

Evaluation of the Influence of the Codon 16 Polymorphism of the Beta-2 Adrenergic Receptor Gene on the Incidence of Arterial Hypotension and Ephedrine Use in Pregnant Patients Submitted to Subarachnoid Anesthesia

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Summary: Magalhães E, Gomes MDA, Barra GB, Govêia CS, Ladeira LCA – Evaluation of the Influence of the Codon 16 Polymorphism of the Beta-2 Adrenergic Receptor Gene on the Incidence of Arterial Hypotension and Ephedrine Use in Pregnant Patients Submitted to Subarachnoid Anesthesia.

Background and objectives: The beta-2 adrenergic receptor gene has several polymorphisms. Recent studies have demonstrated the clinical importance of the latter. The objective of the present study was to evaluate the influence of the Arg16Gli polymorphism on the incidence of arterial hypotension and ephedrine use in pregnant patients submitted to subarachnoid block for Cesarean section.

Method: Healthy parturients (ASA I and II) were submitted to subarachnoid anesthesia for elective Cesarean section (n = 50). Ephedrine was administered in cases of arterial hypotension. The incidence of arterial hypotension and the required dose of ephedrine to correct the arterial pressure were compared between the different genotypes identified.

Results: The most prevalent genotype was Arg16Gli (60%, n = 30) followed by Gli16Gli (26%, n = 13) and Arg16Arg (14%, n = 7). No differences were observed regarding the basic characteristics of the genotypes. In comparison to the Arg16Arg genotype, the Gli16Gli presented a 3.95-fold increase in the hazard ratio of arterial hypotension (95%CI 0.86-18.11; p = 0.076), whereas the Arg16Gli presented a 4.83-fold increase (95%CI 1.13-20.50; p = 0.033). The parturients with the Arg16Arg needed, on average, 6.4 ± 8.5 mg of ephedrine to correct the arterial hypotension, whereas those with the Arg16Gli needed 19.5 ± 15.9 mg (p = 0.0445; 95%CI 0.3325-25.78) and the ones with the Gli16Gli genotype, 19.2 ± 14.3 (p = 0.0445, 95%CI 0.3476-25.26).

Conclusions: The results show that the genetic variant Arg16Arg presents a lower incidence of arterial hypertension and that lower doses of ephedrine were necessary to reestablish normal arterial pressure in the patients with this genetic profile. We conclude that the Arg16Arg genotype confers better pressure stability to the parturients submitted to subarachnoid anesthesia for Cesarean section.

Keywords: ANESTHESIA, Obstetrics, Cesarean section; ANESTHETIC TECHNIQUES, Regional block: subarachnoid; COMPLICATION: hypotension; DRUGS: beta-2 adrenergic receptor.

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INTRODUCTION

In 1892, William Osler said that “If it were not for the great variability among individuals, Medicine might as well be a science and not an art.” Genetic research is providing the necessary responses to unveil this ancient enigma of Medicine and the area that studies the interactions between the genes and drug effects is called pharmacogenetics^{1,2}.

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After the disclosure of the human genome, efforts such as the Hapmap project are trying to map points of variability in the human genome, in an attempt to correlate genetic markers and the predisposition to different pathophysiological processes³. The first results of these studies have shown that we have approximately 10 million “polymorphisms” in our genome – genetic variants in which the gene with the lowest frequency occurs at least once in a population of 100 individuals. The association of some of these polymorphisms has been established for some processes such as the predisposition to some diseases⁴, protection against pathological states and the pharmacological response to drugs used in medical practice^{5,6}.

The term pharmacogenetics has been used to describe the study of the variability in the response to a drug as a result of hereditary factors². Although the term is recent, studies that assessed the similarity of a drug effect in twins or that demonstrated differences in the response to a pharmacological treatment in different populations or ethnic groups are well-known in the medical literature. Currently, studies like these try to correlate the pharmacological response with the genetic variants of the proteins involved with the drug metabolism⁷, their receptors⁸ or even proteins apparently unrelated to the drug action⁹.

The great promise of pharmacogenetics is that based on the variability observed regarding drug effects the therapy for patients with different genetic profiles can be individualized, allowing better treatment outcomes, fewer side effects and better adherence to the treatment regimen⁶. This promise is becoming a reality¹⁰.

Anesthesiology had an important role during the development of pharmacogenetics. Studies carried out in the 1950s demonstrated a low activity of plasma cholinesterases in individuals from the same family¹¹ and even the term "pharmacogenetics" was created by researchers in the anesthesia area¹².

Recent examples of pharmacogenetics applied to anesthesia include studies that assessed genetic variations of the gene of plasma cholinesterase¹³, μ opioid receptor⁸ and CYP2E1 cytochrome¹⁴ and the effects of these modifications on the pharmacological neuromuscular block, opioid drug effect and metabolism of inhaled anesthetic agents, respectively.

Maternal arterial hypotension is a common occurrence after anesthetic blocks for Cesarean sections. Several strategies, such as the uterine displacement¹⁵, infusion of variable amounts of crystalloids and colloids¹⁶, and the use of prophylactic doses of vasopressors¹⁷, have been used to prevent the deleterious effects of decreased arterial pressure on the uteroplacental perfusion and fetal well-being. However, the incidence of arterial hypotension in obstetric anesthesia remains a problem. A new strategy might arise from a pharmacogenetic approach.

The control of arterial pressure and cardiac output depends largely on the activity of beta-2 adrenergic receptors (ADRB2). When activated, the ADRB2 receptor initiates a cell signaling cascade through the G-protein, resulting, among other effects, in smooth muscle relaxation, in order to produce bronchodilation and arterial vasodilation. The ADRB2 receptor gene has been identified as being polymorphic, having at least 10 variations in its genetic sequence¹⁸. Some of these polymorphisms have been identified as being functionally relevant for the arterial pressure control. Among the described polymorphisms, the variation between arginine or glycine in position 16 (Arg16Gly-rs1042713) seems to be the most important one¹⁹.

The effects that these variants of the beta-2 adrenergic receptor can have on the hemodynamic control have yet to be fully defined. A study reported that young, healthy individuals who were homozygous for the Arg16 receptor presented a higher vasodilating response to specific beta-2 agonist and higher counterregulatory sympathetic effects when compared to individuals with the Gly16 receptor²⁰. Another study showed a tendency of individuals homozygous for the Arg16 variation to present higher basal pressure levels when compared to other genotypes of the receptor²¹.

Studies in parturients carrying different isoforms of the beta-2 adrenergic receptor did not show any difference in the incidence of arterial hypotension between the groups, but patients who were homozygous for the Gly16 isoform needed a significantly lower amount of vasopressor drugs to control the arterial pressure²².

Based on the aforementioned studies, which suggest a higher vasodilating activity of the Arg16 isoform when stimulated by an agonist and probably a higher dependence of

the counterregulatory sympathetic stimuli to maintain normal pressure levels, the hypothesis of our study is that individuals with the Arg16 genotype will present a higher incidence of arterial hypotension when compared to the ones with the Gly16 genotype, after the administration of the subarachnoid anesthesia. As the subarachnoid anesthesia results in a sympathetic block, higher vasodilation will probably occur in those patients that depend on a higher basal sympathetic activity.

The objective of the present study was to evaluate the influence of the polymorphism in codon 16 of the beta-2 adrenergic receptor on the incidence of arterial hypotension and the use of ephedrine in pregnant patients submitted to subarachnoid block for Cesarean section.

METHOD

After the study was approved by the Research Ethics Committee of Universidade de Brasilia (REC/UnB) 50 patients treated at the obstetric centers of Hospital Universitário de Brasília (HUB) and Hospital Regional da Asa Sul (HRAS) who received subarachnoid block for elective Cesarean section from January to May 2008 were included in the study after signing the Informed Consent Form.

The patients were assessed regarding the hemodynamic effects of the lower spinal anesthesia through the variations in heart rate and systemic arterial pressure and had their genotype of the beta-2 adrenergic receptor determined.

The exclusion criteria were: gestational age < 37 weeks, twin pregnancy, systemic arterial hypertension, preeclampsia and eclampsia, cardiovascular diseases, ASA physical status III and IV, weight > 130 kg and use of corticosteroids, magnesium sulphate, adrenergic agonists or antagonists during pregnancy.

The patients were monitored through noninvasive arterial pressure (NIBP), continuous electrocardiography (ECG) and saturation of peripheral oxygen (SpO₂) and received intravenous hydration with 2000 mL of Ringer's lactate.

The lower spinal anesthetics were carried out with the patients in the sitting position, between the intervertebral spaces L₃-L₄ or L₄-L₅, using a 25G or 27G Quincke needle, and 0.5% hyperbaric bupivacaine (10 mg) and sufentanyl (3 μ g) were administered. Immediately after, with the patients in the supine position and the operating table on the horizontal position, the level of sensory loss was registered through the pinprick test.

The arterial pressure was measured in the upper limb every three minutes. The incidence of arterial hypotension was defined as a decrease of 20 mmHg in systolic pressure or 20% in the mean basal arterial pressure (mean of three consecutive measurements obtained before the spinal anesthetics was administered with the patient on left lateral decubitus).

The treatment of the arterial hypotension was carried out with ephedrine (concentration of 5 mg.mL⁻¹) with an initial dose of 10 mg and subsequent doses of 5 mg until the pressure response was obtained.

At the end of the surgical procedure, a sample of venous blood (4 mL) was obtained and stored in tubes containing EDTA. DNA was extracted from peripheral blood leukocytes using the

CHELEX-100™²³ method, in which 1 mL of milliQ water was mixed with 50 µL of whole blood and then centrifuged for 3 minutes. After the supernatant was discarded, this procedure was repeated once. Afterward, 200 µL of 5% suspension of CHELEX-100™ was added and, after homogenization, the sample was incubated at 56°C for 30 minutes, at 100°C for 8 minutes and centrifuged for 1 minute at room temperature. The supernatant was collected and stored at -20°C for posterior use.

This DNA was used as substrate for the amplification of the ADBR2 gene, using a pair of ADBR2 5 primer oligonucleotides (5'-CACCCACACCACAGCCGCTGAATGA-3') and ADBR2 6 (5'-ATGACCAGATCAGCACAGGCCAGTGAAGT-3'). The amplification reaction consisted of 5 µL of DNA, 0.2 mM of each deoxynucleotide (dNTP), 50 ng of each primer oligonucleotide, 1.25 U of Taq DNA polymerase enzyme (Fermentas Inc., Hanover, MD, USA), reaction buffer and magnesium sulphate, resulting in a final volume of 50 µL. The amplification was carried out in a TC-312 thermocycler (Techne, Inc. Burlington, NJ, USA), with initial denaturation at 94°C for 3 minutes, followed by 35 cycles of 94°C for 40 seconds and 68°C for 1 minute, ending with a final extension of 5 minutes at 72°C. To confirm the amplification of the fragment of interest (367 base pairs), the PCR products were submitted to 1% agarose gel electrophoresis and stained with ethidium bromide (0.5 µg.mL⁻¹) and visualized under ultraviolet light.

The products of the amplification were purified by an enzymatic method ("EXO-SAP" system) and 1 U of exonuclease I (Biolabs) and 1 U of shrimp alkaline phosphatase (Promega) were added to a 10 µL volume of the PCR product. The product was incubated at 37°C for 30 minutes and then at 80°C for 20 minutes. To the purified PCR products were added 50 ng of the oligonucleotides of interest (sense or antisense) and the material was sent to sequencing. The resulting sequence from each sample was analyzed to identify the Arg16Gly polymorphism after alignment with the reference sequence rs1042713 (http://www.ncbi.nlm.nih.gov/SNP/snp_ref.cgi?rs=1042713) using the Sequencher software (Gene Codes Corporation).

After the sequencing, the patients were allocated to 3 groups with different genotypes. For the statistical analysis, the assessment of the normal distribution was carried out using the Kolmogorov-Smirnov test; the analysis of parametric data was carried out using Student's *t* test and Analysis of Variance (ANOVA) and the non-parametric data were analyzed using the Chi-square test. The survival curves were determined by the log-rank test (Mantel-Cox) and the risk ratio by Cox regression test. The level of

significance was set at $p < 0.05$. The SPSS and GraphPad Prism software for Windows were used for the statistical analysis.

RESULTS

The prevalent genotype was Arg16Gly (60%, $n = 30$), followed by the homozygous Gly16Gly (26%, $n = 13$) and Arg16Arg (14%, $n = 7$). The general characteristics (age, gestational age, weight, height and BMI), basal systolic arterial pressure and mean arterial pressure and the level of the sensory block did not show statistically significant differences between the genotypes (Table I).

The general incidence of arterial hypotension was 76% ($n = 38$), and the incidences in the groups were 77% ($n = 10$) for Gly16Gly, 83% ($n = 25$) for the Arg16Gly and 43% ($n = 3$) for the Arg16Arg s (Figure 1). However, there was no statistically significant difference between the groups ($p = 0.0778$). The variation curve of the systolic arterial pressure when compared to basal values within the first 30 minutes after the block showed that the Arg16Arg genotype showed a significantly lower decrease in the arterial pressure throughout time when compared to the other genotypes ($p < 0.05$) (Figure 2). The analysis of the variation curve of the mean arterial pressure showed similar results. The comparison of the survival curves in which the incidence of arterial hypotension between the genotypes throughout time after the anesthetic block was studied showed significant differences between the genotypes (log-rank = 6.302, $p = 0.043$). Compared with the group with the Arg16Arg genotype, which presented the lowest incidence of arterial hypotension, the group with the Gly16Gly genotype presented a 3.95-fold increase in the incidence (95%CI 0.86-18.11; $p = 0.076$), whereas the Arg16Gly genotype presented a 4.83-fold increase (95%CI 1.13-20.50; $p = 0.033$) (Figure 3).

The mean dose of ephedrine necessary to correct the hypotension after the anesthetic block in the 50 patients was 17.5 mg (SD \pm 15.1), also including the patients that did not need ephedrine. The doses used in the different groups are shown in Table II. The comparison of the doses used between the groups by ANOVA did not show statistical significance ($p = 0.108$). However, when the group that needed less ephedrine (Arg16Arg) was compared individually with the Arg16Gly and Gly16Gly groups, a statistically significant difference was observed regarding the doses used ($p = 0.0445$, 95%CI 0.3325-25.78 and $p = 0.0445$, 95%CI 0.3476-25.26, respectively) (Figure 4).

Table I – General Characteristics of the Patients

Characteristics	Gly16Gly (26%, $n = 13$)	Arg16Gly (60%, $n = 30$)	Arg16Arg (14%, $n = 7$)	<i>p</i>
Age (years)	26.1 \pm 8.2	24.2 \pm 5.8	23.4 \pm 4.6	0.5681
Gestational age (weeks)	39.3 \pm 1.4	39.6 \pm 1.2	39.3 \pm 1.3	0.7322
Weight (kg)	70.1 \pm 11.5	72.4 \pm 11.7	68.1 \pm 6.4	0.6153
Height (cm)	161.5 \pm 7.4	159.7 \pm 7.2	157.4 \pm 6.9	0.4765
BMI (kg.m ⁻²)	27.8 \pm 3.2	28.5 \pm 5.2	27.5 \pm 2.3	0.5013
Level of block (T ₃ -T ₄ /T ₅ -T ₆)	7/6	13/16	2/5	0.5557
Initial SAP (mmHg)	128.1 \pm 18.3	118.9 \pm 14.1	120.3 \pm 4.8	0.2077
Initial MAP (mmHg)	89.9 \pm 15.1	84 \pm 12.9	87 \pm 10.6	0.4142

BMI= body mass index; SAP = systolic arterial pressure; MAP = mean arterial pressure.

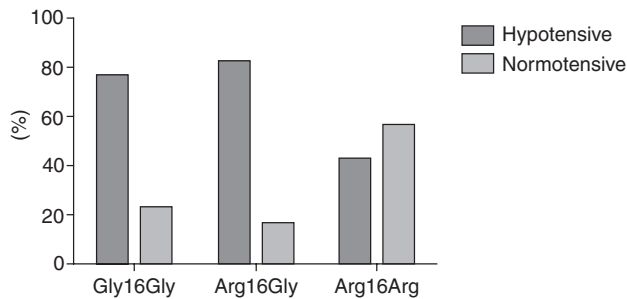


Figure 1 – General Incidence of Arterial Hypotension.

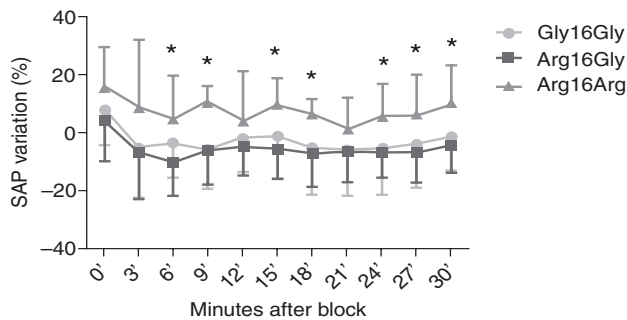


Figure 2 – Variation Curve of Systolic Arterial Pressure.

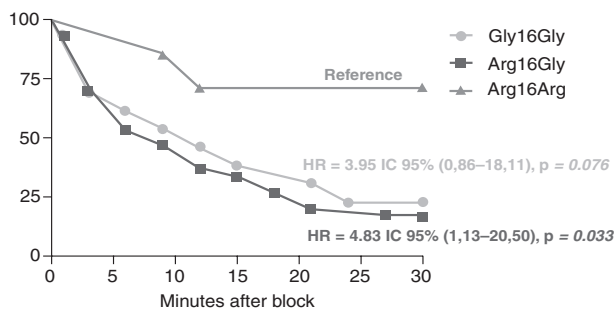


Figure 3 – Survival Curves of Arterial Hypotension Incidence.

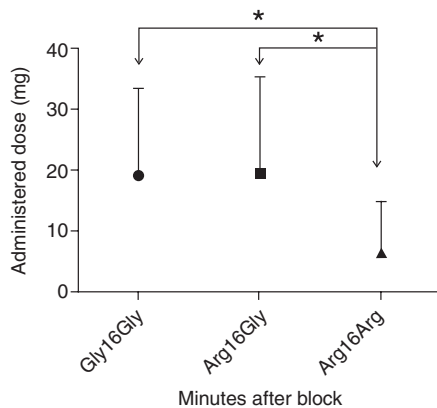


Figure 4 – Individual Comparison of Arg16Arg with Arg16Gly and Gly16Gly.

Table II – Ephedrine Dose (mg) Necessary to Maintain Normal Arterial Pressure After the Anesthetic Block

Genotype	Dose (mg)
Gly16Gly	19.2 ± 14.3
Arg16Gly	19.5 ± 15.9
Arg16Arg	6.4 ± 8.5

DISCUSSION

The studied population presented a high incidence of arterial hypotension which was observed in 38 of the 50 studied patients (76%). Such result is similar to the incidence of arterial hypotension in pregnant women submitted to lower spinal anesthesia for Cesarean section found in the literature^{24,25}.

The genetic analysis showed that 74% of the patients (n=37), homozygous and heterozygous, presented a modification that causes the substitution of arginine for glycine at position 16 of the receptor protein. Studies carried out in populations from the USA showed that among Caucasian individuals the prevalence of the arginine variant is 39%, whereas among African-Americans it is 50%. These values are lower than those observed in the patients of the present study²¹. This fact is probably due to the interbreeding of the Brazilian population. The more genetically diversified a population is, more relevant are the results that associate the presence of a physiological characteristic to a genetic modification. Therefore, the studied population presents an interesting characteristic regarding the genetic studies and especially the studies of polymorphisms of codon 16 of the beta-2 adrenergic receptor. Among the studied genotypes, the patients who were Arg16Arg homozygotes presented a lower degree of hemodynamic instability after the anesthetic block, characterized by a lower incidence of arterial hypotension, less marked decrease in pressure levels after the anesthetic block and lower doses of ephedrine necessary to maintain normal pressure.

Surprisingly, the results obtained did not correspond to those of a similar study carried out in a population of pregnant women in the US²² in which patients with the Gly16Gly genotype presented lower hemodynamic instability and less need for vasopressor to maintain normal pressure.

Some hypotheses can be formulated to explain these facts. The sample size was not calculated, as the observational design of the study did not allow the previous characterization of the genotypic distribution of the selected population. Thus, the sample might have been insufficient to demonstrate the actual association between the polymorphism and the patients' hemodynamic regulation. Another hypothesis is that the differences regarding the genotypic distribution found in this study and those observed in other studies might indicate that the populations have very different genetic profiles. The presence of non-assessed genetic differences (other genes that can influence pressure regulation) might explain the different results.

We conclude that in the Brazilian population the homozygous presence of the polymorphism at codon 16 of the beta-2 adrenergic receptor with the Arg16Arg genotype possibly confers better hemodynamic stability to pregnant patients submitted to subarachnoid block for Cesarean section. However, further studies are necessary to evaluate the degree of the association between the studied polymorphism and the hemodynamic regulation after lower spinal anesthetics in pregnant patients and its effect on arterial hypotension therapy.