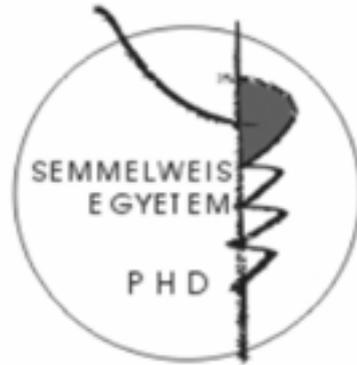


# **Novel antioxidant therapeutic strategies for cardiovascular dysfunction associated with ageing**

Ph.D. Doctoral Thesis

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## Introduction

The populations in the industrial countries in Europe and Northern America are ageing. 16.7 % of the population of the European Union were 65 years of age or older in 2000, 2.4 % more than in 1990. According to epidemiological studies, advanced age acts as the major risk factor of the most important cardiovascular diseases; the incidence and prevalence of hypertension, coronary heart disease, heart failure or stroke increase continuously with advancing age. Because of a physiological ageing process, characteristic structural and functional changes occur in the vasculature and heart, on which pathophysiological disease mechanisms can become superimposed. Thus, these ageing-associated changes become “partners” with pathophysiological mechanisms of diseases to determine the threshold, severity and progression of cardiovascular diseases in older patients.

Recent studies elucidated numerous cellular and molecular mechanisms responsible for the functional decline of the cardiovascular system at old age. The oxidative stress hypothesis (or free radical theory) is currently one of the most favored explanations for how ageing leads to progressive cellular damage at the biochemical level. According to this theory, the cardiovascular dysfunction associated with advanced ageing is related to a progressive and irreversible accumulation of oxidative damage caused by the local formation of reactive oxygen and nitrogen species in the myocardium and coronary vasculature. Ageing organisms are exposed to continuous oxidative injury, due to the higher rate of superoxide ( $O_2^-$ ) and other free radical production from the mitochondrial electron-transport chain. Increases in reactive oxygen and nitrogen species (such as superoxide, hydrogen peroxide ( $H_2O_2$ ), peroxynitrite ( $ONOO^-$ ) or hydroxyl radical ( $OH^\cdot$ )) at old age can elicit oxidative modifications of various cell components, such as lipid, protein and particularly DNA.

A potent oxidant species, peroxynitrite is formed by the reaction of superoxide anion and nitric oxide. Due to its high diffusibility across lipid membranes in the protonated form, peroxynitrite can easily penetrate cells and tends to attack various biomolecules and cellular structures, thereby inactivating functionally important receptors and enzymes, causing lipid-peroxidation and various forms of DNA-injury (strand breaks and base modifications).

Oxidative and nitrosative stress are endogenous inducers of DNA single strand breakage that is the obligatory trigger of activation of the nuclear enzyme poly(ADP-ribose) polymerase (PARP), which mediates the cellular response to DNA injury. Depending on the severity of DNA damage, different cellular pathways can be triggered. In the case of mild DNA damage, PARP facilitates DNA

repair and thus cell survival. Severe DNA injury causes excessive PARP activation that initiates an energy-consuming futile repair cycle by transferring ADP-ribose units from  $\text{NAD}^+$  to nuclear proteins. The excessive nuclear poly(ADP-ribose) formation results in rapid depletion of intracellular  $\text{NAD}^+$  and ATP-pools, slowing the rate of glycolysis and mitochondrial respiration, eventually leading first to cellular energetic crisis and dysfunction, then to cell necrosis. By this route, PARP activation in cardiomyocytes and endothelial cells leads to a cellular energetic crisis, which subsequently causes functional impairment of contractile function at the cellular level and reduced ability of endothelial cells to produce nitric oxide when stimulated by an endothelium-dependent relaxant agonist, such as acetylcholine. Impairment of endothelial function in the coronary arteries may lead to regional or global myocardial ischaemia, which secondarily impairs cardiac performance.

Recent work demonstrated that certain cellular effectors of DNA fragmentation can also be activated by PARP. According to these results, PARP regulates the mitochondrial-to-nuclear translocation of the apoptosis-inducing factor (AIF) in cardiomyocytes and vascular cells. The physiological purpose of this pathway may be that cells with irreparable DNA damage can become safely eliminated.

Enhanced rate of cell death in the ageing myocardium and vessel wall via the necrotic or the apoptotic route by PARP activation results in cardiac and vascular remodeling and impairment of the cardiac and endothelial function.

Pharmacological attempts against nitro-oxidative stress using classic antioxidants, such as vitamin E (which works by scavenging toxic oxidation products), ascorbate or glutathione (which react with peroxynitrite, albeit at a relatively slow rate) resulted in conflicting results in experimental models of disease. According to recent studies, pharmacological inhibition of PARP or rapid catalytic decomposition of peroxynitrite with synthetic metalloporphyrins which block the peroxynitrite - DNA injury - poly(ADP-ribose) polymerase pathway emerge as potent novel antioxidant therapeutic possibilities in multiple pathophysiological conditions.

## **Aim of the study**

Based upon the oxidative stress theory of ageing and recent studies supporting it, we hypothesized that the activation of the nitro-oxidative stress - DNA-injury - poly(ADP-ribose) polymerase (PARP) pathway may play an important pathophysiological role in the functional decline of the cardiovascular system at old age. Thus, interrupting this pathway at different steps may beneficially affect the ageing-associated cardiac and vascular dysfunction.

The aims of the present studies were:

1. In the *in vitro* model of vascular oxidative stress induced by hydrogen peroxide on isolated rat aortic rings:

-Investigation of the possible pathophysiological role of the DNA-damage - PARP pathway in the development of vascular dysfunction induced by oxidative stress

-Testing the effects of pharmacological PARP-inhibition with INO-1001 on endothelial dysfunction induced by hydrogen peroxide and underlying cellular and molecular changes in the vessel wall

2. In the *in vivo* rat model of ageing-associated cardiovascular dysfunction

-Investigation of the possible pathophysiological role of endogenous peroxynitrite overproduction and activation of the PARP pathway in the development of myocardial and endothelial dysfunction associated with advanced ageing

- investigation of the effects of single dose acute PARP-inhibition by INO-1001, and rapid catalytic decomposition of peroxynitrite by FP15 on cardiac performance, vascular functions and the underlying cellular and molecular changes in the heart and in the vessel wall

As a summary, our main goal was to establish novel potent antioxidant therapeutic strategies for ameliorating the cardiovascular dysfunction associated with advanced ageing.

# Methods

## I. Experimental models

### ***In vitro* model of vascular dysfunction induced by oxidative stress**

In organ bath experiments for isometric tension with isolated rat thoracic aortic rings we investigated the effects of *in vitro* hydrogen peroxide exposure on vasoconstriction, endothelium-dependent and -independent vasorelaxation as described detailed below. Endothelial injury was induced by exposing the isolated aortic rings of young male rats to H<sub>2</sub>O<sub>2</sub> (200 and 400 μM) for 30 minutes. In the treatment group, aortic rings were preincubated with the potent PARP-inhibitor INO-1001 (1 μM) before H<sub>2</sub>O<sub>2</sub>-exposure.

### **The rat model of cardiovascular dysfunction associated with advanced ageing**

We investigated ageing (20-24 months old) and young (3 months old) male rats in our experiments as models for ageing-associated dysfunction. Rats were treated with vehicle, or the peroxynitrite decomposition catalyst FP15 for 3 weeks (0.1 mg/kg/day), or with a single dose of the PARP-inhibitor INO-1001 (5 mg/kg). Young rats treated for the same time with vehicle or the corresponding drugs were used as controls.

Systolic and diastolic cardiac performance were investigated by left ventricular catheterisation, endothelium-dependent and -independent vascular functions were determined by *in vitro* vascular reactivity measurements on isolated thoracic aortic rings of the rats as described detailed below.

## II. Hemodynamic measurements

Rats were anesthetized with thiopentone sodium (60 mg/kg i.p.), tracheotomized, intubated and artificially ventilated. Animals were placed on controlled heating pads and core temperature measured via a rectal probe was maintained at 37 °C. The thoracic cavity was opened to permit access to the apex of the heart. All incisions were kept to a minimum to avoid major blood loss. The left ventricle was punctured by a 20 G plastic cannula, through which a 2 F microtip pressure-volume catheter was inserted into the left ventricular cavity. Mean arterial pressure was measured via the right femoral artery. After stabilization for 5 minutes, the signals were continuously recorded using a pressure-volume conductance system, stored and displayed on a computer by the IOX Software System. With the help of a special blood pressure analysis program mean arterial pressure (MAP), maximal left ventricular systolic pressure (LVSP), left ventricular end-diastolic pressure (LVEDP), developed

pressure (DP), mean left ventricular systolic (MSP) and diastolic pressure (MDP), maximal slope of systolic pressure increment (+dP/dt) and diastolic decrement (-dP/dt), time constant of left ventricular pressure decay (Tau) were computed and calculated. Additionally, left ventricular pressure-volume relations were measured by transiently compressing the inferior vena cava. The slope ( $E_{max}$ ) of the left ventricular end-systolic pressure-volume relationships (ESPVR), preload recruitable stroke work (PRSW) and maximal slope of systolic pressure increment – end-diastolic volume relation (+dP/dt-EDV) were calculated as load-independent indexes of left ventricular contractility.

### **III. *In vitro* organ bath experiments for vascular reactivity**

The descending thoracic aorta was carefully removed from the anesthetized animals and placed in cold (+4 °C), oxygenized Krebs-Henseleit solution. The aortae were prepared and cleaned from periadventitial fat and surrounding connective tissue and cut transversely into 4-mm width rings using an operation microscope. Special attention was paid during the preparation to avoid damaging the endothelium.

Isolated aortic rings were mounted on stainless steel hooks in individual organ baths containing 25 ml of Krebs-Henseleit solution at 37 °C and aerated with 95 % O<sub>2</sub> and 5 % CO<sub>2</sub>. Isometric contractions were recorded using isometric force transducers digitized, stored and displayed with the IOX Software System. The aortic rings were placed under a resting tension of 2 g and equilibrated for 60 minutes. During this period, tension was periodically adjusted to the desired level and the Krebs-Henseleit solution was changed every 30 minutes. Maximal contraction forces to potassium chloride (KCl, 100 mM) were determined and aortic rings were washed until resting tension was again obtained. Phenylephrine (PE, 10<sup>-6</sup> M) was used to precontract the rings until a stable plateau was reached, and relaxation responses were examined by adding cumulative concentrations of endothelium-dependent dilator acetylcholine (ACh, 10<sup>-9</sup>-10<sup>-4</sup> M) and endothelium-independent dilator sodium nitroprusside (SNP, 10<sup>-10</sup>-10<sup>-5</sup> M). Contractile responses are expressed as grams of tension, relaxation is expressed as percent of contraction induced by phenylephrine (10<sup>-6</sup> M).

### **IV. Histology**

#### **Immunohistochemical analysis and terminal deoxynucleotidyl transferase-mediated dUTP nick end-labeling (TUNEL) reaction**

Myocardial and aortic samples were fixed in buffered paraformaldehyde solution (4 %) and embedded in paraffin. Three adjacent sections were processed for each of the following types of

immunohistochemical labelling. According to the methods previously described we performed immunohistochemical staining for nitrotyrosine (NT, product of the nitrating effect of peroxynitrite; a marker of nitrosative stress in general, and as “footprint of peroxynitrite” obvious evidence for *in vivo* peroxynitrite generation in particular), for poly(ADP-ribose) (PAR, the enzymatic product of PARP), for apoptosis inducing factor (AIF) and for endothelial nitric oxide synthase (eNOS).

TUNEL assay was performed for detection of DNA strand breaks. The detection was carried out using a commercial kit following the protocol provided by the manufacturer.

### **Quantification of immunohistochemistry and TUNEL**

Semiquantitative histomorphological assessment was performed on all of the stained specimens of the H<sub>2</sub>O<sub>2</sub> and FP15 projects in a blinded fashion using conventional microscopy and the COLIM software package. The results were expressed with a scoring system based on staining intensity and on the amount of positive stained cells.

In the case of apoptosis inducing factor staining the total number of positively stained endothelial cell nuclei was obtained in each section, and an average value was calculated for each experimental group.

For assessment of TUNEL-labelled cells, the number of positive cell nuclei/ microscopic examination field (250x magnification) were counted (four fields characterizing each specimen), and an average value was calculated for each experimental group.

### **V. Statistical analysis**

All data are expressed as means  $\pm$  S.E.M.. Intergroup comparisons were performed by using one-way analysis of variance followed by Student's unpaired t-test with Bonferroni's correction for multiple comparisons. Differences were considered significant when  $p < 0.05$ .

### **VI. Drugs**

Hydrogen peroxide solution was diluted with distilled water. Phenylephrine, acetylcholine and sodium nitroprusside were dissolved in normal saline; FP15 (FeCl tetrakis-2-(triethylene glycol monomethyl ether) pyridyl porphyrin) and INO-1001, the potent indeno-isoquinolinone-based poly(ADP)-ribose polymerase inhibitor were dissolved in 5 % glucose solution.

## Results

### I. Vascular dysfunction induced by H<sub>2</sub>O<sub>2</sub> *in vitro* – effects of PARP-inhibition

#### TUNEL and immunohistochemical analysis

Using the TUNEL assay we found pronounced DNA-damage in the aortic wall in the H<sub>2</sub>O<sub>2</sub> groups as reflected also by the quantitative assessment of TUNEL-positive cells. Pretreatment with INO-1001 tended to decrease H<sub>2</sub>O<sub>2</sub>-induced DNA strand breaks. As expected, the control and the INO-1001 control group showed essentially no TUNEL-positivity.

A marked degree of PARP activation was observed in the aortic wall sections of the H<sub>2</sub>O<sub>2</sub>-groups - when compared to controls - , as evidenced by higher PAR scores. In the case of 200 μM H<sub>2</sub>O<sub>2</sub> we found a tendency towards increased PAR-immunoreactivity, which reached statistical significance in the 400 μM H<sub>2</sub>O<sub>2</sub>-group. Pretreatment with the potent PARP-inhibitor INO-1001 resulted in significantly reduced formation of PAR in the aortic rings exposed to 400 μM H<sub>2</sub>O<sub>2</sub>, while it had no effect on control rings.

An altered pattern was found in the localization of AIF in the intima of the H<sub>2</sub>O<sub>2</sub> groups, whereby a diffuse (mitochondrial) localization of AIF converted into a nuclear localization, consistently with mitochondrial-to-nuclear translocation of this factor. H<sub>2</sub>O<sub>2</sub>-exposure notably increased the number of AIF positive aortic endothelial cell nuclei, which was significantly decreased in the H<sub>2</sub>O<sub>2</sub>-INO-1001 group.

#### Vascular function

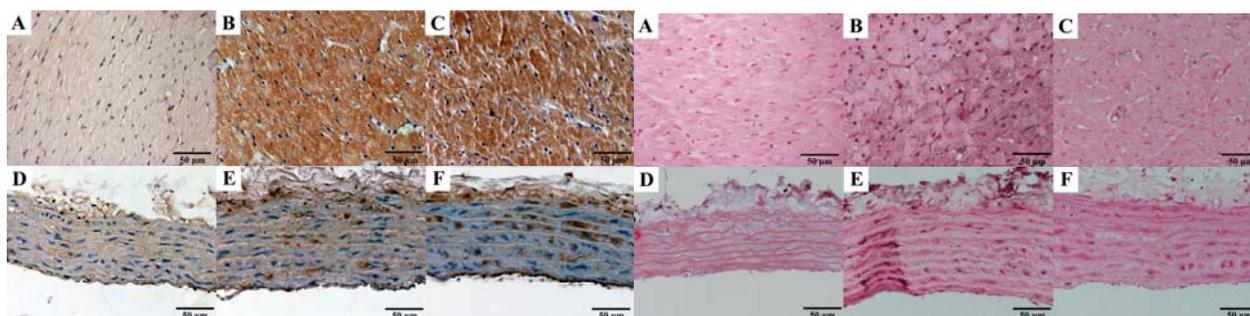
A dose-dependent impairment of endothelial function caused by H<sub>2</sub>O<sub>2</sub> was demonstrated in the *in vitro* organ bath experiments. The endothelial dysfunction induced by the reactive oxidant H<sub>2</sub>O<sub>2</sub> was indicated by the reduced maximal relaxation of isolated aortic rings to acetylcholine (86.2 ± 1.6 % control vs. 72.6 ± 2.0 % 200 μM H<sub>2</sub>O<sub>2</sub> vs. 66.9 ± 2.0 % 400 μM H<sub>2</sub>O<sub>2</sub>, P<0.05), and the H<sub>2</sub>O<sub>2</sub>-dose-dependent rightward shift of the dose-response curve as compared to the control group. The endothelium-independent vascular smooth muscle function indicated by the vasorelaxation of aortic rings to sodium nitroprusside was not impaired by H<sub>2</sub>O<sub>2</sub>. PARP-inhibition by INO-1001 significantly enhanced the acetylcholine-induced, endothelium-dependent, nitric oxide mediated vasorelaxation after exposure with 400 μM H<sub>2</sub>O<sub>2</sub> (maximal relaxation: 77.8 ± 3.0 % 400 μM H<sub>2</sub>O<sub>2</sub> + INO-1001 vs. 66.9 ± 2.0 % 400 μM H<sub>2</sub>O<sub>2</sub>, P<0.05),

indicating improved endothelial function. The same pretreatment had no effect on the endothelium-independent vasorelaxation of aortic rings to sodium nitroprusside. In the INO-1001 control group we found no alterations in both of the acetylcholine- and sodium nitroprusside-induced vasorelaxation when compared to control, INO-1001 did not directly influence the endothelium-dependent and –independent vasorelaxation of aortic rings.

## II. Ageing-associated cardiovascular dysfunction – effects of PARP-inhibition

### Immunohistochemical analysis

Immunohistochemical staining showed increased immunoreactivity for NT and PAR - indicative of nitrosative stress and enhanced activation of PARP - in the left ventricular myocardium and in the aortic wall (mainly in the endothelium) of ageing rats. Single dose treatment with the INO-1001 notably decreased PAR formation both in the myocardium and the aortic wall. NT-immunoreactivity was not affected by acute PARP-inhibition. (Fig. 1.)



**Figure 1. Photomicrographs of nitrotyrosine and poly(ADP-ribose) immunohistochemistry**

Representative immunohistochemical stainings for nitrotyrosine (left side, brown staining) and poly(ADP-ribose) (right side, dark brown/black staining mainly in cell nuclei) in the myocardium (upper panels) and aortic wall (lower panel). Young control group: A, D; ageing control group: B, E; and ageing INO-1001 treatment groups: C, F (magnification: 400X, scale bar: 50 µm).

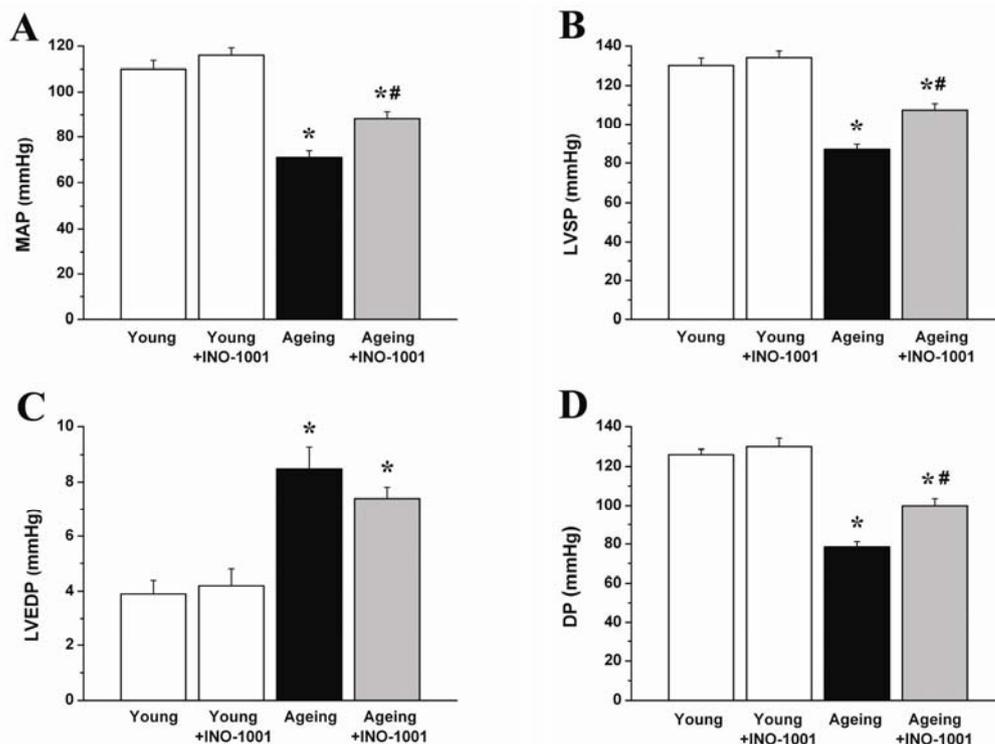
### Vascular function

An impairment of endothelial function in ageing rats was demonstrated in the organ bath experiments. The ageing-associated endothelial dysfunction was indicated by the reduced maximal relaxation of isolated aortic rings to ACh ( $61.2 \pm 2.2$  % ageing control vs.  $80.8 \pm 2.0$  % young control,  $P < 0.05$ ), and the rightward shift of the dose-response curve as compared with the young control group. Single dose treatment with PARP-inhibitor INO-1001 significantly improved the ACh-induced, endothelium-dependent, nitric oxide mediated vasorelaxation in

ageing animals (maximal relaxation:  $69.1 \pm 2.3$  % ageing treatment group vs.  $61.2 \pm 2.2$  % ageing control,  $P < 0.05$ ). The same treatment had no effect in young rats. The endothelium-independent vascular smooth muscle function indicated by the vasorelaxation of aortic rings to SNP was not impaired in ageing rats and was also unaffected by acute INO-1001 treatment. Maximal isometric forces produced by the isolated aortic rings precontracted by KCl (100 mM) and PE ( $10^{-6}$  M) were significantly lower in the ageing control group as compared with young animals, which was not influenced by acute PARP-inhibition.

### Cardiac function

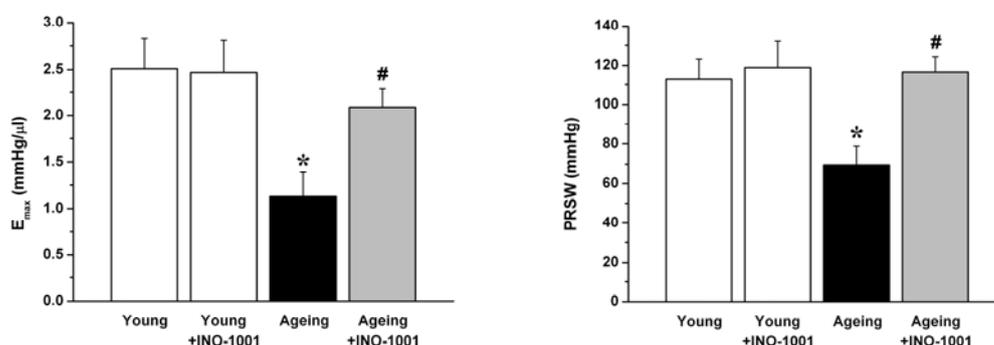
In the ageing control group we found decreased mean arterial pressure (MAP), maximal left ventricular systolic pressure (LVSP), developed pressure (DP) and increased left ventricular end-diastolic pressure (LVEDP). Single dose treatment with INO-1001 in ageing rats significantly improved the hemodynamic parameters MAP, LVSP and DP. (Fig. 2.)



**Figure 2. The effect of ageing and acute PARP-inhibition on arterial and left ventricular blood pressure**

Mean arterial pressure (MAP), maximal left ventricular systolic pressure (LVSP), left ventricular end-diastolic pressure (LVEDP) and developed pressure (DP) are shown in young adult, young treated with INO-1001, ageing and ageing treated with INO-1001 male rats. Values are mean  $\pm$  S.E.M. of 7 experiments in each group. \*,  $P < 0.05$  versus young control; #,  $P < 0.05$  versus ageing control.

Ageing in rats was associated with significantly decreased left ventricular contractility. The load independent, PV-loop derived contractility indexes showed a marked reduction in ageing animals when compared to young controls ( $E_{\max}$ :  $1.13 \pm 0.26$  vs.  $2.51 \pm 0.32$  mmHg/ $\mu$ l; PRSW:  $69.6 \pm 9.2$  vs.  $112.7 \pm 10.6$  mmHg;  $+dP/dt$ -EDV:  $16.73 \pm 2.70$  vs.  $51.9 \pm 8.70$  mmHg/s/ $\mu$ l;  $P < 0.05$ ). After acute PARP-inhibition, we observed significantly increased  $E_{\max}$  and PRSW in ageing rats, ( $E_{\max}$ :  $2.09 \pm 0.49$  vs.  $1.13 \pm 0.26$  mmHg/ $\mu$ l; PRSW:  $116.7 \pm 7.8$  vs.  $69.6 \pm 9.2$  mmHg;  $P < 0.05$ ) indicating the rapid improvement in left ventricular contractility (Fig. 3.). Treatment with INO-1001 in young rats had no effect on any of the hemodynamic parameters studied.



**Figure 3. The effect of ageing and acute PARP-inhibition on cardiac contractility**

The slope ( $E_{\max}$ ) of the left ventricular end- systolic pressure-volume relationships (ESPVR) and preload recruitable stroke work (PRSW) are shown in young adult, young treated with INO-1001, ageing and ageing treated with INO-1001 male rats. Values are mean  $\pm$  S.E.M. of 7 experiments in each group. \*,  $P < 0.05$  versus young control; #,  $P < 0.05$  versus ageing control.

### III. Ageing-associated cardiovascular dysfunction – effects of catalytic peroxynitrite-decomposition

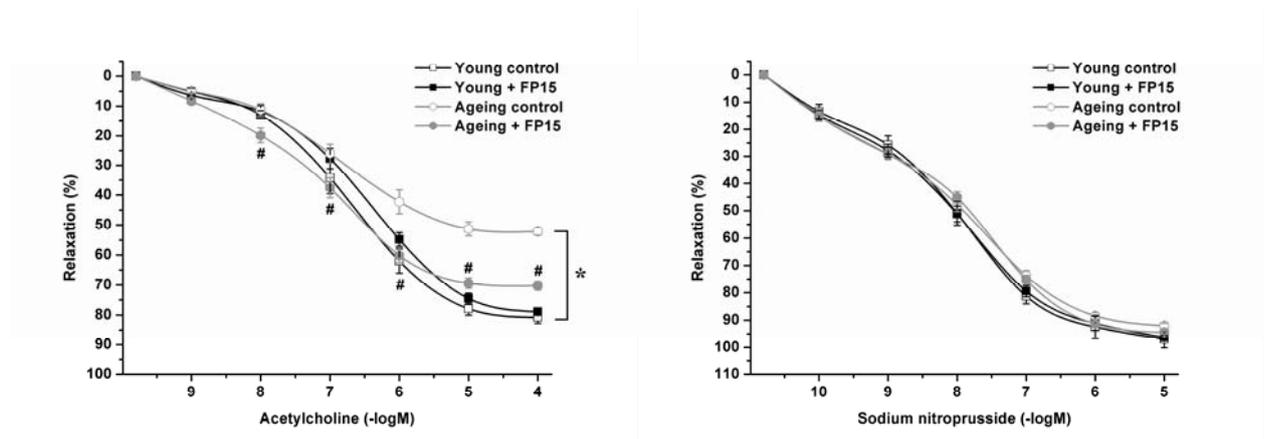
#### Immunohistochemical analysis

Significant immunoreactivity for NT and a marked degree of PARP activation were observed in the aortic wall sections of ageing rats, as evidenced by higher NT and PAR scores, when compared with young animals. Treatment with the peroxynitrite decomposition catalyst FP15 in ageing rats significantly reduced NT immunoreactivity and PAR formation in the aortic intima. Immunohistochemical staining for AIF showed no significant alteration in the localization of this factor in any groups studied. Immunohistochemical score of eNOS was

significantly increased in the aortic endothelium in ageing animals, and was slightly (not significantly) reduced after FP15 treatment.

### Vascular function

Similar to previous studies, the impairment of endothelial function in ageing rats was demonstrated on the thoracic aorta. The ageing-associated endothelial dysfunction was indicated by the reduced maximal relaxation of isolated aortic rings to ACh ( $52.1 \pm 1.3\%$  ageing control vs.  $80.8 \pm 1.5\%$  young control,  $P < 0.05$ ), and the rightward shift of the dose-response curve as compared with the young control group. (Fig. 4.). Treatment with FP15 for 3 weeks significantly improved the ACh-induced, endothelium-dependent, nitric oxide mediated vasorelaxation in ageing animals (maximal relaxation:  $70.3 \pm 1.5\%$  ageing treatment group vs.  $52.1 \pm 1.3\%$  ageing control,  $P < 0.05$ ). The same treatment had no effect in young rats. The endothelium-independent vascular smooth muscle function indicated by the vasorelaxation of aortic rings to SNP was not impaired in ageing rats and was also unaffected by FP15 treatment (Fig. 4.). Maximal isometric forces produced by the isolated aortic rings precontracted by PE ( $10^{-6}$  M) were significantly lower in the ageing control group as compared with young animals, and there were enhanced maximal contraction forces in the ageing FP15 treatment group.

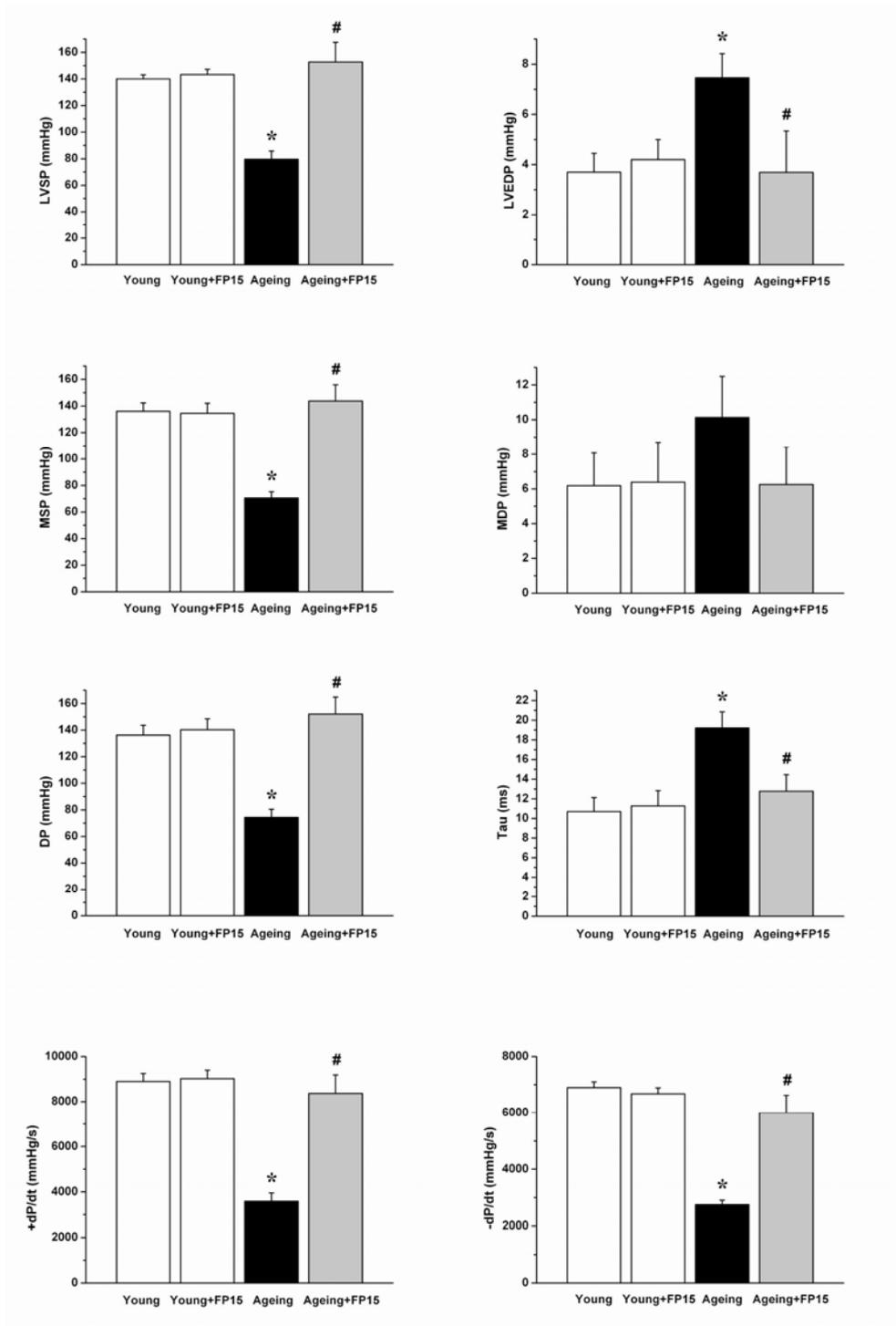


**Figure 4. Reversal of ageing-induced vascular dysfunction by treatment with FP15 in rat aortic rings**

ACh-induced endothelium-dependent relaxation (left side), and SNP-induced endothelium-independent relaxation (right side). Each point of the curve represents mean  $\pm$  S.E.M. of 18-22 experiments in thoracic aortic rings from all 6 animals of all groups. \*,  $P < 0.05$  versus young control; #,  $P < 0.05$  versus ageing control.

### **Cardiac function**

Ageing in rats was associated with significantly decreased maximal left ventricular systolic pressure (LVSP), developed pressure (DP), mean systolic pressure (MSP), maximal slope of systolic pressure increment (+dP/dt) and diastolic decrement (-dP/dt). In contrast, left ventricular end-diastolic pressure (LVEDP) and the time constant of left ventricular pressure decay (Tau) were increased in ageing animals, indicative of diastolic dysfunction. Mean diastolic pressure (MDP) was not significantly altered. (Fig. 5.) Treatment with the peroxynitrite decomposition catalyst FP15 in ageing rats significantly improved the systolic hemodynamic parameters LVSP, DP, MSP, +dP/dt and the diastolic indexes -dP/dt and Tau. (Fig. 5.) Mean arterial pressure (MAP) was decreased in ageing animals ( $77.2 \pm 4.7$  mmHg vs.  $136.1 \pm 4.1$  mmHg in young control rats), and it was significantly improved after FP15 treatment ( $146.3 \pm 16.6$  mmHg,  $P < 0.05$ ). In contrast, in young rats, FP15 had no effect on any of the hemodynamic parameters studied. (Fig. 5.)



**Figure 5. The effect of ageing and FP15 on cardiac function**

Maximal left ventricular systolic pressure (LVSP), left ventricular end-diastolic pressure (LVEDP), developed pressure (DP), mean left ventricular systolic (MSP) and diastolic pressure (MDP), maximal slope of systolic pressure increment (+dP/dt) and diastolic decrement (-dP/dt) and time constant of left ventricular pressure decay (Tau) are shown in young adult, young treated with FP15, ageing and ageing treated with FP15 male rats. Values are mean  $\pm$  S.E.M. of 6 experiments in each group. \*,  $P < 0.05$  versus young control; #,  $P < 0.05$  versus ageing control.

## Conclusions

In the first *in vitro* study we investigated the oxidative injury and impairment of vascular responsiveness induced by hydrogen peroxide in the isolated rat aorta. We explored the pathophysiological role of the H<sub>2</sub>O<sub>2</sub> - DNA-injury - poly(ADP-ribose) polymerase pathway in this impairment and demonstrated favourable effects of pharmacological PARP-inhibition on H<sub>2</sub>O<sub>2</sub>-induced endothelial dysfunction. The current data further support the notion that PARP inhibition may represent a potential therapy approach to reduce vascular dysfunction induced by oxidative stress in several pathophysiological conditions, e.g. in cardiovascular dysfunction associated with advanced ageing.

This is the first study reporting rapid improvement of myocardial and endothelial functions in the *in vivo* rat model of ageing-associated cardiovascular dysfunction by acute inhibition of the PARP enzyme and by catalytic decomposition of peroxynitrite. The current functional and immunohistochemical findings indicate the importance of the endogenous peroxynitrite overproduction and the activation of the peroxynitrite - PARP - pathway, especially its quickly reversible energy depleting aspects in the pathogenesis of myocardial and endothelial dysfunction at old age.

The current work supports the concept that pharmacological PARP-inhibition and/or rapid catalytic peroxynitrite decomposition may represent novel potential therapy approaches to improve cardiovascular dysfunction associated with advanced ageing.

## List of publications

Publications related to the dissertation:

**Radovits T**, Lin LN, Zotkina J, Gerő D, Szabó C, Karck M, Szabó G.

Poly(ADP-ribose) polymerase inhibition improves endothelial dysfunction induced by reactive oxidant hydrogen peroxide in vitro

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