Examined in this article are the protective effects of turmerones from Curcuma longa against UVB-induced oxidative stress – upregulation of cellular defence systems.

The human epidermis represents the largest interface of the body that is constantly in close contact with the environment. Therefore, it is especially vulnerable to oxidative stress, which in turn leads to oxidation of cellular macromolecules such as proteins, lipids and nucleic acids. In order to counteract these harmful effects and consequently ensure the redox status of the cell, a plethora of defence mechanisms exists. Fuelled by new research, activities and expression of enzymes of the anti-oxidative defence line are better understood. Two major factors involved in ageing and anti-oxidative stress mechanisms are the thiol redox systems driven by glutathione peroxidase (GPX1) and thioredoxin reductase (TXNRD1).

Both systems require redox equivalent in the form of NADPH to restore their full anti-oxidative potential. This in turn is generated by another enzyme named NAD(P)H dehydrogenase (NQO1) that generates NADPH from oxidised NADP+ by consuming ATP. While the thioredoxin and the glutathione systems neutralise harmful products emerging from the oxidation and peroxidation of biomacromolecules, the defence for reactive oxygen species (ROS) such as hydrogen peroxide, which are responsible for most of the oxidative stress in cells exposed, for example, to UV-irradiation, depends on the catalase system. This enzyme eliminates hydrogen peroxide by catalysing its decomposition to water and oxygen.

Turmeric (Curcuma longa) is a plant belonging to the Zingiberaceae (ginger) family, which is widely cultivated as vegetables and spices, and for fragrance in Asian countries. Curcuma rhizomes contain essential oils which can be isolated by steam distillation or solvent extraction. The major components in the oil fractions are sesquiterpenes and include α-turmerone, β-turmerone and aromatic (ar)-turmerones. Recently, ar-turmerones have been shown to interfere with platelet aggregation and have also shown PPAR-γ ligand-binding activity, demonstrating bioactivity. In this study we investigated the effects of an enriched, defined turmerone fraction on UV-challenged human skin equivalents by biological techniques, including expression profiling with DNA chips verified by RT-PCR. The bioavailability was assessed by penetration experiments on pig skin followed by an in vivo verification of the effects on human skin out of a delivery optimised vehicle cream.

**Material and methods**

**Turmeric oil**

Turmeric oil is the distilled fraction from the root of Curcuma longa. First step is a solvent free extraction with supercritical carbon dioxide yielding 4%-5% turmeric oil whose main constituents are turmerones (approximately 60%). This is followed by molecular distillation as the second step which improves the colour of the oil (from brown to light yellow), enriches the turmerones, removes the undesired curcumin and reduces the strong odour without altering the efficacy of the product.

**Anti-oxidant activity test**

The anti-oxidant activity was assessed by inhibition of coupled auto-oxidation of linoleic acid and β-carotene as described previously. In brief, to produce the anti-oxidant solution 625 mg turmeric oil was mixed and dissolved in 25 ml methanol. As a positive control tocopherol was utilised while pure methanol served as a negative control. 3 mg β-carotene, 400 mg linoleic acid and 4 g Tween 40 were mixed. 220 mg of this mixture was dissolved in water at 50°C producing the oxidant solution. 13 µl of the antioxidant solution was added to 1 ml of the oxidant solution. The absorbance at 470 nm was measured for 1 hour and the gradient between 20 and 40 min was calculated and expressed as delta E.

**Cell culture**

Reconstituted human epidermis was purchased from SkinEthic (France) and incubated for 24 hours in standard...
maintenance medium at 37°C and 5% CO₂ before start of the experiments. Skin models were incubated with a cream formulation containing 0.5% turmeric oil for 24 hours. Then the cream formulation was completely removed and skin models were UVB irradiated with 350 mJ/cm² followed by a second incubation with a cream formulation containing 0.5% turmeric oil for 12 hours. Subsequently skin models were harvested and stored in RNAlater (Qiagen, Germany) until RNA-isolation.

**RNA isolation**
Total RNA was extracted from cultured skin models using RNeasy Mini Kit (Qiagen, Germany) following the manufacturer’s guide. RNA concentration was assessed spectroscopically with the SmartSpec Plus (Biorad, Germany). Purity and integrity of the RNA was determined by Agilent 2100 bioanlyser with the 6000 Nano LabChip reagent set (Agilent Technologies, USA). RNA samples were stored at -80°C until analysis.

**DNA microarray and data analysis**
Gene expression profiles were determined using Affymetrix HGU133 plus 2.0 GeneChips (Affymetrix, USA) using 2 µg of total RNA pooled from three reconstituted human epidermis skin models. Gene chip assays and initial analysis were carried out as described previously.10 Reverse transcription was performed with the First-Strand-cDNA Synthesis Kit (Super Script III, Invitrogen, USA) according to the guidelines of the manufacturer starting with 100 ng purified RNA from each sample. Quantitative PCR was carried out with a Opticon DNA engine (MJ Research Software, Germany).

**Penetration analysis**
Percutaneous absorption of turmeric oil into fresh dermatomed (1 mm) porcine skin, which has to be reduced to horny layer and epidermis was assessed using Franz diffusion cells as previously described.11 In brief, full thickness skin samples were mounted in modified Franz static dermal penetration cells with the external surface of the stratum corneum facing the donor chamber. A cream formulation with various cosmetic oils (C12-15 alkyl benzoate, PPG-15 stearylether, diethylhexyl carbonate, cyclopentasiloxane/cyclohexasiloxane, ethylhexyl palmitate and petrolatum; Evonik Goldschmidt, Germany) containing 1% turmeric oil was applied onto the stratum corneum for 24 hours. After incubation the remaining cream formulation and the receptor medium (PBS) were collected and processed in HPLC solvent. Chopped porcine skin was extracted with HPLC solvent. Quantitative and qualitative analysis of turmeric oil was carried out by HPLC (Merck-Hitachi, Germany).

**In vivo study**
The in vivo effects of turmeric oil were compared in a placebo controlled study with an O/W cream containing 0.5% turmeric oil. 15 healthy volunteers applied the cream twice daily for 8 weeks. Classical corneometry and assessment of skin tone were carried out before the treatment started and after 8 weeks. Prior to data assessment volunteers had to acclimatise for 15 min at room temperature and 50% relative humidity. For evaluation of the skin tone a special camera (Visioscan VC98, Courage + Penetration analysis was performed using the First-Strand-cDNA Synthesis Kit (Super Script III, Invitrogen, USA) according to the guidelines of the manufacturer starting with 100 ng purified RNA from each sample. Quantitative PCR was carried out with a Opticon DNA engine (MJ Research Software, Germany).
Khazaka, Germany) and software were used. Grey level distribution was calculated to different texture parameters and related to skin tone.

**Results**

Since it has been proposed that the cyto-protective effect of turmeric extracts on hepatocytes may be due to anti-oxidative properties, we tested turmeric oil for an intrinsic anti-oxidative activity in a β-carotene-linoleic acid oxidation assay. As displayed in Figure 1, the golden standard for anti-oxidative substances, tocopherol, completely inhibits auto-oxidation of β-carotene over a time period of 20 min. Without added tocopherol β-carotene is nearly entirely oxidised. In contrast, by adding turmeric oil this auto-oxidation could be decreased by around 90% of the activity displayed by tocopherol indicating a strong intrinsic anti-oxidative activity of turmeric oil.

We further hypothesised that turmeric oil not only possesses an intrinsic anti-oxidative activity but also could trigger cellular defence mechanisms against oxidative stress. To test this hypothesis human reconstituted epidermis treated with turmeric oil was UVB irradiated in order to put oxidative stress on the cells.

After irradiation cells were lysed and gene expression profiling was performed by using Affymetrix gene chip technology. This revealed that turmeric oil enabled UVB irradiated cells to express their own defence mechanisms against oxidative stress in a much stronger way than untreated cells. As verified by RT-PCR technique marked up-regulation was found for CAT, GPX1, NQO1 and TXNRD1 representing main constituents of the glutathione and thioredoxin detoxification systems (Fig. 2).

In order to deploy its strong anti-oxidative abilities to living keratinocytes and therefore to be utilised as a cosmetic active ingredient turmeric oil has to penetrate through the outer cornified layers of the human skin. In the present study the penetration abilities of turmeric oil in dependency of various cosmetic oils with different polarity were assessed in a pig skin penetration assay. As shown in Figure 4, penetration of turmeric oil into pig skin is markedly influenced by the use of an appropriate cosmetic oil obtaining best results with a polar oil such as C12-15 alkyl benzoate.

According to the results of the penetration study, turmeric oil was added to a cosmetic formulation with polar

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**Figure 3:** In vivo effects of a cosmetic formulation containing 0.5% turmeric oil. (A): Skin hydration. (B): Wrinkle reduction. (C+D): Smoothing of uneven skin tone.

**Figure 4:** Penetration of turmeric oil into pig skin.
cosmetic oils at a concentration of 0.5%. In an in vivo study eight week application of this formulation resulted in improved skin moisture as assessed by classical corneometry. Furthermore, this was associated with marked reduction of wrinkles as indicated by decline of surface and volume of the skin. Additionally, treatment with turmeric oil produced significant smoothing of the skin tone as observable by a cumulative increase of various texture parameters related to skin tone (Fig. 5). Especially this finding is in line with the previously demonstrated anti-oxidative properties of turmeric oil, since ROS play a significant role in the melanogenesis in response to UV light exposure. Direct UV irradiation produces ROS in keratinocytes which consequently start oxidation and peroxidation of DNA and membrane phospholipids. The detrimental products of these reactions act as messenger molecules for impairment of the cellular integrity and trigger melanogenesis through activation of tyrosinase which in turn leads to tanning of the skin. In photo-aged skin this might result in an uneven skin tone and sometimes age spots. Therefore, the intrinsic and extrinsic anti-oxidative properties of turmeric oil can counteract oxidative stress in the skin and consequently inhibit irregular tanning.

Conclusions

Good evidence is provided that a turmerone enriched fraction of Curcuma longa is also capable of boosting the cellular protection mechanisms against oxidative stress. It stimulates the expression of GPX1 and TXR which produce efficient ROS scavengers in a NADPH-dependent manner. This activity is safeguarded by an increase in NADPH reductase. Simultaneously catalase directly eliminates the ROS hydrogen peroxide by catalysing its decomposition to water and oxygen. By topical application the anti-aging activity of the turmerone enriched fraction could be demonstrated and translated into a significant improvement of skin physiological parameters including skin hydration, anti-wrinkle activity and skin toning.

References