A CROSS-SECTIONAL STUDY OF WORKERS WITH OCCUPATIONAL EXPOSURE TO PETROLEUM DERIVATIVES

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INTRODUCTION

Human involucrin (hINV) is an early cytoplasmatic protein of the cornified cell envelope (1,2) that is considered as a marker of normal keratinocyte differentiation and maturation (3,4). In keratinocytes the formation of the cornified cell envelope is needed to enable skin to withstand physical, mechanical and chemical agents (5).

In normal orthokeratinized body, hINV expression begins in the upper spinous layer coincident with cell flattening (6); however deeper epidermal staining for hINV has been described in hyperproliferative and diseased epidermis (5).

A significant premature expression in cultured keratinocytes and skin following the exposure to irritant agents have also been described (7). In line with these findings some Authors (8) argued that hINV renders the envelope insoluble and resistant to extraction by irritants. Accordingly, this study was undertaken to assess the pattern of hINV immunohistochemical expression in a group of gasoline storage workers who had undergone to a prolonged exposure to petroleum derivatives.

MATERIALS AND METHODS

A group of healthy male volunteers exposed to gasoline for a period ranging from 10 to 26 years, participated in this study. The subjects had no past or present history of skin diseases. Their age ranged from 35 to 50 years with a mean of 17.2 years. Their skin was of normal appearance with no clear evidence of dermatitis, erythema, dry skin or clinical

reaction. The control group consisted of volunteers, sex and age matched and with unaffected skin who had not any exposure history. All subjects gave written informed consent.

A total of 25 punch biopsies (3 mm diameter) were obtained from the forearm skin of gasoline storage workers (n=20) and control group (n=5). The biopsies were performed using 1% Lidocaine without epinefrine which was infiltrated peripheral to punch sites.

Specimens were fixed in 10% neutral buffered formalin (Bio-Optica, Milan, Italy). After fixation and overnight wash, each specimen was sectioned through its centre along a parasagittal plane, perpendicular to its long axis. Tissue blocks were dehydrated in graded ethanol and embedded in paraffin with anatomic orientation preserved. Sections 3-4 μm thick were cut according to routine procedures, mounted on sialane coated slides and air dried.

Sections were deparaffinised in xylene and rehydrated through a series of graded alcohol, incubated for 30 min in 0.3% peroxidase to quench endogenous peroxidase activity and then rinsed for 20 min with phosphate-buffered saline (PBS). Non-specific binding was attenuated by incubation for 30 min with 5% horse serum.

The antibody employed was a mouse monoclonal antibody (NCL-INV Novocastra Ltd., Newcastle, UK) used at a dilution of 1:200 in PBS. Anti-hINV antibody was applied onto the sections and incubated overnight. 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 Calif., USA).

The immunoreaction was visualized with peroxidase substrate AEC kit (Vectastain AEC kit, Vector Laboratories, Burlingame Calif., USA) yielding a red end-product at the site of the target antigen.

Negative control consisted of parallel skin sections which were incubated with normal rabbit serum, instead of the specific antibody.

To assess involucrin reactivity two representative areas in the epidermis were chosen, one above a dermal papilla (suprapapillary: the narrower

part), the other between two dermal papillae (interpapillary: the wider part), as suggested by Gerritsen et al. (9). The positive staining cells were expressed as a percentage of the total number of cells.
Student's t-test was used for statistical analysis.

RESULTS

In the control group skin immunoreactivity was detected in the keratinocytes of the upper third of the epidermis (granular layer and upper first spinous layer) the horny layer being negative. Stained epidermal cells comprised 19.4% (means) of suprapapillary epidermis and 27.7% (means) of interpapillary epidermis.
In punch biopsies of gasoline storage workers the zone of cells that appeared immunostained by anti-hINV was broader than in normal skin, starting 2-3 cell layers above the basal layer, in a region where the cells were still cuboidal (fig. 1).

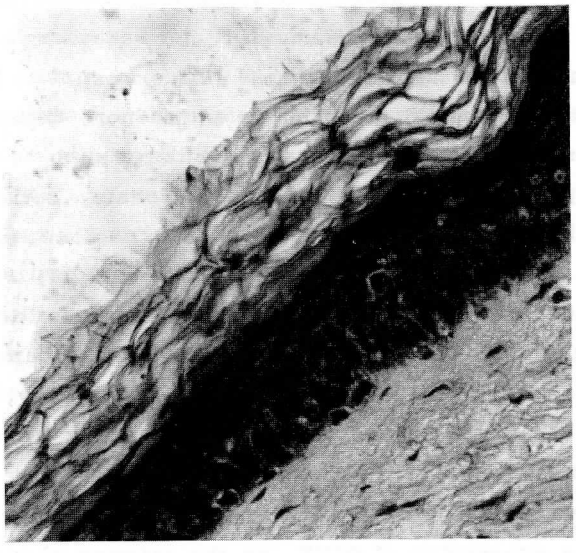

Fig. 1 - HINV immunohistochemical staining, in forearm gasoline storage worker punch biopsy. Immunoreaction products for hINV are detectable from the first-second spinous layer (original magnification (380).

Mean percentage of stained suprapapillary and interpapillary epidermal keratinocytes was 27.7% and 46.8% respectively.
The onset of hINV expression in skin of gasoline workers showed a significant downward shift and an overall increase in number of immunolabeled keratinocytes as demonstrated by t-test values ($P<0.01$).

DISCUSSION

The expression of hINV by epidermal keratinocytes was studied by immunohistochemistry in gasoline exposed workers, in order to assess whether a mild chronic irritant stimulus can lead to changes in epidermal keratinocyte proliferation and differentiation.
As far as skin of subjects never exposed to petroleum derivates our findings agree with those of literature (1,6,10-12). On the other hand skin biopsies of gasoline exposed workers demonstrated a premature synthesis and an increase in number of immunolabeled cells by anti-hINV antibody. In fact, hINV immunoreaction products were detected in the lower part of the stratum spinosum.
Previous investigations have shown that skin exposure to irritants leads to several histopathological reactions in a time-dose dependent manner. Cutaneous application of high doses of irritants such as sodium lauryl sulphate (13), sodium dodecyl benzene sulfonate, cationic detergent cetytrimethylammoniumbromide, sodium laurate, acetone, etc., cause cell death and a strong inflammatory response (7,12,14) while repeated exposure to low doses leads to chronic irritant contact dermatitis (14). The pathway through which these exogenous insults can influence cell biological response, is still incompletely known. Probably, skin irritants could directly impair the barrier function and thereby transduce a signal to the keratinocytes (14). As a consequence of this stimulus the keratinocytes would sense disruption of their barrier function, reacting by a premature expression of hINV, on the attempt of increasing their defence mechanism. It has been hypothesised that these irritants could also influence biological behaviour of keratinocytes (14) and that the premature expression of hINV could be related also to an altered tissue

homeostasis, as observed in hyperproliferative and diseased epidermis (6).

The hINV up-regulation observed in our study seems to demonstrate an involvement of keratinocyte differentiation pathway in the forearm skin of gasoline storage workers. Definitive conclusion can be drawn with a longitudinal study carried out in these workers just before being employed and after the exposition to petroleum derivatives. This approach could be interesting to assess the hypothetical value of the involucrin staining pattern as a pre-clinical sign of skin involvement following gasoline exposure.

Expression of human involucrin (hINV) a protein of the cornified cell envelope, was studied in the skin of gasoline storage workers, in order to evaluate the effects of the exposure to petroleum derivatives.

A total of 25 forearm skin punch biopsies were carried out. Twenty of which were performed on exposed subjects and five on controls. The specimens were processed for immunohistochemistry and hINV expression was evaluated using an anti-hINV monoclonal antibody and the ABC technique. Percentage of immunolabeled keratinocytes was significantly higher in subjects exposed to gasoline with respect to the control sample. A premature hINV expression was detected both in suprapapillary and interpapillary keratinocytes. Such overexpression of hINV seems to be related to an attempt of increasing skin defence mechanism. Therefore it was concluded that also in absence of clinical skin manifestation the exposure to gasoline determines an involvement of keratinocytes on molecular basis.

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