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Arginase Activity and Polyamine Biosynthesis in Psoriasis

J. LAUHARANTA[1] and K. KÄPYAHO[2]

[1]*Department of Dermatology, University Central Hospital, Helsinki, and*
[2]*Department of Biochemistry, University of Helsinki, Helsinki, Finland*

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Polyamine biosynthesis and arginase activity in psoriasis were studied using keratotome strips and suction blister roofs as specimens. In the uninvolved psoriatic skin a slight (1.3-fold; $p<0.05$) increase in the spermidine level was observed compared with control skin. There was also a 1.2-fold increase ($p<0.05$) in the spermidine/spermine molar ratio, which is considered to be an indicator of proliferation activity. No changes were noted in other polyamines or polyamine-synthesizing enzymes ornithine decarboxylase (ODC) and S-adenosyl-L-methionine decarboxylase (AMDC) in the uninvolved psoriatic skin vs. control skin. Neither was there any significant difference in the arginase activity. In psoriatic lesions the levels of all polyamines, as well as the activities of biosynthetic enzymes, were significantly ($p<0.001$) elevated. The putrescine level was elevated to 2.5-fold, spermidine to 2.7-fold and spermine to 1.4-fold. The enzyme activities expressed severalfold increases. The enhancement of arginase activity was less prominent than that of polyamine-synthesizing enzymes, but the increase from 271±88 to 354±126 μmol/g/h (1.3-fold) was statistically significant ($p<0.001$; paired t-test). The increase in arginase activity in suction blister roofs, which represents pure epidermis, was more pronounced than that in keratotome strips, i.e. about double. The results show thus that polyamine biosynthesis is significantly enhanced in psoriatic lesions, but there is only a slight difference between the uninvolved psoriatic skin and control skin. Arginase activity is also increased in psoriatic lesions but less so than polyamine biosynthesis. Arginase activity in normal skin is rather high and it does not seem to be a limiting factor for polyamine biosynthesis. *Key words: Arginase; Polyamines; Ornithine decarboxylase; Adenosylmethionine decarboxylase.* (Received November 9, 1982.)

J. Lauharanta, Department of Dermatology, University Central Hospital, Snellmaninkatu 14, SF-00170 Helsinki 17, Finland.

The biosynthesis of polyamines (putrescine, spermidine, spermine) is markedly enhanced in rapidly proliferating tissues (13). This is seen, for instance, in psoriasis, in which polyamine synthesis is normalized in parallel with clinical improvement (8, 19, 25, 28). After topical application of 12-O-tetradecanoylphorbol-13-acetate (TPA), a tumour promoter, induction of ornithine decarboxylase (ODC) activity is one of the early events preceding the increase in DNA synthesis (30). Studies with specific polyamine antimetabolites suggest that polyamine depletion leads to inhibition of cell proliferation in several experimental systems, including skin (9, 16, 17, 18).

S-adenosyl-L-methionine and L-ornithine are the principal precursors of polyamines. Ornithine is formed mainly by hydrolysis of arginine to ornithine and urea as a part of the urea cycle, which is especially active in liver. The skin shows high arginase activity, which is even elevated in epidermal hyperplasia and hyperkeratinization (26). The other stages in the urea cycle seem to be lacking in the skin (4). The skin arginase has been suggested to play a role in providing ornithine as a substrate for polyamine biosynthesis (3, 26). Ornithine is further decarboxylated by ODC to putrescine, and adenosylmethionine decarboxylase (AMDC) is needed in the formation of spermidine and spermine (Fig. 1) Arginase is apparently also needed in the production of two amino acids, glutamate and proline,

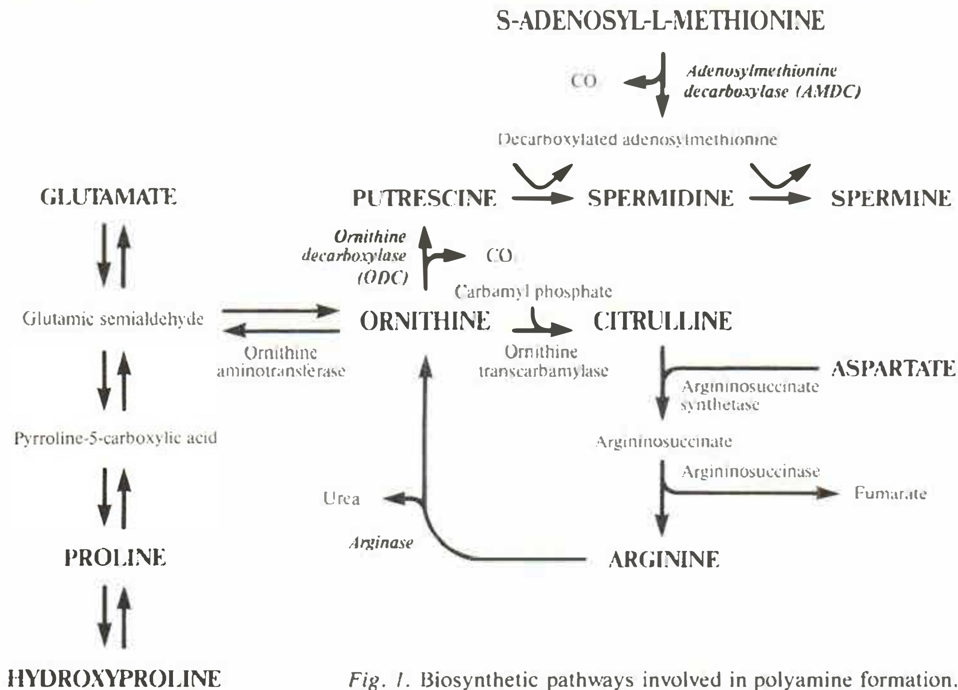

Fig. 1. Biosynthetic pathways involved in polyamine formation.

which are specifically incorporated in keratinous proteins (26, 30). The aim of our study was to investigate still further the polyamine biosynthesis and arginase activity, and their relationship in psoriatic skin.

MATERIALS AND METHODS

Patients and biopsy techniques

The polyamine levels and the activities of ornithine decarboxylase (ODC), S-adenosyl-L-methionine decarboxylase (AMDC), and arginase were determined in the involved and uninvolved skin of 11 psoriatics (1 female, 10 males; mean age 40±18 years). Seven healthy persons served as controls (2 females, 5 males; mean age 38±17 years). In these patients, keratotome strips were used for assays. They were removed with a motor-driven Castroviejo keratotome. The keratotome shim setting for removal of the involved spidermis was 0.5 mm and that for the uninvolved psoriatic epidermis and normal epidermis was 0.2 mm. The skin was anaesthetized by local infiltration of 1% lidocaine without epinephrine. Every specimen was examined histologically. A specimen from the uninvolved psoriatic skin was taken 2–3 cm from the margin of the lesion from which the lesional sample was taken. Pure epidermis was obtained by the suction blister technique (14) from both the involved and uninvolved skin of 4 male psoriatics (mean age 58±17 years) with slightly-infiltrated lesions on the trunk, and assayed for arginase. All specimens were immediately frozen in liquid nitrogen and stored at −50°C until processing.

Polyamine assay

The samples were homogenized in 0.2 N perchloric acid with an all-glass tissue homogenizer. Polyamines were assayed as their fluorescent dansyl derivatives by the method of Seiler (29). The polyamines were allowed to react with dansyl chloride (1-dimethylaminonaphthalene-5-sulphonyl chloride; E. Merck, Darmstadt), and the fluorescent dansyl derivatives were separated by thin-layer chromatography (DC-Alufolien aluminium oxide plates, Merck, art. 5550), using a chloroform-dioxan-*n*-butanol (192 : 4 : 4) solvent system (5). The spots were cut off the plate and extracted into ethanol. The intensity of the fluorescence was assayed against known standards (putrescine, spermidine and spermine hydrochlorides, Calbiochem, San Diego) with a Hitachi MPF-2A fluorescence spectrophotometer (exitation wavelength 335 nm, emission wavelength 515 nm).

*Determination of ornithine decarboxylase (ODC) and S-adenosyl-*L-methionine decarboxylase (AMDC) activities

The samples were homogenized in 25 mM Tris buffer (pH 7.1) containing 0.1 mM EDTA and 5 mM dithiothreitol. The homogenates were centrifuged at 100 000 *g* for 20 minutes and the supernatant fraction was used for the assay. Ornithine decarboxylase activity was determined as in (11) and adenosylmethionine decarboxylase according to (12). L-[1-^{14}C]ornithine (purchased from Radiochemical Centre, Amersham) and S-adenosyl-L-[1-^{14}C]methionine (synthesized as in ref. 24) were used as substrates. The CO_2 liberated was captured into a base (Soluene®) and counted for radioactivity using a Wallac liquid scintillation counter. The activities of both decarboxylases were expressed in pmol CO_2/mg soluble protein/h.

Arginase assay

Arginase activity was assayed by quantitating the conversion of L[U-^{14}C]arginine into ornithine as described by Alhonen-Hongisto et al. (1). The homogenization was performed as in ODC and AMDC assays. Arginase activity was determined from both the supernatant and particulate fractions. The latter was washed and suspended in the homogenization buffer. The final incubation mixture consisted of 50 mM glycine buffer (pH 9.75), 1 mM $MnCl_2$, 50 mM L-arginine, 0.5 μCi of L-(U-^{14}C) arginine and 115 μl of the enzyme preparation in a total volume of 150 μl. After the incubation for 1 hour at 37°C the reaction was halted with 15 μl of 50% trichloroacetic acid containing 0.5 mM L-ornithine. Twenty μl of the acid supernatant was subjected to paper electrophoresis in 0.065 M sulphosalicylic acid buffer (pH 3.2) for 3 hours at 300 V. The ornithine fraction was localized by ninhydrin staining, cut off and counted for radioactivity. Arginase activity was expressed as μmol/g tissue/h.

DNA and protein assays

DNA was measured by the method of Giles & Myers (6) and protein *ad modum* Lowry et al. (22).

RESULTS

Table I shows that the spermidine level in uninvolved psoriatic skin was slightly (1.3-fold; $p<0.05$) elevated, while the levels of putrescine and spermine remained practically unchanged. The spermidine/spermine ratio was also slightly elevated (1.2-fold; $p<0.05$). In involved psoriatic skin all polyamines as well as the spermidine/spermine ratio were significantly elevated ($p<0.001$) compared with healthy controls (unpaired *t*-test) or with uninvolved psoriatic skin (paired *t*-test). The level of putrescine in involved skin was 2.5-fold compared with that in uninvolved skin. The spermidine level was elevated to 2.7-fold and spermine level to 1.4-fold.

Table II gives the activities of the enzymes involved in polyamine biosynthesis. There was no significant difference between the uninvolved psoriatic skin and control skin. The ODC activity was very low in both uninvolved psoriatic skin and control skin, being unmeasurable in many samples, but showed a significantly increased activity in lesional skin. the AMDC showed a slight upward trend in uninvolved skin compared with control skin, but the difference was statistically non-significant. In psoriatic lesions, AMDC activity showed a 7.4-fold increase. In arginase activity the increase in lesional skin was only 1.3-fold, being, however, statistically significant ($p<0.01$). The enhancement of arginase activity was about the same as the increase in the concentration of soluble protein (1.5-fold; data not shown). Table II further shows that in pure epidermis, removed by the suction blister technique, arginase activity was 2.1 times as high in involved as in uninvolved psoriatic skin.

Arginase activity was found in both the supernatant and particulate fractions. The latter could be determined after washing and suspending the 100 000 *g* pellet in the homogenization buffer, and it was found to contain 25–50% of the total activity, with no significant difference between the uninvolved and involved psoriatic skin when keratotome strips were used. However, in suction blister roof a significantly greater fraction (38% vs 17%; $p<0.05$) of the activity was found in the particulate fraction.

Table I. *The levels (mean ± SD) of putrescine (Pu), spermidine (spd) and spermine (Sp), and the spermidine/spermine molar ratio in psoriatic and control skin*

	n	Pu (nmol/ mg DNA)	Spd (nmol/ mg DNA)	Sp (nmol/ mg DNA)	Spd/Sp (nmol/ mg DNA)
Healthy controls	7	20±7	168±23	308±16	0.54±0.05
Psoriatics					
Uninvolved	11	22+9	218±60*	335±124	0.63±0.11*
Involved	11	52±12**	523±123**	407±79**	1.35±0.22**

* $p<0.05$ (uninvolved vs. control skin; unpaired t-test).
** $p<0.001$ (involved vs. uninvolved skin; paired t-test).

DISCUSSION

In agreement with previous reports (2, 20, 21, 25, 28), our study shows that polyamine biosynthesis is markedly enhanced in psoriatic lesions. In uninvolved psoriatic skin, variable changes in polyamine patterns have been observed in different studies. Furthermore, ODC activity has been reported to be normal (28) or elevated (21). Difference in the methods and in the type of the patients may at least partly explain these discrepancies. Our patients had severe plaque-type psoriasis, some even at eruptive stage. For polyamine assay we used the dansylation method in contrast to an amino acid analyzer used by the other groups. The enzyme assays have been performed using nearly identical techniques. We found that only the level of spermidine and the spermidine/spermine ratio were slightly elevated in the uninvolved psoriatic skin. Furthermore, there was a slight upward trend in AMDC activity, but no other notable changes in polyamine contents and enzyme activities. The number of DNA-synthesizing cells is slightly greater in the uninvolved psoriatic skin (7, 10), which according to our results seems to be associated with a slight elevation in the spermidine level of the tissue. A marked increase in polyamine biosynthesis occurs only in clearly visible psoriatic lesions with profoundly elevated proliferative activity.

In keratotome strips the arginase activity of involved psoriatic skin is only moderately increased. In suction blister roofs, which represent pure epidermis, the difference between the involved and uninvolved skin is more pronounced, i.e. about 2-fold. It is known that

Table II. *The activities of ornithine decarboxylase (ODC), S-adenosyl-L-methionine decarboxylase (AMDC) and arginase in psoriatic and control skin*

	n	ODC (pmol/ mg prot/h)	AMDC (pmol/ mg prot/h)	Arginase (μmol/ g tissue/h)	n	Arginase in suction blister roof (μmol/g tissue/h)
Healthy controls	7	0.8±1.0 (0–2.2)	55±13	285±137		
Psoriatics Uninvolved	11	0.7±0.9 (0–2.4)	64±27	271±88	4	273±53
Involved	11	15.8±12.1** (1.9–37.5)	337±135**	354±126**	4	539±105**

** $p<0.001$ (involved vs. uninvolved; paired t-test).

arginase is mostly present in the epidermis (27) and thus dermal contamination in keratotome strips may have diminished the difference. A large proportion of the enzyme resides in the particulate fraction and this "insoluble" fraction seems to increase in hyperproliferative skin.

Arginase apparently provides ornithine for polyamine biosynthesis. Its activity is elevated in psoriatic lesions where the demand for ornithine seems to be increased. Similarly, in a lactating mammary gland, increased arginase activity has been suggested to be associated with enhanced polyamine biosynthesis (23) and in lymphocytes stimulated to proliferation by concanavalin A, increased arginase activity has been suggested to participate in increased polyamine formation (15). The basal arginase activity in human skin is rather high and therefore it does not seem to be a limiting factor in polyamine biosynthesis, since a dramatic increase in polyamine formation may occur with only a moderate increase in arginase activity. This is consistent with the results of Verma & Boutwell (30) who found that a topical application of TPA produces a rapid increase in ODC activity but not in arginase activity.

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