Antihypertensive property of a nickel-piperazine/NO donor in spontaneously hypertensive rats

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Abstract

Aims: The nickel-piperazine/NO donor compound, Ni(PipNONO)Cl, belonging to the family of compounds labelled as "metal-nonoates", due to its promising vasodilating activity, has been considered as a potential drug candidate in anti-hypertensive therapy.

Methods and Results: Drug safety and efficacy have been evaluated in normotensive mice and rats, and in spontaneously hypertensive rats (SHR). In normotensive animals the metal-nonoate maintained blood pressure at basal level both following acute and chronic administration. In SHR, Ni(PipNONO)Cl reduced blood pressure in the dose range of 3-10 mg/kg. When compared with a commercial NONOate, DETA/NO, used at the same doses, Ni(PipNONO)Cl was more effective in the first two weeks, while the effect of the two molecules was similar in the third and fourth week. The degradation and control compound $Ni(Pip)Cl_2$ had no effect on blood pressure and heart rate. Remarkably, the hypotension induced by the new nitric oxide-donor Ni(PipNONO)Cl does not evoke changes in the heart rate and tolerance.

Considering the mechanisms of vascular protection, chronic administration of Ni(PipNONO)Cl improved endothelial function in SHR by increasing eNOS (endothelial NO synthase) expression, attributed to upregulated eNOS and downregulated Cav-1 (Caveolin 1) and by inducing superoxide dismutase 1 (SOD1) expression in aortae.

In cultured endothelial cells Ni(PipNONO)Cl restored the cell functions (cytoskeletal protein expression, migration and proliferation) altered by the inflammatory mediator interleukin-1β (IL-1β), impairing the endothelial to mesenchimal transition.

Conclusion: Ni(PipNONO)Cl showed a safe profile in normotensive mice and rats, and it exerted an anti-hypertensive effects in SHR through the restoration of vascular endothelial protective functions.

Keyword: NO donor, hypertension, nitrate tolerance, endothelial dysfunction

Translational perspective

Characterization of the hypotensive property of a nickel-piperazine/NO donor, Ni(PipNONO)Cl, belonging to the recently developed family of compounds labelled as "metal-nonoates", previously identified as the most effective in preserving endothelial functions. Ni(PipNONO)Cl shows a safe profile in normotensive animals and an anti-hypertensive effect on SHR (Spontaneously Hypertensive Rats) without developing tolerance and endothelial dysfunction. Ni(PipNONO)Cl can be considered as a promising candidate as therapeutic agent, useful in cardiovascular pathologies, as hypertension and atherosclerosis, where endothelial and vascular functions need to be reestablished.

1. Introduction

Resistant hypertension (RH) is defined as uncontrolled blood pressure (BP) despite the use of ≥3 anti-hypertensive drugs, or controlled blood pressure (BP) requiring the use of ≥4 drugs. Recently, a new definition for an extreme phenotype of RH has emerged, the refractory hypertension (RfH) namely uncontrolled BP using at least 5 drugs, characterized by worse cardiovascular outcomes. [1] Cases of multidrug resistant hypertension have also been reported in cancer patients undergoing anti-vascular endothelial growth factor strategies. [2] Drugs which have nitric oxide-mediated vasodilator properties (as angiotensin converting enzyme (ACE) inhibitors or nevibolol), may be effective in treatment of hypertension induced by vascular endothelial growth factor (VEGF) inhibitors that are known to reduce NO synthesis. [3,4] Thus, the possibility exists that new NO donor drugs can be developed to overcome these unmet medical needs.

NO has been shown to be an important signalling molecule in a wide variety of physiological processes, including blood pressure control, neurotransmission, immune response and cell death. [5] Due to its wide range of properties, researchers have been encouraged to develop NO donors that can deliver NO to biological targets to accomplish the desired responses and avoiding relevant side effects as reflex tachycardia. [6,7]

Among the NO donors, the compounds labeled "metal-nonoates" are based on the functionalization of a specific polyamine, N-aminoethylpiperazine (Pip), with a "NONOate" group, and stabilized by complexation to a metal ion, i.e. copper(II), nickel(II), and zinc(II). [8]A slightly modified version of this family of metal-nonoates was obtained through the condensation of the starting N-aminoethylpiperazine with salicylaldehyde. (LUCIA: toglierei questa parte) The metal-nonoate releases NO by reaction with water (2 NO molecules per complex), in a metal-, pH-, and temperature-dependent manner. The nickel(II) containing derivatives Ni(PipNONO)Cl and Ni(SalPipNONO) exhibited the most interesting NO releasing properties, with kinetics characterized by a half-life ($\tau_{1/2}$) of several minutes at physiological pH. [8]

The assays conducted on the vasorelaxation induced by metal-nonoates in rabbit pre-contracted aorta show that these compounds are 10 to 1000 times more potent than the standard reagent sodium nitroprusside. [8] Moreover, recent results on in vitro and in vivo models, show that Ni(PipNONO)Cl exerts a protective effect on the vasculature and induce an anti-atherosclerotic effect in a more efficient manner than DETA/NO. [9]

Based on these promising results, the candidate therapeutic agent Ni(PipNONO)Cl was evaluated in animal models for its safety and efficacy as antihypertensive agent. Ni(PipNONO)Cl was studied in acute and chronic treatment settings in comparison with the degradation and control molecule $Ni(Pip)Cl_2$ or with the commercial NONOate, DETA/NO. The structure of the two metal complexes is reported in Fig.1. Note that the chloride ions of both complexes are immediately exchanged with water molecules upon dissolution.

2. Materials and methods

2.1 Animals

The experiments were done on adult male C57BL/6 mice (50 animals, 6 weeks old), on male Wistar Kyoto rats (WKY, 30 animals, 8 weeks old) or Spontaneously Hypertensive rats (SHR, 40 animals, 8 weeks old) obtained from the Charles River Laboratories (Lecco, Italy). All animals were housed in temperature- and humidity-controlled rooms (22 °C, 50%) with lights on from 07:00 to 19:00, water and food available ad libitum, and left to acclimatize to these conditions for one week. All procedures were carried out in accordance with the Italian law (Legislative Decree no.116, 27 January 1992) and the European Directive 2010/63/UE. All efforts were made to minimize the number of animals used and their suffering. All experimental protocols were approved by Ethical Committee of Florence.

Administration of test compounds was carried out with intraperitoneal injection (i.p.) at 10:00 a.m. every day for 2 or 4 weeks. Control animals were treated with the same volume of vehicle. Body weight, general behaviour, food and water consumption were weekly checked.

Animals were euthanased with an overdose of chloralum hydratum i.p. (Fluka, Sigma Aldrich, St. Louis, MO, USA) decapitated and exsanguinated, and organs were collected. [10] Aorta was removed, immediately frozen in liquid nitrogen and stored at -80 °C until use for biochemical assessments.

2.2 Mean arterial pressure and heart rate measurements

Mean arterial pressure (MAP) and heart rate were monitored every five days in conscious restrained animals by tail-cuff plethysmography using the BP-2000 Blood Pressure Analysis System coupled with BP-2000 analysis software (Visitech Systems, Apex, NC, USA). [11] Animals were conditioned at least four times to the procedure before beginning the experiments. Measurement were repeated every 30 min for 2 hours and data are reported as mean of four separate determinations. MAP is expressed as mmHg and heart rate as bpm (beat per minutes).

2.3 Rat Aorta Vessel Ring

Isolated thoracic aortic rings from SHRs, treated with or without Ni(PipNONO)Cl (10 mg/kg, 30 days) were tested. The aortae were processed as previously described. [9] Changes in tension were recorded by a computerized system (Biopac, Goleta, CA). The value of tension developed after phenylephrine (0.3 mM; Sigma-Aldrich) administration was taken as 100% and the effects of sodium nitroprusside (SNP, cumulative doses) were referred to this value.

2.4 Cell cultures

Human umbilical cord vein endothelial cells (HUVEC) were from Promocell (Heidelberg, Germany) and were maintained in EGM-2 (Lonza, Basel, Switzerland), and 10% FBS (Fetal Bovine Serum, Hyclone, Euroclone, Milan, Italy). Cells were split 1:3 twice a week, and used until they reached passage 10.

2.5 Proliferation assay

HUVEC ($1.5x10^3$ cells/well in 96 multiplates), after adherence, were treated with Ni(PipNONO)Cl (0.1 nM, pre-treatment 30 min) in presence/absence of IL-1β (10 ng/ml, 24 hrs). Cells were then fixed, stained and randomly counted at 20× original magnification in 5 fields as previously reported. [12]

2.6 Scratch assay

Cells ($1×10^5$ cells/well) were seeded into 24-well plates to reach confluence. Cell monolayer was scraped to create a wound of ± 1 mm width. Then, test substances were added with the antimitotic ARA-C (2.5 μg/ml). Images of the wound in each well were acquired from 0 to 18 hrs under a phase contrast microscope, Nikon Eclipse TE 300 (Nikon, Tokyo, Japan), at 20× magnification. Results are expressed as percentage of area of wound.[13]

2.7 Western Blot

Western blot analysis was performed on tissue sample or in cell culture lysates. Aortae were isolated from SHR treated with Ni(PipNONO)Cl or $Ni(Pip)Cl_2$ (10 mg/kg, 30 days) as previously described. [9,14] Tissue specimens were lysed (Cell Lytic, Sigma-Aldrich St. Louis, MO), homogenized and sonicated twice (10 seconds at 25% of power for every cycle).

Cells ($3x10^5$ cells/6 cm diameter plates) were treated with IL-1β (10 ng/ml, 48 hrs) with or without the pre-treatment with Ni(PipNONO)Cl (0.1 nM, 30 min).

Electrophoresis (50 mg of protein/sample) was carried out in 4-12% Bis-Tris Gels (Life Technologies, Carlsbad, CA). Proteins were then blotted onto nitrocellulose membranes, incubated overnight with antibodies (eNOS, Cav-1, SOD1, VE-Cadherin; diluted 1:1000), and then detected by enhanced chemiluminescence system (Bio-Rad, Hercules, CA). Results were normalized to those obtained by using an antibody against β-actin (Sigma-Aldrich, diluted 1:10000). Data are reported as arbitrary densitometric unit (A.D.U.) ± S.E.M.

2.8 Materials

A more detailed description of the Materials is provided in the supplementary material online.

The nickel-piperazine/NO donor compound Ni(PipNONO)Cl and its corresponding decomposition compound $Ni(Pip)Cl_2$ were synthesized by Noxamet Ltd. (Milan, Italy) as previously reported. [8]

2.9 Statistical Analysis

All values are expressed as mean ± SEM. Statistical analysis was performed by using one-way analysis of variance (ANOVA) and Bonferroni as post-test, or two-way ANOVA and Bonferroni as post-test where appropriate (Graph-Pad software, San Diego, CA). Differences were considered statistically significant with a p value < 0.05.

3. Results

3.1 Safety of Ni(PipNONO)Cl in normotensive mice

The main clinical applications of NO donors are hypertension and cardiovascular related disorders. One of the main prerequisite for the safety of antihypertensive drugs should be the maintenance of basal blood pressure and heart rate in normotensive animals.

Firstly, the cardiovascular effects Ni(PipNONO)Cl were evaluated in normotensive C57Black mice. The animals were treated with Ni(PipNONO)Cl, DETA/NO (commercially available NONOate) at increasing doses (0.5, 1 and 3 mg/kg, single i.p. administration) or vehicle (control) and blood pressure and heart rate were evaluated in conscious animals by tail-cuff technique. All the tested compounds maintained blood pressure and heart rate at control values (Fig. 2A and B, respectively), indicating that these doses could be used safely.

To exclude the onset of anti-hypertensive effects after long term treatments, mice were treated for 15 days with test substance (0.5, 1 and 3 mg/kg, administered every day, i.p.) and parameters were evaluated every 5 days. Blood pressure and heart rate did not change during the time at all doses used of both compounds, compared to vehicle group (Fig. 3A and B, and C and D, respectively).

Collectively, these data indicate that Ni(PipNONO)Cl maintained blood pressure and heart rate at basal level after acute treatment and preserved blood pressure and cardiac activity during long term administration.

3.2 Ni(PipNONO)Cl exerts an anti-hypertensive effect in pathologic hypertension model

To assess the anti-hypertensive effect of Ni(PipNONO)Cl in pathological condition, WKY (normotensive rats) and SHR (hypertensive rats) were treated with increasing doses of Ni(PipNONO)Cl (3, 5 and 10 mg/kg, one day). The compound was ineffective on blood pressure

and heart rate of WKY, while it significantly reduced blood pressure in SHR (Fig. 4A and B, respectively). A trend of heart rate reduction with respect to vehicle treatment was observed in WKY, but it was not statistically significant (Fig. 4B)

Long term administration of Ni(PipNONO)Cl (3 or 10 mg/kg, daily i.p. administration for one month) induced reduction of blood pressure (about 17% and 26% at the end of the 30th day, Fig. 5A and C, respectively), while heart rate was not significantly altered (Fig. 5B and D, respectively).

Ni(PipNONO)Cl effects in SHR were then compared with $Ni(Pip)Cl_2$ and DETA/NO, used at the same dose (10 mg/kg). As expected, the compound $Ni(Pip)Cl_2$ had no effect on blood pressure and heart rate (Fig. 6A and B). DETA/NO showed its anti-hypertensive activity only after 15 days of treatment, with an activity which was comparable to the one induced by Ni(PipNONO)Cl after 30 days (Fig. 6A).

These data indicate that Ni(PipNONO)Cl, but not its degradation product $Ni(Pip)Cl_2$, is effective in lowering blood pressure in SHR and not in WKY. The hypotensive effect persists following long term administration with no signs of tolerance and tachycardia.

3.3 Effect of NO donor chronic treatment on body and organ weight

Body weight (BW) increased in both WKY and SHR control groups after one month (16% and 13%, respectively). Ni(PipNONO)Cl, used at highest concentration (10 mg/kg, 30 days) significantly decreased the gain in BW (7.3%) at the end of treatment only in WKY as compared with control rats (16%) and to SHR treated group (10,6%) (Table 1, supplemental material).

Absolute heart, kidney and liver weights were similar in the WKY and SHR control groups. Ni(PipNONO)Cl did not change absolute organ weights in WKY, while liver weight was reduced in SHR treated with Ni(PipNONO)Cl. Absolute and relative kidney weights were not statistically different in the four groups, while an increased liver weight:BW was found in WKY strain, possibly as a result of reduced total BW in the treated animals (Table 1, supplemental material).

When considering the animal and organ weights of SHR treated with Ni(PipNONO)Cl, $Ni(Pip)Cl_2$ and DETA/NO for one month (10 mg/kg/day), body and organ weights (heart, left ventricle, kidney and liver) were unchanged in all animal groups, both as absolute and relative values (Table 2, supplemental material).

These data in the whole support the safe profile of the metal-nonoate following repetitive long-term treatment.

3.4 Ni(PipNONO)Cl improves endothelial functions in SHR and in cell cultures

The maintenance of vascular tone and improvement of endothelial function are added values of anti-hypertensive drugs, as a typical feature of nitrate therapy limitation is the development of tolerance, which consists in phenomena both at systemic level as well as more specific vascular disturbances. [15] Isolated aortic rings from SHRs treated with Ni(PipNONO)Cl (10 mg/kg, 30 days) did not modify the responsiveness to SNP, demonstrating an absence of tolerance to this NO donor (Fig. 7A). Moreover, Ni(PipNONO)Cl (10 mg/kg, 30 days) treatment increased the expression of superoxide dismutase 1 (SOD1) in SHR aortic tissue (Fig. 7B), thus reducing oxidative stress known to contribute to the pathological status of SHR. [16] In order to confirm this protective effect and the reduction of endothelial dysfunction, endothelial NO synthase (eNOS) expression was evaluated in aortae isolated from SHR treated with Ni(PipNONO)Cl (10 mg/kg, 30 days). The compound increased eNOS expression in aortae as compared with the ones treated with vehicle (Fig. 7C). The expression of caveolin-1 (Cav-1), a negative regulator of eNOS activity, [12] was decreased after treatment with Ni(PipNONO)Cl (Fig. 7C), suggesting an activation of eNOS. $Ni(Pip)Cl_2$ (10 mg/kg, 30 days) had no effect both on SOD1 expression or eNOS activation (Fig. 7B and C, respectively).

Considering these results and the previous reported protective effect on endothelium, [8,9] Ni(PipNONO)Cl does not induce tolerance and reduces endothelial dysfunction increasing eNOS activity and upregulating antioxidant enzymes.

Since endothelial dysfunction accompanies conditions that predispose to cardiac fibrosis during chronic pressure overload, we further investigated the capability of the compound to prevent the endothelial-mesenchymal transdifferentiation (EnMT), phenomenon activated by inflammatory stimuli. [17,18] Treatment of endothelial cells with IL-1β (10 ng/ml, 48 hrs) induced loss of VE-Cadherin expression, a pre-requisite for loss of cell-cell contact, the first step of EnMT, [19] which was prevented by Ni(PipNONO)Cl (0.1 nM, Fig. 7D). The loss of cell-cell contacts mediated by IL-1β (10 ng/ml, 18 hrs) induced endothelium to migrate as shown in Fig. 7E (panel c and e). Ni(PipNONO)Cl (0.1 nM) reduced cell migration induced by IL-1 β (Fig. 7B, panel d and e). This protective effect of Ni(PipNONO)Cl on IL-1β treatment was also confirmed on cell proliferation as it prevented cell number reduction induced by the cytokine (Fig. 7F).

In the complex a normalization of cell functions altered by IL-1β is evident in cultured cells treated with Ni(PipNONO)Cl.

4. Discussion

Our experiments provide the first evidence of the hypotensive effect of the metal-nonoate compound Ni(PipNONO)Cl in hypertensive rats after acute or chronic treatment, an effect which is not reproduced in normotensive animals, indicating a safe profile of the compound. The hypotension induced by this novel NO donor does not evoke changes in the heart rate and does not induce tolerance development. Compared to DETA/NO, a commercially available NONOate with a slower rate of NO release, [20,21] the blood pressure lowering effect is evident from the first weeks of treatment and persists during time at the same extent when used at the same concentration.

The doses used in this study (till 10 mg/kg) were chosen on the base of approximate toxic dose, determined in Sanna et al., [14] which was 22.5 mg/kg. At all doses used, Ni(PipNONO)Cl exhibited no effect (hypotension or heart rate modification) on normotensive animals, confirming the safety of the compound in healthy subjects. When it was tested in the pathological animal model, the 3 mg/kg dose, about 10 times lower than the toxic dose, was able to induce a hypotensive effect both after acute and chronic treatment. It is worth noting that other NO donors are used at higher concentrations than Ni(PipNONO)Cl: i.e. 60 mg/kg DETA/NO, [21] 5 mg/kg Terpy, a ruthenium complex derivative, [6] 100 mg/kg nitroglycerin (GTN), [22] 75 mg/kg Isosorbide-5-mononitrate (ISMN). [23] Then, we focused our attention on 10 mg/kg concentration to confirm the hypotensive effect (comparable to the one registered at 3 mg/kg), but mainly to exclude side effects. Acute and repetitive treatments with Ni(PipNONO)Cl at this concentration (10 mg/kg) are safe for normotensive animals since no alteration in systemic blood pressure and heart rate was found. The maintenance of heart rate at control level is of fundamental importance as it is reported among the side effects registered for other NO donors. Indeed, SNP induces a rapid hypotension that leads to reflex tachycardia, which could be an undesirable effect in patients with heart disease, a common feature of hypertension. [6] Finally, the safety of Ni(PipNONO)Cl has been correlated with animal health, and more specifically with body and organ weight. A reduction in body weight could be

measured in WKY after one month of treatment, but not in SHR. Noteworthy, the weight of key vital organs (heart, liver, kidney) does not change following treatment, thus strengthening the safe profile of the metal-nonoate.

The lack of tolerance development is an additive positive feature of Ni(PipNON)Cl. This is documented by the maintenance of blood pressure during time in long term treatment setting of SHR and by the overlapping response to SNP obtained in aortic rings isolated from animals after 1 month of treatment to vehicle and the metal-nonoate.

Beside the NO donor activity of the compound under investigation, previously characterized, [8] the study of the mechanism of action of Ni(PipNONO)Cl at tissue level reveals an upregulation of protective enzymes as eNOS and the antioxidant SOD1, and a downregulation of the eNOS endogenous negative modulator Cav-1. These data fully complement the one found in vitro on the anti-inflammatory and anti-atherogenic effect of Ni(PipNONO)Cl on isolated endothelial and smooth muscle cells. [9] Vascular protection is a prerequisite to avoid tolerance to nitrate which is a feature and a limit of clinically available NO donor drugs. Indeed tolerance consists in a number of phenomena both at the systemic level as well as more specific vascular disturbances implying the induction of endothelial dysfunction through a reduction in NO bioavailability, either as a result of reduced NO production, or increased NO consumption - mainly by ROS. [15,24] All these deleterious features are inhibited by the metal-nonoate.

From a cellular point of view, in this paper we confirm the protective effect of Ni(PipNONO)Cl on endothelial cell functions (seen in Monti et al.) [9] altered by an inflammatory mediator as IL-1β, known to be involved in EnMT. [17,18] Reduced VE-cadherin expression, increased cell migration and altered proliferation monitored in the presence of IL-1β are normalized by Ni(PipNONO)Cl, addressing its potential use in clinical conditions characterized by endothelial dysfunction, cardiovascular fibrosis and atherosclerosis.

All together, these data confirm the safe profile of the recently developed NONOates based on metallic centres on different species and the efficacy of Ni(PipNONO)Cl in reducing blood

pressure in a hypertensive experimental model. Outstanding features which merit to be particularly considered in this class of compounds are the lack of tolerance upon long-term repetitive treatments, the absence of reflex tachycardia, typical of old but still in use NO donors as sodium nitroprusside, and the induction of a cardiovascular protective phenotype at vascular and endothelial level. Among newly described NO donors only some ruthenium-based compound show this positive feature even if they are less potent than commonly available NO donors. [6]

From a clinical point of view, metal-nonates could be attractive NO donor compounds to control systemic blood pressure when changes in the heart rhythm are undesirable, and a general cardioprotective/anti-atherogenic effect is foreseen to reduce the risk of cardiovascular complications associated to endothelial dysfunction.

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Conflict of interest: none declared.

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Figure legends

Figure 1. Chemical structure of Ni(PipNONO)Cl and $Ni(Pip)Cl_2$.

Figure 2. Mice blood pressure and heart rate are unaffected by nonoate acute treatment. A. and B. Effects of NO donors (0 - 0,5 - 1 - 3 mg/kg) on blood pressure and heart rate, respectively, measured in mice after single administration. Measurements were repeated every 30 min for 2 hours. Data are reported as mean arterial blood pressure (MAP, mmHg) or heart rate (bpm) ± SEM (n=6).

Figure 3. Long term administration of nonoate maintains blood pressure and heart rate in normotensive mice. Mice were treated with increasing doses (0.5, 1 and 3 mg/kg, 15 days) of Ni(PipNONO)Cl (A. and B.) and DETA/NO (C. and D.). Measurements were repeated every 30 min for 2 hours. (n=6). Data are reported as MAP (mmHg) ± SEM (A. and C.) or heart rate (bpm) ± SEM (B. and D.).

Figure 4. Effect of increased doses of Ni(PipNONO)Cl on blood pressure and heart rate. A. and B. Effects of Ni(PipNONO)Cl (3, 5 and 10 mg/kg) on blood pressure and heart rate, respectively, measured in WKY and SHR after single administration. All measurements were repeated every 30 min for 2 hours. Data are reported as MAP (mmHg) ± SEM or heart rate (bpm) ± SEM (n=6). *$p<0.05$ and ***$p<0.001$ vs. SHR non treated.

Figure 5. Effect of long-term Ni(PipNONO)Cl administration on blood pressure and heart rate in WKY and SHR. WKY and SHR were treated with or without Ni(PipNONO)Cl (3 mg/kg (A. and B.) and 10 mg/kg (C. and D.), 30 days) and blood pressure (A. and C.) and heart rate (B. and D.) were measured. Values are expressed as MAP (mmHg) ± SEM or heart rate (bpm) ± SEM (n=6). ***$p<0.001$ vs. WKY control group; $^{\#}p<0.05$, $^{\#\#}p<0.01$ and $^{\#\#\#}p<0.001$ vs. SHR control group.

Figure 6. Anti-hypertensive effect of nonoates on SHR. A. and B. SHR were treated with Ni(PipNONO)Cl and respective control compound, $Ni(Pip)Cl_2$, or DETA/NO (10 mg/kg, 30 days)

to measure blood pressure and heart rate, respectively. Values are expressed as MAP (mmHg) ± SEM or heart rate (bpm) ± SEM (n=6). *p<0.05, **p<0.01 and ***p<0.001 vs. control.

Figure 7. Ni(PipNONO)Cl protective effect on vasculature. A. Vascular relaxant responses in pre-contracted aortae isolated from SHRs, treated or not with Ni(PipNONO)Cl (10mg/kg, 30 days), induced by SNP (cumulative doses). Data are expressed as % relaxation ± S.E.M. B. SOD1 expression evaluated in SHR aortic tissue sampled from animals treated with Ni(PipNONO)Cl (10 mg/kg, 30 days). Data are reported as A.D.U. ± SEM (n=6). Representative pictures are shown. **p<0.01 ***p<0.001 vs. Vehicle. C. eNOS and Cav-1 expression in aortic tissue from SHR treated with Ni(PipNONO)Cl (10 mg/kg, 30 days). Data are reported as A.D.U. ± SEM (n=6). Representative pictures are shown. ***p<0.001 vs. Vehicle. D. VE-Cadherin expression in endothelial cells following the treatment with IL-1β (10 ng/ml, 48 hrs) with or without Ni(PipNONO)Cl (0.1 nM). Data are reported as A.D.U. ± S.E.M. ***p<0.001 vs. control; $^{\#\#}$p<0.01 vs. IL-1β. E. Cell migration was assessed in HUVEC pre-treated with Ni(PipNONO)Cl (0.1 nM, 30 min) and then treated with IL-1β (10 ng/ml, 18 hrs). Representative picture are shown (panels a-control, b-Ni(PipNONO)Cl, c-IL-1β, d- Ni(PipNONO)Cl+ IL-1β, 20 x magnification). Data (panel e) are reported as % area of wound ± S.E.M. **p<0.01 and ***p<0.001 vs. control; $^{\#\#}$p<0.01 vs. IL-1β. F. Cell proliferation evaluated in HUVEC treated with IL-1β (10 ng/ml, 24 hrs) with or without Ni(PipNONO)Cl (0.1 nM). Data are reported as number cell counted/well ± S.E.M. **p<0.01 and ***p<0.001 vs. control; $^{\#\#\#}$p<0.001 vs. IL-1β. All experiments above were run in triplicate.