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
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 acetaldelyde, propanal, butanal,
hexanal and 2/3-hexenal, have been used as markers for lipid
peroxidation in olive oils, sunflower oils and other food prod-
ucts [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 Spec-
trometry (GC–NICIMS) to quantify the total of 22 saturated and
unsaturated aldehydes (C2–C12) that produce detectable deriva-
tives. 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 malondi-
aldehyde 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 emis-
sion from the skin itself, using a skin cuvette [18]. VOCs pro-
duced in the skin diffuse into the headspace and can easily be
measured non-invasively and without major interference of com-
pounds 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, noninva-
sive 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 mon-
toring 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 per-
oxidation, 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
H3O+ ions are produced, a drift tube section, a transition cham-
ber 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 H3O+ ions:

$$H_3O^+ + R \rightarrow RH^+ + H_2O$$

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
H3O+ as the reagent ion is that the PA of water is higher that the
PA of the normal constituents of air (cf. NO, O2, CO, CO2 and
N2) 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
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 Boonstra 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 cm² 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 × 10⁻⁴ to 8 × 10⁻⁵ mbar. At 1 × 10⁻⁵ 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 × 10⁻⁸ 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 ∼0.06 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 cm²).

2.2. GC–PTR-MS

A PTR mass spectrum constitutes a total intensity as a function of mass. The product ion mass of proton-transfer reactions only generate singly ionized ions, we simply refer to the mass of the ion instead of to the m/z ratio.

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...
carried out in which the intensity of the UV irradiation was varied
markers. Similar experiments as the ones described above, were
intensity should be reflected in the signal intensity of the potential
UV-induced lipid peroxidation, a variation in the UV intensity
were monitored for 0.5 s per mass, resulting in an acquisition
time of 8 s.

Signals that changed over time were selected and monitored con-
that could be observed. To achieve a high time resolution, only those
acquisition from the headspace of the skin.

The other 0.2 l/h were diluted with 0.3 l/h pure nitrogen and led to
detector were used, a flow of 0.7 l/h was led through the skin
cell was maintained for real-time and on-line analysis of the
of the skin.

During several PTR-MS trial measurements complete mass
spectra were recorded to find the mass values at which a signal
production shows significant correlations (significance of corre-
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 exper-
iments on 16 healthy volunteers

<table>
<thead>
<tr>
<th>Mass</th>
<th>Average concentration (nmol)</th>
<th>Standard deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>45 (Acetaldehyde)</td>
<td>33.3 ± 10.1</td>
<td>23.5</td>
</tr>
<tr>
<td>59 (Propanal)</td>
<td>7.05 ± 2.58</td>
<td>3.32</td>
</tr>
<tr>
<td>73</td>
<td>0.26 ± 0.20</td>
<td>0.25</td>
</tr>
<tr>
<td>87</td>
<td>0.33 ± 0.29</td>
<td>0.29</td>
</tr>
<tr>
<td>Ethene</td>
<td>1.004 ± 0.41</td>
<td>0.64</td>
</tr>
</tbody>
</table>

Acetaldehyde concentrations are calculated from calibration. Propanal concen-
trations 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 × 10⁻⁵ cm³ s⁻¹ was used in calcula-
tions. Integration periods were 55 min, on an illumination area of approximately

3. Results

3.1. On-line UV measurements

During UV exposure of the skin, a clear increase in ion inten-
sity 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-
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.

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
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 propionaldehyde, 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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