

SHORT REPORTS

Inhibition of Arachidonic Acid Oxidation
in vitro by Vehicle Components

Neal S. Penneys

*Department of Dermatology, University of Miami School
of Medicine, Miami, Fla 33101, USA*

Received May 21, 1981

Abstract. In this work, we observed that several vehicle components which are complex lipids inhibited the oxidation of arachidonic acid in the oxygen monitor assay. Inhibition of oxygen consumption in this assay, a relatively non-specific finding, may reflect inhibition of lipoxygenase and/or prostaglandin synthetase activity or possibly sequestration of arachidonic acid. Theoretically, similar effects could occur *in vivo*. Consideration should be given to the formulation of vehicles which maximize the anti-inflammatory effects of vehicle components.

Key words: Vehicles; Prostaglandins; Arachidonic acid

The accessibility of skin has led to a variety of topical therapies directed at limiting cutaneous inflammation. The most widely used preparations contain anti-inflammatory glucocorticoids in either a cream, ointment, gel or liquid vehicle. The anti-inflammatory effects of these preparations have been routinely attributed to corticosteroids and to their protean *in vitro* anti-inflammatory effects (2, 3). Vehicle components are overlooked as possible topical anti-inflammatory agents. Yet it is quite clear that certain topical preparations without "active" ingredients can be soothing and reduce inflammation in the skin.

The products of arachidonic acid oxidation are chemotactic (12L-hydroxy-5, 8, 11, 14-eicosatetraenoic acid) (1) and pro-inflammatory (PGE₂) (6). In this report, a representative sample of the most common compounds in topical vehicles have been evaluated for their ability to interfere with the *in vitro* oxidation of arachidonic acid. Our results indicate that some of the substances in vehicles can inhibit this oxidation and therefore might function *in vivo* as anti-inflammatory agents.

METHODS

1. Preparations of fetal calf skin arachidonic acid cyclooxygenase

All procedures were performed at 4°C. Frozen fetal calf skin was homogenized in 0.154 M KCl (1:3, w/v) in a Waring Blender for one minute at high speed, and then strained. The remaining tissue was re-homogenized and filtered. The pooled homogenates were subjected to sequential centrifugations, the pellets discarded until the last centrifugation which was at 105 000×g for one hour. The microsomal pellet was resuspended in 20 ml of 0.154 M KCl. Fifteen volumes of chilled acetone were added with stirring for 10 min and the precipitate collected on filter paper pre-treated with acetone in a Buchner funnel. The precipitate was washed in acetone, collected as above, extracted twice with chilled pentane, collected, dried, and stored at -50°C until use. For assay, the microsomal preparation was prepared by homogenizing 5 mg of acetone powder in 1 ml of 0.1 M Tris-HCl buffer, pH 8.5, in a small Dounce ball-type homogenizer.

2. Arachidonic acid oxidation assay

Oxygen concentrations were measured with a Model 53 oxygen monitor (Yellow Springs Instrument Company) equipped with a Model 5301 bath assembly as reported by Smith and Lands (5). The oxidation of arachidonic acid by arachidonic acid cyclo-oxygenase was determined by measuring oxygen absorption at a constant temperature of 30±0.5°C. Arachidonic acid (99% purity) was from Sigma Chemical Co., St. Louis, Mo., USA. Its purity was established by gas-liquid chromatography. In most experiments, the total volume of the reaction mixture in the chamber was 3 ml. Additions of enzyme preparations and aliquots of the material to be tested were made through the side of the electrode holder. Control incubations in these studies contained maximal concentrations of arachidonic acid and acetone powder extract of fetal calf skin microsomes. When material to be tested was solubilized in a solvent other than assay buffer, solvent controls were included in the study.

RESULTS AND DISCUSSION

The data are presented in Table I. As representative examples, Fig. 1 and 2 illustrate experiments that test inhibitory and non-inhibitory substances. In all experiments, materials that inhibited the oxidation of arachidonic acid did so in a linear fashion that was proportional to the quantity of material added to the reaction mixture.

Our results suggest the following: 1) waxes (lano-

Table 1. Inhibition of arachidonic acid oxygenation by components of vehicles

1. Substances having little effect	% Inhibition/mg ^a
Zinc oxide	8.4±6.4 (n=15)
Propylene glycol	0.5±3.6 (n=15)
Stearyl alcohol	-3.2±5.1 (n=4)
Potassium sorbate	-2.4±2.0 (n=4)
Dimethylpolysiloxane	0.2±0.4 (n=4)
Potassium aminobenzoate	-1.1±3.1 (n=15)
Polyethylene glycol 6000	1.6±6.3 (n=15)
Camphor	-5.2±4.6 (n=15)
Menthol	2.1±1.1 (n=15)
Lanolin (anhydrous)	-3.7±8.7 (n=18)

2. Substance having an inhibitory effect	% Inhibition/mg ^a
Polyoxyethylene-20-sorbitan monopalmitate	54± 3 (n=15)
Mineral oil	29±11 (n=9)
Polyoxyethylene-80-sorbitan monopalmitate	47± 8 (n=5)
Crisco ^b	34± 8 (n=15)
Aquaphor ^b	29±20 (n=15)
Aveeno, oilated ^b	30± 8 (n=8)
Aloe extract ^b	180±27 (n=23)
Aloe gel (from plant)	94±17 (n=16)
Lubriderm ^b	40± 6 (n=15)
Cetaphil ^b	11± 5 (n=10)

^aData were converted to % inhibition per mg inhibitory substance ± standard deviation so that data from experiments such as those in Fig. 1 and 2 could be presented in table form and compared.

^bCrisco, a mixture of partially hydrogenated soybean and palm oils, monoglycerides, diglycerides and superglycerinated fats; Aquaphor, a mixture of petrolatum, mineral oil, wax 150, and wool wax alcohols; Lubriderm, a mixture of water, petrolatum, lanolin, lanolin alcohol, sorbitol, stearic acid, cetyl alcohol, fragrance, methyl-, propyl-, and butyl parabens, triethanolamine, and sodium chloride; Cetaphil, a mixture of water, cetyl alcohol, propylene glycol, sodium sulfate, stearyl alcohol, and methyl-, propyl-, and butylparabens; Aloe extract, a commercial lyophilized preparation of aloe gel; Aveeno, oilated, a mixture of colloidal oatmeal emollient oils.

lin derivatives) do not interfere with the oxidation of arachidonic acid in our assay system, 2) petrolatum (4) and related compounds (mineral oil) as well as complex vehicles that contain these substances inhibit, 3) certain lipid-containing emulsifiers interfere, and 4) camphor and menthol, common anti-pruritic agents, have no effects, but aloe gel (direct from the plant) and a commercial aloe extract (powder) interfere with the oxidation.

Our data are preliminary and reflect only the ability of a compound to interfere with the oxidation of arachidonic acid in a relatively non-specific in vitro assay. Besides direct enzyme inhibition, addition of relatively insoluble lipids could seques-

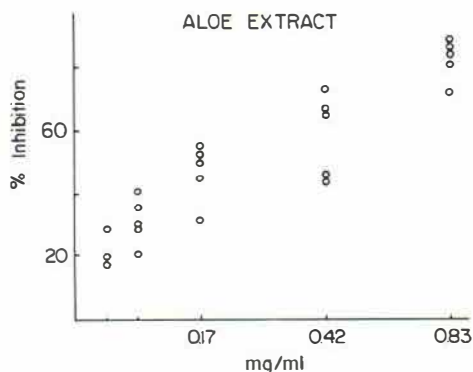


Fig. 1. Inhibition of arachidonic acid oxygenation by increasing quantities of aloe extract.

ter arachidonic acid and indirectly reduce oxygen consumption. Our enzyme preparation probably contains at least two enzymes that are capable of oxidizing arachidonic acid: a lipoxygenase and prostaglandin synthetase. Inhibition of either would have a net anti-inflammatory effect in vivo. Of course, other pathways function in the skin during inflammation and vehicle components could have different effects (even pro-inflammatory) on these pathways. The in vivo effects of a topical agent are an amalgam of poorly understood factors which include penetration of the skin, access to the appropriate microenvironment, and finally specific biochemical effects. If we assume that some of the compounds in vehicles could penetrate into the skin and be anti-inflammatory by inhibiting arachidonic acid oxidation or some other inflammatory mediator-generating pathway, it would be reason-

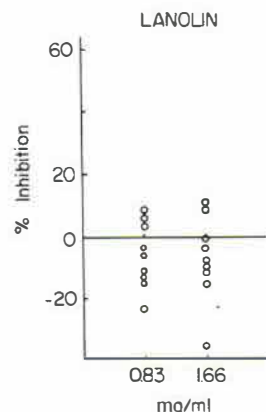


Fig. 2. The effects of anhydrous lanolin on the oxygenation of arachidonic acid.

ble to construct vehicles that contain as much of these components as possible so as to maximize the anti-inflammatory effects of the total preparation.

ACKNOWLEDGEMENT

This study was supported by the Dermatology Foundation of Miami. Dr Penneys is the recipient of Research Career Development Award AM 00203 from the National Institutes of Health.

REFERENCES

1. Goetzl, E. J., Woods, J. M. & Gorman, R. R.: Stimulation of human eosinophil and neutrophil polymorphonuclear leucocyte chemotaxis and random migration by 12L-hydroxy-5, 8, 10, 14-icosatetraenoic acids. *J Clin Invest* 59: 179, 1977.
2. Hammarstrom, S., Hamberg, M., Duell, E. A., Stawiski, M. A., Anderson, T. F. & Voorhees, J. J.: Glucocorticoid in inflammatory proliferative skin disease reduces arachidonic acid and hydroxyicosatetraenoic acids. *Science* 197: 994, 1977.
3. Nijkamp, F. P., Flower, R. J., Moncada, S. & Vane, J. R.: Partial purification of rabbit aorta contracting substance releasing factor and inhibition of its activity by anti-inflammatory steroids. *Nature* 263: 379, 1976.
4. Penneys, N. S., Eaglstein, W. & Ziboh, V.: Petrolatum: interference with the oxidation of arachidonic acid. *Br J Dermatol* 103: 247, 1980.
5. Smith, W. L. & Lands, W. E.: Oxygenation of polyunsaturated acids during prostaglandin biosynthesis by sheep vesicular gland. *Biochemistry* 11: 327, 1972.
6. Søndergaard, J., Helen, P. & Jørgensen, H. P.: Human cutaneous inflammation induced by prostaglandin E. *J Pathol* 109: 239, 1973.

Decreased Lymphocyte Aryl Hydrocarbon Hydroxylase and Glutathione S-Transferase Activities in Patients with Hand Dermatitis

Neeta Singh,¹ Knud Hamann²
and Jørgen Clausen^{1, 3}

¹The Neurochemical Institute, Rådmandsgade 58, DK-2200 Copenhagen N, ²Department of Dermatology, Gentofte Hospital, DK-2900 Hellerup, and ³Roskilde University, Laboratory of Biochemistry and Toxicology, Institute of Biology and Chemistry, DK-4000 Roskilde, Denmark

Received March 26, 1981

Abstract. Aryl hydrocarbon hydroxylase (AHH) and glutathione S-transferase (GST) (EC 2.5.1.18) activity

were measured in human lymphocytes of peripheral blood from 8 patients with irritant or allergic contact dermatitis of the hands, and compared with data from a control group. Both AHH and GST activity was found to be significantly depressed in the allergic and irritant contact dermatitis subjects, as compared with controls. It is suggested that AHH may be a quantitative marker of the inflammatory status.

Aryl hydrocarbon hydroxylase (AHH) is a cytochrome P-450 dependent microsomal enzyme responsible for metabolism of a wide variety of hydrophobic xenobiotics (foreign compounds) (1, 17). P-450 refers to the general class of microsomal heme proteins which serve as the terminal oxygenase in the oxidative metabolism of drugs and various chemicals (1). AHH is inducible in human tissues by 3-methylcholantrene, benzo(a)pyrene and coal tar (1, 3, 12, 16). It has been identified in many mammalian tissues (1, 3).

The transformation products of AHH (e.g. epoxides and hydroxyamines) may further be transformed and eliminated by means of glutathione S-transferase (GST) (EC 2.5.1.18) (7).

Lymphocytes provide a simple method of studying the inducibility of AHH activity to benzo(a)pyrene and 3-methylcholantrene (1, 3). The present communication concerns the determination of levels of both AHH and GST in subjects suffering from irritant or allergic contact dermatitis. Their enzyme levels were compared with those of normal healthy subjects.

MATERIALS AND METHODS

Benzo(a)pyrene, reduced glutathione and 1-Cl-2,4-dinitrobenzene were obtained from Sigma Chemical Co., St. Louis (USA) and NADPH (tetrasodium salt) from Boehringer, Germany. 3-OH-benzo(a)pyrene was a gift from Professor H. V. Gelboin, IIT Research Institute, 10 West 35 Street, Chicago, Ill, USA. All other chemicals were of reagent grade obtained from BDH, S. Merck, and other standard sources.

Control subjects. 28 normal individuals (14 males and 14 females) of 34 years median age and with no history of allergic or toxic skin reactions and free from any infectious disease 3 weeks prior to blood sampling were used as controls (15).

Patients. In the period May-June 1980, 8 patients suffering from contact dermatitis on the hands were referred to the Department of Dermatology, Gentofte Hospital. Their mean age was 47 years (25-61). They were patch-tested with 24 standard allergens as recommended by the ICDRG (International Contact Dermatitis Research Group). Additionally they were patch-tested with supple-