In general, the present results agree with those of Kośmider et al. [6] who have found that Se alleviates nausea, vomiting, diarrhea, abdominal pain and weight loss. However, no influence on hair loss was noted. This symptom seems to be the most persistent during chemotherapy.
Se deficiency, observed in patients with ovarian cancer, increases the toxicity of cytostatics. Recently, Matsuda et al. [7] have suggested that an imbalance in the oxidative system is mainly due to Se deficiency and that Se plays a protective role in functional disturbances of the heart caused by free radical production induced by cytostatics used for chemotherapy.
In conclusion, a sufficiently long supplementation with Se in patients with ovarian cancer subjected to multi-drug chemotherapy results in a number of beneficial biochemical changes and reduction in side effects of chemotherapy.

Experimental

The study group included 31 patients with ovarian cancer, taking Se-Protecton® Zellaktiv (Smith Kline Beecham, Fink Naturarznei GmbH, Germany), 2 capsules 4 times daily. The control group included 31 patients with ovarian cancer who did not receive Se supplementation. The mean age of patients in the study and control groups was 49.4 ± 12.9 and 52.7 ± 12.6 years, respectively. The diagnosis of ovarian cancer was made by laparotomy and histological examination of tumor samples. Clinical staging was according to FIGO criteria. The study group included 15 patients with clinical stage I/II and 16 with stage III/IV. For the control group, these figures were 16 and 15 patients, respectively.
All patients were treated according to the accepted clinical protocol in ovarian cancer: surgery followed by intravenous multi-drug chemotherapy sessions in 21-day intervals. Chemotherapy comprised cisplatin 100 mg/m^2 (Platamine, Farmitalia, Carlo Erba, Italy) and cyclophosphamide 600 mg/m^2 (Endoxan, Asta Medica, Germany). At the start of this trial 23 patients of the study group were during their first and 8 during subsequent sessions. In the control group, 21 patients were during their first and 10 during subsequent sessions. The chemical composition and biological properties of Protecton® Zellaktiv (Smith Kline Beecham, Fink Naturarznei GmbH, Germany) were as follows (per 2 capsules): β-carotene – 15 mg, vitamin C – 200 mg, vitamin E – 36 mg, riboflavin (vit. B_2) – 4.5 mg, niacin (vit. B_3) – 45 mg, selenium yeast – 50 mg (Se = 50 μg). The preparation was administered to patients of the study group during 3 months.
Clinical status was checked every month, using a standard gynecological examination and ultrasound of the abdomen and small pelvis. This was complemented by biochemical tests. The concentration of Se in serum and hair, activity of GSH-P_x, concentration of MDA in serum and platelets were measured before Se supplementation and 4, 8 and 12 weeks thereafter. All biochemical tests were done immediately before the chemotherapy session. In the control group, Se was replaced by placebo. Biochemical tests were done at the same time as in the study group.
The following tests were done before each chemotherapy session: hematology, platelet count, urea, creatinine, bilirubin, aminotransferase activities and CA-125. An evaluation of side effects was performed after each chemotherapy session.

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How relevant is the application of antioxidants in order to avoid UVB-induced photodamages?

H.-P. Podhaisky, S. Riemschneider, T. Galgon and W. Wohlrab

Reactive oxygen species (ROS) are thought to play an important role in mediating UVB-induced harmful skin alterations [1]. For this reason, it is often suggested that a protection from UVB-induced skin damages is possible by the topical use of radical scavengers. However, the idea of cause and effect seems to require a verification in special questions. For example, an erythema induced by UVB irradiation is reduced by topical treatment with antioxidants [2–4]. The frequently used vitamin E has two relevant properties, which are often hardly taken into account: vitamin E absorbs UV light with a maximum at 295 nm and possesses an anti-inflammatory effect via the inhibition of phospholipase [5, 6]. This example is to demonstrate that a causal link between radical scavenger properties and erythema protection cannot be proven by such a study design. Moreover, since ROS play a role in signal transduction too, the clinical benefit of a massive intervention in the oxidative balance remains questionable. It seems conceivable that ROS quenching by overloading the cells with antioxidants leads to an inhibition of physiological processes as well. In this context, the radical nitric oxide should be mentioned [7].
To support our calling into question of the relevance of using radical scavengers to avoid UVB-mediated skin damage we irradiated cultured keratinocytes with UVB light. Experiments were performed using the human keratinocyte cell line HaCaT which has been established as a model for studying mechanisms of UVB-induced cell alterations [8, 9]. Our aim was to investigate the influence of the UV light on the formation of cellular peroxides and cell viability. Moreover, the issue was addressed whether these parameters could be modulated by the pretreatment with the established antioxidants vitamin E or vitamin C and whether thereby an UVB-induced cell damage can be prevented.
In keratinocytes, UVB irradiation (30–240 mJ/cm^2) led to a dose depending raise in the amount of intracellular peroxides. In respect of viability, a drop to 60% of the untreated control was observed (Table). Vitamin C abolished the UVB-induced increase in the formation of peroxides (1000 μM) completely. Vitamin E reduced the UVB-in-

Table: Effect of UVB irradiation on the formation of peroxides and viability in keratinocytes

UVB dosage (mJ/cm^2)	Formation of peroxides (% of untreated control)	Surviving fraction (% of untreated control)
Untreated control	100.0 ± 7.94	100.0 ± 3.76
30	129.83 ± 9.42*	93.61 ± 3.84
60	157.35 ± 3.95*	86.75 ± 2.78*
90	222.37 ± 5.78*	81.80 ± 3.34*
120	234.60 ± 6.95*	74.84 ± 5.86*
150	271.28 ± 4.64*	65.65 ± 4.62*
180	350.31 ± 36.08*	64.20 ± 2.58*
210	395.08 ± 27.53*	63.80 ± 3.48*
240	460.81 ± 22.30*	60.17 ± 2.85*

* $P < 0.05$; UVB vs. untreated control, two-tailed t-test. All data shown are mean ± S.E.M. of n = 6 observations

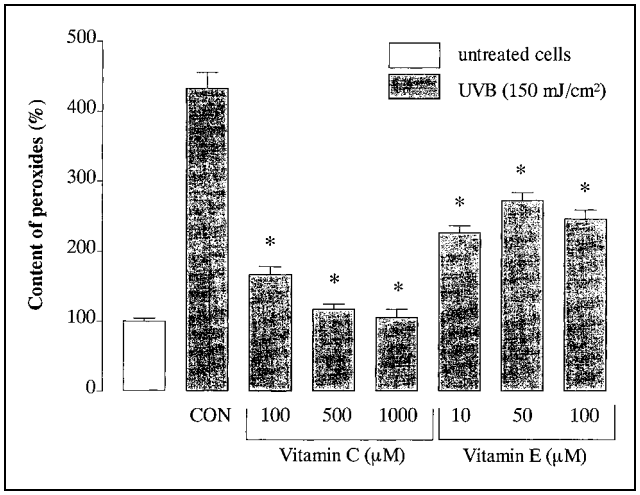

Fig. 1: Effect of vitamin E and vitamin C on the UVB-induced increase in the content of peroxides in HaCaT keratinocytes. Prior to irradiation, cells were incubated for 3 h with vitamin E and vitamin C. * $P < 0.05$, treatment vs. control (CON), two-tailed test. All data shown are mean ± S.E.M. of $n = 6$ observations

duced raise of peroxides to the half (Fig. 1). However both, vitamin E and vitamin C failed to protect keratinocytes from the UVB-induced cytotoxicity (Fig. 2). Vitamin E or vitamin C alone had no effect on the content of peroxides, the proliferation or viability (data not shown).

The present study demonstrates that UVB irradiation clearly induces oxidative stress in keratinocytes. As expected, these cell reaction could be very well diminished by radical scavengers. Considering our results, however, a direct relation between the observed UVB-induced cell damage and ROS could not be shown. Since vitamin C completely suppressed the elevated content of peroxides but still failed to provide any cytoprotection, this discrepancy becomes obvious.

As free radicals are not stable, react immediately and nonspecifically with any biological structure at the place of their generation, a protection from these species is only possible, if the radical scavenger is present at the moment of radical generation in very much higher concentrations than the endogenous target structures of ROS like lipids, proteins and carbohydrates [10]. It seems doubtful whether such a situation is just achievable. Hence, the efficacy of ROS scavengers may be limited from the beginning.

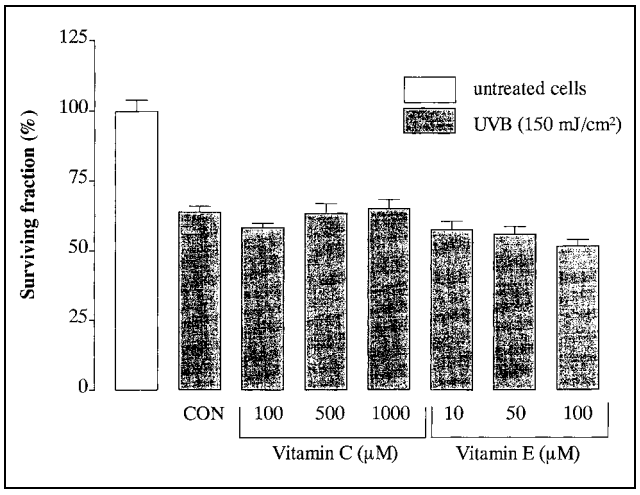

Fig. 2: Vitamin E or vitamin C fail to protect HaCaT keratinocytes from UVB-induced toxicity. Prior to irradiation, cells were incubated for 3 h with vitamin C and vitamin E. All data shown are mean ± S.E.M. of $n = 6$ observations

Damaging alterations in UVB-treated keratinocytes are very complex. Beside indirect DNA injuries by ROS, effects such as direct UVB absorption of the DNA play a role [11, 12]. Moreover, cytokines have been implicated as part of the mechanism responsible for UVB-induced effects [13]. The results of the present study suggest that the relevance of ROS in these processes may be of secondary importance or, at least, that ROS scavengers have limited significance in respect of UVB photoprotection. In conclusion, though there are evidences for the effectiveness of antioxidants as photoprotective agents it remains questionable, whether their mode of action depends on radical scavenger capacity.

Experimental

HaCaT keratinocytes from passages 20–25 were used for the experiments (kindly provided by N. E. Fusenig, DKFZ Heidelberg, Germany). Cells were maintained and subcultured in serum-free medium (SFM supplemented with 0.1 ng/ml EGF and 25 μg/ml bovine pituitary extract, Gibco Life Technologies, Eggenstein, Germany). Keratinocytes were grown in a humidified incubator at 37 °C, 5% CO_2 and 95% air. Keratinocytes were seeded at a density of 5×10^3 cells/well in 96-well microtiter plates in 200 μl of medium. After reaching confluence, cells were incubated for 3 h with vitamin E (+ α tocopherol, Sigma, Deisennhofen) or vitamin C (L-ascorbic acid, Sigma, Deisenhofen, Germany).

Immediately after incubation, UVB irradiation was carried out by means of a UV-irradiation chamber, equipped with the UVB lamp F15T8 (Sankyo Denki, Japan). This lamp emits mainly in the range of 280–320 nm with a peak emission at 310 nm. UVB irradiation was monitored with a UVB sensor RM-11 (Gröbel, Ettlingen, Germany). Prior to the UV treatment, incubation medium was removed, keratinocytes were washed once with phosphate buffered saline (PBS) and then 200 μl of the buffer were added to each well. Untreated control cells were covered by aluminium foil. After the UVB treatment, fresh medium was added. Incubation was continued for 20 h and then viability was assessed.

Cell viability was measured by staining with crystal violet as previously described [14]. Medium was removed and viable cells were fixed with methanol for 10 min and then stained for 10 min with a 0.1% crystal violet solution. Following three washes with tap water, the dye was eluted with 0.1 mol/l trisodium citrate in 50% ethanol for 15 min. Extinction at 540 nm was measured using the microtiter plate reader iEMS (Labsystems, Helsinki, Finland).

UV-induced generation of intracellular peroxides was measured with dihydrorhodamine 123 (purchased from Sigma, Deisenhofen, Germany) [15]. Cells treated with vitamins as described above were loaded with DHR (final concentration 5 μM) 45 min prior to UVB irradiation. After washing with PBS, the irradiation was carried out as described above. Forty five minutes after the end of the irradiation, fluorescence intensity was measured at 538 nm using the microtiter plate reader Fluoroskan Ascent (Labsystems, Helsinki, Finland) with an excitation wavelength of 485 nm.

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Sesquiterpenes from *Ferula hermonis* Boiss

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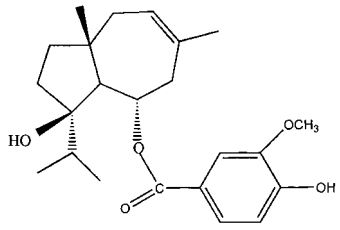
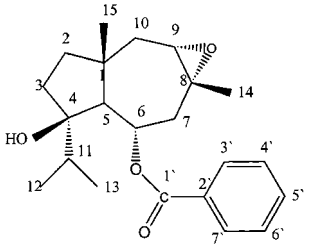
The roots of *Ferula hermonis* Boiss yielded the new 8,9-epoxy derivative of the carotane sesquiterpene jaeschkeanadiol (**2**), together with two other known sesquiterpenes: the less frequently occurring (+)-α-bisabolol and jaeschkeanadiol vanillate (**1**). The identities of the isolated compounds were established from their spectral data and by comparison with published reports.

The genus *Ferula* has been extensively studied, *Ferula hermonis* Boiss was not yet among the investigated species. This note presents the first report on the isolation of epoxyjaeschkeanadiol benzoate (**2**) from nature, besides isolation of the known compounds; jaeschkeanadiol vanillate (**1**) and the less widespread enantiomer (+)-α-bisabolol from *Ferula hermonis* Boiss for the first time.



1



2

The known compound **1** has been reported before in *Ferula eloeochytris* [1], *Ferula jaeschkeana* [2], and in *Ferula rigidula* [3]. (+)-α-Bisabolol was previously isolated from *Atalantia monophylla* Correa [4].

The *n*-hexane extract of the root of *Ferula hermonis* Boiss was fractionated between n-hexane and MeCN. The MeCN fraction was chromatographed on a series of Si gel columns to afford three compounds. The ^{1}H and ^{13}C NMR spectra of **2** (see Experimental section) showed close similarity to the spectra of the previously reported compound jaeschkeanadiol benzoate (the parent compound) [1, 2] except for the presence of two oxygenated carbon signal at δ 56.1 (C-8) and δ 60.8 (C-9), which are typical epoxide carbon signals, instead of the olefinic carbon signals belonging to the parent compound. The occurrence in the ^{13}C NMR of downfield methyl signal at δ 23.3 (Me-14) versus signal at δ 20.2 (Me-14) in the parent compound was a further evidence for the existence of epoxide group between C-8 and 9 in **2**. Inspection of the ^{1}H NMR revealed a signal at δ 1.51 (3 H, s, Me-14) supporting the presence of a methyl group attached to C-8