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On-line monitoring of UV-induced lipid peroxidation products from human skin in vivo using proton-transfer reaction mass spectrometry

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Abstract

Proton-transfer reaction mass spectrometry (PTR-MS) was used to study ultraviolet (UV) light-induced lipid peroxidation in human skin, in vivo. Emissions of volatile organic compounds (VOCs) in the mass range between 20 and 150 amu in the headspace of the skin of 16 healthy volunteers were monitored before, during and after irradiation in an on-line and non-invasive fashion. From these experiments, five volatile substances were found to reflect the damage caused by UV-radiation. The two major compounds (monitored at mass 45 and 59 amu) were identified as acetaldehyde and propanal using a combination of Tenax-based gas chromatographic pre-separation with PTR-MS. The other volatiles (with characteristic ions at, among others, masses 73 and 87 amu) could not be identified. Simultaneous measurement of the established lipid peroxidation biomarker ethene using laser-based photoacoustic trace gas detection revealed a similar pattern and statistically significant correlations between VOC production measured with PTR-MS and ethene. Variations in UV-radiation intensity were reflected by the amount of acetaldehyde and propanal emitted from the skin. Our results show that acetaldehyde and propanal can be used as biomarkers for lipid peroxidation.

Keywords: Proton-transfer reaction mass spectrometry; Volatile organic compound; Lipid peroxidation; Aldehydes; In vivo; Human skin

1. Introduction

Ultraviolet (UV) radiation represents 10% of the solar radiation reaching the surface of the earth and is generally divided into three groups: UV-A (315–400 nm), UV-B (280–315 nm) and UV-C (200–280 nm) of which UV-C radiation is almost completely absorbed by the ozone layer [1]. UV light influences important physiological processes inside the human body, such as vitamin D synthesis and calcium metabolism [2]. However, epidemiological and laboratory based studies have demonstrated that UV light can also cause photo-ageing and is a very important factor in the development of skin cancer [1,2]. The worldwide incidence of melanoma due to sun radiation is increasing rapidly [3].

As a result of UV-irradiation, reactive oxygen species (ROS) are produced in the skin, which subject the body to oxidative stress and increase lipid peroxidation [1,4]. Lipid peroxidation is the oxidative degradation of (poly-) unsaturated fatty acids and is involved in many diseases. Lipid peroxidation can be monitored by measurement of its intermediate or end products [4,5], which can be useful for monitoring diseases and for evaluating the health status of the body and the efficiency of medical treatments [1]. Since lipid peroxidation products differ for different fatty acids [6], the relative amount of the different fatty acids in the skin is very important. Terashi et al. [7] have investigated the composition of skin-cell membranes. Their results show that the most abundant lipids in epidermal keratinocytes were stearic acid (16.0%), oleic acid (16.0%), palmitic acid (17.5%) and linoleic acid (27.4%). Palmitic and stearic acid are saturated fatty acids, which are less susceptible to lipid peroxidation. Linolenic acid, which is the precursor for ethene [5] contributes only 0.1% to the total fatty acid content [7]. It is therefore expected that the most abundant biomarkers will be products of linoleic acid and oleic acid.

Various volatile organic compounds (VOCs) have been proposed as in vivo or in vitro markers of lipid peroxidation. Frankel

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[6] list many lipid peroxidation products from linoleic, oleic and linolenic acid, measured in vitro in vegetative oils, including ethene, ethane, pentane and many different aldehydic products. Several aldehydes, including acetaldehyde, propanal, butanal, hexanal and 2/3-hexenal, have been used as markers for lipid peroxidation in olive oils, sunflower oils and other food products [5,8,9]. However, the emission of aldehydes by biological samples is complex and their concentrations are usually very low [10].

In vitro experiments have shown aldehydic products from lipid peroxidation [11]. Luo et al. [12] have used Gas Chromatography–Negative Ion Chemical Ionization Mass Spectrometry (GC–NICIMS) to quantify the total of 22 saturated and unsaturated aldehydes (C_2–C_{12}) that produce detectable derivatives. They reported the measurement of 20 aldehydes [13] from skin fibroblast cultures using GC–MS.

Most studies on lipid peroxidation measuring volatile (end) products have focused on exhaled ethane and/or pentane [4,10,14,15]. Ethene has also been shown to be a marker for lipid peroxidation [4,16]. Draper et al. [17] have used malondialdehyde adducts with lysine in rat and human urine as a marker for lipid peroxidation in vivo.

Since aldehydes are highly soluble and reactive, they are expected not to be clearly reflected in breath. The most direct way of measuring these compounds is by monitoring the emission from the skin itself, using a skin cuvette [18]. VOCs produced in the skin diffuse into the headspace and can easily be measured non-invasively and without major interference of compounds from the rest of the body.

The drawback of current detection techniques used in the studies mentioned above is that they use intermediate steps and measure markers only indirectly. Furthermore, most methods to measure these secondary volatile products in vitro or in vivo are either invasive or time consuming [10]. Proton-transfer reaction mass spectrometry (PTR-MS) is a technique that is very well suited for measurements of aldehydes and other hydrocarbons in multi-component mixtures of VOCs in an on-line, noninvasive and highly sensitive fashion [19,20]. In fact, PTR-MS has been used in some studies on products of lipid peroxidation in vegetative oils [8,9].

In this paper, we report the use of PTR-MS for measurements on the headspace of human skin for time-resolved, in vivo monitoring of UV-induced lipid peroxidation products. We expect to establish some lower-mass aldehydes to be markers of lipid peroxidation. Laser-based photoacoustic trace gas detection is used to simultaneously measure a known biomarker of lipid peroxidation, ethene. To our knowledge, this is the first report of on-line and in vivo measurements of UV-induced aldehydic lipid peroxidation products.

2. Materials and methods

2.1. PTR-MS

A custom-built PTR-MS system, analogous to the one described in Lindinger et al. [19] was constructed (Fig. 1.) A detailed description of this system can be found in Boamfa et al. [21]. The working principles of PTR-MS have been given in detail elsewhere [19,20,22]. Therefore, only a brief description is given here.

The instrument consists of four parts: an ion source where H_3O^+ ions are produced, a drift tube section, a transition chamber and an ion detection section containing a quadrupole mass spectrometer and a secondary electron multiplier. In the drift tube, the trace gases from the sample gas are ionized by proton-transfer reactions with H_3O^+ ions:

$$H_3O^+ + R \xrightarrow{k} RH^+ + H_2O$$

where k is the reaction rate constant, usually close to or equal to the collision rate constant. This reaction takes place when the proton affinity (PA) of the trace compound R is higher than that of water (166.5 kcal/mol = 7.16 eV). A major advantage of using H_3O^+ as the reagent ion is that the PA of water is higher that the PA of the normal constituents of air (cf. NO, O_2, CO, CO_2 and N_2) and that most of the typical organic compounds are ionized by the proton-transfer (PT) reaction, since their PA are in the range between 7 and 9 eV. The reaction rate can be measured or calculated [23,24] and is known for many of the PT reactions of interest [19,25]. Since the excess energy of the reaction is low, it results in mostly parent ions (RH^+), without the creation of many fragments. Dissociation can occur to form one or two fragments of significant intensity (e.g., alcohols are known to easily split of a water molecule, which results in a fragment ion at molecular

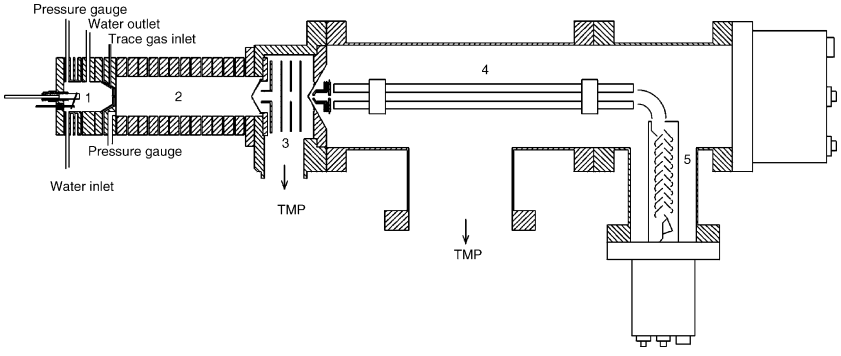

Fig. 1. Schematic view of the Nijmegen PTR-MS instrument. (1) The hollow cathode ion source, (2) the drift tube, (3) the transition chamber, (4) the detection chamber containing the quadrupole and the secondary electron multiplier (5).

mass minus 17). Fragmentation does increase with increasing carbon chain length. However, due to the fact that soft-ionization results in only one or two significant characteristic ions, the matrix of signals is much less complicated than with other mass spectrometry techniques.

Some adaptations are made to the system described in Boamfa et al. [21]. The drift tube ring size was decreased (1.5 cm inner diameter) and the Viton O-rings were replaced by special Teflon PFA rings. Drift tube and inlet tubing are heated to 45 °C. These changes are intended to decrease memory effects and time resolution. The major difference with the previous setup is the decrease in path length the ions have to travel through the transition chamber. This space is principally designed as an intermediate pumping stage between the drift tube and the quadrupole chamber to reduce the pressure in the detection chamber. By increasing the effective pumping window from 24 to 78 cm^2 and decreasing the length of the transition chamber from 8.0 to 3.5 cm, the number of collisions in this transition chamber is reduced significantly. The pressure is decreased from 1–2 $\times 10^{-4}$ to 8 $\times 10^{-5}$ mbar. At 1 $\times 10^{-4}$ mbar, the mean free path of ions is in the order of a centimeter, decreasing with increasing molecular size. The effect of decreasing mean free path can clearly be observed as a mass dependent detection efficiency. The pressure in the quadrupole region is 2 $\times 10^{-6}$ mbar, which is necessary for low detector noise and long detector lifetime.

For medical and biological applications, a small gas flow over the biological sample is necessary in order to detect the extremely low gas emission quantities. The present setup requires a minimal gas load of ~0.6 l/h (STP) to get the optimal 2.25 mbar pressure in the drift tube and an optimal *E*/*N*-value of 120 Td (*E*/*N* is the ratio between electric field and the number density in the drift tube; 1 Td = 10^{-17} V cm^2).

2.2. GC–PTR-MS

A PTR mass spectrum constitutes a total intensity as a function of mass. The product ion mass[1] is a useful, but not unique indicator of the identity of a VOC. VOCs with identical mass cannot be measured separately. Moreover, product ion fragmentation, cluster ion formation and secondary ion–molecule reactions all lead to some degree of mass overlap as has been discussed elsewhere [20,26]. To overcome the problem of mass overlap, a combination of PTR-MS with a gas chromatographic pre-separation technique has been employed [20,22,26,27].

In this study, Tenax TA tubes (20/35 mesh, Chrompack, Middelburg, The Netherlands) are used as an adsorbing agent to adsorb VOCs and function as a GC column. During a GC–PTR-MS experiment VOCs are adsorbed on the absorber's surface (at 35 °C). Afterwards, the tube is flushed with a 1.2 l/h nitrogen flow, and the temperature is slowly ramped (from 35 to 200 °C, at a rate of 5 °C/min). The exit of the tube is connected to the PTR-MS inlet. The headspace of pure gas compounds was used to characterize the Tenax TA tube for identification of the peaks that were observed in the UV experiments.

2.3. Detection of ethene from the skin

Ethene measurements on UV-induced lipid peroxidation were performed using a laser-based photoacoustic trace gas detector, as described in Harren et al. [16]. Briefly, the laser is tuned to a wavelength where the gas under investigation has a high absorption coefficient. By modulating the intensity of the laser light, a periodic heating of the sample gas is caused. This results in pressure variations inside the sample cell of the same frequency as the laser intensity modulation. By carefully choosing the modulation period, an acoustic wave is created inside the closed sample cell. This acoustic wave of known frequency is detected with a microphone. The sensitivity of this technique is enhanced by phase-sensitive detection of the microphone signal with a lock-in amplifier. The amplitude of the acoustic wave is directly proportional to the laser intensity and to the concentration of the absorbing compound. By monitoring the laser power and the intensity of the sound wave, the concentration of the compound under investigation can be derived.

2.4. Subjects

All 16 volunteers were healthy, non-smoking males (20–35 years of age, skin type 2 or 3). The study was carried out with the approval of the Medical Ethical Committee for Research on Human Subjects of the Radboud University Medical Centre, Nijmegen. Written consent was obtained from all participating subjects.

2.5. UV experiments

A circular piece of bare skin (about 5 cm in diameter) of a healthy volunteer was irradiated with UV light. In all experiments, a quartz cuvette (5 cm diameter, volume 20 ml) was placed over the site of exposure (Fig. 2), through which a constant flow of synthetic air was maintained to sample the headspace for trace gas detection.

Fig. 3 shows the spectrum of the UV-source, measured with an intensified CCD camera (Princeton Instruments, ICCD-512-T) mounted on the exit port of a spectrograph (Acton Research Company, SpectraPro 300i). The emission of the light source was mostly in the UV-A region. The transmittance of quartz

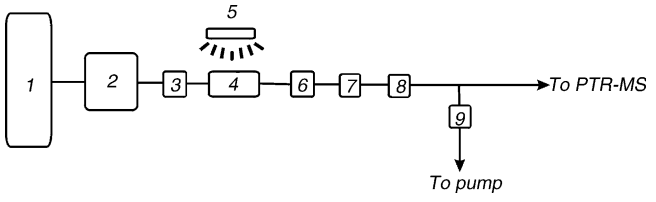

Fig. 2. Experimental setup as used in UV irradiation experiments. (1) Pressurized bottle of medical air, (2) buffer bag, (3) mass flow controller, (4) human subject, (5) solarium, (6) mass flow controller, (7) membrane pump, (8) reduction valve, (9) off-line pressure controller.

[1] Since proton-transfer reactions only generate singly ionized ions, we simply refer to the mass of the ion instead of to the *m*/*z* ratio.

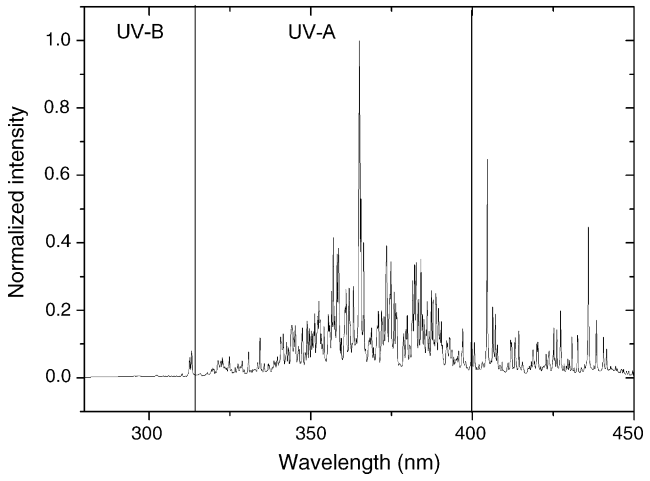

Fig. 3. Spectrum of the UV source.

(SiO_2) in the region of interest (280–400 nm) is nearly constant at ~90%.

In on-line UV experiments, first the background values of VOCs without UV exposure were measured for 10 min. Then, the VOC productions were monitored for a period of 12 min irradiation time, followed by a period of 10 min with the UV light switched off. During the whole experiment, the flow through the cuvette was maintained for real-time and on-line analysis of the headspace of the skin.

Synthetic air was provided to the skin cuvette from a pressurized bottle, via a buffer bag and a mass flow controller (Brooks Instruments, 5850S, Veenendaal, The Netherlands) at a flow rate of 1.0 l/h (STP). The flow rate through the skin cell was kept constant by a small membrane pump and a mass flow controller, the pump created a small pressure drop to keep the cuvette sealed to the skin. The inlet and outlet tubing were shielded with aluminum foil to avoid UV-induced gas emissions from the tubing walls. From the skin cuvette the sample air was transported via Teflon PFA tubing (Fig. 2) to the trace gas detectors. In measurements in which both the PTR-MS and the laser-based ethene detector were used, a flow of 0.7 l/h was led through the skin cuvette, of which 0.5 l/h (STP) was led to the ethene detector. The other 0.2 l/h were diluted with 0.3 l/h pure nitrogen and led to the PTR-MS system. All gas handling parts (tubing, connections, needle valves, etc.), except mass flow controllers, were made of Teflon PFA or Teflon PTFE (PolyFluor Plastics, Hoevestein, The Netherlands).

During several PTR-MS trial measurements complete mass spectra were recorded to find the mass values at which a signal could be observed. To achieve a high time resolution, only those signals that changed over time were selected and monitored consecutively. In on-line experiments ion intensities at 16 masses were monitored for 0.5 s per mass, resulting in an acquisition time of 8 s.

To qualify the VOCs under investigation as biomarkers of UV-induced lipid peroxidation, a variation in the UV intensity should be reflected in the signal intensity of the potential markers. Similar experiments as the ones described above, were carried out in which the intensity of the UV irradiation was varied by changing the distance from the solarium to the skin cuvette, resulting in an intensity change from 2.5 to 16 mW/cm^2.

Reference measurements were performed using a closed quartz cuvette, with an inlet and an outlet (4 mm diameter) for the gas flow. The reference cuvette was exposed to the same intensity UV light to study possible VOCs emitted by the cuvette. Furthermore, the effect of temperature change was tested using an electrical air heater at 50 °C.

To identify the VOCs during UV exposure GC–PTR-MS experiments were carried out in the same time regime as described above. During the last 90 s of UV exposure the flow was led over an adsorbing Tenax TA tube. Afterwards, the tube was analyzed as described above.

3. Results

3.1. On-line UV measurements

During UV exposure of the skin, a clear increase in ion intensity was observed for 7 masses. Fig. 4 shows a typical example of a measurement from the headspace of UV-irradiated skin on the lower inside arm. The time behavior found with PTR-MS was identical to that of the ethene measurements. Due to the low gas flow through the skin cuvette the combined time constant of gas inlet system and drift tube was 1.5 min, The initial rise in concentration can be observed just before 1.7 min after the UV is switched on ($t = 0$). This indicates that lipid peroxidation begins within seconds after the start of the UV-irradiation. After about 3.3 min a stable ion count rate is reached.

The highest ion intensity was found on mass 45 amu, followed by the masses 59, 43, 87, 73, 71 and 69 amu. The intensities at five of these seven masses (masses 43, 45, 59, 73, 87 amu) were found to increase for all 16 volunteers. The average integrated production values and their standard deviations (in nmol) for the compounds monitored on masses 45, 59, 73 and 87 amu together with the corresponding ethene values are given in Table 1. Ethene production shows significant correlations (significance of corre-

Table 1
Average production values and standard deviations from UV-irradiation experiments on 16 healthy volunteers

Mass	Average concentration (nmol)	Standard deviation
45 (Acetaldehyde)	33.3[a]	23.5
59 (Propanal)	7.05	3.32
73	0.26[b]	0.25
87	0.35[b]	0.29
Ethene	1.004	0.64

Acetaldehyde concentrations are calculated from calibration. Propanal concentrations are calculated from acetone calibrations, taking fragmentation and the difference in the k-values given in [19] for acetone and propanal into account. For unidentified ions, the standard value of 2×10^{-9} cm^3 s^{-1} was used in calculations. Integration periods were 35 min, on an illumination area of approximately 15 cm^2.

[a] The values are integrated production values over the whole irradiation period and decay time of the signal back to the background levels.

[b] This ion might represent a fragment of a higher mass neutral and reaction rate values are probably underestimated. Therefore, this value represents the lower limit for the compound monitored on this mass.

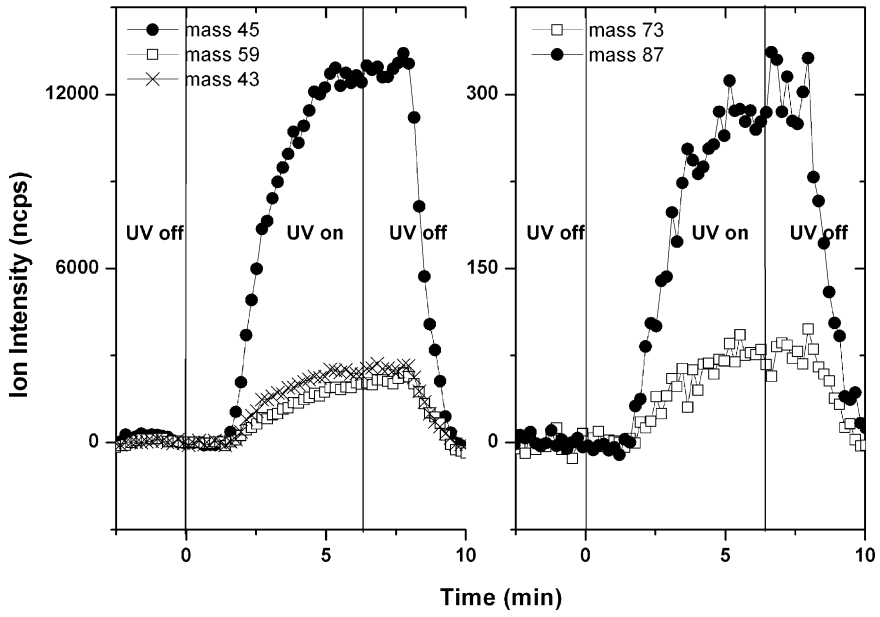

Fig. 4. Result of an on-line measurement of volatile products formed from human skin irradiated with UV light as measured with PTR-MS. UV radiation is switched on at time $t = 0$. The system's time delay is reflected by the ~100 s delay in the rise of the signal.

lations is tested with Student's t-test; $P < 0.05$) with productions of compounds monitored at masses 43, 59 and 73 amu, but not with those indicated by masses 45 and 87. The different compounds monitored by PTR-MS correlate significantly. The large standard deviations for these values reflect the inter-personal variation.

The reference measurements show that all VOC productions originate from the skin. In every reference measurement ions at masses 45 and 59 were observed, but the values in all cases were at least a factor of 10 lower than in the UV experiments. No other compounds were observed in any reference experiment (data not shown).

3.2. GC–PTR-MS measurements

Fig. 5 shows the result of a GC–PTR-MS experiment. Analysis of the content of the Tenax tube resulted in several peaks which were clearly separated in time. Using this method, positive identifications could be obtained for the characteristic ions found at masses 45 and 59 amu, which correspond to acetaldehyde and propanal, respectively. The other compounds, giving rise to ions at masses 73, 69 and 87 amu, could not be identified. Following literature studies, these compounds will be most likely aldehydes or fragments of aldehydes. Possible compounds for the ion at mass 87, with molecular mass 86 (3-methylbutanal and pentanal) show fragmentation on mass 69, but the fragmentation ratios are not in accordance with published values. Moreover, the compounds causing ion signals on masses 69 and 87 do not co-elute. Comparison of retention times of pentanal with the compound observed at mass 87 suggest that mass 87 reflects a fragment of a higher mass compound. The traces on mass 73 and 87 also do not overlap in time, meaning that these ions do not correspond to the same compound.

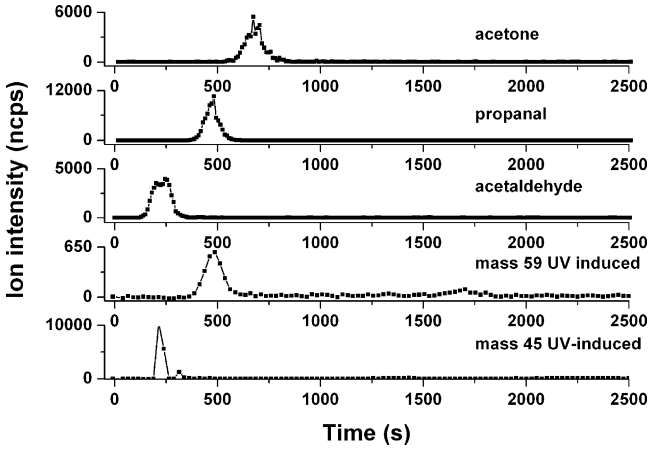

Fig. 5. Result of GC–PTR-MS identification experiments.

3.3. UV intensity dependence

Fig. 6 shows the effect of changes in UV intensity as measured on mass 45 for one subject. With UV-intensity at zero, a stable background value is obtained. For every increase in UV-intensity, a corresponding increased in acetaldehyde concentration is observed. For mass 59 amu, a similar trend was found (data not shown). The signal intensities are approximately linear with the UV intensity. Productions of the other VOCs were too low to observe the complete trend.

4. Discussion and conclusion

From the headspace of UV-irradiated skin from 16 healthy volunteers, seven characteristic ions are found to reflect increase of VOC production when lipid peroxidation is induced in the human skin. Masses 43, 45, 59, 73 and 87 amu are observed in

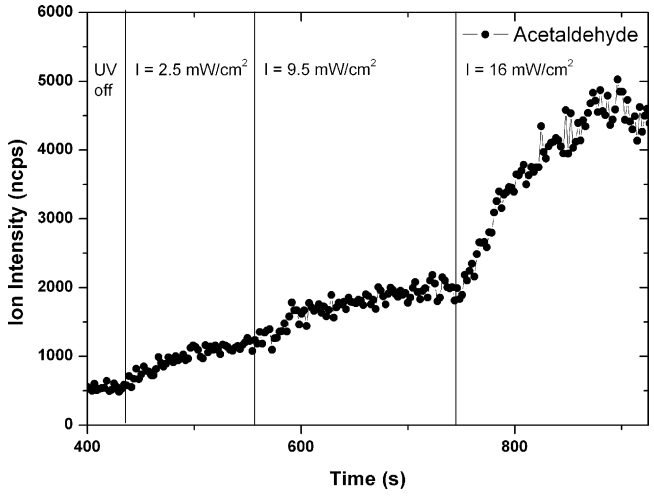

Fig. 6. Acetaldehyde production in human skin as a function of light source intensity.

significant quantities for all 16 subjects. Using a combination of GC–PTR-MS, acetaldehyde and propanal have been identified as the two most prominent compounds (monitored at masses 45 and 59 amu, respectively). Analysis of the pure compounds ruled out the possibility of acetone as a source for the ion trace at mass 59. Both acetaldehyde and propanal depend significantly on UV-light intensity. Simultaneous measurement of the known biomarker ethene using a laser-based photoacoustic trace gas detector show statistically significant correlations with the production of the compounds observed with PTR-MS. The time course of the compounds arising from UV-induced lipid peroxidation corresponds with the pattern of ethene. The identified compounds acetaldehyde and propanal can therefore be regarded as markers of lipid peroxidation.

Signals at masses 73 and 87 amu, which could not be attributed to a known neutral compound with the adopted GC–PTR-MS technique, showed statistically significant correlations and similar time behavior as ethene. Possible sources for the ion at mass 73 are malondialdehyde or butanal [6]. Mass 87 amu most probably reflects a fragment of a higher mass compound, which is also a possibility for mass 73 amu. Future experiments with an improved GC–PTR-MS setup are expected to reveal the identity of these compounds.

Our results confirm that lipid peroxidation is a nearly instantaneous process. Volatile (end) products are observed within a few seconds after the UV light is switched on. PTR-MS proves to be an excellent technique for on-line measurement of UV-induced lipid peroxidation in human skin, since it combines both high sensitivity and high time resolution with non-invasiveness.

It is known that aldehydes are highly soluble, reactive and toxic compounds in the human body [10,28]. The establishing of aldehydic end products of lipid peroxidation in the skin therefore might cause a secondary effect of lipid peroxidation, possibly away from the site of initial damage. It is assumed that aldehydes produced endogenously during lipid peroxidation act as "secondary toxic messengers" for free radical events due to their interaction with essential cell components (e.g., membrane proteins) and thereby cause disturbance of cell functions. It has been shown that aldehydes produce a great diversity of deleterious effects [10], including cross-linking in proteins and DNA [29].

With the fast and on-line PTR-MS measurements of acetaldehyde and propanal and possibly more compounds as biomarkers, it will be possible, e.g., to test the wavelength-dependent effects of UV-A and UV-B light on the skin for various skin parts and skin types, the effect of sun screen protection, or to follow the effects of UV treatment of skin diseases (e.g., psoriasis). Besides, it has a potential to be used for diagnostic purposes related to acute or chronic physiological disorders inside the human body, e.g., in inflammatory processes (acute asthma, inflammatory bowel disease) [30,31] or acute myocardial infarction [32,33].

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