Serum and Tissue Accumulation of Advanced Glycation End-Products Correlates with Vascular Changes

Uremia is associated with increased cardiovascular mortality and accumulation of advanced glycation end-products (AGEs). We designed a cross-sectional single-center, observational study in peritoneal dialysis (PD) patients to investigate correlations between accumulation of AGEs (skin and serum) and vascular changes, as reflected by pulse wave velocity, vascular calcifications and left ventricular mass index.

PATIENTS AND METHODS

The study was approved by the ethics committee of University Hospital of Reims (France). Inclusion criteria were: age > 18 years, ability to sign consent, PD treatment for > 3 months and no history of hemodialysis. Study was

limited to Caucasians because of the assessment of skin autofluorescence (SAF). Exclusion criteria were: history of chronic hemodialysis, PD treatment for < 3 months, PD for cardiac failure, current hospitalization, infection, or neoplasia. Glucose exposure was calculated as followed: $[\Sigma(\text{glucose concentration of the solution} \times \text{volume})] \times \text{dialysis vintage}$. Eleven patients were using conventional solutions Dianeal (Baxter) or stay•safe (Fresenius), 17 were using biocompatible low glucose degradation products (GDP) solutions Physioneal (Baxter) or Balance (Fresenius), and 16 were using icodextrin. Skin autofluorescence was measured with the AGE Reader (Diagnoptics Technologies, Groningen, Netherlands). For serum AGE measurements, carboxymethyllysine (CML), pentosidine, or methylglyoxal hydroxyl-imidazolone 1 (MG-H1) were measured in plasma samples using mass spectrometry coupled with liquid chromatography (LC-MS/MS) (1). Total AGEs (free adducts and protein-linked) were measured. Soluble receptor for AGEs (sRAGE) plasma levels were assessed with commercially available kits (R&D Systems, Minneapolis, MN, USA). Carotid to femoral pulse wave velocity (PWV) was assessed using the SphygmoCor device (AtCor Medical, West Ryde, Australia). All measures were performed by the same operator. For the vascular calcification score (VCS), lateral abdominal plain radiography was used. Calcification score was blindly determined by 2 different physicians as previously described by Kauppila *et al.* Echocardiography was performed using the Acuson Sequoia C256 (Siemens, Munich, Germany). Data were collected and stored in digital format for off-line analysis by 2 trained cardiologists blinded to clinical and biological data, and according to American Society of Echocardiography recommendations.

STATISTICAL ANALYSIS

Quantitative data are described as mean $\pm$ standard deviation (SD) and qualitative data as number (percentage). Group comparisons were done using bivariate analyses (Fisher's exact and Wilcoxon test), a p value < 0.05 was considered significant. Correlation was examined using the Spearman and Pearson tests, as appropriate. Age was strongly correlated with PWV; therefore multiple linear regressions were made to adjust vascular data to patient's age (Bonferroni correction) and a p value < 0.0125 was considered significant. All analyses were performed using SAS version 9.3. (SAS Institute Inc., Cary, NC, USA).

RESULTS

Baseline characteristics of the study population, SAF, plasma AGE levels (CML, pentosidine, and MG-H1), and sRAGE are described in Tables 1 and 2. Results for the evaluation of vascular modifications are shown in Table 2.

We found a significant positive correlation between duration of dialysis and serum concentration of CML, MG-H1, and pentosidine. Cumulative glucose exposure was significantly associated with pentosidine and MG-H1 serum levels (Table 3).

TABLE 1
Clinical and Biological Characteristics of the
Study Population ($n=28$)

Clinical and laboratory data	Mean±SD
Clinical data	
Age (years)	59.3±18.5
Male	19 (67.9%)
Female	9 (32.1%)
Body mass index (kg/m ²)	27±4.5
Systolic blood pressure (mmHg)	145.4±17.8
Diastolic blood pressure (mmHg)	78.8±15.9
Dialysis	
Dialysis vintage (years)	1.64±1.29
Automated PD	17 (64.2%)
Continuous ambulatory PD	11 (42.8%)
Glucose exposure during dialysis (g)	76,102±83,455
Kt/V	2.066±0.54
Total clearance (L/week/1.73 m ²)	78.8±25.9
Urinary output (mL/24 h)	1353±823
Biological data	
Albumin (g/L)	34.6±4.18
CRP (mg/L)	4.66±4.14
PTH (pg/mL)	235.9±221.4
Calcium (mmol/L)	2.3±0.23

PD = peritoneal dialysis; CRP = C-reactive protein; PTH = parathyroid hormone.

TABLE 2
Circulating AGEs (Total), SAF, Soluble RAGE and
Vascular Data

	Mean±SD
Ages and soluble rage	
SAF (AU)	3.19±0.74
CML (mmol/mol lysine)	0.28±0.11
CML, patients with conventional solutions ($n=11$)	0.33±0.14
CML, patients with biocompatible solutions ($n=17$)	0.25±0.09
Pentosidine (μmol/mol lysine)	5.48±2.94
MG-H1 (mmol/mol lysine)	1.98±0.49
sRAGE (ng/mL)	3.21±1.82
Vascular data	
Aortic PWV (m/s)	12.85±4.35
VCS (AU)	3.19±5.05
Left ventricular mass index (g/m ²)	122±30.72
Men	120.9±30
Women	124.2±34.1
Left ventricular ejection fraction (%)	60.1±10.2

AGEs = advanced glycation end-products; SAF (AU = Arbitrary Unit) RAGE = receptor for advanced glycation end-products; CML = carboxymethyllysine; MG-H1 = methylglyoxal derived hydroxyimimidazolone 1; sRAGE = soluble receptor for advanced glycation end-products; PWV = pulse wave velocity; VCS = vascular calcification score.

TABLE 3
Correlations Between AGEs, Vascular Data and Dialysis Parameters

	Patient's age		SAF		CML		Pentosidine		MG-H1		CRP	
	<i>r</i>	<i>p</i>	<i>r</i>	<i>p</i>	<i>r</i>	<i>p</i>	<i>r</i>	<i>p</i>	<i>r</i>	<i>p</i>	<i>r</i>	<i>p</i>
Dialysis vintage	ns		ns		0.579	0.001	0.524	0.004	0.402	0.033	ns	
Glucose exposure	ns		ns		ns		0.509	0.006	0.446	0.017	ns	
LVM index	ns		0.549	0.007	ns		ns		ns		0.579	0.004
PWV	0.468	0.012	ns		0.395	0.037	ns		ns		ns	

AGEs = advanced glycation end-products; SAF = skin autofluorescence; CML = carboxymethyllysine; MG-H1 = methylglyoxal derived hydroxyimimidazolone 1; CRP = C-reactive protein; LVM = left ventricular mass; PWV = pulse wave velocity; ns = not significant.

We also examined the relation between AGE concentrations and cardiovascular changes. First, we found a strong and significant correlation between SAF and left ventricular mass (LVM) index. A modest, albeit non-significant correlation was observed between pentosidine levels and left ventricular ejection fraction ($r = 0.369$, $p = 0.053$). Secondly, modification of the aortic wall, as assessed by PWV, was correlated with plasma CML levels but not with pentosidine levels. Because PWV was also correlated with patient's age, we performed multiple linear regression analysis to adjust vascular change criteria for patient's age. By multiple linear regression, the positive correlation between SAF and left ventricular mass

index remained highly significant ($p = 0.01$) but the association between plasma CML and aortic wall modifications was no longer statistically significant.

We did not observe any correlation between vascular changes and MG-H1 or soluble form of RAGE.

DISCUSSION

The present study shows that tissue and serum accumulation of AGE correlates with vascular changes: SAF correlated with left ventricular mass index, while plasma CML concentrations were associated with vascular stiffness.

We used mass spectrometry coupled with liquid chromatography (LC-MS/MS) to assess serum concentration of 3 different AGEs, whereas most publications to date measured only a single AGE molecule. We found that glucose exposure and duration of dialysis were significantly correlated with serum AGE concentrations. This result is in agreement with a previous publication by Linden *et al.* reporting that the AGE precursor content in PD solutions (3,4-dideoxyglucosone-3-ene) increased with glucose concentrations (3). Taken together, these results suggest that glucose exposure and duration of dialysis are involved in AGE formation and accumulation in PD patients.

Skin autofluorescence (SAF) measures tissue accumulation of AGEs and has been reported to correlate with pentosidine content of skin biopsies. Skin autofluorescence is increased in pathological conditions associated with accumulation of AGEs, such as in diabetic, PD, and hemodialysis patients (4). In the present study, we found a similar increase in SAF values. Furthermore, we found a positive correlation between SAF and myocardial change. Similarly, elevated SAF has been reported to predict cardiovascular mortality in hemodialysis patients (5). Additionally, Koyama *et al.* previously showed that a high concentration of circulating pentosidine was a risk factor for heart failure in the general population (6). These results led us to hypothesize that the biochemical structure of pentosidine may be responsible for increased affinity for myocardial tissue, while CML may have greater vascular affinity. The involvement of CML in modification of the vasculature has been reported in healthy subjects and in microvascular complications (retinopathy) in type 2 diabetes patients (7).

In light of the correlations we observed, and results from the literature, we suggest that tissue accumulation of AGEs may depend on their biochemical structure, with preferential accumulation of larger fluorescent AGEs (such as pentosidine) in the heart, while CML preferentially accumulates in the vasculature. This hypothesis is consistent with the fact that AGEs have a wide range of chemical stabilities, with half-lives that vary from days to years under different physiological conditions. They also have varied biological effects. For instance, some AGEs are protein cross-linkers and therefore may change the structure of a protein or confer resistance to proteolysis. Nevertheless, further clinical and basic research is needed to confirm this hypothesis.

CONCLUSION

This study found clinically relevant correlations between serum and tissue-accumulated AGEs, and vascular changes in a PD population. Our results suggest that (i) reducing accumulation of AGEs may reduce vascular modifications; (ii) AGE accumulation and its adverse effects may vary between tissue types, depending on their biochemical structure. Larger cohort studies are warranted to confirm the possibility of organ affinity of AGEs, to establish the potential utility of biocompatible solutions in preventing AGE-mediated vascular remodeling, and to identify the role of RAGE signaling in these modifications.

DISCLOSURES

The authors have no financial conflicts of interest to declare. The authors would like to thank Dr. Redouane Taama and Fresenius medical care for financial support in this study.

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doi: 10.3747/pdi.2013.00338