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EFFECT OF ACUTE AND CHRONIC NAMI-A ADMINISTRATION ON VASCULAR SMOOTH MUSCLES RESPONSIVENESS *IN VITRO* AND ON BLOOD PRESSURE IN RATS

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NAMI-A is an innovative ruthenium complex endowed with selective activity against lung metastases of solid mouse tumours, and no direct cytotoxic activity on tumour cells. Some experimental evidence showed that, after *in vivo* chronic treatment, NAMI-A may exert toxic effects on kidneys and smooth muscles. Provided that kidney toxicity is typical for a heavy metal-derived compound, no clear role has been attributed yet on the toxicity for smooth muscles. The aim of the study was therefore that of examining the putative short-term toxic effect of NAMI-A on vascular smooth muscle cells on rat aortic rings mounted for isometric tension recording. Cumulative concentration response curves are obtained at 0.01-30µM phenylephrin (PE) in the absence and in the presence of NAMI-A. Where appropriated, aorta rings were pre-incubated with NAMI-A for 10 min. 0.01, 0.1, 1 and 10µM NAMI-A increased maximal PE contraction, and 10 µM also caused a significant shift of the PE curve, with a significant reduction of EC_{50} from 0.18 ± 0.09 to 0.08 ± 0.03 µM. Conversely, 10µM NAMI-A did not affect the contraction induced by 5-100 mM KCl. No significant difference of cumulative KCl concentration response curves was obtained in the presence of NAMI-A, and NAMI-A did not significantly decrease the maximal relaxation induced by acetylcholine, suggesting the lack of interference with the endothelium-dependent, NO-mediated, relaxation. Atomic absorption spectroscopy studies showed an increase of NAMI-A bound in the aorta tissue with increasing times of exposure and concentrations in the bath. The effects of NAMI-A on the cardiovascular system were evaluated *in vivo* on Winstar Hannover rats. Animals were treated acutely with a single *ip* dose of 105 mg/kg NAMI-A, or they were chronically *ip* given 17.5 mg/kg/die (total dose administered 105 mg/kg) for six consecutive days. Variations of systolic pressure *in vivo*, and changes of contraction of aorta rings induced by PE *in vitro* were tested in groups of rats treated with NAMI-A or with physiological saline. Systolic pressure was monitored with the tail cuff-method on "pre-conditioned" and preheated rats, before and 5, 10, 30, 60 minutes after each *ip* treatment. After 2 hours from the single treatment and 24 hours after the 6 days treatment cycle, animals were sacrificed to remove the thoracic aorta for the *in vitro* study of the response to increasing concentrations of PE. After the single dose treatment, systolic pressure had a significant peak at 1 hour (161±15 mmHg *vs* 131±20 mmHg, $p<0.05$ *Student t test*). The increased blood pressure was paralleled by a significant increase of responsiveness of the isolated aorta rings to PE *in vitro*. The chronic 6-day treatment with 17.5 mg/kg/day was free of evident signs of toxicity, as measured by the change in body weight and of serum creatinine levels versus the untreated controls. However, these rats developed a time dependent increase of blood pressure that peaked at the third day of treatment. The study of the effects of the chronic *in vivo* treatment with NAMI-A on the *in vitro* response of aortic rings, harvested 24 hours after the end of treatment, to PE showed a

significant decrease of contractility. These findings suggest the possible correlation between the *in vivo* effects of NAMI-A on blood pressure and the *in vitro* responsiveness of alpha-adrenergic receptors.
This study contributes to explain the mechanism for the renal toxicity of NAMI-A resulting after repeated *ip* treatment cycles since renal vessel are under adrenergic control. The increased constriction of blood vessels in the kidney might justify the glomerular and tubular damage caused by NAMI-A and evidenced by light microscopy examinations.
