

Effects of Chronic Carvedilol Administration on Blood Pressure Variability and Target Organ Injury in Rats with Sinoaortic Denervation

Efectos de la administración crónica de carvedilol sobre la variabilidad de la presión arterial y el daño de órgano blanco en ratas con desnervación sinoaórtica

JULIETA S. DEL MAURO¹, YANINA SANTANDER¹, FACUNDO M. BERTERA¹, ANDREA CARRANZA¹, MARTÍN DONATO^{MTSAC, 2}, SUSANA GORZALCZANY¹, RICARDO GELPI^{MTSAC, 2}, CARLOS TAIRA^{1, 3}, CHRISTIAN HÖCHT^{1, 3}

ABSTRACT

Background: Increased blood pressure variability is a novel risk factor for the development of target organ injury both in hypertensive and normotensive subjects, so its reduction should be considered as a new therapeutic goal.

Objective: The aim of this study was to evaluate the effect of long-term oral carvedilol treatment on blood pressure, blood pressure variability and target organ injury in the left ventricle and thoracic aorta in a model of blood pressure liability.

Methods: Twelve male Wistar rats submitted to sinoaortic denervation were treated during 8 weeks with a single dose of carvedilol 30 mg/kg or vehicle. At the end of treatment, echocardiographic evaluation and blood pressure and short-term variability measurements were performed. Left ventricular and thoracic aortic weights were determined and histological samples were prepared from both tissues. Metalloproteinase MMP-2 and transforming growth factor β (TGF- β) were quantified in the left ventricle and thoracic aorta.

Results: Carvedilol reduced systolic blood pressure and its variability in sinoaortic-denervated rats compared with the control group (126 ± 5 vs. 142 ± 11 mmHg, $p < 0.05$; SD: 2.9 ± 0.5 vs. 6.0 ± 0.5 mmHg; $p < 0.05$). A lower amount of connective tissue was found in carvedilol-treated animals. The expression of TGF- β decreased in both organs after carvedilol treatment.

Conclusions: Chronic carvedilol treatment significantly reduces systolic blood pressure and its short-term variability in sinoaortic-denervated rats, decreasing the degree of left ventricular fibrosis.

Key words: Carvedilol - Target Organ Injury - Sinoaortic Denervation - Blood Pressure Variability - Ventricular Hypertrophy

RESUMEN

Introducción: El incremento en la variabilidad de la presión arterial resulta un nuevo factor de riesgo para el desarrollo de daño de órgano blanco en individuos tanto hipertensos como normotensos, por lo que se postula que su reducción debe considerarse una posible nueva meta terapéutica.

Objetivos: Evaluar el efecto del tratamiento a largo plazo con carvedilol sobre la presión arterial, su variabilidad y el daño de órgano blanco en el ventrículo izquierdo y la aorta torácica en el modelo de la labilidad de presión.

Material y métodos: Se incluyeron 12 ratas Wistar macho sometidas a desnervación sinoaórtica, las cuales fueron tratadas durante 8 semanas con una única administración diaria de carvedilol 30 mg/kg o vehículo. Finalizado el tratamiento se realizó la medición de la presión arterial y de la variabilidad a corto plazo y la evaluación ecocardiográfica. Se determinó el peso del ventrículo y de la aorta torácica y se realizaron preparados histológicos sobre ambos tejidos. Se cuantificó la expresión de metaloproteinasas 2 (MMP-2) y factor de crecimiento transformante β (TGF- β) en el ventrículo izquierdo y la aorta torácica.

Resultados: El carvedilol redujo la presión arterial sistólica y su variabilidad en las ratas con desnervación sinoaórtica en comparación con el grupo control (126 ± 5 vs. 142 ± 11 mm Hg, $p < 0,05$; DE: $2,9 \pm 0,5$ vs. $6,0 \pm 0,5$ mm Hg; $p < 0,05$). Se evidenció menor cantidad de tejido conectivo en los animales tratados con carvedilol. La expresión de TGF- β se encuentra disminuida en ambos órganos luego del tratamiento con carvedilol.

Conclusiones: El tratamiento crónico con carvedilol reduce significativamente la presión arterial y su variabilidad a corto plazo en ratas con desnervación sinoaórtica, disminuyendo el grado de fibrosis del ventrículo izquierdo.

Palabras clave: Carvedilol - Daño de órgano blanco - Desnervación sinoaórtica - Variabilidad de la presión arterial - Hipertrofia ventricular

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Address for reprints: Julieta Sofia del Mauro. Cátedra de Farmacología, Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires - Junín 956 - (C1113AAD) Buenos Aires, Argentina - Tel. +(54-11) 4964-8265. Fax +(54-11) 4508-3645 - e-mail: julidelmauro@hotmail.com

^{MTSAC} Full Member of the Argentine Society of Cardiology

¹ Department of Pharmacology, School of Pharmacy and Biochemistry, Universidad de Buenos Aires,

² Institute of Cardiovascular Pathophysiology, School of Medicine, Universidad de Buenos Aires - CONICET.

³ Institute of Pathophysiology and Clinical Biochemistry, School of Pharmacy and Biochemistry, Universidad de Buenos Aires

Abbreviations

BP	Blood pressure	MMP-2	Matrix metalloproteinase MMP-2
BPV	Blood pressure variability	SAD	Sinoartic denervation
HF	High frequency	SBP	Systolic blood pressure
LF	Low frequency	TGF-β	Transforming growth factor beta
MAP	Mean arterial pressure	VLF	Very low frequency

INTRODUCTION

Blood pressure (BP) is not a constant variable, as it presents short-term (from minutes to days) and long-term (months) periods of deep and spontaneous oscillations. (1) The increase in blood pressure variability (BPV) has been established as a risk factor for the development of target organ injury not only in hypertensive patients but also in normotensive subjects. (1, 2) Clinical studies have determined that 24-hour, day-to-day and between medical visits increased BP fluctuations are associated with higher risk of major cardiovascular events in the hypertensive population. (3) Taking into account this association, it is now postulated that the reduction in BPV should be regarded as a new therapeutic goal of antihypertensive therapy. (3)

Beta-blockers, which until recently had been considered as first line therapy in hypertensive patients without concomitant diseases, are no longer recommended for the initial treatment in uncomplicated hypertensive patients due to their lower protection of cerebrovascular events and higher risk of metabolic disorders. (4) One of the possible reasons associated to beta-blocker reduced target organ protection is the lower attenuation of BPV compared with other antihypertensive agents, especially calcium blockers. (5) However, the clinical evidence associating use of beta-blockers with reduced cardiovascular protection of the hypertensive patient is based to a great extent on clinical studies evaluation atenolol, a beta-blocker with relatively low cardioselectivity and no vasodilator effect. (3, 4) In the last decades, third-generation vasodilating beta-blockers have been developed, among them carvedilol and nebivolol, with better pharmacological properties than atenolol, including the ability to reduce central BP and with neutral metabolic profile. (4) However, the superiority of third-generation vasodilating beta-blockers over atenolol for the reduction of BPV has not been assessed in clinical studies. In a previous work performed in rats submitted to sinoartic denervation, a model of BP liability, we showed that acute administration of carvedilol and nebivolol reduces short-term BPV to a greater extent than atenolol. (6)

Thus, the purpose of this study was to analyze the effects of chronic carvedilol administration on BP and its variability in SAD rats and its possible impact on target organ injury at the left ventricular and aortic levels.

METHODS

Male Wistar rats (200-220 g) were used. They were submitted to sinoartic denervation using the Krieger method consisting in sectioning the aortic nerves at the carotid artery level and the bilateral dissection of carotid bifurcations to remove the carotid nerves. (6) Animals were treated during 8 weeks with carvedilol 30 mg/kg (n=6) or vehicle (n=6), administered orally through a trocar. During the last two weeks of treatment systolic blood pressure (SBP) was measured by the indirect tail-cuff method at 1:00 PM. Mean SBP, intraday variations and interday fluctuations were estimated from these measurements.

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 two-month treatment, the carotid artery was cannulated and connected, 24 hours later, 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), and 2-hour BP recordings were stored and analyzed with Poliview 2.3 software (Astro Med, West Warwick, RI, USA). Mean arterial pressure (MAP), heart rate, and short-term BPV from 3-minute recordings were calculated. In addition, beat-to-beat variability was evaluated from the spectral analysis of BP using Fast Fourier Transform with a Hamming window. Spectral densities were calculated in 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 subject to modulation of neural sympathetic vascular tone, the LF/HF ratio was used as an index of this activity, as this normalization procedure tends to reduce the effect of changes in the absolute values of BPV at LF. (7, 8)

After hemodynamic parameters were measured, all animals were sacrificed by decapitation, and the thoracic aorta and left ventricle were removed to assess target organ injury. The left ventricular weight/body weight ratio and the aortic weight/length ratio were assessed using a precision balance as morphological markers of target organ injury. (9) Presence of fibrosis was determined by left ventricular histopathological evaluation on 4-mm thick histological sections according to previous studies in rats with sinoartic denervation, (9) using Sirius Red to stain collagen fibers pink.

Finally, metalloproteinase MMP-2 (MMP-2) and transforming growth factor β (TGF- β) expressions were assessed by Western blot as biochemical injury markers in left ventricular and thoracic aorta homogenates. The homogenates (20 μ) were run in polyacrylamide gel SDS (12%). Transfer was performed on PVDF membranes and after blocking were incubated overnight with Rabbit anti-MMP-2 (PM 63 kDa), Mouse anti-TGF- β 1 (PM 13 kDa) and Rabbit anti-GAPDH (PM 37 kDa) as loading control. Proteins were detected by

chemiluminescence using photographic film and were quantified with the ImageJ program. (10)

Statistical analysis

The Kolmogorov Smirnov test was used to verify normal distribution of data and variables. Data were expressed as mean \pm standard error of the mean. Statistical comparisons between both groups were performed with Student's *t* test using GraphPad Prism version 5.02 for Windows (GraphPad Software, San Diego, California, USA). Statistical significance was defined as $p < 0.05$.

Ethical considerations

Animal experiments were performed according to the "Guide for the Care and Use of Laboratory Animals" (NIH Publication No. 85-3, revised 1985).

RESULTS

The analysis of tail-cuff BP measurements showed that chronic carvedilol treatment reduced SBP and its intraday variation without modifying SBP fluctuations between interday measurements (Table 1). Central BP measured in the carotid artery of cannulated SAD animals did not show significant differences in MAP between rats treated with carvedilol 30 mg/kg and those that received vehicle (Table 1). However, chronic carvedilol treatment reduced short-term BPV evidenced by a significant reduction of standard deviation compared with the control group (Table 1). Spectral analysis evaluation of BP recordings established that chronic treatment with carvedilol produces a highly significant reduction of beat-to-beat BPV in all the frequency domains compared with the control group (Figure 1). Moreover, the LF/HF index, a marker of sympathetic vascular tone, was lower in SAD rats receiving carvedilol 30 mg/kg compared with animals treated with vehicle.

Target organ injury in the left ventricle and thoracic aorta was different in SAD animals treated with carvedilol or vehicle. The left ventricular weight/body weight ratio was similar in the group of SAD rats receiving vehicle (2.29 ± 0.04 , $n=6$) and those treated with carvedilol 30 mg/kg (2.47 ± 0.12 , $n=6$). Also, no significant differences were detected in the thoracic aorta weight/length ratio between animals treated with carvedilol (2.55 ± 0.35 mg/mm, $n=6$) and the control group (3.07 ± 0.21 mg/mm, $n=6$).

Echocardiographic evaluation did not reveal significant differences in the main echocardiographic systolic and diastolic function parameters between SAD rats treated with carvedilol and the control group treated with vehicle (Table 2).

Preliminary analysis of histological sections evidenced lower degree of interstitial and perivascular fibrosis in the left ventricle of SAD rats with chronic carvedilol treatment compared with the SAD control group receiving vehicle (Figure 2). Finally, the expression of molecular markers of fibrosis established that chronic treatment of SAD rats with carvedilol 30 mg/kg reduces TGF- β expression both in the left

Table 1. Hemodynamic parameters of sinoaortic-denervated rats chronically treated with carvedilol or vehicle.

Hemodynamic parameter	Vehicle SAD rats (n=6)	Carvedilol SAD rats (n=6)
Indirect blood pressure (tail cuff)		
SBP, mmHg	142 \pm 5	126 \pm 2 *
Intraday SD, mmHg	6.0 \pm 0.2	2.9 \pm 0.2 *
Interday SD, mmHg	7.3 \pm 0.9	7.0 \pm 1.3
Direct blood pressure in the carotid artery		
MAP, mmHg	106 \pm 6	93 \pm 8
SD, mmHg	9.2 \pm 0.7	5.8 \pm 1.3 *
Percent coefficient of variation	8.7 \pm 1.0	6.2 \pm 0.5*

Data are expressed as mean \pm standard error of the mean. * $p < 0.05$ vs. vehicle SAD rats. SAD: Sinoaortic denervation. SBP: Systolic blood pressure. SD: Standard deviation. MAP: Mean arterial pressure.

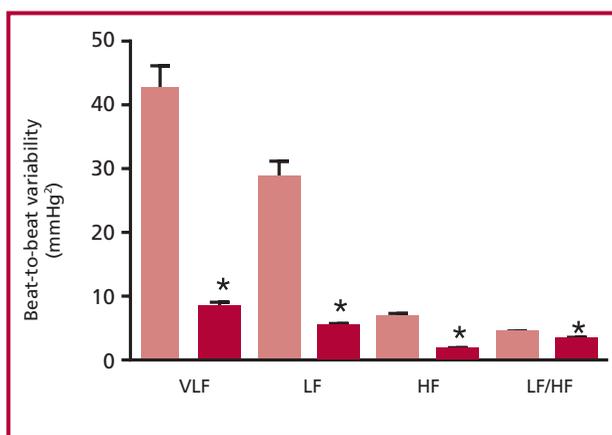


Fig. 1. Beat-to-beat variability at different ranges of frequency in sinoaortic-denervated rats after 8-week treatment with vehicle (light bars) or carvedilol 30 mg/kg (dark bars). * $p < 0.05$ vs. vehicle-treated group. VLF: Very low frequency. LF: Low frequency. HF: High frequency. LF/HF: Low frequency/high frequency ratio

ventricle as in the thoracic aorta compared with the control group treated with vehicle (Figures 3 and 4). Conversely, no significant differences were detected in MMP-2 expression in both tissues between carvedilol and control groups

DISCUSSION

The present study shows that chronic oral treatment with the third-generation vasodilating beta-blocker carvedilol significantly reduces short-term BPV in SAD rats. However, in the carvedilol group, this independent cardiovascular risk marker is only associated with reduction of myocardial fibrosis without changes in morphological measurements and echo-

cardiographic parameters with respect to the vehicle-treated group.

Compared to spontaneously hypertensive SAD rats (SHR-SAD), sinoaortic denervation in normotensive animals represents the ideal experimental model to evaluate only the importance of BPV regulation in the reduction of cardiovascular events and target organ injury. (3) Bilateral resection of carotid nerves increases BP liability without highly significant changes in average BP values. (11) However, previous studies have detected an increase in SBP by the tail-cuff plethysmography method. (11) In addition to hemodynamic changes, elevated BPV in SAD animals is associated with increased target organ injury, including late left ventricular hypertrophy, increased fibrosis and myocardial wall thickening, aortic hypertrophy

with collagen accumulation, impaired arterial distensibility and increased mesangial matrix. (3)

Preclinical evidence in SAD animals and clinical studies in hypertensive patients have led to postulate that increased BPV independently contributes to target organ injury associated with hypertension. (3) It has even been suggested that the poor cardioprotective effect of beta-blockers compared with other antihypertensive drugs would be partly due to their neutral effect on BPV as opposed to the reduction detected with other agents, especially dihydropyridine calcium blockers. (3, 5) In this context, it is necessary to emphasize that the main evidence of the neutral or negative beta-blocker effect on BPV comes from studies evaluating atenolol. It is uncertain whether lack of attenuation of this cardiovascular risk factor with atenolol could be extended to vasodilating beta-blockers as carvedilol. In previous studies in SAD rats, we have reported that acute carvedilol administration produces a greater reduction of short-term BPV than atenolol. (6) Taking into account these studies, we compared chronic carvedilol treatment or vehicle on BPV and target organ injury in SAD rats. The analysis of direct and indirect BP measurements showed that a daily single dose of carvedilol 30 mg/kg can significantly reduce the standard deviation of BP recordings, a well-known parameter of short-term variability. Moreover, as indirect interday BP variation did not differ between the carvedilol and vehicle groups, it can be inferred that the vasodilator beta-blocker did not have a significant effect on day-to-day BPV.

In addition, chronic oral carvedilol administra-

Table 2. Main echocardiographic parameters in sinoaortic-denervated rats after 8-week treatment with vehicle or carvedilol 30 mg/kg.

Echocardiographic parameter	Vehicle SAD rats (n=6)	Carvedilol SAD rats (n=6)
End-diastolic diameter, mm	7.57±0.33	7.30±0.20
End-systolic diameter, mm	5.37±0.37	5.35±0.15
Ejection fraction (%)	64.4±2.8	60.6±1.3
Isovolumic relaxation time, msec	34.7±2.0	34.5±2.5

Data are expressed as mean±SEM. Non-significant p for carvedilol SAD rats vs. vehicle SAD rats.

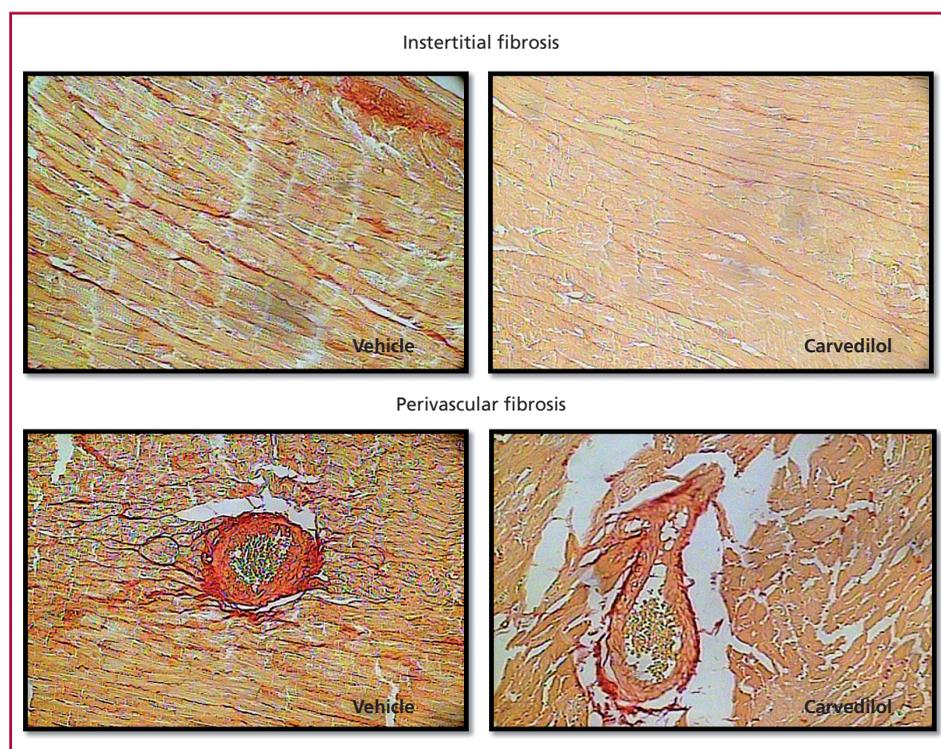


Fig. 2. Representative images (20x) of left ventricular Sirius Red staining, showing interstitial and perivascular fibrosis.

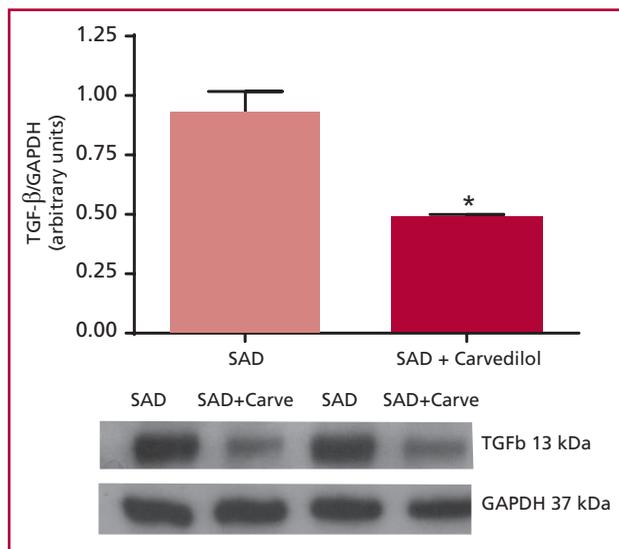


Fig. 3. Transforming growth factor β (TGF- β) expression in left ventricular homogenates of sinoaortic-denervated rats after 8-week treatment with vehicle (light bars) or carvedilol (Carve) 30 mg/kg (dark bars). * $p < 0.05$ vs. vehicle-treated group. GAPDH: Glyceraldehyde-3-phosphate dehydrogenase.

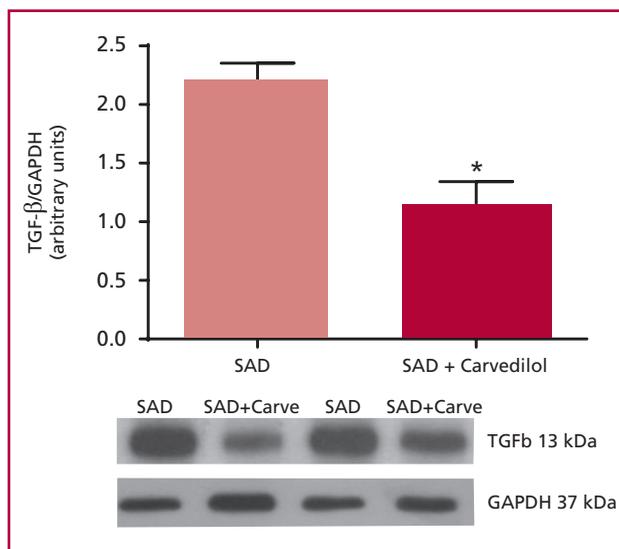


Fig. 4. Transforming growth factor β (TGF- β) expression in thoracic aorta homogenates of sinoaortic-denervated rats after 8-week treatment with vehicle (light bars) or carvedilol (Carve) 30 mg/kg (dark bars). * $p < 0.05$ vs. vehicle-treated group. GAPDH: Glyceraldehyde-3-phosphate dehydrogenase.

tion during 8 weeks significantly reduced beat-to-beat BPV in all the frequency domains. The identification of BPV frequency components by spectral analysis may provide insight into mechanisms involved in BP regulation. (12) Thus, the quantification of variability in the LF, HF domains, and the estimation of the LF/HF ratio allow to indirectly assess BP modulation by endothelial nitric oxide and vascular sympathetic ac-

tivity, respectively. (12) Compared with vehicle treatment, chronic carvedilol administration reduced the LF/HF ratio showing its ability to inhibit sympathetic vascular activity.

The study showed mixed results on target organ injury. Carvedilol did not reduce left ventricular and thoracic aorta morphometric hypertrophy markers or echocardiographic parameters compared with the vehicle group. However, the analysis of left ventricular histological samples and the level of TGF- β expression showed that chronic carvedilol therapy is able to reduce the degree of myocardial and vascular fibrosis in SAD rats. Lack of carvedilol efficacy on morphometric markers could be explained by late left ventricular hypertrophy developing only after 10-16 weeks following SAD. (13)

CONCLUSIONS

In conclusion, chronic carvedilol administration is effective to reduce beat-to-beat and short-term BPV in an experimental model of BP liability. Although BPV attenuation in carvedilol-treated SAD animals did not have beneficial effects on ventricular hypertrophy markers and systolic and diastolic functional parameters, the vasodilating beta-blocker prevented, at least partially, myocardial fibrosis and TGF- β profibrotic factor expression.

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Conflicts of interest

None declared

(See author's conflicts of interest forms in the web / Supplementary Material)

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