smooth muscle cells subsequent to balloon vascular injury in rats. Recently, evidence has been collected showing that novel antioxidants such as superoxide dismutase mimetics, possess protective effect against vascular injury in rats, though the mechanism is still unclear. Here, we have investigated on the protective effect of the peroxinitrite decomposition catalyst Mn (III) tetrakis (4-benzoic acid) porphyrin (MnTBAP) on smooth muscle cell proliferation which follows balloon injury of common carotid artery in rats. In animals undergoing balloon-injury a prominent restenosis and neointima formation occurred. This effect was associated to an elevated production of peroxynitrite, an highly reactive free radical, and to significant elevation of tissue malondialdehyde, a marker of lipid peroxydation. Treatment of rats with MnTBAP (10-40 mg/kg/day, i.p.), dose-dependently reduced post-injury neointima formation, an effect accompanied by decreased peroxynitrite generation and MDA accumulation. The effect of MnTBAP was also associated to decreased expression of NF-κB, an intracellular transduction mediator associated to activation of free radical sensitive genes leading to smooth muscle cell proliferation. These results suggest that novel peroxynitrite antagonist may reduce post-injury neointima formation via inhibition of NF-κB-related intracellular pathway.

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ADENOSINE INHIBITS THE INCREASED ADP-INDUCED FORMATION OF MONOCYTE-PLATELET AGGREGATES IN PATIENTS WITH CARDIAC SYNDROME X

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Background. In patients with cardiac syndrome X (CSX) we showed an increased formation of monocyte-platelet (mono-plt) aggregates in response to adenosine diphosphate (ADP) at rest, which disappeared after exercise. In this study, we assessed whether adenosine may prevent the ADP-induced increase of mono-plt aggregate formation in CSX patients.

Methods. We studied 7 CSX patients (M/F 4/3; age 50±5) and 7 healthy controls (HC, M/F 4/3; age 54±3). Venous blood samples were collected in plastic tubes and mono-plt aggregates were assessed by flow cytometry (BD FACScan) at baseline, after ADP stimulation alone (10^{-7}M final concentration) and after ADP stimulation in presence of adenosine (10^{-5}M final concentration). Mono-plt aggregates were identified using the logical gating facility by combination of binding characteristics of anti-CD14 (monocyte marker) and of anti-CD41 (platelet marker) antibodies. Data were expressed as percentage of monocytes binding platelets (MBP) and as mean fluorescence intensity (MFI) of CD41 in the mono-plt gate.

Results. At baseline there were no differences in mono-plt CD41 MFI and MBP between CSX patients and HC (39.7±17 vs. 44.1±19 mfi, p=0.3; 19.4±19 vs. 17.1±13%, p=1.0, respectively). ADP stimulation increased mono-plt CD41 MFI in CSX patients (to 53.3±17 mfi, p=0.04) and in HC (to 56.1±22 mfi, p=0.02). ADP also increased the percentage of MBP in CSX patients (to 32.0±29%, p=0.03), but not in HC (17.7±16%, p=1.0). In presence of adenosine mono-plt CD41 MFI was not increased significantly by ADP, compared to basal values, both in CSX patients (35.7±10 mfi, p=0.17) and in HC (50.6±19 mfi, p=0.1). Moreover, in presence of adenosine, the percentage of MPB did not increase after ADP stimulation, compared to basal values, both in CSX patients (25.6±21%, p=0.17) and in HC (20.6±16%, p=0.7).

Conclusions. Our data show that ADP induces an increase of platelets bound to monocytes (CD41 MFI) both in CSX patients and in HC. In contrast, an ADP-induced increase of MBP is observed only in CSX patients and is prevented by adenosine, suggesting that this substance may play a role in the reduction of ADP induced mono-plt aggregate formation observed after exercise in our previous study.

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ROLE OF THE INDUCIBILE NITRIC OXIDE SYNTHASE IN INFLAMMATORY PATHOGENESIS OF THE ASCENDING AORTIC ANEURYSM

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Background. Inflammation and over-expression of matrix metalloproteinases (MMPs) contribute to aortic aneurysm formation. We investigate the role of these factors in patients with ascending aortic aneurysm (AAA) undergone surgical resection. The purpose of this study was to determine if nonspecific AAA are associated with macrophage infiltration, increase MMP2-MMP9 and inducibile nitric oxide synthase (iNOS) expression.

Materials. We analyze the expression of MMP2, MMP9 and iNOS in ten patients underwent AAA resection. Fragments of the aneurysmal aortic wall resected during surgical procedures were collected and preserved under a NaCl solution. Normal aortic wall fragments were collected during routine surgical procedures in patients without AAA and analysed as control group. Inflammatory process was detected using the MMP9 polyclonal antibody, monoclonal anti MMP2 and iNOS polyclonal antibody.

Results. By microscopic observation of pathologic and normal fragments, colored with Mallory-Azan, we showed a total disruption of normal architecture of aneurysmal aortic wall. In normal aortic fragments there is not evidence of iNOS activity, but in the aneurysmal fragments has been evidenced an intense reactivity at level of the inflammatory cells presents in the context of aortic wall (fig. 1-2). Reactivity towards MMP2 and MMP9 is found in the normal aortic wall at level of the media where turn out positivity on muscular cells and fibroblasts, mainly where there is an inflammatory infiltration.

Conclusions: Our data showed that iNOS is present only in aneurysmal aortic wall. On the contrary MMP is present even in normal tissue but result increased in the pathologic fragments. Our data support the key roles of the NO in inflammatory pathogenetic process of AAA.

fig.1: Normal aortic wall. Total absence of reaction to iNOS.

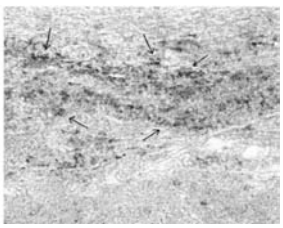

fig.2: Aortic aneurysm: diffuse reaction to iNOS at level of inflammatory process.

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INFLUENCE OF BETA1-ADRENERGIC RECEPTOR POLYMORPHISMS ON EXERCISE CAPACITY IN IDIOPATHIC DILATED CARDIOMYOPATHY

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Beta-adrenergic receptor (b-AR) gene polymorphisms had been associated with adverse outcome in chronic heart failure. We sought to investigate the relationship of b1-AR polymorphisms with peak oxygen uptake (peak VO2) in idiopathic dilated cardiomyopathy (DCM) patients (pts) who were homogeneously treated with betablockers. Forty-nine consecutive unrelated pts (mean age 52±12 years, 37 males, left ventricular ejection fraction, LVEF, 28±5%, left ventricular end diastolic diameter, LVEDD, 65±6 mm, NYHA functional class 1.9±0.6) with DCM (WHO criteria) underwent cardiopulmonary exercise testing. All of them were in stable clinical conditions and had been taking conventional therapy, including ACE-inhibitors and/or AT1R antagonists (100%) and betablockers (100%), for at least 3 months. The Ser49Gly and Arg389Gly polymorphisms of b1-AR gene were characterized by PCR-RFLP analysis. Single locus analysis showed a significant association between b1-AR polymorphisms and exercise capacity. Pts carrying at least one copy of Gly49 allele (n=13) had significantly higher peak V02 as compared with those who were homozygous for Ser49 variant (n=36) (18.7±5.4 vs 15.0±5.2 ml/Kg/min, p=0.034). Carrier status of the Arg389Arg genotype (n=27) was associated with significantly increased peak V02 in comparison with Gly389 allele (n=22) (17.5±5.8 vs 14.0±4.3 ml/Kg/min, p=0.021). At 2-locus analysis, pts carrying Ser49Ser genotype/Gly389 variant combination had the lowest peak VO2 (Figure). These associations remained significant (p=0.014) independently from LVEF and LVEDD. In conclusion, the b1-AR Ser49Ser genotype and Gly389 variant are associated with a significantly depressed exercise performance, thus suggesting their influence on functional limitation and prognosis of DCM pts who were receiving betablockers.