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Examinations of the antioxidative properties of the topically administered drug bufexamac reveal new insights into its mechanism of action

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Abstract

The effect of bufexamac on UV-irradiation-induced lipid peroxidation was investigated. Linolenic acid was used as a model lipid. Bufexamac was shown to be capable of reducing the amount of lipid peroxidation. The quantification was carried out by the thiobarbituric acid assay. Irradiation experiments were also performed using HaCaT keratinocytes as a model system. The oxidative changes were quantified by DNA synthesis measurements and cell viability determinations. Bufexamac was found to act antioxidatively again. To investigate free radical involvement, electron paramagnetic resonance studies were carried out. The influence of bufexamac on the concentration of hydroxyl radicals generated by the Fenton system was examined using the spin trapping technique. Moreover, the hydroxamic acid's ability to react with stable radicals was checked. The quantification assay of 2,2-diphenyl-1-picrylhydrazyl hydrate showed no concentration changes of the stable radical caused by bufexamac. In the Fenton assay antioxidative effects were measured after the addition of the drug. The qualitative changes after irradiating bufexamac were studied at a molecular level by electrospray mass spectrometry. Multiple-stage mass spectrometry experiments enabled the establishment of fragmentation schemes. Phenolic degradation products were detected. The results suggest a new interpretation of the controversially debated mechanism of action of bufexamac and indicate possible reasons for its eczema provoking potential.

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Introduction

Bufexamac is a non-steroidal anti-inflammatory drug (NSAID) and is exclusively used for external therapy of skin diseases (Marsch 2001). The drug combines anti-inflammatory, analgesic and itchiness-reducing effects. Because its anti-inflammatory effects are comparable with those of steroids it is sometimes considered as a steroid surrogate (Baumgartner 1992).

Chemically bufexamac is 4-n-butoxyphenylacethydroxamic acid, an arylacetic acid derivative (Boreham et al 1972). The first step of its synthesis is a Willgerodt-Kindler reaction of butylated 4-hydroxy-acetophenone leading to the thiomorpholide. After basic cleavage, esterification with ethanol and reaction with hydroxylamine hydrochloride, bufexamac is formed (Roth & Kleemann 1982).

The exact mechanism of action of the hydroxamic acid derivative is still under debate. In-vitro bufexamac was shown to act as an inhibitor of prostaglandin synthesis and a stabiliser of rat liver lysosomes. Which of these properties is of importance in-vivo is not clarified yet (Baumgartner 1992).

Bufexamac is indicated for the treatment of acute and chronic eczemas of any genesis, first-degree burns, non-inflammable dermatitis connected with pruritus and anal eczemas (Lubec 1989). However, bufexamac has been shown to evoke skin irritation itself. Studies report these "properties of the anti-eczematic drug bufexamac acting as a frequent and relevant contact sensitizer" (Gebhardt & Wollina 1995; Geier & Fuchs 1989; Kurumaji 1998; Bauer et al 1999). Because of these contradictions in the

literature and a not fully understood mechanism of action, in this article the properties of bufexamac are studied.

Model systems were used to test the effects of bufexamac upon exposure to UV light. The fragmentation of bufexamac and its degradation caused by UV light were studied at a molecular level using electrospray ionisation mass spectrometry (ESI-MS). ESI as a soft ionisation method enables the analysis of bufexamac and its degradation products without artificial fragmentation. However, structural elucidation is possible using the capabilities of an ion trap mass analyser (Papac & Shahrokh 2001), namely tandem mass spectrometry (MS/MS) and multiple-stage mass spectrometry (MS^n).

Electron paramagnetic resonance (EPR) spectroscopy is being used more frequently in pharmaceutical research (Katzhendler et al 2000; Kroll et al 2001) because of the unique information which can be obtained by EPR spectra of both in-vivo and in-vitro experiments (Maeder 1998). The spin trapping method with 5,5-dimethyl-1-pyrroline-1-oxide (DMPO) was used to detect the highly reactive hydroxyl radicals (Gunther et al 1998). The influence of bufexamac on the concentration of these free radicals generated by the Fenton system, as well as the hydroxamic acid's properties regarding the stable 2,2-diphenyl-1-picrylhydrazyl hydrate (DPPH) radical (Trommer et al 2003), were studied in this work.

The aims of this study can be summarised as follows: quantification of the effects of bufexamac on a model lipid after UV exposure in the presence of iron ions by the thiobarbituric acid (TBA) assay; quantification of the effects of bufexamac on Human adult low Calcium high Temperature (HaCaT) cell line keratinocytes after UV exposure evaluated by DNA synthesis measurements and cell viability determinations; use of EPR spectroscopy to test the effects of bufexamac on reactive oxygen species (ROS) and on stable free radicals; investigating the fragmentation of bufexamac and measuring the qualitative changes after UV irradiation by mass spectrometry.

Materials and Methods

Materials

Bufexamac of analytical grade was purchased from Caelo (Hilden, Germany). α-Linolenic acid, ferrous sulfate, ferrous chloride, crystal violet, malondialdehyde-bis-(dimethylacetal), 2-thiobarbituric acid and trichloroacetic acid (all of analytical grade) were obtained from Sigma (Deisenhofen, Germany) as well as 2,2-diphenyl-1-picrylhydrazyl hydrate (DPPH), 5,5-dimethyl-1-pyrroline-1-oxide (DMPO), adenosine 5′-diphosphate sodium salt (ADP) as a chelating agent and 2,2,6,6-tetramethyl-1-piperidyl-*N*-oxyl (TEMPO). Methanol and ethanol of gradient grade were purchased from Merck (Darmstadt, Germany). HaCaT keratinocytes were kindly provided by N. E. Fusenig, Deutsches Krebsforschungszentrum – DKFZ (Heidelberg, Germany). Serum-free medium, epidermal growth factor (EGF) and bovine pituitary extract were obtained from Gibco Life Technologies (Eggenstein, Germany). The bromodesoxyuridine ELISA colorimetric kit for the quantification of DNA synthesis was received from Roche Diagnostics GmbH (Mannheim, Germany).

Sample preparation

Stock solutions of linolenic acid, bufexamac, α-tocopherol (1.0 mM each) and ferrous sulfate (0.5 mM) were prepared immediately before use. The linolenic acid lipid model system was an oil-in-water dispersion of linolenic acid obtained by shaking the system for 120 min using a laboratory flask shaker (GFL 3006, Gesellschaft für Labortechnik, Burgwedel, Germany).

Before UV exposure, 10 μM ferrous sulfate was added to the samples as an electron donor and catalyst of the Haber-Weiss reaction to initiate ROS generation via a Fenton-type reaction (Lambat et al 2002). Previous investigations have shown that there is no effect when irradiating lipid model systems without transition metal catalysts (Trommer et al 2001). All solutions were freshly prepared immediately before use.

For the cell culture experiments HaCaT keratinocytes from the passages 20–30 were used. The cells were maintained and subcultured in serum-free medium supplemented with 0.1 ng mL^{-1} EGF and 25 μg mL^{-1} bovine pituitary extract. The keratinocytes were grown in a humidified incubator at 37 °C, 5% CO_2 and 95% air and seeded at a density of 5×10^3 cells per well in 96-well microtiter plates with 200 μL of the medium (Podhaisky et al 2002). Four days after seeding, the cells were incubated for 3 h with bufexamac. Subsequently, the HaCaT keratinocytes were damaged as described in the following section.

Ultraviolet irradiation

UV irradiation experiments with the linolenic acid lipid model system and the HaCaT keratinocytes were carried out using special irradiation equipment (Dr Gröbel UV-Elektronik, Ettlingen, Germany) enabling a selective exposure to UV-A and UV-B. For UV-A irradiation, the special lamp TL-DK 30W/05 (Philips, Hamburg, Germany) was used. This lamp emits in the range of 320–420 nm with a peak emission at 370 nm. UV-A irradiation was monitored with a UV-A sensor RM-11 (Dr Gröbel UV-Elektronik, Ettlingen, Germany). For the UV-B irradiation experiments, a F15/T8 15 W lamp with a main emission range of 290–320 nm (Sankyo Co., Tokyo, Japan) was employed. For the irradiation experiments before mass spectrometry, an OSRAM Vitalux 300 W equipment (OSRAM, Augsburg, Germany) emitting a solar radiation spectrum was used. The distance between the bulb and the sample was 500 mm.

Before irradiation, the linolenic acid lipid model samples were transferred to open glass dishes (55 mm diameter). Thereby, the optical pathlength was 2.1 mm and a homogeneous exposure was assumed despite light scattering. The samples were treated with a UV-B dose of 0.25 J cm^{-2}, which corresponds approximately to 2–3 times the minimal erythemal dose (MED) in man. This high dosage was required to provoke stress conditions.

HaCaT keratinocytes were irradiated as follows. Before the UV radiation treatment, the incubation medium was removed. The keratinocytes were washed once with phosphate-buffered saline (PBS) and then buffer was added. The untreated control cells were covered with aluminium foil. The temperature during the irradiation was monitored and kept stable at 30 °C by means of the cooling system of the irradiation chamber. After UV treatment, the evaporated medium was added again and incubation was continued for 20 h in the humidified incubator.

Thiobarbituric acid assay

The thiobarbituric acid (TBA) test is a quantitative assay for the detection of malondialdehyde (MDA), and is the most widely used technique to determine lipid peroxidation products.

In this study, the Buege–Aust method was used (Buege & Aust 1978). Briefly, 2 mL of a stock TBA reagent containing 15% (w/v) trichloroacetic acid in 0.25 M HCl and 0.37% (w/v) thiobarbituric acid in 0.25 M HCl was added to 1 mL of the UV-B treated sample. After incubating at 90 °C for 15 min and cooling down, the red TBA–MDA complex (2:1) appears, allowing fluorescence measurement. An HPLC system (Merck-Hitachi, Darmstadt, Germany) equipped with an auto sampler AS-4000A, interface D-6000A, pump L-6200A, UV-VIS-Detector L-4250, fluorescence detector F-1080 was used to quantify the pigment. A reversed-phase column (LiChrospher 100, RP 8, particle size 5 μm) was used with a mobile phase of methanol–water 30:70 for the HPLC procedure (Merck, Darmstadt, Germany). The excitation wavelength was 515 nm and the emission measurement was performed at 555 nm. A calibration curve was generated using malondialdehyde, which was formed from malondialdehyde-bis-(dimethylacetal) under acidic conditions (Trommer et al 2002).

Quantification of DNA synthesis

DNA synthesis was assessed 20 h after UV irradiation by the quantification of bromodesoxyuridine incorporation into the DNA (Muir et al 1990). The cells were labelled with 10 μM bromodesoxyuridine and re-incubated for 2 h in the humidified incubator. Cell supernatant was removed and the cells were fixed and partially denatured for 30 min in one step using the FixDenat solution provided with the ELISA kit. After removing FixDenat, peroxidase conjugated anti-bromodesoxyuridine was added and reacted for 90 min. After washing five times with PBS, 100 μL of a 420 μM tetramethylbenzidine solution was added and the absorbance was measured at 370 nm using the microtiter plate reader i EMS (Labsystems, Helsinki, Finland) and 492 nm as a reference wavelength.

Cytotoxicity assay

Cell viability was measured by staining with crystal violet as described previously (Podhaisky et al 1997). Briefly, after washing with PBS, the cells were fixed with methanol for 10 min and afterwards stained for 10 min with a 0.1% crystal violet solution. After washing for three times with double-distilled water, the dye was eluted with 0.1 M trisodium citrate in 50% ethanol for 1 h. The extinction at 540 nm was measured using the microtiter plate reader again.

Electron paramagnetic resonance (EPR) spectroscopy

EPR studies were carried out in the X-band using a Bruker ESP 380 E FT-EPR spectrometer (Bruker, Rheinstetten, Germany). The quantitative investigations were performed using the double resonator technique in the rectangular cavity ER 4105 DR with corresponding aqueous sample cells ER 160 FC (Rototec Spintec, Spektroskopie Vertriebs-GmbH, Remshalden). The double resonator settings were: microwave frequency 9.78 GHz; microwave power 13.6 mW; modulation frequency 100 kHz; modulation amplitude 0.3 mT; sweep width 15 mT; conversion time 40.96 ms; time constant 10.24 ms; sweep time 41.94 s. For evaluating the hydroxyl radical scavenging activity, the spin trapping method was used. Therefore, the reactive hydroxyl radicals were produced via the Fenton system consisting of hydrogen peroxide and iron (II) ions and trapped by DMPO. Before transferring the specimen into the cavity, 1 mL of each stock solution was added in the following order: firstly, ADP (2 mM), then ferrous sulfate (0.5 mM), test substance (bufexamac) or double distilled water as a blank control, the spin trap DMPO (40 mM) and, finally, H_2O_2 (0.3%).

Exactly 120 s after the addition of hydrogen peroxide the recording of the spectra was started, measuring the specimen in resonator 1 of the cavity and TEMPO (50 μM in methanol) as the reference in resonator 2 of the double resonator.

The double resonator technique is a method used to study aqueous samples in a reproducibly quantitative manner. Every measured signal was set in proportion to a reference signal of TEMPO to avoid analytical artifacts due to changes in the Q factor of the resonator.

DPPH (0.1 mM in methanol) was chosen as a stable free radical. Different concentrations of bufexamac (10–200 μM in 10-μM steps; 2.0 mL of each specimen) were mixed with 3.0 mL of the DPPH stock solution. The spectrum recording was started again after 120 s against TEMPO as a reference sample in the double resonator. All signals were the results of 5 time accumulations.

The quantitative analysis of the EPR spectra were carried out by double integration of the EPR signals using WinEPR software (Bruker, Rheinstetten, Germany).

Mass spectrometry

The mass spectrometric analysis was carried out using a Finnigan LCQ ion trap mass spectrometer with ESI interface and integrated syringe pump (Thermo Finnigan, San Jose, CA).

Electrospray ionisation mass spectrometry was performed in the negative and positive ion mode, respectively, with an ESI voltage of 4.0 kV and a heated capillary temperature of 220 °C. The samples were infused by flow injection via a syringe pump (10 μL min^{-1}). Tandem-MS and MS^n experiments were used to obtain structural information.

In addition, similar experiments were carried out using a hybrid quadrupole orthogonal acceleration-time-of-flight mass spectrometer (Q-Tof 2; Micromass, Manchester, UK) equipped with a standard electrospray source. The samples were infused using a syringe pump at a flow rate of 10 μL min^{-1} and examined in the positive and negative ion mode. For accurate mass measurements, the instrument needed to be calibrated before use, and a lock mass was added serving as internal standard. Thereby a mass accuracy of < 5 ppm was achieved, enabling the determination of elemental composition The structures suggested in the fragmentation scheme of this paper (see Figure 6) refer to the accurate mass data. In MS/MS monoisotopic precursor ions were fragmented using argon as collision gas with a collision energy of 15 eV. The accurate masses of the precursor ions were used to internally calibrate the MS/MS spectra.

Statistical analysis

All quantitative data shown represent the mean values ± s.d. of sextuple measurements (n = 6). Statistical analysis of the effects of bufexamac and tocopherol on the TBA-RP concentration after UV-B irradiation was performed using a one-way analysis of variance. The effects of UV-A dose or bufexamac concentration on DNA synthesis and the effects of UV-B dose or bufexamac concentration on the surviving cell fraction were statistically analysed by a one-way analysis of variance as well.

In all cases, post-hoc comparisons of the means of individual groups were performed using Dunnett's multiple comparison test. A significance level of $P < 0.05$ between groups was accepted as being statistically significant. All calculations were performed using GraphPad Prism 2.0 (GraphPad Software Inc., San Diego, CA).

Results and Discussion

Thiobarbituric acid assay

Figure 1 shows the results of the thiobarbituric acid assay of the linolenic acid lipid model system. The effect of 100 μM bufexamac on the linolenic acid dispersion is shown. Secondary lipid peroxidation products (measured as malondialdehyde units) were significantly decreased by this hydroxamic acid derivative. The TBA reaction product concentration level was decreased by bufexamac from 410 ng mL^{-1} for irradiation without additives to 325 ng mL^{-1} for irradiation in the presence of 100 μM bufexamac. For a better estimation, experiments were carried out additionally in the presence of α-tocopherol as a lipophilic standard antioxidant (Kourounakis et al 2002). α-Tocopherol protected linolenic acid from UV-induced damage as well. The presence of 100 μM α-tocopherol led to a TBA reaction product concentration of 250 ng mL^{-1} after linolenic acid sample irradiation.

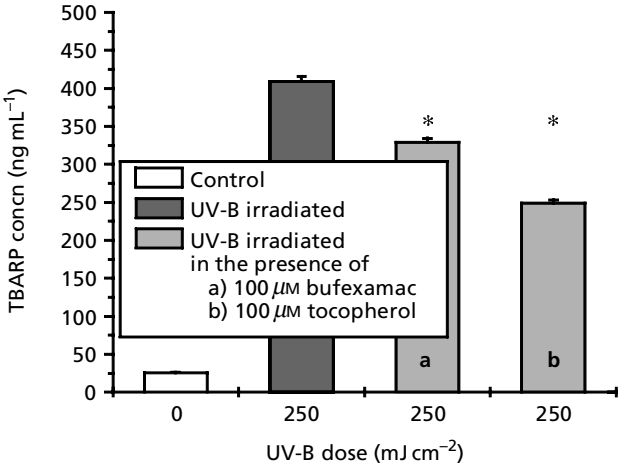

Figure 1 Concentration of the thiobarbituric acid reaction products (TBA-RP) and influences of ultraviolet irradiation, bufexamac and α-tocopherol in the linolenic acid lipid model system. *$P < 0.05$, UV exposed in the presence of bufexamac or α-tocopherol (light grey columns) vs irradiated without additives (dark grey column). Data represent the mean values ± s.d. of sextuple measurements (n = 6).

Bufexamac was able to significantly decrease the amount of linolenic acid lipid peroxidation products measured as MDA content. The antioxidative potential was below that of vitamin E.

To elucidate the mechanism of the lipid protective effects, EPR and MS investigations were applied. Possible mechanisms of antioxidant activity described in the literature are transition metal chelation, direct radical scavenging, reduction by electron donation and enzymatic antioxidation (Voegeli et al 1993).

Cell culture investigations

Before incubation with the test substance, bufexamac irradiation experiments using HaCaT keratinocytes were carried out without the presence of any test drug. This was important for a better evaluation of the effects of UV light on the parameters of DNA synthesis and cell vitality as determined during the cell culture experiments.

Figure 2A shows the influence of UV-A radiation of differing doses on the DNA synthesis of the keratinocytes. The effects of UV-B on the vitality of the cell culture is shown in Figure 2B. It is of interest that there is no additional damaging effect from a UV-B dose of ca. 225 mJ cm^{-2}.

Figures 2C and 2D demonstrate the effects of bufexamac on UV-induced cell damage. A non-irradiated control, as well as an irradiated sample without the presence of bufexamac, was used as reference. Additionally, non-UV-treated samples were incubated with the highest bufexamac concentration used in this study. This was

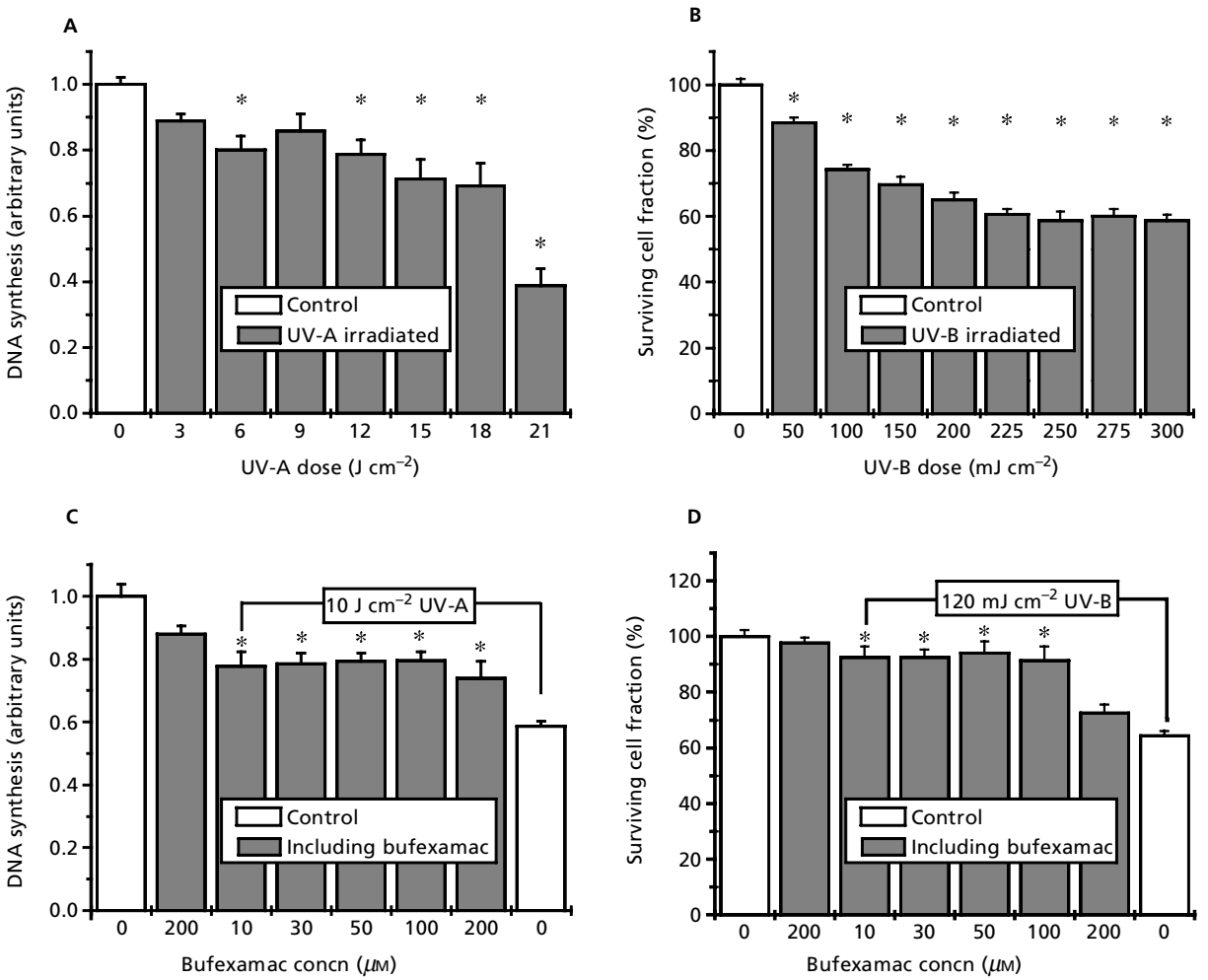

Figure 2 Influence of UV irradiation on HaCaT keratinocytes and effect of bufexamac. A. Influence of UV-A irradiation on DNA synthesis. B. Influence of UV-B irradiation on cell vitality. C. Effect of bufexamac on DNA synthesis after UV-A exposure. D. Effect of bufexamac on cell vitality after UV-B exposure. $^*P < 0.05$ (for A and B, UV exposed (grey columns) vs non exposed (white columns); for C and D, UV exposed in the presence of bufexamac (grey columns) vs irradiated without bufexamac (white columns)). Data represent the mean values ± s.d. of sextuple measurements (n = 6).

necessary to avoid a concealment of toxic drug effects. Figure 2C shows that this study design made sense, since 200 μM bufexamac decreased the amount of DNA synthesis of the HaCaT keratinocytes. The irradiation experiments show that bufexamac was able to partly annihilate the damaging effects of UV-A light on the proliferation behaviour of the cells measured as DNA synthesis. A concentration dependence of this effect was not observed.

In Figure 2D this protective effect is noticeable, too. The UV-B induced reduced vitality of the keratinocytes was nearly on the level of the non-irradiated cells again when bufexamac was added in concentrations of 10–100 μM.

The antioxidative effects observed in the simple linolenic acid lipid model system could be confirmed with the much more complex in-vitro system of the HaCaT cell culture with the help of different analytical methods. Bufexamac shows three absorption maxima (228 nm, 277 nm, 284 nm). Therefore, there was no UV absorption of bufexamac at the used wavelengths and we concluded that simple UV screening was not responsible for the effects described in this study.

Electron paramagnetic resonance (EPR) spectroscopy

Using EPR spectroscopy it was possible to elucidate the radical processes involved in the behaviour of bufexamac.

Therefore, experiments with hydroxyl radicals, using the spin trapping technique with DMPO as a trap for the reactive oxygen species, were carried out. Hydroxyl radicals are the most reactive ROS, hence it is impossible to detect them directly even by EPR because of their high

reaction constants. An indirect determination can be achieved by the reaction with spin traps such as DMPO. The decrease in the signal intensity is regarded as a measure of the antioxidative potential of compounds.

In the Fenton assay, there were no radicals to quantify even after adding bufexamac in the lowest concentration used in this study (10 μM). This could be due to the ability of bufexamac to scavenge Fenton-system-generated hydroxyl radicals faster than DMPO as shown for the antioxidant ascorbyl palmitate in various in-vitro models. In a study by Perricone et al (1999), the lipophilic vitamin C derivative was found to be one order of magnitude faster in radical scavenging than the spin trap. Thus, free radical scavenging has to be added to the mechanisms of action debated in the literature as an explanation of the anti-inflammatory properties of bufexamac. ROS are known to be integrated in inflammation in many cases (Winyard et al 1994; Kaur & Halliwell 2000).

The DPPH radical was used as an example of stable organic free radicals (0.1 mM methanolic solution). Often, the antioxidative properties of compounds are referred to their ability to reduce the concentration of the DPPH free radical.

TEMPO, as another stable free radical, was used as a reference specimen (50 μM methanolic solution) in the double resonator for both DMPO-OH determinations and DPPH studies.

By adding bufexamac to a DPPH solution in several concentrations (10–200 μM), no changes of the EPR signal intensities of the stable radical in relation to the TEMPO reference could be observed. It can be concluded that bufexamac is not able to reduce stable free radicals.

Mass spectrometry

To elucidate the mechanism of lipid and cell protection by bufexamac at a molecular level, mass spectrometric investigations were performed using an ESI-MS device. Figure 3 shows the positive ion mode mass spectra of bufexamac. For the first time tandem MS (MS/MS) and MS^n fragmentation experiments of this antiphlogistic drug were conducted for structural elucidation.

Figure 3A shows the tandem mass spectrum of $m/z = 224$, the $[M+H]^+$ pseudo-molecular ion. Two degradation fragments were detected at $m/z = 163$ and $m/z = 150$. Further fragmentation of the $m/z = 150$ ion in an MS^3 experiment led to the peak at $m/z = 122$ (Figure 3B). The MS^3 experiment of the other main bufexamac fragment detected at $m/z = 163$ gave rise to an ion signal at $m/z = 107$ (Figure 3C).

The negative ion mode fragmentation experiments were successful as well. Figure 4 shows the tandem MS spectrum of the hydroxamic acid derivative bufexamac. The $[M-H]^-$ pseudo-molecular ion was detected at $m/z = 222$. The fragmentation of this species resulted in 5 main peaks at $m/z = 206$, 149, 147, 118 and 93.

Interesting mass spectrometric results were also obtained after the irradiation experiments with bufexamac. Figure 5 shows the positive ion mode spectra after UV exposure. This decomposition is similar to the fragmentation and some photo degradation products are explainable using the data of the MS fragmentation experiments (Figure 5A). The ion signals at $m/z = 163$, 150, 122 and 107 were detected after radiation treatment as well. Peaks at $m/z = 136$ and 114 were measured additionally (Figure 5B).

With the help of experiments using the time-of-flight mass spectrometer the elemental composition of the detected ions was clarified. Figure 6 shows the results of the structural assignments. The generation of phenolic degradation products of bufexamac after irradiating the hydroxamic acid derivative with the OSRAM device can be considered to be the reason for the eczema-provoking potential of the drug.

It was shown in this article that bufexamac exhibits antioxidative effects on a linolenic acid lipid model system and on HaCaT keratinocytes. The results of EPR investigations were useful for detailed studies regarding the radical scavenging properties of bufexamac. The molecular mechanism of the bufexamac fragmentation and degradation after UV exposure was demonstrated by mass spectrometry.

Conclusion

Several analytical techniques were applied to investigate the antioxidative properties of bufexamac using in-vitro systems. The TBA assay was applied to quantify lipid peroxidation products after UV irradiation. It was shown that bufexamac exerts antioxidative effects on the linolenic acid model. The repetition of the experiments using a HaCaT keratinocytes cell culture showed similar results. Bufexamac was able to reduce the damaging effects of UV radiation again.

To learn more about the radical involvement during these processes, EPR experiments were applied. Bufexamac lowered the concentration of spin trapped hydroxyl radicals and had no influence on the content of the stable organic radical DPPH. The first observation results from bufexamac acting as an antioxidant or a free radical scavenger of hydroxyl radicals in the Fenton system. The behaviour in the presence of DPPH can be explained by the non-existence of reducing power of the hydroxamic acid derivative towards DPPH.

To elucidate the mechanism of these antioxidative effects, mass spectrometric studies were carried out, and have shown that bufexamac is degraded after UV exposure. The degradation products of bufexamac were detected. For the first time mass spectrometric fragmentation experiments were carried out which were helpful for structure assignment.

The phenolic bufexamac decomposition products could be the reason for its eczema-provoking properties frequently described in the literature. The scavenging of hydroxyl radicals, as confirmed by the EPR experiments, may explain its controversial mechanism of action. Following this theory, the antiphlogistic properties of bufexamac are due to oxygen radical scavenging because these species are often involved in inflammatory events.

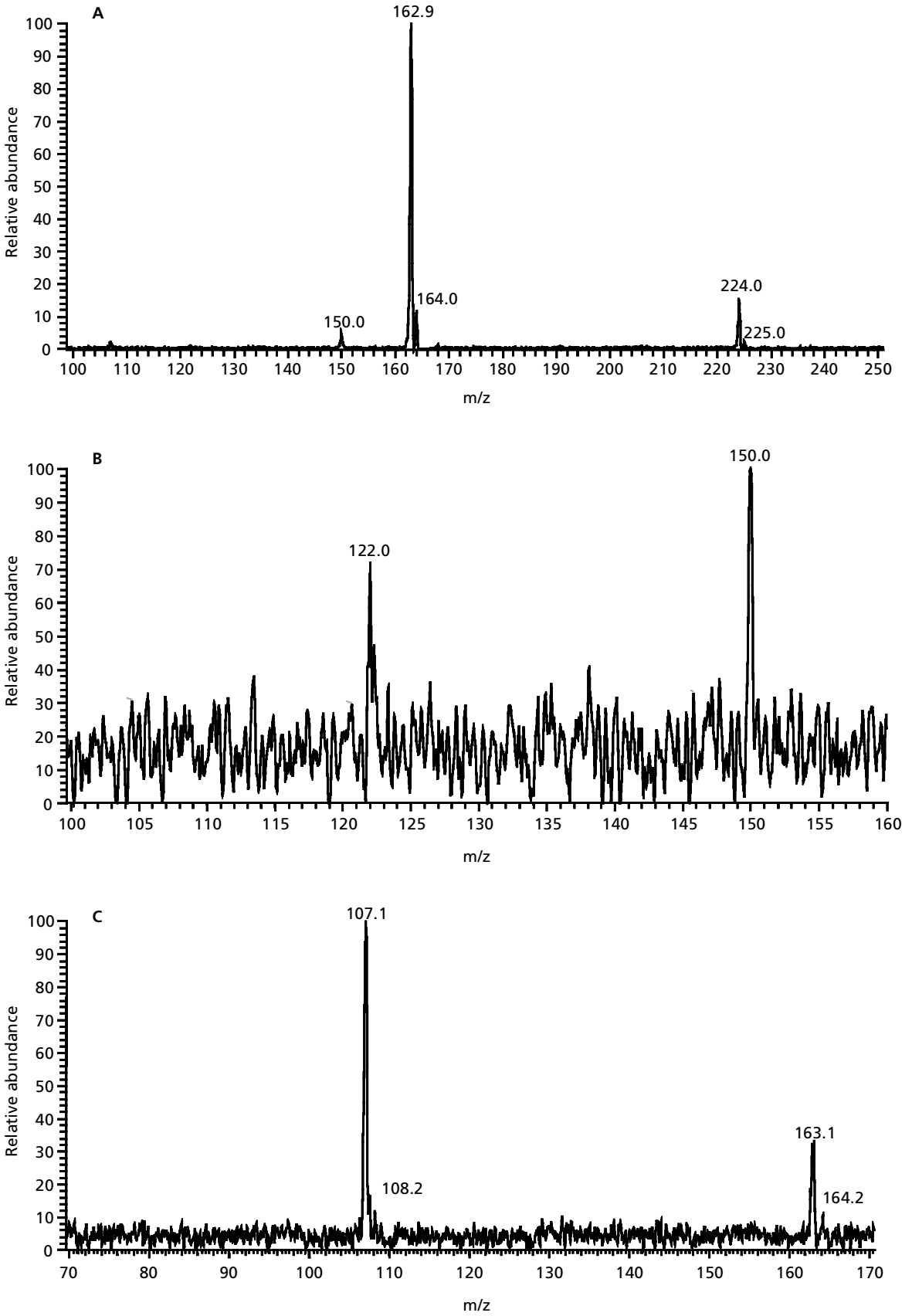

Figure 3 Positive ion mode ESI mass spectra of bufexamac. A. Tandem mass spectrum of m/z = 224. B. MS^3 spectrum of m/z = 224, m/z = 150. C. MS^3 spectrum of m/z = 224, m/z = 163.

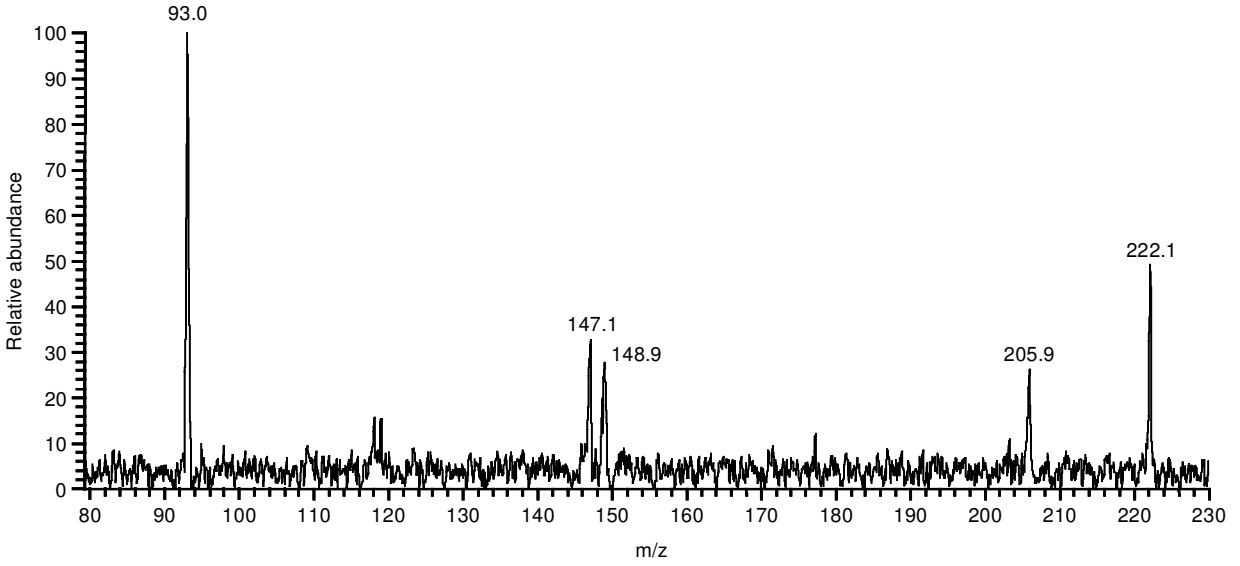

Figure 4 Negative ion mode ESI tandem mass spectrum of bufexamac of m/z = 222.

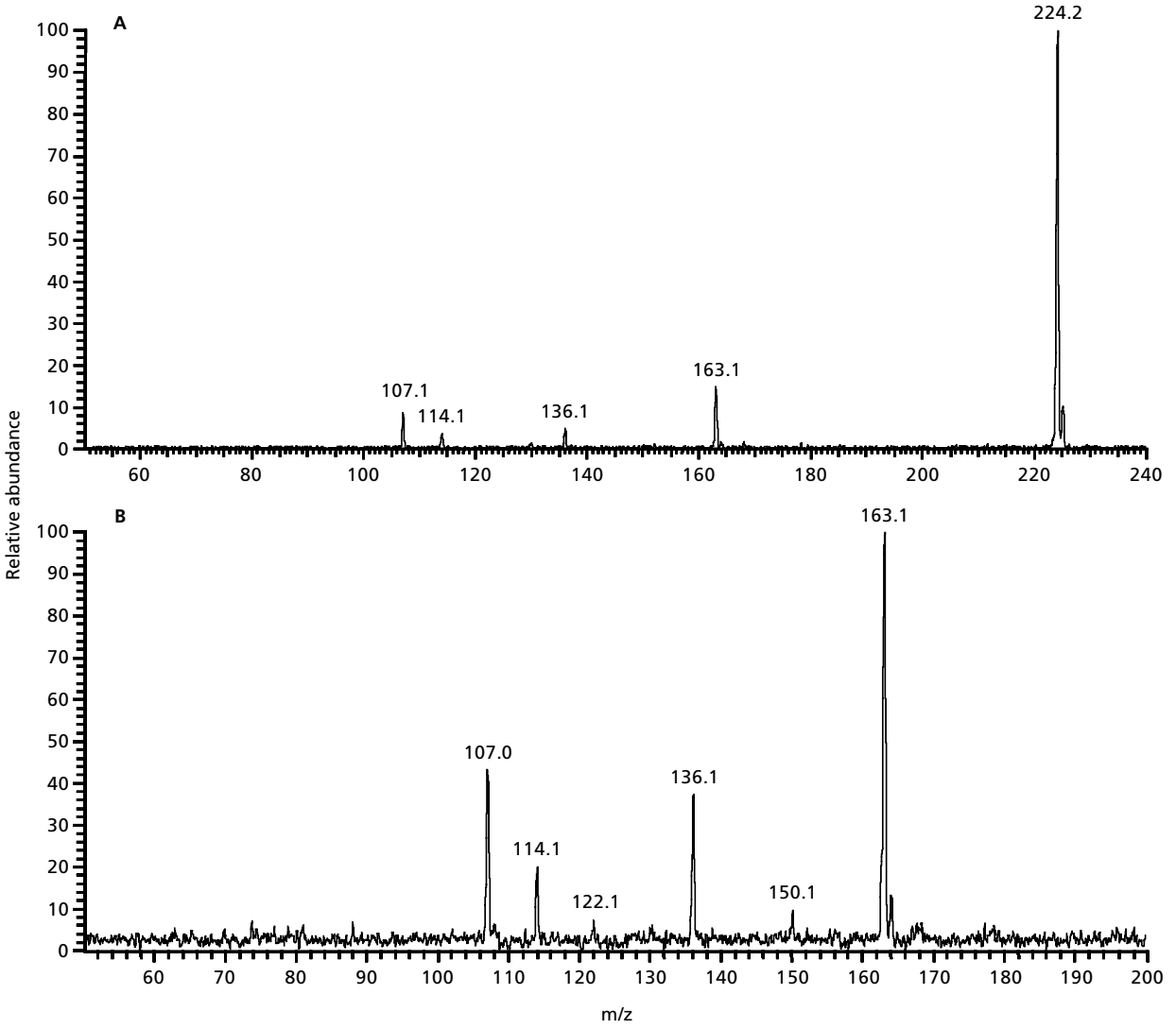

Figure 5 Positive ion mode ESI mass spectra of bufexamac after 120 min UV irradiation by OSRAM VITALUX 300 W emitter. A. m/z = 50–240. B. m/z = 50–200.

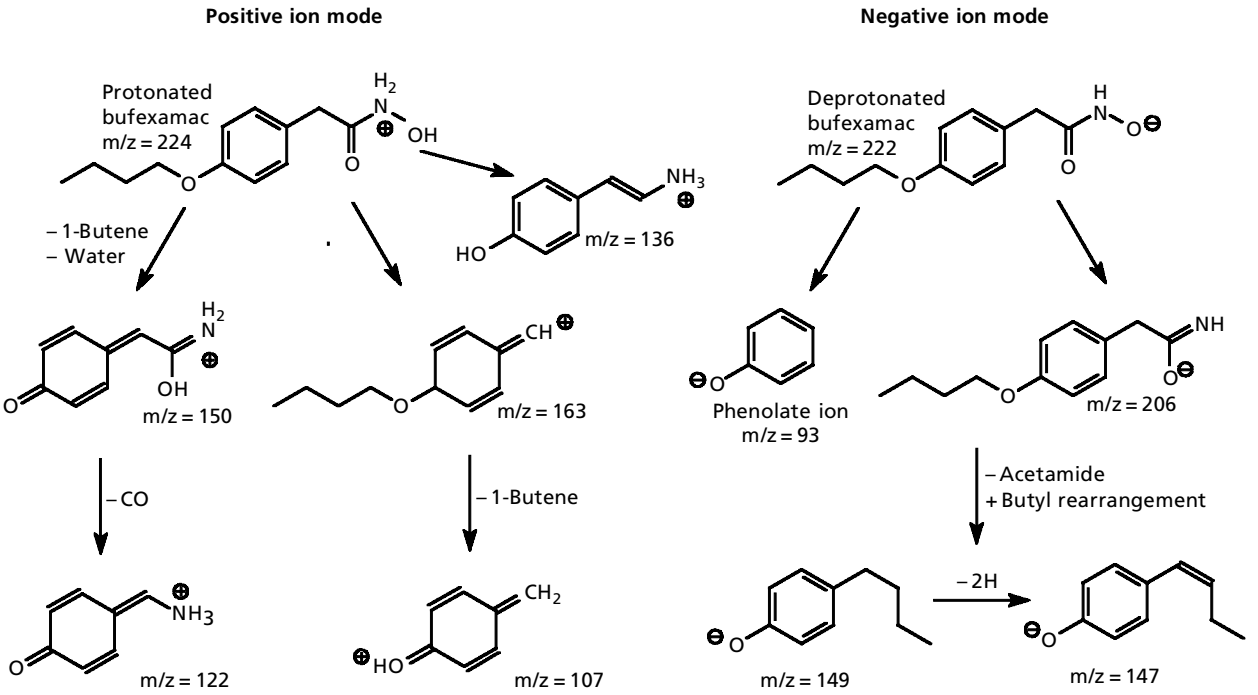

Figure 6 Scheme of the bufexamac decomposition after multiple stage MS experiments and irradiation.

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