Article

Cardioprotective Mechanisms of Interrupted Anesthetic Preconditioning with Sevoflurane in the Setting of Ischemia/Reperfusion Injury in Rats

Mihaela Roxana Popescu 1,2,*, Bogdan Pavel 1,3, Gheorghita Isvoranu 4, Laura Cristina Ceafalan 1,4,5, Anca Maria Panaitescu 1,6, Ruxandra Irina Sava 2, Adelina Vlad 1,3, and Leon Zagrean 1,3,*

1 Cardiothoracic Pathology Department, “Carol Davila” University of Medicine and Pharmacy, 020021 Bucharest, Romania; bogdan.pavel@umfcd.ro (B.P.); laura.ceafalan@umfcd.ro (L.C.C.); anca.panaitescu@umfcd.ro (A.M.P.); adelina.vlad@umfcd.ro (A.V.)
2 Department of Cardiology, Elias Emergency University Hospital, 011461 Bucharest, Romania; ruxandra.sava@yahoo.com
3 Department of Functional Sciences, Division of Physiology and Neuroscience, 010164 Bucharest, Romania
4 INCD “Victor Babes”, 050096 Bucharest, Romania; gina_isvoranu@yahoo.com
5 Department of Cellular and Molecular Biology and Histology, Carol Davila University of Medicine and Pharmacy, 020021 Bucharest, Romania
6 Filantropia Clinical Hospital, 011132 Bucharest, Romania
*
Correspondence: roxana.popescu@umfcd.ro (M.R.P.); leon.zagrean@gmail.com (L.Z.)

Abstract: Background: Anesthetic preconditioning (AP) is known to mimic ischemic preconditioning. The purpose of this study was to investigate the effects of an interrupted sevoflurane administration protocol on myocardial ischemia/reperfusion (I/R) injury. Methods: Male Wistar rats (n = 60) were ventilated for 30 min with room air (control group, CG) or with a mixture of air and sevoflurane (1 minimum alveolar concentration—MAC) in 5-min cycles, alternating with 5-min wash-out periods (preconditioned groups). Cytokines implicated in the AP response were measured. An (I/R) lesion was produced immediately after the sham intervention (CG) and preconditioning protocol (early AP group, EAPG) or 24 h after the intervention (late AP group, LAPG). The area of fibrosis, the degree of apoptosis and the number of c-kit+ cells was estimated for each group. Results: Cytokine levels were increased post AP. The area of fibrosis decreased in both EAPG and LAPG compared to the CG (p < 0.0001). When compared to the CG, the degree of apoptosis was reduced in both LAPG (p = 0.006) and EAPG (p = 0.007) and the number of c-kit+ cells was the greatest for the LAPG (p < 0.0001). Conclusions: Sevoflurane preconditioning, using an interrupted anesthesia protocol, is efficient in myocardial protection and could be beneficial to reduce perioperative or periprocedural ischemia in patients with increased cardiovascular risk.

Keywords: anesthetic preconditioning; sevoflurane; myocardial protection; ischemia/reperfusion injury; apoptosis; fibrosis; c-kit+ cells

1. Introduction

Preconditioning protocols can protect the myocardium against ischemia/reperfusion injury (I/R) by preconditioning protocols. Ischemic preconditioning (IP) by short, repetitive bouts of ischemia represents the most powerful endogenous cardioprotective mechanism [1,2]. Pharmacological preconditioning protocols using volatile anesthetics termed anesthetic preconditioning (AP) represent low-risk procedures with a cardioprotective potency that is similar to IP [3]. Both IP and AP occur in a biphasic pattern, consisting of an early preconditioning phase, within the first 2–3 h, and a late preconditioning phase after 12–24 h [4].

Current experimental and clinical data suggest a probable link between cardioprotection provided by IP and myocardial tissue regeneration induced by bone marrow-derived...
progenitor cells [5,6]. Amongst the population of mesenchymal stromal/stem cells (MSCs), the endothelial progenitor cells (EPC), identified through the specific marker CD34 (also known as flk1), have paracrine activity and play key roles in neovascularization and maintaining endothelial function [7]. IP increases the number of c-kit+ (receptor for stem cell factor) and flk-1+ progenitor cells in the myocardium subjected to ischemia and reperfusion [6]. Given the similarities between IP and AP, similar mechanisms may underlie cardioprotection conferred by AP as well.

Patients with cardiovascular disease exhibit low levels of dysfunctional EPC [8,9], which makes them an interesting target for both diagnostic and therapeutic strategies. Kalenka et al. demonstrated in rat left ventricular samples that sevoflurane, a volatile anesthetic widely used in clinical practice, produces changes in the expression of genes and proteins involved in cardioprotection that persists for 72 h [10]. Sevoflurane has been shown to induce the mobilization of and potentiate the function of EPCs [11,12], possibly through increased cytokine production. After exposure to sevoflurane, human blood monocytes showed overexpression of vascular endothelial growth factor (VEGF) and granulocyte colony stimulating factor (G-CSF) [13]. These factors are known to be involved in the mobilization and recruitment of the MSC [13,14]. In addition, another volatile anesthetic, isoflurane, activates hypoxia induced factor-1 α (HIF-1 α), which in turn stimulates the synthesis of VEGF [15,16] and SDF-1 α, cytokines which have been implicated in MSC mobilization and homing [6]. Sevoflurane has also been shown to modulate the expression of integrins and L-selectin, molecules involved in the acute proinflammatory response and which contribute to local injury [13]. Administration of cytokines such as interleukin 1 (IL-1) is associated with resistance to I/R [17,18]. Interleukin-6 (IL-6) deficient mice are resistant to preconditioning, and that preconditioning-mediated activation of STAT1/3, iNOS, and COX-2 is attenuated in these mice [19,20]. Furthermore, AP has been shown to be associated with reduced apoptosis and fibrosis after I/R injury [21–25].

We hypothesized that sevoflurane preconditioning may mediate cardioprotection via modulation of paracrine activity and MSCs mobilization, which in turn act to decrease apoptosis and fibrosis after I/R injury.

2. Materials and Methods

The study comprised two parts: (1) the cytokine study focused on evaluating the production of cytokine levels known to be implicated in AP-mediated MSC mobilization, while (2) the ischemia/reperfusion study focused on evaluating the extent of the I/R lesion, the recruitment of MSC in the myocardium (immunofluorescence) and the degree of myocardial apoptosis (Figure 1).

We used Wistar adult male rats aged 5–7 months and weighing between 300–400 g. The rats were housed in the INCD “Victor Babeș” animal facility at a constant temperature (22 ± 2 °C) and humidity (55 ± 10%), exposed to artificial lighting with a 12 h/12 h light/dark cycle. The animals were given water (filtered and sterilized with UV light) ad libitum and standard rat chow (granulated concentrated fodder from INCDMI Cantacuzino). All procedures applied to laboratory animals are in accordance with the current national and European legislation specific to working with experimental animals. The protocol was approved by the Ethics committee of UMF “Carol Davila”, 1877/30.01.2012.

All groups were initially anesthetized with 5% chloral hydrate solution (0.8 mL/100 g). Rats were intubated and ventilated with room air for 30 min (control groups), or with a mixture of air and sevoflurane 1 MAC, in cycles of 5 min each, alternating with cycles of 5 min of wash-out during which rats were ventilated with room air (APC groups).
Appl. Sci. 2022, 12, x FOR PEER REVIEW 3 of 15

Figure 1. Ischemia/reperfusion study flowchart. EAPC—early anesthetic preconditioning; I/R—
ischemia reperfusion; LAPC—late anesthetic preconditioning.

2.1. Cytokine Study

After rats underwent either room air ventilation or AP, blood was collected by terminal cardiac puncture to evaluate the level of vascular endothelial growth factor (VEGF), granulocyte colony stimulating factor (G-CSF), IL-1α and IL-6 using the Luminex technique. Samples were collected from control and APC rats \((n = 21\) for each group, \(n = 42\) in total). To study the dynamics of cytokine production, each group was divided into 7 subgroups with rats randomly assigned \((n = 3)\). Each subgroup was defined by the time elapsed between ventilation and moment of blood draw: baseline, 1, 3, 6, 12, 24, and 48 h after the procedure, respectively. An intracardiac collection technique was used. We collected 2 mL of blood on EDTA and transferred it to centrifuge tubes. Blood was centrifuged for 20 min at 1000× g rpm at room temperature (20°C). Approximately 400 μL of plasma was obtained, transferred to Eppendorf tubes, properly labeled and stored at −20°C until processing. We diluted the plasma 10 times and prepared the serum matrix by resuspension in one milliliter of ultrapure water and reconstituted the standard and controls in 0.25 mL of ultrapure water, allowing to stir for 15 min. Tubes containing the capture antibody microspheres are vortexed, sonicated for 15 s, then centrifuged for 30 s at 1000× g. We diluted the microspheres, 25 μL from each tube (3 tubes) with 1175 mL solution for diluting the microparticles. The diluted microparticles are vortexed, 25 μL of the microparticle suspension is placed in the plate, which is covered and incubated overnight at 4°C with continuous stirring. We added 25 μL of detectant antibody solution to each well, incubated for one hour at room temperature with continuous shaking, away from light. A 25 μL measure of streptavidin-phycocerythrin was added to the well with 30 min incubation at room temperature. Resuspension in 150 μL wash buffer with stirring for 2 min was performed. The samples were read using the Luminex 200 (set to analyze at least 100 events for each microsphere population). Multiplex data acquisition and analysis was performed using STarStation 2.3 software (Applied Cytometry Systems, Sheffield, UK).
2.2. Ischemia/Reperfusion Study

For the second part of the study, animals underwent AP or room air ventilation, followed by induction of I/R lesion \((n = 18)\). Rats were randomly allocated to one of three subgroups: (1) Control group (CG) rat were ventilated with room air, followed by I/R injury; (2) early AP group (EAPG)—APC administered immediately before I/R injury; (3) late AP group (LAPG)—APC administered 24 h prior to I/R injury. I/R injury was induced through surgical thoracotomy followed by temporary (30 min) ligation of the left anterior descending artery. Hearts were harvested after 1 day (apoptosis and c-kit\(^+\) subgroup) or 14 days (fibrosis subgroup).

2.2.1. Evaluation of Myocardial Apoptosis and C-kit\(^+\) Cell Infiltration

Twenty-four hours after I/R injury, rats were sacrificed \((n = 9)\) and hearts were removed, frozen in liquid nitrogen, and used to produce 5 µm cryosections to estimate the apoptotic process by TUNEL assay (ApopTag kit—Aptoptosis in situ Detection kit, Chemicon). The nuclei were labeled with DAPI (4′,6-diamidino-2-phenylindiol). Positive TUNEL nuclei in the area of interest were counted, and DAPI-marked nuclei were counted manually. Twenty microscopic fields from 5 independent sections of each heart were studied, and the mean values expressed as number of apoptotic bodies/1000 nuclei were used for statistical analysis. Similar cryosections from the same hearts were alternatively (one section for TUNEL, the next for immunofluorescence (IF) and so on) used for IF labeled with primary anti-flk-1 and anti-c-kit antibodies, followed by appropriate secondary antibodies. The nuclei were labeled with DAPI. Negative controls were obtained using the same protocol, but without the addition of primary antibodies. At least 20 microscopic fields in the area of interest were photographed for each heart. One filter was used for positive TUNEL cells (green) and another to visualize DAPI-stained nuclei. Cells marked in green were manually counted, and the image overlapped with DAPI-colored nuclei (blue) in the same field. These were, in turn, manually counted for the evaluation of the number of double positive nuclei (TUNEL and DAPI positive) out of the total number of blue nuclei (DAPI). C-kit\(^+\) cells were counted in at least 10 fields of 5 independent sections, obtained from three rats for each batch, using a microscope with Nikon TE300 Eclipse fluorescent filters. Two filters were used to visualize c-kit\(^+\) cells and DAPI + nuclei. C-kit\(^+\) cells are reported as percentage of the number of total DAPI+ nuclei. Mean values were used for statistical analysis.

2.2.2. Fibrosis Study

At 14 days after the I/R injury, the rats were sacrificed \((n = 6)\), the heart was removed, passed through a dehydration process and embedded in paraffin. Hematoxylin–eosin and methyl blue stained sections were performed to estimate the degree of fibrosis. A Nikon E200 microscope equipped with a Zeiss camera was used, and the image analysis was done with AxioVision Rel. 4.8., Carl Zeiss Microscopy, White Plains, NY, US. The fibrosis area was represented by the blue colored area out of the entire left ventricular area. The comparisons were performed on a global degree of fibrosis resulting from the average values obtained from at least 2 sections from 5 areas of each heart were analyzed.

2.3. Statistical Analysis

All data analysis was performed using the IBM SPSS software, version 21 (Statistical Package for the Social Sciences) (IBM SPSS Statistics, New York, NY, USA). All data are presented as mean ± standard deviation (SD). Mean values between independent groups were analyzed using the 2-tailed unpaired Student t test. For comparison between the three groups the ANOVA test was used. An \(\alpha\) level of 0.05 was chosen for statistical significance.

3. Results

3.1. Sevoflurane AP Increases Blood Levels of Plasma Cytokines Implicated in Chemotaxis of MSC

AP induced specific cytokine dynamics. We found that the mean VEGF level was elevated 3 h post-exposure at 137.78 pg/mL (preconditioned) vs. 61.15 pg/mL (con-
trol) \((p = 0.021, \text{Figure 2A})\). Mean G-CSF levels were significantly increased at six hours post-exposure at 352.03 pg/mL (preconditioned) vs. 111.35 pg/mL (control) \((p = 0.045, \text{Figure 2B})\). Mean IL-6 levels were significantly increased at 6 h at 1956.07 pg/mL (preconditioned) vs. 777.7 pg/mL (control) \((p = 0.008, \text{Figure 2C})\), and a similar pattern was observed at the same timeline for IL-1\(\alpha\) at 5052.84 pg/mL (preconditioned) vs. 963.36 pg/mL (control) \((p = 0.008, \text{Figure 2D})\). These results support the working hypothesis that AP modulates the plasma level of in a timeframe compatible with EAPC.

Figure 2. The evolution of the plasma level of several cytokines in the first 48 h after the AP stimulus: VEGF (A), G-CSF (B), IL-6 (C), IL-1\(\alpha\) (D), \(n = 3\) for every subgroup. * marks statistically significant results.

3.2. Sevoflurane Preconditioning Protects the Heart against Reperfusion Ischemia Injury by Decreasing the Degree of Apoptosis, Increasing the Number of C-kit\(^+\) Cells in the Damaged Myocardium, and Decreasing the Degree of Fibrosis

3.2.1. Estimation of Myocardial Apoptosis

Apoptosis was assessed by the TUNNEL technique (Figure 3). AP was associated with reduced apoptosis expressed as number of apoptotic nuclei/1000 nuclei, both in the early group (37.83 ± 2.56, \(p = 0.006\)) and in the late group (38.16 ± 2.48, \(p = 0.007\)) compared to the control group (53.16 ± 10.55) (Figure 4). Qualitative analysis also revealed extension of the apoptosis area to the interventricular septum, which was not seen in from the preconditioned groups.
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Figure 3. Immunofluorescence, positive TUNEL cells (green) superimposed on the image of DAPI-labeled nuclei (blue) on the same microscopic field, ×600. Image obtained on myocardial sections harvested 24 h after I/R.

Figure 4. Sevoflurane preconditioning is associated with reduced mean number of apoptotic nuclei/1000 myocardial cells in both early (green) and late (purple) windows of anesthetic preconditioning compared to the control group (blue). * marks statistically significant results.

Figure 5. Graphical representation of the averages in the three groups. Control group (blue), early preconditioning group (green), late preconditioning group (purple).
A more homogeneous distribution of the values obtained for the late preconditioning group was observed, compared to that of the control group (see Figure 5).

Figure 5. Graphical representation of the averages in the three groups. Control group (blue), early preconditioning group (green), late preconditioning group (purple).

3.2.2. Late AP Increases the Number of MSC in the Myocardium Subjected to Ischemia/Reperfusion Injury

We hypothesized that MSC may be involved in the late AP window. To study this hypothesis, we performed a quantitative analysis of c-kit+ flk-1+ cells on hearts harvested 24 h after the I/R injury (Figure 6). We found that c-kit+ cells were significantly increased in the heart following late AP (3.2 ± 0.48 vs. 0.85 ± 0.1, p < 0.0001, Figures 6 and 7).

The flk1+ staining was found to be non-specific, with confounders due to endothelial labeling, thus it was not quantified.

3.2.3. AP Is Associated with a Decreased Area of Myocardial Fibrosis

The degree of fibrosis developed following an acute ischemic event mirrors the extent of myocardial infarction and bares prognostic significance for coronary patients. Qualitative analysis showed reduced fibrotic areas in both AP groups when compared to the control group. The thickness of the infarct area differed between groups, with the largest transmural infarcts being seen in samples obtained from the control group, while samples from the LAPG presented smaller fibrotic areas with the smallest degree of transmurality among the three groups (Figure 8).

Quantitative analysis (Figure 9) revealed that the mean fibrosis percentage was significantly higher in both the EAPG 11.055 ± 0.53 (p < 0.0001) and the LAPG (9.17 ± 1.47, p < 0.0001) when compared to the control group (14.65 ± 0.94).
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**Figure 6.** C-kit+ cells and the image superimposed with the image of the nuclei marked with DAPI, on the same microscopic field, ×600. Image obtained 24 h after the I/R lesion.

**Figure 7.** Graphical representation of the number of c-kit+ cells in the three groups, with an increased number of cells in the preconditioned group compared to the control group. Control group (blue), early preconditioning group (green), late preconditioning group (purple). * marks statistically significant results.
was associated with reduced myocardial apoptosis, increased infiltration of endothelial progenitor cells and reduced fibrotic scars. This has important implications for clinical practice, as volatile anesthetics represent an attractive preconditioning stimulus that benefits coronary patients.

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Figure 8. Optical microscopy, cross section through the heart at the level of the left ventricle. Histological appearance of the infarction area (blue color) at 14 days, atypical aspect of hematoxylin–eosin and methyl blue staining, in the CG (A), EAPG (B) and LAPG (C) using ×10 magnification.

Figure 9. Graphical representation of the degree of fibrosis in the three groups, with a reduced fibrotic area for the preconditioned groups when compared to the control group. CG in blue, EAPG in green, LAPG in purple. * marks statistically significant results.

4. Discussion

In this study, we showed that sevoflurane AP induces changes in cytokine dynamics in rats, and that AP administered 24 h before or immediately before induction of I/R was associated with reduced myocardial apoptosis, increased infiltration of endothelial progenitor cells and reduced fibrotic scars. This has important implications for clinical practice, as volatile anesthetics represent an attractive preconditioning stimulus that benefits from extensive clinical experience and involves fewer risks while being associated with a more predictable cardioprotective response than IP [26].

Clinical trials have involved only the early preconditioning phase [27]. Taking into account the present results, the beneficial effects of late preconditioning in the clinical context are also worth exploring.
The phenomenon of AP is an attractive concept in medical practice because perioperative ischemia and myocardial infarction are a major cause of mortality and morbidity, especially following high-risk interventions. Therefore, the fact that the protective effect of volatile anesthesia extends long after surgery is of real clinical interest. There are data that provide a connection between AP and another interesting topic, namely bone marrow stem cells, with its subpopulation of endothelial progenitor cells [11]. Kamota et al. reported in 2009 that one of the cardioprotective mechanisms of late remote ischemic preconditioning is increased MSC mobilization and recruitment; they also demonstrated that this behavior of the MSC is closely correlated with the increase in the plasma level of some cytokines involved in the dynamics of the respective cells. An interventional study was performed which showed that by blocking the SDF-1α-CXCR4 axis (CXCR4 is a surface receptor for SDF-1) both the mobilization of the BMSC and their related protection are abolished [6].

Sevoflurane AP led to significantly increased levels of VEGF at 3 h and of G-CSF and IL-6 at 6 h. Both VEGF [28] and IL-6 [19] are involved in pro-survival signaling. VEGF is the ligand for flk-1 [29], the marker of endothelial progenitor cells. It plays important roles in angiogenesis and vasculogenesis [29], and has previously been implicated in IP [30]. IL-6 deficient mice are resistant to IP [19]. In humans, CD34+ endothelial progenitor cell mobilization induced by acute myocardial infarction was associated with a rise in G-CSF [31]. Moreover, it is unlikely that the observed changes in cytokine dynamics are attributable to a pro-inflammatory status, as rats suffered minimal manipulation prior to intubation and ventilation and were not subjected to a surgical intervention.

At 24 h following AP, treated rats exhibited decreased numbers of cardiac apoptotic bodies and increased numbers of c-kit+ endothelial progenitor cells. This is highly interesting, as c-kit+ cells have been shown to be critical for the late window of ischemic preconditioning [6]. We acknowledge that our study did not allow us to distinguish between resident cardiac versus bone marrow-derived MSCs.

Fibrotic scar extension represents a clinical prognostic factor in patients recovering after an acute myocardial infarction [32,33]. In our study, the fibrotic area at 14 days was significantly reduced in preconditioned rats, and these findings were consistent for both the early and the late windows of AP, with a more pronounced reduction for the late preconditioned group. Possible mechanisms leading to reduced fibrotic scars are represented by decreased apoptosis during the 24 h following I/R injury, mediated by (1) early increased levels of cytokines such as VEGF, G-CSF, and IL-6, which elicit pro-survival signaling [19,28] during the early phase of AP, as well as induced chemotaxis of MSCs [34–37] and by (2) MSCs infiltration occurring between baseline and 24 h, which have been shown to mediate the late phase of IP [6]. Direct anti-inflammatory effects of sevoflurane have also been described [38]. Furthermore, an important observation was that qualitative inspection of samples showed a reduced gradient of transmurality between the control group, and the early and the late AP groups, the latter displaying the smallest degree of transmurality. Indeed, it is unlikely that cell infiltration plays a major role during EAP, thus LAP may offer more protection than EAP.

The connection between endothelial progenitor cells and cardiovascular risk, overt cardiovascular disease and even the therapeutic options offered by the recognition, isolation and culture of these cells are still the subject of debate. To date, endothelial progenitor cells have been implicated in the whole process of cardiovascular pathology, and many conventional therapies have been shown to positively influence the number and function of endothelial progenitor cells, examples of which are conversion enzyme inhibitors, sartans, or statins [39,40].

More recently, attempts have been made to use the clinical potential of endothelial progenitor cells in the form of stents coated with anti-CD34 antibodies, capable of binding endothelial progenitor cells. For this type of stent, endothelialization occurs in 7 days [41] in experimental studies, but there are also clinical studies in this regard, with promising results [42,43]. In this sense, a preconditioning protocol, similar to the one we used,
that increases the number of circulating endothelial progenitors can be of assistance in stent endothelialization.

The protocol of APC administration is also a key element toward successful I/R injury reduction, as an interrupted protocol was shown to be superior to continuous administration in both APC and IPC through a multi-trigger mechanism [11,44–46].

There are studies that have tried unsuccessfully to show an increase in plasma levels of factors such as VEGF or G-CSF through exposure to sevoflurane [47]. Our study shows an increase in plasma VEGF levels at three hours post-exposure to sevoflurane, and at 6 h a high level of G-CSF appears compared to the control group. This difference from the previous studies is probably generated by the modifications between the protocols used, demonstrating once more the superiority of the interrupted protocol. The early increase after sevoflurane exposure of plasma levels of medullary stem cell mobilizing factors supports the result, with a late increase in the number of endothelial progenitor cells in the bloodstream obtained with the same protocol [11]. These results overlap with existing data in the literature on ischemic preconditioning, which shows a similar trend, with an initial cytokine growth time and only later the multiplication of cell numbers, with the moment overlapping the delayed preconditioning phase [6].

On the other hand, there are studies in which G-CSF is used as a stand-alone therapy [48–51] for its cardioprotective and anti-apoptotic effects, so an increase in its plasma concentration can only bring an additional benefit.

There was also an increase in the level of IL-1α and IL-6 throughout the post-exposure period to sevoflurane, up to 48 h, when their level drops sharply. It could be interpreted that these elevated levels of cytokines with a well-defined pro-inflammatory role occurred in a state of systemic inflammation. It should be noted, however, that the analyzed blood samples were collected from rats that were not subjected to any kind of surgery; they were only intubated and ventilated for 30 min with sevoflurane or atmospheric air. These cytokines have been implicated in myocardial protection against I/R injury, the present experiment indicating that AP would be able to modulate the plasma level of another category of factors involved in cardioprotection.

To further our research, we continued with a study on an in vivo model of AP and I/R induced immediately or 24 h after exposure to sevoflurane, in order to fit into the two protective windows (early and late).

As expected, both phases of preconditioning bring a net benefit, an example being the degree of fibrosis, which was statistically significantly reduced in both groups. AP has a demonstrated anti-apoptotic effect on the myocardium injured by I/R, which is based on several mechanisms [52]. In addition, MSCs recruited in an increased number in the damaged myocardium have a well-established role in increasing the survival capacity of cardiomyocytes [53,54]. Another argument in this regard is the reduction of the number of apoptotic nuclei on the sections obtained from preconditioned animals. It is noteworthy that, for I/R lesions induced during the late preconditioning phase, the number of c-kit+ cells doubles compared to the variant in which the ischemic injury occurs in the early phase. This phenomenon can be justified by a maximum level of BMSC in the blood only after 24 h, which overlaps with the second window of AP and suggests their recruitment to the damaged myocardium participates to the cardioprotective effects of late preconditioning.

Numerous studies report the anti-apoptotic effects of AP through various mechanisms [4,55]. The present study confirms this property of volatile anesthetics, in particular for sevoflurane, and adds to the list of possible mechanisms the direct involvement of medullary stem cells mobilized following AP and recruited into ischemic tissue. Increased myocardial mobilization and survival of medullary stem cells and their paracrine activity with effect on resident myocytes after exposure to sevoflurane have potentially important clinical implications. Perioperatively, medullary stem cells mobilized and recruited without further intervention—considering that the anesthesia was performed with sevoflurane—could have an important role in the functional recovery of the myocardium at risk. On
the other hand, sevoflurane can support stem cell therapies which normally take place under sedation.

5. Study Limitations

Due to the complexity of the study and the many subgroups involved, each subgroup included a low number of animals, and our hypothesis-generating study requires further validation by larger-scale studies.

The lack of functional data is another limitation of our present study, as myocardial function was not evaluated. However, only young, healthy rats were used in the study, which makes pre-existing myocardial ischemia unlikely. In addition, different distributions of the left coronary artery may bias infarction area size [56].

Our study suggests AP with sevoflurane was associated with modulation of plasma cytokines, reduced myocardial apoptosis and fibrosis, and increased myocardial infiltration of ckit+ cells. However, we have not demonstrated causality. Subsequent studies aimed at testing the hypothesis that MSCs mediate these cardioprotective effects may opt to pursue an interventional study to block the mobilization and myocardial recruitment of bone marrow stem cells. Studies using CXCR4 receptor blockers demonstrated significant dampening of cardioprotection conferred by ischemic preconditioning [6].

6. Conclusions

An interrupted anesthetic preconditioning protocol with sevoflurane in rats induced early (3–6 h) cytokine dynamics that were associated with reduced myocardial apoptosis and increased infiltration by endothelial progenitor cells after 24 h, and with a reduced fibrotic area at 14 days after induction of ischemia–reperfusion injury during both the early and the late preconditioning windows. In a clinical setting, AP represents an attractive tool that may reduce ischemic during high-risk surgery and/or in patients at high-risk of periprocedural/perioperative ischemia. In the case of stent implantation, the use of the late preconditioning protocol couldfavor a faster endothelialization.

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Informed Consent Statement: Not applicable.

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