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Mediation analysis for estimating cardioprotection of longitudinal RAS inhibition beyond lowering blood pressure and albuminuria in type 1 diabetes

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Abstract

Objective: We assessed the extent of cardiovascular benefit of renin angiotensin system (RAS) inhibition beyond lowering blood pressure (BP) and albuminuria in type 1 diabetes (T1D).

Methods: This cohort study included 605 T1D participants from the Pittsburgh Epidemiology of Diabetes Complications (EDC) study without baseline coronary artery disease (CAD). Participant follow-up extended through 25 years. We implemented marginal structural models to estimate total effect of and controlled direct effect by isolating the role of BP or albuminuria in mediating the relation between RAS inhibitors and CAD.

Results: Total effect of longitudinal RAS inhibition treatment was associated with 38% decreased CAD risk (HR [95%CI]: 0.62[0.23, 1.77]). The controlled direct effect of RAS inhibition was a 27% risk reduction (HR: 0.73[0.20, 2.59]) when isolating the role of BP and 26% risk reduction (HR: 0.74[0.16, 3.35]) when isolating the role of albuminuria. The mediation proportion for each 10 mmHg systolic BP and each 1 log unit of albumin excretion rate were 34% and 37%, respectively.

Conclusion: Our findings suggest that BP regulation and albuminuria reduction can only partially explain cardiovascular benefit of RAS inhibition on CAD in T1D, supporting the assertion that RAS inhibitors provide additional cardioprotection beyond lowering BP and albuminuria.

Keywords

mediation analysis; longitudinal study design; type 1 diabetes; antihypertensive treatment; coronary artery disease

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Conflict of interests

None

All the authors have no conflict of interest regarding this work.

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Background

Type 1 diabetes (T1D) confers a high risk for cardiovascular disease (1,2). Although the prevention and delay of microvascular complications have improved notably because of diabetes healthcare advances in recent years (3,4), risk of cardiovascular complications remains high (1).

Renin angiotensin system (RAS) inhibition treatment (i.e., angiotensin-converting enzyme [ACE] inhibitors or angiotensin receptor blockers [ARBs]), is known to reduce proteinuria in addition to blood pressure (5–10), and is usually the first-line recommendation of antihypertensive therapy in diabetes patients with elevated blood pressure. However, hypertension guidelines (11–14) have recently changed to recommend any class of antihypertensive agents for diabetes adults, with a preference for RAS inhibitors only in the presence of raised albuminuria. This guideline change is mainly based on evidence from recent head-to-head comparison clinical trials and associated meta-analyses, which have shown RAS inhibitors are not superior to other antihypertensive classes (i.e., thiazides and calcium channel blockers) for decreasing cardiovascular risk (15,16). It is important to note that this evidence was derived mostly from middle-aged or older type 2 diabetes patients who were already on antihypertensive treatment, and therefore may not be generalizable to younger people with T1D.

Clinical trial-based evidence of antihypertensive treatment recommendations in T1D is strikingly lacking. While two previous observational studies suggested cardiovascular protective effect of RAS inhibitor use when controlling for blood pressure (17) or albuminuria (18), these studies only used common conditional models. The conventional approach for treatment effect estimation, which given the observational nature of the data, makes statistical inferences of the treatment effect subject to observed and/or unobserved confounding factors (19). Moreover, both studies examined merely a single time point of RAS inhibitor exposure (either baseline or most recent). Failing to utilize longitudinally available information for evaluating chronic clinical outcomes could lead to misleading results (20).

The aim of the present study was, therefore, to examine the extent of the direct effect of longitudinal RAS inhibition on incident coronary artery disease (CAD), independent of any potential RAS inhibition effects on blood pressure and urinary albumin excretion. A mediation analysis using inverse probability weighted marginal structural models was conducted to estimate the magnitude of the effect of longitudinal RAS inhibition on CAD while isolating the roles of lowering blood pressure and albuminuria.

Methods

Study population

Participants in this analysis were from the Pittsburgh EDC Study, which has previously been described in detail (21). In brief, this is a longitudinal cohort study of childhood-onset T1D. The eligibility for participation contained having been diagnosed with T1D between 1950

and 1980 at Children's Hospital of Pittsburgh, having age <17 years at the diagnosis of T1D, and residing within 100 miles or 2.5 hours from Pittsburgh, Pennsylvania. Of 1,124 eligible participants, 145 (12.9%) died before the study baseline (1986–88); 191 (17%) declined participation; 130 (11.6%) only provided survey information; and 658 (58.5%) participated in baseline clinical examinations in 1986–1988 and the follow-up visits. Of these 658 EDC participants attending clinical examinations, 605 were free from CAD at the start of the study and were selected for the present analysis (Figure 1). Participants were followed for 25 years (2011–2013). Subsequent clinical assessments took place biennially for the first 10 years, with further examinations at the 18- and 25-year follow-up visits. Since baseline, self-reported surveys were undertaken biennially to obtain updated information from the participants, including demographic and medical history, reproductive health, healthcare, and life styles. The EDC cohort has been shown to be epidemiologically representative of the T1D population in Allegheny County, Pennsylvania (22).

Ascertainment of cardiovascular outcomes

CAD was defined as myocardial infarction that was confirmed by Q-waves on an electrocardiogram (Minnesota codes 1.1 or 1.2) or hospital records, angiographic stenosis of 50%, revascularization, EDC physician-diagnosed angina, or ischemic electrocardiograph (ECG) changes (Minnesota codes 1.3, 4.1–4.3, 5.1–5.3, and 7.1).

Medication use assessment

Participants self-reported medication use through questionnaires for the entire duration of the EDC study. Antihypertensive classification was identified using the Anatomical Therapeutic Chemical Classification System/Defined Daily Dose (ATC/DDD) index, which comprises RAS inhibitors (ACE inhibitors and/or ARBs), β receptor blockers, calcium channel blockers, diuretics, and other types of antihypertensives. At each follow-up, a dichotomous indicator variable (yes/no) was created for each antihypertensive class.

Risk factors measurement

Blood pressure was measured by certified personnel according to the Hypertension Detection and Follow-up Program protocol (23) in a sitting position using the right arm after a participant had sat quietly for 5 minutes with an appropriate-sized cuff. The measurement was conducted by a Hawksley random zero sphygmomanometer in the initial 10 years of the study and by aneroid devices in the subsequent follow up visits. An average of the second and third readings were used for data analysis. Pulse rate (beats/min) was determined by palpating the radial pulse for 30s and multiplying by two. Body mass index (BMI) was calculated as the weight in kilograms divided by the square of the height in meters. An ever-smoker was determined as someone who had smoked at least 100 cigarettes in the lifetime. Hypertension was defined as a systolic blood pressure ≥ 140 mmHg, a diastolic blood pressure ≥ 90 mmHg, or the use of blood pressure lowering medications. Overt nephropathy was defined as albumin excretion rate (AER) > 200 $\mu\text{g}/\text{min}$ (300 mg/24h) for at least two of three validated timed urine collections.

Stable glycosylated hemoglobin (HbA1c) was measured by ion-exchange chromatography (Isolab, Akron, OH) for the first 18 months, and the subsequent 10 years by automated high-

performance liquid chromatography (Diamat; Bio-Rad, Hercules, CA); the results of the two methods were highly correlated ($r = 0.95$). For follow-up beyond 10 years, HbA1c was measured with the DCA 2000 analyzer (Bayer, Tarrytown, NY). The DCA and Diamat assays were also highly correlated ($r = 0.95$). The values were then converted to Diabetes Control and Complications Trial (DCCT) - aligned HbA1c using regression equations derived from duplicate assays (24). Total cholesterol and triglycerides were determined enzymatically (25,26). High-density lipoprotein (HDL) cholesterol was obtained enzymatically with a precipitation technique (heparin and manganese chloride) using a modified version of the Lipid Research Clinics method (27). Non-HDL cholesterol was defined as total cholesterol minus HDL cholesterol. An Ectachem 400 Analyzer (Eastman Kodak Co.) was used to measure serum creatinine. The estimated glomerular filtration rate (eGFR) was obtained using the CKD-EPI creatinine equation (28). Urinary albumin was measured by immunonephelometry (29). Urinary albumin excretion rate (AER) was calculated for each of three timed urine samples (24-hour, overnight, and 4-hour collections obtained over a two-week period); the median of the three AERs was used in the analyses. There were 615 EDC participants whose haptoglobin genotype was assessed (haptoglobin 1/1, 2/1 and 2/2).

Statistical methods

Participants were censored if death, lost to follow-up or the end of the 25 years follow up. The analytic strategy was to assess the total adjusted association between longitudinal RAS inhibition treatment and incident CAD, then to define the controlled direct effect of longitudinal RAS inhibition on incident CAD by removing the effect of proposed mediators, blood pressure or albuminuria. The difference between the total association and direct association represents the effect that is explained by the mediator. The mediation of blood pressure and albuminuria was tested individually, using each 10mmHg SBP and each 1 log-AER ($\mu\text{g}/\text{min}$) change for estimating the extent of the mediation effect, respectively. Causal diagrams were used to identify potential confounders of the association between time-varying RAS inhibitor use and CAD and of the association between the mediator of interest (either SBP or AER) and CAD. Variables identified as potential confounders of the RAS inhibition - CAD association included age, sex, diabetes duration, college education (yes/no), family history of myocardial infarction, haptoglobin genotype (1/1, 2/1 and 2/2), smoking, hypertension, body mass index (BMI), systolic blood pressure (SBP), HbA1c, non-HDL and HDL cholesterol, white blood cell count, eGFR, and urinary AER. Models of the SBP - CAD association were adjusted for age, sex, diabetes duration, college education (yes/no), haptoglobin genotype (1/1, 2/1 and 2/2), smoking, non-HDL and HDL cholesterol, eGFR, and urinary AER, and models of the AER - CAD association were adjusted for age, sex, diabetes duration, college education (yes/no), haptoglobin genotype (1/1, 2/1 and 2/2), smoking, BMI, SBP, HbA1c, non-HDL and HDL cholesterol, WBC, and eGFR.

The primary analysis evaluating mediation by blood pressure or albuminuria adjusted for confounders marginally by using inverse probability weighting, i.e., inverse probability weighted marginal structural models (30,31). We chose this approach because it allows the identification of direct effects in the presence or absence of exposure-induced mediator-outcome confounding (32). It also because marginal structural models can account for the

time-varying confounding effect in the presence of time-varying exposure. To execute this analytic approach, we used 2 time-varying Cox proportional hazard models weighted by stabilized inverse probability weights. It is commonly seen that variables change over time during the follow-up period. Such variables can be analyzed with the Cox regression model to estimate its effect on survival time (33–35). In this study, we constructed the Cox models incorporating time-varying covariates using the R package ‘survival’ by Therneau et al (36,37). The weights were generated from modelling RAS inhibition treatment, censoring, mortality and then the mediator (SBP or AER). We used pooled logistic models for estimating these stabilized inverse probability weights (38–40). The equations for weights calculation can be found in the paper by Robins et al. (38). Weights were then incorporated into two time-varying Cox models: (i) modelling the total effect of longitudinal RAS inhibitor use on CAD outcomes; (ii) modelling the controlled direct effect of longitudinal RAS inhibitor use on CAD, independent of effect achieved via blood pressure control or urinary albumin reduction. Results were reported as hazard ratios (HR). Confidence intervals were obtained using robust variance estimators, which account for pseudo-clustering induced by the inverse probability weights (38). Finally, the mediation proportion of blood pressure or albuminuria in the RAS inhibition - CAD association was calculated as the absolute value of: $([\text{Total effect} - \text{Controlled Direct Effect}] / \text{Total effect}) \times 100\%$ (31,41).

As a sensitivity analysis, we implemented mediation analysis using natural effect models (42,43). This approach can be used to test multiple mediators simultaneously; however, since this approach can only accommodate time-invariant data, the analysis was conducted by using the last RAS inhibitor use value as a single measure. The total effect is defined as the sum of the natural direct and natural indirect effect, and the mediated proportion was calculated as the ratio of the indirect effect (of total effect - direct effect) divided by the total effect. Although we suspected the magnitude of the estimated effects (total, direct and indirect effects) would be greater for long term RAS inhibitor use compared with the estimate of a single time point measure, we assume that the mediation proportion of blood pressure or albuminuria remains similar under the two scenarios (longitudinal and final value) as these effects would likely increase proportionally with time. If these assumptions are correct, the natural effect method should yield results that are consistent with the inverse probability weighted approach.

Prior to the analysis, missing data were handled using multiple imputations by chained equations (MICE) (44,45) for all variables that were included in the analyses. A total of 50 datasets were imputed and analyzed; the results were subsequently pooled using Rubin’s combination rules (46).

Of the 605 participants with up to 8 clinic visits, the CAD outcome statuses were completely documented. There were 11.8%, 21.0%, and 25.3% of missing values across visits for time-varying RAS inhibition treatment status, SBP, and urinary AER, respectively. Among covariates included in the analysis, the missing rates were no higher than 28.1% (detailed information about missing data is seen in the supplement: Table S1).

R 3.5.1 (R Core Team, Vienna, Austria) was utilized for statistical analysis.

Results

The mean age and diabetes duration of the 605 EDC study participants free of CAD at study entry were 27 and 19 years, respectively; half of the cohort were females. There were 219 (36.2%) participants who experienced at least one CAD event during the follow-up period. Compared to participants without incident CAD, those who developed CAD were more likely to be older and less educated, have a longer duration of diabetes, smoke, take antihypertensive medications, have family history of myocardial infarction, have hypertension and/or renal disease, and have higher BMI, higher levels of blood pressure, non-HDL cholesterol and urinary AER, and lower eGFR at baseline (Table 1). The median (25th, 75th) follow up was 20.4 (10.7, 24.1) years.

The overall prevalence rate of antihypertensive use increased with longer follow-up time. RAS inhibitors have become the dominant treatment since the early 1990s (EDC study Cycle 3) (Figure 2-A). When examined retrospectively by CAD event status, cases had higher prevalence rate of taking RAS inhibitors compared to non-cases over the entire follow up period even prior to the event occurrence (Figure 2-B).

Associations between time-varying RAS inhibition treatment and CAD are displayed in Table 2. Based on the marginal structural model results, RAS inhibition use over the follow-up period was associated with 38% lower total CAD risk (HR [95%CI]: 0.62 [0.23, 1.77]) although this effect estimate was not statistically significant. When the mediating role of blood pressure was isolated, longitudinal RAS inhibition was associated with a 27% reduced risk of CAD (HR [95%CI]: 0.73 [0.20, 2.59]); 34% of the total effect of RAS inhibition on CAD was explained by lowering blood pressure (mediation proportion). When isolating the role of albuminuria, the controlled direct effect of longitudinal RAS inhibition treatment was associated with 26% CAD risk reduction (HR [95%CI]: 0.74 [0.16, 3.35]), and the proportion of this effect mediated by AER was in 37%.

Results were not meaningfully different when the natural effect model was used for assessing the effect of RAS inhibition at the last time point. When SBP (per 10 mmHg) and urinary AER (per 1 log-unit [$\mu\text{g}/\text{min}$]) were individually tested, the mediation proportion of the total effect attributable to each factor was 38% and 30%, respectively. When testing the combined mediation effect of SBP and AER, the two factors explained 45% of the cardiovascular beneficial effect of RAS inhibition. Of note, however, the estimated total effect for most recent RAS inhibition treatment reduced the CAD risk by only 17% (HR [95%CI]: 0.83 [0.73, 0.96]) (Table 3), compared to 38% in the inversed probability marginal structural model approach modeling time-varying covariates.

Discussion

We used an observational study cohort of individuals with long standing T1D to evaluate the effect of RAS inhibition treatment on CAD outcomes. We observed a trend indicating that RAS inhibition was associated with a clinically meaningful risk reduction for adverse cardiovascular outcomes in the current analysis. By decomposing the association between RAS inhibition treatment and CAD outcomes, we further found that blood pressure and

albuminuria only partially explained the total cardiovascular beneficial effect of RAS inhibition. These results suggest that RAS inhibition protects against cardiovascular disease through additional pathways beyond lowering blood pressure and albuminuria.

The interpretation of the study results warrants caution. Due to the observational nature of the study and the presence of unmeasured confounding, we are unable to definitively conclude that there is a causal effect of RAS inhibition treatment on CAD. The application of inversed probability weighted modeling approach should, however, have partially accounted for differences between participants who were treated and untreated with RAS inhibitors. Also, the medication use information was self-reported, there might be misclassification of using versus not using a given medication, such as RAS inhibitors.

A noteworthy limitation of the study is that the two mediators were tested individually in the primary analysis using controlled direct effect models, and so that we were unable to obtain the total extent of “true” direct cardioprotection of RAS inhibition after accounting for the combined effect of blood pressure and albuminuria. We cannot rule out a synergic or additive effect for blood pressure and albuminuria. However, we were able to test combined mediation effect of these two factors in the natural effect models for most recent RAS inhibition (time-invariant), showing that blood pressure and albuminuria examined approximately half of the total effect (45%) for RAS inhibition on CAD. Given the comparable mediation proportions between time-varying and time-invariant modeling methods when testing the two factors individually, the estimated combined mediation effect might also be similar.

The wide confidence intervals of the results give us caution against definitive conclusions. A relatively small sample size, particularly when using time-varying predictors with multiple time points of measurement, is likely to result in an underpowered analysis and thus wide confidence intervals. Moreover, multiple imputations were employed to handle the missing data prior to the marginal structural modeling, and this may have increased the variability of the data. Nevertheless, sensitivity analyses were conducted and showed a robust cardiovascular protective effect of RAS inhibition with different model specifications in this data (all HRs <0.8).

Several other limitations of the present study should be noted. The study is likely to be underpowered because of a relatively small sample size leading to wide confidence intervals. Consequently, these findings need to be confirmed in future studies with a larger sample size. Moreover, since 98% of the study participants were white in the current study, the results may not be able to generalize to other races and ethnicities. Lastly, one of encountered problems of long-term follow up studies, such as ours, was missing data. Missing data may affect the precision of estimations and introduce bias (47). Although we used multiple imputation to handle the missing data, which may improve the precision of estimations and reduce bias (47), the multiple imputation may increase variability of the dataset.

Conclusion

Our study provides suggestive support for there being additional cardiovascular benefit of RAS inhibitors beyond blood pressure regulation and albuminuria reduction. Although we are unable to conclude a definite independent causal association of RAS inhibition with cardiovascular outcomes, we believe that our results are encouraging and have important clinical implications, supporting expanded use of RAS inhibitors as first choice for hypertension therapy with the aim of decreasing cardiovascular disease in this high-risk population.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.
Flow chart of study participants

Figure 2.

Antihypertensive medication use by Pittsburgh EDC study cycle (C1 - C14)

CAD: coronary artery disease, RASI: renin–angiotensin system inhibitors

C1: 1986–1988, C2: 1988–1990, C3: 1990–1992, C4: 1992–1994, C5: 1994–1996, C6: 1996–1998, C7: 1998–2000, C8/9: 2000–2004, C10: 2004–2006, C11: 2006–2008, C12: 2008–2010, C13: 2010–2012, C14: 2012–2014

Table 1

Baseline clinical characteristics of the study participants

Baseline collection	Total	Incident CAD		Number of Missing
	N=605	Non-cases (n=386)	Cases (n=219)	
Age, years	27.2 (7.7)	24.8 (7.2)	31.3 (6.8) **	0
Age at diabetes onset, years	8.2 (4.1)	8.1 (4.2)	8.3 (3.8)	0
Diabetes duration, year	19.0 (7.4)	16.7 (6.6)	23.0 (7.1) **	0
Female, %(n)	49.8 (301)	51.0 (197)	47.5 (104)	0
College education, %(n)	42.6 (258)	47.7 (184)	33.8 (74) **	0
Family history of MI, %(n)	41.3 (248)	32.6 (124)	56.6 (124) **	4
Haptoglobin 1/1	11.5 (65)	12.3 (44)	10.1 (21)	35
Haptoglobin 2/1	46.0 (260)	47.2 (169)	44.0 (91)	
Haptoglobin 2/2	42.5 (240)	40.5 (145)	45.9 (95)	
Ever smoker, %(n)	37.2 (225)	30.3 (117)	49.3 (108) **	0
BMI, kg/m ²	23.5 (3.2)	23.2 (3.2)	24.0 (3.2) **	0
Hypertension, %(n)	14.4 (87)	7.8 (30)	26.0 (57) **	0
SBP, mmHg	112.9 (14.7)	109.9 (12.3)	118.1 (17.1) **	0
DBP, mmHg	72.5 (10.8)	70.8 (9.8)	75.5 (12.0) **	0
PP, mmHg	40.4 (10.3)	39.1 (9.6)	42.7 (10.7) **	0
HbA1c, %	8.8 (1.5)	8.8 (1.5)	8.7 (1.5)	3
Non-HDLc, mg/dL	135.7 (41.7)	127.6 (38.8)	149.9 (42.9) **	4
HDLc in male, mg/dL	49.5 (9.8)	50.8 (10.0)	47.3 (9.0) **	4
HDLc in female, mg/dL	58.4 (12.9)	58.9 (13.0)	57.5 (12.5)	
Triglycerides, mg/dL	82 (60, 121)	76 (57, 108)	93 (70, 141) **	31
WBC, ×1000/m ³	6.6 (1.9)	6.4 (1.8)	6.9 (2.0) **	5
GFR, mL/min per 1.73 m ²	103.8 (30.8)	108.3 (30.1)	95.9 (30.4) **	3
AER, µg/min	14 (7, 102)	11 (7, 42)	39 (9, 470) **	0
RAS inhibitors,	2.9 (17)	16 (6)	5.1 (11) *	0
β blockers, %(n)	3.3 (19)	2.2 (8)	5.1 (11)	0
Calcium channel blockers, %(n)	0.3 (2)	0.6 (2)	0 (0)	0

Categorical variables were presented as percentage (number) and continuous variables as mean (SD) or median (1st and 3rd quantile)

* $p < 0.05$,

** $p < 0.01$ between comparisons

AER: urinary albumin excretion rate, BMI: body mass index, BP: blood pressure, CAD: coronary artery disease, DBP: diastolic blood pressure, HbA1c: hemoglobin A1c, HDL: high density lipoprotein, PP: pulse pressure, RAS: renin angiotensin system; SBP: systolic blood pressure, SD: standard deviation, WBC, white blood cell count

Table 2

Causal mediation analysis with time-varying exposure and covariates

RAS inhibition effect	Total effect	Controlled direct effect	Mediation proportion *
Mediated by SBP (per 10 mmHg)			
HR (95%CI)	0.62 (0.23, 1.77) (100%)	0.73 (0.20, 2.59) (66%)	34%
Mediated by Albuminuria (per 1 log [AER])			
HR (95%CI)	0.62 (0.23, 1.77) (100%)	0.74 (0.16, 3.35) (63%)	37%

AER: urinary albumin excretion ration, MSM: marginal structural model

* Extrapolated from the total and direct effect. For example of SBP, mediation proportion (%) = $[\ln(0.62) - \log(0.73)] / [\ln(0.62) * 100\%]$

Table 3

Mediation effect of blood pressure and albuminuria on the association between most recent RAS inhibition treatment and incident CAD

RAS inhibition effect	Total	Direct	Indirect
Mediated by SBP (per 10 mmHg)			
HR (95%CI)	0.83 (0.73, 0.96)	0.90 (0.79, 1.02)	0.94 (0.76, 1.11)
Mediation proportion (%)	100%	61.8%	38.2%
Mediated by AER (per 1 log [AER])			
HR (95%CI)	0.83 (0.73, 0.96)	0.88(0.77, 1.00)	0.95 (0.79 1.14)
Mediation proportion (%)	100%	70.5%	29.5%
Mediated by SBP (per 10 mmHg) and urinary AER (per 1 log[AER])			
HR (95%CI)	0.83 (0.73, 0.96)	0.92 (0.79, 1.05)	0.91 (0.75, 1.07)
Mediation proportion	100%	55.2%	44.8%

CAD: coronary artery disease, CI: confidence interval, HR: Hazard ratio, RAS: renin-angiotensin system, SBP: systolic blood pressure

The confidence intervals were obtained using 5000 bootstraps