Advanced glycation end-products (AGE) during haemodialysis treatment: discrepant results with different methodologies reflecting the heterogeneity of AGE compounds

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Abstract

Background. There has been much recent interest in accumulation of advanced glycation end-products (AGE) in uraemic patients. Analysis of AGE has been difficult, because commonly used methodologies, i.e. immunodetection assays or fluorescence measurements, reflect group reactivity and are not specific for chemically defined substances. Some investigators measured individual AGE compounds, e.g. pentosidine, carboxymethyllysine, pyrraline or imidazolone, but a systematic assessment of known compounds using specific HPLC methods in diabetic and non-diabetic end-stage renal disease (ESRD) patients during treatment has not been performed.

Methods. For the present study, the concentrations of early and late products of the Maillard reaction in plasma and ultrafiltrate were monitored during high-flux dialysis sessions in diabetic and non-diabetic patients. AGE were analysed by fluorescence spectroscopy and size exclusion chromatography with fluorescence detection. Specific HPLC methods were used to quantify the Amadori product fructoselysine and the AGE compounds pentosidine and pyrraline in acid or enzymatic hydrolysates.

Results. Using size exclusion chromatography, we confirmed a similar fluorescent peak distribution for diabetic and non-diabetic ESRD patients. Main fractions were found at ~70, ~14 and <2 kDa, confirming results obtained by other authors. In diabetic patients, the fluorescence intensity of the low molecular weight fraction was higher. Uraemic patients differed from controls mainly by the fluorescence of the low molecular weight fraction. The peak spectrum in ultrafiltrates was similar to that in plasma regarding low molecular weight fractions and the 14 kDa peak, but no protein-bound fluorescence was found at 70 kDa. HPLC analysis revealed a significant reduction of plasma pentosidine during high-flux dialysis in non-diabetic (from 9.1±5.1 to 8.5±4.7 pmol/mg protein; P<0.05) and diabetic patients (from 10.0±9.1 to 6.8±4.0 pmol/mg protein; P<0.05). In contrast, plasma fructoselysine showed only a non-significant trend to decrease in diabetic (from 3.24±0.88 to 3.05±0.77 nmol/mg protein) and non-diabetic patients (from 2.69±0.52 to 2.36±0.50 nmol/mg protein). Pyrraline, a non-fluorescent late AGE product derived from reaction of 3-deoxyglucosone with lysine, could not be detected (detection limit ~40 pmol/mg protein). Comparing HPLC and size exclusion analysis, it was found that pentosidine accumulated in the range of low molecular weight substances and was removed by high-flux dialysis.

Conclusions. High-flux dialysis reduces the plasma concentration of fluorescent AGE compounds, i.e. pentosidine, but the Amadori product fructoselysine is not removed, indicating that this compound is protein associated.

Key words: advanced glycation end-products; diabetes; fluorescence; high-flux haemodialysis; HPLC; Maillard products; pentosidine

Introduction

Since the first description of the reaction, the term ‘Maillard reaction’ stands for a complex series of non-enzymatic reactions between reducing sugars (e.g. glucose) and amino groups of proteins, peptides or amino acids [1]. The complexity and variety of the reaction pathways between glucose and amino groups were described in numerous model systems developed in food science because of the importance of heat treatment of sugar and proteins in food processing [2,3].

It has been established that the concentrations of advanced glycation end-products (AGE) increase up to 10-fold during uraemia. This increase is the com-

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bined result of enhanced generation due to oxidative or ζ-dicarbonyl stress [4–6] on the one hand, and of diminished or absent elimination by the kidneys on the other [7]. Only few data are available concerning the effect of chronic haemodialysis on the elimination of chemically defined AGE, presumably because of methodological problems caused by the heterogeneity of AGE compounds.

Odetti et al. [8], using HPLC, failed to find effects of dialysis on plasma pentosidine levels and, even during haemofiltration, reduction was no more than 5%. In contrast, Papanastasiou et al. [9], using a competitive immunological assay, i.e. solid phase-bound AGE–bovine serum albumin (BSA) and polyclonal antibody raised against BSA aged for 3 months, found highly variable effects of haemodialysis on plasma concentrations. The main fraction of AGE was found in the molecular weight range of 1.5–2.0 kDa. Friedlander et al. [10,11], using HPLC for detection of protein-bound pentosidine, found a significant correlation between residual renal function and pentosidine concentrations in plasma of undialysed chronic renal failure patients, peritoneal dialysis patients and haemodialysis patients. The highest concentrations were found for haemodialysis patients. Makita et al. [12] found that in diabetic haemodialysis patients the majority of AGE compounds were in the molecular weight range 2–5 kDa. Significant removal of this fraction by high-flux membranes was noted. We used immunological detection and were unable to demonstrate removal of AGE by haemodialysis using regenerated cellulose membranes [13]. Miyata et al. [14] measured the albumin-bound and free fractions of pentosidine during peritoneal dialysis and haemodialysis. Approximately 80% of free pentosidine was removed by haemodialysis using regenerated cellulose membranes, reflecting substantial diffusive permeability of low molecular weight substances, and a rebound to 80% of the pre-dialysis levels was observed within 24 h.

The present study was undertaken to assess the elimination of chemically defined Amadori and AGE compounds during high-flux dialysis. A spectrum of analytical methods was used, i.e. HPLC for fructoselysine, pentosidine and pyrraline, and total fluorescence and distribution of fluorescence in different molecular weight ranges. We reasoned that simultaneous analysis with different methods should provide more complete insights into the elimination kinetics in the hope of developing rational strategies for removal of Maillard compounds.

Subjects and methods

Patients and controls

Stable chronic dialysis patients, i.e. 10 diabetic and 10 non-diabetic patients, were randomly approached and agreed to participate in the study which had been approved by the local ethics commission. The main characteristics of the study patients are given in Table 1. Plasma taken from healthy volunteers (n=6, female, 26±2 years) having no dietary restrictions served as control.

Treatment modality

Maintenance haemodialysis was performed as follows: blood flow 250–300 ml/min; dialysate flow 500 ml/min using bicarbonate dialysate; treatment time 5 h; steam-sterilized high-flux haemodialysers (Polyflux@ 14S, Gambro Dialysetoren, Hechingen, Germany) containing 1.4 m² synthetic Polyamide S hollow fibre membranes. Patients had no dietary restrictions other than reduced potassium and fluid intake. No food and no coffee or tea were ingested during dialysis sessions.

Study design

EDTA plasma samples were drawn before (i.e. ~10 min after the start), during (i.e. after 2–2.5 h) and at the end of dialysis sessions (5 h). They were processed immediately according to a strict protocol, i.e. storage of EDTA–blood on ice and centrifugation within 30 min at 4°C, 1500 g for 10 min. Plasma aliquots were taken and stored at −80°C before analysis.

For sampling of ultrafiltrate from the dialysate compartment, which was performed immediately after plasma sampling, dialysate tubes were disconnected and the dialysate fluid flushed out while isolated filtration was performed for 10 min. The first fraction (~100–200 ml) was discarded and 50 ml of filtrate was collected. Aliquots were put on ice immediately and stored at −80°C before analysis.

Analytical techniques

For fluorescence spectroscopy, a spectrofluorimeter (LS 50 B, Perkin Elmer, Überlingen, Germany) was used at 350 nm excitation and 430 nm emission wavelengths (see figures). Emission spectra were obtained at 350 nm excitation and 380–600 nm emission. The use of excitation 350 nm/emission 430 nm is based on the spectral fluorescence analysis of patient plasma and ultrafiltrate. A maximum of absorption was found at 350 nm and this wavelength was therefore chosen to increase the sensitivity of detection. Maxima of emission were found at ~440 nm and ~420 nm in plasma and ultrafiltrate, respectively. In order to be able to use a single wavelength of detection for both plasma and ultrafiltrate samples, emission was recorded at 430 nm. Plasma samples, but not ultrafiltrate samples, were diluted 1:25 or 1:50 with phosphate-buffered saline (PBS).

Size-selective gel permeation chromatography (GPC) of

<table>
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<th>Table 1. Clinical data of patients involved in the study groups; values are given as median (range)</th>
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<td>n</td>
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<tr>
<td>Sex (M/F)</td>
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<tr>
<td>Age (years)</td>
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<tr>
<td>Time on dialysis (months)</td>
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<tr>
<td>Residual renal function (creatinine clearance)</td>
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plasma and ultrafiltrate samples was performed using an isocratic HPLC system (Waters, Eschborn, Germany) equipped with a Superdex 75HR 10/30 column (Amersham Pharmacia Biotech, Freiburg, Germany) eluted at 0.5 ml/min with 10 mM PBS pH 7.2, containing 300 mM NaCl. Fluorescence signals (excitation 350 nm; emission 430 nm) were recorded using the LS50B spectrofluorimeter equipped with a flowthrough cuvette for liquid chromatography. For calibration (i.e. the relationship between molecular weight and retention time), the following proteins (Sigma, Deisenhofen, Germany) were dissolved in eluent buffer as standards: BSA (66 kDa), chicken egg albumin (45 kDa), bovine erythrocyte carbonic anhydrase (29 kDa), horse heart myoglobin (17 kDa) and bovine lung aprotein (6.5 kDa). The optimal separation range for the column was 70–7 kDa short oligo- or polypeptides.

The protein content of the plasma samples was calculated as in plasma and ultrafiltrate.

Fluorescence signals were assigned according to their retention times using a linear regression method based on the values found for standard proteins. The accuracy achieved by this size exclusion method is due to the logarithmic calibration characteristics estimated to be in the range of ±20%.

Fructoselysine and pentosidine were quantified by amino acid analysis using HPLC after prior acid hydrolysis. Plasma samples (1 ml) were hydrolysed in 10 ml of 6 M HCl for 23 h at 110°C. After cooling, 1 ml of the hydrolysate was vacuum dried on a Speedvac concentrator (Savant, Formindale, USA) and dissolved in 200 μl of 0.2 M sodium citrate buffer, pH 2.2. Amino acid analysis was performed on an Alpha Plus amino acid analyser (LKB Biocrom, Cambridge, USA), using a stainless steel column (150 × 4 mm) filled with ion-exchange resin, DC4A-spec sodium form (Benson, Reno, Nevada, USA). The composition of elution buffers, ninhydrin reagent as well as the running conditions are described elsewhere [15,16]. Injection volume was 10–80 μl. After separation on the ion-exchange column, amino acids were detected initially using a photodiode array detector PDA 996 (Waters, Eschborn, Germany); for furosine quantification, a detection wavelength of 280 nm was used or a fluorescence detector SFM 25 (Kontron, Eching, Germany); for pentosidine quantification, fluorescence (at excitation/emission wavelengths of 335/385 nm) was measured between the outlet of the column and the ninhydrin reaction coil. Following subsequent ninhydrin derivatization, amino acids were detected at 570 and 440 nm. The protein content of the plasma samples was calculated as the sum of the amino acids. From values of furosine, fructoselysine was calculated by multiplying the molar furosine values by the factor 3.1, i.e. the known conversion factor for the degradation of fructoselysine during acid hydrolysis [17]. For quantification of pentosidine, reference material was isolated as described [16]. Briefly, N-α-acetyllysine, N-α-acetylarginine and ribose were incubated for 48 h at 65°C, followed by acid hydrolysis of the reaction mixture, preparative ion-exchange chromatography and final purification on a semi-preparative reversed-phase HPLC column. The structure and purity of the isolated pentosidine were established with nuclear magnetic resonance, mass spectroscopy and UV spectroscopy. The pentosidine content was determined via elementary analysis. Based on this, a molar absorptivity of 12880 was calculated for the absorption maximum at 325 nm. Purified (purity >98%) furosine was obtained from Neosystem (Strasbourg, France).

Analysis of pyrraline was performed by amino acid analysis using HPLC after prior enzymatic hydrolysis. Enzymatic hydrolysis of plasma samples was performed using four enzymes (pepsin, pronase, aminopeptidase and prolidase) as described previously [18]. For pyrraline detection, amino acid analysis was performed as described above with the combination of the PDA (set at a wavelength of 297 nm) and ninhydrin derivatization [18]. Alternatively, pyrraline analysis was performed via isocratic ion-pair reversed-phase HPLC with UV detection [19]. Pyrraline reference material was synthesized according to Henle and Bachmann [20].

The protein content in plasma and ultrafiltrate samples was analysed using a conventional protein detection assay (Pierce, Rockford, USA) processed according to the manufacturer’s instructions. Highly purified human albumin (Sigma, Deisenhofen, Germany) was used for calibration of the assay. It is important to note that these methods overestimate protein concentration, especially in the presence of short oligo- or polypeptides.

Statistics

The data were not corrected for haemoconcentration and are shown as mean ± standard deviation. The RS/1 software package (release 5.2; BBN Software Products Co., Cambridge, USA) was used for statistical analysis, and statistical significance was assumed for \( P < 0.05 \). Wilcoxon’s signed rank test and Mann–Whitney U-test were applied for comparison of paired and unpaired data, respectively. A linear regression method was used for correlation.

Results

Fluorescence in plasma and ultrafiltrate of dialysis patients

The fluorescence intensity (excitation 350 nm, emission 430 nm) yielded by plasma and ultrafiltrate at various time points of a dialysis session is shown in Figure 1. No significant difference was found when comparing pre-dialysis samples of diabetic and non-diabetic patients. In both groups, a significant exponential decrease by ~30% was observed during high-flux dialysis treatment. Ultrafiltrates obtained during isolated filtration indicate substantial transmembrane passage by convection. There was a significant correlation (\( r = 0.5082, \ P < 0.001 \)) between fluorescence intensities in plasma and ultrafiltrate.

The ratio between the fluorescence intensity and protein concentration, calculated for each patient, decreases by ~30% during treatment (Table 2).

Analysis of the emission spectra (excitation at 350 nm) showed a shift of the emission maximum towards higher wavelengths in plasma samples during treatments. This was statistically significant in non-diabetic, but not in diabetic patients (Table 2). The wavelength of the emission maximum was significantly (\( P < 0.01 \)) lower in ultrafiltrates than in plasma for both patient groups and at all sampling times (Table 2), indicating heterogeneity of fluorescent substances, with different behaviour of the individual substances during treatment sessions. Different wavelengths of emission maxima (\( \lambda_{\text{em}} \)) were also observed when comparing emission spectra of healthy donor serum (\( \lambda_{\text{em}} \) at 455 nm), serum from diabetic patients (\( \lambda_{\text{em}} \) at 444 nm) and aged human serum albumin (\( \lambda_{\text{em}} \) at 425 nm).
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**Fig. 1.** Fluorescence intensity in (top) plasma (diluted 1:25) and (bottom) ultrafiltrate (undiluted) samples taken before, during and at the end of treatment. Ultrafiltrate samples were collected over a period of 10 min after the dialysate flow was stopped and the dialysate compartment was emptied. Data from 10 diabetic and 10 non-diabetic ESRD patients are given as mean ± standard deviation.

**Molecular weight-specific analysis of fluorescent compounds**

Typical GPC chromatograms of plasma of a diabetic ESRD patient compared with plasma of a healthy control are given in Figure 2. Clearly detectable fluorescent peaks were found at mol. wts of ~70 and ~14 kDa for both diabetic and non-diabetic uraemic patients, and these peaks were also found in plasma of healthy controls, although at a lower intensity. In contrast, no fluorescent peaks were detected in the low molecular weight range of plasma samples of healthy controls. As shown in Table 3, the pre-dialysis peak area of the fraction below 2 kDa was lower in non-diabetics compared with diabetics (1.1 ± 0.9 vs 2.1 ± 2.3); however, the difference was not statistically significant (P = 0.432).

Figure 2 also compares GPC for plasma samples taken at the start and end of treatment. Table 3 summarizes the peak areas for the following fractions.

**Table 2.** Changes in fluorescence intensity during dialysis treatment relative to the plasma protein concentration and wavelengths of emission maxima (λ em_max) in plasma and ultrafiltrate

<table>
<thead>
<tr>
<th></th>
<th>Diabetic Start</th>
<th>Diabetic End</th>
<th>Non-diabetic Start</th>
<th>Non-diabetic End</th>
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<tbody>
<tr>
<td>Plasma samples</td>
<td>Fluorescence/ protein (IU/g)</td>
<td>320 ± 124</td>
<td>231 ± 85**</td>
<td>321 ± 169</td>
</tr>
<tr>
<td></td>
<td>λ em_max (nm)</td>
<td>435 ± 6</td>
<td>439 ± 4</td>
<td>437 ± 5</td>
</tr>
<tr>
<td>Ultrafiltrate samples</td>
<td>Fluorescence (IU)</td>
<td>842 ± 454</td>
<td>239 ± 93*</td>
<td>1042 ± 768</td>
</tr>
<tr>
<td></td>
<td>λ em_max (nm)</td>
<td>421 ± 4</td>
<td>422 ± 7</td>
<td>422 ± 4</td>
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</table>

Data are given as mean ± SD; fluorescence was measured at excitation 350 nm and emission 430 nm.

*P < 0.01 vs start; **P < 0.05 vs start.
Table 3. Comparison of peak areas in chromatograms obtained with plasma of diabetic and non-diabetic uraemic patients pre- and post-dialysis

<table>
<thead>
<tr>
<th></th>
<th>Diabetic</th>
<th>Non-diabetic</th>
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<tr>
<td></td>
<td>Pre-</td>
<td>Post-</td>
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<tr>
<td>F1 (50–150 kDa)</td>
<td>12.7 ± 4.9</td>
<td>15.3 ± 9.3</td>
</tr>
<tr>
<td>F2 (10–20 kDa)</td>
<td>5.2 ± 2.3</td>
<td>6.0 ± 9.3</td>
</tr>
<tr>
<td>F3 (&lt;2 kDa)</td>
<td>2.1 ± 2.3</td>
<td>1.2 ± 3.5</td>
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<tr>
<td>F4</td>
<td>0.6 ± 1.5</td>
<td>0.2 ± 1.0</td>
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Peaks were grouped into F1–F4 as indicated. Data are given as mean ± SD. Please note that post-dialysis data are not corrected for haemoconcentration.

*Out of the separation range of the size exclusion column.

(see Figure 2), F1 (50–150 kDa), F2 (10–20 kDa), F3 (<2 kDa) and F4 (peaks beyond the separation capacity of the column). During treatment, the peak area of the low molecular weight fraction (F3) was reduced by ~50%, whereas the peak area of the high molecular weight fractions (F1 and F2) remained constant. In many samples, the low molecular weight fractions were no longer detectable at the end of the treatment period.

The pattern of fluorescent peaks of an ultrafiltrate sample compared with the corresponding plasma sample is shown in Figure 3. In ultrafiltrates, the pattern of fluorescent peaks in the low molecular weight range was similar to that in plasma. The peak areas significantly decreased during the treatment period.

**Fructoselysine**

Fructoselysine concentrations in the plasma of diabetic and non-diabetic ESRD patients are shown in Figure 4. Significantly higher concentrations of the non-fluorescent Amadori product fructoselysine were found pre- and post-dialysis in the plasma of diabetic (pre-dialysis 3.8 ± 0.8, post-dialysis 3.5 ± 0.7 nmol/mg protein) compared with non-diabetic patients (pre-dialysis 2.7 ± 0.5, post-dialysis 2.6 ± 0.5 nmol/mg protein). A non-significant effect of dialysis treatment on fructoselysine concentration was observed in both groups.

**Pentosidine**

No significant differences in plasma pentosidine concentrations were observed between diabetic (10.0 ± 9.1 pmol/mg protein) and non-diabetic (9.1 ± 5.1 pmol/mg protein) patients (Figure 5). In non-diabetic patients, only a slight (7 ± 6%) but statistically significant (P < 0.05) decrease during dialysis (to 8.5 ± 4.7 pmol/mg protein) was noted, while a more pronounced (22 ± 29%, P = 0.078 diabetic vs non-diabetic patients) and significant (P < 0.05) decrease (to 6.8 ± 4.0 pmol/mg protein) was found in diabetic patients. There was a wide scatter of predialysis concentrations in both non-diabetic (from 1.5 to 18.2 pmol/mg of protein) and diabetic patients (from 4.6 to 34.7 pmol/mg of protein). In plasma of...
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Discussion

Our study was designed to assess chemically defined early and late products of the Maillard reaction, i.e. fructoselysine, pentosidine and pyrraline, and, in parallel, global fluorescence intensity, in plasma and ultrafiltrate of diabetic and non-diabetic patients. The theoretical possibility of inhibition by EDTA during enzymatic hydrolysis was excluded by control measurements of heparinized plasma samples.

Pyrraline

Pyrraline concentrations were below the limit of detection (40 pmol/mg protein) in plasma and ultrafiltrate of diabetic and non-diabetic patients. The theoretical possibility of inhibition by EDTA during enzymatic hydrolysis was excluded by control measurements of heparinized plasma samples.

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used for molecular weight analysis and modified proteins cannot be excluded. This would lead to a delayed elution from the column and thereby to an underestimation of the molecular weight of the respective compound.

The presence of two fluorescent high molecular weight fractions in plasma of both healthy donors and uremic patients, but the occurrence of fluorescent low molecular weight fractions in uremic patients only, suggests that the accumulation of the latter is of critical importance for AGE-related complications, in agreement with reports of Makita et al. [12,28]. Recently, Fishbane et al. [29] stressed the importance of lowering AGE-apoB concentrations, which is the reaction product of reactive intermediates of low molecular weight AGE compounds with low density lipoproteins and possibly contributes to dyslipidaemia and related acceleration of arteriosclerosis. Our findings confirm the role of high-flux haemodialysis for an efficient removal of AGE compounds. Although high-flux dialysis effectively removes these low molecular weight substances of <2 kDa, the efficiency of such intermittent treatment has a limited effect on pre-dialysis values, mainly because of rapid rebound [14]. More specific removal may be possible in the future by specific sorption methods [30–32], but it remains questionable whether they will provide additional therapeutic benefit.

Pre-dialysis pentosidine concentrations are lower than those reported by other authors [10,14]. This might be due to the pentosidine reference material used in our study. In contrast to isolation procedures reported in the literature, the content of our pentosidine isolate was determined by UV measurement and molecular weight analysis. Based on this, a molar absorptivity of 12 880 was calculated for the absorption maximum at 325 nm, which is significantly higher than the corresponding value of 4195, which was reported previously [33] without the results of an elementary analysis and which represents, to our knowledge, the only reference for molar absorptivity of pentosidine in the literature. Without control, the use of the lower molar absorptivity might have been responsible for an overestimation of pentosidine previously [10,14]. Sampling of plasma was performed according to a strict protocol in order to exclude variations due to the release of AGE from cell binding sites or cell damage. Plasma concentrations of pentosidine could also be affected by food intake during dialysis [7], which was excluded in our study. As a minor factor, the method used for analysis of protein concentration, i.e. colorimetric measurement of total proteins vs calculation from amino acid analysis, could lead to differences in plasma concentration, when given in pmol/mg protein.

Renal catabolism and clearance of pentosidine in uremic patients were analysed recently by Miyata et al. [34]. Our data are basically in agreement, but we assume that the fraction of ‘free pentosidine’ contains different pentosidine-modified oligopeptides. Free pentosidine, as defined by Miyata et al., was analysed in the non-trichloroacetic acid-precipitable plasma fraction without prior hydrolysis and, therefore, does not include oligopeptide-bound pentosidine. In addition, Miyata’s study was performed with cellulosic low-flux membranes which have a limited permeability for substances in the molecular weight range of 2 kDa (i.e. vitamin B12 clearance is typically in the range of 50–60 ml/min). In contrast, the high-flux membrane used in our study is highly permeable in this molecular weight range (i.e. vitamin B12 clearance = 144 ml/min) and our molecular weight-specific analysis shows the presence of fluorescent compounds in the whole range of molecular weights below 2 kDa in plasma and ultrafiltrate.

Many Maillard reaction products are fluorescent, but one must also consider the possible formation of non-fluorescent products. Fluorescence is not specific for individual compounds; it is a group reactivity which fails to provide quantitative information on concentrations of individual compounds. Nevertheless, the peak areas detected by fluorescence compared pre- vs post-treatment indicate the relative change in the content of fluorescent compounds. The observation of small but significant changes in the emission spectrum in plasma samples during dialysis treatment and the significantly larger shift in emission spectrum when comparing plasma and ultrafiltrate samples underlines the heterogeneous chemical nature of fluorescent products, which are probably removed differently during dialysis.

Our study was designed to focus on the simultaneous measurement of chemically defined compounds formed in vivo during the Maillard reaction in addition to global assessment by fluorescence and to describe the molecular weight-dependent elimination during high-flux dialysis. In summary, our investigations revealed the following novel insights: (i) there are marked differences between pre-dialysis concentrations of various chemically defined Maillard products and their change during high-flux dialysis; (ii) the time course of concentrations of Maillard reaction products might be different in diabetic and non-diabetic patients due to different molecular weight distributions of modified proteins and peptides and/or due to different rates of release from deep compartments; (iii) the elimination of fluorescent compounds by convection is restricted to low molecular weight range compounds, suggesting binding to proteins or AGE modification of higher molecular weight substances; and (iv) changes in fluorescence spectra during treatment and differences between plasma and ultrafiltrate underline the heterogeneous chemical nature of fluorescent AGE which presumably are eliminated differently during high-flux hemodialysis. Clarification of this point requires detailed biochemical analysis of each molecular weight fraction using specific HPLC and mass spectrometry.

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