HEAT SHOCK PROTEIN 27 IS OVEREXPRESSED IN THE SKIN OF BITUMEN EXPOSED WORKERS. EARLY OBSERVATIONS

C. Fenga, C. Loreto, *C. Caltabiano, D. Germanò

Institute of Occupational Medicine, University of Messina
*Institute of Anatomy, University of Catania

INTRODUCTION

Heat Shock Proteins (HSPs) are chaperones involved in cellular defence mechanism against several different types of aggression. The HSP27 is a member of this family (1).

These molecules also participate in essential physiological processes, such as the regulation of cell cycle, differentiation, programmed cell death and tumorigenicity (2).

Physiologically, HSPs are found in several tissues such as breast, uterus, cervix, placenta and skin, as well as in diseases like actinic keratosis, squamous cell carcinoma, seborrheic keratosis, psoriasis and others (3,4). HSP27 has also been demonstrated in normal human epidermis (5). Exogenous stimuli such as physical and chemical stress, high temperatures, heavy metals, ethanol, arsenite, toxins and oxidants as well as bacterial and viral infections (6,7) induce the activation of heat shock gene as a protecting mechanism as regards necrosis and apoptosis (8). In this way HSP27 mediates tolerance against further stress challenge. As far as molecular level is concerned, HSP27 regulates protein synthesis, folding, assembly, degradation, signal transduction and cell proliferation (9). Its role on carcinogenesis is not yet completely understood (10).

The skin of road pavers is exposed to a large number of compounds such as asphalt, bitumen, amines, polymers, oils, solvents, sand, gravel, crushed rock, mineral wad, ultraviolet light and heat (11).

In order to verify an up-regulation of HSP27, determined by the above mentioned stimuli, we studied, immunohistochemically, the forearm skin of a sample of workers exposed to bitumen products.

MATERIALS AND METHODS

A group of 16 males workers, daily exposed to bitumen, has been gathered for the study. The mean age was 41.69 (S.D.=9.45) and their working average age was 13.31 (S.D.=10.74).

A control group of unexposed volunteers was also examined. They were sex and age matched with unaffected skin. They were all investigated from a medical and a working point of view.

Chemical and merceological information, on the compounds present in the working cycle, was collected.

A total of 25 punch biopsies (3 mm diameter) was obtained from the forearm skin of road paver workers (n=16) and a control group (n=5).

Specimens were sectioned at a thickness of 3-4 μm and processed for immunohistochemistry as previously described (12). Briefly speaking, deparaffinized and rehydrated sections were incubated for 30 min in 0.3% H_2O_2/methanol to quench endogenous peroxidase activity, then rinsed for 20 min with phosphate-buffered saline (PBS) (Bio-Optica M107, Milan Italy). Non-specific protein binding was attenuated by incubation for 30 min with 5% horse serum. For localization of HSP27, a mouse anti-HSP-27 monoclonal antibody (Catologue No. NCL-HSP27; Novocastra Laboratories Ltd, Newcastle U.K.) was used at a dilution of 1:20. The antibody was applied directly to the section and the slides were incubated overnight (4°C) in a "humified chamber". Immune complexes were subsequently treated with the secondary antibody and then detected by means of Streptavidin peroxidase, both incubated for 30 min at room temperature (Vectastain ABC kit, Vector Laboratories, Burlingame CA). The immunoreactivity was visualized by development for 2 min with 0.1% 3.3'-diaminobenzidine and 0.02% hydrogen peroxide (DAB substrate kit, Vector Laboratories). Sections were counterstained with Mayer-hematoxylin, mounted with Permount and examined by light microscopy.

Positive controls consisted of tissue specimens with known antigenic positivity and included sections of breast carcinoma tissue.
Negative control consisted of punch skin biopsy sections which were incubated with normal rabbit serum, omitting the primary antibody.
The staining intensity was assigned on a subjective scale as follows: 0 for no reactivity, 1 for weak reactivity, 2 for moderate, 3 for strong and grade 4 for a very strong reactivity. Three observers, independently, assessed the immunohistochemical reaction and, where the findings were divergent, an agreement was reached after discussion.

RESULTS

Sections of normal skin demonstrate a cytoplasmic staining with HSP27 antibody. Keratinocytes of the basal layer were negative but HSP27 was detected in all other layers. Its intensity gradually increased from the suprabasal keratinocytes (grade 1) to the stratum granulosum (grade 2) (1,4).

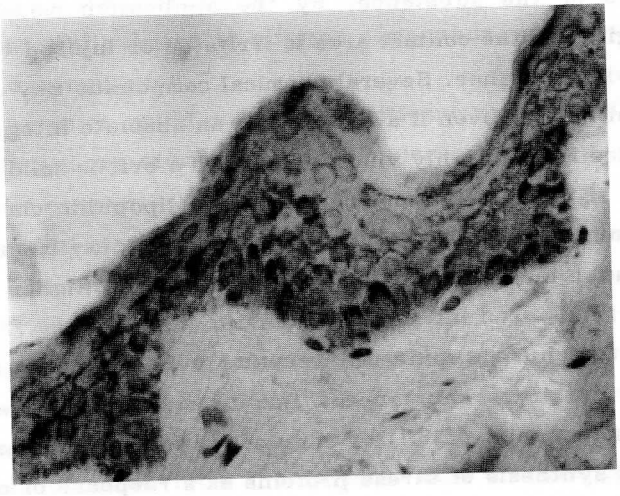

Fig. 1 - HSP27 immunostained skin of workers exposed to bitumen derivatives.

In punch biopsies of bitumen exposed workers, HSP27 immunostaining was homogeneously detected in the whole epidermis including basal cell layer. Immunoreaction products were observed mostly in cytoplasm but also in nuclei. Staining intensity was stronger (grade 3 or 4) than that revealed in normal skin sample (fig. 1).

DISCUSSION

In the present study, it has been demonstrated by immunohistochemistry that chronic exposure of the skin to various deleterious stress factors, such as bitumen compounds, leads to HSP27 epidermis up-regulation. Although HSP27 was detected in normal skin, the extent and distribution of this protein showed considerable up-regulation following bitumen products exposure.

The occupational exposure to bitumen products, found in the road pavers place of work, depends on different factors as manual and mechanical work, lack or presence of protective devices for personnel, smoking, eating habits, work conditions, changing and cleaning of overalls (11). Percutaneous diffusion is influenced by the chemical nature and concentration of the substance, by the application modality and the anatomic region. If the contact area is irritated or injured the cutaneous absorption will be higher. Several chemical compounds may pass through the epidermal barrier even if a barrier has an absolute integrity (11,13). Bitumen varies from a highly viscous liquid to a brittle solid and consists of hundreds of chemicals; most of them have lipophilic characteristics. For this reason they could alter the skin hydrolipidic film and determine pathologies such irritant and allergic dermatitis and higher incidence of skin cancer (11).

It is known that human epidermal keratinocytes represent a target for a variety of cytotoxic substances. Deaton et al. (14) treated human keratinocytes thermally (43°C) or chemically, with sodium arsenite, and pointed out a synthesis of stress proteins as a response of cells to such agents.

The activation of heat shock gene expression is a fundamental and well-

conserved cellular mechanism to protect living organism against various stimuli (8).

HSP27 may protect cells and tissues against stress factors in inflammatory diseases (1), and it has been demonstrated that the expression, and phosphorylation, of this protein is regulated by the inflammatory cytokines tumour necrosis factor-α (TNF-(α), interleukin-1 (IL-1), interleukin-3 (IL-3), interleukin-6 (IL-6), heat shock and other form of oxidative stress (2,15,16). The HSP27 phosphorylation may also be involved in mediating an adaptive response to oxyradical-generating agents such as carcinogens and other xenobiotics.

There is experimental evidence that HSP27 acts as a molecular chaperone and it has a variety of functions including roles in signal transduction, regulation of cell growth, differentiation and tumorigenesis (3,17).

Cells first subjected to mild stress event, sufficient to up-regulate the levels of stress induced protein, are able to survive a subsequent, otherwise lethal, stress event. Both magnitude and duration of the stress determine the amount of damage incurred, and cells die when a threshold of damage is exceeded.

The immunohistological results of the present study, in skin samples of workers chronically exposed to bitumen compounds, point out that there is an HSP27 up-regulation.

This over-expression could reflect a functional response of the skin to deleterious exogenous stimuli present in places of work.

The skin of road pavers is exposed to a large number of compounds such as asphalt, bitumen, amines, polymers, oils, solvents, sand, gravel, crushed rock, mineral wad, ultraviolet light and heat. In order to verify an up-regulation of HSP27, determined by the above mentioned stimuli, we studied, immunohistochemically, the forearm skin of a sample of road pavers occupationally exposed. A total of 25 punch biopsies (3 mm diameter) was obtained from the forearm skin of road paver workers (n=16) and a control group (n=5) not exposed. Specimens were sectioned (thickness: 3-4 μm) and processed for immunohistochemistry. For

localization of HSP27, a mouse anti-HSP27 monoclonal antibody was used. In punch biopsies of bitumen exposed workers, HSP27 immunostaining was homogeneously detected in the whole epidermis including basal cell layer. Immunoreaction products were observed mostly in cytoplasm but also in nuclei. Staining intensity was stronger (grade 3 or 4) than that revealed in normal skin sample.

1) TRAUTINGER F., KINDAS-MÜGGE I., DEKROUT B., KNOBLER R.M., METZE D., Br. J. Dermatol., 1995, 133, 194-202.
2) ARRIGO A.P., Pathol. Biol., 2000, 48, 280-288.
3) CIOCCA D.R., OESTERREICH S., CHAMNESS G.C., McGUIRE W.L., FUQUA S.A., J. Natl. Cancer. Inst., 1993, 85, 1558-1570.
4) GANDOUR-EDWARDS R., McCLAREN M., ISSEROFF R.R., Am. J. Dermatopathol., 1994, 16, 504-509.
5) JANTSCHITSCH C. et al., Br. J. Dermatol., 1998, 139, 247-253.
6) LINDQUIST S., CRAIG E.A., Annu. Rev. Genet., 1998, 22, 631-637.
7) MULTHOFF G., BOTZLER C., ISSELS R., Biol. Chem., 1998, 379, 295-300.
8) LEPPÄ S., SISTONEN L., Ann. Med., 1997, 29, 73-78.
9) HIGHTOWER L.E., Cell, 1991, 66, 191-197.
10) BAYERL C. et al., Eur. J. Dermatol., 1999, 9, 281-284.
11) RIALA R., HEIKKILÄ P., KANERVA L., Int. J. Dermatol., 1998, 37, 27-30.
12) FENGA C., SPATARI G., LORETO C., LEONARDI R., GERMANO' D., Boll. Soc. It. Biol. Sper.-J. Biol. Res., 2000, 76, 67-72.
13) CASTELLINO N., PAOLETTI A., "Dermopatie Professionali", p. 503 in: Medicina del Lavoro, Monduzzi, Bologna, 1993.
14) DEATON M.A., BOWMAN P.D., POWANDA M.C., Fundam. Appl. Toxicol., 1990, 14, 471-476.
15) GUSDON F., SAKLATVALA J., J. Immunol., 1991, 147, 3402-3407.
16) GUSDON F. et al., J. Biol. Chem., 1993, 268, 4236-4243.
17) KINDAS-MÜGGE I. et al., Cell. Growth Differ., 1996, 7, 1167-1174.

KEY WORDS: bitumen products, skin, heat shock protein 27, immunohistochemistry.

Lectured at the meeting held in Catania on September 8, 2000.
Received: September 15; accepted: October 6, 2000.

Address reprint requests/correspondence to Dott. C. Fenga, Ist. di Medicina del Lavoro, Università di Messina, Policlinico Universitario Pad. H, Via Consolare Valeria, Gazzi I-98124 Messina.