to mean concentrations in untreated lesions from our previous study (*3*)].

Changes in the concentrations of arachidonic acid and HETE were correlated in glucocorticoid-treated ($r = .82$; $P < .01$) and control lesions ($r = .81$; $P < .01$). In our previous study (*3*) of untreated psoriasis lesions the changes in arachidonic acid and HETE levels were also correlated ($r = .97$; $P < .001$). Thus a change in arachidonic acid concentration appears to be accompanied by a concomitant change in HETE concentration. This is consistent with the notion that the reduction in HETE content is probably caused by inhibition of arachidonic acid release from cell membranes, and resultant substrate depletion rather than inhibition of lipoxygenase by glucocorticoid (Fig. 2).

Glucocorticoids are known to induce terminal differentiation (*20*) and directly inhibit cell proliferation in epidermis (*21*). This action may in part account for the therapeutic effect of glucocorticoids which usually becomes visibly significant between 4 and 14 days after the initiation of therapy. During this time lesions progressively revert to normal-appearing skin. A molecular correlate of this visible reversion between 4 and 14 days could be the normalization of arachidonic acid and HETE levels. Based on data in other eukaryotic cells we assumed that 28 hours should be ample time for skin penetration, glucocorticoid-receptor activation, messenger RNA transcription (*22*), and molecular but not visible modulation of the lesional phenotype. Therefore, we assayed arachidonic acid and HETE after only 28 hours of glucocorticoid exposure. Our assumption is supported by the fact that 3 days after application the degree of lesional redness in 8 patients and the thickness of the lesions in 9 of the 12 patients on the remaining glucocorticoid-treated site were similar to those observed at the time of initial evaluation (*23*). Thus it is probable that the reduction in arachidonic acid and HETE content is the result of glucocorticoid-induced, reversible, early molecular events because the data were obtained from tissue that had not been visibly improved by glucocorticoid.

It is known that (i) injury to skin tissue can induce psoriasis (*24*), (ii) tissue injury leads to the release of arachidonic acid (*25*), (iii) hydrocortisone (a glucocorticoid) inhibits skin inflammation (*26*) and the release of arachidonic acid in cultured cells (*10*), and (iv) testosterone and estrogen (nonglucocorticoids) do not inhibit skin inflammation (*26*) and have little or no effect on arachidonic acid release in cultured cells (*10*). Therefore, the inhibition of arachidonic acid release and the subsequent cascade of biologically potent molecules shown in Fig. 2 may be an important molecular mechanism for the pharmacologic activity of glucocorticoids. Presumably, nonglucocorticoid inhibitors of arachidonic acid release might also have therapeutic potential.

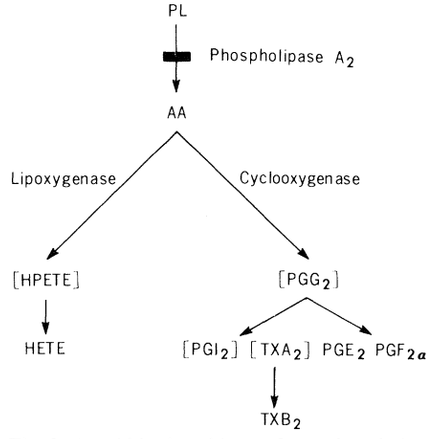

Fig. 2. Arachidonic acid transformations in lesional epithelium of psoriasis patients. The black bar indicates a probable blockade of phospholipase A_2 function as one important molecular event in the glucocorticoid treatment of psoriasis. The compounds within brackets are unstable intermediates which have not yet been directly demonstrated in epidermis (PGG_2, TXA_2, PGI_2, HPETE). The compound TXB_2 has recently been detected in lesional epithelium of psoriasis (*27*). Previously we demonstrated statistically significant increases in the mean lesional concentrations of arachidonic acid (26-fold), HETE (82-fold), PGE_2 (40 percent) and $PGF_{2\alpha}$ (86 percent) in comparison to uninvolved, visually normal psoriasis epidermis (*3*). Abbreviations: *PL*, phospholipid; *AA*, arachidonic acid; *HETE*, 12L-hydroxy-5,8,10,14-eicosatetraenoic acid; *HPETE*, 12L-hydroperoxy-5,8,10,14-eicosatetraenoic acid; PGG_2, prostaglandin G_2 an endoperoxide intermediate in prostaglandin and thromboxane synthesis (*28*); PGI_2, an unstable derivative of arachidonic acid, which has properties opposite to those of TXA_2 on platelet aggregation (*29*); TXA_2, thromboxane A_2 (*28*); TXB_2, thromboxane B_2 (*28*); PGE_2, prostaglandin E_2; $PGF_{2\alpha}$, prostaglandin $F_{2\alpha}$.

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References and Notes

1. E. J. Van Scott, *J. Invest. Dermatol.* **59**, 4 (1972).
2. J. J. Voorhees, *Annu. Rev. Med.* **28**, 467 (1977).
3. S. Hammarström, M. Hamberg, B. Samuelsson, E. A. Duell, M. Stawiski, J. J. Voorhees, *Proc. Natl. Acad. Sci. U.S.A.* **72**, 5130 (1975).
4. D. B. Glass, W. Frey II, D. W. Carr, N. D. Goldberg, *J. Biol. Chem.* **252**, 1279 (1977).
5. D. Wallach and I. Pastan, *ibid.* **251**, 5802 (1976).
6. T. Asakawa, I. Scheinbaum, R. Ho, *Biochem. Biophys. Res. Commun.* **73**, 141 (1976).
7. J. N. Fain and R. E. Shepherd, *J. Biol. Chem.* **250**, 6586 (1975).
8. H. I. Maibach and R. B. Stoughton, in *Steroid Therapy*, D. L. Azarnoff, Ed. (Saunders, Philadelphia, 1975), p. 174.
9. M. W. Greaves and W. McDonald-Gibson, *Br. J. Pharmacol.* **46**, 172 (1972); R. J. Gryglewski, B. Panczenko, R. Korbut, L. Grodzinska, A. Ocetkiewicz, *Prostaglandins* **10**, 343 (1975); Y. Floman and U. Zor, *ibid.* **12**, 403 (1976); V. A. Ziboh and J. T. Lord, *Fed. Proc. Fed Am. Soc. Exp. Biol.* **36**, 851 (1977).
10. S. L. Hong and L. Levine, *Proc. Natl. Acad. Sci. U.S.A.* **73**, 1730 (1976).
11. S. M. Bluefarb, F. M. Howard, E. Leibsohn, C. A. Schlagel, L. Wexler, *J. Int. Med. Res.* **4**, 454 (1976).
12. Diflorasone diacetate is 6α-fluoro-betamethasone 17,21-diacetate, which is to be marketed as Florone Cream, 0.05 percent by The Upjohn Company. The constituents of Florone Cream in addition to diflorasone diacetate are those given in (*13*).
13. The control cream vehicle contains stearic acid, sorbitan monooleate, sorbitan monostearate, polyoxethylene sorbitan monostearate, sorbic acid, citric acid, purified water, and propylene glycol. This control cream and Florone Cream were a gift from C. Schlagel (Upjohn Co.).
14. There were two tubes of cream available for each patient, one containing the glucocorticoid and the other the control cream. Each pair of tubes was randomized and identified with an A or a B. The four lesions chosen for treatment on each patient were numbered 1, 2, 3, and 4 for ease of record keeping during the visual evalua-

tions. By means of a table of random numbers, the two lesions that would receive an application from tube A were determined (two of lesions 1, 2, 3, and 4). The other two lesions received an application from tube B.

15. After the two lesions that were to receive an application from tube A were determined, one was randomly selected to be a biopsy site; likewise, the two lesions that were to receive an application from tube B were selected randomly. Therefore, one of the two lesions from each treatment was scheduled to be biopsied but the code (tube A and tube B) was not broken until after biochemical analyses were completed. The two remaining lesions, one having received a glucocorticoid and one a control cream treatment, were not biopsied but were left to be visually evaluated 2 days later.
16. J. J. Voorhees, E. A. Duell, M. Stawiski, E. R. Harrell, *Adv. Cyclic Nucleotide Res.* **4**, 117 (1974).
17. The surgically removed treated or control tissue used as starting material in this report is designated by several terms which are meant to be synonymous. These terms are lesional epithelium, lesional epidermis, lesional tissue, and diseased tissue. By frozen section histology we estimate that keratinocytic epithelium (epidermis) occupies 80 to 95 percent of the specimen volume. The 80 to 95 percent range is the result of surgical technique, vertical lesional configuration, and the presence of non-keratinocytes (inflammation-associated cells, endothelial cells, fibroblasts, and collagen). Therefore, although 5 to 20 percent of the specimen volume is occupied by collagen and nonepithelial cells, the latter of which probably contain the arachidonic acid transformation cascade (Fig. 2), it seems probable that the data in Fig. 1 are derived mainly from lesional epithelium.
18. J. S. Comaish and J. S. Greener, *Br. J. Dermatol.* **94**, 195 (1976).
19. L. Fry, J. Almeyda, R. M. H. McMinn, *ibid.* **82**, 458 (1970).
20. A. Kojima, M. Sugimoto, H. Endo, *Dev. Biol.* **48**, 173 (1976).
21. C. Marcelo, Y. Kim, I. Pengrin, *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **36**, 773 (1977).
22. P. L. Ballard, J. D. Baxter, S. J. Higgins, G. G. Rousseau, G. M. Tomkins, *Endocrinology* **94**, 998 (1974).
23. Mild lesional pallor in 4 of 12 patients was still evident at 3 days. The degree of redness in 7 patients did not change between the initial application and day 3. There was pallor at day 1 but return to the initial degree of redness in 1 of the 12 patients. Lesional thickness as estimated by the examining finger remained decreased in 3 of 12 patients at day 3. No change in thickness occurred in 7 patients between the initial application and day 3. In 2 of the 12 patients, thickness was decreased at day 1 but returned to a thickness similar to that observed at the time of initial application.
24. D. D. Eddy, E. Aschheim, E. M. Farber, *Arch. Dermatol.* **89**, 579 (1964).
25. C. Pace-Asciak, K. Morawska, F. Coceani, L. S. Wolfe, in *Prostaglandin: Symposium of the Worcester Foundation for Experimental Biology* (Wiley, New York, 1968), p. 371.
26. G. Tonelli, L. Thibault, I. Ringler, *Endocrinology* **77**, 625 (1965).
27. S. Hammarström, M. Hamberg, E. A. Duell, M. A. Stawiski, T. F. Anderson, J. J. Voorhees, in preparation.
28. B. Samuelsson, in *Advances in Prostaglandin and Thromboxane Research*, B. Samuelsson and R. Paoletti, Eds. (Raven, New York, 1976), vol. 1, p. 1; M. Hamberg, J. Svensson, B. Samuelsson, in *ibid.*, p. 19.
29. S. Moncada, R. Gryglewski, S. Bunting, J. R. Vane, *Nature (London)* **263**, 663 (1976).
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Thyroid Hormone Action: The Mitochondrial Pathway

Abstract. *The subcellular compartments have been investigated to compare proteins capable of binding triiodothyronine and thyroxine; specific binders have been found in cytosol, nuclei, and mitochondria from rat liver and kidney. The binding protein from the inner mitochondrial membrane had the highest association constant ($>10^{11}$ liters per mole), suggesting possible direct hormone action on the mitochondria. Binding of hormone analogs was found to be related to known physiological potency, and stereospecific discrimination between* L- *and* D-*thyroxine was observed. The saturable receptor was found in the mitochondrial membranes of rat liver, kidney, myocardium, and skeletal muscle but not in mitochondria from the unresponsive tissues: brain, spleen, and testis. Oxidative phosphorylation by mitochondrial vesicles from hypothyroid rats increased after the addition of physiological concentrations of triiodothyronine, which corroborated direct hormone action on mitochondria.*

Postulated models for steroid hormone action involve nuclear localization of the hormones after binding by a cytoplasmic receptor. A somewhat similar mechanism has seemed plausible for thyroid hormone action in view of reported binding of the thyroid hormones to the cell nucleus or to acidic nuclear proteins (*1*–*4*). We have been examining the subcellular binding of the thyroid hormones to the nucleus, cytoplasm, and the mitochondria as well, to determine the biological relevance of the previously described mitochondrial receptor (*5*) by comparing its binding characteristics to that of other known cellular binding sites and relating binding affinity and hormonal action.

Male Sprague-Dawley rats weighing more than 300 g were obtained from Charles River Breeding Farms. Surgically thyroidectomized rats (Hormone Assay Laboratories) given 0.2 percent $CaCl_2$ in tap water were kept at least 1 month on a low-iodine diet, by which time hypothyroid stigmata had become obvious and serum triiodothyronine (T_3) and thyroxine (T_4) concentrations were in the hypothyroid range. Normal rat serum T_4 concentrations were (mean ± standard deviation) 4.8 ± 1.6 μg/dl in contrast to 1.1 ± 0.6 μg/dl in hypothyroid serums, and T_3 values were undetectable. Isolation and handling of nuclei and cytosol from rat liver and kidney have been described (*1*, *6*). Isolation of mitochondria and preparation of mitochondrial membrane protein were as described elsewhere (*5*), except that the initial homogenate was centrifuged at approximately 620*g* (corresponding to 2250 rev/min in the Sorvall SS-34 head). The mitochondrial membrane protein obtained after Triton X-100 treatment was partially purified. Gel filtration of mitochondrial membrane protein was carried out on a calibrated column of Sephadex G-200 (90 by 1.5 cm) at a flow rate of ~ 15 ml/hour with 0.05*M* tris-HCl buffer, *p*H 7.0, and 3-ml fractions were collected for determinations of absorbance and other analytic procedures. Electron micrographs were interpreted as verifying the high purity of the mitochondria. An electrophoretic study of mitochondrial membrane protein with added labeled T_3 (*5*) showed it to have a mobility greater than that of serum prealbumin, whereas nuclear protein had a mobility less than that of serum albumin, judging from scans of paper strips. Separation of inner and outer mitochondrial membranes was done by the method of Schnaitman and Greenawalt (*7*) and verified by determinations of monoamine oxidase, cytochrome oxidase, and malic dehydrogenase.

Samples of ^{125}I-labeled T_3 and T_4, provided by Abbott Laboratories, were checked for purity by paper electrophoresis and chromatography. Phosphate labeled with ^{32}P was purchased from Squibb. All other chemicals were obtained from commercial sources and were of the highest purity available.

Determination of binding constants. Hormone concentrations in the subcellular constituents studied were: cytosol, 2.3×10^{-2} to 300×10^{-2} μ*M*; intact nuclei or acidic nuclear protein (*1*), 0.75×10^{-4} to 10×10^{-4} μ*M*; and mitochondrial membrane protein, 0.75 to 10 p*M*. The total radioactivity in each tube was determined by counting for ½ to 2 minutes during brief removal from the incubation bath. Incubations were for 1 hour at 0°C for cytosol and mitochondrial preparations and at 37°C for nuclei. The volumes of the protein solutions were 0.4 ml plus 40 μl of isotope solution. The medium consisted of 0.05*M* tris-HCl and 0.01*M* EDTA, at *p*H 7.0 for mitochondria and at *p*H 7.4 for cytosol and nuclei. In studies of mitochondrial protein binding a general range of 1 absorbance (280 nm) unit per milliliter (~ 0.3 to 1 mg/ml) was sought. The actual protein concentrations were determined subsequently and