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# Effect of acute and chronic zofenopril administration on cardiac gene expression

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**Abstract** We investigated whether acute and chronic administration of zofenopril, an angiotensin converting enzyme inhibitor, may modulate the expression of genes which are involved in the pathophysiology of myocardial ischemia and heart failure. We used an acute and a chronic model. In the former isolated rat hearts were perfused for 120 min in the presence or in the absence of 10 μM zofenoprilat, the active metabolite of zofenopril. In the chronic model one group of rats was treated with zofenopril (15.2 mg/Kg die per os) for 15 days, while control rats were treated with the same diet, except that zofenopril was omitted. Total RNA was extracted from hearts, and quantitative reverse transcription-polymerase chain reaction (qRT-PCR) was used to evaluate the expression of α myosin heavy chain, superoxide dismutase, heat shock protein 70 (HSP70), nitric oxide synthase 2 and 3 (NOS2, NOS3), heme oxygenase 1, atrial natriuretic peptide (ANP), muscle phosphofructokinase. Acute or chronic zofenopril administration did not produce any change in hemodynamic variables. qRT-PCR experiments showed that in the acute model ANP expression was slightly although not significantly increased. In the chronic model, significant changes in gene expression were detected: in particular, HSP70 was upregulated ($1.06 \pm 0.38$ vs. $0.72 \pm 0.20$ arbitrary units, $P = 0.025$), while NOS3 was downregulated ($0.66 \pm 0.06$ vs. $0.83 \pm 0.18$ arbitrary units, $P = 0.007$). In the chronic model, liver samples were also assayed, but no significant change in the expression of any gene was detected. We conclude that zofenopril can produce heart-specific effects on gene expression. Persistent changes were detected with regard to specific heat shock protein and nitric oxide synthase subtypes. This action might contribute to the therapeutical response, and particularly to the increased resistance to ischemia.

**Keywords** ACEI · Zofenopril · Cardiac remodeling · Ischemia · HSP70 · NOS3 · ANP

## Introduction

Angiotensin converting enzyme inhibitors (ACEIs) are recommended in the management of heart failure and arterial hypertension because of their cardioprotective effects, as demonstrated both in experimental investigation and clinical trials [1–3]. They have been shown to reduce cardiovascular morbidity and mortality in patients with left ventricular hypertrophy, heart failure, and post-myocardial infarction, and these therapeutical effects do not seem to be entirely related to inhibition of angiotensin II production.

A large body of evidence suggests that a crucial event of heart failure is a deep remodeling of gene expression: several genes were identified whose hypo or hyperexpression was quite constant in heart failure of different aetiologies (ischemic cardiopathy, dilated and hypertrophic cardiomyopathy) [4, 5]. Involved genes include structural genes, coding for components of sarcomere, cytoskeleton, and extracellular matrix (alpha and beta myosin heavy chain, actin, collagen-1α, gelsolin, α-actinin, vinculin),

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calcium homeostasis genes (sarcoendoplasmic reticulum calcium ATPase, calmodulin 1, calsequestrin 2), energy metabolism genes (mitochondrial NADH dehydrogenase, ATP synthase and succinate dehydrogenase; phosphofructokinase and phosphoglucomutase), and natriuretic peptide genes (ANP and BNP).

In addition, studies on the late phase of ischemic preconditioning and on other forms of preconditioning [6–8] suggest the existence of mechanisms that, acting at transcriptional level, induce the development of a phenotype which is more resistant to ischemia. Although the results derived from microarray methodology are quite complex, particular relevance is given to hyperexpression of genes coding for heat shock proteins (mainly HSP28 and HSP70), oxidative stress defense proteins (manganese superoxide dismutase MnSOD, heme oxygenase, thioredoxin), energy metabolism enzymes (mitochondrial ATPase, cytochrome oxidase, adenine nucleotide translocator 1), nitric oxide producing enzymes (nitric oxide synthases NOS2 and NOS3), growth factors (insulin grow factors I and II), and contractile proteins (alpha and beta myosin heavy chain, laminin). In the light of these new findings, the search for drugs capable of modulating gene expression could open interesting perspectives in cardiologic therapy.

Modulation of gene expression may represent a potential effect of some ACEI [9–11]. In particular, we have recently reported [12] that zofenopril can rapidly (2 h) and transiently reduce expression of the gene coding for phospholamban, a physiological inhibitor of sarcoendoplasmic reticulum calcium ATPase (SERCA), in a perfused rat heart model. Zofenopril has been specifically proposed as a cardioprotective agent because a direct anti-ischemic effect has been demonstrated in experimental studies [13–17], whereas the SMILE (Myocardial Infarction Long-Term Evaluation) clinical trial suggested that its beneficial effects in acute myocardial infarction are partly due to reduced infart size [18, 19].

Therefore, the aim of this study was to investigate if acute and chronic zofenopril administration can modulate the expression of genes which play a recognized role in the pathophysiology of ischemia and heart failure. In particular, we selected nine genes among those known to be modulated in late phase of ischemic preconditioning (see Table 1).

## Methods

### Animals treatment and perfusion technique

This investigation conforms to the Declaration of Helsinki and the Guiding Principles in the Care and Use of Animals. The project was approved by the Animal Care and Use committee of the University of Pisa.

**Table 1** Primers used for qRT-PCR

| Accession number | Gene | Primer pairs | | Amplicon length |
|---|---|---|---|---|
| NM_031971 | Heat shock 70kD protein 1A (HSP70) | Sense | 5′-CCGCTGTCGCTGGGTCTG-3′ | 111 bp |
| | | Anti-sense | 5′-GTTGTCCGAGTAGGTGGTGAAGG-3′ | |
| NM_017051.2 | Superoxide dismutase 2, mitochondrial (SOD2) | Sense | 5′-CCTGACCTGCCTTACGACTATGG-3′ | 160 bp |
| | | Anti-sense | 5′-CCTGAGTTGTAACATCTCCCTTGG-3′ | |
| NM_012611.2 | Nitric oxide synthase 2, inducible (NOS2) | Sense | 5′-GAGAAGTCCAGCCGCACCAC-3′ | 149 bp |
| | | Anti-sense | 5′-GAACAATCCACAACTCGCTCCAAG-3′ | |
| NM_021838.2 | Nitric oxide synthase 3, endothelial cell (NOS3) | Sense | 5′-GCCTGAGCAGCACAAGAGTTAC-3′ | 152 bp |
| | | Anti-sense | 5′-CCAGCCCAAACACACAGAACC-3′ | |
| NM_0317151 | Phosphofructokinase, muscle (PFKM) | Sense | 5′-ATCGCCGTGTTGACCTCTGG-3′ | 152 bp |
| | | Anti-sense | 5′-GCCTCCCTGATGTGCTCTCC-3′ | |
| NM_013190.4 | Phosphofructokinase, liver (PFKL) | Sense | 5′-CTACCGTGGACCTGGAGAAGTTG-3′ | 101 bp |
| | | Anti-sense | 5′-CCTGACAGCAGCATTCATACCTTG-3′ | |
| NM_017239.1 | Myosin heavy polypeptide 6, cardiac muscle, Alpha (MYH6) | Sense | 5′-CCTGAACCCAGCAGCCATCC-3′ | 170 bp |
| | | Anti-sense | 5′-GCCTCTCATCTCGCATCTCCTC-3′ | |
| NM_012612.1 | Natriuretic peptide precursor type A (ANP) | Sense | 5′-AGAGTGAGCCGAGACAGCAAAC-3′ | 189 bp |
| | | Anti-sense | 5′-CCAGGTGGTCTAGCAGGTTCTTG-3′ | |
| NM_012580 | Heme oxygenase (decycling) 1 (HMOX1) | Sense | 5′-CACTGCTGACAGAGGAACACAAAG-3′ | 196 bp |
| | | Anti-sense | 5′-ACTGCCACGGTCGCCAAC-3′ | |
| NM_012583 | Hypoxanthine phosphoribosyltransferase 1 (HPRT1) | Sense | 5′-CCCAGCGTCGTGATTAGTGATG-3′ | 126 bp |
| | | Anti-sense | 5′-TTCAGTCCTGTCCATAATCAGTCC-3′ | |

*Acute treatment*

13 male Wistar rats (275–300 g body weight), fed with standard diet, were anesthetized with a mixture of ether and air. After injection of 1000 U sodium heparin in the femoral vein, the heart was quickly excised and perfused according to the working heart technique, as described previously [12]. The standard perfusion buffer included (mM): NaCl 118, $NaHCO_3$ 25, KCl 4.5, $KH_2PO_4$ 1.2, $MgSO_4$ 1.2, $CaCl_2$ 1.5, and glucose 11. Perfusions were carried out using 200 ml of recirculating buffer, which was equilibrated with a mixture of $O_2$ (95%) and $CO_2$ (5%). Temperature was kept between 36.8 and 37°C, and the pH was 7.4.

The heart rate, aortic pressure, aortic flow, coronary flow, and cardiac output (which is the sum of aortic and coronary flow) were monitored for the whole duration of the perfusion. Powerlab 2000 (ADInstruments, Castle Hill, Australia) was used for data acquisition. After an equilibration period of 10 min, two different groups of hearts were perfused for 120 min according to the following protocols: (a) control perfusion: working heart perfusion using standard buffer (six hearts); (b) zofenopril: working heart perfusion using standard buffer with 10 μM zofenoprilat, the active metabolite of zofenopril (seven hearts).

*Chronic treatment*

Eight male Wistar rats assumed zofenopril at the dose of 15.2 mg/Kg/day. Eight control rats assumed the same diet without zofenopril addition, and their daily food consumption was virtually the same as observed in the zofenopril group. After 15 days of treatment, rats were sacrificed and hearts were excised and perfused with the working heart technique for 10 min to assess hemodynamic performance.

Gene expression analysis

Ventricular sections were obtained from freshly dissected hearts from rats of acute and chronic experiment. To assess the specificity of cardiac effects, fresh sections of liver were also dissected from rats subjected to chronic treatment. All tissues were immediately submerged in RNAProtect buffer (Sigma) for an overnight at 4°C. About 50–100 mg of tissue was then homogenized in TRIzol Reagent and RNA extracted following manufacturer protocol.

Elimination of genomic DNA contamination from RNA was obtained by incubating samples with 80 U of DNase I (Sigma), 1× DNase buffer (Sigma), and 20 U of RNase Out (Invitrogen) in 100 μl for 30 min at 25°C and purifying by PureLink Total RNA Purification System (Invitrogen).

1 μg of RNA samples was retrotranscribed with IScript cDNA Synthesis Kit (Bio-Rad) following manufacturer protocol. cDNAs were used for relative quantitative real-time PCR analysis on nine target genes and one housekeeping gene (see Table 1 for sequences, amplicons, and gene bank references). Reactions were performed on iCycler-IQ5 Real-Time PCR System (Bio-Rad) under the following conditions: 2 ng of cDNA, 200 nM primers, 10 μl SyberGreen Master Mix (Bio-Rad) in a total volume of 20 μl; the PCR protocol consistent on initial denaturation at 95° for 3 min and 40 cycles of denaturation at 95°C for 30 s/annealing-extension at 60°C for 30 s followed by a melting protocol of ramping temperature from 65 to 95°C with a 0.5°C increment for every 10 s. All samples, including negative controls, were run in duplicate.

Primers were constructed using Beacon Design software (Premier Biosoft) with a spanning intron strategy excepted for HSP70, a single exon gene. We then verified the absence of contaminating DNA in all the samples amplifying HSP70 by real-time PCR on RNA (2 ng) directly.

Amplification efficiencies were calculated on the basis of standard curves obtained with six 3-fold serial dilutions starting from 50 ng of cDNA.

Relative quantification analysis was performed after Pfaffl method [20]

$$\text{Ratio} = \left(E_{\text{target}}\right)^{\Delta \text{Ct,target}} / \left(E_{\text{ref}}\right)^{\Delta \text{Ct,ref}}$$

where $E_{\text{target}}$ and $E_{\text{ref}}$ are amplification efficiencies of target gene and housekeeping gene, respectively.

Statistical analysis

Results are expressed as mean ± standard error of the mean (SEM). Differences between pairs of groups were evaluated by unpaired $t$ test. The threshold of statistical significance was set at $P < 0.05$. GraphPad Prism version 4.1 for Windows (GraphPad Software, San Diego, CA) was used for data processing and statistical analysis.

## Results

In the acute experiments, baseline values of hemodynamic variables in control hearts averaged as follows: cardiac output 56.8 ± 1.2 ml/min, aortic flow 39.4 ± 0.8 ml/min, coronary flow 17.4 ± 0.9 ml/min, systolic aortic pressure 172 ± 6 mmHg, and heart rate 275 ± 17 beats/min. No significant difference was recorded after acute administration of 10 μM zofenoprilat, since the following average values were observed: cardiac output 53.2 ± 2.5 ml/min, aortic flow 35.9 ± 1.8 ml/min, coronary flow 17.3 ± 1.0 ml/min, systolic aortic pressure 172 ± 8 mmHg, and heart rate 278 ± 17 beats/min. In both groups contractile performance was stable over the whole duration of perfusion.

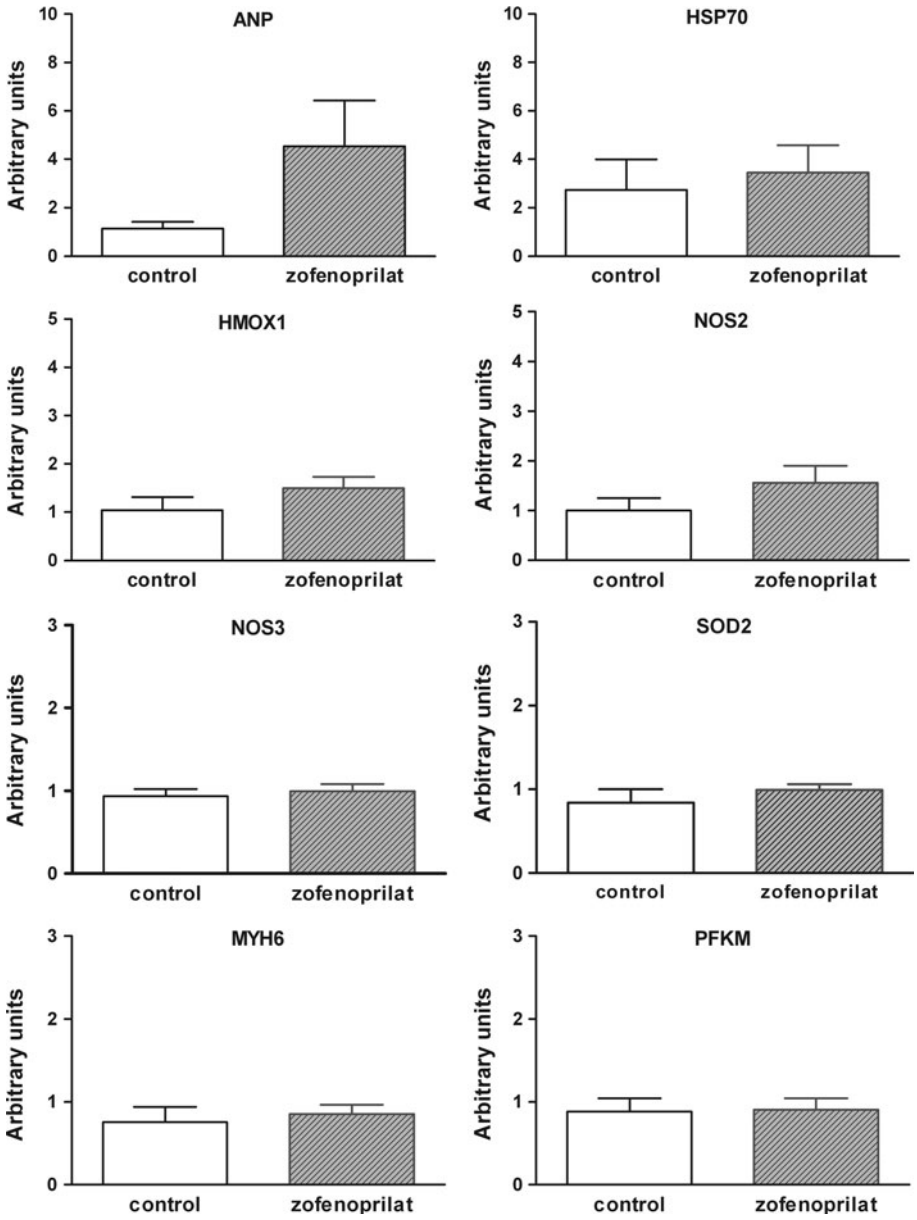


**Fig. 1** Gene expression analysis in rat hearts perfused for 120 min under control conditions (*open columns*) or in the presence of 10 μM zofenoprilat (*shadowed columns*). *Bars* represent mean ± SEM of 6–7 hearts per group. Gene expression was evaluated by quantitative RT-PCR and normalized to HPRT expression. Statistical analysis was performed by unpaired *t* test. See Table 1 for genes symbols-name correspondence

Gene expression studies in hearts derived from acute experiments (see Fig. 1) showed that ANP transcript levels were slightly increased in the zofenoprilat group but the variation did not reach the threshold of statistical significance ($P = 0.126$). In additional experiments, we observed that ANP expression was further and significantly increased if 50 nM nebivolol was associated to 10 μM zofenoprilat ($P = 0.002$, data not shown), while the expression of all other tested genes was still unchanged.

The results described above were obtained after 120 min exposure to a relatively high concentration of zofenoprilat. To determine whether gene expression could be modified also with chronic zofenopril administration at therapeutical dosages, rats were treated with 15.2 mg/Kg zofenopril for 15 days. Chronic treatment did not produce any significant contractile effect, since the hemodynamic variables recorded in excised hearts turned out to be similar in the zofenopril and in the control groups, namely: cardiac output 61.5 ± 0.9 versus 61.0 ± 0.6 ml/min, aortic flow 40.0 ± 0.9 versus 41.0 ± 0.4 ml/min, coronary flow 21.5 ± 1.0 versus 20.0 ± 0.3 ml/min, systolic aortic pressure 161 ± 5 versus 169 ± 1 mmHg, and heart rate 249 ± 5 versus 244 ± 4 beats/min. Such values were also similar to those relative to the acute treatment, showing that after 15 days treatment hearts were still healthy. This conclusion is further supported by the observation that housekeeping gene expression was also unchanged.

In hearts obtained from chronically treated rats, we observed a significant increase in the expression of the HSP70 gene (1.06 ± 0.38 arbitrary units vs. a control

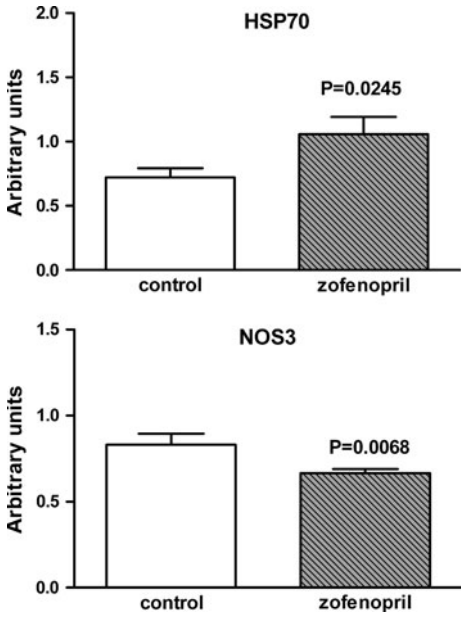


**Fig. 2** Gene expression analysis of hearts derived from control rats (*open columns*) or from rats which had been treated with 15.2 mg/Kg zofenopril for 15 days before sacrifice (*shadowed columns*). Only statistically significant results of HSP70 (upregulated: 1.06 ± 0.38 vs. 0.72 ± 0.20 arbitrary units) and NOS3 (downregulated: 0.66 ± 0.06 vs. 0.83 ± 0.18 arbitrary units) genes are shown. Quantitative PCR experiments were performed twice. *Bars* represent mean ± SEM of eight experiments in each group. Statistical analysis was performed by unpaired *t* test

value of 0.72 ± 0.20 arbitrary units, $P = 0.025$, see Fig. 2) and a significant decrease in the expression of the NOS3 gene (0.66 ± 0.06 arbitrary units vs. a control value of 0.83 ± 0.18 arbitrary units, $P = 0.007$). Notably, no significant change in the expression of the ANP gene was detected.

To assess whether the observed changes in gene expression were specific for cardiac tissue, liver samples were also assayed, and no significant change in the expression of any gene was detected (ANP, NOS2, and NOS3 expression levels in liver were actually below the sensibility threshold of the system).

## Discussion

In this study, we report that zofenopril produced genomic effects after chronic oral administration, while in the acute model zofenoprilat produced only a moderate effect, which did not reach the threshold of statistical significance. In the chronic model, where zofenopril administration (15.2 mg/Kg/day) was close to the therapeutical range [12], the expression of HSP70 and NOS3 was significantly increased (1.5-fold) and decreased (1.3-fold), respectively. These effects occurred in the absence of any haemodynamic effect and were heart-specific, since no change in gene expression variation was detected in liver samples. The time-dependence of such effects is not so obvious, as the two treatments differ not only in timing but also in the mode of zofenopril administration: in the acute treatment, the active metabolite of zofenopril was directly administered ex vivo to the isolated perfused heart, while in the chronic model zofenopril was administered in vivo within the diet. Therefore, the differences in the genomic effect could depend on zofenopril dosage, transport, or site of action.

The assay of protein expression will be necessary to estimate the potential relevance of our findings. However, it is interesting to point out that all the genes mentioned above may have remarkable importance in cardiac pathophysiology. HSP70 belongs to a family of highly conserved stress proteins (heat shock proteins), whose expression is induced by several environmental and intracellular stresses [21]. HSP70 is rapidly regulated under conditions of oxidative stress and has the ability to prevent protein aggregation and to assist with the refolding of denatured or partially folded proteins. HSP70 induction has been implicated in the late phase of ischemic preconditioning, where HSP70 synthesis may be triggered by signals such as reactive oxygen and nitrogen species, which in turn may activate protein kinase C (PKC) and various transcription factors. The cytoprotective properties of HSP70 have been related to inhibition of apoptosis pathways, to interaction with key proteins involved in the regulation of cellular redox balance (like Mn SOD), and to stabilization of SERCA with consequent prevention of cytosolic calcium overload. Although the increase of HSP70 expression observed in our rats after 15 days of zofenopril treatment is modest, it might contribute to the previously reported anti-ischemic effect of this ACEI [17]. Interestingly both the anti-ischemic effect of zofenopril observed in our previous experiments and the activation of HSP70 synthesis seem to be PKC dependent. HSP70 upregulation might also contribute to the increased SR calcium uptake observed in rat myocardium after chronic zofenopril administration [12].

NOS3 is the main NOS isoform in endothelium, and it is also expressed in cardiac myocytes [22]. NOS dysregulation in the early phase of ischemia/reperfusion, consequent to increased Ca2+ uptake in cardiomyocytes and endothelial cells, has been implicated in reperfusion injury. Under both physiological and pathological conditions, regulation of NOS3 occurs at the posttranscriptional level, through the interaction with $Ca^{++}$/Calmodulin or through protein phosphorylation. ACE inhibitors are known to interfere with bradykinin breakdown by ACE, resulting in increased bradykinin concentration and increased nitric oxide production by activation of bradikinin receptors (B1R and B2R) [23]. Enhancement of B2R signaling

activates endothelial NO synthase, yielding a short burst of NO; activation of B1Rs results in a prolonged high output of NO by inducible NO synthase.

NOS3 transcriptional upregulation has been demonstrated in response to shear stress and in late phase of ischemic preconditioning, where NOS3-derived NO plays a major role in protection, although excessive stimulation of NO synthesis may be detrimental [24].

The genomic effect which we have observed with zofenopril might be regarded as a compensatory mechanism establishing a sort of transcriptional feedback. A possible negative feedback mechanism regulating NOS3 expression has been identified by Zhang et al. [25] in a microRNA which seems to directly inhibit transcription.

Interestingly, this genomic effect appears to be specific for zofenopril. In fact, long-term studies on ACEI effect performed in infracted, hypertensive, and healthy rats showed that eNOS expression was activated in myocardium and in arterial endothelium after treatment with imidapril, ramipril, enalapril, and quinapril [11, 26, 27]. These results should be interpreted with great caution, due to differences in dosages and experimental models. Direct assay of NO levels will be necessary to settle this important issue.

A moderate effect of zofenopril on ANP expression was also observed. Interestingly, this effect was accentuated in the presence of beta-blocker nebivolol. Since zofenopril is often associated with beta-blockers in the treatment of myocardial ischemia and heart failure, our observation that ANP expression was further stimulated by their association might have therapeutical importance. ANP is an endogenous hormone released by the heart in response to atrial wall stretch or to neuro-hormone and immune stimuli, and plays important roles in cardiovascular and renal homeostasis [28]. In heart failure, the well known increment of plasma levels of ANP (and BNP) is considered to be a compensative mechanism because of the diuretic, natriuretic, and vasodilating actions of this peptide and its inhibitory effects on renin and aldosterone secretion. Other cardioprotective properties of natriuretic peptides in myocardial ischemia/reperfusion have also been reported from several studies [29]; therefore, acute induction of ANP gene expression may open new therapeutical opportunities in myocardial infarction and heart failure.

## Conclusions

In conclusion, we report that zofenopril can produce heart-specific effects on gene expression. Persistent changes were detected with regard to specific heat shock protein and nitric oxide synthase subtypes. This action might contribute to the therapeutical response, and particularly to the increased resistance to ischemia.

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## References

1. Maggioni AP (2006) Efficacy of angiotensin receptor blockers in cardiovascular disease. Cardiovasc Drugs Ther 20:295–308
2. Prisant LM (2008) Management of hypertension in patients with cardiac disease: use of renin-angiotensin blocking agents. Am J Med 121:S8–S15
3. Pfeffer MA, Frohlich ED (2006) Improvements in clinical outcomes with the use of angiotensin-converting enzyme inhibitors: cross-fertilization between clinical and basic investigation. Am J Physiol Heart Circ Physiol 291:H2021–H2025
4. Sanoudou D, Vafiadaki E, Arvanitis DA, Kranias E, Kontrogianni-Konstantopoulos A (2005) Array lessons from the heart: focus on genome and transcriptome of cardiomyopathies. Physiol Genomics 21:131–143
5. McKinsey TA, Olson EN (2005) Towards transcriptional therapies for the failing heart: chemical screens to modulate genes. J Clin Invest 115:538–546
6. Depre C, Tomlinson JE, Kudej RK, Gaussin V, Thompson E, Kim SJ, Vatner DE, Topper JN, Vatner SF (2001) Gene program for cell survival induced by transient ischemia in conscious pig. Proc Natl Acad Sci USA 98:9336–9341
7. Da Silva E, Lucchinetti E, Pasch T, Schaub MC, Zaugg M (2004) Ischemic but not pharmacological preconditioning elicits a gene expression profile similar to unprotected myocardium. Physiol Genomics 20:117–130
8. Das DK, Maulik N (2006) Cardiac genomic response following preconditioning stimulus. Cardiovasc Res 70:254–263
9. Okada M, Kikuzuki R, Harada T, Hori Y, Yamawaki H, Hara Y (2008) Captopril attenuates matrix metalloproteinase-2 and -9 in monocrotaline-induced right ventricular hypertrophy in rats. J Pharmacol Sci 108:487–494
10. Jin H, Yang R, Awad TA, Wang F, Li W, Williams SP, Ogasawara A, Shimada B, Williams PM, de Feo G, Paoni NF (2001) Effects of early angiotensin-converting enzyme inhibition on cardiac gene expression after acute myocardial infarction. Circulation 103:736–742
11. De Gennaro Colonna V, Rossoni G, Rigamonti AE, Bonomo S, Manfredi B, Berti F, Muller E (2002) Enalapril and quinapril improve endothelial vasodilator function and aortic eNOS gene expression in L-NAME-treated rats. Eur J Pharmacol 450:61–66
12. Frascarelli S, Carnicelli V, Ghelardoni S, Chiellini G, Ronca F, Zucchi R (2009) Effects of zofenopril on cardiac sarcoplasmic reticulum calcium handling. J Cardiovasc Pharmacol 54:456–463
13. Liu X, Engelman RM, Ronson JA, Cordis GA, Das DK (1992) Attenuation of myocardial reperfusion injury by sulfhydryl-containing angiotesin converting enzyme inhibitors. Cardiovasc Drugs Ther 6:437–443
14. Tio AR, de Langen CD, de Graeff PA, van Gilst WH, Bel KJ, Wolters KG, Mook PH, van Wijngaarden J, Wesseling H (1990) The effects of oral pretreatment with zofenopril, an angiotensin-converting enzyme inhibitor, on early reperfusion and subsequent electrophysiologic stability in the pig. Cardiovasc Drug Ther 4: 695–704
15. Przyklenk K, Kloner KA (1991) Angiotensin converting enzyme inhibitors improved contractile function of stunned myocardium by different mechanisms of action. Am Heart J 121:1319–1330
16. Ferrari R, Cargnoni A, Curello S, Ceconi C, Boraso A, Visioli O (1992) Protection of the ischemic myocardium by the converting-enzyme inhibitor zofenopril: insight into its mechanism of action. J Cardiovasc Pharmacol 20:694–704

17. Frascarelli S, Ghelardoni S, Ronca-Testoni S, Zucchi R (2004) Cardioprotective effect of zofenopril in perfused rat heart subjected to ischemia and reperfusion. J Cardiovasc Pharmacol 43:294–299
18. Ambrosioni E, Borghi C, Magnani B (1995) The effect of angiotensine-converting enzyme inhibitor zofenopril on mortality and morbidity after anterior myocardial infarction. The Survival of Myocardial Infarction Long-Term Evaluation (SMILE) Study Investigators. New Engl J Med 332:80–85
19. Borghi C, Ambrosioni E (2003) Double-blind comparison between zofenopril and lisinopril in patients with acute myocardial infarction: results of the Survival of Myocardial Infarction Long-term Evaluation-2 (SMILE-2) study. Am Heart J 145:80–87
20. Pfaffl MW (2001) A new mathematical model for relative quantification in real-time RT-PCR. Nucleic Acids Res 29: 2002–2007
21. Tupling AR, Bombardier E, Vigna C, Quadrilatero J, Fu M (2008) Interaction between Hsp70 and the SR $Ca^{2+}$ pump: a potential mechanism for cytoprotection in heart and skeletal muscle. Appl Physiol Nutr Metab 33(5):1023–1032
22. Darra E, Rungatscher A, Carcereri de Prati A, Podesser BK, Faggian G, Scarabelli T, Mazzucco A, Hallström S, Suzuki H (2010) Dual modulation of nitric oxide production in the heart during ischaemia/reperfusion injury and inflammation. Thromb Haemost 104(2):200–206
23. Erdös EG, Tan F, Skidgel RA (2010) Angiotensin I-converting enzyme inhibitors are allosteric enhancers of kinin B1 and B2 receptor function. Hypertension 55:214–220
24. Balligand JL, Feron O, Dessy C (2009) eNOS activation by physical forces: from short-term regulation of contraction to chronic remodeling of cardiovascular tissues. Physiol Rev 89: 481–534
25. Zhang MX, Ou H, Shen YH, Wang J, Wang J, Coselli J, Wang XL (2005) Regulation of endothelial nitric oxide synthase by small RNA. Proc Natl Acad Sci USA 102:16967–16972
26. Kobayashi N, Hara K, Watanabe S, Higashi T, Matsuoka H (2000) Effect of imidapril on myocardial remodeling in L-NAME-induced hypertensive rats is associated with gene expression of NOS and ACE mRNA. Am J Hypertens 13:199–207
27. Linz W, Jessen T, Becker RH, Schölkens BA, Wiemer G (1997) Long -term ACE inhibition doubles lifespan of hypertensive rats. Circulation 96(9):3164–3172
28. Dietz JR (2005) Mechanisms of atrial natriuretic peptide secretion from the atrium. Cardiovasc Res 68:8–17
29. Nishikimi T, Maeda N, Matsuoka H (2006) The role of natriuretic peptides in cardioprotection. Cardiovasc Res 69:318–328