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.

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

Neeta Singh,[1] Knud Hamann[2] and Jørgen Clausen[1, 3]

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

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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-

Table 1. *Specific activities of AHH and glutathione S-transferase in different subjects*

Subjects	AHH, pmoles/min/mg protein				Glutathione S-transferase, nmoles/min/mg protein			
	Homogenate	% decrease[a]	Microsomes	% decrease[a]	Homogenate	% decrease[a]	Cytosol	% decrease[a]
Controls pooled (28)	2.31		14.36		34.67		60.67	
Contact dermatitis pooled (8)	1.10[b]	52	4.51[b]	69	19.29[b]	44	61.68	
Irritant contact dermatitis pooled (4)	1.21	48	4.86[b]	66	21.45	38	66.74	
Allergic contact dermatitis pooled (4)	0.99[b]	57	4.17[b]	71	17.13[b]	51	56.63	6

The values in parentheses indicate the numbers of subjects taken.
[a] Percentage changes as compared with the respective control values.
[b] The values were statistically significant, using the Wilcoxon's test $p<0.05$.
Each value is the median.

mentary test substances according to the recommendations of the ICDRG. One patient (no. 5) was also tested with 18 standard allergens recommended by the Scandinavian Photodermatitis Research Group (SPDRG). The substances were supplied by Trolab, Copenhagen, and Epikon, Finland respectively. Finn Chambers® were fixed with Scanpor®. The chambers were removed after 24 h and results were read at 1, 2 and 7 days. Reactions were graded according to recommendations of the ICDRG (18). There were 4 patients with allergic contact dermatitis (2 males and 2 females) and 4 patients (3 males and 1 female) with irritant contact dermatitis.

Isolation of lymphocytes and subcellular fractions. 60 ml venous blood was collected from a cubital vein into heparinized glass tubes. The lymphocytes were isolated by the Ficoll–Hypaque sedimentation method of Bøyum (2), giving a purity of 95%.

A homogenate of lymphocytes from 60 ml blood was prepared in 20 ml 0.25 M sucrose by using a Potper Elvehjelm type glass tissue homogenizer (400 rev/min). For the isolation of microsomes and cytosol, mitochondrial supernatant (8000 g_{av} supernatant) was centrifuged at 105000 g_{av} for 60 min in an IEC/B-60 ultracentrifuge. The microsomal pallet thus obtained was suspended in 0.25 M sucrose 0.05 Tris-HCl buffer, pH 7.5. The supernatant was used for estimation of glutathione S-transferase enzyme activity.

Biochemical assays

Protein assay. The concentration of protein in the crude lymphocyte homogenate, microsomes and cytosol was determined according to the method of Lowry et al. (11).

AHH assay. AHH was estimated fluorimetrically by determining the amount of hydroxylated benzo(a)pyrene produced according to the method of Nebert & Gelboin (13). One unit of the enzyme was the amount of enzyme catalysing the formation of hydroxylated product causing fluorescence equal to that of 1 pmole of 3-OH benzo(a)-pyrene per minute.

Glutathione S-transferase assay. This was assayed according to the method of Habig et al (10). One unit of enzyme is equal to the nmole product formed per minute. Specific activities of the enzymes are expressed as units per mg protein.

Statistical evaluation. The data were evaluated statistically by non-parametric assay. The values are presented as rank median. Wilcoxon's test was used to detect the significant difference between groups.

RESULTS

Patients suffering from contact dermatitis, either irritant or allergic, showed a reduced activity of AHH in the crude lymphocyte homogenate as well as in the microsomal fraction, as compared with controls (Table 1). The decrease was 48% and 66% in homogenate and microsomal fraction respectively for irritant contact dermatitis patients. Patients with allergic contact dermatitis showed correspondingly a decrease of 57% and 71% in crude homogenate and microsomal fraction respectively. The decrease was significant for AHH of lymphocytes in patients with contact dermatitis of the hands.

The homogenate fraction of GST was also significantly reduced in contact dermatitis patients (Table 1).

DISCUSSION

Ciaccio & Fruncillo (9) found reduced hepatic AHH in mice after a 15% burn. It appears that AHH decreases in response to inflammatory conditions (4) and that it increases in many tissues (8) in response to xenobiotics such as benzo(a)pyrene.

Bickers & Kappas (1) were the first to measure

AHH in human psoriatic and atopic skin. They examined 6 patients with generalized psoriasis and 3 patients with severe atopic dermatitis and found that in vivo induction by application of coal tar caused induction of cutaneous AHH activity that varied from 2 to 5 fold over the activity in untreated skin areas. There appeared to be no difference in induction between individuals with psoriasis vis-à-vis atopic dermatitis. Subsequently, others have found reduced in vitro AHH activity in epidermis from both psoriatic lesions and clinically normal lesion-free skin from psoriatic patients (5, 6, 15). Induction of epidermal AHH activity by benzanthracene was subnormal. It was proposed that the decreased enzyme activity may be related to a primary genetic abnormality of the disease (5). Decreased AHH activity and inducibility in patients with localized palmo-plantar pustular psoriasis have been presented (15). The decreased AHH activity and inducibility have been demonstrated to be a generalized tissue abnormality in patients with psoriasis (6), though Ruzicka et al. (14) did not find decreased lymphocyte AHH activity in patients with psoriasis.

We found evidence of significantly decreased lymphocyte AHH and GST activity in patients with contact dermatitis on the hands. This change in lymphocyte activity could be explained by the inflammatory response related to the dermatitis of the hands (1, 9). We propose that the decrease in AHH activity is not a specific genetic abnormality of psoriasis, but a uniform reaction in all conditions with inflammatory skin diseases. The inflammation may paralyse the metabolizing system. Further work is needed to confirm that the AHH is a quantitative marker of inflammatory skin status.

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