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Research Article
GCSF Partially Repairs Heart Damage Induced by Repetitive β-adrenergic Stimulation in Mice: Potential Role of the Mobilized Bone Marrow-derived Cells

[1,4]B. Nieto-Lima, [1]A. Cano-Martínez, [2]G. Zarco-Olvera, [3]F.A. Massó-Rojas, [3]A. Páez-Arenas and [1]V. Guarner-Lans

[1]Department of Physiology, Instituto Nacional de Cardiología "Ignacio Chávez", México City, México
[2]Department of Pharmacology, Instituto Nacional de Cardiología "Ignacio Chávez", México City, México
[3]Department of Physiology, Cell Biology Section, Instituto Nacional de Cardiología "Ignacio Chávez", México City, México
[4]Department of Biological Sciences, UNAM, México City, México

Abstract

Background: Granulocyte Colony Stimulating Factor (GCSF) repairs acute heart damage. The main mechanism is its direct action on cardiac tissue. However, the role of the mobilized bone marrow-derived cells by GCSF is less explored. Pathologies such as obesity, mental stress and hypertension trigger chronic heart diseases through stimulation of the β-adrenergic system. Therefore, the effect of GCSF and of isolated mobilized blood marrow cells in a mouse model of heart damage induced by repeated β-adrenergic stimulation with isoproterenol was evaluated. **Materials and Methods:** Two experimental approaches were used: (1) Endogenous mobilization with GCSF was achieved directly in mice with heart damage (5 mg kg^{-1} day^{-1}, 7 days, s.c.). (2) Mobilized bone marrow-derived cells were isolated, labeled and inoculated to other mice with heart damage 1 and 30 days after damage. Ventricular hypertrophy, fibrosis, heart rate and mean blood pressure were measured. Inoculated cells were tracked in the heart. **Results:** GCSF reduced fibrosis; while, inoculated cells diminished fibrosis and mean blood pressure. Inoculation 30 days post-damage reduced fibrosis even more. Ventricular hypertrophy and heart rate were not restored with any treatment. Inoculated bone marrow-derived cells which were enriched in hematopoietic stem and progenitor cells migrated to the area of damage and some were αSMA-positive. **Conclusion:** GCSF partially restores heart damage produced by the repetitive β-adrenergic stimulation. Some mobilized bone marrow-derived cells migrate to the area of damage and are αSMA-positive, a phenotype related to cardiac sarcomerogenesis, cardiac muscle differentiation and cardiomyocyte rhythm, which could contribute to their beneficial effect. However, other mechanisms that could also be synergistically acting remain to be studied. A pool containing a diversity of cell types mobilized by GCSF, diminishes fibrosis and blood pressure in hearts damaged by repetitive stimulation of the β-adrenergic system, independently from the presence of the factor. Stimulation of this system is found in conditions such as obesity, metabolic syndrome or hypertension. Therefore, through this mechanism of GCSF, there exists the possibility of restoration of heart damage by mobilizing a pool of easily accessible cells with the factor, without having to isolate particular cell types nor having to expose the patients to invasive procedures.

Key words: Heart damage, β-adrenergic stimulation, mobilized bone marrow-derived cells, granulocyte colony stimulating factor, fibrosis

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Corresponding Author: V. Guarner Lans, Department of Physiology, Instituto Nacional de Cardiología "Ignacio Chávez" Juan Badiano 1, 14080, Tlalpan, México D.F., México Tel: 55 55 73 29 11/1278 Fax: 55 55 73 09 94

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

A cardiac remodeling process characterized by ventricular hypertrophy, fibrosis, inflammation, oxidative stress, among other compensatory mechanisms, takes place when the mammalian heart tissue is damaged. These changes, although compensatory, eventually generate heart failure and the death of the organisms[1-5]. Therefore, it is important to find new strategies to prevent and repair heart damage. The search for new strategies is even more important due to the increased incidence of obesity, sleep apnea, mental stress and hypertension, which are associated to myocardial infarction, ventricular hypertrophy, heart failure and sudden cardiac death.

Granulocyte Colony Stimulating Factor (GCSF) is a cytokine usually employed to promote the proliferation of neutrophils[6-8]. It can also mobilize other bone marrow cells such as hematopoietic stem cells[9,10] and mesenchymal stem cells[11] to the circulation. All of these cell types help the healing of wounds process[12,13]. The use of this cytokine to restore heart damage has been explored in human trails with controversial results[14-17]. In animal models, it promotes heart repair mainly by direct action on the tissue[18-22]. Although the role of the mobilized cell population by GCSF has been poorly explored, there is evidence that it might have benefic effects, depending on the etiology of the damage[23-25].

The most common strategy employed to study heart damage in animal models is acute heart injury and less information is found in models of chronic heart failure or repetitive β-adrenergic stimulation. The β-adrenergic stimulation resembles situations of chronic stress and also mimics some diseases which can trigger a myocardial infarction[26,27]. Therefore, in the present study we tested if mobilized bone marrow-derived cells by GCSF can repair the heart damage induced by repetitive β-adrenergic stimulation with isoproterenol.

Two experimental approaches were used: (1) Endogenous mobilization with GCSF was achieved directly in mice with heart damage. (2) Blood cells removed from a mouse treated with GCSF were labeled with "PKH26" and injected through the tail artery to another mouse with heart damage. Mobilized cells were injected at two different times after heart damage.

MATERIALS AND METHODS

Materials and equipment: GCSF (Neukine-Filgastrim) was obtained from Accord Farma-INTAS Pharmaceutical LTD, the isoproterenol was from Sigma Aldrich (I5627), Giemsa from Hycel de Mexico (6303), PKH26 from Sigma Aldrich (PKH26). Methocult medium was from stem cell, (M3534) as well as the hematopoietic stem cell enrichment kit (Stemcell, 19756). Masson trichrome Kit was bought from Sigma Aldrich (HT15). Primary antibodies used were rat anti-sca1 (abcam ab25195), rabbit anti-αSMA (alpha smooth muscle actin) (abcam ab5694). Secondary antibodies were alexa fluor 555-conjugated goat anti-rat (abcam ab150158), FITC-conjugated goat anti-rabbit (Santa cruz sc2012). DAPI was from Sigma Aldrich (D4817). All of the material used to prepare the buffers and other solutions was reactive grade. Laboratory equipments used were: AVR-6 apparatus (Honeywell), a Miotome pluss (TBS), an Olympus BX51 fluorescence microscope, an Qcapture digital camera, an ImagePro Premier Software and a Floid Cell Imaging Station.

Animals and groups: Two months old BALB/c mice were used. Animals remained under a 12:12 h light:dark cycle with food and water *ad libitum*. Mice were randomly assigned into 5 groups (n = 4 per group): CONTROL, treated with saline solution; ISO+SS7, treated with daily administrations of isoproterenol (5 mg kg^{-1} day^{-1}, 7 days, s.c.); ISO+GCSF7, treated with isoproterenol for 3 days at the dose previously mentioned followed by 4 coadministrations of isoproterenol and GCSF (300 μg kg^{-1} day^{-1}, 4 days, s.c.); ISO+Cells7, treated with isoproterenol (5 mg kg^{-1} day^{-1}, 7 days, s.c.) and inoculated with mobilized bone marrow-derived cells 24 h after the last administration of isoproterenol. In this group, cells are inoculated when the condition of damage in the heart is present. ISO30+Cells7, treated with isoproterenol (5 mg kg^{-1} day^{-1}, 7 days, s.c.) but inoculated with mobilized bone marrow-derived cells 30 days after the last administration of isoproterenol. At 30 days the adverse conditions created by hypoxia caused by isoproterenol have been compensated allowing for a better survival of the inoculated cells. All animals were sacrificed 7 days after the last treatment (indicated by suffix 7 in all treatments, i.e., ISO+SS7).

Mobilization of bone marrow derived cell: Mobilization was achieved by 4 administrations of GCSF (300 μg kg^{-1} day^{-1}, s.c.). This dose has been previously shown to mobilize hematopoietic stem and progenitor cells to the circulation and it has been used in models of skeletal muscle regeneration[28] and hypertrophy regression[22]. In the present study, the *in vivo* effect of the GCSF was measured by the quantification of the number of neutrophils per 500 leukocytes in peripheral blood

smears (10 µL) stained with 5% Giemsa (Hycel, Mexico, 6303) diluted in NaCl (pH 7.2) for 30 min under continuous slow agitation. Quantifications were done under an Olympus BX51 microscope under a 40x magnification.

Isolation and labeling of the cells: Blood samples were aseptically collected through cardiac puncture with a syringe with 0.1 M EDTA and centrifuged (1200 g, 10 min, 10°C, no break). The white phase containing the White Blood Cells (WBC) was recovered and incubated with warmed red blood cells lysing solution (155 mM NH_4Cl, 12 mM $NaHCO_3$, 0.1 mM EDTA) at 37°C for 7 min. Cells were centrifuged (1000 g, 10 min, 10°C, with break) and washed twice with Hanks solution (5.36 mM KCl, 0.44 mM KH_2PO_4, 4.2 mM $NaHCO_3$, 137 mM NaCl, 0.34 mM Na_2HPO_4) supplemented with 16 mM glucose, 22 mM sucrose and 26 mM non-acid hepes. When red blood cells were still observed, a second lysis was done followed by two washes. Bone marrow samples were obtained by aspiration from the two tibias of the mice. Cells were centrifuged (1000 g, 10 min, 10°C, with break), incubated with warmed red blood cells lysing solution for 5 min and washed twice with hanks solution supplemented with glucose, sucrose and non-acid hepes. In order to track the cells *in vivo*, once the cells were isolated, they were incubated with the red membrane marker "PKH26" (Sigma Aldrich, PKH26) following the protocol suggested by the supplier but by doubling the concentration of marker. These cells were posteriorly inoculated into mice with heart damage.

Colony Forming Unit (CFU) assay: The presence of progenitor cells was estimated by seeding 100,000 WBC isolated from peripheral blood and bone marrow in 6 well plates in Methocult medium (Stemcell, M3534). Cells were incubated for 15 days at 37°C and 5% CO_2. At the end of this time the number of colonies was evaluated. Specifically, for this assay, animals were sacrificed 24 h after the last administration of ISO (ISO) or GCSF (ISO+GCSF).

Separation of cells lin-: The proportion of cells lin- (CD5, CD11b, CD19, CD45R, 7-4, Ly-6G/C (Gr-1), TER119) was obtained through the magnetic separation of the freshly isolated mobilized cells with the hematopoietic stem cell enrichment kit (Stemcell, 19756) according to the manufacture.

Cell inoculation: Once the damage was generated with isoproterenol, freshly isolated mobilized cells (1.2×10^7 cells kg^{-1}) labeled with PKH26 were injected through the tail artery. Two timings of inoculation were tested: 24 h and 30 days post damage.

Hemodynamic and morphological analysis: Hemodynamic (Heart Rate (HR) and Mean Blood Pressure (MBP)) and morphological analysis of myocardial damage were done 7 days after the last administration of saline solution, isoproterenol, GCSF or cells; unless otherwise specified.

Heart rate and mean blood pressure were determined with a VR-6 apparatus (Honeywell). Animals (n = 4 per group) were weighed and anesthetized via an intraperitoneal injection of 50 mg kg^{-1} of sodium pentobarbital to reach a state of surgical anesthesia. Electrodes in the DII position were paced to record the electrocardiogram and a catheter was placed in the right craneal carotid artery to measure mean blood pressure.

For morphometric analysis, after hemodynamic recordings, the heart was removed, washed with TBS (137 mM NaCl, 20 mM tris-HCl, pH 7.4), weighed and fixed in 4% paraformaldehyde for 24 h. The heart was transferred to a 30% sucrose solution for at least 24 h and immersed in tissue-tek for 4 days. Longitudinal sections (10 µm) were serially cut with a cryostat and adhered to slices. Masson trichrome staining (Sigma Aldrich, HT15) was performed to quantify interstitial fibrosis. Briefly, sections were treated according to the manufacturer with slight modification in the time of incubation and the addition of a step with bluing solution: Working Weigert's iron hematoxylin solution (15 min), scott's bluing solution (5 min), Biebrich Scarlet-acid fucshin (2 min), working phosphotungstic/phosphomolybdic acid solution (15 min), aniline blue solution (60 min) and 0.1% acetic acid (30 sec). Cryosections were scanned with a camera attached to an Olympus BX51 microscope under a 40x magnification, transferred to a computer and total area and area positive to fibrosis (color blue) of each section was measured using ImagePro Premier software. The percentage of fibrosis was quantified in the middle sections of the heart of at least 3 animals per group. In inoculated animals, cells were tracked in heart sections under the Floid Cell Imaging Station. Body weight to ventricular weight ratio was quantified as a measurement of Ventricular Hypertrophy (VH)[29,30].

Immunohistological analysis: Immunohistological studies were performed on cells and on heart tissue. In the first case, the isolated cells were adhered to rounded coverslips with poli-l-lysine for two hours (37°C), fixed in 4% paraformaldehyde and washed with TBS. In the second case, hearts were removed from the mice; washed with TBS,

weighed and fixed in 4% paraformaldehyde for 24 h. Longitudinal 10 µm criosections were serially cut. Both cells and tissue sections were blocked for 1 h with 12% fetal bovine serum in KMCT buffer (120 mM KCl, 20 mM NaCl, 10 mM tris-HCl, 1 mM EDTA, 2% triton X-100, pH 8). The following primary and secondary antibodies were used: Rat anti-sca1 (1:100, abcam ab25195), rabbit anti-αSMA (alpha smooth muscle actin) (1:400, abcam ab5694), goat anti-rat conjugated to Alexa Fluor 555 (1:800, abcam ab150158) and goat anti-rabbit conjugated to FITC (1:800, santa cruz sc2012). Primary and secondary antibodies were incubated at 4°C overnight and 1 h at room temperature under agitation, respectively. Nuclei were counterstained with DAPI. Three washes (5 min) with TBS were done between each incubation. Cell and tissue section images were capture with an Olympus BX51 fluorescence microscope (100×) equipped with a Qcapture digital camera and processed with ImagePro Premier software.

Data analysis: Data are presented as Means±Standard Error. Results were analyzed with one-way analysis of variance (ANOVA) followed by Bonferroni post-test using Graph-Pad Prism v.4 .

RESULTS

Cell mobilization with GCSF: A first approach of the effect of the GCSF was achieved by the quantification of neutrophils in smears from peripheral blood, showing a 2.96-fold increase when compared to the control group. Cells isolated from peripheral blood cells of mice treated with GCSF formed 14-fold more colonies compared to control group (Fig. 1a-e). Immunomagnetic separation with the enrichment separation kit revealed that 11.7% of the cells were lin-. Immunohistological analysis of the mobilized bone marrow-derived cells showed that 38.5% were Sca^+.

Treatment with GCSF in mice with heart damage: Mice treated with isoproterenol developed ventricular hypertrophy and had increased heart rate and mean blood pressure. This group also had the highest percentage of fibrosis when compared to the other groups at day 7 post-injury. Mice treated with isoproterenol that received the GCSF, showed no changes in VH (Fig. 2a), heart rate (Fig. 2c) and mean blood pressure (Fig. 2d) compared to ISO+SS7 group. Nevertheless, fibrosis was 35.2% lower than in the ISO+SS7 group but did not reach basal levels (Fig. 2b).

Cell mobilization in mice with and without heart damage: CFU assay from peripheral blood cells and bone marrow cells collected 24 h after the last injection showed that bone marrow cells from ISO group formed the highest number of colonies (250±34 colonies per 10^5 WBC) but when GCSF was administered, the highest number of colonies (64±13 colonies per 10^5 WBC) was observed in cultures from peripheral blood cells (Fig. 3).

Inoculation of mobilized bone marrow-derived cells in mice with heart damage: In order to determine if the

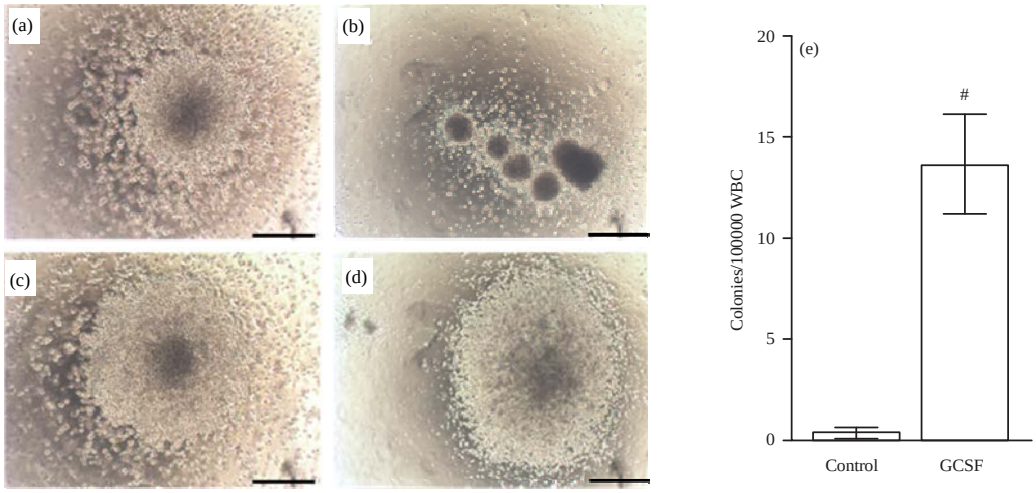

Fig. 1(a-e): Colony forming unit assay, (a-d) Colonies formed from isolated peripheral blood cells of mice treated with GCSF (magnification 4x), 1×10^5 peripheral blood nucleated cells were plated into six-well plates and (e) Colony-forming efficiency was determined by number of colonies per 10^5 peripheral cells plated. Bar = 150 µm. #p<0.05 compared to control

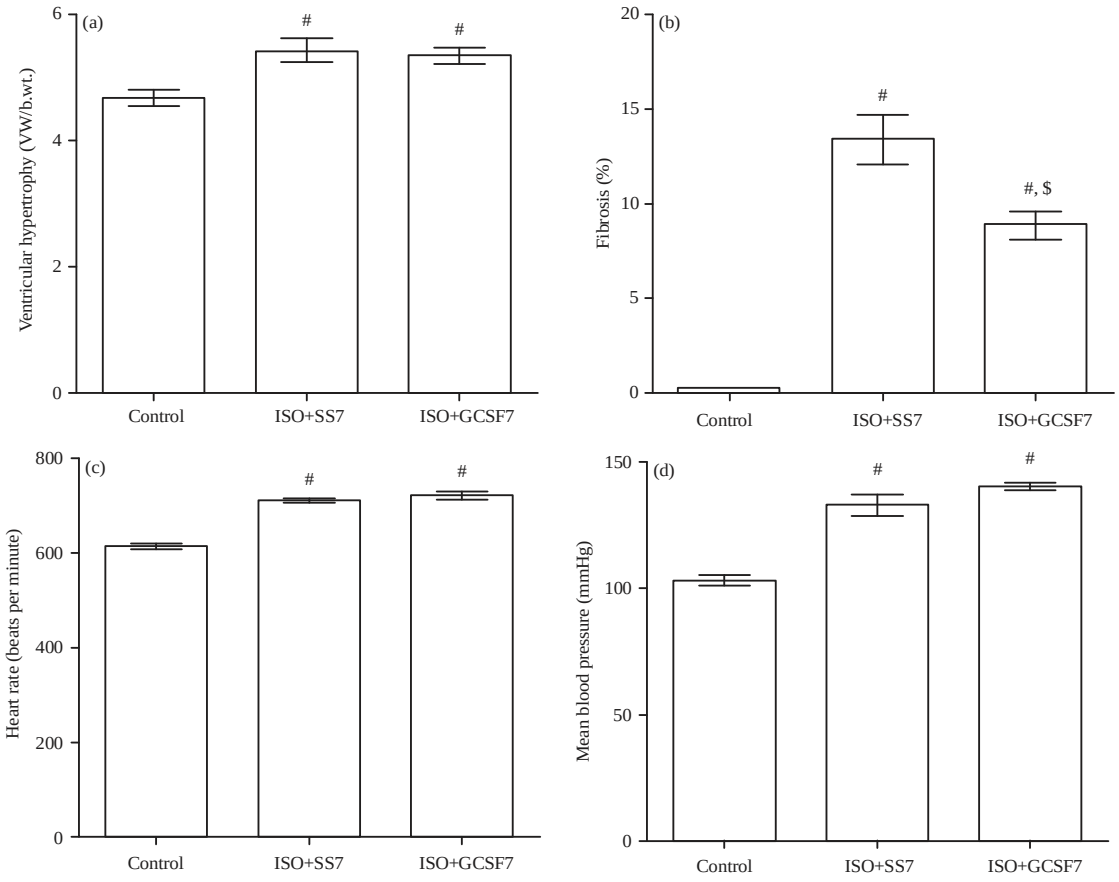

Fig. 2(a-d): Effect of the treatment with GCSF in mice treated with isoproterenol, (a) Ventricular hypertrophy, calculated as ventricular weight/body weight, (b) Quantification of cardiac fibrosis measured by ImagePro Premier software. Animals were sacrificed 7 days after the last treatment (indicated by suffix 7 in all treatments, i.e., ISO+SS7), (c) Mean heart rate and (d) Mean blood pressure. $^{\#}p<0.05$ compared to control and $^{\$}p<0.05$ compared to ISO+SS7. Data represents the Mean±SE

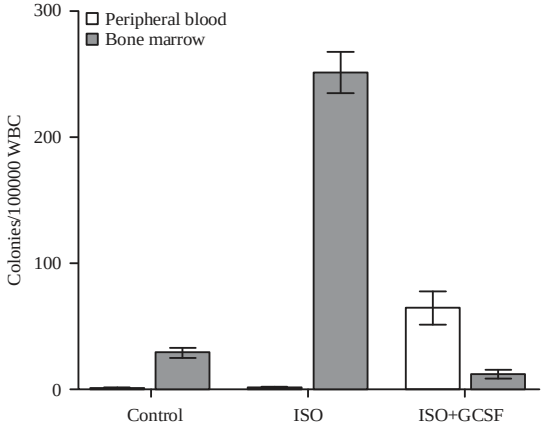

Fig. 3: Colony forming unit assay and heart damage. Quantification of the number of colonies formed from isolated peripheral blood and bone marrow cells from mice treated with isoproterenol (ISO) or with isoproterenol and GCSF (ISO+GCSF)

observed effect of GCSF is due to the mobilized cells, they were isolated, labeled and inoculated into different mice with heart damage. As observed with the treatment with GCSF, inoculation of cells 24 h postinjury (ISO+Cells7) decreased fibrosis (Fig. 4b) and mean blood pressure (Fig. 4g) but did not reverse the effect of ISO on VH (Fig. 4a) and HR (Fig. 4h). When cells were inoculated 30 days after damage (ISO30+Cells7), when adverse conditions are not so pronounced, instead of after 24 h, the same behavior was observed with the only difference that the percentage of fibrosis kept dropping (40.95% vs ISO+Cells7) (Fig. 4). Representative photographs of the heart with fibrosis are shown in Fig. 4c-f.

Finally, the tracking of inoculated cells in heart tissue sections from both conditions (ISO+Cells7 and ISO30+Cells7) revealed that a small proportion of the cells reached the damaged area and some of these cells were positive to SMA (Fig. 5a-h).

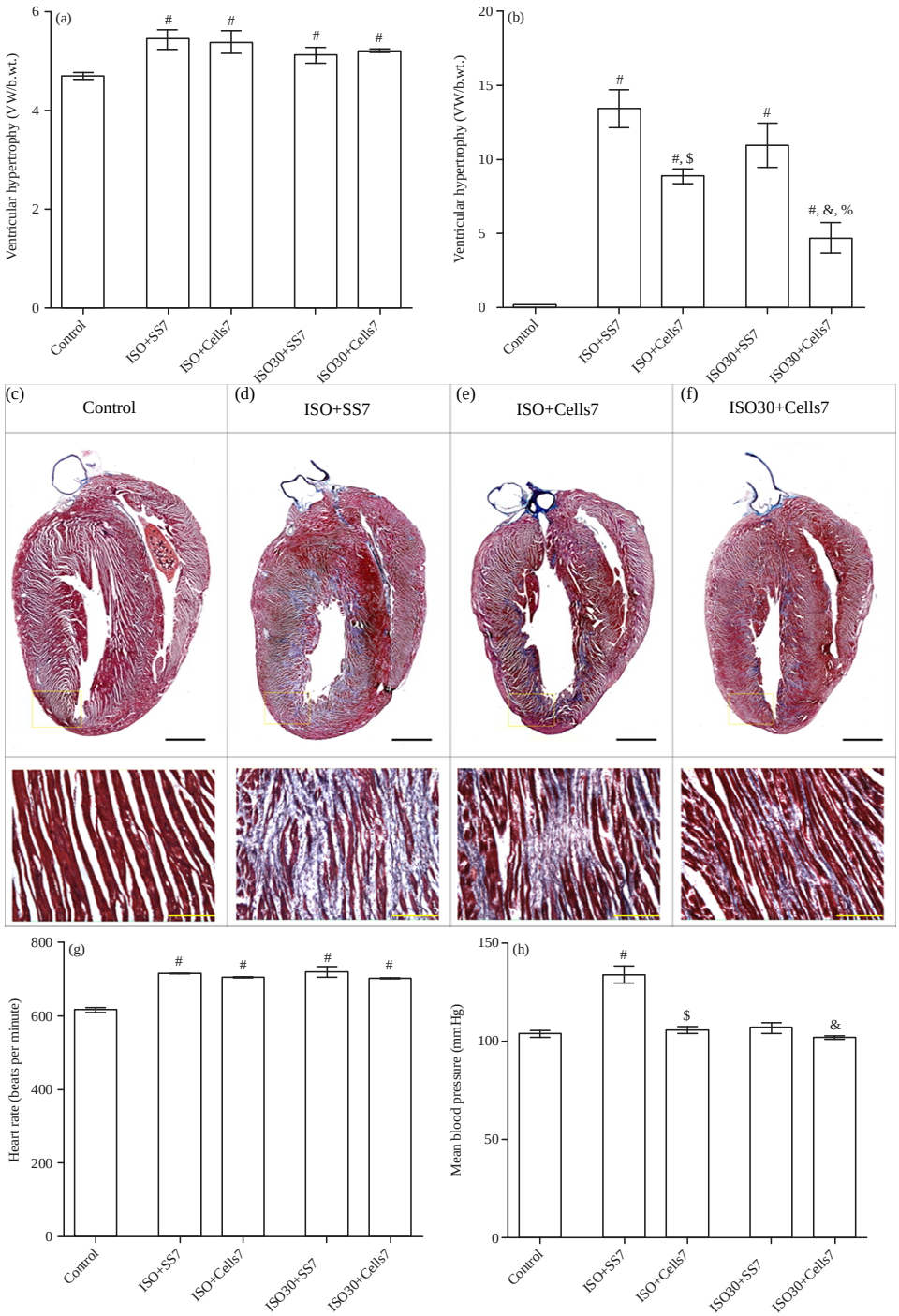

Fig. 4(a-h): Morphometric and hemodynamic changes associated to the inoculation of mobilized bone marrow cells on the heart of mice with heart damage induced by repetitive administrations of isopreoterenol (ISO), (a) Ventricular hypertrophy, calculated as ventricular weight/body weight, (b) Quantification of cardiac fibrosis measured by ImagePro Premier software, (c-f) Representative photographs of heart sections from mice from each treatment stained with Masson's trichrome to identify myocardium in red and fibrosis in blue. Lower panels correspond to high-magnification (10x) photographs of each heart from the upper panel, (g) Heart rate and (h) Mean blood pressure. Animals were sacrificed 7 days after the last treatment (indicated by suffix 7 in all treatments, i.e., ISO+SS7). Black bar = 1500 μm. Yellow bar = 200 μm. $^{\#}p<0.05$ compared to control, $^{\$}p<0.05$ compared to ISO+SS7, $^{\&}p<0.05$ compared to ISO30+SS7 and $^{\%}p<0.05$ compared to ISO+Cells7. Data represents the Mean±SE

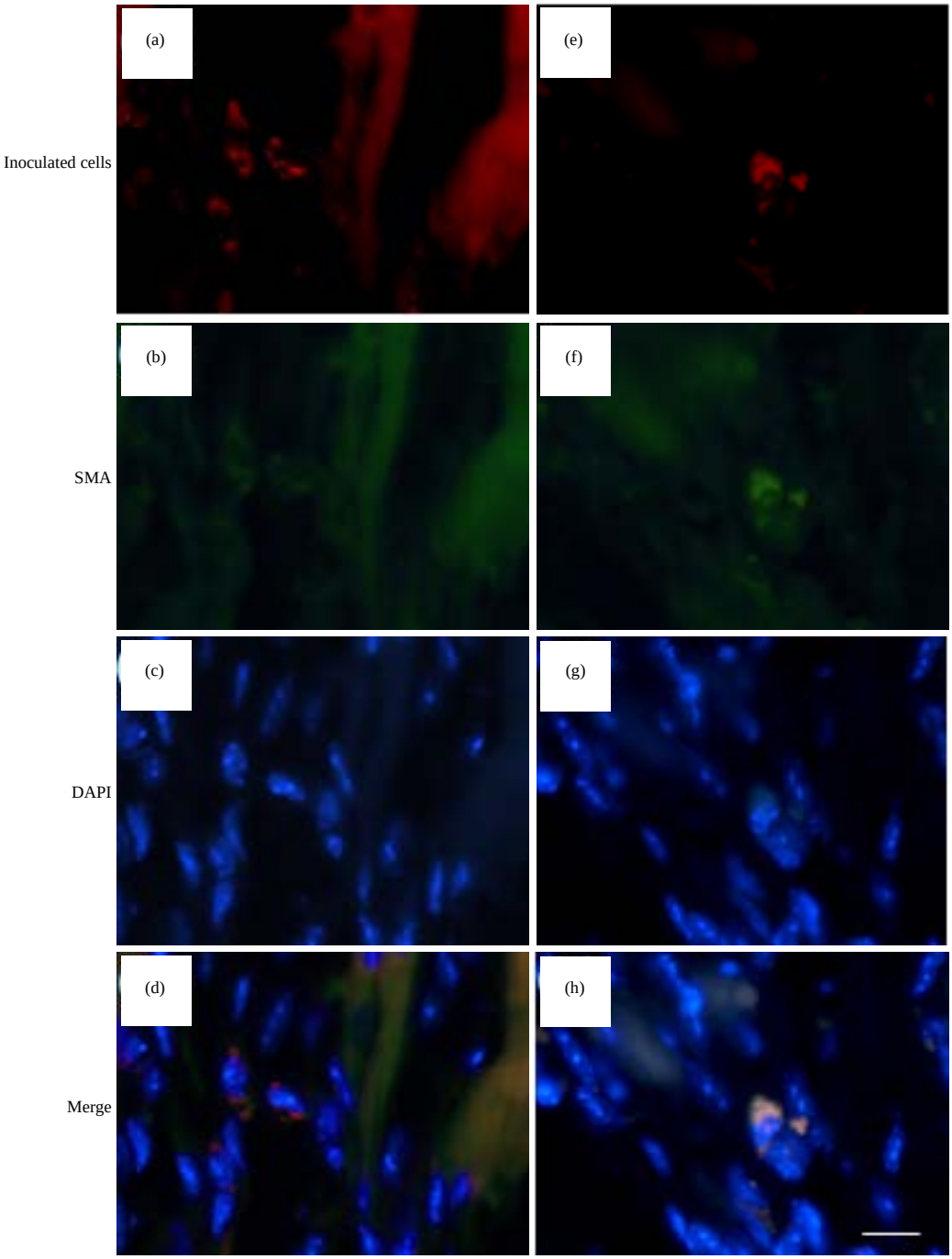

Fig. 5(a-h): Immunofluorescence for αSMA in the heart of inoculated mice. Photographs of the inoculated cells (red) and immunofluorescence for αSMA (green) in heart tissue sections from mice with heart damage. Nuclei were stained with DAPI (blue) (magnification 100x). Left panel (a-d) Inoculated cells negative to SMA. Right panel (e-f) Inoculated cells positive to αSMA. Bar = 30 μm

DISCUSSION

The present study was undertaken to study the effect of mobilized bone marrow-derived cells by GCSF upon morphological and physiological restoration of heart damage induced by repetitive injections of isoproterenol. Daily administration of 5 mg kg^{-1} day^{-1} of isoproterenol for 7 days induced heart damage characterized by ventricular hypertrophy, fibrosis, increased heart rate and mean blood pressure. GCSF repaired this damage possibly through the

mobilization of cells from the bone marrow to the circulation. Based on the CFU assay, the immunomagnetic separation and the immunohistological analysis, these mobilized bone marrow-derived cells were enriched in hematopoietic stem and progenitor cells. Furthermore, some of the inoculated cells arrived to the heart and were positive to αSMA, a protein known to play an important role in cardiac sarcomerogenesis, cardiac muscle differentiation and which also influences cardiomyocyte rhythm[31,32].

Isoproterenol is a cardiotoxic agent successfully used to induce heart damage. Its use has been widely reported in different animal models[33-35]. However, in most studies it is acutely applied in order to mimic a myocardial infarction. Its employment in this manner induces cardiac hypertrophy without increased blood pressure, apoptosis, necrosis, fibrosis, inflammation and oxidative stress[35-43]. ISO has also increased blood pressure and heart rate in other studies. The changes produced in these parameters depended on the type of strain that caused the damage[44,45]. Nevertheless, the mechanisms underlying these changes remain unknown.

In the present study, daily administrations of isoproterenol were given for 7 days mimicking activation of the sympathetic nervous system. Chronic activation of the sympathetic nervous system in humans promotes the development of cardiovascular diseases, such as ventricular hypertrophy, heart failure and sudden cardiac death. Thus, it is important to know the effects of the repetitive stimulation of β-adrenergic receptors[26,27]. More significantly, diseases such as obesity, sleep apnea, mental stress and hypertension chronically increase sympathetic activation[46,47], which is probably the cause of their association to cardiovascular diseases.

Treatment with GCSF promoted the regression of fibrosis in this study, without diminishing hypertrophy or hemodynamic parameters. These results suggest that the cytokine partially repairs cardiac remodeling induced by the repeated β-adrenergic stimulation. The reported results of the appliance of GCSF are still controversial. This cytokine restores acutely-induce heart damage in models of coronary ligation or acute isoproterenol administration in rats. GCSF also reduced fibrosis and restored cardiac function in models of left ventricular hypertrophy induced by transverse aortic constriction[22,48] and in acute stages of myocardial infarction[49]. GCSF also partially reverted fibrosis, cardiomyocyte hypertrophy and left ventricular enlargement induced by the acute administration of isoproterenol in rats[50]. In contrast, in another study hypertrophy and infarct size were not different between rats subjected to myocardial infarction surgery and those treated with two different protocols of administration of the cytokine (50 μg kg^{-1} day^{-1} during 7 days or four cycles of 5 days of 10 μg kg^{-1} day^{-1})[51]. Furthermore, in a model of cryodestruction, ventricular hypertrophy was significantly reduced without a diminution of the scar area[52].

The mechanism by which GCSF repairs heart damage includes activation of anti-apoptotic[53] and anti-autophagic signaling pathways[54]. It stimulates its receptors in the heart to promote cardiomyocyte proliferation[55]. It also modulates the synthesis and degradation of components in the extracellular matrix, such as the MMP2 and MMP9 and it activates sarcomeric proteins such as myosin heavy chain, troponin I and desmin[49].

All of the above mechanisms have been reported in separate studies but they might be taking place at the same time or in a sequential manner. Another possible mechanism, which has not been completely studied, might involve the participation of mobilized cells from the bone marrow to the circulation. These cells might reach the injured area and help its restoration.

In our study, the CFU assay suggested that *in vivo* administration of isoproterenol promotes the proliferation of hematopoietic progenitor/stem cells from the bone marrow cells cultured *in vitro*. However, the adrenergic stimulation with isoproterenol did not release these from the bone marrow to the circulation. In contrast, the administration of GCSF to mice with heart damage produced by isoproterenol released these cells as was demonstrated in the CFU assay performed it this study. These results showed that the hematopoietic progenitor/stem cells were increased in the circulation and decreased in the bone marrow. Once the cells are released from the bone marrow, they may reach the areas of damage and help repair the injured tissue.

However, evidences exist in favor and against this fact. Brunner *et al.*[56] found that GCSF reduces the levels of stem cell factor and stromal-derive factor-1, thus reducing the migration of mobilized bone marrow (ckit+, CXCR4+) cells into the ischemic tissue in a model of coronary artery ligation in mice. GCSF increased the number of resident Sca+ cardiac cells. In contrast, Huber *et al.*[48] found an increased homing of mobilized bone marrow (ckit+, CXCR4+) cells. Li *et al.*[49] reported that bone-marrow derived cardiomyocytes were not detected in hearts from mice treated with GCSF with chronic heart failure. This increased homing was associated with increases the mRNA and protein expression of the stem cell factor in the heart when mice were treated with the GCSF in a model of pressure-induced left ventricular hypertrophy.

A disadvantage of all of these studies is that the mobilized cells were tracked in the same mice treated with the cytokine. This design renders confusing results regarding the precise function of the cells, since it is not possible to discern if the mobilized cells triggered the regression of fibrosis or if it was

the factor that accomplished this role. If this is the case, the cells would have only helped restore the cardiac mass. Even more, the specific role of the cells has not been described when heart damage is induced by repetitive β-adrenergic stimulation.

Here, we tested if the mobilized cells by GCSF participate in heart repair, by mobilizing them in one group of healthy mice and inoculating them into different mice with heart damage. These results show that the inoculation of the mobilized cells had the same effect as GCSF. Additionally, cells helped a faster restoration of the blood pressure. Moreover, some of the inoculated cells were found in the heart. Interestingly, some of these cells were positive to αSMA. All of these data suggest that the cells *per se* have an important role during reparation of the heart and that their effect is independent from the action of the GCSF. The role of the mobilized cells also involves the adoption of a αSMA phenotype, which is associated to cardiac sarcomerogenesis, cardiac muscle differentiation and has influence on cardiomyocyte rhythm[31,32].

Whether these positive cells were hematopoietic stem/progenitor or other kind of cell remains to be studied. Treatment with GCSF mobilizes not only hematopoietic stem cells[9,10] but also mesenchymal stem cells[11]. All of these cell types have been reported to help healing[12,13]; although the amount of cells reaching the injured area and their homing and survival in the damaged tissue are still problems to be solved. Furthermore, GCSF also promotes the proliferation of neutrophils[6-8], which selectively release IL-1B. This interleukin induces the expression of the MMP2 and MMP9 in cardiac fibroblast thereby helping in the regression of fibrosis[22]. Therefore, the inoculation of the whole mobilized cell fraction could represent an advantage over inoculation of just one single population of cells.

Regarding to the time of inoculation, we found that inoculation of cells 30 days post damage induces a more efficient regression of fibrosis, when compared to that found 24 h after damage. It has been questioned whether another moment for cell inoculation could be suitable, although most of the studies have inoculated cells as soon after the damage takes place. Some of the main challenges related to the inoculation of any type of cell are: (a) the low rate of homing and engraftment of cells to the area of damage, (b) their low proliferation rate and (c) the little reparation of heart damage. One of the reasons behind these challenges is the hostile environment found by the cells in the area of damage during the first days or weeks after damage. The environment at this stage is characterized by conditions of hypoxia, inflammation, apoptosis, oxidative stress and extracellular matrix deposition, among others[3,5,37]. These conditions have been reported to modify the properties of the cells, such as their engraftment potential, their ability to differentiate into the desired cell type and their survival rate[57-59]. Therefore, cells inoculated 30 days post damage could benefit from not being subjected to these damaging conditions, thus having better possibilities of arriving and homing in the injured zone. Once in the damaged zone, they could contribute to tissue reparation through the secretion of angiogenic factors, collagenases and metalloproteinases which are capable of restoring the extracellular matrix homeostasis. They could also secrete factors such as IGF-1 which has the potential to activate resident progenitor cardiac cells. Although the rate of differentiation of inoculated cells, mainly stem cells has been reported to be low, they might acquire other phenotypes different from cardiomyocytes, such as the endothelial phenotype, which is another cell type that forms part of the heart. Additionally, a pool of mobilized cells contained not only stem cells but also immune cells. The secretion of IL-1B by these cells might contribute to the regression of fibrosis[22].

CONCLUSION

In conclusion, GCSF is able to partially restore the heart damage produced by the repetitive stimulation of the sympathetic nervous system and the β-adrenergic system. These results show that the mobilized bone-marrow derived cells, migrate to the area of damage and that they acquire an αSMA phenotype, which plays an important role in cardiac sarcomerogenesis, cardiac muscle differentiation and influences cardiomyocyte rhythm. However, other mechanisms that could also be synergistically acting, remain to be studied.

SIGNIFICANT STATEMENT

In this study we report the effects of GCSF and of the mobilized cell pool by this factor on cardiac reparation of damage produced after repetitive administration of isoproterenol that mimic stressful conditions. Isoproterenol models are usually acute and there are few reports on its repeated administration. It is found that the mobilized cells, without the presence of GCSF have an important role on heart reparation since they arrive to the damaged zone and express some molecules of constitutive cells of the heart tissue that help in its physiological functioning. This issue has not been reported.

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