Effects of Rut-bpy (Cis-[Ru(bpy)$_2$(SO$_3$)(NO)]PF$_6$), a novel nitric oxide donor, in L-NAME-induced hypertension in rats[1]

Efeitos do Rut-bpy (Cis-[Ru(bpy)$_2$(SO$_3$)(NO)]PF$_6$), um novo doador de óxido nítrico, na hipertensão induzida com L-NAME em ratos

Marcio Wilker Soares Campelo[I], Ana Paula Bomfim Soares Campelo[II], Luiz Gonzaga de França Lopes[III], Armenio Aguiar dos Santos[IV], Sergio Botelho Guimarães[V], Paulo Roberto Leitão de Vasconcelos[VI]

[I] Fellow PhD Degree, Department of Surgery, Postgraduate Program, UFC, Ceara, Brazil. Technical procedures, acquisition and interpretation of data. The article is part of doctorate degree thesis.
[II] Fellow Master Degree, Department of Surgery, Postgraduate Program, UFC, Ceara, Brazil. Acquisition and interpretation of data.
[III] PhD, Associate Professor, Department of Organic and Inorganic Chemistry, UFC, Ceara, Brazil. Critical revision and analysis of data.
[IV] PhD, Associate Professor, Faculty of Medicine, University de Fortaleza (UNIFOR). Critical revision and analysis of data.
[V] PhD, Associate Professor, Department of Surgery, Head, LABCEX. UFC, Ceara, Brazil. Manuscript writing, statistical analysis, graphics design.
[VI] PhD, Associate Professor, Coordinator, Postgraduate Program, Department of Surgery, UFC, Ceara, Brazil. Tutor, responsible for conception, design, intellectual and scientific content of the study, critical analysis, final approval of manuscript.

ABSTRACT
PURPOSE: To evaluate the effect of Rut-bpy (Cis-[Ru(bpy)$_2$(SO$_3$)(NO)]PF$_6$), a novel nitric oxide donor in *N*ω-nitro-L-arginine methyl ester (L-NAME)-induced hypertensive rats.
METHODS: Twenty-four male Wistar rats were randomly assigned to four groups (n=6), named according to the treatment applied (G1-Saline, G2-Rut-bpy, G3-L-NAME and G4-L-NAME+Rut-bpy). L-NAME (30 mg/Kg) was injected intraperitoneally 30 minutes before the administration of Rut-bpy (100 mg/Kg). Mean abdominal aorta arterial blood pressure (MAP) was continuously monitored.
RESULTS: Mean arterial blood pressure (MAP) in G3 rats rose progressively, reaching 147±16 mmHg compared with 100±19 mm Hg in G1 rats ($p<0.05$). In G4 rats, treated with L-NAME+Rut-bpy, MAP reached 149+11 mm Hg while in G2 rats, treated with Rut-bpy, MAP values were 106±11 mm Hg. In G1 rats these values decreased progressively reaching 87+14 mm Hg after 30 minutes. An important finding was the maintenance of the MAP throughout the experiment in G2 rats.
CONCLUSION: Rut-bpy does not decrease the MAP in L-Name induced hypertensive rats. However, when it is used in anesthetized hypotensive rats a stable blood pressure is obtained.
Keywords: Blood Pressure. Nitric Oxide. Ruthenium. Hypertension. Rats.

RESUMO
OBJETIVO: Avaliar o efeitos do Rut-bpy (Cis-[Ru (bpy)$_2$(SO$_3$)(NO)] PF$_6$), um novo doador de óxido nítrico, em ratos hipertensos induzidos pelo éster metílico de N-nitro-L-arginina (L-NAME).
MÉTODOS: Vinte e quatro ratos Wistar machos foram distribuídos aleatoriamente em quatro grupos (n = 6), nomeados de acordo com o tratamento aplicado (G1-Salina, G2-Rut-bpy, G3-L-NAME e G4-L-NAME+Rut –bpy). L-NAME (30 mg / Kg) foi injetado por via intraperitoneal 30 minutos antes da administração de Rut-bpy (100 mg / kg). A pressão arterial média (PAM) da aorta abdominal foi monitorada continuamente.
RESULTADOS: A pressão arterial média (PAM) em ratos do grupo G3 subiu progressivamente, chegando a 147 ±16 mm Hg, em comparação com 100 ±19 mm Hg em ratos do G1 ($p <0,05$). Em ratos G4, tratados com L-NAME + Rut-bpy, a PAM atingiu 149±11 milímetros de Hg, enquanto no G2 (ratos tratados com Rut bpy) os valores da PAM foram 106 ±11 mm Hg. No G1 esses valores decresceram progressivamente, atingindo 87±14 mm Hg após 30 minutos. Um achado importante foi a manutenção da PAM durante todo o experimento em ratos do grupo G2.
CONCLUSÃO: O uso de Rut bpy não diminui a PAM em ratos hipertensos por L-NAME. No entanto, quando ele é usado em ratos anestesiados, hipotensos, uma pressão arterial estável é obtida.
Descritores*:* Pressão Arterial. Óxido Nítrico. Rutênio. Hipertensão. Ratos.

Introduction

Nitric oxide (NO) modulates the physiological functions of the cardiovascular systems, which include vascular smooth muscle relaxation. It has been widely reported endothelium dysfunction in hypertension[1]. Metal nitrosyl complexes have attained great importance in recent years because of the important role of transition metals in the biological process of NO, as well as the possibility of producing thermodynamically stable and kinetically labile species[2-4]. Moreover, it has been demonstrated that metal nitrosyl complexes may induce a decrease in blood pressure levels in hypertensive rats[5].

The novel metallopharmaceutical Rut-bpy (Cis-$[Ru(bpy)_2(SO_3)(NO)]PF_6$) is a potent vasodilator capable of releasing intracellular NO and activating guanylate cyclase[6]. Besides producing higher maximum relaxation in aortic rings than sodium nitroprusside at similar molar basis, Rut-bpy is associated with higher levels of NO release without being photosensitive or releasing cyanide[6,7]. Based on those premises, this study was aimed to evaluate the effects of a novel nitrosyl-ruthenium complex - NO donor (Rut-bpy), on L-Name induced hypertension in anesthetized rats.

Methods

Approval for experimental use of laboratory animals was obtained on 27/08/2008 (Protocol #62/08) from the Ethics Committee on Animal Research (CEPA) of the Federal University of Ceara, now Ethics Committee on the Use of Animals (CEUA), in view of the Federal Law No. 11794 of October 8, 2008, http://www.planalto.gov.br/ccivil_03/_Ato2007-2010/ 2008/Lei/L11794.htm and Decree No. 6689 of July 15, 2009 that regulated the Law 11794, available at: http://www.planalto.gov.br/ccivil_03/_Ato2007-2010/2009/Decreto/ D6899.htm.

The study was designed so as to minimize the number of animals required for the experiments. Twenty-four male Wistar rats weighing 280-300g and housed under standard living conditions with free access to water and chow, were randomly assigned to 04 groups of 6 animals each, named according to the treatment applied (G1-Saline, G2-Rut-bpy, G3-L-NAME and G4-L-NAME+Rut-bpy).

Chemicals and drugs

Rut-bpy (Cis-$[Ru(bpy)_2(SO_3)(NO)]PF_6$) was synthesized and purified at the Department of Organic and Inorganic Chemistry of the Federal University of Ceara (Brazil), following procedures already described[8]. Nω-Nitro-l-arginine methyl ester hydrochloride (l-NAME) was obtained from Sigma-Aldrich (St. Louis, MO, USA). All other chemicals were purchased from standard commercial sources and were of the highest quality available.

Experimental procedure

The animals were anesthetized with an association of xylazine (10 mg/Kg) and ketamine (90 mg/Kg) applied intramuscularly. Next, a polyethylene catheter (PE10 connected to PE50) filled with heparinized saline was implanted into the abdominal aorta, through of the femoral artery, for blood pressure recording. L-NAME (30 mg/Kg) was injected intraperitoneally 30 minutes before the administration of Rut-bpy (100 mg/Kg). The dosage chosen was based on previous studies[9]. Blood pressure was continuously recorded in anesthetized rats using a pressure transducer and an amplifier (ADInstruments Pty Ltd., Bella Vista, Australia) attached to an intra-arterial polyethylene #10 catheter**.** Mean arterial pressure (MAP) was evaluated in four timepoints: onset of the study l (T0) and during drug administration at 30 (T30), 60 (T60) and 90 (T90) minutes. Values were calculated using the software 5.0 Chart ADInstruments provided by ADInstruments Pty Ltd.

Statistical analysis

Graphpad Prism 5.0 (GraphPad Software, San Diego,CA, USA, www.graphpad.com) was used for statistical analysis and graphics design. All data were tested for distribution (Kolmorogov-Smirnov test with Dallal-Wilkinson-Lilliefor *P* value). Results were expressed as mean+SD. Groups were compared at different timepoints (T0, T30, T60 and T90 minutes) using two-way ANOVA. The level of statistical significance was set at 5%.

Results

Rut-bpy effect on blood pressure

Mean arterial blood pressure (MAP) in G3 rats rose progressively, reaching 147±16 mm Hg compared with 100±19 mm Hg in G1 rats ($p<0.05$). In G4 rats, treated with L-NAME+Rut-bpy, MAP reached 149+11 mm Hg while in G2 rats, treated with Rut-bpy, MAP values were 106±11 mm Hg. In G1 rats these values decreased progressively reaching 87+14 mm Hg after 30 minutes. An important finding was the maintenance of the MAP throughout the experiment in G2 rats (Figure 1).

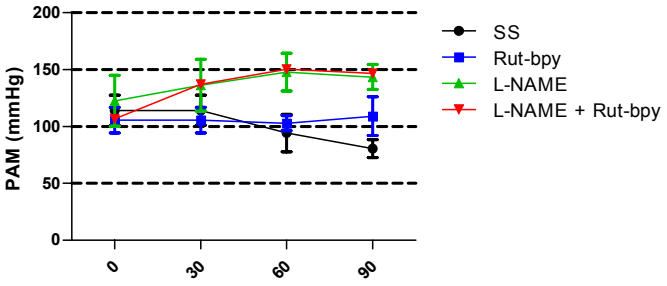

FIGURE 1 - Mean arterial pressures (MAP) in rats treated with saline (G1), Rut-bpy (G24), L-Name (G3) and L-Name+ Rut-bpy (G4). MAP pressure reached 147±16 mmHg after 90 minutes (T90) in G3 and G4. At the same time MAP levels reached a maximum value of 100±19 mm Hg in G2 and 87+14 mmHg in control rats (G1).

Discussion

NO donors produce NO-related activity when applied to biological systems, so they are mainly suited to either mimic an endogenous NO-related response or substitute an endogenous NO deficiency[10]. NO is also the pharmacological principle of a number of drugs collectively labeled nitrovasodilators, which are clinically

used to control hypertensive crisis, protect patients from attacks of angina pectoris, and unload the heart during acute heart failure. Researchers have focused on the development of pharmacological substances capable of releasing NO at specific rates in tissues, in order to overcome NO deficiency.

Over the past years much attention has been given to nitrosyl-ruthenium complexes and their potential pharmacological uses, especially due to their rapid NO release[8] as well as low level of toxicity[9,11-12]. Several ruthenium compounds have been synthesized and purified, but so far none has been tested in studies of experimental hypertension induced by blockade of nitric oxide synthase.

Acute blockade of endogenous NO synthesis with L-arginine analogs such as L-NAME) causes hefty increases in systemic blood pressure (BP) when administered to rats[13]. The rise in BP with L-NAME results entirely from removal of briskly produced NO, thus restoration of NO with the NO-donor sodium nitroprusside (SNP) can reverse the acute hypertensive state[14].

In the present experiment we attempted to acutely reverse the vasoconstrictor actions of systemic NO blockade with L-NAME by Rut-bpy (NO donor) in anesthetized rats. Studies have demonstrated that Rut-bpy induced maximum relaxation in rat aortic rings[6], *in vitro*. In our study the use of Rut-bpy normalized MAP in hypotensive anesthetized rats but was not able to modify the MAP in rats with hypertension induced by L-Name. Studies available in the medical literature, using other ruthenium complexes showed that the use of those substances produced hypotensive effects in animals with hypertension induced by L-NAME[5,15].

It is relevant to point out that the dose of Rut-bpy used in our study was smaller than the lethal dose (LD_{50}) used by other researchers[9]. We believe that these preliminary results must be confirmed with other experiments using different routes of administration and higher doses of Rut-bpy.

Conclusion

Rut-bpy does not decrease the mean blood pressure in L-Name induced hypertensive rats. However, when it is used in anesthetized hypotensive rats an stable blood pressure is obtained.

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Correspondence:
Paulo Roberto Leitão de Vasconcelos
Universidade Federal do Ceará
Rua Professor Costa Mendes, 1608/3º andar
60430-140 Fortaleza – CE Brasil
Tel.: (55 85)288-8063
Fax: (55 85)288-8064
paulo.vasconcelos@ufc.br

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