Mesenchymal Stem Cells Increase Resistance Against Ventricular Arrhythmias Provoked in Rats with Myocardial Infarction

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Abstract

This study evaluated the role of the mesenchymal stem cells derived from adipose tissue (MSCs) in provoked ventricular arrhythmias (VAs) in animals with myocardial infarction (MI). The experimental groups were: sham, subjected to sham surgery and intramyocardial saline injection; MIV, infarcted rats subjected to intramyocardial saline injection; MI + MSCs, infarcted rats subjected to intramyocardial MSCs injection. Injections were performed two days after infarction and the arrhythmogenic inducibility experiment was performed the next day. Only 35% of the MI + MSCs group developed VAs, while the one in the MIV group was 65%. The proportion of nonsustained ventricular tachycardia, sustained tachycardia, and ventricular fibrillation was similar between the infarcted groups, but MSCs animals had shorter duration of nonsustained ventricular tachycardia. However, MSCs increased connexin 43 content in the remote area, even above the levels found in the sham group. MSCs prevented the increase of IL-1β in the different areas of the myocardium. There was higher carbonylation and content of 4-hydroxynonenal (4-HNE, a marker of lipoperoxidation) in the myocardium of infarcted rats, but MSCs attenuated the increase of 4-HNE in the infarcted area. In conclusion, MSCs have a protective effect against the development of arrhythmias, but do not imply a significant benefit for animals that have developed VAs. It is possible to think that the cardioprotection of MSCs involves anti-inflammatory/oxidative actions and improvement in the formation of communicating junctions.

Keywords Cardiac Arrhythmias · Myocardial Infarction · Stem cell

Introduction

An estimated 17.5 million people died from cardiovascular disease in 2012, accounting for 46% of all deaths from noncommunicable diseases [1]. Sudden cardiac death is the most common and often the first manifestation of coronary artery disease, accounting for ≈ 50% of mortality [1].

Ventricular fibrillation (VF) tachyarrhythmias (VTs) often occur early in ischemia and remain a common cause of sudden death in acute MI [2]. Sudden death secondary to sustained VTs or VF accounts for approximately 50% of all deaths in these high-risk patients [3]. Understanding the arrhythmogenic conditions established in the acute and chronic phases of MI plays a key role in improving prognosis and survival [4].

There is a temporal distribution of ventricular arrhythmias (VAs) after acute MI: an early or acute phase of up to 48–72 h, which is a period of very dynamic ischemia/reperfusion. From 72 h to a few weeks to a month post-MI, where remodeling continues [3]. Current therapeutic modalities (e.g., antiarrhythmic drugs, cardioverter/defibrillator implantation, electrophysiological procedures, and revascularization) have reduced the incidence of sustained VAs that occur during the initial phase of post-MI cardiac remodeling [2, 5]. However, these devices may not have the desired therapeutic effect or may not be available at the appropriate time of use. Therefore, there is a great interest and need to search for new therapies that may provide beneficial effects against VAs.

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In light of new therapeutic procedures, stem cell transplantation (SC) has emerged as a promising therapy, with studies suggesting that post-MI cell therapy may prevent VAs [6]. Unfortunately, other lines of evidence have shown that SC has no effect or even triggers VAs [7]. This would be a result mainly related to their common lack of electromechanical integration in the recipient myocardium [8].

The above results show that the arrhythmogenic paradox needs to be investigated to validate the safety of cell therapy. In addition, it is still unclear whether the heart is more resistant to proarrhythmic stimuli after SC therapy. Therefore, the aim of this study was to evaluate the effect of intramyocardial transplantation of adipose-derived mesenchymal stem cells (MSCs) on the inducibility of VAs in the early post-MI remodeling phase in rats.

Materials and Methods

MI Protocol and Experimental Groups

All experimental procedures were performed in accordance with the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health (NIH Publication No. 85−23, revised 1996). The experimental protocol was approved by the Institutional Research Ethics Committee of the Federal University of São Paulo, Brazil (number: 2371290719). All efforts were made to minimize the suffering of animals.

Isogenic Female Fischer-EPM rats (12 weeks old) weighing 170–190 g were housed under a 12/12 h dark/light cycle. The MI was induced as previously described [9, 10]. Briefly, rats were anesthetized with a ketamine/xylazine mixture (50/10, mg/kg, i.p.), intubated, and ventilated (model 683, Harvard Apparatus, 2.0 ml, 80 strokes/min). A left thoracotomy was performed and a 6.0 silk thread was permanently tied around the left anterior descending coronary artery. No coronary occlusion was performed in Sham rats. Rats were randomly assigned to one of the following experimental groups Sham: rats subjected to simulated coronary occlusion and vehicle MSCs injection ($n=12$); MIV: infarcted rats subjected to vehicle MSCs injection ($n=22$); MI + MSCs: infarcted rats subjected to intramyocardial MSCs transplantation ($n=20$).

Echocardiogram

Forty-eight hours after either coronary occlusion, rats were anesthetized with isoflurane (3%, at 2 L/min oxygen flow). Transthoracic echocardiography was performed to determine infarct size using a 12 MHz transducer (Sonos-5500, Hewlett-Packard, MA, USA). Infarct size was assessed on the two-dimensional transverse view of the LV and reported as a percentage of the left ventricular perimeter [9–11]. Only rats with infarct sizes ≥ 37% of the LV were included in the study. MI was defined as the presence of a segment with increased echogenicity and/or change in myocardial thickening or systolic motion. We do not believe it is necessary to perform the test in sham rats, as healthy animals would not be exposed to the electrophysiological study.

MSCs Preparation

MSCs were harvested from male isogenic rats as recently reported [12]. MSCs were phenotyped using BD FACSCalibur flow cytometry (Becton Dickinson, NJ, USA) to identify the following surface markers: CD105+; CD90+; CD29+; CD31-; CD45'. Cell transplantation was performed immediately after echocardiographic examination under isoflurane anesthesia. Thoracotomy and heart exposure were then performed. A total of 5×10^5 MSCs were diluted in 100 μL sterile phosphate buffer and injected into five different peri-infarct regions. The sham and MIV groups received only sterile phosphate buffer.

Arrhythmogenic Inducibility Protocol

The study was performed 24 h after MSCs transplantation. Arrhythmia induction was performed only in infarcted animals because Sham animals rarely develop VAs [13]. Rats were anesthetized with 3-5% isoflurane (1 L/min, O_2) and maintained with 1–2% isoflurane. Body temperature was maintained at 36ºC with a heating pad. The six-lead body surface electrocardiogram was recorded with a computerized acquisition system (Emka Technologies, Paris, France). Ventricular intracardiac electrograms were recorded with a 1.6 F octopolar catheter (EPR −802, Millar Instruments, Houston, USA) inserted into the left external jugular vein. Right ventricular pacing was performed with 2 ms currents and pulses delivered by an external stimulator (STG-3008, Multi-Channel Systems, Reutlingen, Germany). To induce VAs, electrical stimulation was programmed in cycles of 10 ms for 10 s and an additional stimulus at shorter coupling intervals. VAs were characterized as the product of three or more spontaneous ventricular beats and, if sustained, lasted for 30 s or more after the programmed electrical stimulation. Sustained (STV)/nonsustained (NSTV) ventricular tachycardia and VF were then assessed. All animals were euthanized at the end of the study.

Myocardial MSCs Detection

Detection of MSCs in LV tissue samples was evaluated by sex-determining region Y (SRY) chromosome of male donor

cells using real-time polymerase chain reaction (qPCR) [14]. The qPCR was performed on Applied Biosystems equipment (model 7500, USA). Primers were: Sry: sense 5`CTTTGCAGGGGTGAAGTTGCG-3`, antisense 5'-AGC CCAGTCCCTGTCCGTAT-3`; β-Actin: sense 5'-TTGCT GACAGGATGCAGAAG-3', antisense 5'-ACATCTGCT GGAAGGTGGAC-3'. The 7500 v2.06 software (Applied Biosystems) was used for data processing.

Tissue Sample Preparation

A total of 50 mg of infarcted and remote myocardium were homogenized for 30 s, respectively (buffer: 1.15% KCl solution; phenyl methyl sulfonyl fluoride (100 mmol/L isopropanol; 10 µL/mL KCl). The homogenate was centrifuged at 3000 rpm for 10 min at 0 and 4 °C, and the supernatant was used for protein dosage by the Bradford method.

Western Blot

A total of 30 µg was subjected to SDS-PAGE as described elsewhere [15]. Rabbit primary antibodies (Abcam, Cambridge, MA; 1:1000 dilution) were: collagen 1, collagen 3, connexin 40, connexin 43, connexin 45, and glyceraldehyde-3-phosphate dehydrogenase (GAPDH; 1:2000).

Table 1 Left ventricular echocardiographic data

	MIV (n = 12)	MI + MSCs (n = 22)	p-value
Body mass (g)	154 ± 3	153 ± 3	0.6
Infarct size (%)	37 ± 1	41 ± 1	**0.002**
Heart rate (bpm)	275 ± 5	289 ± 3	**0.02**
DALV (cm²)	0.31 ± 0.01	0.28 ± 0.009	**0.01**
SALV (cm²)	0.19 ± 0.01	0.17 ± 0.009	0.1
EDD (mm)	6.18 ± 0.17	5.81 ± 0.13	0.09
ESD (mm)	3.84 ± 0.14	3.87 ± 0.13	0.8
EF (%)	68 ± 2	67 ± 2	0.7
ASF (%)	39 ± 2	39 ± 2	0.8
EDT (ms)	41 ± 1	40 ± 2	0.7
IRT (ms)	19 ± 1	19 ± 1	0.8
E wave (m/s)	0.51 ± 0.01	0.54 ± 0.01	0.1
A wavw (m/s)	0.18 ± 0.01	0.16 ± 0.01	0.2
E/A ratio (a.u)	3 ± 0.22	4 ± 0.24	0.1

DALV, diastolic area of the left ventricle; SALV, systolic area of the left ventricle; EDD, left ventricular end-diastolic diameter; ESD, left ventricular end-systolic diameter; EF, left ventricular ejection fraction; ASF, area shortening fraction; EDT, E wave deceleration time; IRT, isovolumic relaxation time.

Membranes were washed five times and incubated with horseradish peroxidase-conjugated goat anti-rabbit antibody (1:2000; Cell Signaling Technology, Danvers, MA) for 60 min. Membrane bands were imaged using an Amersham
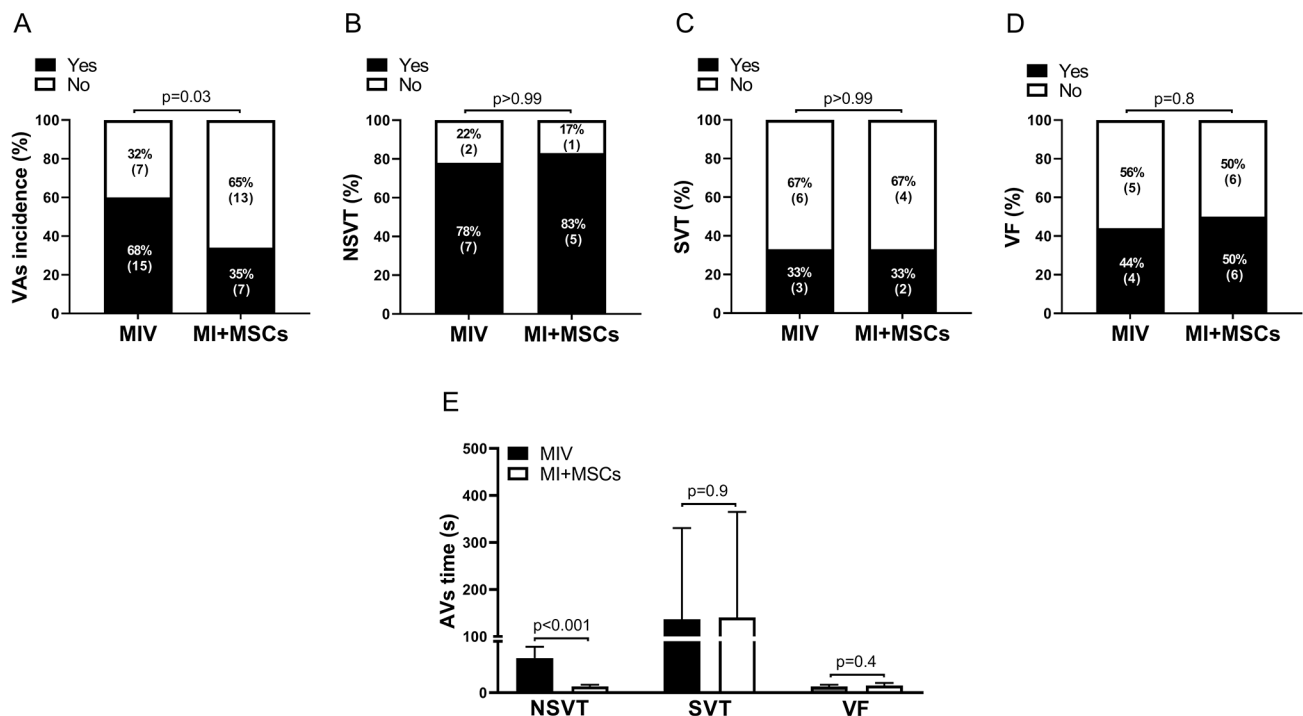

Fig. 1 Influences of mesenchymal stem cells derived from adipose (MSCs) tissue transplantation on ventricular arrhythmias (VAs) in infarcted rats. **(A)** The incidence of VAs after the induction of electrical stimuli was reduced in infarcted rats after MSCs transplantation. **(B)** non-sustained ventricular tachycardia (NSTV). **(C)** Sustained ventricular tachycardia (STV). **(D)** ventricular fibrillation (VF). **(E)** Duration of VAs. Comparisons with significant differences between groups are illustrated above the bars. Real number of animals is shown in parentheses in the columns
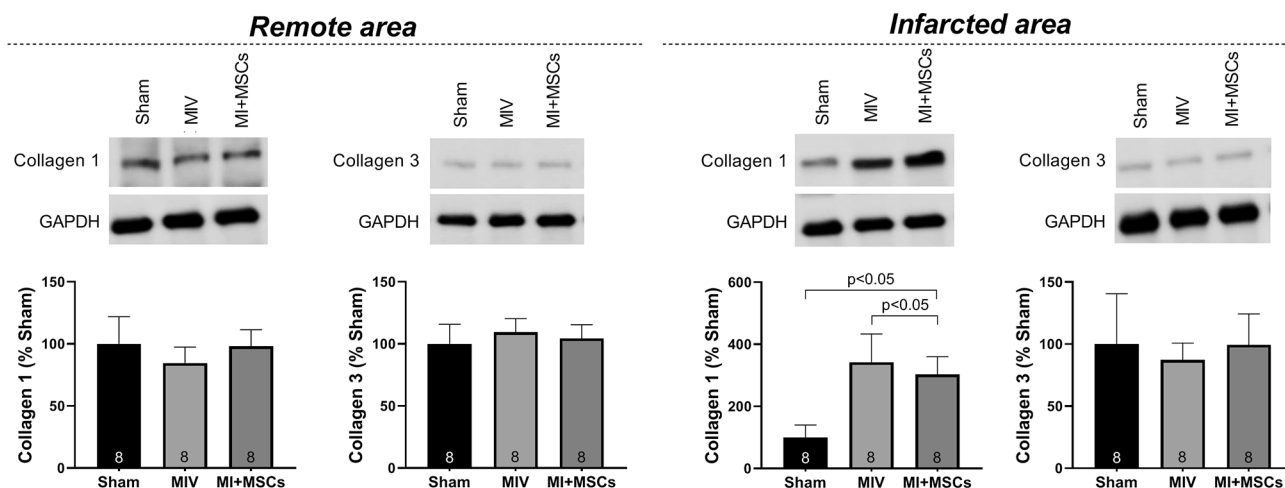
Fig. 2 Quantitative analysis for collagen 1 and collagen 3 expression. Collagen signal intensity was assessed in the remote and infarcted zone. Comparisons with significant differences between groups are illustrated above the bars. GAPDH, Glyceraldehyde 3-phosphate dehydrogenase. The sample size is shown within the columns

Imager 600 system (GE Health Care, Little Chalfont, Pittsburgh, PA, USA).

Enzyme-Linked Immunosorbent Assay (ELISA)

ELISA assays were performed according to the manufacturer's instructions (R&D Systems, USA) to the following mediators: tumor necrosis factor-alpha (TNF-α); interleukin 1β (IL-1β), interleukin 6 (IL-6) and interleukin 10 (IL-10). The plate was read using a SpectraMax M5 spectrophotometer (Molecular Devices, CA, USA).

Oxidative Stress Markers

4-Hydroxynonenal (4-HNE) was used as a marker of myocardial lipoperoxidation for an equal protein load (20 μg) with rabbit antibody (1:2000 dilution; Abcam, Cambridge, MA, USA) on Western blot. Carbonyl groups introduced into proteins by oxidative reactions were assessed using the Abcam kit ab178020 (Abcam, Cambridge, MA, USA). Experiments were performed as described elsewhere [16].

Antioxidant Enzymatic Activity

Catalase (CAT), glutathione peroxidase (GPX) and superoxide dismutase (SOD) enzyme activities were measured as previously described elsewhere [17].

Statistical Analysis

Data are presented as mean ± standard error of the mean. The normality of the data was analyzed using the Shapiro-Wilk test. Data with normal distribution were analyzed by unpaired t-test or one-way ANOVA (Newman-Keuls posttest), respectively. Non-parametric data were analyzed by Mann-Whitney or Kruskal-Wallis followed by Dunns. Chi-squared test was used for contingency data. Analyses were performed with GraphPad Prism software (version 9.3.1). The significance level was set at $p \leq 0.05$.

Results

Mortality

A total of six rats died within 24 h of MI and there were no deaths in the Sham group. All surviving animals were examined by echocardiography and electrophysiology.

LV Morphology and Performance

Infarct size and heart rate had significantly higher mean values in the MI + MSCs group compared to the MIV group (Table 1). In addition, the LV diastolic area was significantly smaller in the MI + MSCs group vs. the MI group. It is worth mentioning that the mean values of various markers of LV function are consistent with previous reports from our laboratory, indicating systolic and diastolic functional abnormalities [9, 10, 14, 18].

MSCs Detection and Arrhythmogenic Inducibility

Using real-time gene expression analysis, we observed that our intramyocardial transplantation procedure was efficient. The results of SRY expression show that there were no significant differences in MSCs content between the remote

$(1.27\pm0.29$, a.u.) and infarcted $(1.3\pm0.42$, a.u.) areas of the LV.

MSCs therapy had a cardioprotective effect on the induction of VAs. As shown in Fig. 1, only 35% of the MI + MSCs group developed VAs after electrical stimulation, while the incidence of arrhythmias in the IM group was 68%. In animals that developed VAs, NSVT/SVT and VF ratios were not significantly different between experimental groups. However, the NSVT time was significantly shorter in the MI + MSCs group.

Collagen, Connexin, and Cytokines Expression

Infarcted rats had higher levels of collagen 1 in the infarcted area compared to sham rats without the effect of cell therapy (Fig. 2).

There was no effect of infarction or MSCs on collagen 3 expression. Connexin 40 increased similarly in both regions of the myocardium in the infarcted groups, but MSC therapy resulted in a higher level of connexin 43 in the infarcted area (Fig. 3). A lower level of connexin 43 in the remote area was
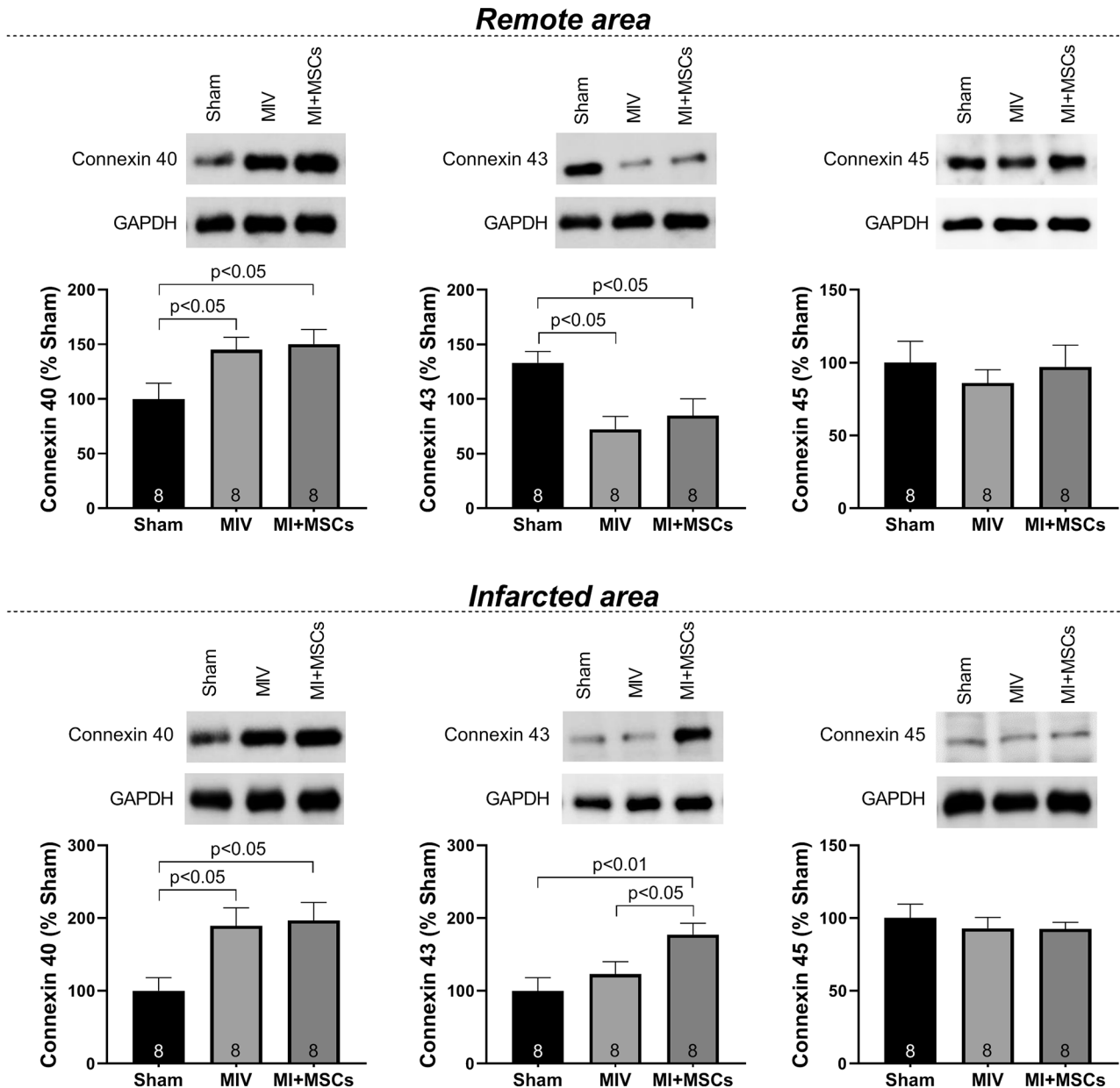

Fig. 3 Quantitative analysis for connexin 40, 43, and 45 expression. Connexin signal intensity was assessed in the remote and infarcted zone. Comparisons with significant differences between groups are illustrated above the bars. GAPDH, Glyceraldehyde 3-phosphate dehydrogenase. The sample size is shown within the columns
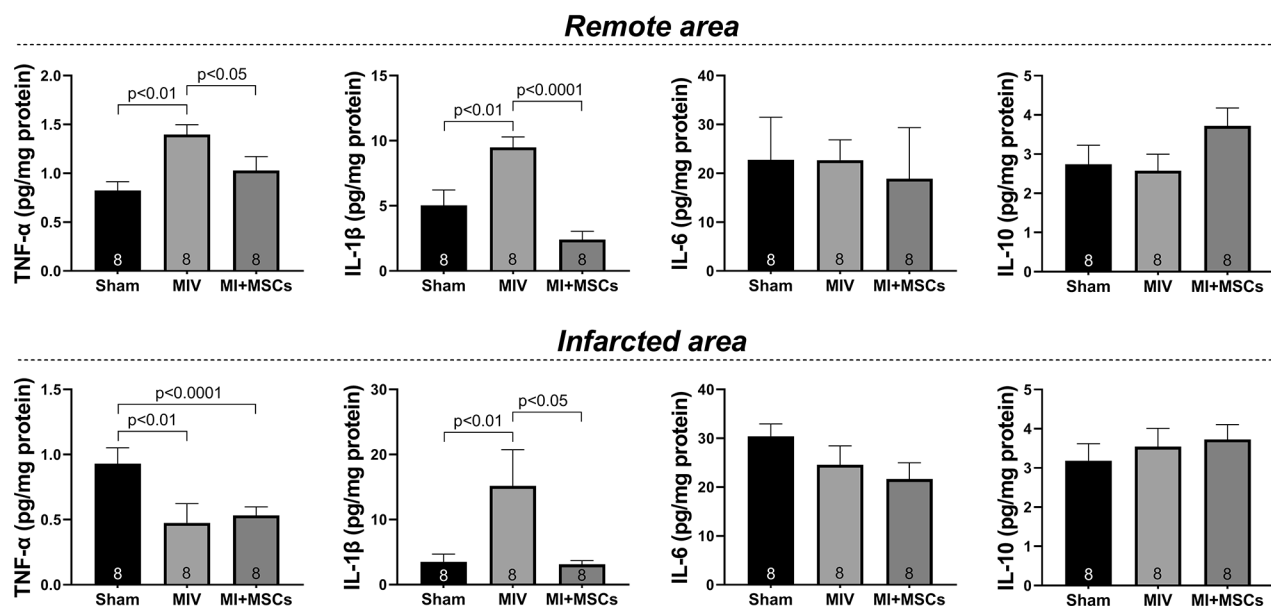
Remote area

Infarcted area

Fig. 4 Quantitative analysis for tumour necrosis factor alpha (TNF-α), interleukin-1 beta (IL-1β), interleukin-6 (IL-6), and interleukin-10 (IL-10) expression. Cytokine signal intensity was assessed in the remote and infarcted zone. Comparisons with significant differences between groups are illustrated above the bars. GAPDH, Glyceraldehyde 3-phosphate dehydrogenase. The sample size is shown within the columns

documented in the infarcted groups. There was no effect of infarction or MSCs on connexin 45 expression.

Figure 4 shows that infarcted rats had higher levels of TNF-α and IL-1β in the remote area. In the infarcted area, TNF-α levels were lower in the infarcted groups, whereas IL-6 expression remained higher than in the sham group. Notably, the MI + MSCs group had significantly lower levels of TNF-α and IL-1β than the MIV group in remote areas and IL-1β in infarcted areas. There was no effect of infarction or MSCs on the expression of IL-6 and IL-10.

Oxidative Stress

Carbonylation levels in remote and infarcted areas of the myocardium were significantly increased in the infarcted groups, and increased lipoperoxidation was reported only in the infarcted area (Fig. 5). This increase in lipoperoxidation was prevented by MSCs therapy. MI + MSCs animals had lower and higher GPx and SOD activities in the remote infarct area compared to other experimental groups. However, in the infarct area, MSCs therapy exacerbated the activity of CAT, GPx and SOD compared to other experimental groups.

Discussion

Data on the antiarrhythmic effects of MSCs are inconclusive. In our experiments, MSCs prevented the occurrence of VAs in infarcted rats. However, cell therapy did not alter the proportion of NSVT, SVT, and VF in animals that developed VAs. A shorter duration of NSVT was the only benefit of MSCs.

The increase in collagen content, especially types I and III, is known to adversely affect myocardial function. In addition, myocardial fibrosis is a direct substrate for triggering VAs and contributes to re-entries [19]. Therefore, our data suggest that the protective electrophysiological effects of MSCs do not appear to involve prevention of an increase in myocardial collagen content, at least in the acute phase of the disease.

Our findings corroborate previous data for increased connexin 40 and decreased connexin 43 expression in failing myocardium [20]. Connexin 40 upregulation was found at the endocardial surface, at and adjacent to Purkinje fibers, highlighting the Purkinje/working ventricular myocyte interface/junction as a potential site of altered electrical coupling that could trigger arrhythmogenesis [20]. It is well known that reductions in connexin 43 correlate with the presence of arrhythmias and ventricular dysfunction [20, 21]. Our findings of higher connexin 43 expression corroborate previous data showing higher connexin 43 expression in the border zone, remote zone, and infarcted zone of rats
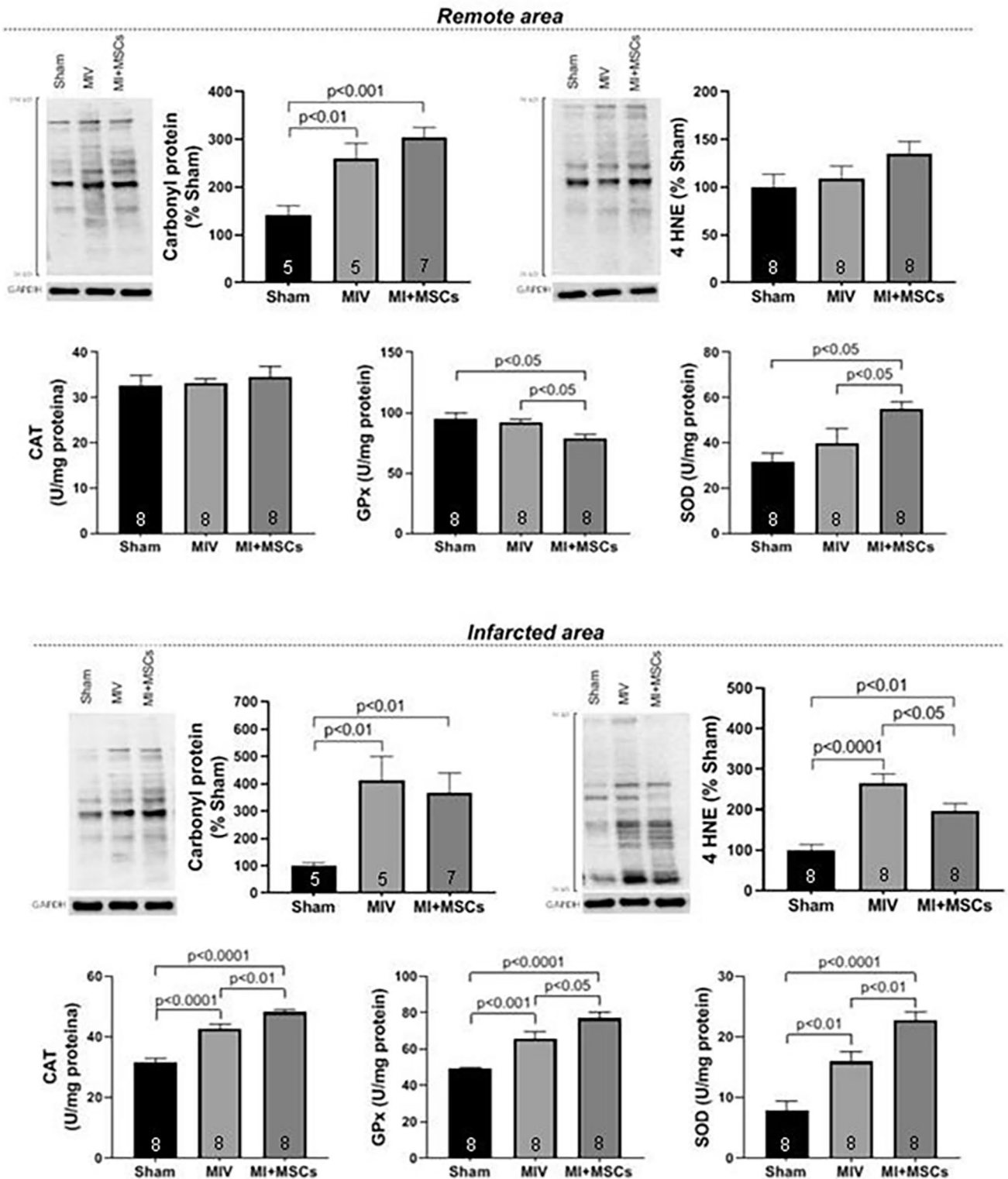
Fig. 5 Quantitative analysis for markers of oxidative stress (protein carbonylation and 4-hydroxynonenal aldehyde (4-HNE) and antioxidant enzyme activity. Analyzes were carried out in the remote and infarcted zone. Comparisons with significant differences between groups are illustrated above the bars. GAPDH, Glyceraldehyde 3-phosphate dehydrogenase. The sample size is shown within the columns

subjected to cardiac SC transplantation [22 and 23]. Other investigators also reported that bone marrow-derived MSC transplantation promoted higher connexin 43 content in the ischemic zone in rats with four weeks of coronary occlusion [3]. In our study, connexin 43 levels were not reduced in the infarcted area in animals without MSC transplantation, but were overexpressed in animals subjected to cell therapy. These findings are consistent with the concept that MSCs can form culminating junctions among themselves and between neighboring cardiomyocytes [24].

There is substantial evidence implicating inflammation in the etiology of VAs. For example, the induction of inflammatory cytokines as a consequence of systemic inflammation can affect the function of ion channels in cardiomyocytes, leading to prolonged action potential duration [25]. Considering the cardioprotective role of MSCs, it is possible to speculate that our finding of normalization of TNF-α expression in the remote infarct area may contribute to electrical stability in the myocardium. Experimental studies have shown that transgenic animals overexpressing TNF-α have more severe VAs [26]. TNF-α may alter the electrical activity of cardiomyocytes through various mechanisms to induce VAs. TNF-α can decrease Ito and Kv4.2 protein expression, inhibit the cardiac delayed rectifier K current via the protein kinase A pathway, and alter cellular Ca^{2+} release and uptake [26]. The lower expression of TNF-α in the infarcted area is noteworthy, but the significance of the genesis of post-infarction VAs remains to be explained.

Finally, our findings of higher IL-1β expression after MI are consistent with a previous study by our group [14], and attenuation of IL-6 by MSCs may be involved in the prevention of VAs. IL-6 may play a pivotal role in the modulation of ICa, L and IK currents, and both factors may contribute to cardiac instability [26].

There is limited information on the activity pattern of antioxidant enzymes after MI, especially in the early stages of cardiac remodeling. However, it is known that alterations in lipoperoxidation, protein carbonylation, N-glycosylation, and S-nitrosylation can adversely affect transmembrane and intracellular ion homeostasis, culminating in electrical instability [27]. Here, we investigated the 4-HNE content in the remote and infarcted areas. The involvement of 4-HNE in the genesis of cardiac electrical instability has been well documented in isolated cardiomyocytes. Bhatnagar [28] showed that perfusion of cardiomyocytes with 400 μmol/L 4-HNE induced prolongation of the action potential, progressive depolarization of the resting membrane potential, and loss of excitability. Therefore, our findings of lower 4-HNE expression in the infarcted area may have contributed to the antiarrhythmic effects of MSCs.

Oxidative stress results from an imbalance between pro-oxidant molecules and the antioxidant defense system [17, 29]. Therefore, a higher antioxidant status in the myocardium may be indicated as a way to prevent or attenuate the VAs [30]. In our study, we identified a distinct response of antioxidant enzymes in the infarcted heart. It was observed that there was greater activity of SOD, CAT, GPx and SOD in the infarcted area. There is limited information on the activity pattern of antioxidant enzymes after infarction, especially in the early stages of cardiac remodeling. We identified only one study that showed an upward trend in SOD, CAT and GPx activity within one week after MI in rats [31]. Interestingly, MSCs cells have exacerbated greater enzyme activity. SOD activity was higher in the MI + MSCS group compared to the other experimental groups. It is difficult to understand the translation of this finding to the prevention of AVs, but it is possible to consider that the increase in activity of antioxidant enzymes occurs as an antioxidant response to counteract oxidative stress. It is speculated that the additive effect of MSCs in increasing the activity of antioxidant enzymes may explain, at least in part, the lower level of 4-HNE in the infarcted area.

In conclusion, although there are controversial studies regarding the benefits of MSCs in intramyocardial transplantation, in our study we were able to verify that they are capable of forming a protective action against the development of VAs, but there is no significant benefit for animals that have developed VAs. It is possible to think that the cardioprotection of MSCs involves anti-inflammatory and antioxidant actions and improvement in the formation of gap junctions. The relevant aspect of our experiments is to demonstrate that the antiarrhythmic effect of MSCs was present in the early stage of post-infarction cardiac remodeling. Consequently, it is possible to think that from a clinical point of view, the use of MSCs therapy can be considered as an antiarrhythmic measure in patients in the early MI phase, especially in those for whom other resources have not had the expected results.

Declarations

Competing Interests The authors declare that they have no conflict of interest.

Ethics Approval The experimental protocol was approved by the Institutional Research Ethics Committee of the Federal University of São Paulo, Brazil (number: 2371290719).

Consent to Participate Not applicable.

Consent to Publication All authors give consent for publication of the manuscript.

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