CME

Pharmacokinetic and Pharmacodynamic Profile of Nebivolol in an Animal Model of Metabolic Syndrome

Perfil farmacocinético y farmacodinámico del nebivolol en un modelo animal de síndrome metábolico

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ABSTRACT

The cardiovascular and pharmacokinetic effects of nebivolol were evaluated in hypertensive fructose-fed and control rats, analyzing the effect of intravenously administered nebivolol 3 or 10 mg/kg on blood pressure, heart rate, and short-term and beat-to-beat blood pressure variability. The enantioselective pharmacokinetic profile of d- and l-nebivolol enantiomers was evaluated. Short-term and beat-to-beat blood pressure variability was assessed using standard deviation and blood pressure spectral analysis, respectively. The hypertensive state altered the pharmacokinetics of nebivolol, evidenced by reduction of nebivolol clearance in the fructose group compared to the control group after administration of the highest dose. The antihypertensive effect of nebivolol was similar in both groups, while the bradycardic effect was greater in control rats. Although no significant differences were found in beat-to-beat blood pressure variability, short-term blood pressure variability showed greater reduction after nebivolol administration in fructose-fed rats compared to control normotensive animals (-57.9%±11.8% vs.-19.6%±9.2%; p<0.05). In conclusion, although nebivolol reduces blood pressure and blood pressure variability in both groups, no significant differences were found in the pharmacokinetics and cardiovascular effects of fructose-fed rats, except for lower bradycardic efficacy and greater reduction in short-term blood pressure variability.

Key words: Nebivolol – Fructose-Fed Rats - Enantioselective Pharmacokinetics - Blood Pressure - Blood Pressure Variability.

RESUMEN

Con el objetivo en este estudio de evaluar los efectos cardiovasculares y la farmacocinética del nebivolol en ratas hipertensas por sobrecarga de fructosa y en ratas control, se registraron los efectos de la administración intravenosa de nebivolol, 3 mg/kg o 10 mg/kg, sobre la presión arterial, la frecuencia cardíaca y la variabilidad de la presión arterial a corto plazo y latido-a-latido, y se evaluó la farmacocinética enantioselectiva a partir del análisis de la concentración plasmática de los enantiómeros d-nebivolol y l-nebivolol. La variabilidad de la presión arterial a corto plazo y latido-a-latido se evaluó mediante la desviación estándar y el análisis espectral del registro de la presión arterial, respectivamente. El estado hipertensivo alteró la farmacocinética del nebivolol, evidenciado por una reducción en el aclaramiento del nebivolol en el grupo fructosa respecto del grupo control luego de la administración de la dosis más alta. El efecto antihipertensivo del nebivolol fue similar en ambos grupos, en tanto que el efecto bradicardizante fue mayor en las ratas del grupo control. Aunque no se observaron diferencias significativas en la variabilidad de la presión arterial latido-a-latido, la reducción de la variabilidad de la presión arterial a corto plazo inducida por el nebivolol fue significativamente superior en las ratas del grupo fructosa en comparación con los animales normotensos (-57,9% ± 11,8% vs. -19,6% ± 9,2%; p < 0,05). En conclusión, si bien el nebivolol reduce la presión arterial y la variabilidad de la presión arterial en ambos grupos, no se encontraron diferencias significativas en las ratas con sobrecarga de fructosa en cuanto a la farmacocinética y los efectos cardiovasculares, a excepción de una eficacia bradicardizante menor y una reducción mayor de la variabilidad de la presión arterial a corto plazo.

Palabras clave: Nebivolol - Ratas con sobrecarga de fructosa - Farmacocinética enantioselectiva - Presión arterial - Variabilidad de la presión arterial

Abbreviations

HF	High frequency	NO	Nitric oxide
LF	Low frequency	BP	Blood pressure
SC	Systemic clearance	MAP	Mean arterial pressure
SD	Standard deviation	Vss	Steady state volume of distribution
HR	Heart rate	BPV	Blood pressure variability
VLF	Very low frequency		

INTRODUCTION

Metabolic syndrome is a group of metabolic conditions –insulin resistance, abdominal obesity, hypertension and abnormal lipid panel- that increase the risk of cardiovascular events. (1) In addition to these disorders, metabolic syndrome and type II diabetes have been associated in the last few years with adverse abnormalities in blood pressure variability (BPV), an emerging cardiovascular risk factor. (2, 3) Although the pathophysiological mechanisms in metabolic syndrome are largely unknown, increased sympathetic activity seems to have a major role in the development of several components, among them, visceral obesity, increased blood pressure (BP) and insulin resistance. (4, 5)

The participation of sympathetic hyperactivity in metabolic syndrome components suggests that beta-adrenergic receptor blockers would be effective antihypertensive agents to control hypertension in patients presenting this disorder. However, most beta-blockers have a negative effect on insulin sensitivity and the metabolism of carbohydrates and lipids, and are therefore not recommended in hypertensive patients with metabolic syndrome. (6) It is important to point out that the metabolic adverse effects are not shared by all beta-blockers. Nebivolol, a third generation beta-blocker, is free from deleterious effects on carbohydrate metabolism and insulin sensitivity and may be used in prediabetic patients. (7)

The purpose of this study was the integral pharmacokinetic and cardiovascular evaluation of nebivolol in in vivo fructose-fed rats, an experimental model of metabolic syndrome.

METHODS

Animals

Male Sprague-Dawley rats (220-250 g) were used. Animals were randomly assigned to receive tap water (control rats, n=18) or fructose solution (10% w/v) (fructose rats, n=18) during 6 weeks. The development of the metabolic abnormalities characteristic of fructose overload was verified 5 weeks later by previously described triglyceride and blood glucose tests. (8)

The left carotid artery and the left femoral vein were catheterized under ether anesthesia and then the catheters were tunneled below the skin to emerge at the back of the neck. Experiments were performed in awake animals 24 hours after catheter insertion. On the day of the experiment, the arterial catheter 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 (Polyview, PVA 1, Grass-Astro Med, West Warwick, Ri, USA), and BP recordings were stored and analyzed with Polyview 2.2 software (Astro-Med, West Warwick, RI, USA). Mean arterial pressure (MAP), calculated as the sum of diastolic pressure and one third of pulse pressure, and heart rate (HR) were monitored during 60 minutes prior to drug administration.

After determining baseline BP, intravenous nebivolol 3 mg/kg (n=6) or 10 mg/kg (n=6) or vehicle (n=6) were injected during 30 seconds. After nebivolol administration, a continuous 3-hour BP recording was performed and blood samples (100 μl) were obtained at 5, 10, 15, 30, 60, 90, 120 and 180 minutes.

The concentration of both nebivolol enantiomers –d-nebivolol and l-nebivolol- in plasma samples was quantified using a previously described enantioselective chromatographic method. (9)

Estimation of blood pressure variability

The effect of intravenous nebivolol administration was quantified on beat to beat and short-term BPV. Beat to beat BVP was evaluated through spectral analysis of 3-minute periods taken from the continuous BP recording at baseline and at regular intervals after nebivolol administration. As in previous studies (8, 10) data spectral analysis was performed using the Fast Fourier Transform with a Hamming window. Spectral densities were calculated at the very low frequency (VLF) (0.1-0.2 Hz), low frequency (LF) (0.2-0.7 Hz) and high frequency (HF) (0.7-2.5 Hz) ranges. Although it is well known that LF variability is affected by the modulation of sympathetic vascular tone, the LF/HF ratio was used as variability index, since this normalization minimizes the effect of changes in the absolute values of LF variability (10, 11)

Pharmacokinetic analysis

A two-compartment model with first order elimination was applied for enantiomer pharmacokinetic analysis. TOPFIT pharmacokinetic software (version 2.0, Dr Karl Thomae GmbH, Schering AG, Gödecke AG, Germany) was used to estimate the area under the curve (AUC), systemic clearance (SC) and steady state volume of distribution (Vss) pharmacokinetic parameters. (12)

Statistical analysis

The Kolmogorov Smirnov test was used to assess normal distribution of data and study variables. Data were expressed as mean ± standard error of the mean. Baseline cardiovascular parameters in both groups were compared using Student´s t test. A two-way ANOVA followed by the Bonferroni post-hoc test was used to compare nebivolol effects on MAP, HR, standard deviation (SD) and the LF/HF ratio. Logarithmic transformation of pharmacokinetic parameters was performed to reduce variance heterogeneity in two-way ANOVA followed by Bonferroni post-hoc test. GraphPad prism version 5.02 for Windows (GraphPad Software, San Diego, California, CA) was used for statistical analyses. Statistical significance was established at p < 0.05.

Ethical considerations

Experiments were performed in accordance to the Guide for the Care and Use of Laboratory Animals (NIH publication, No. 85-3, revised 1985).

RESULTS

Compared with the control group (n=18), animals with fructose overload showed a significant increase of blood glucose (1.61±0.02 vs. 1.32 ± 0.06 mg/ml; p<0.05), tryglicerides (1.01±0.08 vs 0.49 ± 0.05 mg/ml; p<0.05), MAP (114±2 vs. 102±2 mmHg; p<0.05) and MAP SD (4.00±0.16 vs. 3.27±0.21; p<0.05). These findings agree with the metabolic and hemodynamic profiles previously reported in this experimental model of metabolic syndrome. (8, 13) Conversely, no significant differences were detected in baseline

HR and in the different parameters of beat-to-beat BPV between both groups (data not shown).

Plasma levels of both nebivolol enantiomers evidenced a biexponential decrease compatible with a two-compartment pharmacokinetic model (Figure 1). Pharmacokinetic analysis established that nebivolol presents a linear enantioselective pharmacokinetic profile, considering that l-nebivolol SC and Vss were significantly higher compared with d-nebivolol in both experimental groups (data not shown). Pharmacokinetic parameters in both experimental groups showed that SC of both enantiomers was significantly lower in the fructose group (l-nebivolol: 127.8±39.3 ml/min, n=6; d-nebivolol: 38.3±13.6 ml/min; n=6) vs. the control group (l-nebivolol: 170±27.2 ml/min, n=6; d-nebivolol: 64.2±10.1 ml/min; n=6) (p<0.05) after administration of the highest dose.

The evaluation of treatment hemodynamic effects established that racemic nebivolol, at both dose levels, is able to reduce HR, MAP and MAP SD in both experimental models. Vehicle injection did not induce significant cardiovascular effects both in control animals as in the fructose group. Intravenous nebivolol injection reduced HR in a dose-dependent manner in both experimental groups. The chronotropic response induced by nebivolol 3 mg/kg and 10 mg/kg was significantly lower in rats with fructose overload compared with the control group (Figure 2). The intravenous administration of nebivolol 3 mg/kg or 10 mg/kg induced a biphasic hypotensive response in both experimental models, characterized by a fast decrease of MAP followed by its normalization and a prolonged reduction of the pressure level (Figure 3). The hypotensive nebivolol response, expressed as percent MAP reduction with respect to baseline, was similar in the fructose and control groups (see Figure 3). Nebivolol also reduced short-term BPV, estimated by means of MAP SD, both in control normotensive rats as in the fructose-fed hypertensive group. Moreover, the decrease in MAP SD was significantly higher in the fructose group than in the control group after intravenous injection of nebivolol 10 mg/kg (Figure 4).

Spectral analysis of beat-to-beat BPV established that nebivolol reduces similarly the VLF and LF domains in fructose-fed rats and in the control group (Table 1). The LF/HF ratio, a marker of sympathetic vascular activity, decreased in both experimental groups with no significant differences between them. (see Table 1)

DISCUSSION

The study findings establish that the fructose overload hypertension model modifies the enantioselective pharmacokinetic and pharmacodynamic properties of nebivolol, with reduction in the plasma clearance of both enantiomers, a decrease in the bradycardic response and greater reduction of short-term BPV compared to normotensive control animals. Previous studies have shown that nebivolol has enantioselec-
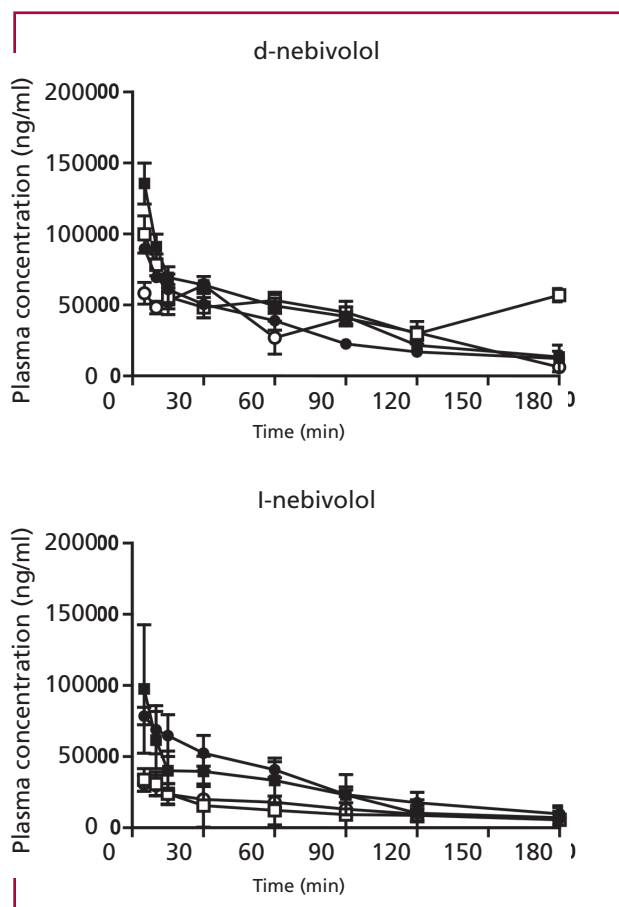

Fig. 1. Plasma concentration of d-nebivolol and l-nebivolol as a function of time in control rats (circles) and fructose-fed rats (squares) after 3 mg/kg (white symbols) or 10 mg/kg (black symbols) nebivolol administration. Each point shows the mean ± standard error of the mean of 6 animals.

tive pharmacodynamic properties due to the great selectivity of d-nebivolol for the beta1-adrenergic receptor and the vasodilator effect of both enantiomers. (14, 15) The mechanisms involved in drug-dependent vascular tone reduction include NO synthase stimulation and the inhibition of endothelial dysfunction through an antioxidant effect attributed to the interaction of nebivolol with the membrane. (14, 15) The pharmacokinetic assessment in humans has shown that nebivolol undergoes high hepatic extraction to be mostly metabolized by the P450 2D6 cytochrome to its active hydroxylated metabolite. (14) Nebivolol has stereoselective pharmacokinetic properties, considering that d-nebivolol peak and minimum plasmatic concentrations were greater than those of the l-nebivolol enantiomer. (16) The present work confirmed the enantioselective pharmacokinetic profile of nebivolol in the metabolic syndrome model, evidenced by greater l-nebivolol clearance compared to the d- enantiomer. The comparison of the pharmacokinetic parameters shows that there are differences in nebivolol clearance between both experimental groups, as rats with fruc-
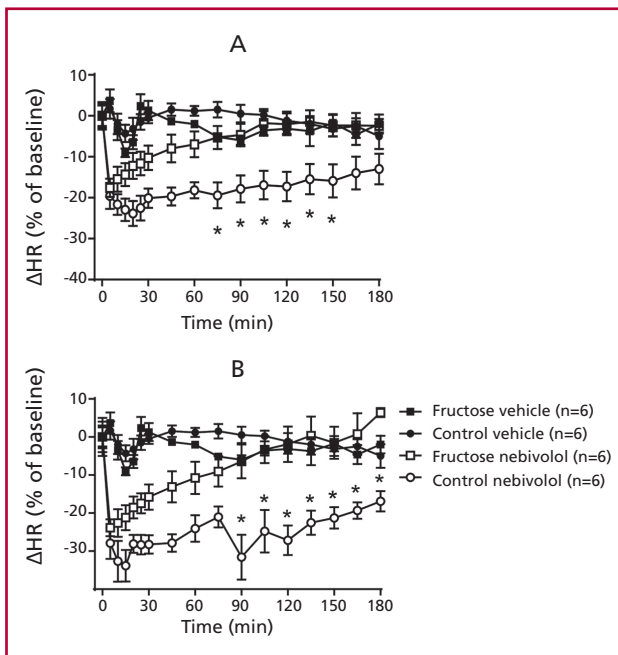
Fig. 2. Time course of heart rate changes (ΔHR, % of baseline) after vehicle (black symbols) or 3 mg/kg (A) or 10 mg/kg (B) nebivolol (white symbols) administration in control rats (circles) and fructose-fed animals (squares). Each point shows the mean ± standard error of the mean of 6 animals. * p<0.05 vs. control rats.

Fig. 4. Time course of changes in short-term blood pressure variability (ΔSD, % of baseline values), after vehicle (black symbols) or 3 mg/kg (A) or 10 mg/kg (B) nebivolol (white symbols) administration in control rats (circles) and fructose-fed animals (squares). Each point shows the mean ± standard error of the mean of 6 animals. * p<0.05 vs. control rats.
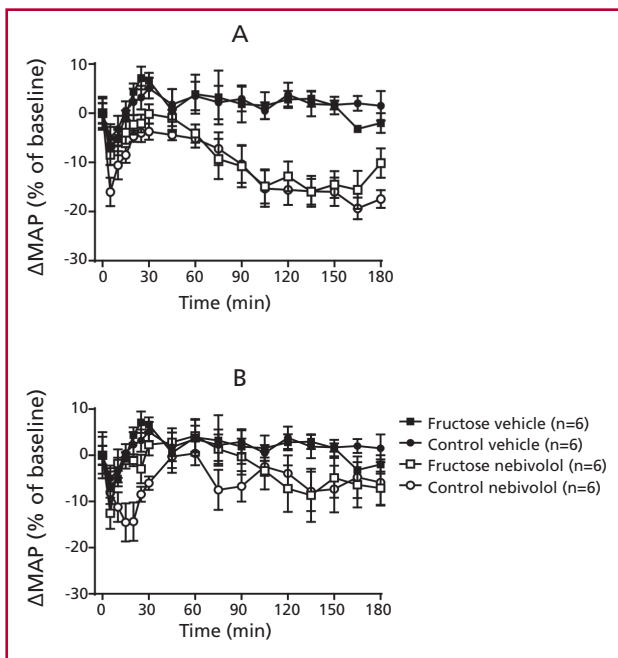

Fig. 3. Time course of changes in mean arterial pressure (ΔMAP, % of baseline), after vehicle (black symbols) or 3 mg/kg (A) or 10 mg/kg (B) nebivolol (white symbols) administration in control rats (circles) and fructose-fed animals (squares). Each point shows the mean ± standard error of the mean of 6 animals. * p<0.05 vs. control rats.

tose overload had lower clearance than control rats at both loading doses. Although the mechanisms implied in this finding are not clear, taking into account
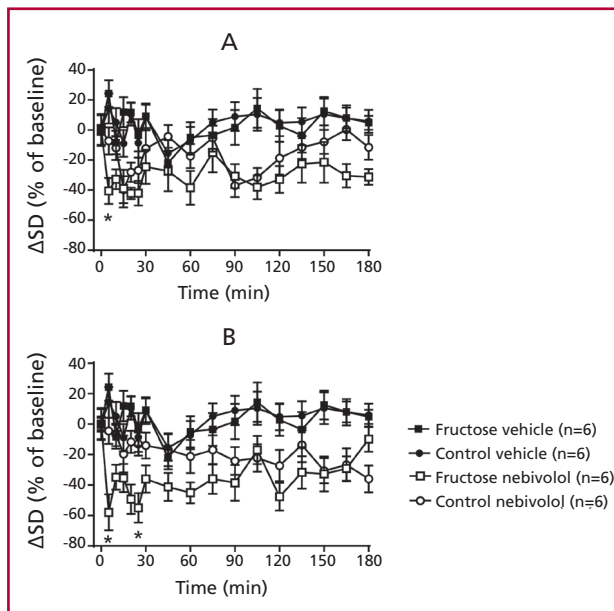
that nebivolol clearance depends on hepatic blood flow, (16) our results suggest lower hepatic perfusion in the model of metabolic syndrome compared with control animals after nebivolol 3 mg/kg and 10 mg/kg administration. In agreement with this assumption, in a previous study performed at our laboratory, we found a similar behavior when evaluating the pharmacokinetic characteristics of nebivolol in spontaneously hypertensive animals. (17)

Intravenous administration of nebivolol induced MAP reduction independently of the dose, without significant differences in the magnitude of the hypotensive effect between fructose-fed and control animals. According to the pharmacokinetic profile, the antihypertensive action of nebivolol would depend both on the reduction of cardiac output associated to selective beta1-adrenergic receptor blockade as on vascular relaxation produced by increased NO availability. However, previous studies have established that nebivolol reduces peripheral vascular resistance preserving cardiac output in hypertensive patients, suggesting that the vascular action outweighs the cardiac mechanisms in the antihypertensive effect of this third generation beta-blocker. (14) The evaluation of intravenous nebivolol effects on HR and the spectral analysis of beat-to-beat BPV confirm the greater relevance of nebivolol vascular action in the antihypertensive response of control and fructose-fed animals, since BP reduction was similar between both experimental groups despite a lower bradycardic response in fructose-fed rats.

Table 1. Effect of nebivolol on beat-to-beat blood pressure variability in different frequency domains expressed as percent of baseline value.

	Fructose rats		Control rats	
	Nebivolol 3 mg/kg (n=6)	Nebivolol 10 mg/kg (n=6)	Nebivolol 3 mg/kg (n=6)	Nebivolol 10 mg/kg (n=6)
VLF	-17.,6± 8.1	-15.3±6.2	-15.3±5.0	-18.6±7.1
LF	-32.2±7.8	-29.6±5.5	-29.2±6.3	-26.4±5.2
HF	-18.1±8.2	-20.2±6.2	-17.3±7.8	-16.5±6.3
LF/HF ratio	-12.2±4.6	-17.0±5.6	-7.1±3.9	-30.7±6.0

Data are expressed as mean ± standard error of the mean. VLF: Very low frequency. LF: low frequency. HF: High frequency

The identification of BPV frequency components using spectral analysis may provide information on the mechanisms involved in BP regulation. (18) Thus, variability quantification in the VLF and LF domains and the estimation of the LF/HF ratio allows the indirect assessment of BP modulation by endothelial NO and vascular sympathetic activity, respectively. (18) Nebivolol administration induced a similar BPV reduction in the range of VLF, LF and the LF/HF ratio in fructose-fed compared to control rats. These findings suggest that the efficacy of nebivolol in terms of endothelial NO increase and vascular sympathetic activity reduction is similar in fructose-fed hypertensive rats compared to normotensive animals, explaining the absence of significant differences in MAP reduction.

Finally, we evaluated the effect of a single dose of nebivolol on short-term BPV. Nebivolol administration reduced SD in BP recordings in both experimental groups, although the decrease was significantly higher in fructose-fed animals compared with the normotensive group. Short-term BPV reduction induced by nebivolol is not related to its hypotensive effect, considering that the administration of both doses produces the same BP fall, but differs in the magnitude of BPV decrease in this model of metabolic syndrome.

CONCLUSIONS

The third generation beta-blocker nebivolol significantly reduces MAP and short-term BPV in fructose-fed rats. Assuming that prediabetes is associated with increased MAP as well as its fluctuations, the findings of this study suggest that nebivolol would be a good therapeutic option to attenuate both cardiovascular risk factors in the population with metabolic syndrome.

Conflicts of interest

None declared
(See author´s conflicts of interest forms in the web / Supplementary Material)

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