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Endothelial nitric oxide synthase polymorphisms affect the changes in blood pressure and nitric oxide bioavailability induced by propofol

Gustavo H. Oliveira-Paula[a], Riccardo Lacchini[b], Lucas C. Pinheiro[a], Graziele C. Ferreira[a], Marcelo R. Luizon[a], Waynice N.P. Garcia[c], Luis V. Garcia[c], Jose E. Tanus-Santos[a,*]

[a] Department of Pharmacology, Ribeirao Preto Medical School, University of Sao Paulo, Ribeirao Preto, SP, Brazil
[b] Department of Psychiatric Nursing and Human Sciences, Ribeirao Preto College of Nursing, University of Sao Paulo, Ribeirao Preto, SP, Brazil
[c] Department of Biomechanics, Medicine and Rehabilitation of the Locomotor System, Ribeirao Preto Medical School, University of Sao Paulo, Ribeirao Preto, SP, Brazil

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ABSTRACT

Propofol anesthesia is usually accompanied by hypotension, which is at least in part related to enhanced endothelial nitric oxide synthase (NOS3)-derived NO bioavailability. We examined here whether *NOS3* polymorphisms (rs2070744, 4b/4a VNTR, rs3918226 and rs1799983) and haplotypes affect the changes in blood pressure and NO bioavailability induced by propofol. Venous blood samples were collected from 168 patients at baseline and after 10 min of anesthesia with propofol 2 mg/kg administered intravenously by bolus injection. Genotypes were determined by polymerase chain reaction and haplotype frequencies were estimated. Nitrite concentrations were measured by using an ozone-based chemiluminescence assay, while NOx (nitrites + nitrates) levels were determined by using the Griess reaction. We found that CT + TT genotypes for the rs3918226 polymorphism, the ba + aa genotypes for the 4b/4a VNTR and the CTbT haplotype were associated with lower decreases in blood pressure and lower increases in nitrite levels after propofol anesthesia. On the other hand, the TCbT and CCbT haplotypes were associated with more intense decreases in blood pressure and higher increases in nitrite levels in response to propofol. Our results suggest that *NOS3* polymorphisms and haplotypes influence the hypotensive responses to propofol, possibly by affecting NO bioavailability.

1. Introduction

Propofol is an intravenous agent extensively used for the induction and maintenance of anesthesia in several clinical settings [1]. The advantages of propofol anesthesia include rapid onset of effects, prompt recovery, and reduced incidence of nausea and vomiting [1]. However, propofol use is frequently associated with exaggerated hypotensive responses [2], which are consequences of its effects on sympathetic nervous system, myocardial contractility or vascular tone [3–5].

The vasodilation produced by propofol is related, at least in part, to increased formation of nitric oxide (NO) [6], a small gaseous and lipophilic molecule that plays a major role in vascular homeostasis [7,8]. NO is synthesized from L-arginine by at least three different synthases: neuronal (NOS1), inducible (NOS2) and endothelial (NOS3) NO synthase, which is the dominant isoform in the vasculature and is crucial for the control of blood pressure [7,8]. Interestingly, studies have shown that propofol induces NOS3 activation, possibly via phosphorylation of Ser1177, resulting in enzyme activation, NO production and vasodilation [9,10]. In agreement with these studies, we have recently reported that propofol increases nitrite levels [11], which sensitively reflect endothelial NO formation, since approximately 70% of plasma nitrite is derived from NOS3 activity [12,13]. Therefore, given the effects of propofol on NOS3 regulation, it is possible that variations in the gene encoding NOS3 affect the hypotensive responses induced by this anesthetic.

Among several polymorphisms in *NOS3* gene, the single nucleotide polymorphisms (SNPs) rs2070744, rs3918226 and rs1799983, and a VNTR in intron 4 have been widely studied due to their functional implications and clinical relevance [7,8]. Indeed, studies have demonstrated that these polymorphisms affect NOS3 expression or activity and modify the levels of markers of endogenous NO formation in humans [14,15]. Moreover, these polymorphisms influenced the susceptibility to cardiovascular and metabolic diseases, as well as the response to drugs that affect NO bioavailability [7,16,17]. Indeed, a recent study observed a lack of association of the rs2070744 polymorphism with changes in hemodynamic parameters after propofol anesthesia in Chinese subjects [18]. However, no previous study has evaluated the effects of additional *NOS3* polymorphisms and the

* Corresponding author. Department of Pharmacology, Ribeirao Preto Medical School, University of Sao Paulo, Av. Bandeirantes, 3900, 14049-900 Ribeirao Preto, SP, Brazil.
E-mail addresses: tanus@fmrp.usp.br, tanussantos@yahoo.com (J.E. Tanus-Santos).

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combined effect of haplotypes formed by these *NOS3* polymorphisms on changes in blood pressure and NO formation after propofol administration.

In this study, we examined whether the *NOS3* polymorphisms rs2070744, rs3918226, 4b/4a VNTR and rs1799983 affect the changes in blood pressure and NO bioavailability induced by propofol anesthesia. In addition, we have also examined whether *NOS3* haplotypes influence the variations in blood pressure and NO markers levels induced by propofol.

2. Materials and methods

2.1. Subjects and study design

This study was approved by the Institutional Review Board at the Ribeirao Preto Medical School, University of Sao Paulo, Brazil. All volunteers were recruited at the Endoscopy Division from the University Hospital of the Ribeirao Preto Medical School and each patient signed an informed consent.

We studied 168 patients scheduled for colonoscopy procedures. Eligibility criteria included American Society of Anesthesiologists (ASA) physical status I or II, age between 20 and 80 years and body mass index (BMI) ≤ 30 kg/m^2. We excluded patients with evidence of severe hypertension, uncontrolled respiratory, renal, hepatic and hematological diseases and history of stroke or myocardial. Subjects with hypotensive episodes requiring administration of vasopressor drugs were also excluded from this study given that the administration of these drugs affects hemodynamic parameters evaluated in the present study.

After the initial examination, a venous blood sample was collected (baseline) and propofol 2 mg/kg was administered intravenously by bolus injection. This dose is commonly required for induction of anesthesia [19] and was associated with hypotensive effects in previous studies [20,21]. Ten minutes after the induction of anesthesia, a new blood sample was collected. At each blood sampling, systolic (SBP), diastolic (DBP), and mean (MBP) blood pressure, and heart rate (HR) were recorded. Blood samples were immediately centrifuged at 1000 g for 3 min, and plasma aliquots were stored at −70 °C until analysis. To avoid any influence on hemodynamic and biochemical parameters, colonoscopy procedures commenced only after the second blood sample collection, when the study period ended. All patients were continuously monitored for cardioscopy at DII and V5, HR, blood pressure, and blood oxygen saturation until full recovery. Changes in hemodynamic and biochemical parameters were calculated by subtraction of values observed 10 min after propofol anesthesia from values observed at baseline.

2.2. Measurement of plasma nitrite and plasma nitrate concentrations

Plasma aliquots were analyzed for their nitrite content using an ozone-based reductive chemiluminescence assay as previously described [22]. Plasma NOx (nitrite + nitrate) concentrations were measured by using the Griess reaction as previously described [23]. Nitrate concentrations were obtained by subtraction of nitrite concentrations determined by chemiluminescence from plasma nitrite + nitrate levels measured by Griess reaction.

2.3. Genotyping

Genotypes for the *NOS3* rs2070744, rs3918226 and rs1799983 polymorphisms were determined by Taqman Allele Discrimination assay using real-time polymerase chain reaction (PCR). Primers and probes for the rs2070744 polymorphism were customized as follows: forward primer 5′-ACCAGGGCATCAAGCTCTTC-3′, reverse primer 5′-GCAGGTCAGCAGAGAGACTAG-3′ and probes 5′-CAGGGTCA GCC[G/A]GCCA-3´. Primers and probes for the rs3918226 polymorphism were customized as follows: primer forward 5′-AGCGT GCGTCACTGAATGA-3′, reverse 5′-ACACCCCCATGACTCAAGTG-3′ and probes 5′-CAGGAAGCT[G/A]CCTTC-3'. Primers and probes for the rs1799983 polymorphism were designed by Applied Biosystems (assay ID: C_3219460-20). TaqMan PCR was carried out in a total volume of 10 µl (5 ng of template DNA, 1× Taqman Genotyping Master Mix and 1× Taqman Allele Discrimination Assay) placed in 96-well PCR plates. Thermal cycling was performed in standard conditions and fluorescence was recorded by StepOnePlus Real Time PCR equipment (Applied Biosystems, Foster City, CA, USA). The results were analyzed with manufacturer's software. Genotypes for the 4b/4a VNTR were determined by PCR and fragment separation by electrophoresis in 8% polyacrylamide gels as previously described [23]. A representative gel to demonstrate the genotypes for this polymorphism is shown in Supplementary Figure S1.

2.4. Haplotype inference

Haplotypes frequencies were inferred using the Haplo.stats software (version 1.4.4; http://cran.r-project.org/web/packages/haplo.stats/index.html). The possible haplotypes including the rs2070744, rs3918226, 4b/4a VNTR and rs1799983 polymorphisms were: TCbG, CCbT, TCaG, CCaG, CTbT, TCbT, CCbG, CTbG, CTaT, TCaT, CTaG, CCaT, TTbG, TTaG, TTbT andTTaT. Only the haplotypes with observed frequencies > 1% were included in subsequent analysis.

2.5. Statistical analysis

Continuous data were tested for normality. Clinical and laboratory characteristics of the patients are expressed as means ± SD, whereas the changes in hemodynamic and biochemical parameters after the induction of anesthesia with propofol are expressed as means ± SEM. Deviation from the Hardy-Weinberg equilibrium was evaluated bychi squared test. The effects of *NOS3* genetic markers on hemodynamic and biochemical parameters were tested by one way ANOVA followed by Tukey post-hoc test or unpaired *t*-test. To confirm our findings, we carried out a multiple linear regression analyses to account for possible confounding factors that could influence changes in hemodynamic and biochemical parameters induced by propofol. Age, use of angiotensin converting enzyme (ACE) inhibitors, *NOS3* genotypes, and *NOS3* haplotypes were included as independent variables in multiple linear regression models to explain changes in SBP, MBP, DBP, and HR, as well as nitrite and nitrate levels after propofol anesthesia using JMP® software (SAS Institute, Cary, NC). A probability value < 0.05 was considered significant.

Given the sample size of this study, we used G*Power 3.1.9.2 [24,25] to calculate the statistical power taking into account three independent variables (age, use of ACE inhibitors and *NOS3* genotypes/haplotypes), with an α value of 0.05. Power analysis of the effects of genotypes ($f^2 = 0.15$) and haplotypes ($f^2 = 0.10$) on changes in MBP in response to propofol revealed a statistical power of 99%and 94%, respectively.

3. Results

The clinical and laboratorial characteristics of the 168 patients enrolled in this study are shown in Table 1. Propofol induced decreases in SBP, MBP, DBP ($P < 0.0001$) and HR ($P < 0.05$) and increases in nitrite and nitrate levels ($P < 0.0001$).

The genotype distributions for *NOS3* polymorphisms did not deviate from Hardy-Weinberg equilibrium ($P > 0.05$). No difference in baseline values of SBP, MBP, and DBP was found among the genotypes of each polymorphism ($P > 0.05$; Supplementary Figure S2). Fig. 1 shows the effects of *NOS3* genotypes on changes in SBP, MBP and DBP after propofol anesthesia. Interestingly, patients carrying the CT + TT genotypes for the rs3918226 polymorphism showed lower decreases in