Sevelamer reduces endothelial inflammatory response to advanced glycation end products

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Abstract

Background: Advanced glycation end products (AGEs) have been related to the pathogenesis of cardiovascular diseases (CVD), chronic kidney disease (CKD) and diabetes mellitus. We sought to investigate the binding capacity of sevelamer to both AGEs and uremic serum in vitro and then test this pharmaceutical effect as a potential vascular anti-inflammatory strategy.

Methods: AGEs were prepared by albumin glycation and characterized by absorbance and electrophoresis. Human endothelial cells were incubated in culture media containing AGEs and uremic serum with or without sevelamer. Receptor for advanced glycation end product (RAGE) expression was evaluated through immunocytochemistry and western blot to explore the interactions between AGEs and the endothelium. Inflammatory and endothelial dysfunction biomarkers, such as interleukin 6 (IL-6) and IL-8, monocyte chemoattractant protein-1 (MCP-1), plasminogen activator inhibitor-1 (PAI-1) and serum amyloid A (SAA) were also measured in cell supernatant. The chemotactic property of the supernatant was evaluated.

Results: AGEs significantly induced the expression of RAGE, inflammatory and endothelial activation biomarkers [IL-6, (P < 0.005); IL-8, MCP-1, PAI-1 and SAA (P < 0.001)] and monocyte chemotaxis as compared with controls. In addition, AGEs increased the levels of inflammatory biomarkers, which were observed after 6h of endothelial cell incubation with uremic serum [IL-6 (P < 0.001) IL-8, MCP-1 and PAI-1 (P < 0.05)]. On the other hand, after 6h of endothelial cell treatment with sevelamer, RAGE expression (P < 0.05) and levels of inflammatory biomarkers [IL-6 and IL-8 (P < 0.001), MCP-1 (P < 0.01), PAI-1 and SAA (P < 0.005)] significantly decreased compared with the AGEs/uremic serum treatment alone.

Conclusions: Sevelamer decreased both endothelial expression of RAGE and endothelial dysfunction biomarkers, induced by AGEs, and uremic serum. Further studies are necessary for a better understanding of the potential protective role of sevelamer on uremic serum and AGEs-mediated endothelial dysfunction.

Key words: AGEs, chronic kidney disease, endothelial dysfunction, inflammation, sevelamer, uremic serum
Introduction

Advanced glycation end-products (AGEs) are a heterogeneous group of molecules such as sugars, lipids and nucleic acids, formed by nonenzymatic glycosylation reactions through a complex sequence of reactions referred to as the Maillard reactions [1]. The most prevalent AGE in vivo is carboxymethyl-lysine, better known as CML [2]. AGEs have a high renal clearance in the body and the main form of excretion is through urine [3]. They are considered medium-sized uremic toxins that accumulate in the bloodstream of patients with chronic kidney disease (CKD). Their origin is endogenous, occurring mainly when the body is exposed to high sugar levels, such as in diabetes [4].

The excessive intake of dietary AGEs has been linked to endothelial dysfunction, insulin resistance and the progression of atherosclerosis [5, 6]. Furthermore, recent studies have suggested that the increased intake of AGEs leads to their accumulation in the brain of diabetic and obese patients [7, 8] and thus it contributes to cognitive decline in these patients [9]. To exert their cellular effect, AGEs bind to cell surface receptors, such as AGE-R1 or, most importantly, to the receptor for advanced glycation end products (RAGE) [10, 11]. The RAGE-AGEs linkage stimulates several pro-inflammatory cytokines, including tumour necrosis factor alpha (TNF-α), interleukin (IL)-6 and IL-8, as well as profibrotic cytokines such as transforming growth factor beta (TGF-β) [12] through activation of the transcription factor nuclear factor kappa B (NF-κB), which in turn leads to gene expression and release of pro-inflammatory molecules as well as the release of reactive oxygen species (ROS) [13]. Furthermore, studies led by our group showed that endothelial cell exposure to a uremic environment increases monocyte chemotactant protein-1 (MCP-1), IL-8 and vascular adhesion molecule-1 (VCAM-1) expression, which suggests a relationship between vascular injury, systemic inflammation and uremic toxicity [14].

Sevelamer is a phosphate binder used in CKD patients to control hyperphosphatemia. In addition to its effects on phosphate levels, sevelamer seems to have pleiotropic effects that include a reduction of lipid levels and clearance of some uremic toxins in the bloodstream [15, 16]. Clinical studies enrolling CKD patients have shown that sevelamer may reduce serum levels of some low-molecular-weight uremic toxins [17]. We have previously demonstrated that sevelamer reduces systemic inflammatory response both in an animal model of CKD and in hemodialysis (HD) patients [18, 19]. Other studies have also demonstrated that sevelamer may decrease systemic inflammation in patients with CKD, as it reduces circulating levels of uremic toxins such as AGEs [20] as well as indoxyl sulfate, and p-cresol [17, 21]. Moreover, sevelamer can reduce the expression of adhesion molecules intercellular adhesion molecule-1 (ICAM-1) and VCAM-1 (markers of vascular injury) consequently it reduces circulating and cellular levels of AGEs, increases antioxidant defense, and decreases circulating levels of pro-oxidants molecules [22-24]. Taken together, these studies indicate that sevelamer may help remove uremic toxins via nondialytic process.

Thus, the present study aimed to investigate the potential protective endothelial effects of sevelamer through its binding capacity on other uremic toxins beyond phosphate, particularly AGEs. The potential chelation effect of sevelamer on uremic serum of patients with CKD was also investigated. Human endothelial cells and monocytes were used to mimic the early model of endothelial dysfunction. Hence we suggest a novel approach for the use of sevelamer, which could hypothetically contribute to the decrease in inflammatory cellular response and, consequently, avoid the development of CVD in AGEs-elevated pathologies such as CKD and diabetes.
amounts from donors’ total blood. The U-937 cells were cultured in RPMI 1640 (Gibco) supplemented with 10% FBS (Gibco), 100 U/mL of penicillin and 50 mg/mL of streptomycin (Gibco). As the cells grew in suspension, a ratio of 10^5 monocytes/mL was maintained in the culture medium for 3 days. The cells were kept in culture flasks and incubated at 37°C in a 5% CO2 atmosphere.

Synthesis and characterization of AGEs
AGEs and bovine serum albumin (BSA) (Sigma-Aldrich, St Louis, MO, USA) were prepared as previously described [25]. In short, AGEs and BSA were characterized by spectrophotometry (Tecan, Männedorf, Switzerland) by comparing the absorbance at 330, 360, 400 and 420 nm, as previously described [26, 27]. For cell treatment, AGES and BSA were applied to the culture media at 0.2 mg/mL. AGEs and BSA were also characterized by basic protein polyacrylamide gel electrophoresis (PAGE). Then, 15 µg of the sample was electrophoresed in 10% resolving gel at 100 V for 4 h at room temperature with inverted poles and the bands were visualized after Coomassie blue staining.

Endotoxin assay
To exclude endotoxin contamination of AGEs and BSA, a limulus amebocyte lysate (LAL) assay test (Thermo Fisher Scientific, Rockford, IL, USA) was performed according to the manufacturer’s recommendations. The absorbance was measured at 410 nm (Tecan) and all measurements were taken in duplicate.

Cell viability assay
Cell viability was assessed using the 3-(4,5-dimethyl-thiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) (Sigma-Aldrich) assay according to Mosmann [28]. The endothelial cells were plated in 96-wells culture plates at a density of 10^4 cells/well. After 24 h of incubation, the medium was replaced and cells were treated with BSA and AGEs with or without 3% sevelamer for 24 h. All analyses were performed in triplicate.

Treatment of endothelial cells with AGEs, uremic pool and sevelamer
Endothelial cells were cultured at 10^5 cells/well in 96-well plates as described and incubated at 37°C in a 5% CO2 atmosphere overnight. For cell treatment, AGEs and BSA were diluted at a concentration of 0.2 mg/mL in DMEM with or without 3% sevelamer. To prepare the uremic medium, serum from patients (1 ml) was mixed to form a single uremic pool. Subsequently the endothelial cells were incubated with uremic medium (10% uremic pool) with or without 3% sevelamer. Finally, the endothelial cell supernatants were recovered at 0 and 6 h by simple aspiration of the contents of the wells and stored at −20°C for subsequent quantification of IL-6, IL-8, MCP-1, plasminogen activator inhibitor-1 (PAI-1) and serum amyloid A (SAA) as well as for the chemotaxis assay. Three experiments were performed in triplicate for all cell culture models used.

Nuclear magnetic resonance
Spectra were obtained with a 1D^{31}P nuclear magnetic resonance (NMR) spectrometer (Avance III; Bruker, Billerica, MA, USA) operating at 14.1 Tesla (600 MHz for 1H and 242 MHz for ^{31}P) equipped with a reverse pentaneurcular observation probe of 5 mm field gradient z (QX1) axis. We evaluated the phosphate concentration in samples containing phosphate (4 mM), with or without 3% sevelamer, to validate in vitro the chelating effect of sevelamer to phosphate. The samples were solubilized in 600 µL of a solution of D2O·H2O (1:9 v/v) at 30°C. The ^{31}P decoupled spectra were obtained with a 30° angle, with a magnetization window of 400 ppm and an acquisition time of 0.17 s. The relaxation time of 0.200 and 1024 transients was processed with the help of the TopSpin program (Bruker, Karlsruhe, Germany) applying exponential multiplication on the acquired free induction decays (FIDs). All analyses were performed in triplicate.

RAGE immunostaining
RAGE expression in cells treated with AGEs was as previously described by Rempel et al. [27]. The images were captured using an AxioImager Z2 light microscope (Carl Zeiss, Jena, Germany) equipped with an automated scanner slide (Metasystems, Altussheim, Germany). All analyses were performed in triplicate.

Western blot analysis
Growth-arrested cells cultured in 100-mm dishes were stimulated for 24 h with BSA and AGEs at 0.2 mg/mL, with or without 3% sevelamer, as previously described [27]. RAGE antibody (1:1000; Santa Cruz Biotechnology, Dallas, TX, USA) and horse-radish peroxidase-conjugated sheep anti-rabbit IgG polyclonal antibody (1:10000) (Sigma-Aldrich) were used as primary and secondary antibodies, respectively. The reaction was visualized by enhanced chemiluminescence western blotting reagents (GE Health Care Life Sciences, Little Chalfont, UK). Band intensity was analyzed using Image Studio Lite 5.0 (LI-COR Biotechnology, Lincoln, NE, USA). All analyses were performed in triplicate.

Biomarker assays (IL-6, IL-8, MCP-1, PAI-1 and SAA)
Endothelial supernatant levels of IL-6, IL-8, MCP-1, PAI-1 and SAA were measured by the Luminox methodology in combination with the xPONENT 3.1 software package (Luminox, Austin, TX, USA). The MAGPIX system (Darmstadt, Germany) with 14 markers was used following the manufacturer’s instructions (adipokine human magnetic 14-plex panel for Luminox platform). Results were expressed through a standard curve (in pg/mL). All samples were analyzed at the same time under standardized experimental conditions and measurements were performed in triplicate.

Monocyte migration assay
Cell migration assays were performed using modified Boyden chambers with a 6.5-mm diameter, 10-µm thickness, porous (8.0 µm) polystyrene membrane separating the two chambers (GE Healthcare Bio-Sciences, Westborough, MA, USA). Briefly, the endothelial cells were treated with AGEs, with or without 3% sevelamer, and the cell supernatant was added to the lower compartment of the chamber to evaluate the chemotactic effect of this supernatant on the monocytes. Subsequently, 10^5 U-937 cells/mL were added to the top of the chamber over the membrane. The cells were incubated at 37°C and 5% CO2 for 90 min. The chemotactic ability of the supernatant (migration) was measured by counting the number of cells that were in the lower chamber compartment with a Neubauer chamber.

Data analysis
Statistical analyses were performed using the statistical packages JMP (version 8.0; SAS Institute, Cary, NC) and SigmaStat (version 3.5; Systat Software, Erkrath, Germany). The determination of significant differences was performed by either Student’s t-test or analysis of variance (ANOVA) for parametric data and Mann–Whitney and ANOVA on ranks for nonparametric data. The values were expressed as mean ± standard error of
the mean (SEM) of three or five independent experiments. P < 0.05 was considered statistically significant.

Results
The main clinical and laboratory characteristics of the 26 patients enrolled in the study are described in Tables 1 and 2, respectively. The mean age was 52 ± 2 years and 38% were male. Hypertensive nephrosclerosis was the main cause of CKD and all patients in the sample were hypertensive. Only 11% of the patients had diabetes mellitus as an associated disease. The patients were treated with statins, aspirin and antihypertensive drugs in 30%, 43% and 100% of cases, respectively. The median values found for total calcium, PTH, Kt/V, albumin and total cholesterol were within the reference range for patients in stage 5 CKD.

AGEs characterization
To verify AGE formation after albumin glycation, AGEs were characterized by ultraviolet (UV) absorption (Figure 1A), as previously described elsewhere [26, 27]. Figure 1B shows the electrophoretic migration of AGES by PAGE.

AGEs endotoxin levels
An LAL assay (Thermo Fisher Scientific) was performed to exclude the possibility that AGE and BSA were contaminated by endotoxins. The levels of endotoxins in BSA and AGES were 1.65 and 1.04 EU/mL, respectively, which are not considered relevant.

Cell viability assay
Endothelial cell viability was assessed by MTT. Endothelial cells were cultured with BSA and AGES at 0.2 mg/mL, with or without 3% sevelamer. Neither AGES nor sevelamer had a significant effect on cell viability (Figure 2).

NMR
To verify if the binding capacity of sevelamer was preserved in vitro, the phosphate concentration on media samples containing phosphate (4 mM) was evaluated with and without sevelamer (3%). A reduction in phosphate concentration in samples treated with sevelamer was noted, confirming that its in vitro chelating action was preserved (Figure 3).

Effect of AGES on RAGE immunostaining and protein expression
Endothelial cells were treated with AGES and specifically stained to detect this receptor. As shown in Figure 4, positive staining was visualized in AGE-treated cells (Figure 4D). Endothelial cells treated with either media alone (Figure 4A) or BSA alone (Figure 4C) were used as negative controls (Figure 4B). The staining was RAGE specific because the secondary antibody using phosphate (4 mM) was evaluated with and without sevelamer (3%). A reduction in phosphate concentration in samples treated with sevelamer was noted, confirming that its in vitro chelating action was preserved (Figure 3).

Effect of AGES, uremic serum and sevelamer on biomarkers expression
IL-6, IL-8, MCP-1, PAI-1 and SAA expression increased significantly in a time-dependent manner when the endothelial cells were exposed to AGES (Figure 5). However, when the cells were exposed to the uremic serum pool for 0 and 6 h, only SAA did not have a significant increase (Figure 6). In contrast, a significant time-dependent decrease in the expression of these biomarkers was observed in cells incubated with AGES or the uremic serum pool in the presence of sevelamer. Nonetheless, sevelamer was not effective in reducing PAI-1 levels after uremic serum treatment.

Monocyte migration assay
Figure 7 illustrates the effect of BSA and AGES, with or without sevelamer, on monocyte migration. Media alone (DMEM) was used as a negative control to evaluate the spontaneous movement of monocytes. MCP-1, IL-8 and MCP-1 with IL-8, known chemoattractant molecules, were used as positive controls at 50 ng/mL. After 90 min of incubation, a significant increase in

Table 1. Main clinical characteristics of the study population

| Parameters                            | Value       |
|---------------------------------------|-------------|
| Number of patients                    | 26          |
| Age (years)                           | 52 ± 2      |
| Gender (% men)                        | 38          |
| Ethnicity (% Caucasian)               | 81          |
| Comorbidities (%)                     |             |
| Diabetes mellitus                     | 11          |
| Cardiovascular diseases               | 15          |
| Hypertension                          | 100         |
| Etiology of CKD (%)                   |             |
| Hypertensive nephrosclerosis          | 30          |
| Diabetic nephropathy                  | 11          |
| Chronic glomerulonephritis            | 50          |
| Other                                 | 9           |
| Vitamin D (% used)                    | 45          |
| Statins (% used)                      | 30          |
| Aspirin (% used)                      | 43          |
| Antihypertensive agents (% used)      | 100         |
| Time on dialysis (months)             | 17 ± 3      |

Values expressed as mean ± SD.

Table 2. Main laboratory characteristics of the study population

| Parameters                | Value       |
|---------------------------|-------------|
| Cholesterol (mg/dL)       | 180 (108–248) |
| Low-density lipoprotein cholesterol (mg/dL) | 106 (42–176) |
| High-density lipoprotein cholesterol (mg/dL) | 44 (21–80) |
| Triglycerides (mg/dL)     | 150 (73–240) |
| Hemoglobin (g/dL)         | 11.5 (10.8–12.0) |
| Albumin (g/dL)            | 3.9 (3.3–4.7) |
| Calcium (mg/dL)           | 9.0 (7.6–10.3) |
| Phosphatase (mg/dL)       | 6.7 (4.1–9.6) |
| PTH (pg/ml)               | 446 (11–1666) |
| Alkaline phosphatase (UI/L) | 149 (65–602) |
| Kt/V                      | 1.5 (1.1–1.8) |
| CRP (mg/ml)               | 4.9 ± 4.8   |
| IL-6 (pg/ml)              | 6.7 ± 8.1   |
| IL-8 (pg/ml)              | 128.2 ± 206.2 |
| SDF-1 (pg/ml)             | 2625.9 ± 1288.6 |

Values expressed as mean ± SEM or median (25th-75th percentiles). SDF-1, stromal cell-derived factor-1.
Cell migration was noted in the media enriched with MCP-1 (254.2 ± 46.8%; P < 0.05) and in the supernatant of the endothelial cells treated with AGEs (402.3 ± 163.8%; P < 0.01) compared with the negative controls (DMEM alone, taken as 100%). Conversely, the sevelamer-enriched supernatant led to a significant decrease in cell migration induced by AGE-enriched media (402.3 ± 163.8 versus 33.3 ± 7.4%; P < 0.005) and BSA-enriched media (277.7 ± 70.87 versus 62.5 ± 4.1%; P < 0.05).

Discussion

Vascular inflammation plays a crucial role in the development of CVD complications and comorbidities in CKD [29, 30]. AGEs are a heterogeneous group of compounds responsible for the activation of inflammatory responses, causing deleterious effects on vascular tissue [31]. Moreover, AGEs may accumulate in arteries, thus increasing the risk of endothelial dysfunction and atherosclerosis progression [5, 32].

Sevelamer is a phosphate binder with potential pleiotropic effects used in CKD patients for hyperphosphatemia treatment [33]. In addition, sevelamer may decrease the circulating levels of uremic toxins [24], which reduces the possibility of uremic-related cardiovascular complications such as atherosclerosis [34]. In this context, Nikolov et al. [35] demonstrated that sevelamer combined with lanthanum carbonate leads to a decrease in serum phosphate, vascular calcification and atherosclerosis in mice with CKD.

The administration of sevelamer for 3 weeks reduced the circulating levels of CML, methylglyoxal (MG), phosphatemia and lipid levels in diabetic HD patients, which resulted in a reduction in inflammatory biomarkers and oxidative stress [36]. Interestingly, some studies on HD and pre-dialysis patients and recent meta-analysis studies have suggested that sevelamer use was associated with lower all-cause mortality compared with calcium-based binders [37–39], suggesting a potential advantage of the former binder over the latter.

To the best of our knowledge, this is the first in vitro demonstration using endothelial cells in which sevelamer reduced systemic and vascular inflammation. The reduction of inflammation was evaluated through the decreased expression of RAGE, inflammatory biomarkers (IL-6, IL-8, MCP-1 PAI-1 and SAA) and monocyte migration induced by AGES. Taken together, these findings suggest a possible role of sevelamer in attenuating inflammatory pathways stimulated by uremic serum and AGES. Exposure of the endothelium to uremic toxins leads to a series of changes in vascular homeostasis and its functions [39]. We demonstrated previously—using this same model—that...
uremic plasma activates inflammatory pathways by inducing the production of chemokines and adhesion molecules, usually present in early events of atherosclerosis in CKD patients [14, 40]. We also observed, using endothelial cells and monocytes treated with AGEs, that both cells were activated by AGEs via RAGE through protein kinase C beta pathway, producing MCP-1 and VCAM-1 [27]. In addition, AGEs impaired the production of nitric oxide (NO), in part by NO synthase downregulation by increasing both mRNA degradation [41] and nicotinamide adenine dinucleotide phosphate oxidase expression, which suggests a link between AGEs and endothelial dysfunction [42]. Thus there is growing interest among the scientific community in developing ways to minimize the deleterious effects of uremic toxins, such as AGEs.

The involvement of RAGE on endothelial cell activation after AGE exposure was previously demonstrated [13, 27]. In contrast, we found a significant decrease in RAGE expression in the presence of sevelamer, which indirectly suggests that this chelator can act by binding in vitro to AGEs, therefore reducing the expression of RAGE. This finding was also observed in a study by Yubero-Serrano et al. [24] in patients with type 2 diabetes mellitus in which sevelamer reduced the circulating levels of AGEs. Other studies have also demonstrated the pleiotropic binding capacity of sevelamer in the reduction of circulatory levels of p-cresol and AGEs [18, 20].

Our study demonstrated that the longer the exposure of endothelial cells to both AGEs and uremic serum, the more systemic (IL-6 and SAA) and vascular (IL-8, MCP-1 and PAI-1)
Biomarkers are expressed. These results further confirm the link between vascular activation, systemic inflammation and uremic toxicity [2, 14]. Recent studies have implicated a causal role of SAA as a pro-inflammatory and pro-thrombotic mediator in the pathogenesis of atherosclerosis [43]. Moreover, others have shown that SAA is a potent pro-atherogenic chemokine on the endothelium, inducing the expression of cytokines and adhesion molecules such as IL-6, IL-8, MCP-1, ICAM-1, VCAM-1 and tissue factor [40, 44, 45]. Furthermore, AGEs increase the endothelial cell levels of mRNA coding for PAI-1 mediated by RAGE [46]. Our results demonstrated that sevelamer led to a significant decrease in the expression of IL-6, IL-8, MCP-1, and SAA, molecules related to systemic and vascular inflammation and induced by AGEs and uremic serum, suggesting a

Fig. 5. The effect of AGEs on systemic inflammatory biomarker production (IL-6 and SAA) and vascular inflammatory biomarker (IL-8, MCP-1 and PAI-1) production by endothelial cells. Endothelial cells were incubated with AGEs or BSA (0.2 mg/ml), with or without sevelamer (Sev.) (3%), for 0 and 6 h. Data are expressed as mean ± SEM of three independent experiments. (A) IL-6: "****P < 0.001, AGEs 0 h versus AGEs 6 h; "***P < 0.001, AGEs 6 h versus AGEs + Sev. 6 h. (B) SAA: "**P < 0.01, BSA 0 h versus BSA 6 h; "***P < 0.001, AGEs 6 h versus AGEs + Sev. 6 h; "****P < 0.001, AGEs 0 h versus AGEs + Sev. 6 h. (C) IL-8: "P < 0.05, control 0 versus control 6; "***P < 0.001, AGEs 0 h versus AGEs + Sev. 6 h; "****P < 0.001, AGEs 6 h versus AGEs + Sev. 6 h. (D) MCP-1: "P < 0.05, BSA 6 versus BSA + Sev. 6 h; "**P < 0.01, AGEs 6 h versus AGEs + Sev. 6 h; "***P < 0.001, control 6 h versus control 0 h, BSA 0 h versus BSA 6 h. (E) PAI: "***P < 0.001, AGEs 6 h versus AGEs + Sev. 6 h; "****P < 0.001, control 0 h versus control 6 h, BSA 0 h versus BSA 6 h, AGEs 0 h versus AGEs 6 h. **P < 0.01, AGEs 6 h versus control 0 h.

Sevelamer reduces endothelial inflammatory response to AGEs
potential protective role of sevelamer on endothelium dysfunction beyond its classic effect via phosphate binding. On the other hand, sevelamer was not effective in reducing PAI-1 levels after uremic serum treatment, suggesting that the high serum levels of PAI-1 found in CKD patients [47] may be due to activation of other pathways different from the ones activated by phosphate and AGEs.

Finally, the cell migration assay was performed to mimic the early events of atherosclerosis development. Our findings reveal that the binding capacity of sevelamer to AGEs reduced monocyte migration, suggesting a possible early effect of sevelamer in preventing monocyte recruitment and attachment to the endothelium (thus all the activation of pathways) and finally plaque formation. Taken together, these findings suggest that sevelamer has a protective effect by binding nonspecifically to uremic serum and AGEs and therefore reduces the expression of inflammatory biomarkers present in the atherosclerosis process.

One of the limitations of our project is that it was not possible to measure the AGE levels in vitro by the NMR technique because it lacks the sensitivity and specificity to detect which specific molecule of the heterogeneous group of AGEs was bound to sevelamer. To overcome this limitation, the measurements of RAGE and inflammatory biomarker expression were used to indirectly assess sevelamer’s action on reducing inflammation mediated by AGEs. Additionally, even though U-937 cells are commonly used as a model of monocytes, it would also be interesting to investigate the effect of AGEs and sevelamer in other cell lines, such as THP-1, or in monocytes extracted from the peripheral blood of CKD patients.

**Conclusion**

In conclusion, this study demonstrates, in vitro, that the anti-inflammatory effects of sevelamer on endothelial cells and monocytes may be due not only to its classic effect on phosphate, but also to its binding capacity to AGEs and possibly other uremic toxins. Sevelamer might be considered a therapeutic option to reduce the high AGE levels observed in pathological conditions such as CKD and perhaps diabetes, regardless of the presence of hyperphosphatemia. In the future, clinical studies should be designed to validate the potential capacity of sevelamer to decrease systemic AGEs and uremic serum concentrations. Thus it is hypothesized that this clinical treatment could provide a beneficial effect on vascular function, yielding relevant clinical outcomes.

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**Conflict of interest statement**

None declared.

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