

The peroxynitrite decomposition catalyst FP15 improves ageing-associated cardiac and vascular dysfunction

Tamás Radovits^a, Leila Seres^a, Domokos Gerő^b, Li-ni Lin^a,
Carsten J. Beller^a, Song-He Chen^a, Julia Zotkina^a, Irina Berger^c,
John T. Groves^d, Csaba Szabó^b, Gábor Szabó^{a,*}

^a Department of Cardiac Surgery, University of Heidelberg, INF 326 OG 2, 69120 Heidelberg, Germany

^b Department of Human Physiology and Experimental Research, Semmelweis University, Üllői út 78/A, 1082 Budapest, Hungary

^c Department of Pathology, University of Heidelberg, INF 220/221, 69120 Heidelberg, Germany

^d Department of Chemistry, Princeton University, Princeton, NJ 08544, USA

Received 21 July 2006; received in revised form 11 September 2006; accepted 18 September 2006

Available online 20 November 2006

Abstract

Overproduction of oxidants and free radicals in ageing tissues induces nitro-oxidative stress, which has recently been implicated in the pathogenesis of cardiovascular dysfunction associated with ageing. Peroxynitrite, a strong cytotoxic oxidant damages proteins and DNA and activates several pathways causing tissue injury, including the peroxynitrite–poly(ADP-ribose) polymerase (PARP) pathway. In this study, we investigated the effectiveness of the peroxynitrite decomposition catalyst FP15 on ageing-associated cardiac and vascular dysfunction.

Young and ageing rats were treated with vehicle or FP15 intraperitoneally. Using a microtip Millar pressure catheter we performed left ventricular blood pressure analysis to assess systolic and diastolic function. Endothelium-dependent and -independent vasorelaxation of isolated aortic rings were investigated by using acetylcholine and sodium nitroprusside.

Ageing animals showed a marked reduction of systolic and diastolic cardiac function and loss of endothelium-dependent relaxant responsiveness of aortic rings. FP15-treatment significantly improved cardiac performance and endothelial function. Immunohistochemical staining confirmed that FP15 effectively reduced nitrosative stress and prevented the activation of PARP in the aortic wall of ageing rats.

Our results demonstrate the importance of endogenous peroxynitrite-overproduction in the pathogenesis of ageing-associated cardiovascular dysfunction. Pharmacological decomposition of peroxynitrite by FP15 may represent a novel therapeutic utility to improve cardiac and vascular dysfunction associated with ageing.

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Keywords: Ageing; Nitro-oxidative stress; Peroxynitrite; Ventricular function; Endothelial function

1. Introduction

There is growing evidence that ageing is an important risk factor in the development of ischaemic heart disease. This may be due to an age-associated increase in coronary vascular resistance leading to reduced myocardial blood supply and flow reserve (Hachamovitch et al., 1989). Numerous studies suggest that ageing is associated with impaired function of endothelium in

laboratory animals (Tschudi et al., 1996) and humans (Egashira et al., 1993) and this endothelial dysfunction makes the ageing population prone to cardiovascular complications and microthrombus formation. Recent studies demonstrate that the cardiovascular dysfunction associated with advanced ageing is related to the local formation of reactive oxygen and nitrogen species in the myocardium and coronary vasculature (Bejma et al., 2000; van der Loo et al., 2000; Csiszar et al., 2002).

Ageing organisms are exposed to continuous oxidative injury, due to the higher rate of superoxide and other free radical production from the mitochondrial electron-transport chain (Sohal and Sohal, 1991). Increases in reactive oxidant species (ROS) and other oxidants at old age can elicit oxidative

* Corresponding author at: Laboratory of Cardiac Surgery, Department of Cardiac Surgery, University of Heidelberg, INF 326 OG 2, 69120 Heidelberg, Germany. Tel.: +49 6221 566246; fax: +49 6221 564571.

E-mail address: dzsi@hotmail.com (G. Szabó).

modifications of various cell components, such as lipid, protein and particularly DNA (de la Asuncion et al., 1996).

A potent oxidant species, peroxynitrite (ONOO^-) is formed by the reaction of superoxide anion ($\text{O}_2^{\bullet-}$) and nitric oxide (NO) and has been established as a pathophysiological relevant endogenous trigger of DNA single-strand breakage. Peroxynitrite-induced DNA damage leads to the activation of nuclear enzyme poly(ADP-ribose) polymerase (PARP), which initiates an energy-consuming metabolic cycle by transferring ADP-ribose units from NAD^+ to nuclear proteins. This process results in rapid depletion of intracellular ATP-pools and impaired mitochondrial respiration, eventually leading to cellular dysfunction and death (Virag and Szabo, 2002). Pharmacological inhibition of PARP in a rodent model has been demonstrated to improve cardiovascular dysfunction associated with ageing (Pacher et al., 2004b) showing the involvement of this pathway in the pathophysiology of cardiac and vascular ageing.

Peroxynitrite is also known to impair vasoregulatory functions. Peroxynitrite formation ($\text{O}_2^{\bullet-} + \text{NO}$) strongly reduces NO-bioavailability by scavenging the vasorelaxant nitric oxide, while nitration of tyrosine by peroxynitrite inactivates among others the prostacyclin synthase enzyme, the manganese superoxide dismutase, src-kinases, mitochondrial complex I, sarcoplasmic reticular Ca-ATPase; all resulting in vascular dysregulation (van der Loo et al., 2000).

Pharmacological attempts against 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. Recent studies with pharmacological inhibition of PARP (Pacher et al., 2004b; Szabo et al., 2003, 2004b; Soriano et al., 2001a) or decomposition of peroxynitrite (Szabo et al., 2002; Pacher et al., 2003), which block the peroxynitrite–DNA injury–poly(ADP-ribose) polymerase pathway emerge as novel antioxidant therapeutic possibilities in multiple pathophysiological conditions.

Peroxynitrite reacts very rapidly and efficiently with synthetic metalloporphyrins. One of them, FP15 an N-PEGylated-2-pyridyl iron porphyrin has shown a superior performance as a peroxynitrite decomposition catalyst (Szabo et al., 2002).

In order to examine whether cardiac and vascular dysfunction at old age can be beneficially affected by rapid pharmacological decomposition of peroxynitrite, in this study we investigated the effects of the novel potent peroxynitrite decomposition catalyst FP15 on nitro-oxidative stress, left ventricular performance and the vascular function of isolated aortic rings in a rat model of ageing-associated cardiovascular dysfunction.

2. Materials and methods

2.1. Animals, treatment protocols

The investigation conforms with the *Guide for the Care and Use of Laboratory Animals* published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996). All procedures and handling of animals

during the investigations were reviewed and approved by the Ethical Committee of the Land Baden-Württemberg for Animal Experimentation.

Young adult (3-month-old, 230–250 g) and ageing (24-month-old, 320–370 g) male dark agouti (DA) rats (Harlan Winkelmann, Germany) were housed in a room at a constant temperature of $22 \pm 2^\circ\text{C}$ with 12 h light/dark cycles and fed a standard laboratory rat diet and water ad libitum.

Ageing rats were treated with vehicle (ageing control group, $n = 6$), or the peroxynitrite decomposition catalyst FP15 intraperitoneally (i.p.) for 3 weeks ($0.1 \text{ mg kg}^{-1} \text{ day}^{-1}$, a dose found to be effective in previous studies (Pacher et al., 2003) (ageing treatment group, $n = 6$). Young rats treated for the same time with vehicle (young control group, $n = 6$), or same dosed FP15 (young treatment group, $n = 6$) were used as controls.

2.2. Hemodynamic measurements

Rats were anesthetized with thiopentone sodium (60 mg kg^{-1} 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 20G plastic cannula, through which a 2F Millar-microtip pressure catheter (SPR-838, Millar Instruments, Houston, TX, USA) was inserted into the left ventricular cavity. Mean arterial pressure was measured via the right femoral artery. After stabilization for 5 min, the pressure signals were continuously recorded using an A/D converter (EMKA Technologies, Paris, France) at a sampling rate of 1000 s^{-1} , stored and displayed on a computer by the IOX Software System (EMKA Technologies, Paris, France). With the help of a special blood pressure analysis program (EMKA Technologies, Paris, France) 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.

2.3. In vitro organ bath experiments

After the hemodynamic measurements, the descending thoracic aorta was carefully removed from the open-chest animals and placed in cold ($+4^\circ\text{C}$) Krebs–Henseleit solution (118 mM NaCl, 4.7 mM KCl, 1.2 mM KH_2PO_4 , 1.2 mM MgSO_4 , 1.77 mM CaCl_2 , 25 mM NaHCO_3 , 11.4 mM glucose; pH 7.4). The aorta were prepared and cleaned from periadventitial fat and surrounding connective tissue and cut transversely into 4 mm width rings ($n = 3$ or 4 from each animal) using an operation microscope.

Isolated aortic rings were mounted on stainless steel hooks in individual organ baths (Radnoti Glass Technology, Monrovia, CA, USA), containing 25 ml of Krebs–Henseleit solution at 37°C and aerated with 95% O_2 and 5% CO_2 . Special attention was paid during the preparation to avoid damaging the endothelium.

Isometric contractions were recorded using isometric force transducers (Radnoti Glass Technology, Monrovia, CA, USA), digitized, stored and displayed with the IOX Software System (EMKA Technologies, Paris, France).

The aortic rings were placed under a resting tension of 2 g and equilibrated for 60 min. Phenylephrine (10^{-6} 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^{-9} to 10^{-4} M) and endothelium-independent dilator sodium nitroprusside (SNP, 10^{-10} to 10^{-5} M). Contractile responses are expressed as grams of tension, relaxation is expressed as percent of contraction induced by phenylephrine (10^{-6} M).

2.4. Immunohistochemical analysis

After the preparation of the descending thoracic aorta, one aortic ring from each animal was fixed in buffered paraformaldehyde solution (4%). Three adjacent sections were processed for each of the following types of immunohistochemical labelling.

According to the methods previously described (Liaudet et al., 2000), 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 (van der Loo et al., 2000; Halliwell, 1997), and for poly(ADP-ribose) (PAR, the enzymatic product of PARP). Primary antibodies used for the stainings were polyclonal sheep anti-nitrotyrosine antibody (OXIS, Portland, OR, USA) and mouse monoclonal anti-poly(ADP-ribose) antibody (Calbiochem, San Diego, CA, USA). For immunohistochemical detection of apoptosis inducing factor (AIF) we applied the procedure described previously (Xiao et al., 2004) using rabbit polyclonal anti-AIF antibody (Chemicon International, Temecula, CA, USA). To detect endothelial nitric oxide synthase (eNOS), a routine immunohistochemical procedure was applied using the avidin biotin method (Gross et al., 2005). Primary antibody was rabbit polyclonal anti-eNOS antibody (Dinova, Hamburg, Germany).

Quantitative histomorphological assessment was performed by the COLIM software package (Pictron, Budapest, Hungary) based on the intensity and distribution of labelling. The results were expressed with a scoring system. On the base of densitometrically measured intensity the colour classes were coupled with intensity score values as follows: 0 = no positive staining; 1–3 = increasing degrees of intermediate staining; 4 = extensive staining. The program automatically measured the area of the stained objects, assigned an area score (1 = up to 10% positive cells; 2 = 11–50% positive cells; 3 = 51–80% positive cells; 4 = >80% positive cells), and calculated an average score (0–12) for the whole picture (intensity score multiplied by area score).

2.5. 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 $p < 0.05$.

2.6. Drugs

Phenylephrine, acetylcholine and sodium nitroprusside (Sigma–Aldrich, Germany) were dissolved in normal saline, FP15 (FeCl tetrakis-2-(triethylene glycol monomethyl ether) pyridyl porphyrin) was dissolved in 5% glucose solution.

3. Experimental results

3.1. Immunohistochemical analysis

As shown in Fig. 1, significant immunoreactivity for nitrotyrosine 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 potent peroxynitrite decomposition catalyst FP15 in ageing rats significantly reduced nitrotyrosine immunoreactivity and PAR formation in the aortic intima (Fig. 1). Fig. 2 shows representative stainings for NT and PAR in the ageing control and FP15-treatment groups.

Immunohistochemical staining for AIF showed no significant alteration in the localization of this factor in any groups studied (Fig. 1).

Immunohistochemical score of eNOS was significantly increased in the aortic endothelium in ageing animals, and was slightly (not significantly) reduced after FP15-treatment (Fig. 3).

3.2. Vascular function

Similar to previous studies, the impairment of endothelial function in ageing rats was demonstrated in our in vitro organ bath experiments. The ageing-associated endothelial dysfunction was indicated by the reduced maximal relaxation of isolated aortic rings to ACh ($52.1 \pm 1.27\%$ ageing control versus $80.8 \pm 1.49\%$ young control, $p < 0.05$), and the rightward shift of the dose-response curve as compared with the young control group. (Fig. 4B). Treatment with FP15 for 3 weeks significantly improved the ACh-induced, endothelium-

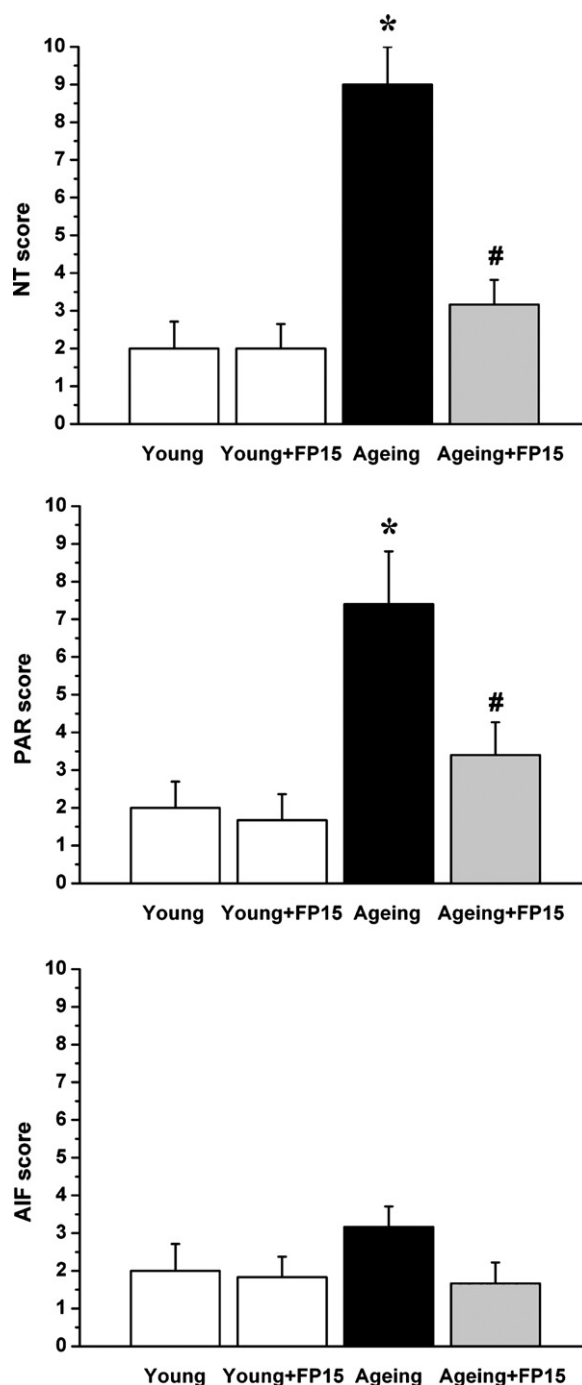


Fig. 1. Scoring of NT-, PAR- and AIF-immunohistochemistry. Immunohistochemical scores for nitrotyrosine (NT), poly(ADP-ribose) (PAR), and nuclear apoptosis-inducing factor (AIF) in the intima of aortic wall in young, young treated with FP15, ageing and ageing treated with FP15 male rats. * $p < 0.05$ vs. young control; # $p < 0.05$ vs. ageing control.

dependent, nitric oxide mediated vasorelaxation in ageing animals (maximal relaxation: $70.3 \pm 1.49\%$ ageing treatment group versus $52.1 \pm 1.27\%$ ageing control, $p < 0.05$). The same treatment had no effect in young rats (Fig. 4B).

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. 4C).

Maximal isometric forces produced by the isolated aortic rings precontracted by phenylephrine (10^{-6} M) were significantly lower in the ageing

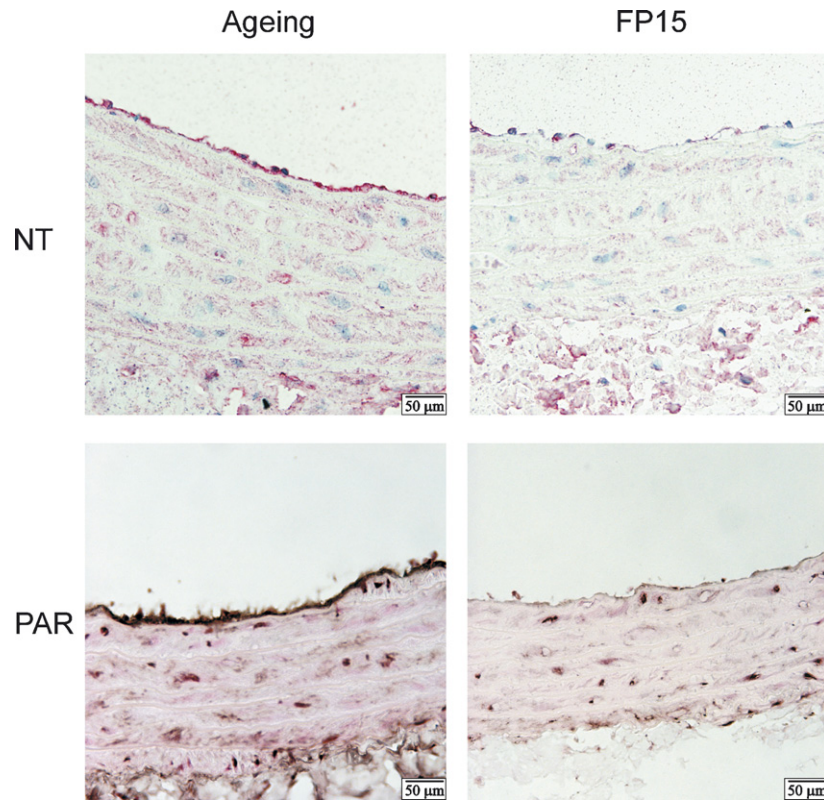


Fig. 2. Photomicrographs of NT- and PAR-immunohistochemistry. Representative immunohistochemical stainings for nitrotyrosine (NT) and poly(ADP-ribose) (PAR) in the ageing control, and ageing FP15-treatment groups. Scale bar: 50 μm .

control group as compared with young animals, and there were enhanced maximal contraction forces in the ageing FP15-treatment group (Fig. 4A).

3.3. 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 (77.24 ± 4.66 mm Hg versus 136.11 ± 4.05 mm Hg in young control rats), and it was significantly improved after FP15-treatment (146.31 ± 16.61 mm Hg, $p < 0.05$).

In contrast, in young rats, FP15 had no effect on any of the hemodynamic parameters studied (Fig. 5).

4. Discussion and conclusions

In the current study, we demonstrate that pharmacological decomposition of peroxynitrite with the novel potent metalloporphyrin peroxynitrite decomposition catalyst FP15 remarkably reduces nitrosative stress and inhibits the peroxynitrite–PARP pathway, resulting in improved systolic and diastolic cardiac function and enhanced endothelium-dependent vasorelaxation in a rat model of ageing-associated cardiovascular dysfunction.

Recent studies elucidated numerous cellular and molecular mechanisms responsible for the functional decline of the cardiovascular system at old age (Csiszar et al., 2005). The oxidative stress hypothesis (or free radical theory, as it was originally proposed) (Harman, 1956) is currently one of the most favoured explanations for how ageing leads to progressive cellular damage at the biochemical level. According to this theory, age-related loss of physiological function and ageing is caused by the deleterious effects of progressive and irreversible accumulation of oxidative damage. Several previous studies have demonstrated that ageing organisms have a higher rate of mitochondrial free radical production ($\text{O}_2^{\bullet-}$ and H_2O_2) due to the incomplete terminal oxidation at older age (Sohal and Sohal, 1991; Nohl et al., 1978; Kim et al., 1996).

Large amounts of the superoxide anions, which are produced in ageing tissues interact with the physiological mediator nitric oxide, forming the potent oxidant peroxynitrite ($\text{O}_2^{\bullet-} + \text{NO} \rightarrow \text{ONOO}^-$) (Halliwell, 1997). 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 (van der Loo et al., 2000), and causing 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 (Virag and Szabo, 2002).

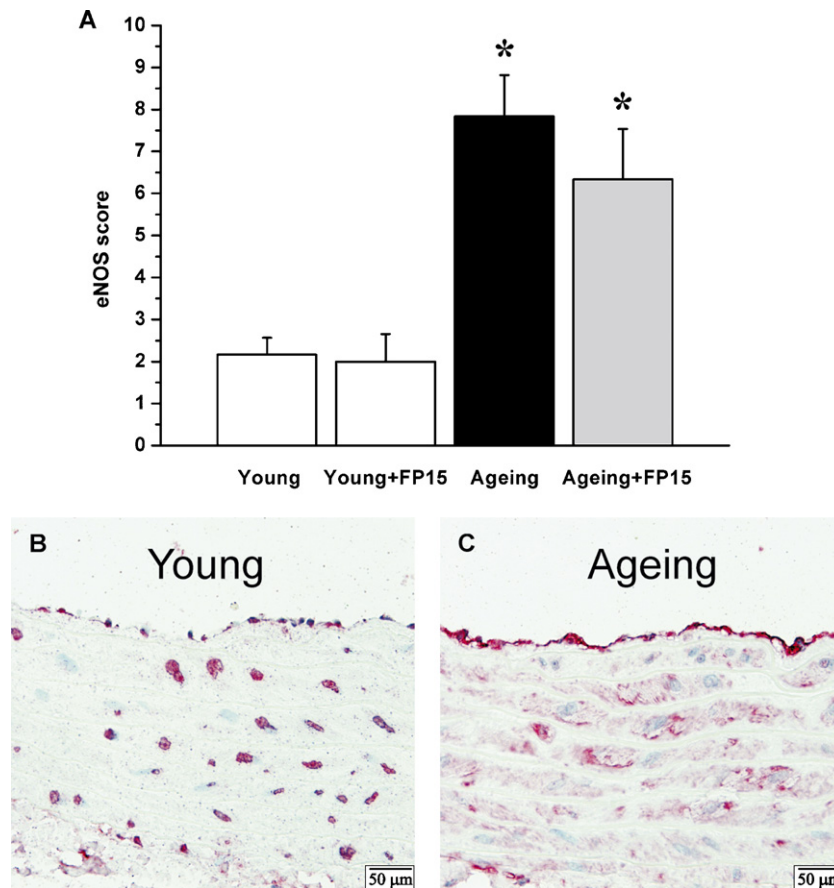


Fig. 3. eNOS-immunohistochemistry. Immunohistochemical scores for endothelial nitric oxide synthase (eNOS) in the aortic intima of young, FP15-treated young, ageing and FP15-treated ageing rats (A), and representative immunohistochemical stainings for eNOS in young (B) and ageing control animals (C). * $p < 0.05$ vs. young control. Scale bar: 50 μm .

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 NAD^+ to nuclear proteins. The excessive nuclear poly(ADP-ribose) formation results in rapid depletion of intracellular 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 (Soriano et al., 2001b). 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 (Soriano et al., 2001b; Szabo et al., 1997, 2004a; Pacher et al., 2002a,b,d,e; Szabo and Bahrle, 2005). Impairment of endothelial function in the coronary arteries may lead to regional or global myocardial ischaemia, which secondarily impairs cardiac performance (Pacher et al., 2004b).

Recent work demonstrated that certain cellular effectors of DNA fragmentation could also be activated by PARP. According to these results, PARP regulates the mitochon-

drial-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 (Xiao et al., 2004).

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 (Pacher et al., 2002b,e; Capasso et al., 1990).

The increased peroxynitrite formation and PARP activation together with the above-discussed downstream molecular and intracellular mechanisms are considered to play an important role in the pathogenesis of various forms of chronic heart failure (Pacher et al., 2002c, 2005, 2006).

We demonstrated increased immunoreactivity for nitrotyrosine and activation of PARP in the aortic intima of ageing rats (Fig. 1), which confirm the nitrosative stress and the activation of the peroxynitrite–poly(ADP-ribose) polymerase pathway at two characteristic levels, and are consistent with previous studies (van der Loo et al., 2000; Csiszar et al., 2002, 2005) and with the above-discussed “free radical theory” of ageing. However, our immunohistochemical data for AIF showed only a tendency towards more intense nuclear staining (indicative of mitochondrial-to-nuclear translocation of this factor) in ageing rats without reaching the level of significance.

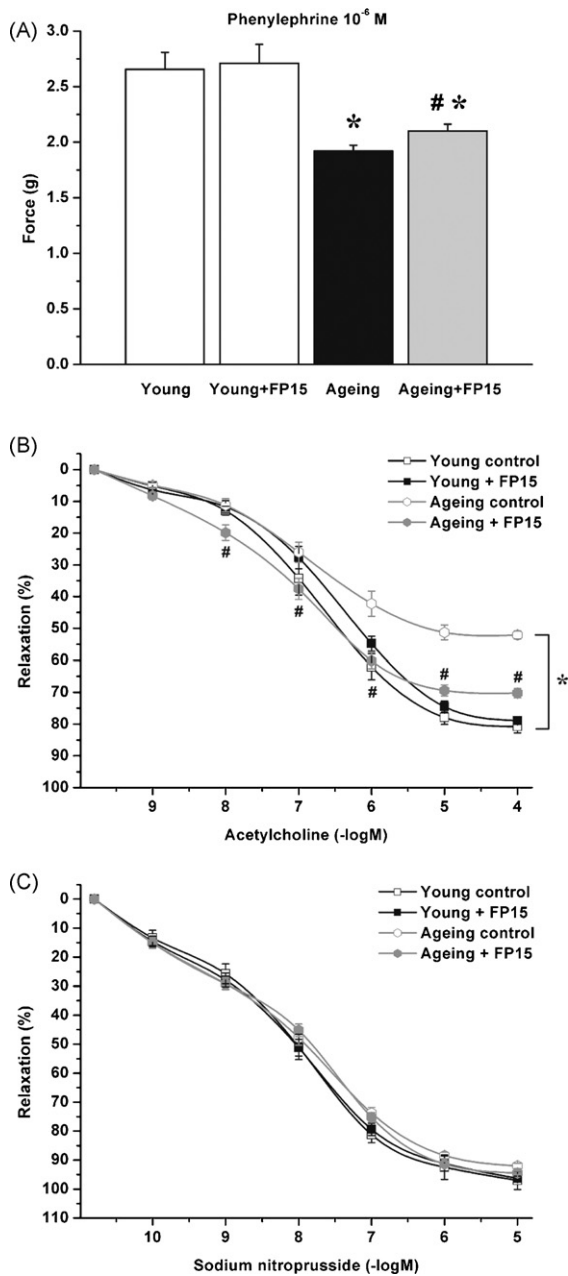


Fig. 4. Reversal of ageing-induced vascular dysfunction by treatment with FP15 in rat aortic rings. Contraction forces induced by phenylephrine (10^{-6} M) (A); ACh-induced endothelium-dependent relaxation (B); SNP-induced endothelium-independent relaxation (C). Each point of the curve represents mean \pm S.E.M. of 18–22 experiments in thoracic aortic rings from all six animals of all groups. * $p < 0.05$ vs. young control; # $p < 0.05$ vs. ageing control.

After treatment with the peroxynitrite decomposition catalyst FP15 in ageing rats we found significantly reduced nitrotyrosine and PAR formation in the aortic intima (Fig. 2). Similar to other studies with PARP-inhibitors (Xiao et al., 2004; Pacher et al., 2002f), our immunohistochemical data after FP15-treatment indicate the inhibition of the peroxynitrite–PARP pathway, the main downstream mechanism of nitro-oxidative stress. Consistent with our current study, WW85 (another peroxynitrite decomposition catalyst) has also been shown to prevent PARP activation in a different experimental

model of cardiovascular injury (heart transplantation and rejection) (Pieper et al., 2005).

In ageing vessels, the nitro-oxidative damage of the vascular smooth muscle layers were found to be less pronounced, when compared to the endothelium. These immunohistochemical findings are in line with recent works, which demonstrate that the vascular superoxide-overproduction at old age occurs mainly in the endothelial cells (van der Loo et al., 2000), which are in addition more vulnerable to oxidative injury.

Endothelial dysfunction associated with advanced ageing is a well-known phenomenon: the underlying intracellular pathways and molecular mechanisms have been subject of intensive investigations (van der Loo et al., 2000; Pacher et al., 2004b, 2002e,f). In accordance with these studies we report here impaired endothelium-dependent acetylcholine-induced relaxation of isolated aortic rings of ageing rats. The endothelium-independent relaxation induced by the exogenously administered NO-donor SNP was unaffected by ageing, indicating the normal dilative capacity of the vascular smooth muscle. These functional data are consistent with our immunohistochemical findings showing signs of severe nitrosative stress mainly in the endothelium of ageing vessels. In contrast with a previous work using epinephrine for precontraction (Pacher et al., 2002e), we found a significant decrease in contraction forces induced by phenylephrine in ageing animals which was in line with the results of another study investigating vascular function of diabetic rats (Soriano et al., 2001a) and may be due to alterations in receptor density and/or receptor/effector coupling.

Similarly to the effects of pharmacological PARP inhibitors (Pacher et al., 2004b, 2002e,f), FP15-treatment significantly enhanced the endothelium-dependent vasorelaxations (i.e. improved the endothelial function in rats with advanced ageing), but did not affect the normal vasorelaxation to SNP or the vascular function of young rats (Fig. 4).

The endothelial dysfunction in ageing can be explained by the reduced ability of endothelial cells to produce nitric oxide when stimulated by an endothelium-dependent relaxant agonist, such as acetylcholine (Soriano et al., 2001b). Interestingly, we found significantly enhanced immunoreactivity for endothelial nitric oxide synthase (eNOS) in the aortic endothelium of ageing rats (Fig. 3), suggesting intensive NO-generation. Thus, we hypothesize that the reduced endothelium-dependent relaxation associated with ageing is not due to the downregulation of NO-production of endothelial cells, but rather to the increased NO-inactivation by superoxide anions resulting in altered NO bioavailability, as NO is removed in the reaction of peroxynitrite formation ($O_2^{\bullet-} + NO \rightarrow ONOO^-$). The paradoxical increase in eNOS expression and activity (demonstrated also by another recent study) may serve as an insufficient compensatory mechanism attempting to counteract the increased inactivation of NO (van der Loo et al., 2000).

Previous studies performing invasive hemodynamic measurements in ageing rats report decreased cardiac performance and development of progressive heart failure after the age of 20 months (Pacher et al., 2004a,b; Anversa et al., 1989). A recent study provided detailed echocardiographic evidence of a progressive decrement in multiple aspects of systolic and

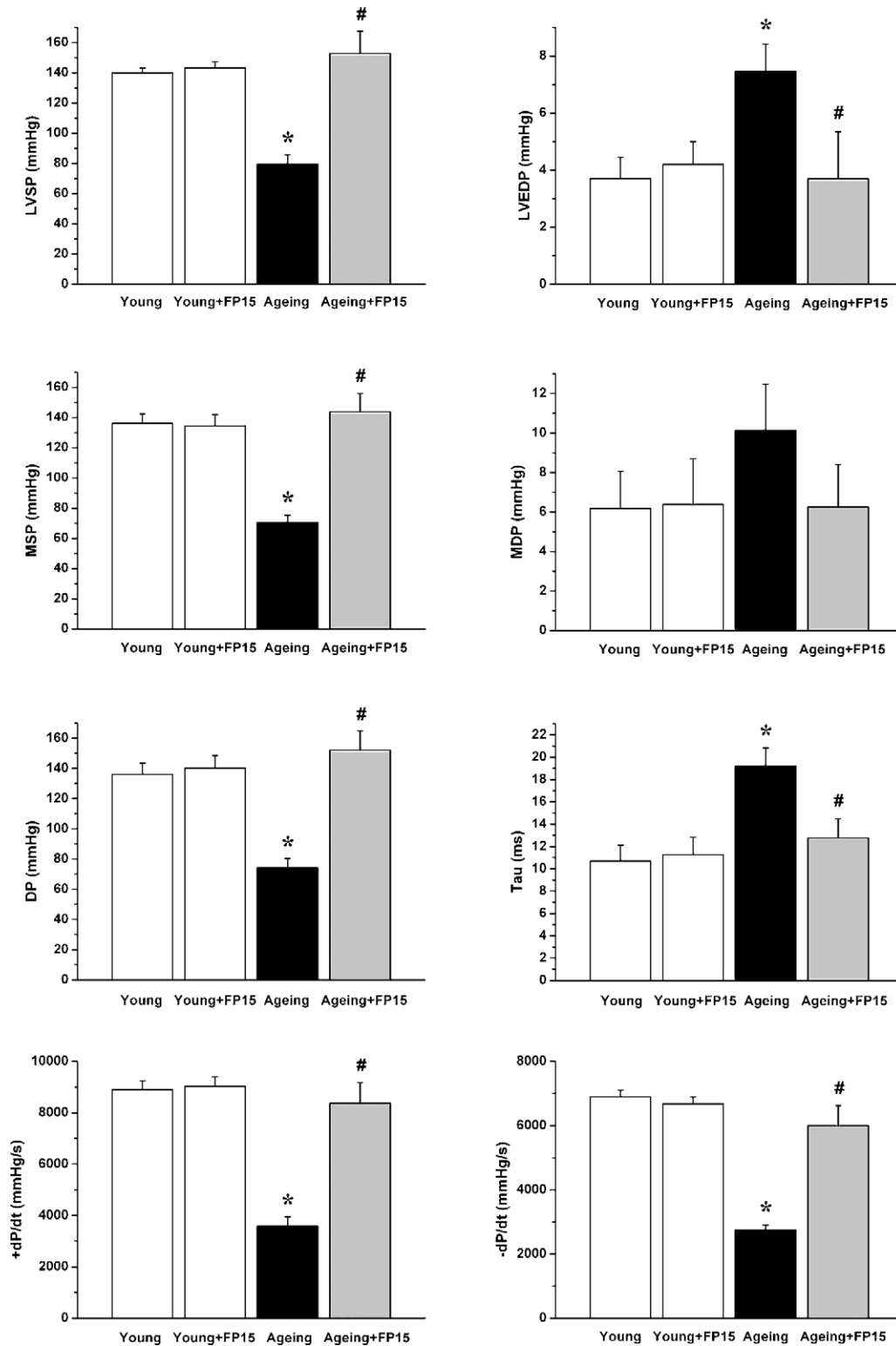


Fig. 5. The effect of ageing and FP15 on cardiac function. Maximal left ventricular systolic pressure (LVSP), left ventricular end-diastolic pressure (LVEDP), developed pressure (DP), mean left ventricular systolic (MSP) and diastolic pressure (MDP), maximal slope of systolic pressure increment ($+dP/dt$) 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 six experiments in each group. * $p < 0.05$ vs. young control; # $p < 0.05$ vs. ageing control.

diastolic LV function in ageing rats (Boluyt et al., 2004). Consistent with these results, we demonstrated that advanced ageing is associated with impaired cardiac relaxation and diastolic dysfunction, as reflected by prolonged time constant

of pressure decay (Tau), increased LVEDP and decreased $-dP/dt$, and a marked depression of systolic pressure development, as indicated by decreased MAP, LVSP and depressed contractility index $+dP/dt$ (maximal slope of systolic pressure

increment), a widely used cardiac contractile parameter. $+dP/dt$ is independent from ventricular volume and heart size and therefore it is especially informative in assessing cardiac contractility in models, where the size of the heart is altered (in the rat the size of the heart is age-dependent).

Pharmacological decomposition of peroxynitrite with FP15 ameliorated both systolic and diastolic cardiac function in ageing animals (Fig. 5), as indicated by the improvement of all hemodynamic parameters measured in our study. Our data are also consistent with the results of a recent study investigating the beneficial effects of the PARP-inhibitor INO-1001 in age-related cardiac dysfunction (Pacher et al., 2004b). Two other reports with the PARP-inhibitor PJ34, however, demonstrated improvement in only the diastolic (but not systolic) cardiac function (Pacher et al., 2002e,f).

Regarding the improved cardiac function, rapid pharmacological decomposition of peroxynitrite by FP15 in our model seems to be comparable to or better than the efficacy of blocking the pathway by PARP-inhibition. By eliminating peroxynitrite nitro-oxidative stress can be reduced (as shown by reduced nitrotyrosine formation) and severe damage of DNA can be effectively prevented. Additionally, using this concept we can avoid peroxynitrite-induced modifications of enzymes, receptors and structural proteins and may help to restore the normal bioavailability of the crucial physiological mediator nitric oxide (as confirmed by the enhanced endothelium-dependent vasorelaxation).

Based on the data presented in the current report, we propose that pharmacological decomposition of peroxynitrite may represent a potential therapy approach to improve cardiovascular dysfunction associated with ageing.

5. Limitations

In this study, we investigated the effects of pharmacological peroxynitrite decomposition in a rat model of ageing-associated cardiovascular dysfunction. In respect of human cardiovascular ageing, the major limitation of this rodent model is that arterial blood pressure – as reported also by other recent studies (Pacher et al., 2004a,b) – falls with age, the opposite of what generally happens in human beings.

This phenomenon, and the found increase in blood pressure after FP15 therapy in this rat model should be kept in mind, and further preclinical studies are necessary to elucidate the possible clinical benefit of this novel therapeutical approach.

Acknowledgements

This work was supported by a Grant from the German Research Foundation (SFB 414) to GS and by the Hungarian Research Fund (OTKA AT049488) to CS.

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