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Ventricular Arrhythmias and Mortality Associated with Isoflurane and Sevoflurane in a Porcine Model of Myocardial Infarction

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Ischemia of the myocardium can lead to reversible or irreversible injury depending on the severity and duration of the preceding ischemia. Here we compared sevoflurane and isoflurane with particular reference to their hemodynamic effects and ability to modify the effects of acute severe myocardial ischemia and reperfusion on ventricular arrhythmias and mortality in a porcine model of myocardial infarction. Female Large White pigs were premedicated with ketamine, midazolam, and atropine. Propofol was given intravenously for the anesthetic induction, and anesthesia was maintained with isoflurane or sevoflurane. Endovascular, fluoroscopy-guided, coronary procedures were performed to occlude the midleft anterior descending artery by using a coronary angioplasty balloon. After 75 min, the balloon catheter system was withdrawn and the presence of adequate reperfusion flow was verified. The pigs were followed for 2 mo, and overall mortality rate was calculated. The isoflurane group showed lower arterial pressure throughout the procedure, with the difference reaching statistical significance after induction of myocardial ischemia. The ventricular fibrillation rate was higher in isoflurane group (81.3%) than the sevoflurane group (51.7%; relative risk, 1.57 [1.03 to 2.4]). Overall survival was lower in the isoflurane group (75%) than the sevoflurane group (96.4%). In conclusion, in this porcine model of myocardial ischemia and reperfusion, sevoflurane was associated with higher hemodynamic stability and fewer ventricular arrhythmias and mortality than was isoflurane.

Ischemia and the subsequent reperfusion of the myocardium can lead to reversible or irreversible injury, depending on the severity and duration on the preceding ischemia. This damage has different clinical manifestations, including the development of ventricular arrhythmias with high incidence of ventricular fibrillation.[34,42,51]

Many factors can influence the incidence and severity of the arrhythmias associated with myocardial ischemia, from isolated premature ventricular beats to ventricular fibrillation: location and distribution of the occluded coronary artery, extension of myocardial infarction, duration of the coronary occlusion, anesthetic protocol, and heart rate.[4,13]

The porcine model of myocardial infarction has been used in experimental cardiology for many decades because of its proved reproducibility and similarity with key human anatomic characteristics and pathophysiologic events known to occur in patients with myocardial infarction.[1,16,52,55,60,67] One of the undisputed complications of this model is its high incidence of ventricular arrhythmias and fibrillation, which complicates anesthetic protocols and postoperative managment.[29,32,34,42,58,66]

Myocardial ischemia–reperfusion injury can lead to severe complications; measures to minimize myocardial damage have been an important target of research. Because anesthetics may provide protection against this damage,[36,53] improved understanding of the role of anesthetics in the prevention of myocardial injury may provide anesthesiologists with strategies to improve outcome. The use of particular anesthetics for the induction and maintenance of general anesthesia is one such approach proposed to protect against the adverse effects of ischemia.[18,17]

Experimental evidence suggests that volatile anesthetic agents have direct protective properties against ischemic myocardial damage.[33,35,41] These cardioprotective effects of inhalant anesthetics can be attributed to several properties exhibited by these agents: preservation of myocardial energy stores (ATP concentrations),[45,53] free radical scavenging,[19,61,62] and negative inotropic and chronotropic actions.[10] Decreases in heart rate, systolic pressure, and, therefore, the rate–pressure product would lower myocardial energy demand and oxygen consumption, thereby reducing myocardial metabolic requirements.[53] In addition, halogenated anesthetics reduce the incidence of ventricular arrhythmia, as compared with intravenous agents.[18,19,45] Some authors[33] have suggested that halogenated anesthetics have an antiarrhythmic effect similar to that of verapamil. The ability of these agents to alter Ca^{2+} homeostasis in myocardium could explain this effect.[28,31,41]

In the present study, we compared sevoflurane with isoflurane with special reference to their hemodynamic effects and ability to modify the effects of acute severe myocardial ischemia and reperfusion on ventricular arrhythmias and mortality. Therefore, this study had as its main objective the development of a suitable and safe anesthetic protocol in swine as an animal model to study acute myocardial infarction after induction of acute myocardial ischemia. Improving survival rates in animal models not only increases the efficiency of these models but also the ethical acceptability of the procedures. Using the most appropriate anesthetic protocol ensures animal welfare, reduces morbidity, optimizes performance, and increases the quality of research.

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Materials and Methods

Animals. Female Large White pigs (n = 45; weight [mean ± 1 SD], 25 ± 3 kg) were included into this study. Experiments conformed to the Council of the European Communities (86/609/EU)[15] and Spanish regulations (RD 223/1988),[50] both of which regulate the protection of animals used for experimental and other scientific purposes. The experimental protocol was approved by the Ethical Committee of the University of Leon. All efforts were made to minimize animal suffering and the number of animals used. The pigs underwent dietary restriction from solids and liquids for 24 h and 2 h, respectively, to eliminate gastric contents before the intervention.

Anesthesia protocol. The isoflurane group comprised 16 pigs, and the sevoflurane group had 29 pigs. All animals were premedicated with ketamine (5 mg/kg IM), midazolam (0.35 mg/kg IM), and atropine (0.02 mg/kg IM). Intravenous access was maintained throughout the procedure by using 2 intravenous catheters that were introduced in marginal ear veins, and the pigs received 10 mL/kg/h normal saline throughout the procedure. Propofol (4 mg/kg IV) was given to induce anesthesia, and anesthesia was maintained by using isoflurane or sevoflurane. Once sufficiently sedated, pigs underwent tracheal intubation and subsequently were ventilated with 100% oxygen by using a circle system with a carbon dioxide (CO_2) absorber in the circuit and a fresh gas flow of 2 L/min (Julian, Dräger, Lübeck, Germany). The initial tidal volume was 10 mL/kg at 12 breaths per minute. The tidal volume was adjusted to maintain the end-expired CO_2 concentration between 35 to 45 mm Hg. Body temperature was maintained between 36.5 and 37.5 °C by using an under-table heating device. Analgesia by fentanyl was induced by using an initial bolus (5 µg/kg IV) followed by constant intravenous infusion of 5 µg/kg/h. Isoflurane or sevoflurane was administered by using calibrated vaporizers (AG model, Dräger). Anesthetic concentrations were monitored (Julian, Dräger) and the vaporizer frequently adjusted to maintain an end-expired agent concentration of 1 MAC.

Continuous electrocardiographic monitoring during the procedure allowed us to determine the incidence and type of ventricular arrhythmias (extrasystoles, fibrillation). Respiratory and hemodynamic parameters were recorded every 10 min: CO_2 expired fraction, expired anesthesia gases (Julian, Dräger), heart rate, partial saturation of oxygen, and invasive arterial pressures (M1166 A Model 66S, Hewlett-Packard, Palo Alto, CA).

Endovascular surgical technique. The endovascular surgical technique was performed as previously described elsewhere.[50] The left carotid artery was surgically dissected and cannulated with a 7-French arterial introducer (Avanti+, Cordis Europa NV, Roden, Netherlands). By using a portable radiologic source (GE Stenoscop, GE Medical Systems, Piscataway, NJ) for fluoroscopy guidance, the left main coronary artery was intubated with a 6-French guiding catheter (length, 40 cm; JR 3.5, Cordynamic, Iberhospitex, Barcelona, Spain) that was custom-designed for the protocol. A baseline coronary angiography was performed. A standard angioplasty guidewire (Hi-Torque Balance Middle-Weight 0.014", Guidant Europe, Diegem, Belgium) then was introduced to the segment most distal to the anterior descending artery. A coaxial angioplasty balloon (diameter, 3 mm; length, 15 mm; Cordynamic, Iberhospitex) was placed in the middle segment of the left anterior descending artery (distally to the first diagonal branch) and inflated to a nominal pressure of 8 atm to achieve complete occlusion (Figure 1). The artery was occluded for 75 min, after which the occlusive balloon catheter was deflated and withdrawn, and the reperfusion was verified. A premounted stainless steel stent (3 × 18 mm; Apolo, Iberhospitex) was implanted into occlusion zone (Figure 1). After coronary angiography to confirm adequate flow in the epicardial artery, all catheters were withdrawn from the endovascular system, the incision surgically closed, anesthesia was suspended, and pigs were weaned from mechanical ventilation and transferred to the postsurgery recovery suite. The animals were euthanized after 2 mo of follow-up.

Drug protocol. Antiplatelet therapy consisted of daily oral doses of clopidogrel (75 mg) and acetylsalicylic acid (125 mg), beginning the day before surgery and continuing until the end of follow-up. Anticoagulation was achieved with 50 UI/kg heparin IV bolus on accessing the artery followed by an infusion rate of 10 UI/kg/h through the guiding catheter. Prophylactic antibiotics used were amoxicillin–clavulanic acid with a single preintervention dose of 12.5 mg/kg IM and subsequently continued by 350 mg PO every 12 h for 5 d. All pigs received the same antiarrhythmic protocol. Amiodarone (5 mg/kg/h) was administered by continuous infusion throughout the procedure and, in case of ventricular fibrillation, lidocaine (2 mg/kg IV) was administered to decrease later incidence of fibrillation and external electrical defibrillation was applied (43100A Defibrillator, Hewlett-Packard) by using initial loads of 300 J (as much as 360 J in cases of repeat applications). Postoperative analgesia was maintained with buprenorphine (0.01 mg/kg IM every 8 h) for 3 d.

Statistical analysis. All data are expressed as mean ± 1 SD or 95% confidence interval. To identify significant differences in hemodynamic variables (arterial pressure, saturation of peripheral O_2) between groups, one-way ANOVA was applied by using the Epi Info 3.4.3 program (Centers for Disease Control, Atlanta, GA). Comparisons between individual pigs at different time points were made by using one-way repeated-measures ANOVA technique. Mortality and incidence of ventricular fibrillation were compared among the 3 groups by using the χ^2 test and Fisher exact tests. A P value of less than or equal to 0.05 was considered statistically significant.

Results

All procedures for transient occlusion of the left anterior descending coronary artery were done according to the prespecified protocol. No pigs had noteworthy complications from anesthesia.

Hemodynamic data. After the occlusion of the middle segment of the left anterior descending artery, all animals experienced a gradual decrease both in heart rate and in arterial pressures, with partial recovery at reperfusion (Table 1). However, compared with those that received isoflurane, pigs in the sevoflurane group showed better hemodynamic stability during the procedure. Mean arterial pressure was significantly (($P < 0.005$ for all comparisons) higher throughout the entire procedure in the sevoflurane group as compared with the isoflurane group (Table 1). Heart rate was also slightly higher in the sevoflurane group, reaching statistical significance at the end of arterial occlusion (sevoflurane, 78.7 ± 12.22 bpm; isoflurane, 70.6 ± 7.71 bpm; $P < 0.05$) and at reperfusion (sevoflurane, 87.7 ± 14.57 bpm; isoflurane, 76.1 ± 9.96 bpm; $P < 0.05$).

Incidence of ventricular arrhythmias. In our study, all pigs experienced ventricular extrasystoles after induction of acute myocardial ischemia. The presence of these episodes was much more frequent ($P < 0.001$) in 3 different periods: within the first 10 min after coronary occlusion (13.9% of all episodes), within the first 20 to 40 min after occlusion (52.8%), and within the first 10 min after reperfusion (13.8%); 19.5% of ventricular

Table 1. Hemodynamic data (mean ± 1 SD) before and during occlusion and after reperfusion.

	Before occlusion	Time after occlusion (min)			After reperfusion
		0	30	60	
Isoflurane (n = 16)					
Heart rate (b/min)	89.6 ± 11.47	80.1 ± 12.10	73.5 ± 8.98	70.6 ± 7.71[a]	76.1 ± 9.96[a]
Systolic blood pressure (mm Hg)	90.3 ± 8.63[b]	75.4 ± 9.64[b]	68 ± 7.91[b]	67.8 ± 8.06[b]	69.8 ± 10.88[b]
Diastolic blood pressure (mm Hg)	63.2 ± 8.63	53.6 ± 10.49	47.3 ± 6.71[b]	47.5 ± 5.63[b]	49.5 ± 6.95[b]
Median arterial pressure (mm Hg)	75.3 ± 8.30[a]	62.9 ± 10.49[b]	56.6 ± 7.05[b]	56.8 ± 6.07[b]	59.2 ± 8.83[b]
Sevoflurane (n = 29)					
Heart rate (bpm)	84.6 ± 7.65	79.3 ± 7.00	76.2 ± 11.19	78.7 ± 12.22	87.7 ± 14.57
Systolic blood pressure (mm Hg)	102.4 ± 11.12	86 ± 10.70	82.9 ± 6.51	83.6 ± 7.11	83.7 ± 9.37
Diastolic blood pressure (mm Hg)	72.7 ± 12.19	62.3 ± 9.22	59.3 ± 5.86	61.5 ± 6.90	59.1 ± 4.16
Median blood pressure (mm Hg)	88 ± 12.33	72.3 ± 9.81	70.6 ± 6.91	70.4 ± 4.63	70.8 ± 5.15

[a]Value significantly ($P \leq 0.05$) different from that for sevoflurane group
[b]Value significantly ($P \leq 0.005$) different from that for sevoflurane group

extrasystoles occurred at various other times throughout the monitoring period.

The global incidence of ventricular fibrillation in our study was 62.2% (28 of 45 pigs). In the isoflurane group, ventricular fibrillation occurred in 13 of the 16 pigs (81.3%); these episodes were distributed as follows: 19% within the first 10 min after the occlusion of the artery, 38.1% within the first 20 to 40 min of ischemia, 23.8% within the first 10 min after reperfusion, and the remaining 19% arbitrarily distributed during the rest of the time. In the sevoflurane group, ventricular fibrillation occurred in 15 of the 29 animals (51.7%), with all episodes occurring during the occlusion period: 6.6% within first 10 min of ischemia, 73.4% within the first 20 to 40 min of ischemia, and the remaining 20.0% arbitrarily distributed during the rest of the time. In addition, no episodes of ventricular fibrillation took place after reperfusion (Figure 2).

The risk of suffering ventricular fibrillation was 1.57 times higher in the isoflurane group than in the sevoflurane group (relative risk, 1.57 [95% confidence interval, 1.03 to 2.40]; $P < 0.05$). In addition, the number of ventricular fibrillation episodes per animal was significantly ($P = 0.008$) higher in the isoflurane group, in which 2 pigs (12.5%) had 2 ventricular fibrillation episodes and 3 (18.75%) experienced 3 episodes throughout the procedure, but no recurrent ventricular fibrillation episodes were detected in the sevoflurane group (Figure 3).

Mortality. In the isoflurane group, 4 premature deaths occurred: 2 pigs died due to ventricular fibrillation during the procedure, and 2 pigs died due to heart failure within 24 h after the procedure. However, in the sevoflurane group, only one animal died (at 2 h after myocardial reperfusion); the cause of death was irreversible cardiogenic shock without ventricular arrhythmias. There is a strong trend ($P = 0.04$) toward a lower survival rate in the isoflurane group (75.0%) as compared with the sevoflurane group (96.4%).

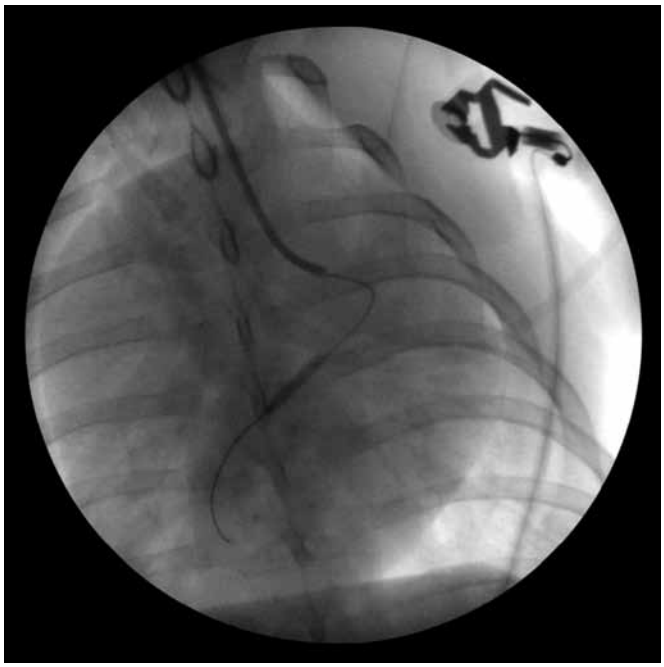

Figure 1. Occlusion of the left anterior descending artery (distally to the first diagonal branch) by using coaxial angioplasty balloon.

Discussion

In general, sufficient anesthesia results in a well-balanced combination of sedation, analgesia, hypnosis, and muscle relaxation. Selection of an appropriate anesthetic regimen requires consideration of the goals of the investigation as well as the possible side effects of the chosen anesthetic drugs.[30,59]

Myocardial ischemia–reperfusion injury can lead to severe complications, and measures to minimize myocardial damage have been an important target of research. Because some anesthetics may provide protection,[36,53] increased understanding of the role of anesthetics in the prevention of myocardial injury may provide anesthesiologists with strategies to improve outcome. The use of particular anesthetics for the induction and maintenance of general anesthesia is one such approach proposed to protect against the adverse effects of ischemia.

In our study, arterial pressures differed between the 2 groups of pigs before ischemia and during the procedure; the values throughout the procedure were higher in the sevoflurane group. This beneficial cardiovascular effect of sevoflurane over isoflurane has been confirmed in pigs and other species.[5,63] Swine anesthetized with isoflurane had significantly lower arterial pressures than did those that received equipotent concentrations of sevoflurane.[37] The authors of another study[21] concluded that mean arterial pressure in horses is better maintained with sevoflurane than isoflurane. Nevertheless, other studies did not confirm this difference between sevoflurane and isoflurane in terms of hemodynamic effects[8,9,26,39]

We noted a decrease in the heart rates of both groups as acute myocardial ischemia was provoked. This phenomenon has been

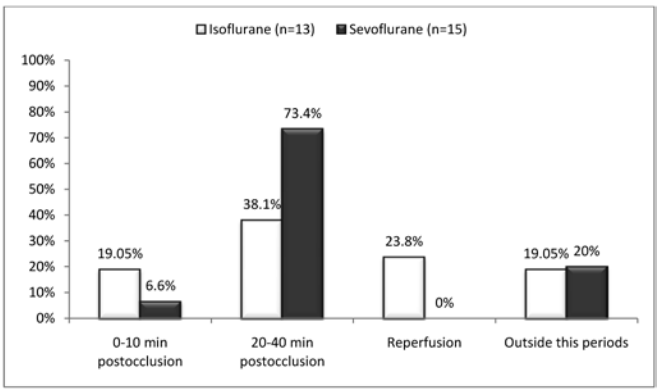

Figure 2. Distribution of episodes of ventricular fibrillation (%) relative to time after occlusion and reperfusion in the 2 groups.

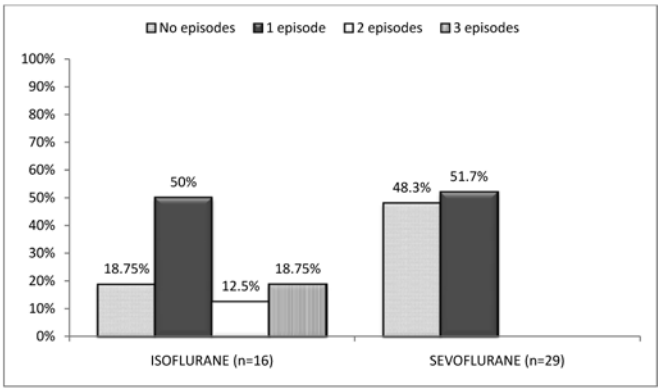

Figure 3. Number of episodes of ventricular fibrillation per animal (%) in both groups.

described previously[2] and attributed to pronounced increase in vagal tone.[38] Conversely, other authors have demonstrated a significant increase in heart rate[2,22,64] and decrease in arterial pressure[2,22] with myocardial reperfusion, which results are consistent with our findings.

The increased hemodynamic stability we noted with sevoflurane could explain its potential cardioprotective effect. In addition, halogenated anesthetics exhibit cardioprotective properties at therapeutic doses, independent of their anesthetic and hemodynamic effect, leading to the concept of anesthetic preconditioning.[35]

As previously described,[24,57] all of our pigs showed premature ventricular complexes associated with the induction of ischemia. These premature complexes clustered around 3 concrete periods: during the first minutes after coronary occlusion, at approximately 30 min after occlusion, and after myocardial reperfusion. Other authors[4,7] have described similar results, although the clustering of episodes around the periods we observed was not characterized completely. The incidence of ventricular fibrillation after occlusion of the left interventricular artery in the pig is very high,[29,32,34,42,58,60,66] with observed rates ranging from 50% to 75%[3,4,25,34,39,42] to 100%.[6,11,47,65]

Numerous authors indicate that volatile anesthetic cause reduction of cardiac arrhythmias.[14,45] In our study, the lower rates of ventricular fibrillation that we obtained (51.7%) were due to administration of sevoflurane to a concentration of 1 MAC throughout anesthetic maintenance, a fundamental condition to obtain myocardial protection, according to various studies.[43-45] In addition, none of our sevoflurane pigs experienced episodes of ventricular fibrillation after reperfusion, a result that supports the claim that administration of sevoflurane before, during, and after ischemia reduces the incidence of ventricular fibrillation after reperfusion.[44,45] The antiarrhythmic effects of sevoflurane have been confirmed in multiple studies, in which the agent was implicated in decreasing the calcium concentration within the myocyte, myocardial contractility,[43,44,46,56] and myocardial oxygen demand.[54]

Due to the high susceptibility of pigs to developing fatal arrhythmias during coronary occlusion, rates of mortality can get to be high. A 33% mortality rate due to ventricular fibrillation is common in this porcine model of myocardial ischemia.[32] In the present study, total mortality in the isoflurane group was 25% (12.5% during the myocardial ischemia and 12.5% within 24 h after procedure). However, in the sevoflurane group, total mortality was 3.57%, with only a single pig dying (due to cardiogenic shock). Reported rates of mortality in similar studies of myocardial ischemia in pigs vary: 10%,[69] 16%,[58] 20%,[20,23,25,34] 30% to 35%,[27,49] 75%,[40] and even 90%.[7] Therefore, in the current study, administration of sevoflurane during anesthetic maintenance yielded a survival rate of 96.4%, surpassing those in other similar studies of myocardial ischemia.

The cardioprotective properties of volatile anesthetics have been studied for several decades and currently constitute powerful tools in the management of patients with coronary artery disease. Volatile agents consistently reduce perioperative myocardial infarction and mortality in cardiac surgery when compared with total intravenous anesthesia.[36] Some studies in patients undergoing coronary artery surgery have found some evidence showing that sevoflurane has better myocardial protection than do intravenous agents like propofol.[12,18,17,68]

Our study has several potential limitations. First, young and healthy animals are probably not the best model to reproduce all the conditions present in patients with ischemic heart disease. Further studies are warranted to assess the cardioprotective properties of volatile anesthetics in more representative models (for example, models of atherosclerotic animals). Second, the study design did not follow a randomized assignment of subjects to each group but sequential analysis of 2 different protocols of anesthesia. As we soon detected a potential benefit of sevoflurane, all subsequent procedures were performed by using this agent. Although doing so can be deemed a potential flaw of the described results, the rest of the procedure was performed exactly in the same way in both groups. In conclusion, in this porcine model of acute and severe myocardial ischemia and reperfusion, sevoflurane was associated with higher hemodynamic stability, fewer ventricular arrhythmias, and less mortality than was isoflurane.

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