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ORIGINAL RESEARCH ARTICLE

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β-blockers interfere with cell homing receptors and regulatory proteins in a model of spontaneously hypertensive rats

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Abstract

Aim: To examine the interference of β-blockers with the chemokine stromal cell-derived factor-1 (SDF-1) found in cell homing receptors, C-X-C chemokine receptor type 4 (CXCR-4) and CXCR-7, and regulatory proteins of homing pathways, we administered atenolol, carvedilol, metoprolol, and propranolol for 30 days using an orogastric tube to hypertensive rats.

Method: We collected blood samples before and after treatment and quantified the levels of SDF-1 with enzyme-linked immunosorbent assay (ELISA). On day 30 of treatment, the spontaneously hypertensive rats (SHR) were euthanized, and heart, liver, lung, and kidney tissues were biopsied. Proteins were isolated for determining the expression of CXCR-4, CXCR-7, GRK-2 (G protein-coupled receptors kinase 2), β-arrestins (β1-AR and β2-AR), and nuclear factor kappa B (NFκB).

Results: We found that the study drugs modulated these proteins, and metoprolol and propranolol strongly affected the expression of β1-AR ($P = .0102$) and β2-AR ($P = .0034$).

Conclusion: β-blockers modulated tissue expression of the proteins and their interactions following 30 days of treatment. It evidences that this class of drugs can interfere with proteins of cell homing pathways.

KEYWORDS

β-blockers, SDF-1, spontaneously hypertensive rats, stem cell homing

1 | INTRODUCTION

Arterial hypertension is a major risk factor for heart, cerebrovascular, and kidney diseases. Neural, humoral, and myogenic factors are involved in the development of hypertension, and they are associated with increased vasomotor tone, decreased vasodilatory ability, and internal remodeling of blood vessels.[1]

β-blockers are a class of drugs used to treat hypertension and its potential consequences.[2] They act by blocking the effect of β1- and β2-adrenergic receptors, which are functionally coupled to G protein receptors.[3] They interfere with signaling pathways triggered by these receptors through molecular interactions, second messenger activation and signal transduction by kinases,[3] thus neutralizing receptor overstimulation and restoring heart function.[4] β-blockers currently available for clinical use include atenolol and metoprolol that are β1-selective blockers;[5,6] propranolol that is a first-generation β-blocker with nonselective action on β1- and β2-receptors;[7] and carvedilol that inhibits β1-, β2-, and α1-receptors and has antioxidant effects.[8]

Evidence suggests that drugs used to treat heart conditions may interfere with major cell signaling pathways[9] such as activation of cell migration and cell proliferation and differentiation—the so-called cell homing mechanisms.[10] The homing pathway is activated by the chemokine stromal cell-derived factor-1 (SDF-1/CXCL-12) when it binds to its receptors CXCR-4 and CXCR-7.[10] Both CXCR-4 and β-adrenergic receptors interact with subunits of G protein leading to the activation of parallel mechanisms such as receptor desensitization, signal transduction, and transcription activation of target genes,[11] which may lead to activation of the cell cycle and chemotaxis.[12] It is recognized that some β-blockers can inhibit cell proliferation and that signaling pathways of β-adrenergic receptor antagonists and cell homing share common molecules such as G protein-coupled receptors (GPCRs) and β-arrestins (β-AR).[13]

Various animal models have been used to study the pathophysiology of arterial hypertension including Dahl salt-sensitive rats to study renal hypertension, animal models of neurogenic hypertension, and the model of spontaneously hypertensive rats (SHR).[14] SHR are suitable to study the development of hypertension because it is a model that reproduces essential hypertension in humans.[15] These rats share a genetic predisposition to hypertension without specific etiology, increased total peripheral resistance without volume expansion, heart hypertrophy, and similar responses to drug treatment.[16,17]

Spontaneously hypertensive rats lineage reproduces the long-term deleterious effects of hypertension in humans and can be treated with β-blockers. This study aimed to examine potential interferences of continuous β-blocker administration with the activation of cell homing pathways (SDF-1, CXCR-4, and CXCR-7), GPCR kinase 2 (GRK-2), desensitization of GPCRs, β-arrestins (β1-AR and β2-AR), and nuclear factor kappa B (NFκB) in the heart, liver, lung, and kidney tissues in this animal model (SHR).

2 | METHODS

2.1 | Study design and sample

This in vivo experimental study was approved by the Ethics Committee for Animal Use (CEUA) at Instituto de Cardiologia do Rio Grande do Sul/Fundação Universitária de Cardiologia (protocol number 4655/11). Animal experiments were performed conform the NIH guidelines (Guide for the care and use of laboratory animals) and standard operating procedures set by the Department of Animal Production and Experimentation and the ethical principles of animal experimentation (Brazilian Society for Laboratory Animal Science, SBCAL/COBEA) in accordance with Brazilian Law No. 11,794/08.

The experimental animals were kept under conventional vivarium conditions (ventilated, controlled temperature cages, 12/12-hour light and dark cycles), and were given access to water ad libitum and food (Nuvilab CR1) during the experimental protocol. This lineage of rats shows between the 6th and the 24th weeks of life without any intervention systolic blood pressure (SBP) levels from 145 to 200 mm Hg.[18,19] The study sample comprised SHR males, mean age of 6 months. They were divided into 5 groups (7 rats per group) to receive the following through an orogastric tube (gavage): atenolol (AT, 0.33 mg/animal/d); carvedilol (CV, 0.26 mg/animal/d); metoprolol tartrate (MT 0.53 mg/animal/d); propranolol (PP, 0.48 mg/animal/d); or dimethyl sulfoxide (DMSO) vehicle (control, 0.26 mg/animal/d). They were on this schedule for 30 consecutive days. Drug doses were allometrically estimated.[20]

After completion of the protocol (day 30), the rats were anesthetized with high-dose ketamine (80 mL, 180 mg/kg ip) and xylazine (2%, 16 mg/kg ip), their heart, liver, lungs, and kidneys were removed and then they were euthanized. Plasma samples were collected by caudal (at baseline and day 30) and cardiac puncture (day 30).

2.2 | Blood pressure measurement

Blood pressure (BP) measurements in the study SHR were taken directly using femoral artery catheterization 24 hours following this procedure, which was performed on day 30 of the protocol. The agents used to anesthetize the rats during the implantation of the femoral artery catheters for the blood pressure measurements were ketamine (90 mg/kg) and xylazine (10 mg/kg) by intraperitoneal administration. To allow a free movement of the animals in the case during BP recordings the arterial cannula was hooked up to a 20-cm length of tubing that was connected to a calibrated signal transducer (Model 041-500503A, CDX III Transducer, TX, USA) connected to a signal amplifier (General Purpose, IL, USA). Systolic blood pressure (SBP) and diastolic blood pressure (DBP) measurements were recorded using WinDaq software (version 2.19; DATAQ Instruments, sample rate of 2000 Hz per channel).

2.3 | Systemic levels of SDF-1

Blood samples collected from the animals were centrifuged at 450 *g* for 10 minutes for separating plasma. Plasma samples were then stored at −20°C until use. Expression levels of SDF-1 isoforms α were determined using ELISA, with the detection of specific antibodies through antibody-antigen interaction using a commercial kit (Cloud-Clone Corp.) following the manufacturer's instructions. Optical densities were then measured using a spectrophotometer (SpectraMax M2e, Molecular Devices) and quantified through a 4-parameter linear regression (Excel, Microsoft). The results were expressed as picograms of proteins per milliliter (pg/mL).

2.4 | Analysis of tissue proteins

We collected tissue samples of the heart, liver, lungs, and kidneys after the animals were euthanized. Proteins were isolated following an adapted assay protocol (Sambrook and Russell, 2001),[21] and analyzed by Bradford colorimetric assay and then stored at −20°C until use. Proteins that were isolated from the tissue samples were analyzed using Western blot and CXCR-4, CXCR-7, GRK-2, β1-AR, β2-AR, and NFκB were quantified. Protein extracts underwent prior separation using polyacrylamide denaturing gel electrophoresis and transferred to nitrocellulose membranes (GE Healthcare). Membranes were incubated in primary antibodies

to the above-mentioned proteins and secondary antibodies (anti-IgG/host peroxidase conjugated, Millipore; Santa Cruz Biotech). Incubation times ranged from 3 to 72 hours. Hybridization was revealed by peroxidation reaction (ECL, GE Healthcare), exposed to X-ray films (GE Healthcare) and then digitized (HP). Positive images were analyzed by digital densitometry (Scion Image Software), and the results were expressed as arbitrary units (AU) taking into account the amount of protein applied on the gel, total protein amount from the tissues and normalization strategies. The normalization procedures included comparisons between positive radiographic images and total protein amount extracted from each tissue for each treatment, transferred to the membranes, stained with Ponceau red (Nuclear) and quantified using digital densitometry.

2.5 | Statistical analysis

We conducted all analyses using the Statistical Package for Social Sciences (SPSS, version 23.0), and further analyses were performed using a statistical software (BioEstat version 5.0). Nonparametric continuous data were expressed as medians and interquartile ranges. We used the Student's *t* test and ANOVA for comparisons between means of parametric variables, followed by Tukey's multiple comparison test. For quantitative comparison of protein expression between treatment groups for each time point (baseline and day 30) we conducted the Kruskal-Wallis test, followed by the Student-Newman-Keuls multiple comparison test. We analyzed protein expression for each group and time point using the Wilcoxon-Mann-Whitney test. We assessed the measure of correlation between protein expression and β-blocker use using the Spearman's rank correlation coefficient (nonparametric data) and Pearson's correlation coefficient (parametric data). The statistical significance was set at $P < .05$.

3 | RESULTS

3.1 | β-blockers modulate molecular interactions involved in cell homing

Anthropometric (weight) and hemodynamic parameters (BP variables) of the sample rats treated β-blockers are summarized in Table 1. Weight measures were taken at baseline (prior to treatment) and on day 30 with no significant difference ($P = .213$) (mean end weight of 339.7 g in the treatment groups and 353.3 g in the control group). There were no differences in the hemodynamic variables between treatment groups (SBP, $P = .154$; DBP, $P = .202$, and heart rate - HR, $P = .921$).

To assess systemic and tissue effects of the study drugs, we correlated mean values of arbitrary units of the target molecules measured in the tissues examined. Table 2 shows the correlations between the proteins analyzed for each drug treatment. Carvedilol and metoprolol may have interfered with the most among molecular interactions largely because these interactions were not preserved compared to the control group. The molecular interactions were then measured for each tissue as presented below.

3.2 | β-blockers do not interfere with systemic levels of SDF-1 following long-term drug administration, but they do interfere with the interaction of chemokines and other target molecules

To assess whether long-term β-blocker administration interfere with systemic levels of SDF-1, we measured the levels of this chemokine at baseline and on day 30 of drug administration (Figure 1). The levels of SDF-1 varied according to the drug administered and time point; however, these differences were not significant ($P = .138$, Figure 1A). Median SDF-1 levels were 1573.4 pg/mL ± 275.8 at baseline and 1441.2 pg/mL ± 197.2 on day 30 of treatment. These results suggest there is no change in the systemic levels of chemokine due to long-term administration of β-blockers. However, when we compared plasma levels of SDF-1 with the expression of its receptors in the tissues examined, atenolol, carvedilol, and propranolol were shown to interfere either negatively or positively with molecular interactions (Figure 1B). In addition, the levels of SDF-1 were lower after the administration of metoprolol and propranolol (1378 and 1305 pg/mL, respectively), and they were inversely correlated with β-arrestins ($r = -1.0$, $P < .001$, Figure 1B).

3.3 | Atenolol, carvedilol and propranolol interfere with the expression of CXCR-4 and CXCR-7 in several tissues

We analyzed tissue expression of SDF-1 receptors CXCR-4 and CXCR-7 with Western blot on day 30 of drug administration in the heart, lung, liver, and kidney tissues of SHR (Figure 2). The expression of these receptors varied depending on the drug used, but these differences were not significant in any of the tissues examined (CXCR4, Figure 2A - heart, $P = .660$; liver, $P = .346$; lung, $P = .599$;

TABLE 1 Anthropometric and hemodynamic characteristics of SHR animals following β-blocker administration

Variables	C	AT	CV	MT	PP	*P*-value
SBP 30 d (mm Hg)	171.1 ± 28.8	199.0 ± 11.0	196.2 ± 18.6	174.0 ± 11.9	198.0 ± 39.0	.154
DBP 30 d (mm Hg)	112.7 ± 21.7	135.2 ± 9.0	136.1 ± 16.0	120.2 ± 14.6	132.3 ± 30.4	.202
HR 30 d (bpm)	306.1 ± 13.4	304.6 ± 29.9	305.5 ± 14.8	307.1 ± 25.4	294.9 ± 29.0	.921
Weight (g)	353.3 ± 7.5	334.6 ± 11.8	337 ± 18.0	347.9 ± 18.2	339.4 ± 14.5	.213

Data presented as mean ± standard deviation. SBP 30 d: systolic blood pressure on day 30 following drug administration; DBP 30 d: diastolic blood pressure on day 30 following drug administration; mm Hg: millimeters of mercury; HR 30 d: heart rate on day 30 following drug administration, bpm, beats per minute; g, grams.

TABLE 2 Molecular interactions affected by β-blockers in SHR following 30 d of drug administration

	Control	Atenalol	Carvedilol	Metoprolol	Propranolol
CXCR-4 + CXCR-7	r = .82; P < .001	r = .76; P = .001	r = .88; P < .001		r = .67; P = .008
CXCR-4 + β-Ar-2	r = .77; P = .001	r = .67; P = .009		r = .61; P = .019	r = .79; P = .001
β-Ar-2 + GRK-2	r = .86; P = .006			r = .86; P = .007	r = .85; P = .007
CXCR-7 + β-Ar-1	r = .50; P = .048	r = .57; P = .022	r = .62; P = .010		r = .56; P = .024
CXCR-7 + β-Ar-2	r = 0.81; P = .001	r = .64; P = .008			r = .68; P = .004
CXCR-7 + GRK-2	r = .76; P = .029	r = .72; P = .046			r = .71; P = .046
β-Ar-1 + β-Ar-2	r = .50; P = .048	r = .69; P = .003	r = .60; P = .014	r = .54; P = .032	r = .59; P = .016

β1-AR: β-arrestin 1; β2-AR: β-arrestin 2; GRK-2, G protein-coupled receptor kinase 2; r, correlation; P < .05.

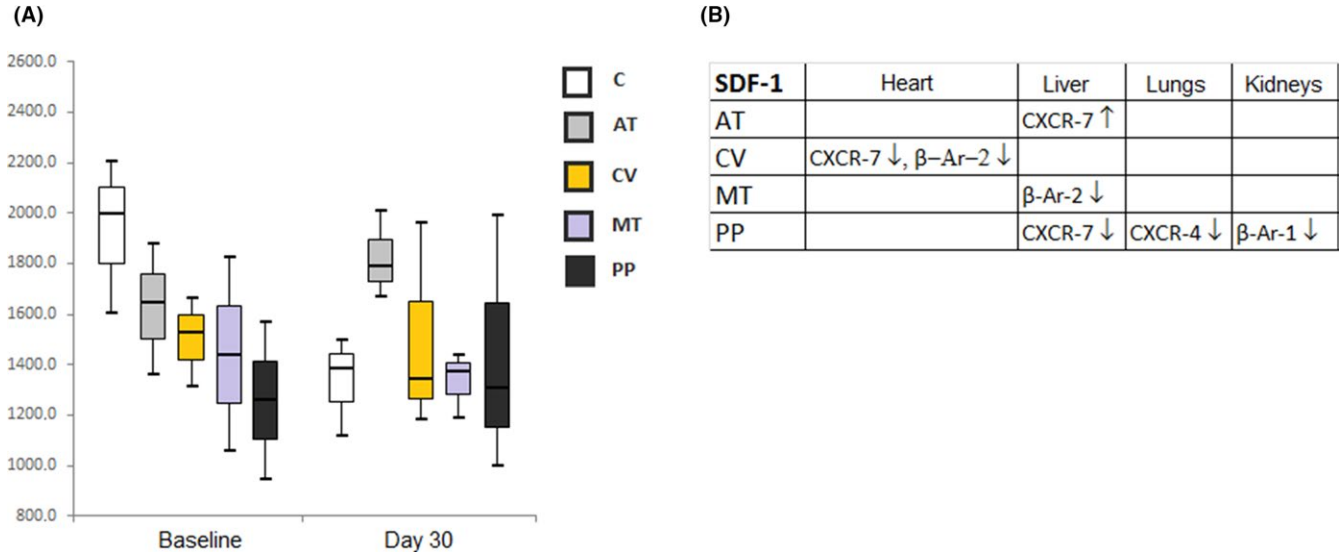

FIGURE 1 β-blockers do not interfere with systemic levels of the chemokine SDF-1 following long-term drug administration, but they do interfere with the interaction of SDF-1 and its receptors. SDF-1 levels in the control (C), atenolol (AT), carvedilol (CV), metoprolol (MT), and propranolol (PP) groups at baseline and on day 30 following drug administration. Results expressed as picograms of protein per milliliter (pg/mL), $P \leq .05$. Direct correlation (↑) r = 1.00, and inverse correlation (↓) r = −1.00; $P \leq .01$. 7 rats per group

kidney, P = .997; CXCR-7, Figure 2B - heart, P = .523; liver, P = .814; lung, P = .366; kidney, P = .516). The expression of CXCR-4 was associated with CXCR-7 in the kidney tissue of SHR treated with carvedilol (r = 1.0, P < .001). CXCR-7 showed a direct correlation when we compared its expression in the heart and kidney tissues following atenolol administration (r = 1.0, P < .001), and an inverse correlation in these same tissues following propranolol administration (r = −1.0, P < .001). Atenolol and carvedilol also interfered with the interaction of CXCR-7 and SDF-1, either directly in the liver tissue (r = .96, P = .042) and inversely in the heart tissue (r = −.99, P = .001), respectively (Figure 2B). Atenolol, carvedilol, and propranolol interfered with the interaction of homing receptors affecting the activation and desensitization of regulatory proteins as described below.

3.4 | β-blockers do not interfere with tissue expression of GRK-2, but they do interfere with the interaction of GRK-2 with target molecules

Figure 3 shows tissue expression of the regulatory protein GRK-2 of homing and β-adrenergic receptors in the heart, liver, and kidney tissues following β-blocker administration. There was no direct interference with protein expression (P = .617 in the heart, P = .359 in the liver, P = .549 in the kidney). When we compared the expression of GRK-2 and homing receptors, we found that propranolol interfered in the heart tissue and GRK-2 was inversely correlated with CXCR-4 (r = −.99, P = .013).

3.5 | β-blockers interfere with homing receptor desensitization

Figure 4 shows the expression of β-arrestins in several tissues of the animals treated with β-blockers. There was no differential modulation in the heart (β1-AR, P = .241; β2-AR, P = .871), liver (β1-AR, P = .208; β2-AR, P = .861), and kidney tissues (β1-AR, P = .908; β2-AR, P = .614). However, in the lung tissue, metoprolol increased the expression of β1-AR when compared to the control group (P = .005), the atenolol group (P = .042), the carvedilol group (P = .031), and the propranolol group (P = .010) (Figure 4A). In contrast, propranolol was associated with reduced expression of β2-AR when it was compared to the atenolol (P = .012) or metoprolol groups (P = .003) (Figure 4B).

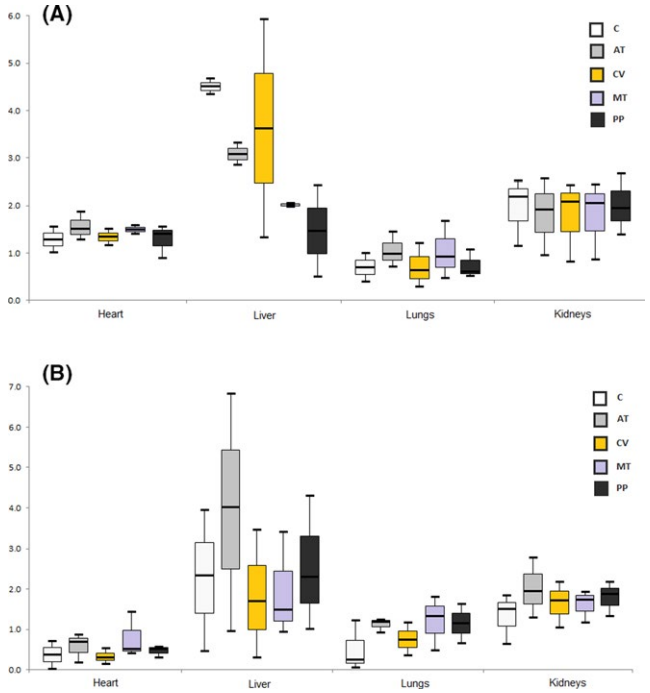

FIGURE 2 Atenolol, carvedilol, and propranolol interfere with the expression of CXCR-4 and CXCR-7 in different tissues. Expression of CXCR-4 A, and CXCR-7 B, in the heart, liver, lung, and kidney tissues in the control (C), atenolol (AT), carvedilol (CV), metoprolol (MT), and propranolol (PP) groups following 30 d of drug administration. Data expressed as arbitrary units (AU), $P \leq .05$. Direct correlation (↑) $r = 1.00$, and inverse correlation (↓) $r = -1.00$; $P \leq .01$. 7 rats per group

As for tissue expression, we found that, for the metoprolol group, β1-AR was inversely associated in the liver and lung tissues with an increase in arbitrary units in the lung ($r = -1.0$, $P < .001$). Carvedilol showed a direct association with protein expression in the kidney and liver tissues ($r = .97$, $P = .028$). Tissue expression of β2-AR was

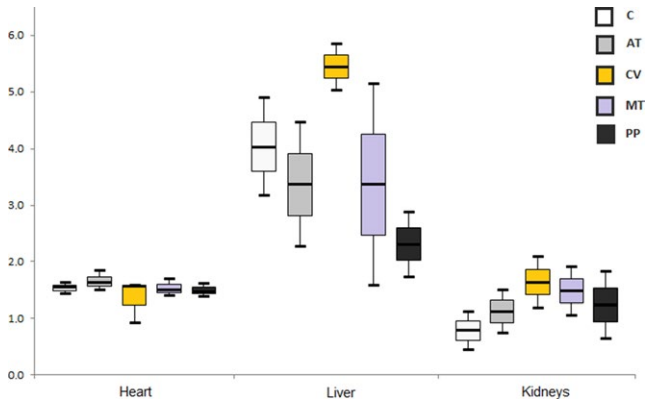

FIGURE 3 β-blockers do not interfere in the tissue expression of GRK-2, but they do interfere with the interaction of GRK-2 with target molecules. Expression of G protein-coupled kinase receptor (GRK-2) in the heart, liver, and kidney tissues in the control (C), atenolol (AT), carvedilol (CV), metoprolol (MT), and propranolol groups following 30 d of drug administration. Data expressed as arbitrary units (AU), $P \leq .05$. Direct correlation (↑) $r = 1.00$, and inverse correlation (↓) $r = -1.00$; $P \leq .01$. 7 rats per group

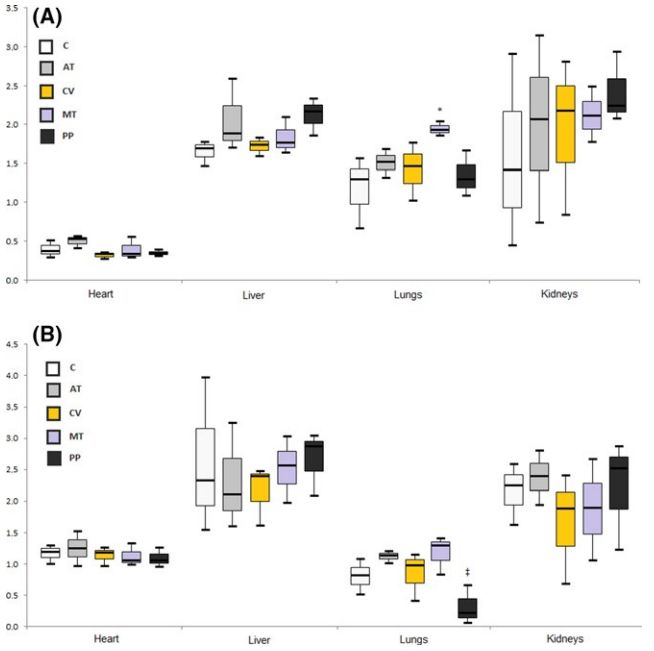

FIGURE 4 β-blockers interfere with homing receptor desensitization. Expression of β-arrestin 1 (β1-AR) and β-arrestin 2 (β2-AR) in the heart, liver, lung, and kidney tissues in the control (C), atenolol (AT), carvedilol (CV), metoprolol (MT), and propranolol (PP) following 30 d of drug treatment. Data expressed as arbitrary units (AU), * ‡ $P \leq .05$. Direct correlation (↑) $r = 1.00$, and inverse correlation (↓) $r = -1.00$; $P \leq .01$. 7 rats per group

associated with atenolol administration in the heart and the kidney tissues ($r = .99$, $P = .013$). In addition, the two arrestins were directly correlated in the kidney tissue ($r = .95$, $P = .046$) in the carvedilol group and inversely correlated in the liver tissue in the propranolol group ($r = -1.0$, $P < .001$). The expression of β2-AR was increased in the liver tissue compared to all other groups (controls 2.3; atenolol 2.1, carvedilol 2.4, metoprolol 2.6, and propranolol 2.9 AU).

In the analysis of the interactions of β-arrestins with other pathway molecules without any drug action, the expression of β1-AR was associated with CXCR-4 in the lung tissue ($r = 1.0$, $P < .001$). Only carvedilol interfered with the interaction of β1-AR with CXCR-7 in the lung tissue ($r = .97$, $P = .028$). It also affected the interaction of β2-AR with CXCR-7 in the heart tissue ($r = 1.0$, $P < .001$). In the atenolol group, β2-AR was associated with CXCR-4 in the heart and lung tissues ($r = 1.0$, $P < .001$), and this same correlation was seen in the kidney tissue in the propranolol group ($r = 1.0$, $P < .001$). These results evidence the interference of β-blockers with the interaction of receptors and desensitizing proteins. β2-AR was also correlated to GRK-2 in the heart tissue in the atenolol group ($r = .97$, $P = .034$).

3.6 | β-blockers do not interfere directly with NFκB

We analyzed the expression of NFκB in the heart tissue of rats treated with β-blockers for 30 days (Figure 5). None of the study drugs, regardless of their effects on proteins downstream in the

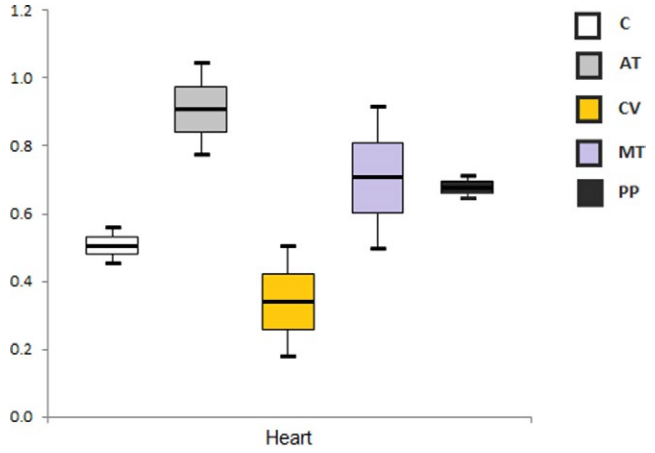

FIGURE 5 β-blockers do not interfere directly with NFκB. Expression of nuclear factor kappa B (NFκB) in the heart tissue in the control (C), atenolol (AT), carvedilol (CV), metoprolol (MT), and propranolol (PP) groups following 30 d of drug administration. Data are expressed as arbitrary units (AU), $P \leq .05$. Direction correlations (↑) $r = 1.00$, and inverse correlation (↓) $r = -1.00$; $P \leq .01$. 7 rats per group

pathway that could lead to changes in their expression, showed any significant effects on the translation of NFκB ($P = .216$). We found no significant correlation between this protein and all others.

4 | DISCUSSION

Assuming that drugs may interfere with cell trafficking needed for tissue regeneration, our study examined β-blocker interference with a major chemokine of cell homing and the expression of its receptors, as well as GRK-2, GPCR desensitization proteins, β-arrestins (β1-AR and β2-AR), and a downstream molecule involved in transcription. Figure 6 summarizes all these interactions.

In this study, the SHR model was used as it is an established model of human hypertensive disease; these rats develop hypertension from the 5th week of life.[18] In the clinical practice, β-blockers are used to treat hypertension.[3] The control group was intended to provide baseline values for the parameters studied to contrast with the results from the treatment groups. All animals were 6-month-old and therefore mimicked the long-term deleterious effects of hypertension similar to those found in humans. Baseline weight and hemodynamic values (SBP, DBP, and HR) on day 30 of drug administration were consistent in all groups with no remarkable tendency.

Except in the lung tissue, β-blockers did not show significant direct effects either increasing or decreasing the amount of the proteins analyzed. However, all drugs showed modulation effects on these molecules, either positive or negative (they affected the expression of paired proteins). Atenolol and propranolol showed interactions that were closest to those seen in the control group, whereas metoprolol and carvedilol caused disruption of most of these interactions (Table 2). These were the effects when considering all tissues jointly, but β-blockers affected differently the interactions of receptors and regulatory proteins in each type of tissue analyzed.

First, our analysis of the levels of SDF-1 in SHR at baseline and on day 30 of atenolol, carvedilol, metoprolol, and propranolol administration showed that these drugs did not interfere with SDF-1 levels. It is known, however, that SDF-1 levels are increased in hypertensive states,[16] and that the greater the cardiovascular involvement, the

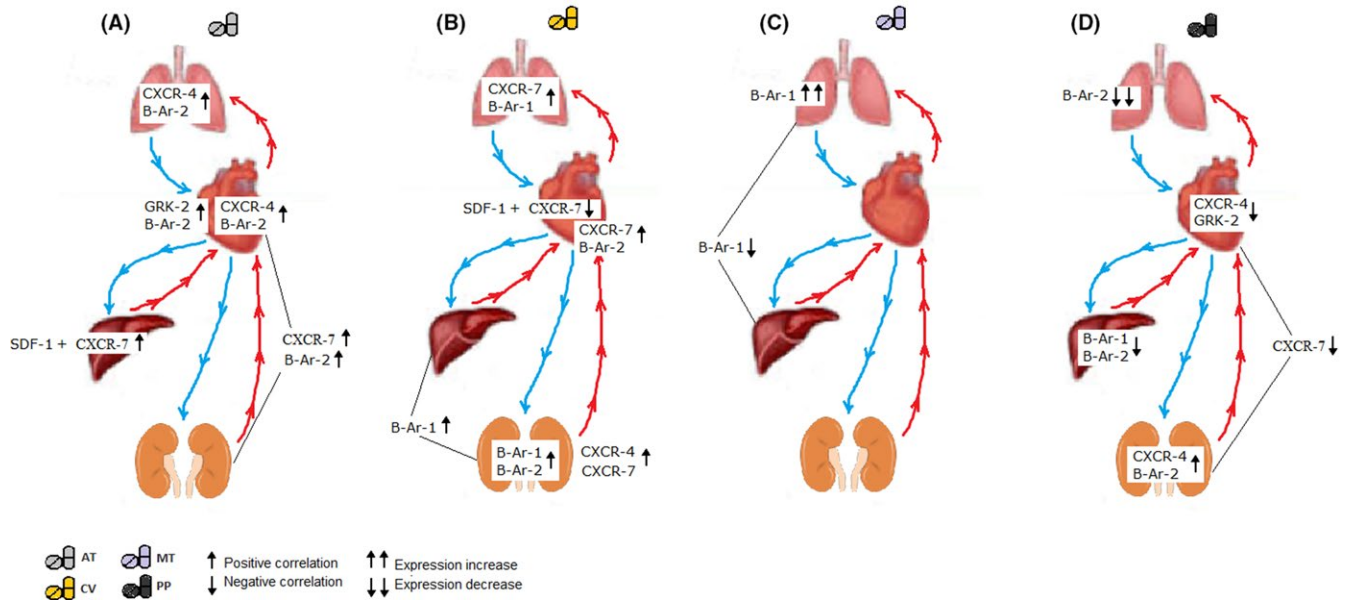

FIGURE 6 β-blocker action on the expression of homing proteins, desensitization, and regulation in SHR. β-blockers effects were assessed on systemic levels of SDF-1 and CXCR-4, CXCR-7, β-arrestin 1 (β1-AR), β-arrestin 2 (β2-AR), protein-coupled kinase (GRK-2), and nuclear factor kappa B (NFκB) in several tissues following 30 d of drug administration. The correlations between the molecules are displayed in the legend of this figure. In each tissue, A, atenolol [AT], B, carvedilol [CV], C, metoprolol [MT], and D, propranolol [PP] interfered with the various interactions between molecules. Seven rats per group

higher SDF-1 levels.[22] Increased levels of SDF-1 are also associated with adverse cardiovascular outcomes in individuals with coronary artery disease.[23] Furthermore, increased plasma levels of SDF-1 are associated with increased mobilization of hematopoietic stem cells,[24] which apparently was not impaired in our study, though other studies have shown a negative effect of propranolol on in vitro cell proliferation.[25]

We found no significant differences when we examined specific homing receptors, CXCR-4 and CXCR-7. However, in the lung tissue, CXCR-4 was inversely associated with SDF-1 in the propranolol group. This finding corroborates that reported by Zou et al (2013) that propranolol inhibits homing of endothelial progenitor cells through the SDF-1 pathway thus suppressing the expression of CXCR-4 possibly via protein kinase B (PKB/Akt) and mitogen-activated protein kinases (MAPKs).[25] As for CXCR-7, SDF-1 was inversely associated with this receptor in the heart tissue in the carvedilol group and in the liver tissue in the propranolol group. However, in the liver tissue, atenolol showed an opposite effect. It is worth mentioning that CXCR-7 seems to play a nonsignaling role by removing SDF-1 from the extracellular space and indirectly controlling signaling of CXCR-4.[26] Würth et al (2014) reported a direct interaction between CXCR-4 and CXCR-7 that promoted chemotaxis and cell proliferation.[12]

We then examined the interference of β-blockers with GRK-2. We found an inverse association with CXCR-4 in the heart tissue in the propranolol group and a direct association with β2-AR in the atenolol group. GRK-2 phosphorylates the intracellular region of the activated receptor promoting β-arrestin binding that in turn causes G protein-coupled receptor internalization.[27,28] Because of its involvement in the regulation of β-adrenergic receptors and its role in the development of heart failure, Huang et al (2014) suggested that GRK-2 inhibition in the heart tissue should be explored as a potential treatment approach.[29]

In our analysis of arrestins, we found evidence that long-term administration of metoprolol affected the expression of β1-AR and β2-AR in the lung tissue. In addition, metoprolol was the single β-blocker that lost its molecular interactions after 30 days of drug administration. Studies have shown that metoprolol promotes cardioprotective signaling through the activation of β-adrenergic receptors, β-arrestins, and extracellular signal-regulated kinase (ERK) 1/2.[30-32] In an impressive study, Rajagopal et al (2010) demonstrated that the interactions between CXCR-7 and β-arrestins may trigger, after desensitization, the activation of MAPK through the recognition of complex receptor internalization.[33] Further investigations are necessary on the effects of GPCR receptor blockers and their interference with receptor internalization processes.

Besides the mechanisms discussed above, G protein-coupled receptors comprise the largest superfamily of receptor proteins encoded by the human genome.[34] These receptors are membrane proteins involved in signal transduction pathways through the activation of G proteins intracellularly.[35] This activation is mediated by the interaction of the agonist with the extracellular receptor domain and propagates intracellularly by activating several signaling cascades in different physiological processes such as neurotransmission, cell growth, metabolism, differentiation, proliferation and secretion and immune defense.[35,36] These drug interactions may interfere with the normal functioning of these pathways and greatly affect the body's response, as in tissue regenerative and renewal capacity, which in turn may induce compensatory cellular mechanisms.

Our analyses of the main interactions between SDF-1 and CXCR-4 and CXCR- 7, GRK-2, β1-AR, and β2-AR evidenced that β-blockers interfere with expression and systemic release mechanisms. Recent studies have shown the interactions of homing proteins and receptors with G protein-coupled receptor kinases (desensitization) and regulatory proteins of G protein-coupled receptors and transcription factor,[33,37-39] and how these interactions are most likely crucial for treatment protocols requiring intact cell signaling to take effect in the tissue regeneration. Our analyses of NFκB in the heart tissue showed higher levels in the atenolol group, and NFκB activation was dependent on CXCR-4 being activated. Therefore, the stimulation of the phosphatidylinositol-3 kinase (PI3K)-Akt pathway leads to cell survival and proliferation.[12]

In conclusion, β-blockers may have modulated the expression of proteins and their interactions in the tissues examined following long-term administration. It shows that this class of drugs may interfere with proteins of cell homing pathways. Understanding molecular mechanisms involved in these receptors is crucial for improving our knowledge on cell signaling and the actual effects of treatment protocols acting on cell homing pathways such as cell therapy.

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CONFLICT OF INTERESTS

The authors declare no conflict of interest.

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