LETTER TO THE EDITOR

Modulation of serum levels of sRAGE by bromelain in patients with chronic kidney disease: a pilot study

Advanced glycation end-products (AGEs) are a heterogeneous group of nonenzymatic adducts of proteins, lipids, and nucleic acids. They are generated in pro-oxidant and pro-inflammatory conditions, in particular, in sustained hyperglycemia, and accumulate during aging and, in an accelerated manner, in diabetes and renal insufficiency. Clinical consequences include cardiovascular complications and inflammatory / immune processes. The deleterious effects of AGEs are induced in particular by an interaction with the receptor for AGEs (RAGE), the transmembrane receptor of the immunoglobulin superfamily.[1] RAGE is also activated by non-AGE molecules such as members of the S100-calgranulin superfamily, high-mobility group box-1 protein, amyloid-β peptide, β-sheet fibrils, glycated β_2-microglobulin, and advanced oxidation protein products. The upregulation of RAGE expression is assumed to be involved in the pathogenesis and progression of numerous diseases such as atherosclerosis, vascular calcification, complications of diabetes, rheumatoid arthritis, neurodegenerative diseases, cancer progression, transplant rejection, and chronic kidney disease (CKD).[2] Therefore, the ligand-RAGE interaction has become a promising therapeutic target for preventing or retarding the above diseases. One potential approach is the competitive inhibition of RAGE by soluble RAGE (sRAGE). It acts as a decoy of different agonistic ligands, thereby preventing their interaction with RAGE. In the human serum, there are 2 truncated forms of sRAGE: the spliced variant (endogenous secretory RAGE [esRAGE]) and the enzymatically cleaved form arising from the shedding of the external domain of the receptor by matrix metalloproteinase 10.[3]

The administration of recombinant sRAGE in diabetic and nondiabetic animal models counteracted progressive atherosclerosis. Very high levels of sRAGE were observed in healthy centenarians (aged 100–104 years), while low levels have been reported in patients with components of metabolic syndrome, atherosclerosis, coronary artery diseases, Alzheimer's disease, and rheumatoid arthritis. Various pharmaceutical drugs augment the circulating sRAGE levels such as angiotensin-converting enzyme inhibitors in type 1 diabetics or cerivastatin in patients with hypercholesterolemia. It was found that serum sRAGE increases in patients with impaired renal function; however, it remains unclear whether such increase is caused just by reduced renal removal or is a protective reaction against toxic AGEs.[4]

Because the circulating sRAGE level depends mainly on the shedding of the cell surface RAGE and because RAGE activity is enhanced in CKD, we investigated the acute effect of cysteine protease, bromelain, in patients with impaired renal function. Previous research has shown that proteases – trypsin and bromelain – interfere with the ligand-RAGE interaction resulting in the lowering of oxidative stress in endothelial cells and suppression of genotoxicity in renal tubular cells. Therefore, we found it interesting to investigate the potential involvement of sRAGE.

We enrolled 11 Caucasian patients (10 men, 1 woman) aged 29 to 70 years (mean, 53.7 ±15.9 years) with CKD. The estimated glomerular filtration rate (eGFR) was calculated using the Modification of Diet in Renal Disease formula and ranged between 11.2 and 47.6 ml/min (mean, 31.9 ± 10 ml/min). The exclusion criteria were as follows: diabetes mellitus, pregnancy, symptoms of acute inflammation, and immunosuppressive treatment 6 months prior to enrolment. The basic characteristics of the patients as well as blood chemical data are summarized in the TABLE. Routine blood counts and the plasma electrolyte concentration were within the normal range. Blood lipid values showed a trend for lower high-density lipoprotein concentration (36.7 ±2.0 mg/dl) and elevated levels of triglycerides (214.3 ±150.0 mg/dl) while plasma low-density lipoprotein cholesterol concentration was normal (103.5 ±21.6 mg/dl). Concomitant medications included diuretics (11 patients), angiotensin converting enzyme inhibi-

TABLE Clinical and biochemical characteristics of patients before bromelain treatment

No.	Age	Sex	Cause of CKD	eGFR, ml/min	Serum creatinine, mg/dl	Urea, mg/dl	Uric acid, mg/dl	Fasting glucose, mg/dl
1	34	M	unknown	20.7	3.6	130	13	110
2	42	M	hypertension	39.1	2.0	45	4.2	84
3	79	M	unknown	30.8	2.2	73	9.3	94
4	43	M	APKD	34.9	2.2	52	6.5	88
5	59	M	APKD	36.5	2.0	83	ND	98
6	70	M	hypertension	40.4	1.78	19,3	6.8	84
7	58	F	IN	11.2	4.3	144	6.3	96
8	49	M	sclerodermia	26.8	2.7	64	7.8	106
9	71	M	nephrectomy	35.2	2.0	63	6.3	111
10	29	M	FSGS	27.5	2.9	80	8.9	84
11	57	M	hypertension	47.6	1.6	54	6.8	98
mean	**53.7**			**31.9**	**3.2**	**73.4**	**7.6**	**95.8**
SD	**15.9**			**10**	**1.3**	**36.2**	**2.4**	**10.1**

Abbreviations: APKD – adult polycystic kidney disease, CKD – chronic kidney disease, eGFR – estimated glomerular filtration rate, F – female, FSGS – focal segmental glomerulosclerosis, IN – interstitial nephritis, M – male, SD – standard deviation

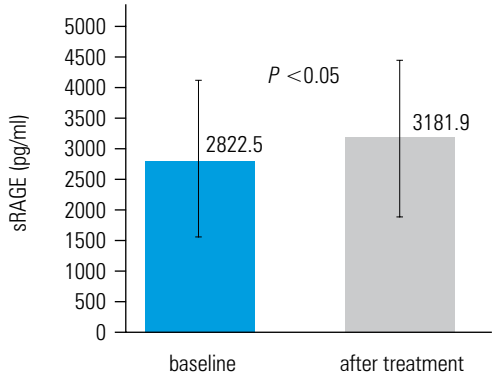

FIGURE 1 Serum soluble receptor for AGE (sRAGE) in 11 patients with chronic kidney disease before and after 3 days of bromelain treatment; data are presented as mean ± standard deviation

tors (10), allopurinol (8), β-receptor blockers (1), statins (6), and vitamin D_3 (5).

Bromelain (Wobenzym®, Mucos Pharma GmbH, Berlin, Germany) was administered orally at a daily dose of 1200 mg (3 times 400 mg) for 3 consecutive days. Serum AGE and sRAGE were evaluated before and 3 days after the administration. Venous blood was collected simultaneously with routine control examinations in the morning of day 0 and on day 4, after a fasting period of 10 to 12 hours after the last bromelain administration.

AGE-associated fluorescence of the serum samples was measured by fluorescence spectroscopy (activation/emission wave-lengths: λex 355 nm / λem 460 nm, Perkin-Elmer), as described previously. Although fluorescence is not specific, the similar fluorescence spectra of various synthetic AGEs (including pentosidine and vesperlysine) are generally used to evaluate circulating AGEs.[5]

We measured serum sRAGE with a sandwich enzyme-linked immunosorbent assay using standard kits (Quantikine, RD Systems, Minneapolis, United States), according to the manufacturer's protocol. In this assay, the plate is coated with a monoclonal antibody against RAGE (extracellular domain) and a polyclonal antibody is used for detection. Mean minimal detectable dose of RAGE is 4.12 pg/ml.

The results are expressed as mean ± standard deviation. The *t* test for paired samples was used for the evaluation of the differences within the group. The Pearson correlation coefficient was evaluated as a measure of the linear dependence between the variables. All results were considered as statistically significant at a *P*-value of less than 0.05.

All patients completed the study and no side effects of bromelain (Wobenzym®) were recorded. After 3 days of bromelain administration, the AGE-associated fluorescence of the plasma remained unchanged (54.05 ±20.26 and 53.69 ±20.09 arbitrary units, respectively). However, the concentration of sRAGE increased significantly after bromelain treatment (mean, 3181.9 ±1293 pg/ml) compared with the baseline values (mean, 2822.5 ±1288.3 pg/ml), $P = 0.015$ (FIGURE 1). Significant negative correlations between sRAGE and eGFR were observed before and after bromelain administration. Interestingly, after 3 days of bromelain treatment, the correlation between sRAGE and eGFR was even more pronounced than at baseline (FIGURE 2). eGFR was not affected by bromelain administration.

In this pilot study, we observed significantly increased sRAGE by bromelain in patients with CKD stages 3–4. The baseline circulating levels of sRAGE observed in our study are in line with the data of Kalousova et al.[4] who reported increased values in CKD patients compared with normal healthy subjects. The inverse relationship of sRAGE and eGFR suggests the key role of the kidney in the removal of sRAGE. Administration of bromelain for 3 days resulted in a small but

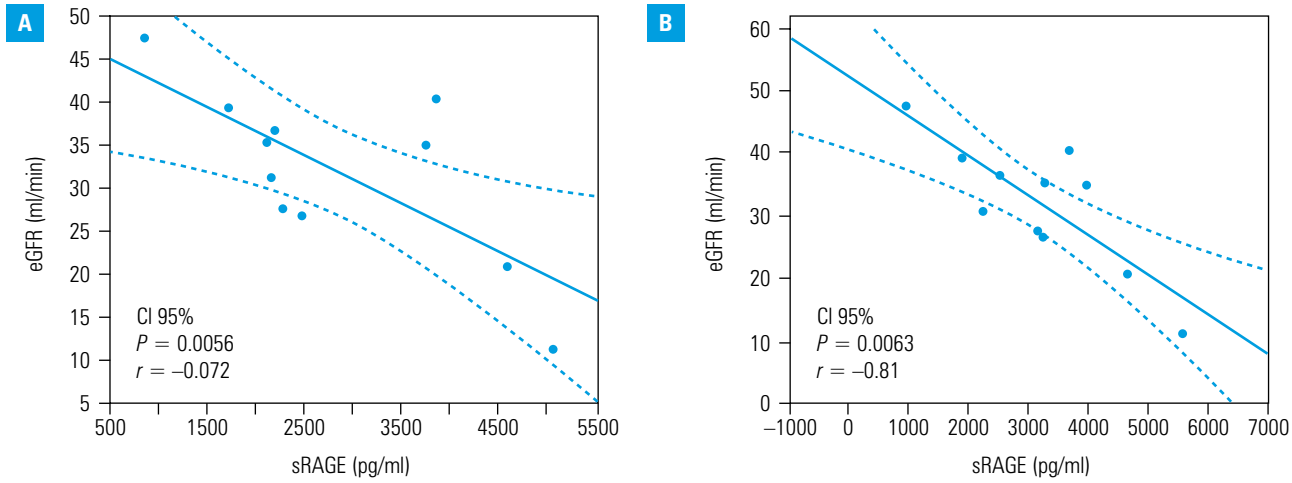

FIGURE 2 Correlations between serum soluble receptor for AGE (sRAGE) and estimated glomerular filtration rate (eGFR) before (**A**) and after 3 days (**B**) of bromelain therapy
Abbreviations: CI – confidence interval

significant rise of its serum concentration, while AGE-associated fluorescence remained unchanged. Since circulating AGEs are an important determinant of sRAGE levels we assumed that the observed rise was a consequence of an enhanced shedding of the cell surface RAGE induced by bromelain. However, we did not measure the secreted isoform of sRAGE, esRAGE, which has a much lower concentration.

The clinical relevance of the altered sRAGE level is a matter of discussion. Due to the large amount of circulating AGEs and non-AGE ligands in CKD, much higher levels of sRAGE than the observed ones are needed for an effective decoy. This critical aspect was considered recently by Bierhaus and Nawroth.[2] The final proof for the effectiveness of bromelain in patients with CKD can only be demonstrated if the RAGE-mediated activation of the nuclear factor κ-light-chain-enhancer of activated B cells is downregulated by the action of this protease. We assume that our previous positive experience with bromelain in experimental renal diseases (in combination with trypsin)[6] are a consequence of higher doses of bromelain, which may induce elevated levels of sRAGE. Moreover, sRAGE-independent mechanisms could be involved in the beneficial effects of bromelain.

In conclusion, our pilot study demonstrates that short-term administration of bromelain is safe and causes a small but significant rise in circulating sRAGE. Bromelain is an extract derived from the stems of pineapples. If its beneficial sRAGE-mediated effects can be confirmed in a larger number of CKD patients over a longer period, bromelain may be a useful treatment in this patient population.[7,8]

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