The angiotensin II receptor type 2 polymorphism influences haemodynamic function and circulating RAS mediators in normotensive humans

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Abstract

Background. The haemodynamic responses to angiotensin II type 1 (AT1) receptor blockade may be mediated in part by interactions between angiotensin II and the angiotensin II type 2 receptor (AT2R). An AT2R G1675A gene polymorphism has been described, but the functional effects of this polymorphism are unknown.

Methods. Haemodynamic function, circulating renin–angiotensin system mediators and norepinephrine were measured in young healthy subjects at baseline and at 2 and 4 weeks after treatment with irbesartan. Subjects were divided into two groups on the basis of the AT2R G1675A gene polymorphism: GG subjects (n = 12) and AA/GA subjects (n = 22).

Results. AA/AG subjects exhibited hypotensive and renal vasodilatory responses to irbesartan at 4 weeks, but GG subjects did not. In accord with haemodynamic effects, circulating aldosterone levels were suppressed in AA/AG, while circulating norepinephrine levels were augmented only in GG subjects. In contrast, increases in circulating renin, angiotensin II and plasma renin activity after irbesartan were exaggerated in AA/AG subjects.

Conclusions. The AT2R G1675A polymorphism is a determinant of haemodynamic responses to AT1 receptor blockade, an effect that may be due to influences on aldosterone escape.

Keywords: aldosterone escape; angiotensin II type 2 receptor gene polymorphism; renal haemodynamic function; renin–angiotensin–aldosterone system

Introduction

Haemodynamic responses to angiotensin II type 1 (AT1) receptor (AT1R) blockade may in part be influenced by interactions between angiotensin II and the angiotensin II type 2 (AT2) receptor. Activation of the angiotensin II type 2 receptor (AT2R) results in vasodilatation and antiproliferative effects on vascular smooth muscle cells [1]. It has also been postulated that the AT2R plays a role in mediating the ‘aldosterone escape’ phenomenon after blockade of the AT1R [2]. Specifically, the rise in circulating angiotensin II that follows AT1R blockade can activate the AT2R, leading to an increase in catecholamine release and enhanced secretion of aldosterone [2–4]. Although previous experimental work has therefore suggested an interaction between the AT2R, the sympathetic nervous system, and aldosterone activation, the relevance of these interactions in humans is incompletely understood.

Recently, a G1675A polymorphism was identified in intron 1 of the AT2R gene that influences AT2R transcription [5–7]. This variant has been associated with left ventricular hypertrophy [5–9]. Although this polymorphism has not been associated with differences in acute haemodynamic responses to infused angiotensin II in hypertensive subjects, data regarding its influence on blood pressure and vascular responses to AT1R blockade in normotensive individuals are controversial [10–12]. Accordingly, we examined the effect of the AT2R G1675A gene polymorphism on renal and systemic haemodynamic function responses to AT1R blockade in young healthy subjects during strictly controlled and standardized experimental conditions.

Materials and methods

Candidates who fulfilled the following criteria were recruited to participate: normoalbuminuria (albumin excretion rate <20 μg/min), blood pressure <140/90 mmHg, absence of chronic illness and no regular medications. The Toronto General Hospital Research Ethics Board approved the protocol. All subjects gave informed consent.

As in previous protocols, subjects adhered to a sodium replete (>150 mmol/day) and moderate protein diet (<1.5–2.0 g/kg/day) for 7 days before each study [13]. Female subjects were studied during the follicular phase of the menstrual cycle and were again studied during the same phase of a subsequent menstrual cycle.

On the first day, two peripheral veins were cannulated in the usual fashion, and a syringe infusion pump was used for infusions of inulin (glomerular filtration rate, GFR) and para-aminobipurate (PAM—effective renal plasma flow, ERPF), as previously described [14–16]. Filtration fraction (FF), renal blood flow (RBF) and renal vascular resistance (RVR) were derived using standard calculations [14–16]. Norepinephrine and circulating renin–angiotensin system (RAS) components were measured using standard techniques [15,16]. Subjects were then started on...
Fig. 1. a-i. Plasma norepinephrine and renin–angiotensin system responses to irbesartan in subjects with the AT2R G1675A gene polymorphism (mean ± SD). *P < 0.05 for effect of irbesartan at 2 weeks vs. baseline; †P < 0.05 for effect of irbesartan at 4 weeks vs. baseline; ‡P < 0.05 for effect of irbesartan in AA/AG vs. GG on blood pressure at 4 weeks; §P < 0.05 for levels of circulating mediators in AA/AG subjects vs. GG subjects at 4 weeks.
AT2R polymorphism and vascular function

Table 1. Dietary parameters and blood pressure responses to irbesartan in subjects with the AT2R G1675A gene polymorphism (mean ± SD)

<table>
<thead>
<tr>
<th></th>
<th>Baseline 4 weeks</th>
<th>Baseline 4 weeks</th>
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<tbody>
<tr>
<td>24-h sodium (mmol/day)</td>
<td>204 ± 25</td>
<td>226 ± 16</td>
</tr>
<tr>
<td>24-h urea (g/kg/day)</td>
<td>1.13 ± 0.09</td>
<td>1.09 ± 0.05</td>
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<thead>
<tr>
<th></th>
<th>2 weeks</th>
<th>4 weeks</th>
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<tbody>
<tr>
<td>HR (beats per minute)</td>
<td>61 ± 13</td>
<td>64 ± 9</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>117 ± 3</td>
<td>109 ± 3</td>
</tr>
<tr>
<td>MAP (mmHg)</td>
<td>86 ± 3</td>
<td>80 ± 3</td>
</tr>
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<table>
<thead>
<tr>
<th></th>
<th>AA/AG group</th>
<th>GG group</th>
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<tbody>
<tr>
<td>GFR (mL/min/1.73 m²)</td>
<td>118 ± 6</td>
<td>112 ± 5</td>
</tr>
<tr>
<td>FF</td>
<td>0.18 ± 0.01</td>
<td>0.15 ± 0.01*</td>
</tr>
<tr>
<td>RBF (mL/min/1.73 m²)</td>
<td>1097 ± 68</td>
<td>1215 ± 82</td>
</tr>
<tr>
<td>RVR (mL/L/min)</td>
<td>0.083 ± 0.006</td>
<td>0.070 ± 0.005*</td>
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*P < 0.05 for effect of irbesartan at 4 weeks vs. baseline.
†P < 0.05 for effect of irbesartan at 2 weeks vs. baseline.
‡P < 0.05 for effect of irbesartan at 4 weeks in AA/AG vs. GG subjects.

Table 2. Renal haemodynamic function responses to irbesartan in subjects with the AT2R G1675A gene polymorphism (mean ± SD)

<table>
<thead>
<tr>
<th></th>
<th>Baseline 4 weeks</th>
<th>Baseline 4 weeks</th>
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<tbody>
<tr>
<td>ERPF (mL/min/1.73 m²)</td>
<td>697 ± 45</td>
<td>749 ± 53*</td>
</tr>
<tr>
<td>GFR (mL/min/1.73 m²)</td>
<td>118 ± 6</td>
<td>112 ± 5</td>
</tr>
<tr>
<td>FF</td>
<td>0.18 ± 0.01</td>
<td>0.15 ± 0.01*</td>
</tr>
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<td>RBF (mL/min/1.73 m²)</td>
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<td>RVR (mL/L/min)</td>
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<td>0.070 ± 0.005*</td>
</tr>
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</table>

*P < 0.05 for effect of irbesartan.

Irbesartan 75 mg PO daily. Two weeks later, circulating norepinephrine, RAS mediators and blood pressure measurements were obtained. After an additional 2 weeks, circulating norepinephrine, RAS mediators, blood pressure and renal haemodynamic function measurements were repeated. As in previous experiments, peripheral blood pressure was measured in the right brachial artery with an automated DINAMAP® sphygmomanometer (Critikon, FL, USA) [14–16]. At baseline and 4 weeks, reported blood pressure values represented the mean of readings obtained at four time points over the course of a morning (8 am, 10 am, 10:30 am and 11 am), with duplicate measurements taken at each reading, for a total of eight readings over 3 h. At 2 weeks, three blood pressure readings were obtained over 2 h (8 am, 9:30 am and 10 am) for a total of six readings. All experiments were performed in a quiet, temperature-controlled room in the supine position.

The data were analysed on the basis of the presence or absence of the AT2R G1675A gene polymorphism. Based on previous work examining the effect of RAS inhibition on blood pressure and a 10% standard deviation, our study had a >75% power to detect 10% differences in blood pressure at the 0.05 level of significance [15,17]. Results are presented as mean ± SD. Between-group comparisons of all parameters at baseline were made using parametric methods (unpaired t-test). Within-subject and between-group differences in the response to irbesartan were determined by repeated measures analysis of variance (ANOVA) (SPSS for graduate students, version 14.0).

Results

At baseline, GG and AA/AG subjects were similar in age (29 ± 6 vs. 31 ± 6 years) and body mass index (24.8 ± 1.0 vs. 24.5 ± 0.9 kg). In women, oestrogen levels were consistent with the follicular phase of the menstrual cycle (73 ± 31 vs. 90 ± 70 pmol/L). Dietary parameters and body weight remained stable throughout the study (Figure 1a).

At 2 weeks, irbesartan was associated with similar blood pressure declines in AA/AG and GG subjects (Table 1, Figure 1b and c). At 4 weeks, blood pressure effects remained significant in AA/AG subjects, and renal vasodilatory effects were also observed (Tables 1 and 2, Figure 1b and c). In contrast, at 4 weeks, blood pressure effects were significantly blunted compared with AA/AG subjects, and renal haemodynamic function did not change in the GG group (Tables 1 and 2, Figure 1b and c). In accord with haemodynamic effects, in AA/AG vs. GG subjects, circulating aldosterone levels were suppressed throughout the study, while circulating norepinephrine levels were augmented in GG subjects (Figure 1d and e). In contrast, increases in circulating renin, angiotensin II and plasma renin activity after irbesartan were exaggerated in AA/AG subjects (Figure 1f–i).

Discussion

The AT2R G1675A gene polymorphism influences protein expression of the AT2R, but its influence on haemodynamic function has not been fully elucidated. Our first major observation was that in subjects with the A allele, we observed persistent blood pressure and renal vasodilatory effects, despite the exaggerated rise in circulating RAS mediators; these effects were not observed in GG subjects. These findings suggest that the polymorphism may in part account for heterogeneity in clinical responsiveness to AT1R antagonists. Exaggerated effects on circulating RAS mediators in the AA/AG group suggest that greater RAS-negative feedback inhibition may have contributed to enhanced haemodynamic effects.

‘Aldosterone escape’ has been postulated to limit the efficacy of AT1R blockade [2]. Our second major observation was that in conjunction with greater haemodynamic...
effects in AA/AG subjects, AT1R antagonism was associated with a persistent decline in circulating aldosterone. This finding suggests that subjects with the A allele did not exhibit aldosterone escape, and that the failure to achieve a sustained decline in blood pressure or renal vasodilation in the GG group might be due, at least in part, to the blunted effect of irbesartan on aldosterone levels in this group. Finally, blunted haemodynamic and aldosterone escape effects in GG subjects may have been due to the significant rise in norepinephrine after irbesartan that was only observed in this group. Sympathetic nervous system activation can cause direct renal microvascular vasoconstriction and a rise in systemic blood pressure, and enhance aldosterone section through paracrine mechanisms [3,4]; both of these mechanisms may have blunted the vasodilatory effects of irbesartan in GG subjects compared with the AA/AG group.

This study has limitations. Firstly, the sample size was small, and may have limited our ability to detect some differences in renal haemodynamic or blood pressure parameters. Furthermore, our results cannot determine if the AT2R G1675A polymorphism affects circadian changes in blood pressure since ambulatory monitoring was not used. We attempted to minimize the effect of the small sample size by utilizing homogeneous study groups and by careful pre-study dietary preparation. We also decreased variability by using a study design that allowed each subject to act as his or her own control.

In conclusion, the AT2R G1675A polymorphism affects the haemodynamic response to AT1R inhibition in young healthy subjects. Although the responsible mechanism remains to be elucidated, this may be due in part to different effects on aldosterone escape.

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Conflict of interest statement. None declared.

References


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