EFFECTS OF CLONIDINE, ISOFLURANE, AND THE CLONIDINE+ISOFLURANE ASSOCIATION IN ISOLATED HEARTS

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ABSTRACT

Background: Isoflurane has been consecrated as an anesthetic drug of choise in heart surgeries, as it presents characteristics that ensure the preservation of cardiac indexes and myocardial stability. Recently, alpha-adrenergic agents, mainly Clonidine, have been added to this anesthetic arsenal in an attempt to prevent adrenergic discharges, increase cardiac stability, reduce myocardial ischemia and improve anesthetic induction and recovery.

Objective: The aim of this study was to evaluate the cardiovascular effects of Clonidine, Isoflurane, and Clonidine+Isoflurane association in isolated hat hearts preparations, as well as to evaluate direct heart effects of the clonidine, possibly masked by its central action.

Method: This study used twenty four, male, albino, Wistar rats from the Biotherium of the Federal University of Minas Gerais (UFMG). The animals were anesthetized with 100 mg of ketamine + 10 mg of xylazine intraperitoneally, and, after a full thoracotomy, their hearts were isolated and placed in coronary perfusion using a Krebs-Henseleit nutrient-rich solution, according to the Langerdorff method. The parameters of Heart Rate, Systolic Blood Pressure, Coronary Flow, and Myocardial Contractility were evaluated at times of O, 1, 2, 3, 5, 10, and 15 minutes.

Results: Heart rate – No statistically significant difference was observed among the Clonidine, Isoflurane, Clonidine+Isoflurane, and Control groups. Systolic Blood Pressure – No statistically significant difference was identified among the Clonidine, Clonidine+Isoflurane, and Control groups. In the group that received only Isoflurane, the systolic blood pressure proved to be equal to the control group and presented, on average, 18.3 more units than did the Clonidine group. In the groups that received Clonidine+Isoflurane, the systolic blood pressure was, on average, 22.5 units less than the

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control group and, on average, 32.7 units less than the group that received only Isoflurane. Coronary Flow – No statistically significant difference was found regarding the coronary flow among the Clonidine, Isoflurane, Clonidine+Isoflurane, and Control groups. Myocardial Contractility – No statistically significant change was found in this parameter among the Clonidine, Isoflurane, Clonidine+Isoflurane, and Control groups.

Conclusion: Clonidine, when used in an isolated manner, produced no significant effect on the hemodynamic behavior of the isolated rat hearts. When used in association with Isoflurane, Clonidine was capable of diminishing the effects of this drug, demonstrating an apparent protective effect upon the heart. The observed effects occurred directly upon the heart, considering that this study was conducted on isolated hearts, that is, with no connection to the central nervous system.

INTRODUCTION

Among the recently developed halogenated anesthetics, Isoflurane has stood out for its use in heart surgeries, as it presents great reliability and characteristics that can improve myocardial stability. Isoflurane was synthesized in 1965 in the United States by Dr. Ross Terrel during his research aimed at identifying an anesthetic drug with properties that were superior to halothane and other such inhaled anesthetics of the day. This drug consists of 1-chloro-2, 2, 2-trifluoroethyl-difluoromethyl ether (CF3 CHCL-O-CHF2), a formula that ensures high grade stability. While 56% of halothane will break down in six months, when exposed to a strong base, Isofluane undergoes no reaction when exposed to the same base over the same period of time. This stability eliminates the need for a preservative, as is the case with halothane, which requires the addition of butylated hydroxytoluene (BHT), which can leave residues inside the vaporizer and result in small quantities of decomposing toxic products when exposed to soda lime or ultraviolet light.[1] Molecular structure and *in vivo* stability are of utmost importance for the metabolic standards of volatile anesthesia, and Isoflurane has proven to be the most stable in this respect.[2] Rivenes et al.,[3] in their research on congenital heart defect patients, showed the cardioprotective effects of Isoflurane concerning the preservation of cardiac indexes and myocardial contractility in basal values. Johnston et al.,[4] stated that the greatest advantage of Isoflurane is that it does not sensitize the myocardium to clinical doses of catecholamine. Three to five fold higher doses of adrenaline are necessary to produce arrythmias when using Isoflurane, as compared to halothane.

Moreover, Linde et al.[5] reported that Isoflurane has proven to be a safe drug, especially in elderly patients.

Recently, alpha-adrenergic pharmacos, whose most well-known examples are Clonidine and Dexmedetomidine, have been added to the existing anesthetic arsenal, with additional advantages due to high selectivity to alpha receptors, with preference for Clonidine due to its pharmacological and economic advantages. The use of these agents is intended to prevent adrenergic discharges, increase cardiac stability, reduce myocardial ischemia and improve anesthetic induction and recovery. Clonidine is an alpha-adrenergic agent that is 200 times more specific than adrenaline for alpha-2 receptors. It is able to reduce the central discharges of adrenaline in the synaptic cleft, at the brainstem level, mainly in the locus coeruleus.[6] It can also act by following an anti-nociceptive pathway, which originates in the brainstem, contributing to control the pain, suppressing the centripetal transmission of impulses. This pathway is activated by stimulating the locus coeruleus and the dorsal raphe nucleus, which is mediated by the release of noradrenaline. Clonidine diminished the variations in hemodynamic responses to intubation and during surgery itself, reducing the quantity of anesthetics in up to 40%. Its use reduces the plasmatic concentration of postoperative noradrenaline.[7] It has been used both as an anesthetic adjunct and in the treatment of chronic pain, because of its anesthetic and analgesic activities.

One of the main objectives of anesthesia, especially in those patients at risk of suffering ischemia during surgery, is to maintain the balance of myocardial oxygenation. This can be achieved by diminishing the sympathetic activity and the hyperdynamic response. The ability of alpha-2 adrenergic drugs in modulating the sympathetic tonus leads to a desirable hemodynamic profile that can aid in maintaining the balance between the demand for and supply of myocaridal oxygenation.[8,9,10]

In addition, a significant reduction in perioperative ischemia was also observed, by monitoring the ST segment depression, in patients who have undergone cardiac revascularization and received 5 mcg/kg of Clonidine.[11,12] De Kock et al.[13] reported a reduction in hemodynamic events in 350 patients who had undergone abdominal operations and who received 4 mcg/kg of intravenous clonidine upon the induction of anesthesia, followed by an infusion of 2 mcg/kg/h. Bernard et al.[14] demonstrated that pre-medication with clonidine diminished undesirable effects seen during anesthetic recovery, in which alpha-adrenergic drugs played an important role in patients with a high anesthetic risk of myocardial ischemia. Honarmand and Safavi[15] reported that the oral intake of Clonidine, in the preoperative stage (3mcg/k), significantly prevents the increase in systemic blood pressure

and heart rate, induced by the use of a tourniquet, in general anesthesia with Isoflurane. Patients who received a prior dose of Clonidine presented an important reduction in the need for Isoflurane to maintain an adequate anesthetic plan.[16]

Isoflurane has been widely used in heart surgeries, even in patients with poor cardiovascular conditions. Clonidine was recently added to the anesthetic arsenal in an attempt to improve hemodynamic stability and diminish side effects. It is a well-known fact that the effects of a combination of drugs can be greater than that expected from the mere addition of the same drugs.[17]

OBJECTIVE

The present study aims to evaluate the cardiovascular effects of Clonidine, Isoflurane, and the Cloridine+Isoflurane association in isolated rat hearts preparations. This study also aims to evaluate possible direct cardiac effects of clonidine, which can be masked by its central action.

METHOD

This study used 24 male albino Wistar rats, from the Biotherium of the Federal University of Minas Gerais (UFMG), with an average body weight of 363.4 ± 25.9 g. The weight of the studied hearts varied between 0.95 and 1.84 grams, with an average of 1.21 ± 20.0 g.

This work followed the guidelines set forth by the Brazilian College of Animal Experimentation (COBEA), and the Guide to the Care and Use of Experimental Animals.

The animals were anesthetized with the association of 100 mg of Ketamine + 10 mg of Xylazine, administered intraperitoneally. After the animal had been immobilized on an appropriate surgical table, a full thoracotomy was performed, with the sectioning of all costal arches at the midaxillary line and elevation of the sternal portion in the cephalic direction, for complete exposure of the intrathoracic organs. 500UI of heparin sodium was injected into the posterior vena cava in an attempt to promote anti-coagulation. The cardiac basal vessels were dissected and the aorta was repaired with a 2.0 cotton thread. The aorta was opened and catheterized with an appropriate 22 G caliber metallic device, maintaining the tip of the catheter above the aortic valve, in order to ensure coronary perfusion. A second 18 G caliber fenestrated plastic catheter was introduced into the left atrium, passing through the mitral

valve and exiting at the tip of the left ventricle, in order to drain these chambers and serve as a support while removing the heart. During these procedures, special attention was given to avoid lesions to the other cardiac structures. After sectioning the cardiac basal vessels, above the catheter introduced into the aorta, the heart was removed and placed in a coronary perfusion system, following the Langendorff method.

For the perfusion of the coronary arteries, the present study used the Krebs-Henseleit Nutrient-Rich Solution, gasified with 95% oxygen and 5% carbon gas, obtaining an average pH of 7.35±0.05. The perfusion pressure was maintained at a constant level of 90 cm column of water and at a temperature of 37.5ºC±0.5ºC, using a heating and circulation water system (NOVUS N960). Perfusion was performed equally in all hearts for 20 min, using only a nutrient-rich solution. This procedure was conducted in order to obtain the recovery and hemodynamic stabilization of the hearts from the surgical trauma. Electrodes were placed in the myocardium to measure the heart rate and a flexible stem catheter balloon[18] was placed inside the left ventricle to determine the systolic blood pressure. After having completed these procedures, the chosen parameters chosen for this study were measured at the following times: t0 – immediately after the mentioned 20 min; t1 – 1 min later; t2 – 2 min later; t3 – 3 min later; t5 – 5 min later; t10 –10 min later; and t15 –15 min later.

The animals were divided into four study groups:
Group I – Control (n = 6) hearts were perfused with only a nutrient-rich solution, with a constant perfusion pressure of 90 cm of H_2O and at a temperature of 37.5ºC±0.5ºC.

Group II – Clonidine (n = 6) – hearts were perfused with 2 mcg of Clonidine diluted in 1 ml of nutrient-rich solution and injected into the ascending aorta for 1 min under constant perfusion pressure of 90 cm of H_2O and at a temperature of 37.5ºC±0.5ºC.

Group III – Isoflurane (n = 6) – hearts were perfused with 1.5 MAC (Minimal Alveolar Concentration) of Isoflurane diluted in a nutrient-rich solution and injected into the ascending aorta for 1 min under constant perfusion pressure of 90 cm of H_2O and at a temperature of 37.5ºC±0.5ºC.

Group IV – Clonidine+Isoflurane association (n = 6) – hearts were perfused simultaneously with 2 mcg of Clonidine, diluted in 1 ml of nutrient-rich solution, and 1.5 MAC of Isoflurane,

diluted in a nutrient-rich solution and injected into the ascending aorta for 1 min, under constant perfusion pressure of 90 cm of H_2O and at a temperature of 37.5ºC±0.5ºC.

All of the animals were submitted to the same procedures until the beginning of the tests, as described above.

The present study analyzed the variables of Heart Rate (HR), through the electrodes implanted directly in the myocardium; systolic blood pressure (SP), through the intraventricular flexible stem catheter balloon; coronary flow (CF), through the collection and measurement of the nutrient-rich solution from the coronary sinus; and myocardial contractility (MC), by measuring the maximum speed of the rise in intraventricular pressure (dP/dt).

The data were statistically analyzed using the Univariate Analysis Method, by the software R of public domain. All analyses used a significance level of 5%.

RESULTS

The results showed:

Regarding Heart Rate.

No statistically significant difference was identified ($p<0.001$), regarding the HR, among the Clonidine, Isoflurane, Clonidine+Isoflurane, and Control groups (Fig. 1).

Regarding Systolic Pressure.

In the groups that received Clonidine, the SP showed no statistically significant difference from the Control and Clonidine+Isoflurane groups ($p > 0.05$).

In the groups that received only Isoflurane, the SP showed no statistically significant difference from the Control group ($p > 0.05$) and presented, on average, 18.3 more units than did the group that received only Clonidine.

In the Clonidine+Isoflurane group, the SP presented, on average, 22.5 units less than did the control group and, on average, 32.7 units less than did the group that received only Isoflurane.

Thus, it can be concluded that the isolated hearts submitted to treatment with Isoflurane present a greater SP than do the Clonidine and Clonidine+Isoflurane association groups. It was also observed that the hearts in the Clonidine+Isoflurane association group presented a lower SP than did the hearts from the Control and Isoflurane groups (Fig. 2).

Regarding Coronary Flow.

No statistically significant difference was identified ($p > 0.05$) regarding CF among the Clonidine, Isoflurane, Clonidine+Isoflurane, and Control groups (Fig. 3).

Regarding MC (dP/dt).

No statistically significant difference was identified ($p>0.05$) in the measurement of the dP/dt among the Clonidine, Isoflurane, Clonidine+Isoflurane, and Control groups (Fig 4).

These results show that clonidine can intensify the Isoflurane potential, because when it was associated with Isoflurane, this association presented results that were different from those presented by these same drugs, when used in an isolated manner.

By contrast, the results illustrate that these pharmacos, when used in association, can act directly upon the heart, due mainly to the fact that this study was conducted on isolated hearts, that is, devoid of any link to the central nervous system.

DISCUSSION

The different aggressions that a patient can suffer are reflected in the production of high levels of adrenaline. The adrenaline acts in the adrenergic receptors, stimulating the sympathetic system, thus leading to a new adrenaline discharge.

The peak of adrenergic stimulation is also known as the "Cannon Reaction"[19] or the "Fly or Fight Situation". In this condition, peripheral vasoconstriction leads to an accentuated pallor due to the deviation of blood to the muscles and brain. The cardiac debt is increased, mainly due to the reflex tachycardia and the release of endogenous catecholamines. In the respiratory system, bronchodilation and tachypnea appear, which are necessary to increase blood oxygenation. This reaction is systemic and usually present before, during, and after surgeries. It is mediated by the "Reticular Formation".

The Reticular Formation consists of a series of neurons distributed sparsely throughout the brain stem and constitute one of the oldest brain structures, whose primordial function is to alert the animal when in the face a danger, preparing it to escape.

To accomplish this, the Ascending Reticular Activating System (ARAS) is used, which places the brain on maximum alert. One of the nuclei of the Reticular Formation is specifically involved in this task, the "Locus Coeruleus". Located on the floor of the fourth ventricle, this structure contains the greatest concentration of adrenaline release cells and is capable of modulating the level of consciousness through the large quantity of alpha-2 adrenergic receptors.[20] These receptors are normally occupied by adrenaline; however,

Clonidine can also occupy them with a selectivity of 200 times that of adrenaline. In this manner, Clonidine can impede the action of adrenaline, blocking the sympathetic receptors and reducing stress. The blocking of the sympathetic discharge diminishes anxiety by reducing the plasmatic concentration of perioperative adrenaline. This combination of factors creates favorable hemodynamic conditions and improves the supply of oxygen to the myocardium.

These data show that, when acting centrally, Clonidine can protect the heart. However, there are still many questions related to these effects, mainly concerning its location of action. The bradycardia that occurs subsequent to the use of clonidine can be a consequence of its central action but it can also be a result of a direct Clonidine heart effect. Some authors considered that Bradycardia, commonly seen after the administration of alpha-2 adrenergic drugs, can occur due to the sympatholytic action of these agents, leading to an increase in vagal activity or to be a result of the pre-synaptic reduction of the release of noradrenaline or even due to direct parasympathomimetic action. Angus et al.[21] believe that the alpha-2 adrenergic receptors release a relaxing factor in the endothelium but that their effects on human coronary blood vessels are still rather unknown. Nakane et al.[22] investigated the responses to alpha-adrenergic drugs in the isolated coronary arteries of dogs, using the inserted cannula method, and reported that Clonidine produced light vasoconstriction, not related to the dose. By contrast, vasodilation was observed when Clonidine was applied in extremely large doses.

Recently, researchers have found alpha-2 adrenergic receptors in heart tissues. Sohngen et al.,[23] studying dogs that had been anesthetized and pre-treated with propranolol to block the beta-adrenergic agents, reported a coronary vasodilation induced by Clonidine, suggesting its direct action upon the alpha-2 receptors of the coronary arteries. In a study on isolated rat hearts, Mukaddam-Daher et al.[24] demonstrated that Clonidine acts directly upon the heart, reducing the CF during bradycardia, while Akers et al.[25] reported that the administration of Clonidine significantly reduced the cardiac release of norepinephrine.

As regards Isoflurane, the majority of studies have shown that this agent produces a cardioprotective effect. Johnston at al.[26] claimed that the greatest advantage of Isoflurane is that it does not sensitize the myocardium to clinical doses of catecholamine. Three to five fold higher doses of adrenaline are necessary to produce arrhythmias with Isoflurane, as compared to halothane, whereas.

In the present study, Clonidine, Isoflurane, and the Clinidine+Isoflurane association showed no change in HR among these groups or in the Control group at any of the evaluated times.

Badoer et al.[27] observed a reduction in blood pressure, in the cardiac output, and in the peripheral resistance when using Clonidine, most likely due to the reduction in the sympathetic tonus and increase in the vagal activity. According to these authors, the potential specific receptors, responsible for the hypotensive action of Clonidine, as well as is its localization, are still uncertain. These authors suggest the possibility of Clonidine diminishing the common point around which blood pressure is normally regulated and increasing the baroreceptor activity.

The choice of Isoflurane for patients with coronariopathy is due to the protective action exerted upon the myocardium. In a study with a group of volunteers, It was reported a reduction in both blood pressure and peripheral vascular resistance, yet maintaining the cardiac output, in turn guaranteeing for the increase of the HR. Its systemic effects were observed, yet with a significant increase in the left ventricular ejection time, most likely due to the reduction in the HR.[28]

In the present investigation, the isolated use of Clonidine produced no changes in the SP. The isolated use of Isoflurane increased the SP, when compared to the control group, and when Clonidine was associated with Isoflurane, this association significantly reduced the SP. These results demonstrate that Clonidine can attenuate the effect of Isoflurane on SP. The results also show a direct action upon the heart, since the effects were found in isolated hearts, that is, devoid of any connection to the central nervous system

Related to the effect of Clonidine on CF, the results are not as similar, most likely due to the lack of uniformity in the different study designs. Sohngen et al.[29] described a coronary vasodilation; while Chlopicki et al.[30] affirmed that the role of alpha-2 receptors in the coronary bed is still unclear. Regitz et al.[31] described a self-regulation of the beta receptors in denervated hearts, while Finkel et al.[32] suggested that the same phenomenon can occur with alpha-2 adrenergic receptors. Crystal[33] reported that the coronary vasodilator effect of Isoflurane was considerably greater than that of enflurane, halothane, sevoflurane, and desflurane, whereas Coetzee et al.,[34] studying isolated rat hearts submitted to cardioplegia and reperfusion, also reported a cardioprotective effect resulting from the use of Isoflurane. Agnew et al.[35] reported that Isofulrane protects the myocardium, limiting ischemic areas and enhancing functional recovery.

In the present study, the isolated use of Clonidine, Isoflurane, and the association of these two agents showed no changes in CF among these and the Control group at any of the studied times.

Studies related to the behavior of MC when using Clonidine generally point to the reduction of inotropism. Hermiller et al.[36] reported a reduction in the isovolumetric ventricular time of contraction and an increase in the pre-ejection period, while Hamilton,[37] despite reporting a significant reduction in contractility when using alpha-2 adrenergic agents, refers to a clonidine as a drug exempt from collateral effects on the heart.

Regarding Isoflurane, the results are more consistent and suggest a reduction in inotropism and chronotropism. Kemmotsu et al.[38] studied papillary muscles of felines and found in vitro depressor effects when using Isoflurane. These authors reported a reduction in both the frequency and the contractile strength, wich is dose dependent, and affirmed that the effect was more evident in muscles that had been removed from hearts with artificially provoked congestive heart failure. Merim[39] showed that Isoflurane decreases the left ventricular first derivative of blood pressure in function of the time (dP/dt), however with a maintenance of output, due to the decrease in the HR and vasodilation.

In the current study, no significant changes in MC were observed among the Clonidine, Isoflurane, Clonidine+Isoflurane, and Control groups.

Respecting the limits of the present investigation, it can be concluded, based on the results, that Clonidine, when used in an isolated manner, produced no significant effect on the hemodynamic behavior of the isolated rat hearts in any of the evaluated parameters. Isoflurane, when used in an isolated manner, showed a tendency to increase the SP. By contrast, the Clonidine+Isoflurane association significantly diminished the SP in relation to the Control group and the isolated use of Isoflurane.

CONCLUSION

Clonidine, when used in an isolated manner, produced no significant effect on the hemodynamic behavior of the isolated rat hearts. However, when used in association with Isoflurane, this agent was able to diminish the effects of this pharmaco, illustrating an apparent protective effect on the heart.

The effects were observed directly upon the heart, considering that this study was conducted on isolated hearts, that is, with no link whatsoever to the central nervous system.

Potential Conflict of Interests

The authors declare that there are no pertinent conflicts of interest.

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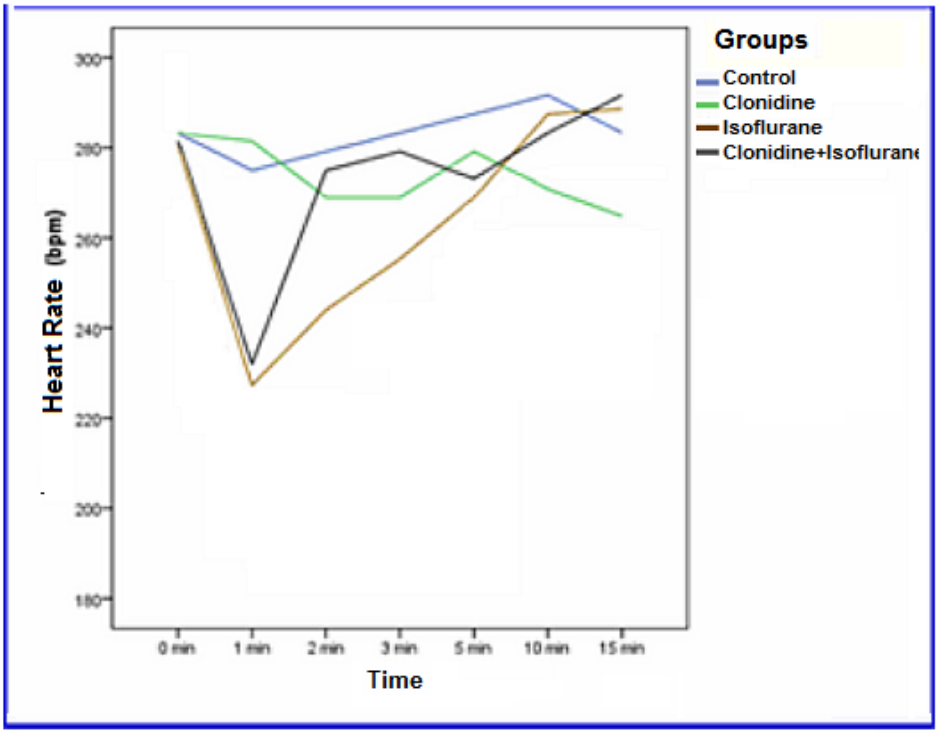

Figure 1 – Effects of Clonidine, Isoflurane, and the Clonidine-Isoflurane association on HR. ($p<0,001$).

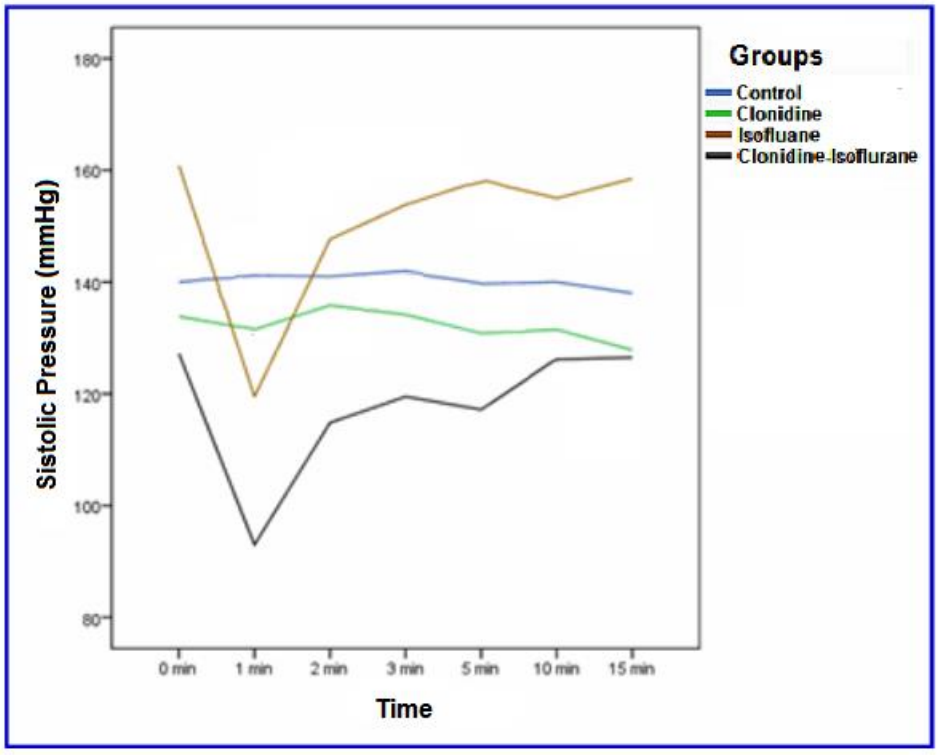

Figure 2 – Effects of Clonidine, Isoflurane, and the Clonidine-Isoflurane association on SP ($p > 0,05$).

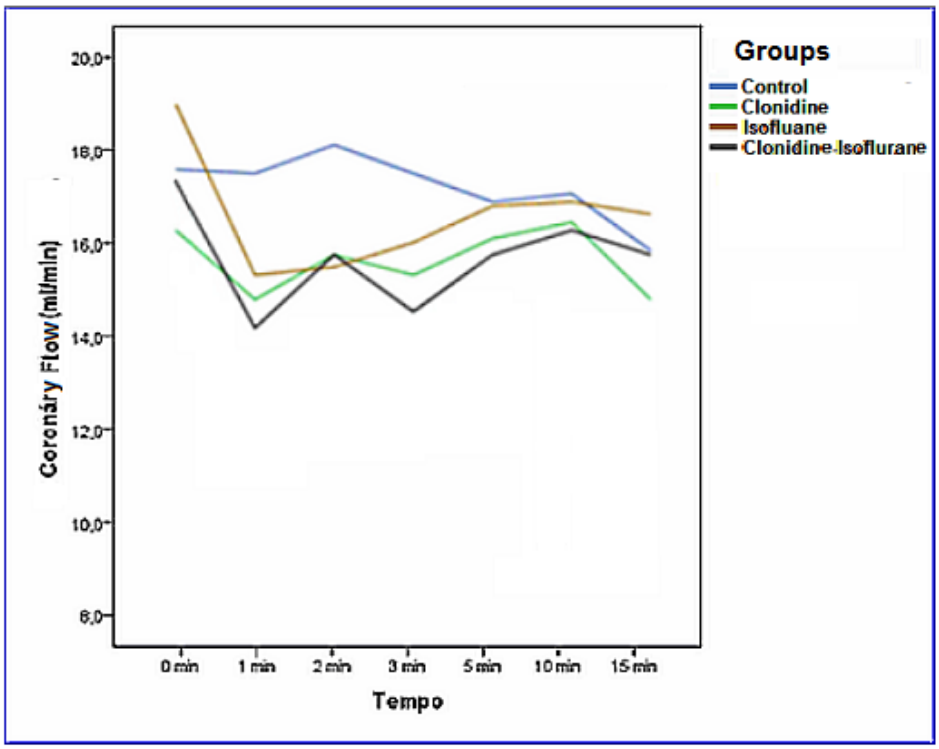

Figure 3 – Effects of Clonidine, Isoflurane, and the Clonidine-Isoflurane association on CF ($p > 0,05$).

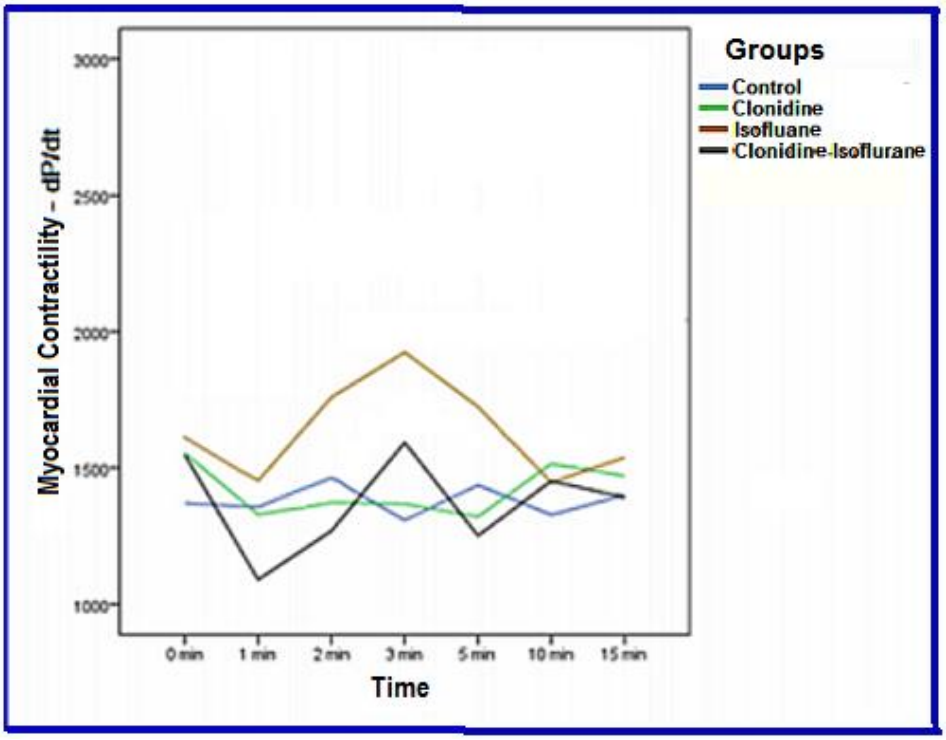

Figure 4 – Effects of Clonidine, Isoflurane, and the Clonidine+Isoflurane association on MC ($p>0,05$)