

Effect of 5 HT3-Receptor Antagonists on QTc Interval Prolongation in Patients with Common NOS1AP Variant

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Abstract

Purpose: 5 HT3-receptor antagonists (5HT3RA) are frequently used for treatment and prophylaxis of postoperative nausea and vomiting (PONV) and can also produce prolongation of the QTc interval. Here, we investigate if a common SNP (rs10494366) within the human NOS1AP gene (previously associated with extremes in the QTc interval measurements) could be associated with QTc interval changes in patients who were administered 5HT3RA. **Patients & methods:** Genotyping (TaqMan real-time PCR) and electrocardiographic measurements were performed in 132 surgical patients, who obtained intraoperatively 5HT3RA (dolasteron or granisetron). **Results:** The statistically significant post-drug prolongation of QTc interval was observed for the carriers of the major (T) allele. The corresponding absolute risk increases were 0.08 in heterozygous and 0.15 in the homozygous carriers of T allele. **Conclusion:** Our data suggest that carriers of the rs10494366 major allele may demonstrate increased risk of QTc interval prolongation following administration of 5HT3RA.

INTRODUCTION

An undesirable feature of many perioperative medications is their potential to interfere with cardiac repolarization¹. Measured clinically as a prolonged QT interval on electrocardiogram (ECG), such a delay creates electrophysiological environments that favor the development of arrhythmias²³. Because of an inverse relationship between heart rate and the QT interval, for analytical purposes, cardiac repolarization time is routinely normalized to a heart rate independent or “corrected” value known as the QTc interval. An association between prolonged QTc interval and perioperative morbidity as well as mortality is well established⁴⁵.

Several therapeutic agents indicated and widely used for prophylaxis and treatment of postoperative nausea and vomiting (PONV) have been implicated in cases of prolonged QTc interval⁶⁷⁸. This represents a unique challenge in terms of perioperative management, since anywhere from 10-30% of asymptomatic individuals can demonstrate a prolonged baseline QTc interval⁶⁹¹⁰. As such, the use of selective 5-hydroxytryptamine type 3 (5-HT3) receptor antagonists (5HT3RA) should be used with caution in this patient population. However, these antiemetics are used routinely in the perioperative setting - often without extensive cardiac monitoring in otherwise asymptomatic

patients owing to a void in our ability to reliably predict which individuals will experience clinically significant QTc interval prolongation following exposure to such medications⁶¹¹.

Although a number of genetic targets associated with prolonged QTc intervals have been identified and studied, the utility of this information in terms of perioperative risk reduction is limited¹²¹³¹⁴¹⁵¹⁶. Recently, Arking et al. reported that the gene NOS1AP (CAPON), coding for a regulator of neuronal nitric oxide synthase, constitutes a new genomic target that modulates cardiac repolarization¹⁷. Individuals at extremes of the QTc interval exhibited a common single nucleotide polymorphism (SNP) in intron 1 of this gene (rs10494366), which influenced the length of the QTc interval. Furthermore, the investigators reported that approximately 60% of subjects of European ancestry in their study population carried at least one minor allele of the NOS1AP gene variant. Our goal was to investigate whether this SNP in the NOS1AP gene could affect the QTc interval changes associated with administration of 5HT3RA in surgical patients.

PATIENTS AND METHODS

Approval for retrospective, clinical study was obtained through the Institutional Review Board of the Pennsylvania

State University College of Medicine. Blood DNA samples were obtained from an existing genomic database of patients who were part of a completed protocol that had investigated the efficacy of 1 mg granisetron and 12.5 mg dolasetron for the prevention of PONV₂₀. In total, 150 patients treated with either 5HT3RA were recruited for a pharmacogenomic arm of the study. All patients included in the study were of European ancestry. As approved by our Institutional Review Board, potential study participants were contacted via telephone by the investigators and following a description of the proposed research, additional consent for the analysis of previously collected DNA samples was obtained. Subjects had agreed to such contact as an option in their participation in the previous study. In addition to the demographic and PONV-related outcomes analyzed during the previously published study, ECG recordings were collected on all subjects before and 10 min after 5HT3RA administration. This ECG data although collected, had not been extensively analyzed and/or reported in the initial study and thus, for the purposes of our current investigation, baseline and post-drug QTc intervals were evaluated. The QT interval was measured as described by Charbit et al., and was corrected for heart rate (QTc) according to Bazett's formula ($QTc = [QT] / [\sqrt{R-R}]$; where $\sqrt{R-R}$ represents the square root of the R-R interval)₁₉.

GENOTYPING PROCEDURES

Whole blood samples (approximately 0.12 mL) were spotted on IsoCode Cards (Schleicher & Schuell Inc, Keene, NH), dried overnight, placed in airtight foil bags with desiccant, and stored at room temperature until extraction. DNA analysis of the samples (in duplicate) was performed using the Assay-on-Demand™ SNP genotyping product (Applied Biosystems, Foster City, CA). Isolated DNA was incubated with two flanking primers (forward and reverse) for amplification of the sequence of interest and two TaqMan™ MGB probes for detecting specific alleles containing a fluorescent reporter dye (VIC and FAM) at the 5' end of each allele specific probe and non-fluorescent quencher at the 3' end of the probe. The real-time PCR analysis was performed using TaqMan Universal Master Mix, No AmpErase™ UNG (Applied Biosystems, Foster City, CA) in 96-well optical plate using an ABI Prism 7700 Sequence Detection System (Applied Biosystems, Foster City, CA). The amplification conditions included, holding (10 min at 95°C), followed by 40 cycles of 15 sec denaturation at 92°C, and annealing/extension at 60°C. After PCR amplification, endpoint fluorescence measurements from each well were

obtained. Based on the observed fluorescence measurements, the presence or absence of either one of two copies of the SNP rs10494366 NOS1AP T or G allele was determined in each sample.

STATISTICAL ANALYSIS

All data was entered into an SPSS spreadsheet for analysis (SPSS Version 15.0). Prolonged QTc interval was defined as a QTc > 440 msec in men and > 450 msec in women₁₉. Preliminary power analysis indicated that at least 120 patients were required in order to perform meaningful statistical calculations (power = 0.8, alpha 0.05 for clinically relevant difference in QTc of 20 msec within groups). A paired T-test was used to assess intra-group changes in QTc interval. Frequency data were compared utilizing the χ^2 test. A value of $p < 0.05$ was considered significant. In addition, the absolute risk (AR) with 95% confidence intervals was calculated for each genotype by analyzing the number of patients with prolonged QTc before and after 5HT3RA administration utilizing the following formulae:

$$AR_{TT} = \text{Prolonged QTc} \rightarrow TT_{\text{post}} - \text{Prolonged QTc} TT_{\text{pre}} \quad AR_{TG} = \text{Prolonged QTc} \rightarrow TG_{\text{post}} - \text{Prolonged QTc} TG_{\text{pre}} \quad AR_{GG} = \text{Prolonged QTc} \rightarrow GG_{\text{post}} - \text{Prolonged QTc} GG_{\text{pre}}$$

From these values, the absolute risk increase (ARI) and number needed to observe clinical effect (NNOCE) for the genotypes expressing the SNP rs10494366 NOS1AP allele (TT and TG) were calculated relative to the non-expression group (GG) utilizing the following formulae:

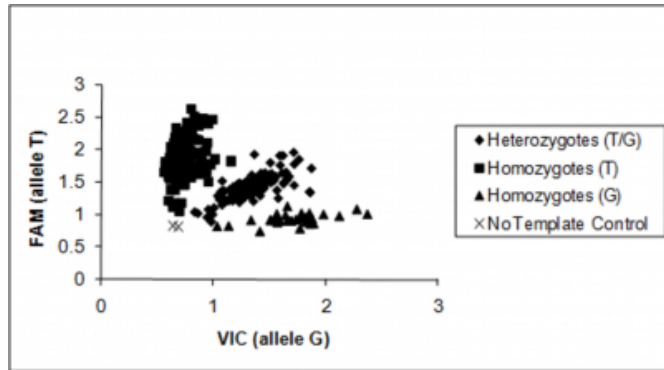
$$ARI_{TT:GG} = AR_{TT} - AR_{GG} \quad ARI_{TG:GG} = AR_{TG} - AR_{GG} \\ NNOCE_{TT:GG} = 1/ARI_{TT:GG} \quad NNOCE_{TG:GG} = 1/ARI_{TG:GG}$$

RESULTS

Out of 150 patient samples from the existing genomic database, we were able to contact and obtain additional consent for further genetic testing from 132 patients (Table 1). The TaqMan real-time PCR assay used in this study produced satisfactory allelic discrimination for rs10494366 SNP in DNA samples collected from all patients (Fig. 1).

Figure 1

Figure 1: Allelic discrimination of the investigated DNA samples for SNP rs1049366 NOS1AP using the TaqMan real-time PCR assay. Vertical axis represents relative fluorescence for FAM label (marker of T - major allele); horizontal axis represents relative fluorescence for VIC label (marker of G - minor allele).



The SNP rs1049366 NOS1AP allelic distribution was in Hardy Weinberg equilibrium (χ^2 ; $p = 0.9346$). The frequencies of allele T (major allele) and G (minor allele) were 0.63 and 0.37, respectively. The relative frequencies of the pertinent genotypes were: TT 39.4%, TG 46.9%, and GG 13.7%. Although more females than males were enrolled in the study (109 vs. 23, respectively), no statistically significant differences ($\chi^2 = 1/76$, $p = 0.845$) were observed in the gender distribution pattern among the SNP rs1049366 NOS1AP genotypes. No differences in the age, ASA status and use of granisetron vs. dolasetron were noted between investigated genotypes (Table I).

The baseline mean QTc for all investigated patients was 428.2 ± 47.2 msec.

Figure 2

Table 1: Characteristics of the 132 investigated patients obtaining 5HT3-receptor antagonists

Characteristics	Genotypes			Statistics
	TT	TG	GG	
Number of patients (N)	52	62	18	$N_{TOTAL} = 132$
Age (mean \pm SD)	49.14 ± 13.8	44.97 ± 10.3	49.5 ± 13.8	ANOVA; $p = 0.107$
Female	41 (78.8%)	54 (87%)	14 (77.7%)	χ^2 ; $p = 0.851$
Genotype frequency (%)	21	46.9	13.7	χ^2 ; $p = 0.927$
ASA status (Number of patients)				
I	6	8	6	χ^2 ; $p = 0.089$
II	37	48	9	
III	9	5	3	
Number of patients treated with granisetron	21	33	10	χ^2 ; $p = 0.324$
Number of patients treated with ondansetron	31	29	8	
Baseline QTc (sec)	0.429 ± 0.05	0.432 ± 0.04	0.419 ± 0.04	ANOVA; $p = 0.632$
Number of patients with prolonged post-drug QTc	28 (53.8%)	21 (33.9%)	5 (27.8%)	χ^2 ; $p = 0.046$
ARI (compared to GG genotype)	0.15	0.08	0	
NNOCE (compared to GG genotype)	7	13	-	

Prolonged QTc was defined as > 440 msec for men and > 450 msec for women. ARI was used to abbreviate the 'absolute risk increase' and NNOCE was used to abbreviate 'number needed to observe clinical effect.'

According to gender-referenced thresholds, a prolonged QTc interval was found in 41 of the 132 patients at baseline, representing a global percentage of 31% (95% CI: 23.1%, 38.9%). The administration of 5HT3RA produced statistically significant ($p < 0.001$ by paired t test) prolongation of the QTc to 445.3 ± 42.2 msec when measured in the entire group of patients. The global incidence of the prolonged QTc measured at 10 min after administration of 5HT3RA was 41% (95% CI: 32.6%, 49.4%). No statistically significant differences were observed in the genotypic distribution of patients with prolonged QTc interval at baseline among the observed genotypes ($\chi^2 = 2.22$, $p = 0.332$). On the other hand, the post-5HT3RA distribution of number of patients with prolonged QTc was markedly different between the observed genotypes ($\chi^2 = 6.15$, $p = 0.046$). No statistical difference was observed in the pre- and post-drug treatment mean QTc for the GG group (419 ± 45 msec vs. 437 ± 38 msec; $p = 0.059$ by paired t test), whereas statistical difference was observed between pre- and post-drug QTc mean values for the TG (427 ± 51 msec vs. 447 ± 38 msec; $p = 0.003$) and TT groups (432 ± 42 msec vs. 446 ± 37 msec; $p = 0.017$).

AR for prolonged QTc interval after the 5HT3 receptor antagonist treatment was $AR_{TT} = 0.15$ (95% CI: 0.05, 0.25),

$AR_{TG} = 0.08$ (95% CI: 0.01, 0.15), and $AR_{GG} = 0$ for groups TT, TG, and GG, respectively. Compared to homozygotes for the minor allele (GG), both heterozygotes and homozygotes for the major allele (TT and TG) demonstrated ARI for QTc prolongation post-drug administration of $ARR_{TT} = 0.15$ (95% CI: 0.05, 0.25) and $ARR_{TG} = 0.08$ (95% CI: 0.01, 0.15) respectively (since AR_{GG} in our study was found to be 0). The resulting NNOCE were calculated as $NNOCE_{TT} = 7$ (95% CI: 4, 20) for the TT genotype and $NNOCE_{TG} = 13$ (95% CI: 7, 100).

DISCUSSION

The discovery of a SNP (rs10494366) within the NOS1AP gene provides a novel genetic target to investigate the regulation of QTc intervals at the molecular level. To our knowledge, no prior studies have reported on the effect of 5HT3RA on the QTc interval in relation to the allelic variation of this SNP. Our data suggest that individuals who are homozygous or heterozygotes for the major SNP rs10494366 allele (T) are at an increased risk for developing QTc interval prolongation following the administration of granisetron or dolasetron when compared with homozygous carriers of the minor allele (G).

In reporting our findings, we have coined a relatively new term, the NNOCE or the ‘number needed to observe clinical effect. Although it is calculated in a manner consistent with ‘number needed to treat’ (NNT) or ‘number needed to harm’ (NNH), we felt that it was important to make such a distinction, since the administration of 5HT3RA in this case was not being compared to any benefit or direct harm, but rather a potential for harm (i.e., greater risk of developing prolonged QTc interval). Our data suggest that in a similar population, one in every 7 homozygotic patients and one in every 13 heterozygotic patients for the major allele (T) exposed to either granisetron or dolasetron would develop significant QTc prolongation within 10 minutes of drug administration. It is interesting to note however, that although the 95% confidence interval for NNOCE in homozygotes of the dominant (T) allele is relatively tight, a rather wide interval exists for the NNOCE in heterozygotes. This finding supports the notion that various environmental and other genetic factors may augment the risk for developing perioperative QTc interval prolongation to a greater degree in heterozygotes than in homozygotes for the dominant (T) allele.

While Arking et al. identified the presence of at least one

copy of the NOS1AP gene variant in 60% of patients of European origin, our data demonstrated that 86% of patients carried at least one copy of this allele₁₈. Selection bias may have contributed to this higher observed frequency in that the original PONV study population was comprised predominantly of middle-aged females (reflected in the current study demographics), both of which (age and sex) are independent risk factors for prolonged QTc interval₂₀₂₁.

A number of other study limitations must also be addressed in order to guide future research on the relationship between the gene variant in NOS1AP and cardiac repolarization time. First, our study was retrospective in nature and thus, prospective investigations should be undertaken to validate our findings. Second, only two 5HT3RA were evaluated, and as a consequence, our results may not be generally applicable to other drugs even within the same class. Future investigations should not only address another commonly utilized agent from this group (i.e. ondansetron), but also medications such as droperidol, metoclopramide, promethazine, prochlorperazine, and the volatile anesthetics should all be studied. Third, our genomic analysis was limited to the examination of a single variant SNP within the NOS1AP gene. Future work should examine the interplay between this newly described allelic variant and other well known mutations implicated in prolonged QTc syndromes in order to determine whether these mutations represent independent and/or synergistic risk factors for developing clinically significant QTc interval prolongation in the perioperative period.

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