Control of oral advanced glycation endproducts (AGEs) by Sevelamer carbonate improves glucose metabolism and albuminuria in stage 2-4 diabetic kidney disease (DKD)

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Background

Reducing the amount of advanced glycation endproducts (AGEs) in the diet of experimental animals reduces inflammation, oxidative stress (Infl/OS), albumin excretion rate (AER), and diabetic kidney disease (DKD). A preliminary study with diabetes and DKD showed that Sevelamer Carbonate (SC) treatment reduces Infl/OS, AGEs and HbA1c(1). Therefore, the current study proposed to validate these findings and determine if Sevelamer reduces levels of inflammation and oxidative stress markers and benefits kidney function.

Methods

Adult patients treated for type 2 diabetes with at least one diabetes medication, HbA1c>6.5%, with albuminuria (>200 mg urinary albumin/gram creatinine) on a spot urine, and stage 2-4 DKD were recruited from the Mount Sinai Medical Center and Mount Sinai Beth Israel. Exclusion criteria included hypophosphatemia, hyperphosphatemia, hypercalcemia, biopsy proven renal disease other than DKD, gastrointestinal disorders or significant gastrointestinal surgery, and concomitant inflammatory diseases. Of 865 clinic records screened, 146 subjects met the inclusion criteria, 120 were invited to participate, and 117 agreed to participate and were randomized, the number calculated to give an 80% chance of finding a 20% change in AGEs at study end with 5% type I error, based on the previous cross-over trial results (1). Of those randomized, 91 completed the 6 month study (Hurricane Sandy reduced the number finishing the trial by interrupting all communication with 12 subjects in mid-study). Fasting blood samples, anthropometric parameters, 3-day food records, and the first morning (spot) urine were obtained at randomization, 3 and 6 months of study. In addition, blood samples were obtained at 1 and 2 weeks after randomization to assess serum calcium and phosphate levels as a safety measure.

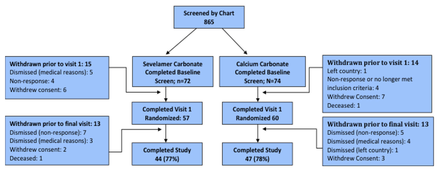
The study (NCAT01493050) was approved by the Mount Sinai and Beth Israel Medical Center Institutional Review Boards and all patients signed informed consent forms, consistent with the Declaration of Helsinki.

Study Design

This was a two center, randomized, single-blinded, ITT study. Medical care was not altered by the study team. Most subjects were receiving insulin, oral anti-diabetic drugs, metformin (Met) (~50%), statins, and aspirin. All subjects received one or more antihypertensive drug (including angiotensin converting enzyme inhibitors or angiotensin receptor blockers). Daily multivitamin supplements, containing 400 IU cholecalciferol (Natures Bounty Inc., Bohemia, NY), were provided as a safety measure, and to guard against Vitamin D deficiency, because of the possibility that it could influence albuminuria (2). Subjects were randomized to receive either SC (1600 mg t.i.d. with meals) or calcium carbonate (CC) (1200 mg t.i.d. with meals).

Compliance was checked by pill count on a weekly basis by telephone. Compliance was further quantitatively defined in the SC arm by a LDL reduction of ≥10% (3), by urine phosphate levels in both treatment groups, and by assessing HbA1c changes from baseline to 6 months. In addition, a study research coordinator contacted the study subjects on at least a weekly basis to encourage compliance.

Methods



Dietary Intake

A study dietitian obtained a detailed dietary assessment from all subjects at baseline, 3 and 6 months. The dietitian also placed weekly telephone calls to ensure constant intake of dietary intake of calcium, inorganic phosphates and nutrients. The content of nutrients, minerals and AGE intake was estimated from food records using a nutrient software program (Food Processor version 10.1; ESHA Research, Salem, Oregon) and a food-AGE database (4). AGEs intake was expressed as equivalents (Eq), where 1 Eq=1X106 units (4).

Routine blood tests

The Mount Sinai Medical Center laboratory performed clinical blood and urine measurements for all participants. GFR was estimated using the Cystatin C-Creatinine CKD-Epi-Cys-Cr formula and presented as eGFR (5, 6).

Measurement of AGEs and circulating biomarkers.

Serum, urine, and intracellular N-carboxymethyl-lysine (CML) and methylglyoxal (MG) derivatives were quantified by ELISA using two non-cross-reactive monoclonal antibodies that recognize lipid and protein AGEs, but not free AGEs. Leptin and adiponectin were tested in duplicate by commercial ELISA kits. Cystatin C and FGF-23 were measured in duplicate by the Mount Sinai Medical Center Clinical Laboratories and random samples were retested to ensure reproducibility. All analyses were performed on blinded samples.

Quantitative RT-PCR assay

Freshly collected peripheral blood mononuclear cells (PMNC) were separated by Ficoll-Hypaque Plus gradient (American Biosciences, Uppsala, Sweden). Proteins were extracted from cell lysates. Total RNA was extracted using Trizol (Molecular Probes, Inc). The extracted RNA OD280/260 ratio was 1.8-2.0. Total RNA was reverse-transcribed using Superscript III RT (Invitrogen). Nrf2, AGER1, SIRT1, TNFR1, and RAGE mRNA expression was analyzed by quantitative SYBR Green real-time PCR.

Western analysis

As described (1) Cell lysates were prepared by sonication in 500ml lysis buffer (New England Biolabs), cell proteins were separated on 8% SDS-PAGE gels and transferred onto nitrocellulose membranes and visualized by chemiluminescence (Roche). Bound immune complexes in RIPA lysis buffer were used for immunoblotting after SDS-PAGE and NT transfer.

Statistical methods

Extreme outliers (two values [one for SIRT1, and one for ACR], which exceeded more than four times the next largest value) were deleted. Data from baseline variables that were approximately symmetric in distribution were summarized using their mean and standard standard deviation; highly skewed variables were summarized using their three quartiles; and binary variables were summarized by percentages. Spearman rank correlations, adjusted for age, were used to summarize relationships between study variables. Deltas were defined, for each study variable, as the differences between values at end of study (6 months) and values at baseline. The differences in deltas between the treatments (SC and CC) were the primary endpoints in the study; these were tested using Student's t-tests. Pre-specified secondary analyses were sub-group analyses by age (split at the median: 65 years) sex, race (Caucasian vs. non-Caucasian), and use of metformin at baseline. Sub-group by treatment interactions were tested using general linear models with treatment, sub-group and their interaction as explanatory variables. Only variables for which there was evidence of an interaction between sub-group and treatment were reported by level of sub-group.

The pre-specified analysis plan was to analyze according to a modified intention-to-treat protocol that only retained subjects with some study data at both baseline and the end of the study. Lack of compliance to treatment and cross-overs were not allowed in the analyses. No correction was to be made for multiple significance testing; significance was to be determined by a p value below 0.05 in all cases. Analyses were performed with SAS version 9.3 and Stata version 12.

Table 1. Baseline Summary Statistics: Mean (standard deviation) Unless Stated

	All study subjects (n = 91)		Sevelamer carbonate (n = 44)		Calcium carbonate (n = 47)	
Sex, % men	58		55		62	
Race, % white	46		55		38	
Metformin use, % yes	49		50		49	
Age (years)	64.0	(9.9)	64.5	(10.7)	63.5	(9.2)
Systolic blood pressure (mmHg)	137	(17)	138	(17)	137	(17)
Diastolic blood pressure (mmHg)	76.5	(10.7)	74.4	(9.6)	78.4	(11.5)
Total cholesterol (mg/dl)	168	(52)	175	(53)	161	(51)
Triglycerides (mg/dl)*	128	(94, 181)	131	(94, 208)	126	(90, 179)
HDL cholesterol (mg/dl)	49.9	(16.3)	53.6	(17.8)	46.7	(14.2)
LDL cholesterol (mg/dl)	88.0	(43.5)	89.4	(40.0)	86.9	(47.0)
Waist/hip circumference	0.976	(0.071)	0.979	(0.068)	0.974	(0.074)
BMI (kg/m^2)	33.8	(7.6)	34.0	(7.4)	33.6	(7.8)
HbA1c (%)	8.59	(1.84)	8.97	(1.69)	8.23	(1.93)
Glucose (mg/dl)	157	(72)	169	(79)	145	(64)
Cystatin C (mg/dl)	1.55	(0.50)	1.51	(0.47)	1.59	(0.53)
eGFR (ml/min/1.73m^2)	49.1	(18.8)	51.2	(21.2)	47.1	(16.4)
AER (µg/mg)*	224	(48, 1003)	409	(66, 1222)	138	(45, 799)
FGF-23 (pg/dl)*	13.5	(8.2, 22.5)	13.4	(7.9, 21.0)	13.5	(8.9, 23.0)
Adiponectin (µg/ml)	9.31	(6.65)	9.54	(7.1)	9.09	(6.28)
Leptin (ng/ml)*	10.4	(3.7, 17.6)	10.1	(3.7, 16.8)	10.4	(3.7, 20.9)
Nrf2 (mRNA)*	0.15	(0.06, 0.34)	0.135	(0.04, 0.33)	0.17	(0.08, 0.36)

Table 2. Changes (final minus baseline) by Intervention Group

	Sevelamer carbonate Mean (standard error)		Calcium carbonate Mean (standard error)		P value
HbA1c (%)	-0.09	(0.26)	0.13	(0.17)	0.48
Glucose (mg/dl)	12.68	(17.57)	0.32	(10.15)	0.54
Cystatin C (mg/L)	0.20	(0.07)	0.19	(0.10)	0.90
eGFR (ml/min/1.73m^2)	-3.6	(13.1)	-1.7	(12.2)	0.50
ACR (mg/mg)	-128.7	(78.9)	95.6	(67.6)	0.03
FGF 23 (mg/ml)	-2.72	(1.87)	7.94	(6.31)	0.12
Adiponectin (mg/ml)	2.23	(0.72)	-0.09	(0.55)	0.01
Leptin (ng/dl)	-1.18	(1.61)	0.90	(1.47)	0.34
Nrf2 (mRNA)	0.36	(0.13)	-0.17	(0.10)	0.002
Sirt1 (mRNA)	0.12	(0.03)	-0.02	(0.02)	0.0004
AGER1 (mRNA)	0.16	(0.04)	-0.10	(0.07)	0.002
ER-a (mRNA)	0.24	(0.16)	-0.19	(0.10)	0.03
Serum CML (U/ml)	-8.74	(1.03)	-2.27	(1.74)	0.002
Serum MG (nmol/ml)	-0.93	(0.12)	-0.03	(0.14)	<0.0001
iCML (U/mg protein)	-2.33	(0.40)	-0.87	(0.48)	0.02
iMG (nmol/mg protein)	-0.11	(0.03)	-0.02	(0.04)	0.07
RAGE (mRNA)	-0.08	(0.03)	0.15	(0.05)	0.0004
TNFR1 (mRNA)	-0.27	(0.20)	0.09	(0.03)	0.08
8-isoprostanes (pg/ml)	-17.3	(12.1)	29.8	(13.8)	0.01
VCAM-1 (ng/ml)	-72.8	(53.8)	42.5	(51.7)	0.13

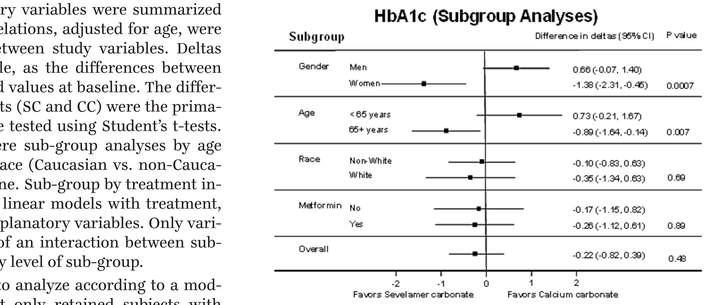


Figure 1: HbA1c levels reduced by Sevelamer Carbonate (SC) are also influenced by sex and age. Forest plot of the overall and sub-group analyses of data from subjects with T2DM and diabetic kidney disease (DKD) treated with SC or calcium carbonate (CC) for 6 months. Data is shown as the change (delta) (M±SEM) from baseline (visit 6 compared to visit 1). Significant changes after 6 months on SC compared to CC were found in women (p=0.007) and participants older than 65 y.o. (p=0.013). Overall and sub-group analysis data based on race and co-treatment with other anti-AGE agents (Metformin) revealed non-significant changes (absolute values are shown in Table 2)

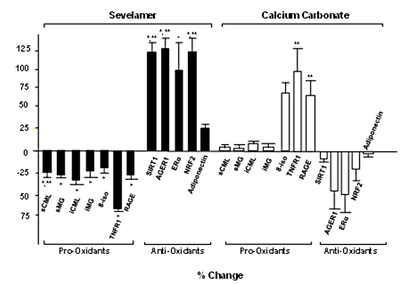


Figure 2: Sevelamer Carbonate (SC) reduces circulating and intracellular AGEs and pro-oxidant and inflammatory factors and improves anti-oxidant/anti-inflammatory factors in T2DM with DKD. After 6 months on SC (closed bars) or CC (open bars), changes are shown as percentage from baseline (mean ± SEM). Levels of circulating AGEs (sCML, sMG) are assessed in fasting sera; 8-isoprostanes and adiponectin in fasting plasma; intracellular CML (iCML) and MG (iMG) levels in PMC lysates; TNFR1, SIRT1, AGER1, RAGE, ERα, NRF2 mRNA levels by RT-PCR; *p denotes statistical significance in % changes between SC and CC groups; **p denotes significance between baseline and end within each group (absolute values are shown in Table 2).

Table 3. Overall ITT analysis

	Baseline	Visit 6	Delta	p value*	Baseline	Visit 6	Delta	p value*	p values
HbA1c (%)	8.9±1.7	8.8±2.2	-0.11±1.69	0.965	8.2±1.2	8.4±2.1	0.15±1.16	0.740	0.375
Glucose (mg/dl)	168±78	182±124	13.2±115.3	0.332	145±63	145±67	-0.54±70.12	0.991	0.490
Cystatin C (mg/L)	1.5±0.4	1.7±0.2	0.28±0.68	0.470	1.6±0.5	1.7±0.8	0.11±0.49	0.326	0.183
CKD-Epi-CystC (ml/min/1.73m^2)	52±20	48±22	-3.6±13.1	0.639	48±16	46±20	-1.7±12.2	0.660	0.495
AER (ng/mg)	764±554	635±342	-128±217	0.830	506±342	601±279	95±453	0.709	0.035
FGF 23 (pg/ml)	19.9±19.1	16.4±12.2	3.8±45.1	0.160	19.4±18.9	20.7±45.4	1.3±12.1	0.320	0.716
Adiponectin (pg/ml)	9.5±7.1	11.7±7.6	2.3±4.8	0.242	9.1±6.3	8.8±5.2	-0.27±3.62	0.653	0.004
Leptin (ng/dl)	12.5±11.1	11.3±9.5	-1.2±10.5	0.241	15.1±15.1	16.2±16.9	1.1±10.2	0.911	0.293
Nrf2 (mRNA)	0.28±0.43	0.64±0.34	0.36±0.85	0.024	0.39±0.81	0.23±0.26	-0.17±0.69	0.125	0.001
Sirt1 (mRNA)	0.10±0.15	0.22±0.12	0.12±0.21	0.037	0.14±0.15	0.12±0.12	-0.02±0.12	0.314	0.133
AGER1 (mRNA)	0.12±0.23	0.28±0.41	0.16±0.27	0.015	0.24±0.52	0.14±0.13	-0.10±0.47	0.097	0.002
ER-α (mRNA)	0.25±0.58	0.50±0.67	0.24±0.71	0.120	0.34±0.84	0.15±0.36	-0.19±0.70	0.126	0.023
Serum CML (U/ml)	35.9±5.5	27.1±5.8	-8.7±6.8	0.679	36.1±10.6	33.8±6.9	-2.3±11.9	0.312	0.002
Serum MG (nmol/ml)	3.6±0.7	2.7±0.7	-0.90±0.70	0.070	3.6±0.9	3.5±0.8	-0.03±0.99	0.143	0.001
iCML (U/mg protein)	7.0±2.4	4.7±1.9	-2.3±2.6	0.022	6.9±2.4	5.9±3.0	-0.9±3.3	0.331	0.018
iMG (nmol/mg protein)	0.51±0.23	0.40±0.17	-0.10±0.23	0.345	0.52±0.28	0.55±0.18	0.05±0.26	0.210	0.078
RAGE (mRNA)	0.28±0.33	0.20±0.26	-0.08±0.22	0.273	0.13±0.22	0.28±0.31	0.15±0.34	0.005	0.001
TNFR1 (mRNA)	0.38±1.37	0.11±0.19	-0.27±1.30	0.282	0.08±0.14	0.16±0.22	0.09±0.20	0.038	0.072
8-isoprostanes (pg/ml)	98±72	81±84	-17±80	0.514	69±38	89±104	30±94	0.270	0.010
VCAM-1 (ng/ml)	1147±589	1074±548	-72±356	0.482	1017±638	1060±545	42±354	0.388	0.231
Systolic BP (mmHg)	137±16	144±20	6.3±20	0.329	137±16	142±20	7.3±28	0.432	0.398
Diastolic BP (mmHg)	74.4±9.6	74.9±13.3	0.5±13.2	0.732	78.4±11.5	78.1±10.5	1.3±14.2	0.876	0.659
Total cholesterol (mg/dl)	175±53	172±73	-2.9±49.5	0.788	161±50	162±49	-0.91±39.54	0.761	0.827
Triglycerides (mg/dl)	180±140	267±331	87.4±231.8	0.043	146±101	144±78	-1.9±103.7	0.622	0.021
HDL cholesterol (mg/dl)	53.6±17.8	49.1±17.6	-4.5±10.2	0.192	46.7±14.2	44.7±8.8	-1.8±8.9	0.117	0.202
LDL cholesterol (mg/dl)	89.1±39.9	77.4±36.6	-11.7±34.4	0.022	86.9±47.0	86.5±17.6	-0.42±36.8	0.866	0.148
Waist/hip ratio	0.98±0.06	0.97±0.06	-0.01±0.07	0.372	0.98±0.07	0.96±0.09	-0.02±0.06	0.105	0.330
Body mass index (kg/m^2)	33.9±7.4	34.0±7.4	0.06±1.92	0.959	33.6±7.8	33.5±7.7	-0.10±1.27	0.564	0.652

†Values are means ± SD

* Statistical significance between last (visit 6) and first visit (last minus first visit) after Sevelamer Carbonate or Calcium Carbonate

‡ Statistical significance between deltas (last minus first visit) after Sevelamer Carbonate compared to Calcium

* For nomenclature see Uribarri J1, Cai W, Pyzik R, Goodman S, Chen X, Zhu L, Ramdas M, Striker GE, Vlassara H. Suppression of native defense mechanisms, SIRT1 and PPAR γ, by dietary glycoxidants precedes disease in adult humans; relevance to lifestyle-engendered chronic diseases. Amino Acids. 2014 Feb;46(2):301-9. doi: 10.1007/s00726-013-1502-4

Results

SC decreased HbA1c in women (p=0.0007) and in subjects >65y.o. (Fig. 1). SC reduced AGEs, 8-isoprostanes and VCAM1; increased anti-inflammatory gene expression (Nrf2, AGER1, SIRT1, ERα); and reduced pro-inflammatory genes (RAGE and TNFR1) and albuminuria (fig.2) independently of baseline eGFR or rate of decline. Co-treatment with Met did not affect the outcome variables tested.

Conclusion

SC reduced AGEs and cellular pro-inflammatory responses, whereas anti-Infl/OS defenses were increased. HbA1c was reduced in women and those >65y.o. age.

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