The following full text is a publisher's version.

For additional information about this publication click this link.
http://hdl.handle.net/2066/23491

Please be advised that this information was generated on 2019-03-13 and may be subject to change.
Mai Le • Joost Schalkwijk • Georges Siegenthaler
Peter C. M. van de Kerkhof • Jacques H. Veerkamp
Pieter G. M. van der Valk

Changes in keratinocyte differentiation following mild irritation by sodium dodecyl sulphate

Received: 30 October 1995

Abstract Although the induction of acute irritant dermatitis by detergents has been studied extensively in recent years, our understanding of the cell biological events in the repair phase, and its relevance for the development of chronic irritant dermatitis is limited. Here we studied the reaction pattern of human skin to short-term application of sodium dodecyl sulphate (SDS) in a model that induced a minimal acute inflammatory reaction (absence of polymorphonuclear leukocytes, PMN) and did not have cytopathic effects on the epidermal keratinocytes as determined by histological investigation. All parameters were measured up to 14 days after exposure to SDS. Application of SDS caused disturbances of barrier function as measured by transepidermal water loss and had vascular effects as judged by erythema. Several cell biological markers for epidermal growth and differentiation were examined by immunohistochemistry. A rapid and strong induction of the cornified envelope precursor protein involucrin was seen in the stratum spinosum, with a peak at 24 h. Within 24 h a strong upregulation of epidermal fatty acid binding protein (E-FABP) was noted, with a peak at 7 days after injury. Cellular proliferation in the basal layer was increased fivefold as assessed by nuclear staining for the Ki-67 antigen, showing a peak at 48 h. Surprisingly, no significant induction of cytokeratin 16 and SKALP/elafin expression, two markers associated with epidermal hyperproliferation and inflammation, was seen. These findings suggest that the cellular changes following exposure to detergent are distinct from those seen in other forms of skin injury. We would speculate that the epidermal response to detergent exposure is primarily directed at restoration of barrier function.

Key words Irritant dermatitis • Keratinocytes • Sodium Dodecyl sulphate

Introduction

Repeated exposure of the skin to irritants may lead to chronic irritant contact dermatitis [8, 24, 25]. Although impairment of the horny layer barrier function and cytotoxic damage to epidermal cell populations are thought to play a role, the pathogenetic process remains poorly understood [24, 25, 34-38]. Application of standard irritants such as sodium dodecyl sulphate (SDS) has been used as a model to study the effects of irritants on physicochemical properties of the skin such as transepidermal water loss (TEWL), electrical capacitance (stratum corneum hydration), percutaneous drug transport, and skin colour reflectance (erythema) [7, 30-33]. Recently, cell biological changes following barrier disruption by SDS, acetone or tape stripping have been reported [16, 20, 39]. Using a model with 48-h exposure to 5% SDS, a strong inflammatory response was found characterized by a dense infiltrate consisting of polymorphonuclear leucocytes (PMNs) and mononuclear cells [38]. Others have shown that Langerhans cell class II antigen expression is downregulated when toxic concentrations of SDS are applied [35].

The relevance of these findings for the development of chronic irritant dermatitis is not clear because the application of high doses of SDS causes cell death and a strong inflammatory response. Therefore, the effects of barrier disruption on epidermal cells cannot be dissociated from inflammatory changes or even wound healing processes in the skin. Another model for barrier disruption that does not seem to have cytopathic effects on the epidermal keratinocytes is the usage of low doses of SDS, which may serve as a model for chronic irritant dermatitis. This study was designed to address the question of whether SDS exposure results in changes in differentiation of keratinocytes similar to those seen in other dermatitis models.
tinocytes is tape stripping [16]. Tape stripping induces an increase in TEWL [16] and induction of a hyperproliferative/inflammatory phenotype in the keratinocytes which includes the expression of cytokeratin 16 and the epidermal proteinase inhibitor skin-derived antileucoprotease (SKALP) elafin as we have shown previously [1, 14]. The amount of cellular inflammatory infiltrate induced by tape stripping is variable [4, 16]. It has been established that barrier repair correlates with increase in lipid synthesis and lipid deposition, or with enzymes associated with these processes [9, 17]. In chronic irritant dermatitis, repeated exposure to mild irritation rather than toxic dosages is thought to be the mechanism. We therefore wanted to investigate the effect of a mild irritant stimulus on epidermal growth and differentiation.

In the present study we titrated the SDS applications to induce skin irritation (as assessed by erythema and TEWL) but avoiding significant amounts of infiltrating inflammatory cells and gross damage to the epidermis. We studied the expression of several markers for normal and abnormal keratinocyte growth and differentiation. In addition to the envelope precursor protein involucrin, and the inflammation/hyperproliferation-associated molecules CK16 and SKALP/elafin [13, 21, 29], we studied the expression of epidermal fatty acid binding protein (E-FABP) which is a recently described cytoplasmic lipid transport protein [22, 23, 27], otherwise known as psoriasis-associated fatty acid binding protein [12]. We showed that the epidermal response to mild irritation by SDS is characterized by an absence of proteins normally found under inflammatory/hyperproliferative conditions (e.g. CK16 and SKALP/elafin). In contrast there was a strong upregulation of involucrin and E-FABP. These findings suggest that the epidermis can respond differentially to trauma, depending on the inciting injury.

Materials and methods

Subjects

A group of 11 healthy volunteers (eight males and three females) with no past or present history of skin disease, participated in this study. Their age ranged from 21 to 23 years with a mean age of 22 years. Permission of the Medical Ethics Committee was obtained prior to the experiments. All subjects gave written informed consent.

Chemicals and antisera

A mouse monoclonal antibody (M752) against lucecyte elastase and peroxoide-conjugated secondary antisera were obtained from Dakopatts, Glostrup, Denmark. A polyclonal antibody directed against recombinant SKALP/elafin was prepared as described previously [15]. The monoclonal antibody Ks8.12 was used to detect cytokeratin 16, and was obtained from Sigma Chemical Co, St Louis, Mo. The monoclonal antibody MIB-1, which recognizes the proliferation-associated Ki-67 antigen in formalin-fixed material, was obtained from Immunotech, Marseille, France. The monoclonal antibody MON-150, directed against human involucrin, was prepared and characterized as described previously [5]. A polyclonal rabbit antiserum against E-FABP was prepared as described previously [25]. The Vectastain kit was obtained from Vector, Burlingame, Calif. and the SDS was from Bio-Rad, Richmond, Calif.

SDS application

Previous experiments had shown mild erythematous responses with a 5% w/v SDS patch test applied for 4 h [26]. Our self-made patches consisted of a 1.5 cm² piece of absorbent non-woven fabric on a 4.0 cm² piece of impermeable plastic foil. A 200 µL aliquot of SDS solution was pipetted on to each patch. The patches were fixed to the skin of the upper back parallel to the vertebral column with tape (Fixomull Stretch, Beiersdorf, Hamburg).

Clinical grading and TEWL measurement

Visual readings and TEWL measurements were performed at 0 h, 4 h, 10 h, 24 h, 48 h, 96 h, 168 h (day 7) and 336 h (day 14) after removal of the patches. The erythema was graded using the following visual scoring: 0, no response; 1, mild patchy erythema; 2, diffuse mild erythema; 3, moderate erythema; 4, intense erythema; 5, intense erythema with oedema. TEWL measurements were done with a Tewameter TM 210 (Courage & Khazaka, Germany), according to standard guidelines [19]. Before the actual assessment, there was a waiting period of 15 min to reduce the effects of sweating [2]. During the measurements the room temperature was kept constant at 20°C. The relative humidity varied from 35% to 49% (mean 39.3%).

Biopsy procedures

Punch biopsies (3 mm diameter) were taken from each of the patch test sites of the 11 volunteers after visual reading and TEWL measurements. A maximum of four biopsies per volunteer were taken and a total of four biopsies were obtained per time-point. After 24 h fixation in formalin the samples were embedded in paraffin, sectioned at 6 µm and prepared for immunohistochemistry.

Immunohistochemical methods

The slides were deparaffinized in xylene for 20 min, followed by dipping in an ethanol series from 100% to 50%. After washing in phosphate-buffered saline (PBS) the sections were incubated with the normal rabbit serum (20%) for antileucastase, MON-150, Ks8.12, and with normal swine serum (20%) for anti-SKALP for 15 min. Before this preincubation the sections were pretreated with citrate buffer in the case for Ks8.12 and MON-150. Antigen retrieval in a microwave oven was necessary for MON-150. The sections were then incubated with the following primary antibodies: antileucastase, anti-SKALP, Ks8.12 and MON-150 at the dilutions of 1:250, 1:500, 1:10 and 1:100 in PBS ude with 1% bovine serum albumin (BSA), respectively. Sections incubated with antileucastase, Ks8.12 and MON-150 were stained with peroxidase-conjugated rabbit antimouse immunoglobulins in PBS containing 5% human serum for 30 min. All slides were developed with 3-amino-9-ethylacbazol as the chromogenic substrate for 10 min at 37°C. After two washes in demineralized water, the slides were mounted in glycerol-gelatine.

An avidin-biotin immunoperoxidase technique was used for MIB-1 and anti-E-FABP according to standard procedures. After deparaffinizing and washing in PBS the slides were preincubated with 20% normal horse serum and 20% normal goat serum for MIB-1 and anti-E-FABP, respectively. This preincubation was followed by incubation with primary antibodies MIB-1 and anti-E-FABP at dilutions of 1:50 and 1:100 in PBS containing 1% BSA, respectively. After washing in PBS the sections were incubated in antirabbit IgG-biotinated antibody and antihorse IgG-biotinated antibody for MIB-1 and anti-E-FABP. The sections were then incubated in avidin-biotin complex diluted (1:50/1:50) in PBS with 1% BSA. All slides were developed with a metal-enhanced
DAB substrate kit (Pierce, Rockford, Ill., USA) for 10 min at 37°C. After washing in demineralized water and dipping in 100% ethanol and xylene, the slides were mounted with Permount (Fisher-Scientific, NJ, USA). Haematoxylin-eosin staining was performed according to the method of Mayer.

Histological grading

Cycling keratinocytes were determined by counting the number of MIB-1-positive nuclei per millimetre length of section. The quantifications of involucrin and E-FABP expression were similar: two representative locations (supra- and interpapillary) on the epidermis were taken for assessing the number of positively stained layers. The ratio of the number of positive layers to the total number of cell layers was calculated.

Results

Clinical assessment and TEWL measurements

On the basis of a pilot dose-finding experiment, a 4 h exposure to 5% SDS was chosen. This regimen induced mild to moderate erythema which peaked at 24 h and then sharply declined. Figure 1 shows the time course of the visual grading of erythema. No strong reactions were observed. As shown in Fig. 2, TEWL values reached a maximum on day 1 after exposure. Thereafter TEWL values gradually decreased to normal values by day 14.

Histological findings

H & E staining

On days 1 and 2 after SDS application nearly all sections showed a very mild perivascular infiltrate composed of mononuclear cells. No PMNs were observed, either in H & E-stained sections or in sections stained with the PMN-specific marker leucocyte elastase (not shown). The upper dermis showed slight vascular dilatation and oedema (Fig. 3). No cytopathic effects were noted in the epidermis. On days 4–14 parakeratosis was found (not shown). No apparent acanthosis was seen.

Epidermal proliferation

The MIB-1 antibody was used to stain the Ki-67 antigen in cycling cells. Keratinocytes with positively stained nuclei were located in the basal and the first suprabasal layers of the epidermis (Fig. 4a–c). In normal unstimulated skin the number of MIB-1-positive nuclei was 58.9 ± 15.5 per mm (mean ± SEM). The cutaneous reactions to SDS were characterized by a marked increase in the number MIB-1-positive nuclei and reached a maximum of 251.3 ± 44.4 (mean ± SEM) on day 2 after application. On day 14 the number of Ki-67-positive cells had reached baseline.

---

**Fig. 1** The time course of erythema scores after challenge. 0, no reaction; 1, patchy mild erythema; 2, diffuse mild erythema; 3, moderate erythema; 4, intense erythema

**Fig. 3** H&E staining of a section 10 h after SDS application showing slight vascular dilatation in the upper dermis (scale bar 100 μm)

**Fig. 2** The time course of TEWL. (■ baseline TEWL of normal skin, ▲ TEWL of SDS-treated skin is demonstrated)
volunteers, which is a precursor protein of the cornified en-dermis. After thiourea, we further studied the expression of involucrin. SDS-PAGE analysis (Fig. 6) of normal skin and 24 h after application revealed that the involucrin gene was expressed in one or two layers of the stratum granulosum only 24 h after application. Some authors have extensively characterized the involucrin gene and many others have extensively characterized in the involucrin gene in the epidermis. The involucrin gene is expressed in normal epidermis. These include previous studies have shown that the involucrin cells of the suprabasal and the basal layers of the epidermis express involucrin. A number of studies have shown that the involucrin cells of the suprabasal and the basal layers of the epidermis express involucrin.

Fig. 5. The data on K-67 expression are summarized in the graph. The data on K-67 expression are summarized in the graph. The data on K-67 expression are summarized in the graph.
E-FABP expression

Fig. 8 E-FABP expression up to 14 days after challenge. Values are the proportions of epidermal cell layers stained expressed as a percentage of the total epidermal cell layers.

Fig. 9 Series of sections showing staining of involucrin (scale bar 100 μm). a normal skin; b day 1 after stimulus; c day 14 after stimulus.

E-FABP are found throughout the stratum spinosum [5, 12, 28]. Figures 7 and 8 show the time curve of involucrin and E-FABP expression after SDS application. The peak of involucrin expression was found very early (on day 1), whereas the peak of E-FABP induction was found on day 7. Figures 9a–c and 10a–c show the immunohistochemical localization of involucrin and E-FABP. Both involucrin and E-FABP expression were found in the stratum spinosum up to day 7 after SDS application. By day 14 the expression pattern of both involucrin and E-FABP had returned to normal.

Discussion

Chronic irritant dermatitis is thought to evolve from repeated and cumulative exposure to irritants [8, 24, 25]. Therefore the pathogenesis of chronic dermatitis is difficult to study using experimental models in humans. Several models have been used to study the physicochemical events during acute irritant reactions, such as skin barrier disruption by SDS or acetone application, or tape stripping [16, 30, 32, 33]. The cell biological mechanisms underlying the pathogenetic events of these acute models,
and their contribution to the development of chronic irritant contact dermatitis, have received little attention. Recently, studies in humans [35, 37] have indicated that exposure to chemicals can lead to specific cutaneous reactions depending on the nature of the chemical. Studies in mice using acetone as an agent to disrupt barrier function have shown an increase in cell proliferation and, more importantly, an increase in enzymes involved in lipid synthesis [11, 20]. These studies indicate that the cutaneous reaction to injury does not necessarily follow one defined common pathway. In addition to the nature of the stimulus, the severity of the inciting injury also determines the epidermal response. When the injury disturbs the dermoepidermal junction, or induces epidermal necrosis, the response will be very similar to that seen in the healing of partial thickness skin wounds. This will include a strong inflammatory response, an increase in connective tissue synthesis/turnover, and an epidermal response directed at closure of the wound including the induction of anti-inflammatory mechanisms, as we have shown previously [3].

It is evident that the cell biological processes involved in wound healing will be different from those resulting from a mild epidermal trauma such as perturbation of barrier function. Therefore, in this study, we used a model which left the epidermis intact and induced a very mild inflammatory reaction. This was confirmed at the histological level: no cytopathic effects on the keratinocytes were noted, and cellular infiltration was restricted to a few mononuclear cells that were seen in the initial phase, around the blood vessels. Surprisingly, a clear reaction was seen clinically (erythema) and by physicochemical measurement (TEWL), despite the absence of a significant cellular infiltrate at the histopathological level. Immunohistochemical staining for various parameters known to be altered during disturbance of epidermal integrity (as seen in wound healing, psoriasis, tape stripping) were studied. Remarkably, no induction of keratin 16, and only a very slight induction of SKALP/elafin, two molecules known to be upregulated during wound healing and psoriasis [13, 21], were seen. Using elastase as a marker, the presence of neutrophils at any stage was excluded.

The cell biological response of the keratinocytes was shown to include an increased proliferative rate as measured by Ki-67-positive cells, the induction of involucrin and E-FABP, a protein putatively associated with high lipid traffic. Involucrin is one of the earliest proteins to be incorporated in the cornified envelope [10] and has been shown to be a substrate for transglutaminase type 1 [18]. E-FABP, also known as psoriasis-associated fatty acid binding protein, is a recently discovered member of the cytoplasmic fatty acid binding protein family [23, 27] which has a high affinity for the C-18 fatty acids. In psoriasis [12], E-FABP expression has been found to be upregulated both at the mRNA and protein levels. In a recent study the expression of E-FABP was examined following retinoid application or SDS application, using northern blot analysis. Because of the variable E-FABP mRNA expression in normal skin, no clear induction by SDS or correlation with barrier disruption were found [6]. Interestingly the kinetics of E-FABP induction in our model were different from the kinetics of involucrin. Involucrin is rapidly induced and shows a peak on day 1. E-FABP was also induced early (day 1) but peaked on day 7. We would speculate that the increased expression of E-FABP reflects a temporary increase in lipid traffic which appears to be associated with abnormal keratinocyte differentiation (Masouyé I et al., manuscript in preparation). The question as to whether E-FABP is essential for skin barrier function awaits further mechanistic studies.

We hypothesize that SDS could influence the epidermis in two ways. First, SDS could directly impair the barrier function (as measured by increasing TEWL) and thereby transduce a signal to the keratinocytes. Following this stimulus the keratinocytes would sense disruption of barrier function and the epidermis could react by overexpression of involucrin. Second, SDS could also directly influence the keratinocytes. In this case the overexpression of cell biological markers and the increase in TEWL are correlated events, but not necessarily causally linked.

Clearly the model described here is a form of mild acute injury which appears to be fully reversible within 1 or 2 weeks. We can only speculate on the relevance of these findings with respect to the development of chronic irritant dermatitis. Repeated exposure to low-dose irritancy could lead to a chronic overstimulation of the repair processes seen in this model, such as continuous hyperproliferation and prolonged overexpression of involucrin and E-FABP. How this relates to the clinical features of chronic irritant dermatitis (inflammation, lichenification, fissures) is unclear. Further studies on the effects of continuous barrier disruption and the associated repair processes are needed to gain more insight into the pathogenetic process of chronic irritant dermatitis.

Acknowledgement This investigation was supported, in part, by the Swiss National Science Foundation (grant no. 32-31338.91 to GSI)

References