Published in final edited form as:

Dermatitis. 2019 ; 30(2): 155–161. doi:10.1097/DER.0000000000000454.

***Euphorbia myrsinites* sap-induced phytodermatitis: A prototype of irritant contact dermatitis?**

Benedikt Weber, MD, PhD¹, Christoph Sinz, MD¹, Wolfgang M. Bauer, MD¹, Stefanie Kancz, Msc¹, Harald Kittler, MD¹, Tamar Kinaciyan, MD¹, and Alessandra Handisurya, MD¹

¹Department of Dermatology, Medical University of Vienna, 1090 Vienna, Austria

Abstract

Background—A hallmark of *Euphorbia myrsinites* (EM), a member of the widespread perennial *Euphorbia* species, is the extrusion of a poisonous latex-like sap irritant to skin and eye after contact. The exact mechanisms underlying these effects have not been unraveled so far.

Objectives—Allocation of EM sap-induced phytodermatitis to irritant or allergic contact dermatitis; investigation of mechanism(s) causing keratinocyte damage.

Methods—Cutaneous effects of EM sap on healthy human skin were investigated by clinical scoring and reflectance confocal microscopy (RCM) analyses and compared to allergic contact dermatitis (ACD). In addition, the effects of sap exposure to keratinocytes were analysed *in vitro* using histological analyses and flow cytometry.

Conclusions—We report on two cases of EM sap-induced phytodermatitis. Patch testing with fresh EM sap induced dermatitis in 100% of the tested sites with a clinical course following a decrescendo pattern. Compared to ACD the lesional phenotype was more severe and epidermal disruption more pronounced. Exposure of human skin tissues and cultivated keratinocytes to EM sap *in vitro* resulted in a dose-dependent increase in keratinocyte-apoptosis. The reported findings support the primarily toxic irritant nature of EM sap-induced phytodermatitis. The contribution of ingenol mebutate to (non-toxic) proinflammatory effects remains to be elucidated.

Keywords

Euphorbia myrsinites; sap; contact dermatitis; reflectance confocal microscopy

Correspondence: Assoc. Prof. PD Dr. med. Alessandra Handisurya, Medical University of Vienna, Department of Dermatology, Waehringer Guertel 18-20, 1090 Vienna, Austria. Tel: +43 1 40400 77020; Fax: +43 1 40400 78050. alessandra.handisurya@meduniwien.ac.at.

Conflict of interest: The authors have no conflicts of interest to declare pertinent to the present paper.

Author contributions:

Study conception and design: B. Weber, A. Handisurya

Acquisition of data: B. Weber, C. Sinz, W. M. Bauer, S. Kancz, A. Handisurya

Analysis and interpretation of data: B. Weber, C. Sinz, W. M. Bauer, A. Handisurya

Drafting of manuscript: B. Weber, A. Handisurya

Critical revision: C. Sinz, W. M. Bauer, H. Kittler, T. Kinaciyan

All authors consented to the final, submitted version.

Introduction

Euphorbiaceae comprise a large and diverse family of plants with more than 8000 different species found all around the globe (1,2). The sub-species *Euphorbia myrsinites* (also known as myrtle spurge) is an evergreen perennial native mainly to Europe and central Asia where it is grown as ornamental garden plant or found as invasive weed.

A defining characteristic of the *Euphorbia* species is the extrusion of a poisonous, latex-like sap after damage of stems or leaves, which serves as an intrinsic wound-healing mechanism and as a deterrent to herbivores. The milky sap contains diterpene esters (3), such as phorbol and ingenol mebutate, which are toxic when ingested and irritant to eyes (4) and skin after contact. The clinical presentation of *Euphorbia* sap-induced toxic contact dermatitis can vary from mild to severe depending on the amount and quality of sap, the duration of exposure, and the host's skin condition. Symptoms, in general, start two to eight hours after contact and can increase in intensity within the next twelve hours. Skin manifestations range from erythema, edema and vesicles to development of bullae, ulcers and necrosis and are often accompanied by painful burning or itching sensations (2,5,6). While eruptions are usually confined to the contact area, in sporadic cases skin lesions were observed at distant sites (2,7). In some cases delayed onset and development of new lesions several days after initial contact have been reported (6,7,8). Reactions typically resolve within three to four days without sequelae.

Although the available reports demonstrate the irritant effects of *Euphorbia* sap to skin (1,2, 5,6,8), the exact mechanism behind these inflammatory reactions have not been unraveled so far. One possible mechanism is the direct corrosive effect of phorbol esters contained in the plant's sap with direct pH-induced cytotoxicity leading to necrotic damage of keratinocytes. These effects can be augmented by ingenol mebutate via induction of a proinflammatory milieu and cell death (9). However, given the ubiquitous nature of these plants a previous allergic sensitization cannot always be excluded. Considering the delayed response in several patients, a type IV hypersensitivity reaction to sap proteins may represent an alternative explanation (6,10,11) and also immediate type I hypersensitivity reactions have been reported previously (11,12,13). In addition, combinations of irritant and allergic components may be present (6) further complicating the assignment of the skin eruptions observed in *Euphorbia* sap-exposed skin.

To this end, identification of the primary mechanism causing keratinocyte damage and better discrimination of non-allergic versus allergic causes of *Euphorbia* sap-induced toxic contact dermatitis could guide diagnostic, therapeutic and preventive interventions in affected patients. In this study we report on the clinical spectrum of contact dermatitis elicited by the sub-species *Euphorbia myrsinites* and investigated the impact of *Euphorbia* sap on skin keratinocytes by histology and flow cytometry. In addition, to evaluate a non-invasive tool that enables detailed studies on contact dermatitis, we conducted a comparative *in vivo* analysis of *Euphorbia myrsinites* sap-exposed skin to allergic contact dermatitis (ACD) induced by the established allergen nickel sulfate using reflectance confocal microscopy (RCM).

Methods

This study was approved by the Ethics Committee of the Medical University of Vienna, Austria (EK 2015/2017, EK 071/2005) and performed in accordance with the Declaration of Helsinki principles.

For all procedures fresh sap of a commercially available *Euphorbia myrsinites* plant (Stauden Ring, Oldenburg, Germany) (Figure 1A) was obtained by puncturing the stems with an 18 gauge needle and collecting the emitted milky liquid (Figure 1B) under sterile conditions.

For patch testing, healthy volunteers (n=7) were exposed to freshly obtained undiluted Euphorbia sap, controls consisting of patients with positive type IV hypersensitivity to nickel sulfate (n=5) to the respective allergen (SmartPractice Europe, Barsbüttel, Germany). Four participants of the test group who had a previous sensitization to nickel sulfate were exposed to both, Euphorbia sap and nickel sulfate, allowing for intraindividual comparison. The substances were applied to skin on the volar side of the lower arm, occluded with Finn Chambers on Scanpor (SmartPractice Europe GmbH) and evaluated on day (D) 1 and D2 after application by clinical inspection and RCM. Clinical scoring was performed according to the guidelines of the International Contact Dermatitis Research Group (14): weakly positive (+) when erythematous maculae were observed; strongly positive (++) in the presence of papulovesicles; very strong reaction (+++) when coalescing vesicles or erosions had formed; or negative (-). For RCM analyses (VivaScope® 3000 confocal laser scanning microscope; Caliber Imaging & Diagnostics Inc., Rochester, NY, USA) horizontal mapping of each skin site was performed and at least 10 images were captured throughout the entire epidermis mainly focusing on the target zones, the stratum granulosum and stratum spinosum. Images taken from the mid-epidermal region (suprabasal epidermal layers) were evaluated for the following parameters: 1) maximum size of vesicles (mean diameter per image field calculated according the following formula: $x = \text{inner maximum diameter of a vesicle} / \text{total length of the field}$); 2) maximum number of multi-chambered vesicles per image field; 3) epidermal necrosis (maximum necrosis observed in each scan according to a visual score of 0-4 with 0 = none to 4 = severe); 4) spongiosis (maximum spongiosis observed in each scan according to a visual score of 0-4; 0 = none to 4 = severe).

Histological analyses were performed on normal human skin obtained from elective breast reductions and abdominoplasties. 4 mm punch biopsies were immediately placed into a well of a 24-well plate containing RPMI 1640 supplemented with 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin. Ten-fold serial dilutions of freshly obtained Euphorbia sap (ranging from 0.001 to 10 µl) were added to the wells. For control purposes, biopsies not exposed to sap were incubated in parallel. After a 24-hour incubation samples were formalin-fixed and embedded in paraffin. Tissue sections at 4 µm thickness were stained with hematoxylin-eosin and analysed using conventional light microscopy (Zeiss AxioObserver Z1, Carl Zeiss Microscopy, Jena, Germany).

Cells of the spontaneously immortalized human keratinocyte line HaCaT were cultivated in quadruplicates in the absence or presence of ten-fold serially diluted fresh Euphorbia sap

(range 0.1–10 µl). After a 24-hour incubation HaCaTs were stained with 7-aminoactinomycin D (7-AAD, dilution 1:10; Merck Millipore, Darmstadt, Germany) and a phycoerythrin (PE) labelled-annexin V antibody (PE Annexin V Apoptosis Detection Kit I, BD Biosciences, Erembodegem, Belgium) for flow cytometric analyses (FACSCalibur flow cytometer; BD Biosciences) and the data analysed using FlowJo v10.2 software (FlowJo; LLC., Ashland, OR, USA). The percentages of apoptotic cells in the presence of increasing amounts of sap are given in relation to the untreated controls.

All obtained values were compared using a Mann-Whitney test using the SPSS statistics software (SPSS 24.0, IBM Statistics). All P values were two-tailed and values of 0.05 considered statistically significant. In the case of multiple testing the alpha level was corrected post hoc using the Bonferroni method.

Results

Clinical presentations after contact with *Euphorbia myrsinites*

A female 24 year-old gardener presented with sharply demarcated erythema, edema, heat and burning sensations in the face, on her ears and hands (Figure 1C–F) two hours after having pruned *Euphorbia myrsinites*. In the perioral region scattered, partly yellowish, papulovesicles of 0.5–2 mm in size were noted (Figure 1C), while the periorbital region was spared. The patient denied excessive sun-exposure, use of new cosmetics or ointments, ocular and systemic symptoms, and co-morbidities except mild type-I-allergies to plant pollens other than *Euphorbia* species, dust mites, and pet hair. In contrast, her co-worker, also a female of 24 years, who had worked alongside her, presented with a very faint and discrete erythema on the forehead and scattered small blisters on the mucosa of the upper oral vestibule. However, her main symptoms were burning pain and the feeling of ‘plastic-like’ skin. These two cases exemplify the diverse clinical spectrum of *Euphorbia myrsinites* sap-induced skin reactions.

Macrostructural (clinical) presentation of *Euphorbia myrsinites* sap-induced dermatitis in comparison to ACD

In order to investigate the clinical picture and course of *Euphorbia myrsinites* sap-induced local skin reactions healthy skin (Figure 2A) was exposed to freshly obtained sap. At all application sites of all volunteers (7/7; 100%) sap exposure induced the development of a sharply demarcated dermatitis confined to the contact sites that was either papulovesiculosis (Figure 2B) or erosive (Figure 2C) and accompanied by discrete to moderate itching. Application of different amounts of *Euphorbia myrsinites* sap (diluted in 0.9% sodium chloride solution) resulted in increased severity of dermatitis with increasing sap concentrations (data not shown). Remission started shortly after removal of the sap-occluding patch with clearance of symptoms after additional one to three days, albeit a discrete residual hyperpigmentation persisting for more than four weeks could be observed in two of the seven volunteers (28.6%; not shown).

In contrast, in the skin areas of the controls with known type IV hypersensitivity to nickel sulfate the clinical response was dominated by erythematous macules and small papules

(Figure 2D) after re-exposure to the allergen and continued to worsen after removal of the allergen with a maximum reaction observed after several days.

For semi-quantitative comparison clinical scoring on D2 after exposure revealed mean values of $2.9 (\pm 0.38)$ for *Euphorbia myrsinites* sap-induced lesions and $2.0 (\pm 0.71)$ for nickel sulfate-induced ACD (Figure 2E). The differences on the clinical severity between *Euphorbia myrsinites* sap-induced and nickel sulfate-induced lesions were borderline statistically significant ($P = .048$).

Microstructural evaluations by RCM of *Euphorbia myrsinites* sap-induced contact dermatitis and ACD

To evaluate the structural and cellular changes non-invasively *in vivo*, all skin application sites were additionally evaluated on D2 by RCM. Exposure to fresh *Euphorbia* sap resulted in the presence of large intraepidermal vesicles with accumulation of cellular debris and inflammatory cells, parakeratosis, spongiosis and necrosis in the suprabasal epidermal layers (Figure 3A, B). In comparison, RCM revealed numerous multi-chambered vesicular bodies, spongiosis and incipient epidermal necrosis at suprabasal epidermal levels in ACD (Figure 3C, D).

Overall semi-quantitative scoring of RCM showed a significantly higher maximal size of epidermal vesicular bodies in *Euphorbia* sap-induced compared to ACD lesions ($P = .028$; Figure 3E). However, the absolute numbers of multi-chambered vesicular bodies were significantly higher in ACD ($P = .008$; Figure 3F). The scores for necrosis (Figure 3G) and spongiosis (Figure 3H) were higher in *Euphorbia* sap-induced dermatitis than in ACD, the differences, however, were not statistically significant ($P = .095$ and $P = .55$, respectively).

Cellular changes following exposure to *Euphorbia myrsinites* sap

Cultivation of skin biopsies in the presence of freshly obtained sap for 24 hours resulted in cytoplasmic vacuolization of keratinocytes predominantly in the basal layers of the epidermis accompanied by dyskeratotic (apoptotic) cells (Figure 4A, B). The observed changes were dose-dependent with higher numbers of vacuolated cells when higher amounts of sap had been added. In contrast the epidermis of control skin not exposed to *Euphorbia* sap remained unaffected after the cultivation period (Figure 4C).

Furthermore, keratinocyte viability was significantly impaired by addition of *Euphorbia myrsinites* sap. Cultivation of HaCaT cells in the presence of *Euphorbia* sap for 24 hours induced a significant increase in the numbers of apoptotic cells that was dose-dependent ($P = .021$). Compared to untreated HaCaT cells (cultivated in the absence of sap, 7.1% apoptotic cells) the number of apoptotic cells increased by 143.1% and 40.1% in the presence of 10 and 1 $\mu\text{l/ml}$ sap, respectively, (Figure 4D).

Discussion

Due to the ubiquitous presence of many species of euphorbiaceous plants, contact can occur during outdoor activities or when handling ornamental plants. So far mostly reports on ocular manifestations and only few reports on skin manifestations after contact with fresh

sap of *Euphorbia myrsinites* have been published (2,5,6). Presumably, the incidence is much higher due to numerous unreported or misinterpreted cases. In addition, if the onset of symptoms occurs with delay or the plant is unknown to physician or patient, a causative association between exposure and appearance of skin eruptions can easily be missed. Although an irritant-toxic origin of the *Euphorbia* sap-induced dermatitis has been suspected (2), but not confirmed previously, ACD represents a valid differential diagnosis especially when considering the ubiquity of these plants. Unfortunately the clinical picture is not pathognomonic and histological evaluation of tissues taken from affected sites may not always allow distinction between ICD from ACD, as they share similar features making a definite diagnosis based on histomorphology challenging. However, ICD typically shows more severe damage to the superficial layers with signs of intracellular vacuolation, nuclear pyknosis, and epidermal hyperproliferation (15,16).

Prompted by two cases of *Euphorbia myrsinites* sap-induced dermatitis, we investigated the effects of the plant's sap on human skin and compared the clinical symptoms and the natural course of the skin eruptions with ACD elicited by the common and established allergen nickel sulfate in order to allow exact differentiation. While the European Society of Contact Dermatitis guideline for diagnostic patch testing recommend two days of occlusion (14), it was decided to limit exposure to EM sap and the control allergen to one day due to the level of discomfort experienced after application of EM sap. We refrained from open testing to avoid accidental smearing of the sap to other skin sites and the eyes. Eruptions at sites of sap exposure were induced in all tested individuals, even in those who denied frequent outdoor activities and contact to greens. The range of dermatitis was variable ranging from clinically barely apparent to sunburn-like rash including the development of vesicles, papules and erosions and followed by decrescendo dynamics as known for toxic/irritant (non-allergic) dermatitis. While the reason for the clinical variability is not exactly known, the observed differences might be attributable to individual differences in the immunomodulatory response to ingenol mebutate or to individual differences in the epidermal barrier function. Overall, *Euphorbia myrsinites* sap induced a more severe phenotype of dermatitis than ACD by nickel sulfate in our test persons, including the participants who were concomitantly exposed to sap and nickel sulfate. RCM analyses confirmed the macroscopical observations with regard to more substantial epidermal disruption in *Euphorbia* sap-induced irritant dermatitis in comparison to the ACD controls. Furthermore, larger, partly multichambered epidermal vesicular bodies filled with necrotic cells as well as inflammatory cells were observed in ICD and the degree of necrosis and spongiosis was higher. This is in accordance with a previous report where ICD was elicited by exposure to sodium lauryl sulfate and compared to ACD to nickel sulfate and standard allergens (17) and supports the usefulness of RCM in distinguishing ICD from ACD.

The reported findings further support the primarily toxic irritating nature of *Euphorbia myrsinites* sap. In addition, re-exposure to *Euphorbia myrsinites* sap did not result in an augmented response in our test persons (data not shown) as it would be typical to allergic contact reactions. Cross-reactivity to latex was excluded as demonstrated by a negative patch test (not shown). One limitation is that the evaluation was performed at certain time points after removal of the test substances, but not longitudinally over a course of several days when lesions or sequelae were still visible.

Finally, the *in vivo* observations were confirmed *in vitro* by a dose-dependent increase in apoptotic suprabasal keratinocytes in the epidermal compartment and significantly higher rates of apoptosis after exposure of human skin tissues derived from healthy donors and human keratinocytes to *Euphorbia myrsinites* sap, respectively. No morphological evidence for an allergic component relevant to the development of *Euphorbia myrsinites* sap-induced contact dermatitis was observed. Due to ethical considerations we have refrained from taking biopsies from the patch test sites, hence the histomorphological changes were observed on human skin necropsies, hampering investigation of cellular infiltrate/chemotaxis, that might have occurred *in vivo*.

According to previous reports *Euphorbia myrsinites* contains abundant amounts of diterpene and phorbol esters, which have both been associated with irritative and pro-inflammatory epithelial changes in humans (2). Thus, these substances represent potential culprit components that might be responsible for the development of the irritant cutaneous changes. However, besides these toxic effects, the pro-inflammatory effect of the phorbol ester ingenol mebutate present in the plant's sap might also contribute to the inflammatory changes without being primarily cytotoxic (18). Therefore, it can be hypothesized that *Euphorbia myrsinites* sap-induced dermatitis is the result of a complex directly cytopathogenic as well as proinflammatory cocktail resulting in a spongiotic, papulovesicular dermatitis. The most obvious mechanism is the corrosive effect of phorbol esters with direct pH-induced cytotoxicity leading to necrotic damage of keratinocytes. These effects are further augmented by ingenol mebutate via site-specific recruitment of immune cells (18). These considerations also have therapeutic implications as the barrier defect in toxic dermatitis will most likely not profit from topical corticosteroid use, which may further compromise the epidermal barrier and delay healing processes. However, lipid-rich moisturizers are effective in the short-term treatment of experimentally induced ICD (19). Future investigations will have to elucidate in how far ingenol mebutate contributes to the inflammatory changes in *Euphorbia myrsinites* sap-induced dermatitis and whether the cytotoxic effects also play a role in the anticarcinogenic effect of ingenol mebutate when used for cancer treatment.

Given the detrimental ocular and cutaneous effects of *Euphorbia myrsinites* sap and the wide dissemination of the plant, protective measures are mandatory. However, it seems that, especially in Europe where *Euphorbia myrsinites* often serves as an ornamental plant, the awareness of its toxicity is rather low. This is also exemplified by our case series where even trained florists were unaware and did not take protective measures. The attachment of warning labels could be considered to raise the awareness of persons handling *Euphorbia myrsinites* and may thus prevent future toxic incidents.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

We thank Katharina Strasser, PhD for excellent technical support.

Funding information: Funding information: Austrian Science Fund (FWF), Austria. Grant Numbers P29619-B30 and P-30615-B30.

References

1. Webster GL. Irritant plants in the Spurge family (Euphorbiaceae). *Clin Dermatol.* 1986; 4:36–45. [PubMed: 3719513]
2. Huerth KA, Hawkes JE, Meyer LJ, et al. The Scourge of the Spurge Family-An Imitator of Rhus Dermatitis. *Dermatitis.* 2016; 27(6):372–381. [PubMed: 27775975]
3. Vasas A, Hohmann J. Euphorbia diterpenes: isolation, structure, biological activity, and synthesis (2008-2012). *Chem Rev.* 2014; 114(17):8579–8612. [PubMed: 25036812]
4. Eke T, Al-Husainy S, Raynor MK. The spectrum of ocular inflammation caused by euphorbia plant sap. *Arch Ophthalmol.* 2000; 118(1):13–16. [PubMed: 10636407]
5. Spoerke DG, Temple AR. Dermatitis after exposure to a garden plant (Euphorbia myrsinites). *Am J Dis Child.* 1979; 133(1):28–29. [PubMed: 760508]
6. Cammu G, De Boule K, De Cleene M, et al. Toxic effects of cushion spurge after exogenous contact. *Eur J Emerg Med.* 2000; 7(2):155–157. [PubMed: 11132079]
7. D'Arcy WG. Letter: severe contact dermatitis from poinsettia. *Arch Dermatol.* 1974; 109(6):909–910.
8. Asilian A, Faghihi G. Severe irritant contact dermatitis from Cypress spurge. *Contact Dermatitis.* 2004; 51(1):37–39. [PubMed: 15291835]
9. Braun SA, Baran J, Schrupf H, et al. Ingenol mebutate induces a tumor cell-directed inflammatory response and antimicrobial peptides thereby promoting rapid tumor destruction and wound healing. *Eur J Med Res.* 2018; 23(1):45. [PubMed: 30266096]
10. Santucci B, Picardo M. Occupational contact dermatitis to plants. *Clin Dermatol.* 1992; 10:157–165. [PubMed: 1393949]
11. Ibanez M, Fernandez-Nieto M, Martinez J, et al. Asthma induced by latex from 'Christmas flower' (Euphorbia pulcherrima). *Allergy.* 2004; 59(10):1127–1128. [PubMed: 15355476]
12. Hausen BM, Schulz KH. Occupational contact dermatitis due to croton (Codiaeum variegatum (L.) A. Juss var. pictum (Lodd.) Muell. Arg.). Sensitization by plants of the Euphorbiaceae. *Contact Dermatitis.* 1977; 3(6):289–292. [PubMed: 146585]
13. Paulsen E, Skov PS, Andersen KE. Immediate skin and mucosal symptoms from pot plants and vegetables in gardeners and greenhouse workers. *Contact Dermatitis.* 1998; 39(4):166–170. [PubMed: 9817221]
14. Johansen JD, Aalto-Korte K, Agner T, et al. European Society of Contact Dermatitis guideline for diagnostic patch testing - recommendations on best practice. *Contact Dermatitis.* 2015; 73(4):195–221. [PubMed: 26179009]
15. Krasteva M, Kehren J, Sayag M, et al. Contact dermatitis II. Clinical aspects and diagnosis. *Eur J Dermatol.* 1999; 9(2):144–159. [PubMed: 10066966]
16. Willis, CM. Irritant Dermatitis. Berlin Heidelberg: Springer; 2006. 3452006
17. Astner S, González E, Cheung AC, et al. Non-invasive evaluation of the kinetics of allergic and irritant contact dermatitis. *J Invest Dermatol.* 2005; 124(2):351–359. [PubMed: 15675954]
18. Rosen RH, Gupta AK, Tying SK. Dual mechanism of action of ingenol mebutate gel for topical treatment of actinic keratoses: rapid lesion necrosis followed by lesion-specific immune response. *J Am Acad Dermatol.* 2012; 66(3):486–493. [PubMed: 22055282]
19. Saary J, Qureshi R, Palda V, et al. A systematic review of contact dermatitis treatment and prevention. *J Am Acad Dermatol.* 2005; 53(5):845. [PubMed: 16243136]

Figure 1. *Euphorbia myrsinites* and clinical presentation of sap-induced contact dermatitis. (A) *Euphorbia myrsinites*. (B) Extrusion of the characteristic white, milky sap (arrows) after puncturing the plant's stem. A patient presented with sharply-demarcated erythema and edema with heating and burning sensations on the skin of the face, the retroauricular region and the dorsal parts of the fingers after contact with *Euphorbia myrsinites* sap (C-F). In addition, in the submental region scattered, partly yellowish papulovesicles were noted.

Figure 2.

Patch test results after exposure of normal skin (A) to *Euphorbia myrsinites* sap (B, C). In all application sites application of *Euphorbia* sap resulted in a papulovesiculous or erosive dermatitis that was confined to the contact areas. Positive reaction elicited after re-exposure of controls with known type-IV hypersensitivity to nickel sulfate to the allergen (D). Semi-quantitative comparison of clinical scores on D2 revealed a tendency towards a more severe response in *Euphorbia myrsinites* sap-induced lesions (EM) than in allergic contact dermatitis (ACD) (E).

Figure 3.

Reflectance confocal microscopy imaging of the suprabasal epidermis of *Euphorbia myrsinites* sap-induced contact dermatitis (A, B) and of allergic contact dermatitis (ACD) elicited by re-exposure of previously sensitized skin to nickel sulfate (C, D). After sap exposure reflectance confocal microscopy revealed the presence of large intraepidermal vesicles (arrows, A) with accumulation of cellular debris and inflammatory cells (arrows, B). The images taken of ACD showed spongiosis (arrows, D), multi-chambered vesicular bodies and initial epidermal necrosis. Semi-quantitative scoring of images taken of Euphorbia sap-

induced contact dermatitis (EM) and ACD revealed significantly larger intraepidermal vesicles in sap exposed skin (**E**). Vesicles, however, were significantly more numerous in ACD (**F**). The scores for necrosis (**G**) and spongiosis (**H**) were relatively similar in both.

Figure 4.

Exposure of healthy human skin biopsies for 24 hours to 10 µl/ml (A) or 1 µl/ml (B) of freshly obtained sap *in vitro* induced a degenerative vacuolization of keratinocytes. In contrast, the control biopsies cultivated in the absence of sap showed epidermal integrity with no signs of epidermal cellular necrosis (C). Hematoxylin-eosin stains (magnification 40x).

Determination of cellular viability of HaCaT cells after 24 hour exposure to *Euphorbia myrsinites* sap by flow cytometry (D). Addition of freshly obtained sap to HaCaT cells resulted in an increase in the numbers of apoptotic cells (143.1% and 40.1% increase in the presence of 10 µl/ml and 1 µl/ml sap, respectively) compared to cells cultivated in the absence of sap (untreated controls), which was set at 0% to exclude physiological, sap-unrelated cell death after cultivation.