Gas Chromatographic and Mass Spectroscopic Analysis of Excimer and Erbium: Yttrium Aluminum Garnet Laser-Ablated Human Cornea

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The photoablative products from human cadaver corneas treated with lasers were investigated using gas chromatography and mass spectroscopy. The photoablations were done separately with an excimer laser (193 nm) and with an erbium: yttrium aluminum garnet (Er:YAG) laser (2.94 μm). More than 20 different types of molecules with a molecular weight of 40–400 mass units could be identified, most of which were found to be alkanes. The comparison of the two types of laser ablations showed larger fragments and fewer types of molecules present after excimer laser treatment than after Er:YAG photoablation. Invest Ophthalmol Vis Sci 33:2180–2184, 1992

Photoablation of the cornea currently is being used in clinics. Potential applications of the excimer laser (193 nm) in corneal surgery include anterior lamellar keratectomy to smooth an irregular corneal surface and modifying refractive errors by reshaping the anterior surface.1,2

Alternate techniques to the use of the excimer laser to generate photoablation have been investigated for several reasons (eg, possible mutagenicity,3 technical complexity, and high costs). Lasers emitting a wavelength of approximately 3 μm (erbium: yttrium aluminum garnet [Er:YAG] and hydrogen fluoride [HF] laser) also can be used for photoablation4 because of their strong corneal absorption. However, a totally different laser–tissue interaction has been postulated.5,6 Hitherto, there is only limited information in the literature about the molecular mechanisms and reaction products of excimer laser photoablation. By contrast with previous publications showing the use of gas chromatography (GC) alone to separate the ablation products,7 we report the application of a combination of GC and mass spectroscopy (MS). We compared and identified qualitatively the molecular fragments produced by photoablation of human cornea with the excimer laser (193 nm) and the Er:YAG laser (2.94 μm).

Materials and Methods

Excimer laser photoablation was done with a commercial laser (ExciMed UV 200; Summit, Watertown, MA) that emitted laser pulses at a wavelength of 193 nm with an internally controlled fluence of 180 mJ/cm² and a circular beam size of 3.5 mm in diameter. Before the ablations, the fluence and beam homogeneity were tested using a joulemeter (Gentec ED 500; Canada) and black photographic paper. The ablation rate in the stroma was 0.4 μm per pulse (window corrected), and the repetition rate was 10 Hz. The pulse duration was less than 40 nsec.

The midinfrared (2.94 μm) Er:YAG laser (Schwartz, Orlando, FL) was used in a non-Q-switched mode with a pulse duration of 150 μsec at a repetition rate of 4 Hz. In this configuration, the circular beam had a diameter of 1.6 mm with a Gaussian-like beam profile in TEM00 mode. The average fluence amounted to 2.7 J/cm² (by Gentec ED 500) with an ablation rate of 17 μm per pulse (window corrected).4

We identified the molecules produced by laser photoablation by combining GC and MS analysis (Fig. 1). Different chemical products of laser treatment were separated on a GC column before they entered and passed the ion source of the MS. Transferred into positively charged molecules, they were focused electrically into the quadrupole filter where they were separated and detected depending on their mass-to-electrical charge (m/e) ratio.

Pilot experiments showed that four corneas from fresh human cadaver eyes were necessary to harvest enough material for the analysis. The cadaver eyes
were enucleated up to 5 hr postmortem, and the corneas were found to be clear. After manual debride-
ment of the epithelium, the corneas were ablated (one after the other) over the whole cornea by moving the
eye. Immediately before perforation occurred (vi-
sually controlled under a slit lamp), the eye was re-
moved and replaced by the next one. For each laser,
we repeated the experiment three times. Photoabla-
tion was done in a special chamber with a quartz win-
dow (Fig. 2) fitted with an electrical air pump so that
all photoablative products were sucked through a
moisture absorbent substance and then into a Tenax
(Supelco, Germany) tube for adsorption of the pho-
toablative molecules. The adsorbent substance (in-
cluding the photoablative products) was inserted into
the injector of the GC/MS (Finnigan MAT 1020,
70eV, Quadrupole; Miami, FL). Based on a com-
puter-supported library, the interpretation of the
spectra and identification of more than 44,000 differ-
ent molecules was possible.

Results
To exclude signals from CO, CO₂, N₂, O₂, and SO₂
from the surrounding air, molecular fragments with a
m/e ratio less than 40 were not examined. Because of
the low volatility of molecules with a molecular
weight greater than 400, these cannot pass through
the GC column and be transferred into the MS for
analysis. Thus, we limited the evaluation range of mo-
lecular weight from 40 to 400.

Figure 3 (trace 4) is a plot of an original mass chro-
matogram of the photoablative substances produced
by the excimer laser. Trace 3 shows the original mass
chromatogram after Er:YAG photoablation. Trace 2
represents products from column-bleeding sub-
stances that have nothing to do with the sample.
These column-bleeding substances are localized in
the Tenax tube (Fig. 2, part 5). They also are superim-
posed in traces 3 and 4 and excluded from the analy-
sis. Trace 1 shows typical fragments from alkane frag-
mentation, which have been raised by standards as a
part of the Finnigan MAT 1020.

Single peaks were analyzed on their mass spectra.
Figure 4 shows the analysis of one peak in trace 4 as
an example (m/e = 1131; Fig. 3, arrow). All other
peaks were analyzed identically. These mass spectra
were compared with the “library” in the data-process-
ing system incorporated into the Finnigan 1020 de-
vice (Fig. 5). The fit score, which shows the concor-
dance of the sample spectrum with the library spec-
trum, ranges from 1–1000. Scores better than 700
express a very good fit, indicating that a molecular
structure chosen by the library is probably identical
to the measured molecule. Therefore, every signifi-
cant peak (eg, in Fig. 3, trace 4, for the excimer) repre-
sents one type of identified molecule.

All molecules detected and identified by GC/MS
analysis as photoablative products are listed in Table
1. These all had a fit score of 700 or higher. As shown
in Figure 3, most of the identified compounds are
alkanes, some of which contain R-NH₂ or R-SH
groups probably originating from amino acids. Others
form cyclic products like oxacyclotetradecane or are
methylated or esterified. In all three experiments, the
same products were identified, indicating a sufficient
reproducibility of the method. Differences occurred
in molecule detection and identifications only in
structures with fit qualities less than 700. The qualita-
Fig. 3. Original plot of a mass chromatogram of molecular fragments produced by photoablation. Ordinate: relative units; abscissa: mass/charge (for further explanation, see text). Arrow points out the peak 1131, an analysis of which is shown in Figure 4. Trace 1: typical alkane fragmentation raised by internal standards. Trace 2: column-bleeding substances originating from the tenax tube (see Fig. 2, part 5). Trace 3: original mass chromatogram of the EnYAG photoablative products. Trace 4: original mass chromatogram of the excimer photoablative products.

tive comparison of the ablative products of the two laser types showed a tendency toward larger fragments and less different types of molecules after excimer than after Er:YAG photoablation.

Discussion

The corneal stroma consists of two major classes of macromolecules: glycoproteins and proteoglycans. The glycoproteins contain one or more sugar molecules covalently bound to an amino acid chain. One of the best known glycoproteins is collagen, which comprises up to 71% of the dry weight of the cornea. Proteoglycans are characterized by the presence of a large polysaccharide moiety, frequently containing oligosaccharides or glucosaminoglycans attached to a relatively small protein structure. Glycosaminoglycans and collagen form the extracellular matrix of the cornea and are involved in corneal hydration.

The mechanisms of both types of laser photoablation still are unclear. For the excimer laser (193 nm), it was postulated that a nonthermal mechanism existed, leading to the term “ablative photodecompensation.” Experimental data has shown some evidence of a thermal mechanism. However, other investigators could not detect histologic signs of coagulation effects accompanying photoablation with the excimer laser. The histologic evaluation of corneas treated with Er:YAG and excimer lasers found greater ther-

Fig. 4. Original plot of a mass spectrum analyzed from peak no. 1131. Ordinate: relative units; abscissa: molecular weight.
Fig. 5. Comparison of an original mass spectrum with spectra from the “library.” Trace 1 represents the original mass spectrum. Trace 2 shows the closest spectrum from the library (fit score 820). Trace 3 and 4 are other alternatives with fit scores smaller than 700.

There is little data concerning the molecular products expelled during excimer laser photoablation, and none is available about Er:YAG laser ablation to our knowledge. This minimal information results from GC analysis; this technique primarily is a method of separation, and it is less appropriate for identification of structural properties. There are no data concerning the molecular products expelled during erbium:YAG laser ablation to our knowledge. This minimal information results from GC analysis; this technique primarily is a method of separation, and it is less appropriate for identification of structural properties.

Both laser wavelengths investigated are capable of producing photoablation of the cornea. This suggests that there is a photochemical-photothermal mechanism where laser photons supply enough energy to break intramolecular bonds directly or indirectly. The resulting molecular fragments are expelled as a gas; otherwise, we would not have found any molecules in our samples.

Table 1. Photoablative fragments

<table>
<thead>
<tr>
<th>Excimer</th>
<th>Erbium: YAG</th>
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<tr>
<td>1. C₆H₁₂O₂NS₂</td>
<td>1. C₆H₁₂O₂NS₂ Thiosulfuric acid S-2 aminoethyl ester</td>
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<tr>
<td>2. C₅H₁₀O₄</td>
<td>2. C₅H₁₀O₄ 1,2,3,4 Cylopentanetrol</td>
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<tr>
<td>3. C₆H₁₃ON</td>
<td>3. C₆H₁₃ON N-ethynyl, N-methyl-acetamide</td>
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<tr>
<td>5. C₈H₁₄O₃N₂NN</td>
<td>5. C₈H₁₄O₃N₂ NN Methylenebis-2 propenamide</td>
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<tr>
<td>6. C₉H₁₆O₂</td>
<td>6. C₉H₁₆O₂ Buteniodicotic acid (Z) Monobutyl ester</td>
</tr>
<tr>
<td>7. C₉H₁₇3,5,5'</td>
<td>7. C₉H₁₇ 3,5,5' Trimethyl-1-hexene,</td>
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<tr>
<td>8. C₁₀H₁₈H</td>
<td>8. C₁₀H₁₈H 4 Methyl 2 propyl-pentan(1) ol</td>
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<tr>
<td></td>
<td>9. C₁₁H₂₀O₂ 2 Propenoic Acid (Z)-octyl ester</td>
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<td></td>
<td>10. C₁₁H₂₂ Undecane 9 methyl</td>
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<td></td>
<td>11. C₁₂H₂₄ 2,4 Dimethyl-decane</td>
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<td></td>
<td>12. C₁₂H₂₄O₂ 2,2 Dioxepane</td>
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<tr>
<td></td>
<td>13. C₁₂H₂₄O₂P Phosphoric acid tributyl ester</td>
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<td></td>
<td>14. C₁₂H₂₄O 4 Dodencanol</td>
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<td></td>
<td>15. C₁₃H₂₆ 3,5 Dimethyl-undecane</td>
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<td>16. C₁₃H₂₈ Octadecene</td>
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Analysis by GC and MS is the most precise qualitative method available. Because of its qualitative nature, GC-MS does not deliver percentages of each product. However, combining this technique with
other quantitative chemical methods could provide more insight into the different physical processes occurring during photoablation with excimer and infrared lasers.

**Key words:** excimer laser, Er:YAG laser, photoablative products, GC–MS analysis

**Acknowledgments**

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**References**