Acta Dermatovener (Stockholm) 62: 221–224, 1982

METHYLGLYOXAL *BIS* (GUANYLHYDRAZONE) AND α-DIFLUOROMETHYLORNITHINE-INDUCED POLYAMINE DEPRIVATION IN PSORIATIC LESIONS

Merja Kousa,[1] Kirsti Käpyaho,[2] Jorma Lauharanta,[1] Kirsti Linnamaa,[2]
Juhani Jänne[2] and Kimmo Mustakallio[1]

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

Abstract. Intradermally injected methylglyoxal *bis* guanylhydrazone) (MGBG), an inhibitor of S-adenosyl-l-methionine decarboxylase, and α-difluoromethylornithine (DFMO), an inhibitor of ornithine decarboxylase, induced polyamine deprivation in psoriatic lesions. The effect was dose-dependent. An injection of MGBG in 200 μmol concentration lowered the mean spermidine and spermine levels significantly. A significant reduction in the mean putrescine level was achieved by DFMO in mmol concentration.

Key words: Polyamines in psoriasis; Methylglyoxal *bis* (guanylhydrazone); α-difluoromethylornithine

Tha natural polyamines—putrescine, spermidine and spermine—play an essential role in cell proliferation and differentiation (8, 14). The precursors of polyamines are L-ornithine and L-methionine. Putrescine is synthesized from ornithine by decarboxylation catalysed with ornithine decarboxylase (ODC). Putrescine-stimulated S-adenosyl-L-methionine decarboxylase (AMDC) is required for the synthesis of decarboxylated adenosylmethionine, the precursor of spermidine and spermine.

The polyamine metabolism is increased in both benign and malignant proliferating tissues. Recent studies have shown an increased polyamine metabolism in psoriasis (1, 4, 10, 11, 12, 13, 15). Effective antipsoriatic treatment with topical corticosteroid (15), with dithranol (1, 4), with Ingram regimen (13), with PUVA (4, 10) and with an aromatic retinoid (10) have lowered the polyamine levels in psoriatic skin markedly.

The accumulation of polyamines in psoriasis and their normalization during treatment has suggested that an antipsoriatic effect might be achieved by using specific polyamine inhibitors. In recent years several chemical compounds which can block polyamine synthesis have been discovered. In cell and tissue culture studies polyamine antimetabolites have led to a significant inhibition of growth and cell proliferation (8). The most extensively examined synthesis inhibitors of polyamines are methylglyoxal *bis* (guanylhydrazone) (MGBG) and α-difluoromethylornithine (DFMO). In the present study we have examined the effect of intradermally injected MGBG and DFMO on polyamine levels in psoriatic skin.

PATIENTS AND METHODS

The patient series consisted of 17 persons, treated at the Department of Dermatology, Helsinki University Central Hospital. They all suffered from severe psoriasis. Control specimens were from 10 healthy persons.

MGBG (Aldrich Europe, Beerse, Belgium) and DFMO (Merrell International, Strasbourg, France) were dissolved in physiological saline, neutralized and injected intradermally in untreated psoriatic lesions. The MGBG solution was used in 100 μmol in 4 and in 200 μmol concentrations in 6 patients. The DFMO solution was used in both 25 mmol and in 100 mmol concentrations in 4 patients and in 50 mmol concentration in 3 patients. The injections were performed by means of Dermo-Jet® (Akra, France).

The 4 mm whole-skin punch biopsies from the injection site were taken before and both one and 2 or 3 days after the injection. Samples were frozen in liquid nitrogen and stored at -50°C until processed.

For polyamine determinations the samples were homogenized in 0.2 N perchloric acid using a Potter-Elvehjelm tissue homogenizer. The extracted polyamines were determined as their fluorescent DANS derivatives by Seiler's method (16) with the modification described by Dreyfuss (2). Putrescine, spermidine and sperimine, used as standards, were obtained from Calbiochem (San Diego, Calif.). 1-Dimethyl-5-naphthalene sulphonyl chloride (DANS-Cl) was a product of E. Merck (Darmstadt). The thin-layer chromatographic separation of

Table I. *Mean polyamine levels (nmol/g skin ± SEM) in involved skin of 17 patients with psoriasis, versus 10 healthy controls*

Polyamines	Psoriatic lesions	Normal controls
Putrescine	56± 6*	29± 2
Spermidine	344±21*	100±10
Spermine	283±20**	132±13
Spermidine/spermine ratio	1.22± 0.40*	0.77± 0.04

* $p<0.001$. ** $p<0.02$.

polyamines was performed using d.c.-Alufolien aluminium oxide plates (Merck, Art 5550).

The statistical significance was tested by means of Student's *t*-test.

RESTULTS

Table I presents the mean polyamine levels in involved skin of the patient series versus 10 healthy controls. The putrescine and spermidine contents of psoriatic skin were highly significantly greater ($p<0.001$) and spermine content significantly greater ($p<0.02$) than that in the skin of healthy controls. An intralesional injection of MGBG, 100 μmol, caused only a slight decrease in the levels of all three polyamines, but after an injection of 200 μmol MGBG a significant decrease occurred in spermidine and spermine levels, as shown in Table II and illustrated in Fig. 1. Furthermore, spermidine decreased more than spermine, resulting in a reduction in the spermidine/spermine ratio, which fell from 1.26±0.08 to 1.01±0.04 ($p<0.05$) in the group receiving MGBG 100 μmol and from 1.26±0.04 to 1.06±0.04 ($p<0.05$) with MGBG 200 μmol.

There was virtually no effect with an injection of 25 mmol DFMO. The polyamine levels after 50 mmol and 100 mmol DFMO are given in Table III. A distinct decrease was seen in the putrescine and spermidine contents, whereas the mean spermine content increased slightly. Fig. 2 demonstrates the curves of polyamine levels after injection of DFMO in 100 mmol concentration.

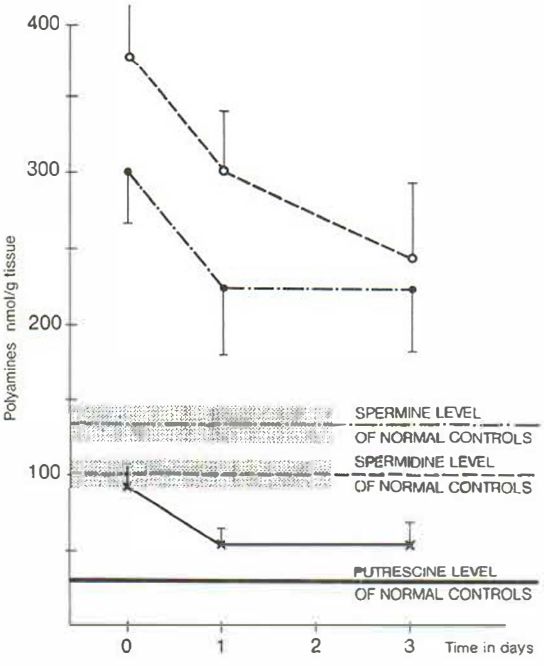

Fig. 1. Reduction of polyamine levels in psoriatic lesions in 6 patients after injections of MGBG, 200 μml.

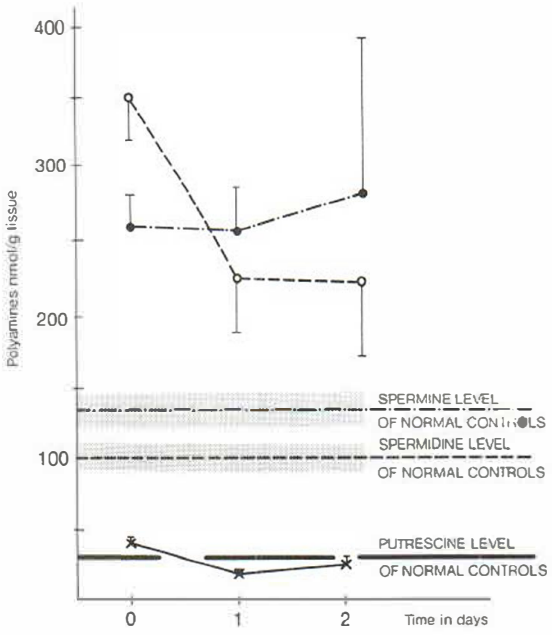

Fig. 2. Reduction of polyamine levels in psoriatic lesions of 4 patients after injections of DFMO, 100 mmol.

DISCUSSION

It has been suggested that the use of inhibitors of polyamine biosynthesis may have practical application in the treatment of proliferative disorders of the skin (10, 13). Of the four enzymes necessary for polyamine biosynthesis the decarboxylase of

Table II. *Polyamine levels (nmol/g skin ± SEM) in psoriatic lesions after injections of MGBG in 100 and 200 μmol concentration*

MGBG concentr.	*n*	Time (days)	Putrescine	Spermidine	Spermine
200 μmol	6	0	81±16	376±36	302±33
		1	59±11	302±40	225±45
		2	61±16	244±50*	226±41**

* $p<0.001$. ** $p<0.01$.

ornithine and adenosylmethionine are the rate-limiting enzymes. Consequently the effective inhibitors of polyamine synthesis discovered for the present are inhibitors of ODC or AMDC (5, 8).

MGBG is one of the most effective inhibitors of polyamine biosynthesis. Several in vivo and in vitro studies have been published concerning its mode of action (5, 8). It acts as a competitive inhibitor for the substrate adenosylmethionine and has caused putrescine accumulation in cell cultures. Moreover MGBG is known to prevent oxidative phosphorylation, RNA polymerase activity, and to cause mitochondrial damage. It has been shown that adding MGBG to cell cultures completely prevents thymidine incorporation into DNA.

MGBG has been used as a cytostatic agent in malignant diseases, with promising effects (9, 17). In the present work we studied the effect of intradermally injected MGBG and DFMO on polyamine metabolism in psoriatic lesions.

The mean polyamine levels of psoriatic skin in this study are in agreement with that of Böhlen et al. (1) and Grosshans et al. (4) who also used whole-skin biopsies. There seems to be great individual variation, depending on the type and state of the disease.

The mean levels of putrescine, spermidine and spermine were decreased by 26%, 37% and 32% 3 days after an injection of 200 μmol MGBG. The decrease in spermidine was statistically significant ($p<0.05$).

The spermidine/spermine ratio, considered to be an indicator of proliferation activity (8), fell significantly after injection of 100 μmol MGBG ($p<0.05$). The decrease after 200 μmol was also distinct, but did not reach statistical significance. In previous studies addition of MGBG has resulted in a continued accumulation of putrescine both in cell cultures (3), and in animal experiments (6). However, this was not seen in the present study. The putrescine level decreased to almost the same extent as the spermidine level. This may be due to an inhibitory effect on the increased oxidative phosphorylation and mitochondrial damage which indirectly influences the polyamine metabolism.

ODC activity can be competitively inhibited with several substrate analogues such as several diamines, α-hydrazino-ornithine, α-methylornithine, α-difluoromethylornithine, 1.3-diaminopropane, 1.3-diaminopropanol, 1.5-diaminopentane and 1.6-diaminohexane (5). α-Difluoromethylornithine (DFMO), used in the present study, causes an irreversible inhibition of the enzyme. Recently Grosshans and co-workers (4) have investigated the

Table III. *Polyamine levels (nmol/g skin ± SEM) after injections of DFMO in 50 and 100 mmol concentrations*

DFMO concentr.	*n*	Time (days)	Putrescine	Spermidine	Spermine
50 mmol	3	0	62±14	327±35	369±102
		1	35± 6	261±43	317±87
		3	38± 6	239±40	265± 13
100 mmol	4	0	42± 4	350±31	266± 22
		1	19± 2*	224±38	262± 30
		2	25± 6*	223±50	280±113

* $p<0.01$.

effect of two ODC inhibitors, α-methylornithine and DFMO, on psoriatic skin. Application of DFMO (10% in a cream base) caused a significant reduction of putrescine and spermidine levels in the skin. However, this was not associated with a distinct clinical improvement.

In the present experimental work, 2 days after an injection of DFMO in 100 mmol concentration, the mean level of putrescine was reduced by 40% and that of spermidine by 36%, whereas the mean level of spermine increased slightly. This is compatible with a previous report that prevention of the accumulation of putrescine but not that of spermine by DFMO occurs in cell culture studies (7).

This study shows that both MGBG and DFMO prevents polyamine accumulation in psoriatic skin. However, it is not yet possible to draw any conclusion concerning the possible therapeutic applicability of local MGBG or DFMO because of the short (3 days) follow-up. Further studies are needed to define the role of polyamines in the pathophysiology of psoriasis and the possible therapeutical usefulness of polyamine inhibitors.

REFERENCES

1. Böhlen, D., Grove, J., Beaya, M. F., Koch-Weser, J., Henry, M. H. & Grosshans, E.: Skin polyamine levels in psoriasis: the effect of dithranol therapy. Eur J Clin Invest *8:* 215, 1978.
2. Dreyfuss, G., Dvir, R., Harell, A. & Chayen, R.: Determination of polyamines in urine. Clin Chim Acta *49:* 65, 1973.
3. Fillinghame, R. H. & Morris, D. R.: Accumulation of polyamines and its inhibition by methylglyoxal *bis* (guanylhydrazone) during lymphocyte transformation. *In* Polyamines in Normal and Neoplastic Growth (ed. D. H. Russel), pp. 249–260. Raven Press, New York, 1973.
4. Grosshans, E., Henry, M., Tell, G., Schechter, P. J., Böhlen, P., Grove, J. & Koch-Weser, J.: Les polyamines dans le psoriasis. Ann Dermatol Venereol *107:* 377, 1980.
5. Heby, O. & Jänne, J.: Polyamine antimetabolites: Biochemistry specificity and biological effects of inhibitors of polyamine synthesis. *In* Polyamines in Biology and Medicine (ed. D. R. Morris & L. J. Marton), pp. 243–310. Marcel Dekker, New York, 1981.
6. Hölttä, E., Hannonen, P., Pispa, J. & Jänne, J.: Effect of methylglyoxal *bis* (guanylhydrazone) on polyamine metabolism in normal and regenerating rat liver and rat thymus. Biochem J *136:* 669, 1973.
7. Hölttä, E., Pohjanpelto, P. & Jänne, J.: Dissociation of the early antiproliferative action of methylglyoxal *bis* (guanylhydrazone) from polyamine depletion. A comparison of the effects of DL-alfa-difluoromethylornithine and methylglyoxal *bis* (guanylhydrazone) on the growth of human fibroblasts. North-Holland Biomedical Press *97:* 9, 1979.
8. Jänne, J., Pösö, H. & Raina, A.: Polyamines in rapid growth and cancer. Biochim Biophys Acta *473:* 241, 1978.
9. Knight, W. A., Livingston, R. B., Fabian, C. & Constanzi, J.: Methylglyoxal *bis* (guanylhydrazone) (methyl-GAG, MGBG) in advanced human malignancy. Proc Am Assoc Cancer Res *20:* 319, 1979.
10. Lauharanta, J., Kousa, M., Käpyaho, K., Linnamaa, K., & Mustakallio, K.: Reduction of increased polyamine levels in psoriatic skin by retinoid and PUVA-treatments. Br J Dermatol *105:* 267, 1981.
11. Lowe, N. J., Breeding, J. & Russell, D.: Cutaneous polyamines in psoriasis. Psoriasis. Proceedings of III International Symposium, Stanford, 1981 (ed. E. M. Farber & A. J. Cox).
12. Proctor, M. S., Fletcher, H. V., Shaklau, B. & Rennet, O. M.: Elevated spermidine and spermine levels in the blood of psoriasis patients. J Invest Dermatol *65:* 409, 1975.
13. Proctor, M. S., Wilkinson, D. J., Orenberg, E. K. & Farber, E. M.: Lowered cutaneous and urinary levels of polyamines with clinical improvement in treated psoriasis. Arch Dermatol *115:* 945, 1979.
14. Russel, D. H. & Durie, ?. G. M.: Polyamines as biochemical markers of normal and malignant growth. *In* Progress in Cancer Research and Therapy, vol. 8. Raven Press, New York, 1978.
15. Russel, D. H., Combest, W. L., Duell, E. A., Stawinski, M. A., Anderson, T. F. & Voorhees, J. J.: Clucocorticoid inhibits elevated polyamine biosynthesis in psoriasis. J Invest Dermatol *71:* 177, 1978.
16. Seiler, N.: Use of the dansyl reaction in biochemical analysis. Methods Biochem Anal *18:* 259, 1970.
17. Seppänen, P., Alhonen-Hongisto, L., Siimes, M. & Jänne, J.: Quantitation of methylglyoxal *bis* (guanylhydrazone) in blood plasma and leukemia cells of patients receiving the drug. Int J Cancer *26:* 571, 1980.

Received October 16, 1981

Merja Kousa, M.D.
Department of Dermatology
University Central Hospital
Snellmaninkatu 14
SF-01700 Helsinki 17
Finland