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ARYL HYDROCARBON HYDROXYLASE ACTIVITY AND PSORIASIS

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Abstract—Aryl hydrocarbon hydroxylase (AHH) has been measured in the skin, jejunum and liver of normal and psoriatic individuals. We have been unable to confirm previous reports of an abnormality in AHH activity in patients with psoriasis. Re-examination of the laboratory records on which the original reports were based leads us to doubt their veracity and validity.

Microsomal cytochrome P-450 dependent mono-oxygenases mediate the biotransformation of a wide range of xenbiotics and endogenous substrates [1]. The mono-oxygenase system includes at least two distinct cytochrome species with individual substrate specificities, which serve as the terminal oxidase for the electron transport chain that operates in the hydroxylation of lipophilic substrates [2]. In general cytochrome P-448 dependent activities are induced by polycyclic hydrocarbons such as benzanthracene, whereas cytochrome P-450 dependent activities are induced by drugs such as phenobarbitone [3]. The principal organ for mono-oxygenase activity is the liver, although activity has been shown to be present in a variety of extrahepatic tissues including kidney, lung, intestine, and skin [4]. Aryl hydrocarbon hydroxylase (AHH), as measured by the hydroxylation of benzpyrene [5], is a mono-oxygenase activity that has been extensively studied in both hepatic and extra-hepatic tissues [6], where it has been shown to be inducible both *in vivo* [6] and *in vitro* by various drugs, carcinogens and insecticides [7].

AHH activity has recently been measured in human epidermal suction blisters, and has been reported to be lower and less inducible than normal in both the clinically normal skin [8] as well as in the lesions of skin [9–12] from patients with psoriasis. In addition it has been reported that the induction of AHH activity by benzanthracene, *in vitro*, is also defective in the epidermis and other tissues of patients with psoriasis [10, 11]. These findings have been taken to explain the suspected decrease in susceptibility to cancer of patients with psoriasis [12], in addition to differences in the response of skin to topical drugs [13], and the hepatic metabolism of antipyrine [14]. Changes in AHH activity in response to cellophane tape stripping of the skin [15] also suggest that AHH activity is implicated in epidermal cell regulation, an abnormality of which is a major feature of the plaques of psoriasis.

The present study began as an investigation into the nature of the defect in AHH activity and how it might lead to an increase in cell proliferation of psoriasis. The inability to reproduce the initial findings led to a reinvestigation of the role of AHH in psoriasis.

METHODS

Human liver wedge biopsies were obtained from patients undergoing surgery; jejunal biopsies were obtained using a Crosby capsule and epidermal suction blisters were obtained from the forearm using 2 blister cups that gave five blisters of 5 mm dia per cup. Microsomal fractions were prepared from each of these tissues as follows: samples were homogenised using a glass/glass homogeniser, and centrifuged at 9000 *g* for 20 min. The resulting supernatant was further centrifuged at 100,000 *g* for 1 hr and the microsomal pellet resuspended in 0.1 M phosphate buffer pH 7.4. The microsomal fraction from mouse skin was prepared according to established procedures [16]. Mono-oxygenase activity was also determined in human jejunum samples using whole tissue homogenates obtained by glass/glass homogenisation. Protein concentration was determined according to the method of Lowry *et al.* [17].

Enzyme assays. Mono-oxygenase activity was measured using benzpyrene, ethoxycoumarin and ethoxyresorufin as substrates. All fluorescence measurements were made using a Perkin–Elmer model LS3 fluorescence spectrometer.

(a) *Benzo[a]pyrene hydroxylation*

AHH activity, as measured by the hydroxylation of benzo[a]pyrene (BP) was determined by three methods:

Method 1—Nebert and Gelboin [5]

This was the method used in the original work [8–12, 15]. In this assay the phenolic products of mono-oxygenase action on BP are extracted into sodium hydroxide and determined fluorimetrically. The reaction mixture in a final volume of 1.0 cm^3 contained 100 μmoles sodium phosphate buffer pH 7.4; 1.2 μmoles NADPH; 3.0 μmoles $MgCl_2$; and varying amounts of tissue preparation. Incubations were performed in round bottom test tubes in a shaking water bath at 37°. The reaction was started by the addition of 100 nmoles of benzpyrene in 25 μl

acetone and terminated after 30 mins (3 mins for liver) by the addition of 2.0 cm^3 of acetone. The resulting acetone aqueous phase was extracted with 3.0 cm^3 of hexane and after centrifugation, 2.0 cm^3 of the upper hexane layer transferred to another tube and extracted with 3.0 cm^3 of 1.0 M sodium hydroxide. The fluorescence of the sodium hydroxide phase was measured at 522 nm emission, 396 nm excitation. Zero time incubations to which acetone was added prior to the addition of benzpyrene, and minus tissue blanks which contained all assay ingredients except tissue preparations were used as controls. The results were expressed as picomole (10^{-12}) equivalents of 3-OH BP using a calibration curve obtained by the addition of known amounts of 3-OH BP to zero time incubations, followed by extraction in the usual manner.

Method 2—Dehnen, Taningas and Roos [18]

This method is based on the direct fluorimetric measurement of BP metabolites after the addition of Triton X 100 and triethylamine to the incubation mixture. Incubations were performed in a shaking water bath at 37° in a final volume of 300 μl containing; 0.1 M sodium phosphate buffer pH 7.4; 3 mM $MgCl_2$; 1.2 μmoles of NADPH and various amounts of microsomal preparation. The reaction was started by the addition of 100 nmoles of BP and terminated after 30 mins by the addition of 25 μl of 10% (w/v) Triton X 100 in triethylamine. The fluorescence of the sample was recorded between 450 and 550 nm at an excitation wavelength of 435 nm. Zero time incubations were used as controls. Results were expressed as picomole (10^{-12}) equivalents of 3-OH BP using a calibration curve obtained by the addition of known amounts of 3-OH BP to zero time incubations.

Method 3—Yang and Kicha [19]

In this method the oxidative metabolism of BP is monitored by recording the NADPH dependent decrease in fluorescence of benzpyrene bound to microsomes. Incubations were performed in a final volume of 300 μl in a micro-cuvette at 37°. Microsomes were suspended in 0.1 M sodium phosphate buffer pH 7.4 containing 5 mM $MgCl_2$ and 0.1 mM EDTA. 0.05–0.5 nmoles of BP were added in 2 μl of acetone and after allowing the contents to equilibrate the fluorescence reading of the sample was adjusted using the autoconcentration scale of expansion facility. The reaction was started by the addition of 0.2 μmoles of NADPH in 5 μl of phosphate buffer. Fluorescence readings were integrated on a 16.8 second time cycle and the decrease in fluorescence at 387 nm excitation 407 nm emission was monitored for 10 min. Results were expressed as picomoles (10^{-12}) of BP metabolised.

(b) *7-Ethoxycoumarin O-de-ethylation*

Ethoxycoumarin O-de-ethylase activity was measured using the method of Greenlee and Poland [20].

(c) *Ethoxyresorufin O-de-ethylation*

The de-ethylation of ethoxyresorufin was measured using the method described by Pohl and Fouts [21]. Results were expressed as picomoles (10^{-12}) of resorufin produced per minute per g tissue using Rhodamine B as a standard.

3. In vitro *induction of BP metabolism by benzanthracene*

The tissue culture system used to investigate the induction of AHH activity was identical to that used in previous work [8–12, 15]. Whole liver pieces approximately equivalent to a total percutaneous needle biopsy sample (3 mm dia and 3–5 cm in length), jejunal biopsies obtained using a Crosby capsule; and whole epidermal blisters were placed in the culture media and incubated for 18 hr in 95% O_2 and 5% CO_2 with or without benzanthracene.

RESULTS

1. *Assay characteristics*

The detection limits for each assay are shown in Table 1. The results have been expressed in two ways, absolute sensitivity and tissue sensitivity. The absolute sensitivity gives an indication of the smallest amount of chemical change that has to occur before significant differences between samples and blanks can be detected, and therefore relates to the magnitude and reproducibility of blank values. The absolute sensitivity was not affected by variations in parameters such as final extraction volumes and fluorimeter sensitivity. For example, although decreasing the final volume used for the extraction of metabolites in the method of Nebert and Gelboin increases the fluorescence of the sample, the values for the blank are increased by an identical magnitude. The exception to this is the direct, in cuvette, method of Yang and Kicha [19] where absolute sensitivity is determined primarily by the stability of fluorimeter readings at high sensitivity. The tissue

Table 1. The sensitivity of the three methods used to determine AHH activity

Method	Absolute sensitivity	Tissue sensitivity
1. Nebert and Gelboin [5]	1 pmole	25 mg
2. Dehnen, Taningas and Roos [18]	0.2 pmole	2 mg
3. Yang and Kicha [19]	0.2–0.5 pmole*	1.0 mg

* Variable sensitivity depending upon the scale expansion of fluorimeter readings.

Tissue sensitivity is the minimum amount of skin from hairless mice needed to detect AHH activity by each method.

Table 2. Mono-oxygenase activity in microsomal preparations from mouse skin and human skin

	Mouse	Human
AHH activity Method 1 pmol/min/g tissue	1.68 ± 0.32 n = 20	*n.d. (50 mg) n = 8
AHH activity Method 2 pmol/min/g tissue	4.58 ± 0.97 n = 6	n.d. (30 mg) n = 7
AHH activity Method 3 pmol/min/g tissue	42 ± 11 n = 6	n.d. (20 mg) n = 5
Ethoxycoumarin O-de-ethylation pmol/min/g tissue	14.6 ± 3 n = 20	n.d. (20 mg) n = 4
Ethoxyresorufin O-de-ethylation pmol/min/g tissue	12.3 ± 4.2 n = 12	n.d. (20 mg) n = 2

Results are expressed as the mean ± S.E. Maximum amounts of tissue wet weight used for the determination of activity in human skin are given in parentheses.
* n.d. = not detectable.

sensitivity gives an indication of the amount of skin needed to produce changes equivalent to the absolute sensitivity. The data shown in Table 1 is based on the amount of skin from adult hairless mice needed to detect activity using each method. In general AHH activity in mouse skin is reported to be between 10 to 15 times higher than in human skin [22].

2. *Mono-oxygenase activity in microsomes from mouse and human skin*

The microsomal fraction of mouse whole skin exhibited mono-oxygenase activity towards all three substrates studied (Table 2). In agreement with previous workers the actual value for microsomal AHH activity varied widely depending on the assay used [5, 18, 19]. In contrast to mouse skin, microsomal fractions prepared from human epidermis of 11 normal and 19 psoriatic individuals showed no detectable mono-oxygenase activity, even when using tissue amounts up to 20 times greater than those needed to detect activity in mouse skin (Table 1). In addition no detectable mono-oxygenase activity could be demonstrated using whole epidermal homogenates, or nuclear or soluble fractions from human epidermis obtained from suction blisters.

3. In vitro *induction of BP metabolism by benzanthracene (BA)*

All attempts to induce AHH activity in liver, jejunum or skin *in vitro* failed. AHH activity in jejunum and liver declined sharply on incubation in the tissue culture system used, and activity after 18 hr of incubation was invariably 1% or less of the activity of fresh tissue for liver, and not detectable in jejunum (Table 3). The inclusion of BA in the tissue culture media had no significant effect on the loss of activity (Table 3). No detectable AHH could be demonstrated in human epidermal suction blisters after incubation in the presence or absence of BA (Table 3).

4. *Mono-oxygenase activity in fresh human jejunal biopsies*

Values for AHH and ethoxycoumarin O-de-ethylase (EOD) activity in whole homogenates of jejunum, without prior tissue culture, are shown in Table 4. Values for both AHH and EOD activity varied widely for both normals (AHH 120–1400 pmol/min/g tissue; EOD 80–1200 pmol/min/g tissue) and psoriatics (AHH 120–1600 pmol/min/g tissue; EOD 40–1120 pmol/min/g tissue). No sig-

Table 3. AHH activity in human jejunum, human liver and epidermal blister samples after incubation in tissue culture

	AHH activity pmol/min/g tissue		
	Fresh	Incubated 18 hr	Incubated 18 hr + BA 100 μM
Liver n = 4	627	5	7
	500	4	2
	320	3	4
	800	8	4
Jejunum n = 2	160	n.d.	n.d.
	240	n.d.	n.d.
Skin n = 4	n.d.	n.d.	n.d.

Activity was determined using the method of Nebert and Gelboin [5].
* n.d. = not detectable.

Table 4. Aryl hydrocarbon hydroxylase (AHH) and ethoxycoumarin O-de-ethylase (EOD) activity in homogenates of human jejunal mucosa without prior incubation

	Normal $n = 6$	Psoriasis $n = 5$
AHH pmol/min/g	610 ± 564	483 ± 524
EOD pmol/min/g	210 ± 139	236 ± 233

Results are expressed as the mean ± S.D. AHH activity was determined using the method of Nebert and Gelboin [5] and EOD using the method of Greenlee and Poland [20].

nificant differences in either AHH or EOD activity were evident between normal and psoriatic individuals.

DISCUSSION

The first reports of a defect in AHH activity in patients with psoriasis were based on the observations that basal and induced levels of AHH activity in epidermal blisters were lower in individuals with psoriasis [8]. However, we have been unable to demonstrate detectable AHH activity in human epidermal blisters when assayed fresh, or after incubation with BA. In the present study the average wet weight of epidermal blisters obtained by the suction technique was in the order of 20 mg, with a microsomal protein content of 0.1–0.4% of the wet weight. Therefore in a typical experiment, using half the blister sample for basal AHH, and half for induced, an average of 20 μg of microsomal protein is available for each assay. Allowing for protein determinations and triplicate determinations of AHH activity, the average protein content for each tube in the AHH assay is in the order of 5 μg. Using the previously reported values for AHH activity in epidermal blisters of 3 pmoles/mg microsomal protein/hr [8–12, 15], it can be calculated that the amount of 3-OH BP formed in each assay is in the order of 0.01 pmoles. However, the detection limit originally reported by Nebert and Gelboin for the AHH assay is 1 pmole of 3-OH BP [5], and we have confirmed this figure. It appears therefore that the figures previously reported for levels of AHH activity in human epidermis obtained from blisters are about a hundred times below the detection limits for the assay [8–12, 15]. Therefore we cannot confirm the previous reports of a defect in AHH activity in epidermal suction blisters from patients with psoriasis [8–12, 15]. Furthermore we could not detect AHH activity in human epidermal suction blisters even when using methods considerably more sensitive than that of Nebert and Gelboin. In addition we have been unable to demonstrate microsomal cytochrome P-450 dependent activity in epidermal suction blisters using ethoxycoumarin and ethoxyresorufin as substrates. In this respect our results with ethoxycoumarin are in keeping with those of Damen and Mier [23] for human epidermis.

In contrast to epidermis, AHH activity has been measured in whole human skin obtained from neonatal circumcision [22] and punch biopsies of abdominal skin [24]. Using the latter technique no significant differences in basal or induced levels of AHH activity were evident between normal and psoriatic individuals [24] using the method of Nebert and Gelboin [5]. However, although it was stressed that measurements were being made at the limits of detection for the method [24], these studies used a whole skin homogenate at a protein concentration of 2–5 mg per incubation, whereas studies using epidermal blisters are limited to a homogenate protein concentration of about 100 μg. Furthermore, in contrast to previous reports that AHH activity in human skin is exclusively epidermal [15] our evidence now suggests that AHH activity in skin is located predominantly in the dermis (unpublished). Using methods currently available, the measurment of AHH in human epidermis requires larger quantities of tissue than can be obtained from suction blister cups which only provide up to 20 mg of tissue.

In addition to the defect in AHH activity in epidermal blisters from psoriatic patients [8–12], it has also been reported that basal and induced levels of AHH activity in jejunal and liver biopsies from psoriatic individuals are lower than those from normal individuals [9]. In these studies biopsy samples were divided in half and incubated, *in vitro*, for 18 hr [9]. Levels of AHH activity in fresh biopsy samples were not reported. Our present results show that under the incubation conditions used in previous work, AHH activity in jejunal and liver biopsies declines so greatly from values obtained with fresh tissue that the technique cannot be used to examine induction by BA. In this technique needle biopsies approximately 3 mm dia and 3 cm in length are put straight into incubation medium and it is well known that necrosis occurs rapidly even with liver slices as small as 0.5 mm dia [25]. Furthermore, the loss of microsomal cytochrome P-450 and P-450 dependent mono-oxygenases occurs even during the culture of freshly isolated hepatocytes [26–28], although the induction of AHH activity in isolated hepatocytes grown in primary monolayer culture has been demonstrated [26]. Thus, the loss of AHH activity in the tissue culture system used in the present study is probably a result of the severely anoxic conditions that result from incubating whole tissue pieces, as well as the decrease in activity caused by the culture media [28]. Because of the remarkable decline in AHH activity upon incubation *in vitro* the technique used in the previous studies cannot be used to study inducibility of AHH activity in jejunum and liver and reports of a defect in patients with psoriasis [9] should therefore be disregarded. Furthermore we have now found no differences in basal levels of AHH or ethoxycoumarin O-de-ethylase in fresh jejunal biopsies from normal and psoriatic individuals. Although the previous work was done on microsomal preparations whereas the present study was done on whole tissue homogenates it is unlikely that the differences previously found were due to extra-microsomal activity. Intestinal AHH activity in laboratory animals has been shown to be dependent on the diet [29], and it is possible that the wide variation in activity found in the present study is due to dietary factors. Although we have only made

observations of basal microsomal AHH activity in fresh liver biopsies from two patients with psoriasis, and two normal subjects, the values obtained, using the method of Dehnen *et al.* [18], were similar, (normals 2762 and 1204 pmol/min/g tissue and psoriatics 1896 and 1349 pmol/min/g tissue: the results were comparable when expressed per microsomal protein). Our present findings are thus in keeping with those for lymphocytes [30] and monocytes [31] of patients with psoriasis.

In attempting to explain the earlier findings of an abnormality in tissues from patients with psoriasis, [8–12] we examined the original laboratory data. We found that a number of records had been altered several years after their original entry. Moreover although we no longer have the original chart readings, it appears from recalculation that the activity reported in the previous work was attributed, in some cases to differences as little as 0.3 mm in chart recordings of fluorescence. AHH activity was expressed per mg of microsomal protein and another source of anxiety concerns the quantity of microsomal protein which was recorded as zero in some cases and in others was improbably high. In restrospect it is unfortunate that the original laboratory data were not inspected earlier: had it been, the unreliability of calculated results would have been detected much sooner. In attempting to understand how such unsatisfactory data was arrived at it may be of relevance that whereas the tissue samples were obtained by a number of different people in the Department of Dermatology all measurements of AHH were executed or supervised and all the results calculated by one person in the Department of Clinical Pharmacology. Whatever the true explanation for the data on which the previous reports were based, and with which two of the present authors were associated, [8–12, 15] we now doubt their veracity. Thus both from a review of past work and from the present studies, we can find no evidence of a generalised tissue abnormality of AHH in psoriasis.

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