Expression of the 8 kDa Heat Shock Protein (Ubiquitin) in Psoriasis*

Introduction

All cells show a heat shock or stress response when exposed to heat or other influences such as toxic substances, heavy metals, treatment with ethanol, amino acid analogues etc. The response induces the specific proteins called heat-shock proteins (1,2). Human skin is at the front line with respect to insults from the environment and it is therefore of interest to understand its stress response. In addition, heat and cold treatment is used in a number of medical treatments for skin diseases. It is therefore of interest to know what the heat-shock response is and whether it contributes to or is deleterious to the medical treatment. Moreover, it is important to know whether the disease condition stimulates the heat shock itself.

Ubiquitin (8 kDa) is a 76 amino acid protein and is perhaps the most conserved gene product in evolution (3). It modulates degradation of abnormal or damaged proteins and belongs to the class of heat-shock proteins induced in conditions of cell stress (4,5). Many studies show that ubiquitin is involved in several chronic degenerative diseases characterised by the formation of cellular inclusion bodies. The ubiquitin response to cell injury appears to be cytoprotective and is particularly important in diseases of the nervous system (6-8). The present study was performed to investigate the pattern of ubiquitin expression in human epidermis and dermis with psoriasis, and to correlate ubiquitin expression with proliferative activity and degree of differentiation of cells. In addition it is known that psoriasis, a hyperkeratotic skin condition the aetiology of which is unknown, may be

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Sixteen biopsy samples from patients with psoriasis who were treated by PUVA and the same samples from these patients before PUVA treatment were investigated by immunohistochemistry on formalin-fixed paraffin-embedded tissue sections, using anti-ubiquitin antibody.

In psoriatic epidermis, ubiquitin was mostly expressed in basal layers with a cytoplasmic staining pattern. Various cells of the dermis layer with psoriasis were stained with anti-ubiquitin antibody. Staining was again confined to the cytoplasmic region and lesions with or without PUVA treatment did not exhibit a different staining pattern. It did, however, seem to have higher staining intensity in PUVA-treated psoriatic lesions than untreated ones. We conclude that ubiquitin might be regarded as a useful marker of cell injury during the stressful conditions of tissue and as an indication of cell vitality and viability.

Key Words: Ubiquitin, psoriasis, immunohistochemistry.

Received: December 1, 1999

Turk J Med Sci
31 (2001) 69-72 © TUBITAK

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treated by PUVA (psoralen plus UV light, A band). This treatment may also easily induce hsb production. Formalin-fixed, paraffin-embedded tissue samples from skin with psoriasis which were treated with PUVA, and untreated samples were investigated by immunohistochemistry, using a polyclonal antibody specific for ubiquitin (8 kDa hsb).

Materials and Methods

The study was conducted on 16 patients with psoriasis who were exposed to PUVA therapy for almost 10 or 15 sessions (total mean dosage of PUVA 22-38 joule/cm²). Biopsy samples for each patient was taken afterwards for immunohistochemical analysis.

Tissues were fixed in formalin and embedded in paraffin. Paraffin-embedded blocks were cut at a thickness of 5 mm on a vibrotome. Sections were dried in an incubator overnight at 40°C and deparaffinised in xylol and dehydrated through graded alcohol. Sections were later left in phosphate buffered saline (PBS). Sections were incubated with anti-ubiquitin antibody (1:250 dilution in PBS) for 4 hours at room temperature. Subsequent incubations were performed with peroxidase conjugated rabbit anti-mouse immunoglobulin antibody (Dakopatts, Copenhagen, Denmark) (1:500 dilution in PBS). The final colour product was developed by incubating for 10 min in diamino benzidine alone (DAB; 0.5 ng/ml in PBS buffer containing 0.01 H₂O₂) for a brown reaction product. Counterstaining was performed with Harris’s haematoxylin (Merck). Sections were coverslipped and examined under a light microscope.

Results

Keratinocytes of the basal layer, spinozum layer and granulozum layer were positive with anti-ubiquitin antibody (Fig. 1). There was, however, no reaction on the corneum layer with the antibody (Fig. 2). Staining intensity appeared not to vary from the upper layers of epidermis to the dermis.

Staining or labelling was heterogeneously distributed throughout the cells, corresponding to a cytoplasmic expression of hsb. All ubiquitin staining was confined to the cytoplasmic site of cells (Fig. 3).

In spite of the fact that ubiquitin is found in all eukaryotic cells and is ubiquitious, the immunohistochemical staining of it within the cells can only take place as its concentration is elevated. Control normal human skin sections (5 volunteers) were almost free of staining with anti-ubiquitin antibody (unstained normal skin sections are not shown).

It was interesting to discover that biopsy sections stained with anti-ubiquitin antibody did not display a distinct staining pattern between psoriatic lesions and PUVA-treated psoriatic lesions, in spite of having stronger antibody staining with PUVA-treated sections than not untreated ones. Furthermore, biopsies taken from the patients covered non-lesional regions where there was no significant ubiquitin staining after PUVA treatment (data not shown).
Discussion

In this study, we demonstrated by immunohistochemistry that ubiquitin is expressed in human skin with psoriasis and might be expressed with PUVA treatment as well. Most of the basal layer of epidermal cells displayed higher levels of ubiquitin than cells of the dermis. This could be an indication of stress effects on the first lines. The psoriatic cases we used in our study had been treated by PUVA. PUVA has the potential to induce stress in epidermal cells since the skin is exposed to increasing temperature during the time in the PUVA cabinet. When the stress response is triggered, the synthesis of most of the cellular proteins is switched off temporarily; consequently cell proliferation would be inhibited at least temporarily and it is known that PUVA treatment is basically antiproliferative. These effects may be more important if local PUVA becomes more widely accepted. The biopsies in the present work were taken from the skin of patients with psoriasis, before and after treatment with PUVA.

It is worth noting that the non-psoriatic regions of the biopsies with PUVA did not show anti-ubiquitin antibody staining. However, the staining of the sections with PUVA treatment appeared to be much denser than untreated biopsy sections. This difference remains unsolved, whether PUVA increases the ubiquitin activities in psoriatic lesions where the cells are highly susceptible to the disease and the cells have already had elevated ubiquitin activity or non-psoriatic regions with PUVA response to synthesis of ubiquitin, but it is not as detectable as the disease stimulation.

Ubiquitin is a critical factor in the ATP-dependent pathway for protein degradation in eukaryotic cells (9). The discovery that ubiquitin is a hsp suggests an important role for protein breakdown in the protection of cells from high temperatures and other types of stress, for instance inflammatory effect and radiation effect.

Recent studies demonstrated that other hsp were induced following PUVA treatment (10,11) or other disease conditions (12-14). Changes in the expression of hsp often occur under conditions that are ultimately lethal to the cells, indicating protein damage and denaturation as the common mechanism of induction (15).

There is concern that long-term PUVA may generate activated oxygen species, such as the superoxide radical anion (O$_2^-$), the hydroxyl radical (OH$^-$), or hydrogen
peroxide, in the epidermal cells, including DNA strand breaks and peroxidation of protein. Most protein oxidation is due to the oxidation of sulphydryl groups causing disulphide crosslink changes, which inactivate or denature proteins (16,17).

In conclusion, using a polyclonal anti-ubiquitin antibody, we demonstrated the presence of the disease and PUVA-induced ubiquitination. The ubiquitin produced may be a response which prevents cell degeneration.

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