ORIGINAL ARTICLE

Biological activity produced by ruthenium chloride on perfusion pressure, left ventricular pressure and heart failure using an isolated rat heart model

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Abstract

Background: Several drugs have been used to treat heart failure, including digoxin, spironolactone, captopril, and valsartan; however, some of these drugs can produce different secondary effects. *Aim and Objectives*: The aim of this investigation was to evaluate the biological activity of ruthenium chloride on perfusion pressure, left ventricular pressure and heart failure. *Material and Methods*: Effects produced by ruthenium chloride (0.001 to 100 nM) on perfusion pressure was evaluated using an isolated rat heart model. Besides, the biological activity of ruthenium chloride (1 to 100 nM) on Left Ventricular Pressure (LVP) was determined in the absence or presence of nifedipine, indomethacin, prazosin, and WB-4101 at a dose of 1 nM. Finally, biological activity of ruthenium chloride (1 to 100 nM) against heart failure was evaluated using an ischemia/reperfusion injury model. *Results*: The results showed that ruthenium chloride increased the perfusion pressure from 1 to 100 nM; ruthenium chloride increased LVP in a dose-dependent manner, whose effect was inhibited by prazosin and WB-4101; and the ruthenium derivative significantly decreased the infarct area (p = 0.05) compared with the control conditions. *Conclusion*: These data suggest that ruthenium chloride may act as α_{1B}-adrenoreceptor activator and could be a good agent to treat heart failure.

Keywords: Ruthenium chloride, heart failure, α_{1B}-adrenoreceptor, receptor

Introduction

Heart failure is one of the main health problems worldwide and has increased in recent years, resulting in a decrease in the quality of life of patients [1]. It is noteworthy that several drugs such as bisoprolol, carvedilol, captopril, candesartan, spironolactone, and furosemide [2-4] have been used to treat heart failure; however, some drugs can produce secondary effects such as hypotension [5], hyperkalemia [6], cough [7], and others. In the search for some therapeutic alternatives, several compounds have been used to treat heart failure; for example, a study showed that a ruthenium (II) complex can produce a cardioprotective effect in diet-induced prediabetic rats [8]. Besides, a study displayed that the nitrosyl-ruthenium complex may produce an increase in nitric oxide levels, translated as a vasodilatory effect in an isolated rat aortic ring model [9-10]. Another report indicated that Ru360 (an oxo-bridged dinuclear ruthenium ammine complex) reagent can prevent Ca^{2+} overload and angiotensin

II-induced remodeling through inhibition of the mitochondrial calcium uniporter in shMock cells [11]. Furthermore, other data indicated that ruthenium red inhibited mitochondrial Ca^{2+} uptake in individual rat cardiomyocytes [12]. Other study displayed that ruthenium red can decrease myocardial dysfunction through USP33 (ubiquitin-specific peptidase 33) inhibition using a rat model of cardiac arrest [13]. Other studies showed that a ruthenium derivative (oxo-bridged dinuclear ruthenium amine complex) can prevent heart failure in post-ischemic rat hearts through mitochondrial calcium uniporter inhibition [14]. Furthermore, other reports indicate that RuBPY (*cis*-[Ru(bpy)2 (py)NO2](PF6)) reagent can decrease Ca^{2+} concentration through the guanylyl cyclase-cGMP-GK pathway activation [15].

Contrary to these data, a study showed that ruthenium (II)-NHC complex produced oxidative injury using some biochemical parameters such as malondialdehyde, superoxide dismutase, reduced glutathione, and catalase levels [16]. All these data suggest that some ruthenium derivatives (Figure 1) can produce changes in the cardiovascular system; however, their biological activity on heart failure is not clear; perhaps this phenomenon could be due to different experimental approaches used or to different molecular mechanisms involved in the effect produced by ruthenium derivatives. Analyzing these hypotheses, the objective of this research was to evaluate the biological activity of ruthenium chloride on heart failure using an ischemia/reperfusion injury model.

Material and Methods

All the procedures used in this investigation complied with the research ethics rules used in the guide for the care and use of laboratory animals (Washington, DC: National Academy Press, 1996 [17].

Animals

Wistar male rats weighing 200-50 g (n = 42) were obtained from the Laboratory of Pharmacochemistry Research of the University Autonomous of Campeche.

Reagents

All agents were acquired from Sigma-Aldrich Co. Ruthenium chloride and other compounds were dissolved in methanol, and from this solution, dilutions were made by adding Krebs-Henseleit solution (v/v). Krebs-Henseleit solution was prepared using a previously reported method [18] with the following reagents: NaCl (117.8 mM); KCl (6 mM); $CaCl_2$ (1.75 mM); NaH_2PO_4 (1.2 mM); $MgSO_4$ (1.2 mM); $NaHCO_3$ (24.2 mM); glucose (5 mM); and sodium pyruvate (5 mM). The solution was actively bubbled with an O_2/CO_2 (95:5) gas mixture, regulated to pH 7.4 and 37°C.

Anesthesia

Pentobarbital (50 mg/kg) was intraperitoneally injected into rats to induce anesthesia. Then, the chest was opened, and a loose ligature was passed through the ascending aorta. Following this, the heart was removed, and a cannula was inserted into the aorta. The cannula was linked to an acrylic chamber, which in turn was bound to a Graham condenser. This apparatus was used to retrogradely perfuse the heart with Krebs-Henseleit solution at a constant flow (10 ml/min).

Heart extraction

The heart was removed from the anesthetized animal and clipped to a cannula on the perfusion apparatus. The cannula was attached to the outflow of a reservoir containing an oxygenated perfusion solution.

Perfusion pressure

The biological activity produced by ruthenium on the perfusion pressure was determined using a pressure transducer connected to the chamber where the hearts were mounted. The pressure transducer was bound to a computerized data system (Biopac).

Left Ventricular Pressure (LVP)

Contractile activity was determined by measuring LVP using a saline-filled latex balloon (0.01 mm, diameter) inserted into the left ventricle via the left atrium. The latex balloon was bound to the cannula, which was linked to a pressure transducer that was connected to the MP100 data acquisition system.

Ischemia/reperfusion model

After removing and mounting the heart in the chamber, the heart was perfused with a Krebs-Henseleit solution for 15 minutes to restore its function. Then, the heart was subjected to a period of ischemia for 30 minutes. We turned off the perfusion system in the absence (control conditions) or in the presence of ruthenium chloride. The doses were administered before the ischemia period (for 10 minutes) and during the entire period of reperfusion. At the end of each experiment, the perfusion pump was turned off, and 0.5 ml of fluorescein solution (0.10%) was slowly injected through the cannula inserted into the aorta. This process aimed to differentiate damaged areas from healthy tissue. The heart was then removed from the perfusion apparatus and cut into two transverse sections at a right angle to the vertical axis to determine the infarct area (expressed as a percentage) using a previously reported method [19].

Biological activity

Biological activity produced by ruthenium on perfusion pressure: The effects of ruthenium chloride (0.001 to 100 mM) on perfusion pressure over time (3-21 min) were determined. It is important to mention that biological activity produced by ruthenium chloride was obtained in isolated hearts perfused at a constant flow rate of 10 ml/min.

Effect produced by ruthenium chloride on LVP through type-L calcium channel activation: Boluses (50 µl) of ruthenium chloride at a dose of 0.001 to 100 mM were administered, and the effect on the LVP was evaluated. The dose-response curve (control) was repeated in the presence of nifedipine at a concentration of 1 mM (the duration of preincubation with nifedipine was a 10-minute equilibration period).

Effects produced by ruthenium chloride on LVP through prostaglandin's activation: Boluses (50 µl) of ruthenium chloride at a dose of 0.001 to 100 mM were administered, and their biological activity exerted on LVP was evaluated. The dose-response curve (control) was repeated in the presence of indomethacin at a concentration of 1 nM (the duration of preincubation with nifedipine was a 10-minute equilibration period).

Effects produced by ruthenium chloride on LVP through α_1-adrenoreceptor activation: Boluses (50 µl) of ruthenium chloride at a dose of 0.001 to 100 mM were administered, and their biological activity exerted on LVP was evaluated. The dose-response curve (control) was repeated in the presence of either prazosin or WB-4101 at a concentration of 1 mM (the duration of preincubation with nifedipine was a 10-minute equilibration

period). It is important to mention that the doses of indomethacin, nifedipine, prazosin, and WB-4101 have already been reported in other types of studies [20-21]. For this reason, these doses were used in this study.

Statistical analysis

The obtained values are expressed as Mean ± SEM. The data obtained were subjected to Analysis of Variance (ANOVA) with the Bonferroni correction factor using the SPSS 12.0 program [22]. The differences were considered significant when the value of *p* was equal to or smaller than 0.05.

Results

The results of biological evaluation exerted by ruthenium are shown as follows:

First stage

The results showed that ruthenium chloride increased perfusion pressure ($p = 0.05$) at doses of 1 to 100 nM compared to control conditions (Figure 2).

Second stage

Figure 3 shows that ruthenium chloride increased LVP at the dose of 1 to 100 nM and this effect was not inhibited in the presence of either indomethacin or nifedipine at a dose of 1 nM.

Third stage

Figure 4 shows that ruthenium chloride produced changes in LVP levels in a dose-dependent manner (1-100 nM); however, this effect was inhibited by either prazosin or WB-1401 reagents.

Fourth stage

Other results showed that ruthenium chloride significantly decreased ($p = 0.05$) the infarct area at dose of 1 to 100 nM compared with compounds control conditions (Figure 5).

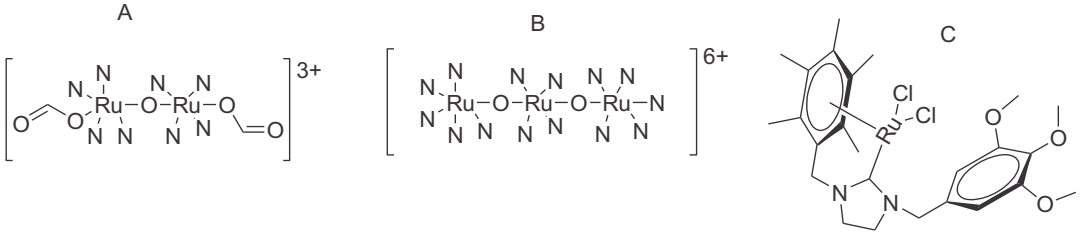

Figure 1: Chemical structure of ruthenium derivatives. Ruthenium red (A); oxo-bridged dinuclear ruthenium amine complex (B) and Ruthenium (II)-NHC complex (C). N = NH_2

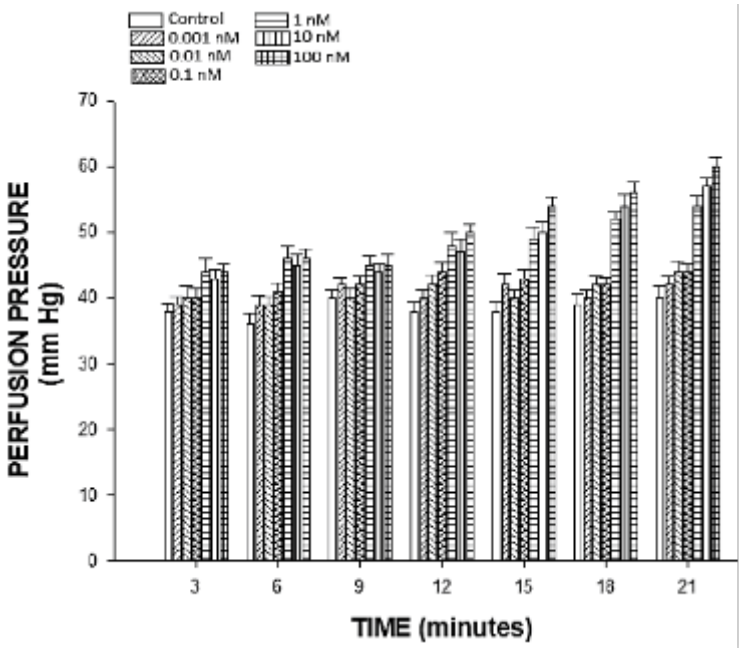

Figure 2: Biological activity exerted by ruthenium chloride on perfusion pressure through of time. Boluses (50 µl) of ruthenium chloride (0.001 to 100 nM) were administered and their effects were determined. The results showed that ruthenium chloride significantly increased (p = 0.05) the perfusion pressure at a dose of 1 to 100 nM in a time-dependent manner compared to control conditions. Each bar represents the mean ± SEM of six experiments.

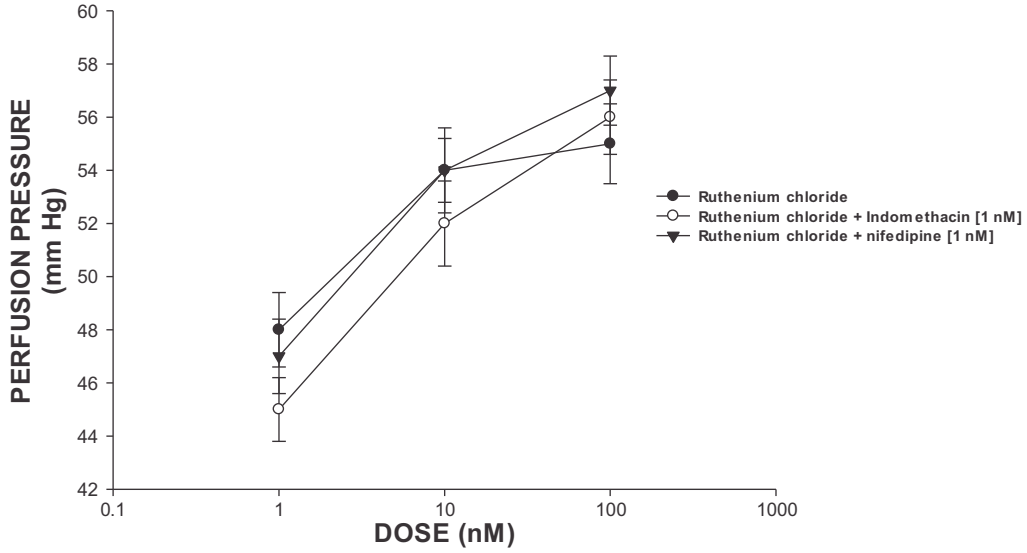

Figure 3: Effect produced by ruthenium chloride on left ventricular pressure (LVP) via either prostaglandin synthesis or calcium channel activation. Intracoronary boluses (50 µl) of ruthenium chloride [1 to 100 nM] were administered and the corresponding effect on the LVP was determined in the absence and presence of either nifedipine or indomethacin at a dose of 1 nM. The results showed that ruthenium chloride increased the LVP in a dose-dependent manner and this effect was not inhibited by nifedipine or indomethacin. Each bar represents the mean ± SEM of six experiments.

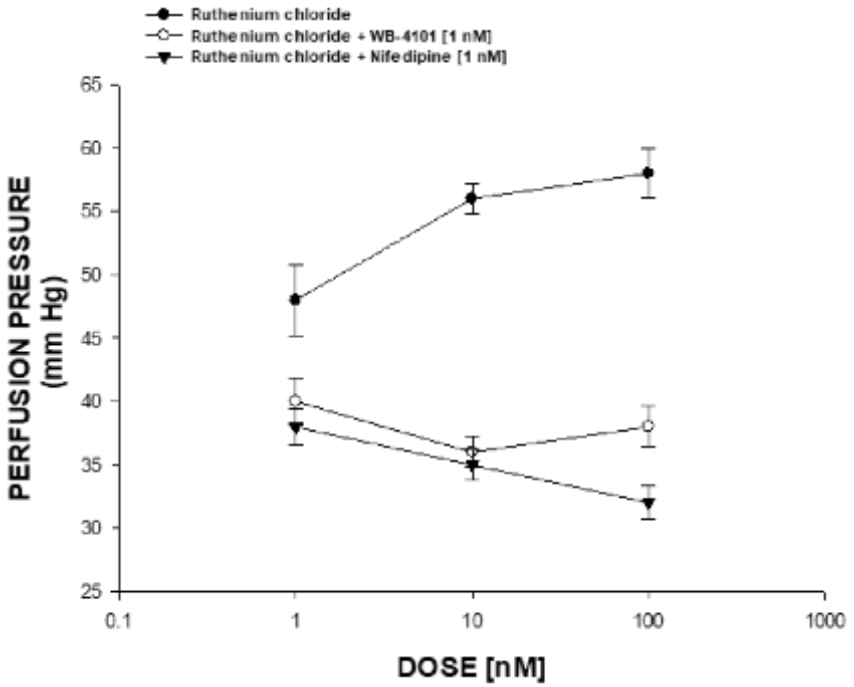

Figure 4: Biological Activity exerted by ruthenium chloride on left ventricular pressure (LVP). Intracoronary boluses (50 µl) of ruthenium chloride 1 to 100 nM were administered and the corresponding effect on the LVP was determined. The results showed that ruthenium chloride increased the LVP in a dose-dependent manner ($p = 0.05$) and this effect was inhibited in the presence of either prazosin or WB-1401 drugs. Each point represents the mean ± SEM of six experiments.

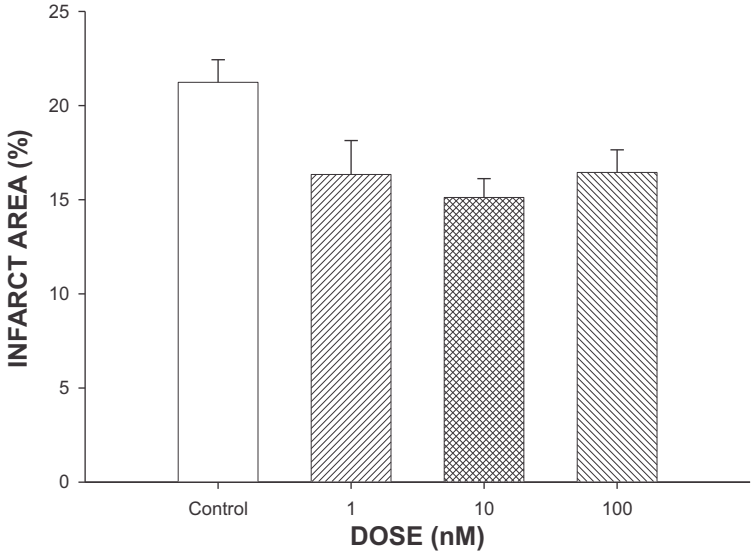

Figure 5: Effect produced by ruthenium chloride at dose of 1 to 100 nM on infarct area. The results showed that ruthenium chloride significantly decreased ($p = 0.05$) infarct size compared with the control conditions. However, there was no significant difference between doses 1 to 100 nM. The values indicate the mean ± SEM of six experiments.

Discussion

In the literature, there are reports on the effects that some drugs exert on perfusion pressure [23]. However, there is little evidence of the biological activity exerted by some ruthenium derivatives on either perfusion pressure or blood pressure [11, 24]. Analyzing these data, the effect produced by ruthenium chloride at doses of 0.001 to 100 nM on perfusion pressure was determined using an isolated rat heart model. The results showed that ruthenium chloride at doses of 1, 10, and 100 nM increased perfusion pressure; these data suggest that the minimum dose to produce changes in perfusion pressure was 1 nM, possibly due to either release or activation of some biomolecule. Analyzing these data and other studies indicated that some ruthenium derivatives can induce changes in blood pressure by increasing intracellular calcium [25]; for this reason, in this study, the biological activity of ruthenium chloride on left ventricular pressure was determined in the presence of nifedipine (a calcium channel blocker) [26]. The results displayed that ruthenium chloride increased LVP in a dose-dependent manner, and this effect was not inhibited by nifedipine. These data indicated that the biological activity of ruthenium chloride on left ventricular pressure was not via calcium channel activation.

On the other hand, we also analyzed some reports indicating that some ruthenium derivatives can produce changes in prostaglandin levels [27]. For this reason, this study evaluated the biological activity of ruthenium chloride on LVP through prostaglandin activation. The results showed that ruthenium chloride increased LVP in a dose-dependent manner, and this effect was not blocked by indomethacin. These results suggest that the effect produced by ruthenium chloride on LVP was not via prostaglandin activation. Analyzing these data and other reports indicated that some ruthenium derivatives produced changes in vascular tone by altering the adrenergic system [28]; therefore, in this research, the biological activity exerted by ruthenium chloride on LVP in the absence or presence of either prazosin (α_1-adrenoreceptor inhibitor) or WB-4101 (α_{1B}-adrenoreceptor antagonist) drugs [29, 30] was evaluated. The results showed that ruthenium chloride increased LVP in a dose-dependent manner, and this effect was inhibited by both prazosin and WB-4101 drugs. These results suggest that the effect produced by ruthenium chloride on LVP was via α_{1B}-adrenoreceptor activation.

Second stage

Several studies indicated that some ruthenium derivatives produced changes in the cardiovascular system [9–16]. However, the biological activity exerted by ruthenium derivatives on heart failure is not very clear; perhaps this phenomenon could be due to the different experimental approaches or the different chemical characteristics of each ruthenium derivative. This research aimed to evaluate the biological activity produced by ruthenium chloride (1 to 100 nM) on heart failure using an ischemia/perfusion injury model. The results showed that the ruthenium chloride decreased infarct size in the different doses compared with the control conditions. It is important to mention that there was no significant difference between doses of 1 to 100 nM; perhaps this phenomenon could be since only the minimum dose is required to produce beneficial effects in the infarct area. However, it is

important to mention that there is a possibility that increasing the dose of the ruthenium derivative could activate other biological systems, so it would be interesting to perform alternative experiments in the future.

Conclusion

Overall, the results indicate that ruthenium chloride reduces infarct area and increases both perfusion pressure and left ventricular pressure through α_{1B}-adrenoreceptor activation. Besides, ruthenium chloride can act as a positive inotropic agent, giving it good prospects for treating heart failure.

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