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Comparative analysis of polyamine metabolism in benign and neoplastic keratinocytic proliferations

Valentina Broshtilova[1] ✉, Valentin Lozanov[2], Ljubka Miteva[3]

Abstract

Introduction: Polyamines (putrescine, spermidine, and spermine) are polycationic compounds that play a central role in keratinocytic proliferation, differentiation, and regulation. The objective was to elucidate the polyamine metabolic changes that occur in various benign and neoplastic skin proliferations.
Methods: The study included 58 patients: 31 with the plaque form of psoriasis vulgaris and 27 with non-melanoma skin tumors. The levels of putrescine, spermidine, and spermine were detected in lesional and non-lesional skin samples.
Results: Findings were representative ($p < 0.05$). Psoriatic lesions showed a twofold elevation of all polyamines in lesional skin compared to non-lesional skin. Spermine had the highest concentration, which suggested a leading position of propylamine synthesis in psoriatic pathogenesis. Results on the polyamine metabolism of basal cell carcinoma represented basic characteristics similar to those of psoriasis. Conversely, squamous-cell carcinoma lesions showed the highest concentration of putrescine, suggesting a crucial role of spermidine-spermine acetyltransferase in their pathogenesis.
Discussion: Our findings showed different polyamine metabolic changes in lesions from benign and neoplastic keratinocytic proliferations. Basal-cell carcinoma polyamine metabolism revealed a closer relationship to psoriasis than to squamous-cell carcinoma, which might explain its long-term benign course and non-metastatic nature.

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Introduction

The polyamines putrescine (PU), spermidine (SMD), and spermine (SM) are some of the major cations in eukaryotic cells. The majority of polyamine molecules are bound to polyanionic macromolecules such as DNA, RNA, and phospholipids (1), resulting in far-reaching effects on cellular processes including DNA replication, transcription, and translation. It is not surprising that numerous studies using specific inhibitors of polyamine biosynthesis have documented that these small ubiquitous molecules are absolutely required for cell growth and differentiation (2-4). The cellular functions of polyamines and their interaction with cellular components that play a key role in promoting the process of controlled proliferation and uncontrolled hyperproliferation (tumorigenesis) remain largely unknown (2).

The polyamines are variously distributed throughout the epidermal and dermal compartments of normal skin (2). Baze et al. indicated that polyamine concentrations varied significantly between the epidermis and dermis, both quantitatively and qualitatively: SMD and SM levels were much higher in the epidermis than in the dermis, and PU/SMD and SMD/SM ratios were much lower in the epidermis. These differences characterized the very well-known specificity of cellularity, proliferative activity, and differentiation between the two main cutaneous compartments. The higher SMD and SM concentrations in the epidermis suggested that both substances might play a special role in epidermal metabolism (3).

Various skin inflammations express a proliferative nature. Psoriasis (Pso) is the most common example of a hyperproliferative keratinocytic process. Histologically, it is defined by parakeratosis, acanthosis, and papillomatosis. Pso is considered a model of benign keratinocytic proliferation with a higher synthetic rate and faster turnover of epidermal cells. Non-melanoma skin cancers – basal-cell and squamous-cell carcinomas (BCC and SCC, respectively) – are models of malignant keratinocytic proliferation.

The polyamine concentration in proliferating tissues was found to be elevated. Blood samples of Pso patients had levels of both SMD and SM that were two times as high as those of healthy controls (3). Polyamine levels in blood, urine, and other biological fluids fluctuated in accordance with Pso activity (4). The SMD/SM ratio, which is considered an indicator of proliferation activity, was found to be increased in Pso lesions compared to non-lesional skin of patients affected by the disease (5). Interestingly, Pso patients receiving etretinate showed a significant drop in all urinary polyamines, with the greatest prevalence of SM (6). Lowe et al. demonstrated that Pso lesions had increased ornithine decarboxylase (ODC) activity in both lesional and non-lesional skin, compared to healthy controls (7). Patients with eruptive forms of Pso had significantly higher levels of ODC than those with chronic-recalcitrant forms. Due to technical challenges all these investigations were carried out on small cohorts, which did not allow proper statistical analysis.

On the other hand, elevated ODC activity in K6/ODC and K5/ODC transgenic mice was constitutively targeted to either the outer root sheath of the hair follicle or the basal epidermal layer in the skin with keratin-5 or keratin-6 promoters to yield increases in polyamine pools, especially PU levels (8). Bitransgenic mice expressing both skin-targeted ODC and the v-Ha-ras oncogene developed spontaneous keratoacanthomas and SCC (9), whereas no tumors were found in the monotransgenic mice (10). BCC devel-

[1]Faculty of Medicine, Department of Dermatology and Venereology, 1, Georgi Sofiisky st., 1431 Sofia, Bulgaria. [2]Faculty of Medicine, Department of Medical Chemistry and Biochemistry,1, Georgi Sofiisky st., 1431 Sofia, Bulgaria. [3]Faculty of Medicine, Department of Dermatology and Venereology, 1, Georgi Sofiisky st., 1431 Sofia, Bulgaria. ✉**Corresponding author: broshtolova@mail.bg**

oped in mice that were both heterozygous null for the patched tumor suppressor gene and over-expressed ODC in follicular cells (11). The formation of skin tumors in these mice depended on polyamine biosynthesis, especially PU, because treatment with the specific inhibitor of ODC activity, alpha-difluoromethylornithine, blocked skin tumor formation and caused rapid regression of existing tumors (12). Even modest reductions in ODC expression reduced skin tumor susceptibility, as demonstrated by the reduced skin tumor yield in ODC +/– haploinsufficient mice subjected to a two-stage initiation-promotion protocol (13). To date, all the investigations on polyamine biochemical changes in neoplastic keratinocytic proliferations have been carried out on transgenic animal models or tissue cultures. Our study hypothesized the pre-existing trends in vivo on morbid tissues obtained from affected subjects.

The objective was to detect and comparatively analyze the levels of the three basic polyamines (PU, SMD, and SM) from epidermal skin samples taken from patients with Pso, BCC, and SCC.

Methods

Eighty-five patients were enrolled in the study. The study was undertaken after receiving approval from a local ethical committee. Informed consent was obtained from each subject.

Thirty-one patients with mild to moderate plaque-form psoriasis vulgaris were enrolled in the study. The inclusion criteria were: 18 to 64 years old, onset of the disease from 6 months to 30 years, and PASI ranging from 2 to 16. No patients with psoriatic arthritis, pustular psoriasis, or psoriatic erythroderma were investigated. All subjects were generally healthy. Obesity, hypertension, metabolic syndrome, thrombophlebitis, and other dermatological diseases were specifically excluded. Pso lesions were not triggered or exacerbated by drug intake or infectious agents. The patients had a 1-month period of washing out any systemic and topical treatments prior to the investigation.

Epidermal shave biopsies, taken according to the standardized technique described by Lowe et al. (1982) (7) and based on Arthur and Shelley's method (1959) (14), were used. The non-lesional skin samples were taken from patients' backs, from areas a minimum of 5 cm from a Pso lesion. These were called non-affected psoriatic lesions (NAPso). The lesions were frozen at –196 °C and stored at –80 °C.

Twenty-seven patients with non-melanoma skin tumors were studied: 15 with BCC and 12 with SCC. Their age ranged from 30 to 68 years. The patients were generally healthy, with no other skin disease. The lesions were precisely excised. For each tumor, a central 2 mm section was punched and frozen at –196 °C in cryogenic nitrogen. The rest was sent for routine histology analysis. Control skin samples were taken from each patient's back via the epidermal shave technique described above and processed in cryogenic nitrogen. These samples were referred to as non-affected skin of cancer patients (NAS).

A high-performance liquid chromatography method for the simultaneous analysis of amino acids and biogenic polyamines using a pre-column derivatization of amino groups with N-(9-fluorenylmethoxycarbonyloxy)succinimide was used. The method was originally described by Lozanov et al. (15) with linearity calculated for each polyamine with a correlation coefficient higher than 0.991, in concentrations ranging from 0.2 to 50 M, except for SM, for which the correlation coefficient was r = 0.984. The limit of quantitation was estimated to be around 50 pM in a 50 µl sample injection. The repeatability of the method, expressed as R.S.D., ranged from 1.1 to 6.7%. A proper adjustment of binary time / pH gradient was the main technical pitfall as skin samples were investigated for the first time. The quantitative determination was carried out using the external calibration method.

Statistical analysis was performed using SYSTAT v. 7 (SPSS, Chicago, USA, 1996).

Results

A total of 31 Pso patients were included. The mean age was 42.13 years, with the mean onset of the disease 8.61 years. All patients had mild to moderate forms of the disease with mean PASI scores of 7.58. The mean age of the non-melanoma skin cancer patients was 27. The mean age of the 15 patients with BCC was 55.8 years, while the 12 SCC patients had a mean age of 54.82 years. There was no gender preponderance in the various patient categories.

The results of the polyamine levels are summarized in Table 1.

The three basic polyamines showed twofold greater concentrations in lesional skin (Pso) compared to non-lesional skin in patients with psoriasis (NAPso). In lesional skin, SM had the highest concentration, followed by SMD and SM. The level of SM increased most (2.5 times), followed by PU (2.3 times) and SPD (2.0 times). The SMD/SM ratio was 1.09 in Pso non-affected skin, and under 1 in lesional skin. This result did not prove the significance of the SMD/SM ratio in evaluating the proliferation rate of keratinocytes in benign inflammatory proliferations.

More interesting results were obtained comparing NAPso with the samples from non-affected skin of patients with BCC and SCC (NAS). No difference in PU, SMD, and SM levels was found ($p > 0.05$), suggesting that Pso polyamine metabolic dysregulation was only local, on the sites of existing lesions. Thus, our findings repudiated the hypothesis that the entire skin surface in Pso patients demonstrates a latent morbid state with a lower sensitivity threshold. In both categories SMD had the highest concentration. The SMD/PU and SM/PU ratio was close to 4 in both groups, revealing that under normal conditions the healthy keratinocytes synthesized four times more SMD and SM than PU.

PU, SMD, and SM were different in all 15 specimen pairs of BCC ($p = 0.00$). The results in the BCC group showed equal SM and SMD levels. PU demonstrated minimal concentration, which was

Table 1 | Polyamine levels detected in affected and non-affected skin specimens from patients with psoriasis and basal- and squamous-cell carcinoma.

	Pso	NAPso	NAS	BCC	BCC c	SCC	SCC c
Specimens	31	31	27	15	15	12	12
PU	0.44 (± 0.28)	0.19 (± 0.07)	0.19 (± 0.07)	0.34 (± 0.08)	0.17 (± 0.05)	0.42 (± 0.08)	0.18 (± 0.06)
SMD	1.71 (± 0.36)	0.85 (± 0.20)	0.87 (± 0.14)	1.8 (± 0.07)	0.87 (± 0.19)	1.21 (± 0.23)	0.82 (± 0.18)
SM	1.96 (± 0.43)	0.78 (± 0.13)	0.69 (± 0.08)	1.8 (± 0.27)	0.65 (± 0.11)	1.10 (± 0.38)	0.67 (± 0.08)

Pso - samples from psoriatic patients, NAPso - non-affected skin of psoriatic patients, NAS - non-affected skin of non-melanoma skin cancer patients, BCC - samples from basal cell tumors, BCCc - control samples from patients with basal cell carcinomas, SCC - samples from squamous cell tumors, SCC c - control samples from patients with squamous cell carcinomas, PU - putrescine levels, SMD - spermidine levels, SM - spermine levels.

5.2 times lower than SM and SMD.

In the SCC group only the PU results were significant (p = 0.03). PU was detected at the highest level compared to SMD and SM. The PU rate of synthesis was most increased. SMD and SM levels demonstrated the lowest absolute values compared to all the cohorts investigated. These data corresponded to the results of previous studies conducted on tissue cultures and transgenic animal models, demonstrating the crucial role of PU and its synthetic enzyme ODC in SCC pathogenesis.

Discussion

Our results demonstrated a twofold concentration of the basic polyamines in tissue samples of patients with Pso compared to NAPso and NAS. There was no difference in polyamine metabolism in NAPso and NAS. This disproved the hypothesis of the latent morbid state of non-lesional skin in Pso subjects. The highest level of SM in Pso skin proved its essential role in epidermal metabolism and, in particular, the process of keratinization. SM demonstrated the highest proliferative trend in both Pso and BCC specimens. The SMD/SM ratio in all Pso patients was below 1, and was close to 1 in BCC patients. Thus, the hypothesis of positive correlation of SMD/SM ratio with the tissue proliferation activity was not proven.

The enzyme S-adenosyl-L-methionine decarboxylase (AMDC), which regulates SMD and SM biosynthesis, probably plays a major role in the process of chronic benign keratinocytic proliferation. This suggestion is based on the positive correlation found between SMD and SM levels in Pso lesions. The utilization of a higher number of propylamine groups correlates with the lower levels of adenosine triphosphate (ATP) in Pso lesions explaining this well-known phenomenon. Moreover, Weinstein et al. (1981) proved the beneficial therapeutic role of a potent topical AMDC activity inhibitor, methylglyoxal-bis-guanylhydrazone, which reduced Pso plaques, induration, and erythema (16).

We demonstrated similar polyamine metabolism changes in BCC and Pso patients. The closer relation of BCC polyamine metabolism to benign keratinocytic proliferations than to SCC might explain its long-term benign course and non-metastatic nature. It is unlikely that polyamine abnormalities represent a primary defect in Pso and BCC pathogenesis because changes in polyamines have been noted in several other conditions of epidermal hyperplasia. It is possible, however, that the polyamines are important as cell regulatory factors and that some treatments may partly act via modulation of polyamine metabolism. Manipulation of polyamine metabolism is a realistic target for therapeutic or preventive strategy in the treatment of Pso and BCC. Future therapeutic approaches should focus on reducing exogenic SM intake, utilizing new SM blockers, and synthesizing AMDC inhibitors.

On the other hand, SCC polyamine metabolism crucially depended on PU and ODC activity. The lowest levels of SMD and SM suggested the utilizing role of their catabolic enzyme, spermidine-spermine acetyltransferase (SSAT). These findings corresponded with the hypothesis by Pietila et al., who speculated that SCC tumorogenesis could be induced by continuously lower keratinocytic levels of SMD and SM (17). New SCC therapeutic strategies should focus reducing PU dietary intake and synthesizing ODC inhibitors and SSAT blockers.

Although our results demonstrated different polyamine metabolic changes in lesions from benign and neoplastic keratinocytic proliferations, further studies are needed to elucidate the role of polyamines in the processes of cell proliferation, differentiation, and tumorigenesis, thus opening new therapeutic horizons.

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