ORIGINAL ARTICLE

Cardioprotective Effects of Chronic Release Formulations of Subcutaneous Implants of Carvedilol in Spontaneously Hypertensive Rats

Cardioprotective Effects of Chronic Release Formulations of Subcutaneous Implants of Carvedilol in Spontaneously Hypertensive Rats

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ABSTRACT

Background: In our laboratory, we have developed subcutaneous implants of carvedilol capable of maintaining stable concentrations of the β-blocker during 3 weeks.
Objective: The aim of this study was to evaluate the in vivo release and the cardioprotective efficacy of subcutaneous implants of carvedilol developed with poly-epsilon-caprolactone (PCL) and Soluplus (SP) polymers in spontaneously hypertensive rats (SHR).
Methods: Twelve spontaneously hypertensive male rats (250-300 g) underwent placement of subcutaneous implant of PCL:SP 100:50 mg (control group, n = 6) or carvedilol:PCL:SP (100mg:100mg:50mg) (carvedilol group, n = 6), every 3 weeks. The plasma profile of each implant and its effect on systolic blood pressure (SBP) was evaluated for 62 days. At the end of treatment, echocardiographic parameters were determined, and direct SBP and direct mean arterial pressure (MAP) were measured.
Results: The group that received the implant containing 100 mg of carvedilol presented plasma concentrations of the drug in the range of 100- 500 ng/mL throughout 62 days of treatment, after which the SBP was 20 mmHg lower than in the control group (217±3 mm Hg vs. 237 ± 6 mm Hg; p <0.05). Direct SBP and MAP were significantly lower in the treated group than in the control group. The implant loaded with carvedilol 100 mg reduced short-term blood pressure variability (BPV) in SHR compared with the control group. Echocardiographic parameters as left ventricular ejection fraction (LVEF), shortening fraction and E/A ratio were significantly greater in treated rats. Left ventricular weight was lower in the rats with carvedilol implant.
Conclusion: Implants containing CAR/PCL/SP (100:100:50) mg provide therapeutic and stable plasma levels of carvedilol during treatment, which correlate with a significant and sustained decrease in indirect BP values. Treatment with carvedilol implants attenuated direct BP values and blood pressure variability in SHR. Treatment with implant produced cardioprotective effects evidenced in the echocardiogram by a reduction in left ventricular hypertrophy.

Key words: Carvedilol - Subcutaneous implants – Blood pressure – Echocardiography

RESUMEN

Introducción: En nuestro laboratorio hemos desarrollado implantes subcutáneos de carvedilol capaces de mantener niveles lasmáticos sostenidos del β-bloqueante durante 3 semanas.
Objetivo: Evaluación de la liberación in vivo y la eficacia cardioprotectora de implantes subcutáneos de carvedilol desarrollados con poliepsilon- caprolactona (PCL) y Soluplus (SP) en ratas espontáneamente hipertensas (REH).
Materiales y métodos: Se utilizaron 12 REH macho (250-300 g), a las cuales se colocó un implante subcutáneo cada 3 semanas de PCL: SP 100:50 mg (control, n = 6) o carvedilol: PCL:SP (100mg:100mg:50mg) (carvedilol, n = 6). Se evaluó el perfil plasmático y el efecto sobre la presión arterial sistólica (PAS) durante 62 días. Al final del tratamiento, se realizaron determinaciones ecocardiográficas y la medición de la PAS y. la presión arterial media (PAM) directas.
Resultados: El grupo que recibió el implante conteniendo 100 mg de carvedilol presentó concentraciones plasmáticas del fármaco en el rango de 100- 500 ng/mL a lo largo de 62 días de tratamiento, luego del cual la PAS fue 20 mmHg menor que en el grupo control (217 ± 3 mmHg vs. 237 ± 6 mmHg; p <0,05). Las PAS y PAM directas fueron significativamente menores el grupo tratado que en el control. El implante de carvedilol 100 mg redujo la variabilidad de la presión arterial (VPA) de corto plazo en comparación con el

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control. Parámetros ecocardiográficos como la fracción de eyección del ventrículo izquierdo (FEVI), fracción de acortamiento, y relación E/A fueron significativamente mayores en las ratas tratadas. El peso del VI fue menor en las ratas que recibieron el implante con carvedilol.

Conclusión: Los implantes conteniendo CAR/PCL/SP (100:100:50) mg aportan niveles plasmáticos terapéuticos de carvedilol y estables durante el transcurso del tratamiento, los cuales se correlacionan con una disminución significativa y sostenida de los valores de PA indirecta. El tratamiento con los implantes de carvedilol logró atenuar los valores de PA directa y su variabilidad en las REH. Se demostró que el tratamiento con los implantes ejerció un efecto cardioprotector evidenciado en el ecocardiograma y por una reducción de la hipertrofia ventricular izquierda.

Palabras claves: Carvedilol - Implantes subcutáneos - Presión arterial - Ecocardiografía

INTRODUCTION

Hypertension (HTN) is the leading cause of mortality worldwide and represents the main risk factor for cardiovascular disease. (1) Prevention of cardiovascular events associated with HTN requires not only effective peripheral or brachial blood pressure (BP) reduction, but also the attenuation of central BP and 24-hour blood pressure variability (BPV). (2-5) A key aspect of antihypertensive therapy is to achieve and maintain persistent BP control during 24 hours to ensure attenuation of the blood pressure morning surge. (6)

Recent updates of the recommendation of international guidelines on the management of HTN have downgraded the use of β-blockers for the treatment of uncomplicated HTN. (7) However, β-blockers are a heterogeneous group of agents which include third-generation agents with a more favorable hemodynamic and metabolic profile compared with conventional β-blockers as atenolol. Carvedilol is a nonselective ß-blocker with vasodilating activity secondary to β1-adrenergic blockade and additional pleiotropic actions, including antioxidant, anti-inflammatory and antiapoptotic effects. (9)

One limitation of carvedilol for the treatment of hypertension is its low oral bioavailability and rapid systemic clearance, which adversely affect sustained BP reduction and BPV. In this setting, carvedilol encounters first-pass metabolism after oral administration resulting in low bioavailability (about 25%) and short elimination half-life of only 2 hours. (10)

In view of these facts, different formulations of carvedilol have been developed to increase bioavailability and prolong the duration of the β-blocker in the organism, as carvedilol nanoparticles using chitosan or Eudragit® RS100 polymer. (11,12) Although these formulations have improved the pharmacokinetic profile of carvedilol, its impact on the control of hemodynamic variables and on prevention of target organ damage has not been evaluated. (11,12) In a previous study, we have developed and evaluated subcutaneous implants of carvedilol which included different proportions of the SoluPlus (SP) hydrophilic polymer and poly-epsilon-caprolactone (PCL). The pharmacokinetic and pharmacodynamic evaluation of these formulations has established that those implants with a higher proportion of SoluPlus provide stable plasma concentrations of carvedilol allowing a sustained reduction of systolic blood pressure (SBP) in spontaneously hypertensive rats (SHR) during at least 2 weeks. (13)

Considering these facts, the aim of this study was to evaluate the in vivo release and the cardioprotective efficacy of a subcutaneous implant of carvedilol developed with PCL and SP polymers in SHR.

METHODS

Subcutaneous implants of carvedilol

Subcutaneous implants were prepared with PCL: SP 100:50 mg alone or loaded with carvedilol 100 mg using the melt-molding-compression method. To prepare the PCL and SP implants loaded with carvedilol 100 mg, the polymers and the drug powder were mixed in different weight ratios, placed in an 11-mm diameter stainless steel mold and firmly compressed with a punch, then heated in a preheated oven (70 ºC, 1 h) and compressed (0.713 kg/cm^2) during the entire heating treatment. Next, the molds were cooled (4 ºC, 30 minutes) and, finally, the samples were removed to obtain disk-shaped implants (11 mm diameter).

Animals

Twelve spontaneously hypertensive male rats (250-300 g) were trained during 2 weeks for SBP measurement using the previously described indirect tail-cuff method. (14) After estimating baseline SBP for 3 days, the rats were anesthetized with intraperitoneal ketamine (75 mg/kg) and xylazine (10 mg/kg). One of the following subcutaneous implants was placed under the skin at the back of the neck: PCL:SP 100:50 mg (n = 6) or PCL:SP 100:50 mg loaded with 100 mg of carvedilol (n = 6), every 3 weeks.

During a follow-up period of 62 days, indirect SBP was measured and 100 μL of venous blood were drawn from the lateral vein of the tail to determine carvedilol levels. Systolic blood pressure was measured by the non-invasive indirect tail-cuff method using a sphygmomanometer coupled to a Grass 7C polygraph (Grass Instrument Co., Quincy, MA, USA) described in previous studies. The animals were first conditioned in a thermostated room for 60 min and then transferred to a special acrylic tube heated at 37 ± 1 ºC to measure SBP. Each day, indirect SBP was calculated as the average of six separate measurements.

Plasma levels of carvedilol were measured by liquid chromatography with fluorometric detection using an analytical method validated in the laboratory. (15)

In the last week of treatment, echocardiographic measurements were performed in anesthetized rats with a combination of ketamine/xylazine using an Acuson Sequoia C512 ultrasound system, equipped with a 7-14 MHz transducer. At the end of the 2-month follow-up period, the carotid artery

was cannulated and 24 hours later was connected to a Spectramed P23XL pressure transducer (Spectramed, Oxnard, CA, USA) coupled to a Grass 79D polygraph (Grass Instruments, Quincy, MA, USA). The polygraph was connected to a digital converter (Poliview, PVA 1, Grass-Astro Med, West Warwick, RI, USA). Blood pressure records were obtained during 2 hours and were analyzed with Poliview 2.3 software (Astro Med, West Warwick, RI, USA). Systolic blood pressure, diastolic blood pressure (DBP) and mean arterial pressure (MAP), which represents central BP due to the place where it was measured, were calculated. Short-term BPV was calculated by assessing the standard deviation (SD) of consecutive 3-minute SBP recordings. (14)

After the hemodynamic parameters were measured, the animals were sacrificed by decapitation and the left ventricle (LV) was removed for morphometric analysis of the level of hypertrophy by determining LV weight/body weight ratio using a precision balance. (14)

Statistical analysis

The Kolmogorov-Smirnov test was used to evaluate the normal distribution of data and study variables. Results were expressed as mean ± standard error of the mean (SEM). Statistical comparison between treatment groups was performed using one-way ANOVA followed by Tukey's method as post-hoc test using GraphPad Prism version 8.0 (GraphPad Software, San Diego, California, USA). A p value < 0.05 was considered statistically significant.

Ethical considerations

The experiments with animals were carried out in accordance with the "Guide for the Care and Use of Laboratory Animals" (NIH publication 85-3, 1985 Revision) and were approved by the Ethics Committee of the School of Pharmacy and Biochemistry of the University of Buenos Aires (EXP-UBA No. 0037832/2019).

RESULTS

The different disk-shaped carvedilol implants had a diameter of 11 mm (Figure 1). The insertion of the carvedilol implants under the skin at the back of the base of the neck was successful in all cases with no signs of pain or behavioral changes in the animals during the assessment period. During the 62-day follow-up period, carvedilol plasma levels measured every 3 days remained stable in the range of 100-500 ng/mL (Figure 1). Indirect SBP was significantly lower in the group with implants loaded with carvedilol 100 mg compared with control SHR with implants of PCL:SP without carvedilol (Figure 2). In this way, at the end of follow-up, SBP was 20 mm Hg lower in SHR with implants loaded with carvedilol 100 mg versus those assigned to the control group (217 ± 3 mm Hg vs. 237 ± 6 mm Hg; $p < 0.05$).

Direct BP measurement showed a significant reduction of SBP and MBP in SHR with implants loaded with carvedilol 100 mg compared with the control group (Table 1). There were no statistically significant differences in DBP between both experimental groups (Table 1). The analysis of the SD of direct BP recording established that the implant loaded with carvedilol 100 mg reduced short-term BPV in the SHR compared with the control implant (Table 1).

The echocardiographic examination revealed that treatment with the subcutaneous implant loaded with carvedilol 100 mg improved systolic and diastolic function in SHR compared with the control implant. Specifically, the group of animals treated with carvedilol implants presented higher left ventricular ejec-

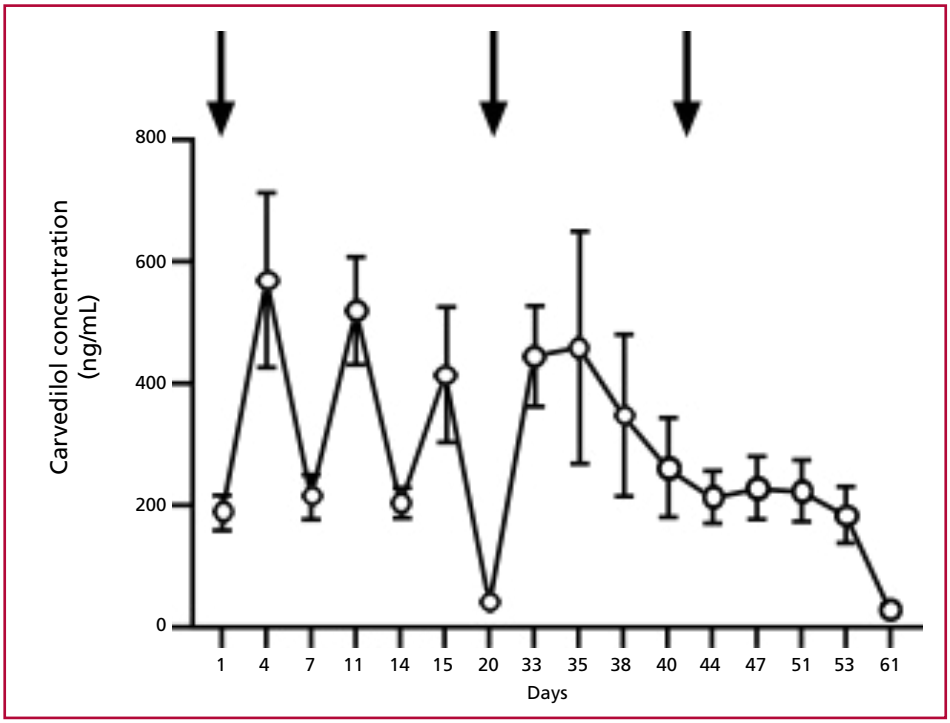


Fig. 1. Carvedilol plasma levels after placement of implants of carvedilol 100 mg in SHR. Each data point represents mean ± SEM

The arrows indicate the moment the implants of carvedilol 100 mg were placed

tion fraction (LVEF) and shortening fraction than the control group of SHR (Table 2). The subcutaneous implant of carvedilol was also associated with a reduction in isovolumetric relaxation time and increased E/A index (Table 2). In SHR treated with the subcutaneous implant of carvedilol 100 mg during 2 months, left ventricular mass index expressed as LV weight/body weight ratio showed a significant reduction compared with the control group (2.99 ± 0.15 mg/g vs. 3.40 ± 0.05 mg/g; $p < 0.05$).

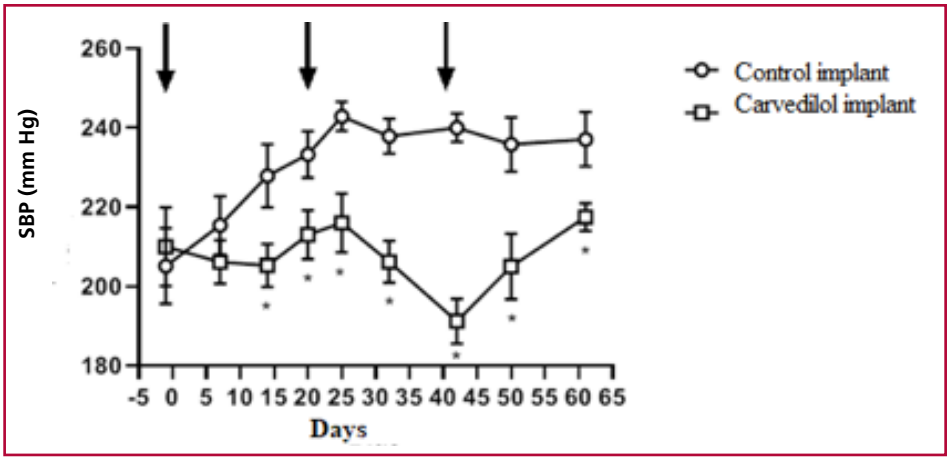
DISCUSSION

The present study demonstrated that the development of subcutaneous implants of carvedilol 100 mg represents an effective strategy for maintaining stable long-term levels of the β-blocker, resulting in a sustained reduction of peripheral and central SBP in SHR with cardioprotective effects documented by improvement in systolic and diastolic function.

The effective prevention of target organ damage associated with HTN requires effective control of both central BP and BPV without generating adverse metabolic effects. (8) Overactivation of the sympathetic nervous system plays a key role in the development and maintenance of HTN, so modulating sympathetic overactivity represents an important goal of treatment. (16) In fact, increased sympathetic activity represents the main pathophysiological mechanism involved BP morning surge. (17)

Despite the recognized importance of the sympathetic nervous system in the development of hypertension, β-blockers are no longer recommended as first-line treatment due to clinical trial evidence suggesting reduced cardioprotection because of their limited effects on central BP and BPV, and the development of metabolic adverse effects. (18-20) However, these limitations are typical of conventional β-blockers, especially atenolol, and do not seem to be extrapolated to vasodilator β-blockers with pleiotropic action, such as carvedilol. (8, 21) The results of clinical trials and evaluations conducted in experimental models of HTN have established that chronic treatment with carvedilol is more effective than with atenolol in reducing central BP and BPV, hemodynamic properties that suggest greater protection from target organ damage associated with HTN. (8)

Despite the improved hemodynamic profile of carvedilol, a limitation of this vasodilator β-blocker is its rapid systemic elimination affecting its ability to maintain a sustained reduction in blood pressure for 24 hours. (9) A clinical measure that quantifies the 24-hour therapeutic coverage provided by an antihypertensive agent is the trough-to-peak ratio, which represents the relationship between maximal BP decrease and its reduction before the administration of the next dose. (6) A higher trough-to-peak ratio indi-



*p < 0.01 vs. control implant
The arrows indicate the moment the implant was placed.
SBP: systolic blood pressure

Fig. 2. Changes in indirect SBP in SHR after placement of the control implant or implant of carvedilol 100 mg.

	Control implant (n = 6)	Carvedilol 100 mg implant (n = 6)	p value
SBP (mm Hg)	176 ± 8	143 ± 11	<0.05
DBP (mm Hg)	128 ± 7	111 ± 11	NS
MAP (mm Hg)	131 ± 11	159 ± 6	<0.05
SD (mm Hg)	7.10 ± 0.46	5.22 ± 0.17	<0.05

Data indicate mean ± standard error of the mean.
SBP: systolic blood pressure. DBP: diastolic blood pressure.
MAP: mean arterial pressure. SD: standard deviation

Table 1. Hemodynamic parameters obtained during direct BP measurement in SHR with control implant or implant of carvedilol 100 mg.

Table 2. Echocardiographic parameters from SHR with control implant or implant of carvedilol 100 mg.

Echocardiographic parameters	Control implant (n = 6)	Carvedilol 100 mg implant (n = 6)	p value
Anterior wall thickness in diastole (mm)	1.78 ± 0.05	1.86 ± 0.05	NS
Anterior wall thickness in systole (mm)	2.55 ± 0.18	2.68 ± 0.12	NS
Posterior wall thickness in diastole (mm)	1.78 ± 0.11	1.84 ± 0.06	NS
Posterior wall thickness in systole (mm)	2.38 ± 0.05	2.66 ± 0.09	NS
Isovolumetric relaxation time (ms)	37.3 ± 1.5	32.1 ± 1.8	<0.05
End-diastolic diameter (mm)	7.60 ± 0.30	7.96 ± 0.33	NS
End-systolic diameter (mm)	5.40 ± 0.34	5.00 ± 0.23	NS
Ejection fraction (%)	63.9 ± 3.6	75.1 ± 1.6	<0.05
Shortening fraction (%)	29.0 ± 2.4	37.2 ± 1.4	<0.05
E/a ratio	0.76 ± 0.02	0.98 ± 0.04	<0.05

Data indicate mean ± standard error of the mean.

cates a longer duration of action that provides a better risk-to-benefit ratio through optimal therapeutic coverage during the early morning hours, a day period associated with greater incidence of major cardiovascular events. (6, 17)

Comparative clinical trials have demonstrated that antihypertensive agents with a trough-to-peak ratio of 0.9 ensure greater attenuation of the early morning BP surge than those with a trough-to-peak ratio of around 0.5. (22) In the case of carvedilol, the trough-to-peak ratio of commercially available immediate-release and extended-release formulations is less than 0.8. (6)

Considering these limitations, in our laboratory we have developed subcutaneous implants of carvedilol using melt-molding-compression with PCL and SP polymers, which represent two of the main excipients used in the development of extended-release formulations. (23,24) Since PCL limits the access of the surrounding aqueous medium into the implant, which slows down the dissolution of the drug and thus decreases its release, we evaluated the impact of replacing a fraction of PLC by SP, a polymer with amphiphilic properties that improves dissolution and bioavailability of many poorly soluble drugs. (24) Thus, we have obtained a subcutaneous implant of PCL:SP 100:50 mg containing carvedilol 100 mg, which, when implanted every 3 weeks, allows stable and persistent carvedilol levels in the range of 100-500 ng/mL.

The hemodynamic evaluation established that subcutaneous implants of carvedilol were effective in reducing peripheral and central SBP and in attenuating short-term BPV, hemodynamic parameters that contribute to target organ damage associated with HTN. These findings are similar to those previously obtained in our laboratory after chronic oral administration of carvedilol in SHR. (25) In other words, maintaining stable plasma levels in the range of 100-500 ng/mL over time with subcutaneous implants of carvedilol 100 mg, has hemodynamic benefits that are at least equivalent to chronic daily oral administration of the β-blocker.

Subcutaneous implants of carvedilol also produced cardioprotective effects in the SHR, as evidenced by a reduction in left ventricular hypertrophy and improvement in systolic and diastolic function compared with the control group. In previous echocardiographic examinations performed in SHR chronically treated with carvedilol we have not documented an improvement in systolic and diastolic function compared with untreated SHR. (25) These findings would suggest that sustained carvedilol levels from subcutaneous implants would be more beneficial in maintaining systolic and diastolic function in SHR compared with intermittent oral administration.

CONCLUSIONS

The development of subcutaneous implants of PCL:SP:carvedilol 100:50:100 mg allows stable and sustained plasma levels of the β-blocker in the range of 100-500 ng/mL and generates a reduction in peripheral and central SBP and in short-term variability. Compared with the control group, subcutaneous implants of carvedilol had cardioprotective effects, prevented left ventricular hypertrophy and improved systolic and diastolic function in SHR.

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Conflicts of interest

None declared.

(See authors' conflict of interests forms on the web/Additional material.)

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