Clinical Research Article

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The effect of beta1-adrenergic receptor gene polymorphism on prolongation of corrected QT interval during endotracheal intubation under sevoflurane anesthesia

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Background: The hemodynamic responses to endotracheal intubation are associated with sympathoadrenal activity. Polymorphisms in the beta1-adrenergic receptor (β_1AR) gene can alter the pathophysiology of specific diseases. The aim of this study is to investigate whether the Ser49Gly and Arg389Gly polymorphism of the β_1AR gene have different cardiovascular responses during endotracheal intubation under sevoflurane anesthesia.

Methods: Ninety-one healthy patients undergoing general anesthesia were enrolled. Patients underwent slow inhalation induction of anesthesia using sevoflurane in 100% oxygen. Vecuronium 0.15 mg/kg was given for muscle relaxation. Endotracheal intubation was performed by an anesthesiologist. The mean arterial pressure (MAP), heart rate (HR), and the corrected QT (QTc) interval were measured before induction, before laryngoscopy, and immediately after tracheal intubation. Genomic DNA was isolated from the patients' peripheral blood and then evaluated for the β_1AR-49 and β_1AR-389 genes using an allele-specific polymerase chain reaction method.

Results: No differences were found in the baseline values of MAP, HR, and the QTc interval among β_1AR-49 and β_1AR-389, respectively. In the case of β_1AR-49, the QTc interval change immediately after tracheal intubation was significantly greater in Ser/Ser genotypes than in Ser/Gly genotypes. No differences were observed immediately after tracheal intubation in MAP and HR for β_1AR-49 and β_1AR-389.

Conclusions: We found an association between the Ser49 homozygote gene of β_1AR-49 polymorphism and increased QTc prolongation during endotracheal intubation with sevoflurane anesthesia. Thus, β_1AR-49 polymorphism may be useful in predicting the risk of arrhythmia during endotracheal intubation in patients with long QT syndrome. (Korean J Anesthesiol 2011; 61: 117-121)

Key Words: Beta1 adrenergic receptor, Endotracheal intubation, Polymorphism.

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Introduction

Endotracheal intubation using laryngoscopy increases arterial pressure, heart rate, and the incidence of cardiac arrhythmias, which usually cause little consequence in healthy patients but may be detrimental to patients with cardiovascular diseases [1-3]. The hemodynamic responses to laryngoscopy and tracheal intubation are caused by a catecholamine discharge associated with sympathoadrenal activity. The corrected QT (QTc) interval can also be prolonged during rapid injection of catecholamine, brief stimulation of the sympathetic nervous system, and an imbalance of the cardiac sympathetic tone [4-7].

Stimulation of the sympathetic nervous system, due to exercise or emotional stress, causes activation of cardiac β-adrenoceptors. Beta1-adrenergic receptor (β_1AR) is an important mediator of the sympathetic cascade and regulates numerous physiologic events, including heart rate and contractility. Polymorphisms in the β_1AR gene can affect responses to drugs in patients with hypertension or heart failure and alter the pathophysiology of specific disease states, where sympathetic activation plays a major role. The β_1AR gene is localized to chromosome 10 and two common polymorphisms, Ser49Gly and Arg389Gly, were identified in 1999 [8].

The Ser49Gly polymorphism is located in the extracellular amino-terminal region of the receptor and the Gly49 variant correlates with the development of cardiomyopathy and heart failure [9,10]. Arg389Gly is located in the intracellular cytoplasmic tail near the seventh transmembrane region of the receptor, which is a putative Gs-protein binding domain. Patients homozygous for the Arg389 allele are at an increased risk for developing hypertension [11]. Given these findings, endogenous catecholamine stimulation of β_1AR during endotracheal intubation may result in enhanced cardiovascular response in one genotype over another. No study has been made on β_1AR polymorphism with cardiovascular responses to endotracheal intubation under laryngoscopy.

The aim of the present study is to investigate whether the functionally important Ser49Gly polymorphism and the Arg389Gly polymorphism of the β_1AR gene have different cardiovascular responses during endotracheal intubation under sevoflurane anesthesia.

Materials and Methods

After obtaining approval from our Institutional Review Board and receiving written informed consent from the participants, 100 patients (American Society of Anesthesiologists physical status class 1) between the ages of 20 and 50 were enrolled in this study. Patient exclusion criteria were: abnormal serum electrolyte values, a QTc interval duration greater than 440 ms, taking medication affecting QTc interval duration (tricylic antidepressant agents, antidysrhythmics, beta adrenergic antagonists, calcium channel blocking agents), the existence of valvular cardiac disease, and any cardiac rhythm other than sinus rhythm, diabetes mellitus, pregnancy, or obesity.

All study data were collected in the morning (8:00−11:00) to prevent the effects of day-night changes on the QTc interval. Patients received no premedication. After being taken into the operating room, electrocardiogram monitoring, pulse oximetry, non-invasive blood pressure, fraction of inspired oxygen and end-tidal sevoflurane, and carbon dioxide concentration monitoring were begun. Blood pressure was measured with an automatic oscillographic device every 2 minute during the study period. After the monitoring equipment had been attached, the patients were allowed to rest for 5 minutes while lactated Ringer's solution 4 ml/kg was infused before inducing anesthesia. A standard real-time automated three-lead electrocardiogram was continuously recorded using a data acquisition system (PowerLab; AD Instruments, Colorado Springs, CO, USA). The QT interval was measured in lead II from the onset of the QRS complex to the end of the T wave, which was defined as a return to the T-P baseline. When U waves were present, the nadir between the T and U waves was regarded as the end of the QT interval. Biphasic T waves were considered to end with the final return to baseline. The values of the QT interval of four successive beats were averaged. The QT interval was corrected using the Fridericia formula: $QTc = QT / \sqrt[3]{(R - R \text{ interval})}$.

Patients underwent slow inhalation induction of anesthesia with a facemask using sevoflurane in 100% oxygen to avoid the confounding effects of other anesthetic agents. Anesthesia was induced by initially administering 1.0% sevoflurane and increasing the inspiratory concentration after every fifth breath by 0.5% until a maximum of 6% sevoflurane was reached. After induction, the anesthesia was maintained with sevoflurane and ventilation was assisted using a facemask at an end-tidal concentration of 3% sevoflurane to provide an adequate depth of anesthesia. As spontaneous breathing diminished, patients were manually assisted via the facemask while an exhaled tidal volume of 8 ml/kg was maintained. The respiratory rate was adjusted to maintain an end-tidal carbon dioxide partial pressure of 35 mmHg. Vecuronium 0.15 mg/kg was given for muscle relaxation at 10 min after induction with sevoflurane. Laryngoscopy was attempted 5 min after vecuronium injection. Laryngoscopy and tracheal intubation were performed by one anesthesiologist, and then the sevoflurane end-tidal concentration was reduced to 2%. Data for patients with a failed intubation on the first attempt or intubations which took more than 40 sec were also excluded from the analysis. The mean arterial pressure (MAP), heart rate (HR), and QTc interval were

Table 1. Primer Sets and Tm for the SNaPshot Assay

	β_1-AR S49G (rs1801252)	β_1-AR G389R (rs1801253)
Forward	GACAGCGCTCGGCTCCT	GAGCAGAAGGCGCTCA
Reverse	GTAGCGGAAGGGCGAGGT	GTGGCCCCRACGACATC
SNP Primer	GCTGAGACAGCGGCTCGGGGC	TGCGCGCGCAGCAGAGCAGTC
Tm (°C)	60	60

Table 2. Baseline Parameters in Patients for β_1*AR-49* and β_1*AR-389*

Characteristics	β_1-AR Ser49Gly genotype		P	β_1-AR Arg389Gly genotype		P
	Ser/Ser	Ser/Gly		Arg/Arg	Gly allele	
Sex (M/F)	27/41	11/12	0.495	24/35	14/18	0.777
Age (yr)	35.7 ± 9.3	36.0 ± 8.7	0.898	35.3 ± 9.7	36.6 ± 8.1	0.505
Height (cm)	166.4 ± 9.0	167.1 ± 8.0	0.756	166.4 ± 9.1	166.7 ± 8.1	0.873
Weight (kg)	63.6 ± 11.0	66.7 ± 10.8	0.250	64.5 ± 10.4	63.4 ± 12.0	0.641
MAP (mmHg)	91.6 ± 11.9	89.4 ± 9.9	0.432	92.3 ± 11.6	88.8 ± 11.1	0.165
HR (bpm)	76.4 ± 12.1	74.0 ± 13.9	0.454	74.5 ± 12.4	78.2 ± 12.7	0.187
QTc (msec)	368.2 ± 24.4	373.3 ± 22.4	0.403	370.9 ± 24.9	366.6 ± 22.0	0.436

Data are presented as mean ± SD. Baseline parameters were assessed before anesthesia. MAP: mean arterial pressure, HR: heart rate, QTc: corrected QT.

Table 3. Changes in Arterial Pressure, Heart Rate, and QTc Interval

Study variable	T_0	T_1	% change
MAP (mmHg)	91.0 ± 11.5	106.6 ± 23.3	18.4 ± 27.7*
HR (bpm)	75.8 ± 12.6	101.2 ± 19.9	37.8 ± 37.8*
QTc (msec)	369.5 ± 23.9	412.9 ± 28.4	12.0 ± 7.2*

Data are presented as mean ± SD. MAP: mean arterial pressure, HR: heart rate, QTc: corrected QT. T_0: prior to induction of anesthesia, T_1: immediately after laryngoscopy and tracheal intubation, % change: $(T_1 - T_0) \times 100 / T_0$. *P < 0.001 by paired t-test.

measured before induction, before laryngoscopy, and immediately after tracheal intubation.

All patients underwent peripheral blood sampling for isolation of genomic DNA. Samples were stored at −20°C until DNA extraction. Genomic DNA was prepared using a nucleic acid isolation device, QuickGene Mini-80 (FUJIFILM, Tokyo, Japan). The genotyping was screened using a single base primer extension assay using ABI PRISM SNaPShot Multiplex kit (ABI, Foster City, CA, USA) according to the manufacturer's recommendations. Table 1 shows the primer sequences and annealing temperatures used for the SNaPshot assay. The polymerase chain reaction (PCR) was performed on an ABI 9700 ThermalCycler (ABI, Foster City, CA). After amplification, the PCR product was purified using shrimp alkaline phosphatase (SAP) (USB Corporation, Cleveland, OH, USA) and exonuclease I (USB Corporation, Cleveland, OH, USA). One μl of the purified amplification products was added to a SNaPshot Multiplex Ready reaction mixture containing 0.15 pM of genotyping primer for the primer extension reaction. Then, the sequences were analyzed on an ABI Prism 3730xl DNA analyzer (Applied Biosystems, USA). Analysis was carried out using Genemapper software (version 4.0; Applied Biosystems).

Statistical analysis was performed with SPSS 12.0 (SPSS Inc., Chicago, IL, USA). All values were expressed as mean ± SD. Differences in MAP, HR, and QTc interval were determined using a paired t-test. Comparisons between the two groups were analysed using Student's t-test or a paired t-test where applicable. A P value of < 0.05 was considered significant.

Results

Ninety-one of 100 patients completed this study. The nine patients were excluded from the analysis due to failed intubation on the first attempt or delayed intubation which took more than 40 sec. Ninety-one patients (38 men and 53 female, age 35.8 ± 9.1, weight 64.3 ± 10.9 kg, and height 166.6 ± 8.7 cm), were enrolled into the study.

The allelic frequencies of the mutant Gly49 and Gly389 were 12.6% and 19.8%, respectively. Table 2 shows the baseline values of MAP, HR, and the QTc interval of the β_1*AR-49* and β_1*AR-389* gene polymorphism. No differences were found in the baseline values of MAP, HR, and the QTc interval for β_1*AR-49* and β_1*AR-389*, respectively. Significant increases were detected in MAP, HR, and the QTc interval following laryngoscopy and tracheal intubation (Table 3).

When the percentage change of MAP, HR and the QTc interval was examined by genotype immediate after intubation, only in β_1*AR-49*, was the change of the QTc interval significantly greater

Table 4. Percentage Change of Mean Arterial Pressure, Heart Rate, and QTc Interval According to the Genotype of β_1-AR Polymorphisms

Characteristics	β_1-AR Ser49Gly genotype		P	β_1-AR Arg389Gly genotype		P
	Ser/Ser	Ser/Gly		Arg/Arg	Gly allele	
% Increase of MAP	14.7 ± 28.4	29.9 ± 22.2	0.067	16.6 ± 26.0	21.6 ± 30.8	0.886
% Increase of HR	33.3 ± 36.5	51.4 ± 39.5	0.347	39.5 ± 38.4	34.7 ± 37.1	0.601
% Increase of QTc	13.0 ± 7.0	9.1 ± 7.1	0.005	12.0 ± 6.9	12.1 ± 7.7	0.614

Data are presented as mean ± SD. MAP: mean arterial pressure, HR: heart rate, QTc: corrected QT.

in Ser/Ser genotypes than Ser/Gly genotypes. No differences were observed immediately after tracheal intubation in the change of MAP and HR for β_1*AR-49* and β_1*AR-389*, respectively (Table 4).

Discussion

We found that the allelic frequencies of the Gly49 and Gly389 single-nucleotide polymorphism (SNP) were 12.6% and 19.8%, respectively, which were similar to the 15% and 27% found in studies on Caucasians ($P > 0.05$) [8]. The increase in the QTc interval was greater in Ser/Ser than Ser/Gly for β_1*AR-49*, whereas no difference in the percentage change of MAP and HR was observed for β_1*AR-49* and β_1*AR-389*.

In the human heart, β_1*AR* and β_2*AR* coexist, and β_1*AR* predominates. β_1*AR* actively participates in the regulation of heart rate and contractility in cardiomyocytes. The β_1*AR* couples to the Gs-protein thereby elevating the intracellular level of cyclic AMP and causing positive inotropic and chronotropic effects, in vitro as well as in vivo [12].

Several studies have investigated a possible impact of the Ser49Gly and Arg389Gly β_1*AR* polymorphism on resting hemodynamics and hypertension, but the results were variable [13-20]. The Gly49 variant demonstrated characteristic features of a constitutively active receptor. In cells expressing the Gly49 β_1*AR*, basal and agonist-stimulated adenylyl cyclase activity was higher than that of Ser49 β_1*AR*. The Gly49 β_1*AR* was more sensitive to the inhibitory effects of antagonists, such as metoprolol, and displayed increased affinities for the agonist [13,14]. However, other studies on the phenotypic effects of the β_1*AR* polymorphism have revealed controversial data. The Gly49Gly receptor showed greater long-term agonist-promoted down-regulation than the Ser49Ser receptor and subjects with Gly49Gly had significantly lower resting heart rates than patients carrying the 1 or 2 Ser alleles [15]. In our study, the increase of the QTc interval was greater in Ser/Ser than Ser/Gly for β_1*AR-49*, which was consistent with Paavonen's findings showing that patients with the Ser49Ser genotype had a longer QT interval during exercise than patients with other genotypes [16]. Our findings suggest that Ser49 homozygotes are more active and risky alleles than the other variants in the QTc prolongation associated with endotracheal intubation.

Isoprenaline-induced adenylyl cyclase activation was 3−4 times higher in the Arg389 receptor than in the Gly389 receptor [17]. These differences were due to a greater coupling of the Arg389 receptor to the Gs-protein than was found in the Gly389 receptor. Greater inotropic and cyclic AMP responses to catecholamine were reported in Arg389 homozygotes when the dobutamine stress test was performed [18,19]. The Arg389 β_1*AR* exhibited greater short-term agonist-promoted desensitization than the Gly389 β_1*AR* [20]. However, some studies found no differences in the increase in exercise-induced heart rates and contractility in Arg389- and Gly389 β_1*AR* subjects, whereas dobutamine evoked greater increases of heart rate and contractility in Arg389- than in Gly389 β_1*AR* subjects [21]. The reason for this discrepancy in cardiac responses to exercise with dobutamine is not completely understood. However, exercise may induce more physiologic responses, which are dependent on the physical fitness of the subjects, while dobutamine infusion may induce more pharmacologic responses [22]. According to our results, no difference in cardiovascular phenotypes immediately after tracheal intubation was found in β_1*AR-389* gene polymorphism. The exact mechanism for this was not clear, but the intubation-induced cardiovascular response was more similar to exercise-induced cardiovascular change than the change due to catecholamine infusion. Our results suggest that the Arg389Gly polymorphism of the β_1*AR* made little or no contribution to the difference in cardiovascular changes from endotracheal intubation during sevoflurane anesthesia.

Our study has a limitation in that endotracheal intubation was not the only factor that influenced the QTc interval. We cannot rule out the effect of sevoflurane on the QTc interval prolongation during endotracheal intubation, although no differences in the QTc interval before endotracheal intubation were found for Ser49Gly and Gly389Arg polymorphism (data not shown). Most anesthetic agents, including sevoflurane, prolong the QTc interval [23,24], which was consistent with the results of this study (data not shown). In this study, inhalation induction with sevoflurane was performed without premedication or intravenous induction agents to avoid the complicating effect of other anesthetic drugs on the QTc interval. This is because the effect of some anesthetics on the

QTc interval remains controversial. To facilitate endotracheal intubation, vecuronium was administered, because it lacks an autonomic effect and causes no significant change in the QTc interval [25].

In conclusion, we found an association between the Ser49 homozygote gene of β_1AR-*49* polymorphism and increased QTc prolongation during endotracheal intubation with sevoflurane anesthesia. Thus, β_1AR-*49* polymorphism may be useful for predicting the risk of arrhythmia during endotracheal intubation in patients with congenital or acquired long QT syndrome.

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