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A low-dose atorvastatin and losartan combination directly improves aortic ring relaxation and diminishes ischaemic-reperfusion injury in isolated rat hearts

Authors' Contribution:
A Study Design
B Data Collection
C Statistical Analysis
D Data Interpretation
E Manuscript Preparation
F Literature Search
G Funds Collection

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Summary

Background: The cardiovascular pleiotropic effects of statins and angiotensin receptor blockers (ARBs) could be of interest for innovative preventive approaches. We aimed to investigate whether low-dose atorvastatin and losartan, separately not possessing protective cardiovascular pleiotropic effects, express them when combined.

Material/Methods: Forty-five adult male Wistar rats were anaesthetized and their thoracic aortas and hearts were isolated. Relaxation of aortic rings, coronary flow rate and the extent of myocardial ischaemic-reperfusion injury were measured. Different concentrations (0.01, 0.1, 1.0 μM) of atorvastatin and losartan added to a perfusion medium were first tested. The separate drugs, which were ineffective, were then combined at the same concentrations and the concentration was tested in the same model.

Results: Low concentrations of atorvastatin or losartan (0.1 and 1 μM, respectively) produced no effects in isolated aorta. However, surprisingly, when these drug concentrations were combined, a significantly improved endothelium-dependent relaxation of the thoracic aorta was observed. Similarly, when combining individually ineffective concentrations of atorvastatin or losartan (0.01 and 0.1 μM, respectively), significantly increased coronary flow and a decreased extent of myocardial injury were observed. By using a nitric oxide-synthase inhibitor, we demonstrated that the vasodilatory effects obtained were nitric oxide-dependent. The degree of effectiveness by the combination was comparable to that obtained by 10-fold (atorvastatin) or 100-fold (losartan) higher concentrations of the separate drugs.

Conclusions: Our results revealed that remarkable additive/synergistic effects exist between low-doses of a statin (atorvastatin) and an ARB (losartan), resulting in important cardiovascular protection. This new concept could be valuable in cardiovascular prevention.

key words: **atorvastatin • losartan • low-dose • combination • cardiovascular prevention • vasodilation**

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Background

Beyond their primary mode of action of reducing plasma cholesterol levels or lowering blood pressure, statins and inhibitors of the renin-angiotensin system are known to have independent beneficial, so-called pleiotropic effects [1–3]. These effects are exerted on the cardiovascular system, as well as other organ systems. Several studies have revealed that statins enhance the bioavailability of nitric oxide (NO) and consequently improve endothelial function. They also have anti-oxidative, anti-inflammatory, immunomodulatory and antithrombotic effects [4–7]. Similar effects were proved for inhibitors of the renin-angiotensin system (angiotensin receptor blockers [ARB] and angiotensin-converting enzyme inhibitors) which also improve endothelial function, further possessing anti-oxidative and anti-inflammatory properties [8–10].

While dose-response curves for the therapeutic effects (lipid lowering and blood pressure reducing, respectively) of the above-mentioned drugs are well-known, there are no firm data confirming associations between their doses and (different) pleiotropic effects. In clinical studies, pleiotropic effects were documented mostly at therapeutic doses. Nevertheless, pleiotropic effects also exist at lower doses than those necessary for achievement of their primary effects [11–14]. Furthermore, the dose-response curves for primary action and the dose-dependent curves for pleiotropic effects might not be similar. Moreover, different drugs that possess similar pleiotropic effects (but different primary effects) might act additively or even synergistically when combined.

In the present study we aimed to explore this issue, which might be of clinical value – whether a combination of low-dose atorvastatin and low-dose losartan (both at doses insufficient to produce any detectable effects when applied separately) is capable of inducing protective vascular effects on the isolated rat thoracic aorta and rat myocardial ischaemic-reperfusion injury model.

Material and Methods

Drugs and solutions

Krka Pharmaceuticals (Krka, d. d., Novo mesto, Slovenia) generously provided atorvastatin (Atorvastatin Calcium) and losartan (Losartan Potassium). They were diluted in distilled water to prepare solutions later used in experiments. Phenylephrine, acetylcholine and N(ω)-nitro-L-arginine (L-NNA) (all Sigma-Aldrich Chemie, Steinheim, Germany) were also dissolved in distilled water prior to their use.

All hearts were perfused with Krebs-Henseleit (K-H) solution for isolated hearts (composition in mM: 118.6 NaCl; 4.7 KCl; 11.1 glucose; 25 $NaHCO_3$; 1.66 $MgSO_4$; 1.2 NaH_2PO_4, and 2.52 $CaCl_2$) (all Merck Darmstadt, Germany). Isolated aortic rings were perfused with K-H solution for arterial perfusion of the following composition (in mM): NaCl 118, KCl 4.7, $CaCl_2$ 2.5, $NaHCO_3$ 25, $MgSO_4$ 1.2, KH_2PO_4 1.2, and glucose 11 (all Merck, Darmstadt, Germany).

Animals

Adult male Wistar rats (n=45) weighing 260–300 g were obtained from the Faculty of Medicine, Ljubljana. They were bred under constant housing conditions and fed with standard rat chow in the form of pellets (Altromin No. 1320, Lage, Germany); a regular circadian cycle lasting for 12 h was maintained. Five to seven animals were kept in each cage at a controlled environmental temperature. All animal procedures were conducted in accordance with the guidelines set by the Veterinary Administration of the Republic of Slovenia (permit No. 34401-23/2009/3).

Animal preparation and organ isolation

Rats were anaesthetized using an *i.p.* injection of Urethane (130 mg Urethane/100 mg body weight; Sigma-Aldrich, St. Louis, USA) mixed with heparin in a dosage of 1000 I.U. per animal (Krka, Novo mesto, Slovenia). Thoracotomy was then performed.

Isolated heart preparation

A cannula filled with cold K-H solution with the addition of heparin (2500 I.U./100 ml K-H) was introduced into the aorta above the semilunar valve, followed by heart isolation. The hearts were then mounted on a Langendorff apparatus and perfused with oxygenated K-H solution (95% O_2 + 5% CO_2; pH 7.4 at 37.5°C) under constant pressure. A pressure catheter (SPR-524, Millar, Houston, TX, USA) was introduced through the left atrium and mitral valve to the left ventricle. An electrocardiogram (ECG) was recorded from the surface of the heart by two silver electrodes (ITIS, Ljubljana, Slovenia), positioned in the direction of the heart's electrical axis and the signals were preamplified. Hearts were protected with a glass coat and Parafilm to maintain constant temperature (38.5°C) and humidity. The temperature of the experimental environment was kept at 23°C to 25°C at all times.

Isolated heart protocol

All hearts were randomized into several groups; each was composed of 5 hearts, and subjected to different perfusion protocols. Experiments lasted for 120 minutes. The control group was perfused with oxygenated K-H solution during the first 30 min (perfusion phase), followed by 40 min of global zero flow ischaemia with complete flow cessation of K-H solution to the isolated heart. After that, hearts were perfused with oxygenated K-H solution for 50 min (reperfusion period). In the test groups, hearts were perfused with oxygenated K-H for the first 20 min only, followed by 10 min perfusion of K-H solution with the added drugs atorvastatin or losartan in different concentrations of 0.01 μM, 0.1 μM or 1 μM, or a low-dose combination of atorvastatin (0.01 μM) and losartan (0.1 μM). Then 40 min of global zero flow ischaemia was applied, followed by 50 min of reperfusion with K-H solution with added drugs. One group of hearts was perfused with L-NNA (0.1 mM) alone for 10 minutes followed by the low-dose combination (0.01 μM atorvastatin and 0.1 μM losartan) added to the L-NNA (0.1 mM) until the end of the experiments (during the perfusion and reperfusion period).

Measured variables

In order to measure *coronary flow rate*, the coronary effluent was collected in a calibrated test tube at various time

intervals during the perfusion and reperfusion periods, recording the effluent volume. Coronary flow rate was expressed in mL/min. Effluents were further used for biochemical analysis of *lactate dehydrogenase* (LDH) release rate activity. LDH release rate activity was determined by the modified Wroblewski-LaDue method [15] and expressed in μkat g^{-1}·min^{-1}. *Heart rate* was obtained from oscillations detected in the electrocardiogram, aligned to ventricular pressure values and expressed in beats per min. *Left ventricular pressure,* expressed as the difference between systolic and diastolic pressures, was measured continuously with a Millar pressure catheter-transducer as described previously [16].

Rat thoracic aorta isolation

The thoracic aorta was isolated, rinsed of blood, dissected, cleansed of fat and connective tissue, and cut transversally into cylindrical rings (3–4 mm in length). In order to preserve the endothelium, very cautious ring dissection was performed. On average, 5–7 arterial rings were prepared from each thoracic aorta. Aortic rings were immediately mounted in standard organ baths filled with K-H solution, as previously described [17].

Thoracic aorta experimental protocol

After mounting, equilibration of rings at 20 mN resting tension was performed, periodically adjusting it to the desired level and changing the K-H solution every 10 minutes. The equilibration period lasted for 60 min. Later, rings were contracted with 60 mM KCl until reproducible contractile responses were obtained. Then the rings were precontracted with 1 μmol/L phenylephrine (Phe) before addition of 1 μmol/L acetylcholine (ACh) to check the presence of a functional endothelium.

After washout, the first set of experiments was made. The cumulative relaxation tests for atorvastatin, losartan or their combination (all in concentrations from 10 nM to 0.1 mM) were performed in rings precontracted with 1 μM Phe. To test the involvement of the nitric oxide pathway in the relaxation response to the atorvastatin and losartan combination, one group of rings was preincubated in L-NNA before contraction with Phe and cumulative relaxation with the combination.

In another set of thoracic aorta experiments, the influence of incubation in atorvastatin, losartan or their low-dose combination on endothelial dependent relaxation was tested. Following washout, rings were then incubated for 30 min in atorvastatin (0.1 μM), losartan (1 μM), and their combination or in K-H solution (control group). After another washout the endothelium dependent relaxation was remeasured, and rings were precontracted with 1 μM phenylephrine before addition of 1 μM acetylcholine (ACh) to measure the endothelium dependent vasorelaxation.

Data acquisition

Vascular responses and data for all parameters measured in isolated hearts were recorded and processed on a Dewetron acquisition system (Dewetron, Graz, Austria) after analogue-digital conversion (National Instruments, NI PCI-6013, Austin, USA) on the hard disk of a personal computer by Dewesoft 6.0 software (Dewetron, Trbovlje, Slovenia).

Data analysis

Statistical analysis was performed using GraphPad Prism 5.0 (GraphPad Software, San Diego California, USA). Values were expressed as means ±SEM for *n* observations, where *n* represents the number of animals/hearts and *m* represents the number of aortic rings used in each of the studied groups. In isolated heart experiments and comparison of endothelium-dependent vasorelaxation, one-way analysis of variance (ANOVA) with Bonferroni post-test was used to compare the studied groups. Thoracic aorta relaxations were compared by two-way analysis of variance (ANOVA) with Bonferroni post-test used to compare the studied groups. A value of $P<0.05$ was considered significant.

RESULTS

Thoracic aorta relaxation

Relaxation tests were carried out in aortic rings precontracted with 1 μM phenylephrine. The separate drugs induced vasorelaxation significantly compared to the control group; atorvastatin in a concentration equal to and above 0.3 μM and losartan in a concentration equal to and above 3 μM (both $P<0.05$; Figure 1). The combination of both drugs was even more effective; vasorelaxation was induced significantly compared to the control, even in lower concentrations than when the separate drugs were used (from 0.03 μM concentration; $P<0.05$; Figure 1). Notably, the combination of drugs induced significantly higher vasorelaxation compared to the separate drugs (Figure 1A).

The possible influence of atorvastatin, losartan or their low-dose combination on thoracic aorta endothelium-dependent relaxation was measured with acetylcholine in aortic rings precontracted with phenylephrine after incubation in different concentrations of the drugs. Only a 1 μM concentration of atorvastatin and a 10 μM concentration of losartan were effective; endothelium-dependent relaxation improved by 21.1% ($P<0.001$) and 17.0% ($P<0.01$) compared to the control, respectively (Table 1). Lower doses of atorvastatin or losartan were ineffective. The low-dose combination of atorvastatin (0.1 μM) and losartan (1 μM) was effective in improving the endothelium-dependent relaxation of aortic rings compared to the control (by 26.3%; $P<0.001$; Table 1). The separate drugs, used in the same concentration as in the combination, did not influence the endothelium-dependent relaxation of thoracic aorta rings.

In order to test the role of NO in the vasorelaxation obtained, the following experiment was done: the rings were incubated in L-NNA, a nitric oxide-synthase (NOS) inhibitor; in this case relaxation was absent (Figure 1B).

Isolated heart experiments

Coronary flow

Maximal coronary flow values were achieved in the 75^{th}–77^{th} minute of the experiments, compared to the control group. Atorvastatin was effective in 0.1 or 1 μM concentrations, while losartan was effective only in 1 μM concentration (Figure 2A, B); lower doses were ineffective. Atorvastatin or losartan in 1 μM concentration produced an increase in coronary flow compared

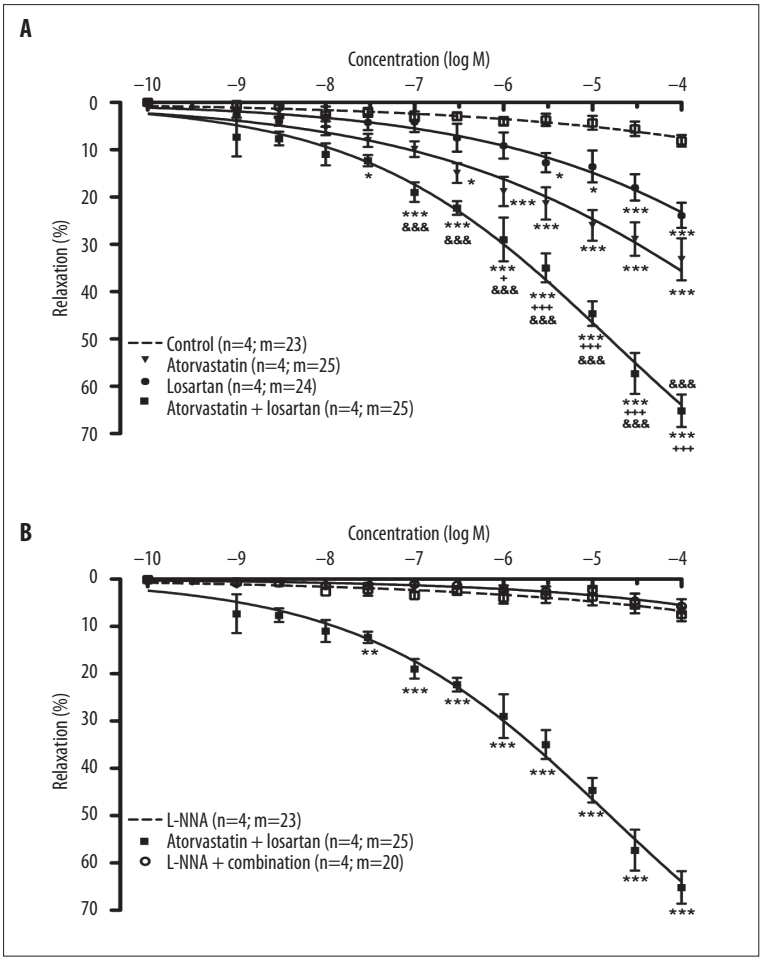

Figure 1. (**A**) Effects of cumulative addition of atorvastatin, losartan or their combination on isolated rat aortic rings precontracted with 1 μM phenylephrine. (**B**) Cumulative relaxation curve of the atorvastatin and losartan combination after ring incubation in N(ω)-nitro-L-arginine (0.1 mM, L-NNA), Relaxation is expressed as the percentage of the precontraction occurring in phenylephrine. All data are means ±SEM. ** signifies P<0.01; *** P<0.001 *vs.* control; &&& signifies P<0.001 for the combination *vs.* losartan; + signifies P<0.05; +++ P<0.001 for the combination vs. atorvastatin. N is the number of animals in a separate group; m is the number of aortic rings.

Table 1. Acetylcholine-induced endothelium-dependent relaxation values of isolated rat aortic rings after 30-minute incubation in K-H solution (control group) and in different concentrations of atorvastatin (0.01, 0.1 and 1 μM), losartan (0.1, 1 and 10 μM) or the low-dose atorvastatin and losartan combination (0.1 μM and 1 μM, respectively).

	Atorvastatin	Losartan	Combination (0.1 μM A+ 1 μM L)	Control
0.01 μM	75.3±3.0	76.3±1.4	/	/
0.1 μM	84.6±1.1	78.2±2.6	96.0±1.6***	/
1 μM	92.0±2.7***	80.2±2.4*		/
10 μM	/	88.9±3.3**	/	/
K-H only	/	/	/	76.0±3.6

All data are means ±SEM. ** P<0.01; *** P<0.001 *vs.* control; * signifies P<0.05 for combination *vs.* losartan.

to controls by 117.4% or 102.1%, respectively (both P<0.001; Figure 2A, B). When using the low-dose combination of both drugs, coronary flow was increased maximally by 80.3% compared to controls (P<0.001; Figure 3C). The low-dose combination also increased coronary flow significantly in comparison to the separate drugs when used in the same concentration (Figure 2C). Comparison of the area under the coronary flow curves showed that the separate drugs significantly increased coronary flow compared to the control group only at higher concentrations – atorvastatin in a concentration of 0.1 μM by 52.9% (P<0.05; Table 2) and in a concentration of 1 μM by 82.5% (P<0.001; Table 2); losartan in a concentration of 1 μM by 67.3% (P<0.001; Table 2). The low-dose combination of atorvastatin (0.01 μM) and losartan (0.1 μM) also significantly increased coronary flow by 68.9% (P<0.001; Table 2) compared to the control group. The increase in the low-dose combination group was similar to that of atorvastatin or losartan in the highest concentration used (1 μM). The combination also significantly increased coronary flow compared to the separate drugs at the concentration of 0.01 μM (atorvastatin) or 0.01

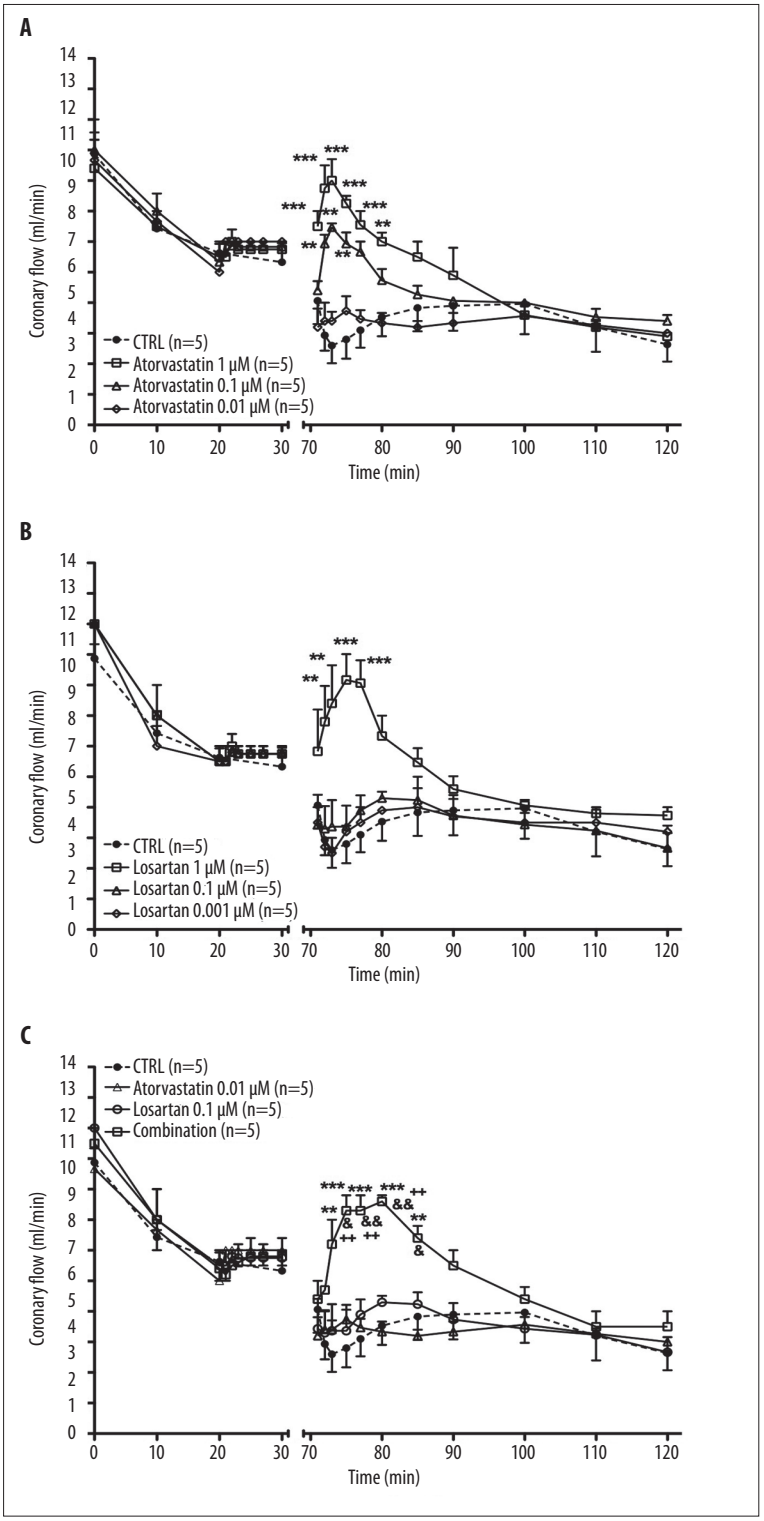

Figure 2. Coronary flow (ml/min) values in isolated rat hearts in experiments with reperfusion following a 40-minute ischaemia period. In the control group (CTRL) hearts were perfused with K-H only. In other experimental groups perfusion was done with (**A**) atorvastatin (0.01; 0.1 and 1 μM); (**B**) losartan (0.01; 0.1 and 1 μM) or (**C**) the low-dose combination of atorvastatin (0.01 μM) and losartan (0.1 μM). n = number of hearts in the experimental group. All data are means ±SEM. * signifies $P<0.05$; ** $P<0.01$; *** $P<0.001$ *vs.* control; $^{++}$ signifies $P<0.01$ *vs.* atorvastatin; $^{\&}$ signifies $P<0.05$ $^{\&\&}$ $P<0.01$ *vs.* losartan.

and 0.1 μM (losartan). The drugs used separately, but in the same doses as in the combination, produced no influence on the coronary flow compared to controls (Table 2).

Lactate dehydrogenase (LDH) release rate

LDH is a cytosolic intracellular enzyme released due to cell membrane damage. From analysis of coronary effluent, LDH release rates were obtained in order to assess the degree of cardiac tissue injury. At the beginning of the experiments, LDH release rates were increased due to the preparation procedure and decreased by the 30th minute, when constant and approximately equal values were reached in all heart groups. The values of LDH release rates steadily rose in the control group during reperfusion, reaching a maximum in the 110th and 120th minute of the experiments.

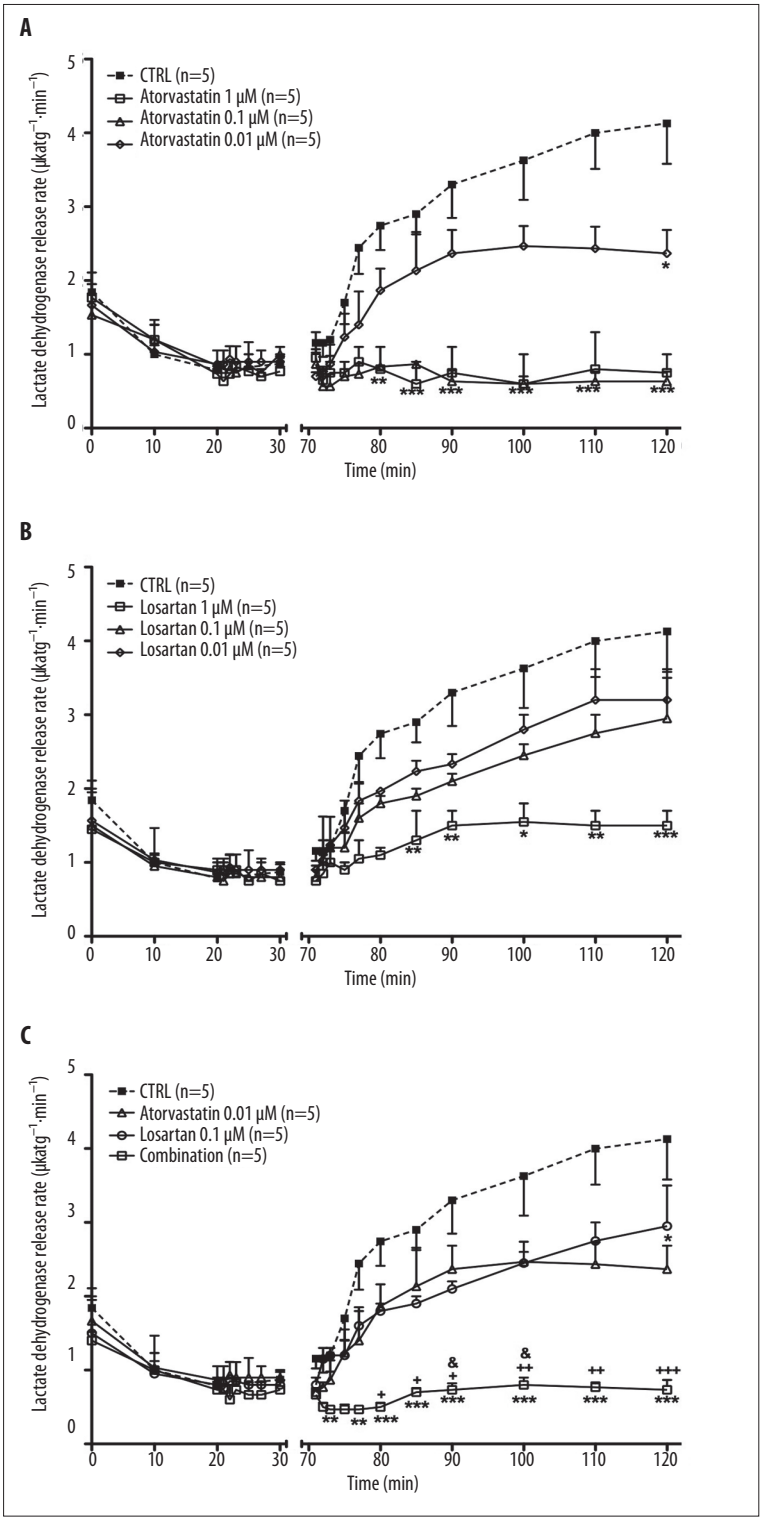

Figure 3. Lactate dehydrogenase (LDH) release rate (μkat g^{-1}·min^{-1}) values of isolated rat hearts in experiments with 40-minute ischaemia, followed by reperfusion. In the control group (CTRL) hearts were perfused with K-H only. In other experimental groups perfusion was done with (**A**) atorvastatin (0.01; 0.1 and 1 μM); (**B**) losartan (0.01; 0.1 and 1 μM) or (**C**) the low-dose combination of atorvastatin (0.01 μM) and losartan (0.1 μM). n = number of hearts in the experimental group. All data are means ±SEM. * signifies P<0.05; ** P<0.01; *** P<0.001 *vs.* control; $^{++}$ signifies P<0.01; $^{+++}$ P<0.001 *vs.* atorvastatin; $^{\&}$ signifies P<0.05 *vs.* losartan.

The maximal decrease of LDH release rates in the treated groups of hearts was observed at the end of the reperfusion period (in the 110th and 120th minute). Atorvastatin was effective in 0.1 or 1 μM concentrations and losartan only in 1 μM concentration (Figure 3A, B); lower doses were ineffective. Atorvastatin or losartan in 1 μM concentration produced a maximal decrease in LDH release rates compared to the control of 471.4% or 166.7%, respectively (both P<0.001; Figure 3A, B). When using the low-dose combination of both drugs, LDH release rates were decreased by 403.1% compared to controls (P<0.001; Figure 3C). The decrease in LDH release rate produced by the low-dose combination of both drugs was statistically significant compared to controls or the same concentrations (as in their combination) of the separate drugs (Figure 3C).

Table 2. Area under the coronary flow curves (AUC) after perfusion with low-dose combination solution and separate drugs solutions. In the control group hearts were perfused with K-H only. In other experimental groups perfusion was done with atorvastatin (0.01; 0.1 and 1 μM); losartan (0.01; 0.1 and 1 μM), the low-dose combination of atorvastatin (0.01 μM) and losartan (0.1 μM) or the low-dose combination of atorvastatin (0.01 μM) and losartan (0.1 μM) with the addition of N(ω)-nitro-L-arginine (0.1 mM, L-NNA).

	Atorvastatin	Losartan	Combination (0.01 μM A+ 0.1 μM L)	Combination + L-NNA	Control
0.01 μM	579.0±25.2^{+}	564.2±32.4$^{\&\&}$	898.4±38.3***	614.7±28.2$^{\#\#\#}$	/
0.1 μM	813.8±5.1*	600.6±32.3$^{\&}$			/
1 μM	970.6±25.7***	890.0±90.6***	/	/	/
K-H only	/	/	/	/	531.9±51.3

All data are means ±SEM. Each experimental group comprised five hearts. Statistical significance *vs.* control (*) or *vs.* the combination ($^{\&}$/$^{+}$/$^{\#}$) is shown.

Table 3. Area under the lactate dehydrogenase (LDH) release rates (AUC) after perfusion with low-dose combination solution and separate drugs solutions. In the control group hearts were perfused with K-H only. In other experimental groups perfusion was done with atorvastatin (0.01; 0.1 and 1 μM); losartan (0.01; 0.1 and 1 μM), the low-dose combination of atorvastatin (0.01 μM) and losartan (0.1 μM) or the low-dose combination of atorvastatin (0.01 μM) and losartan (0.1 μM) with the addition of N(ω)-nitro-L-arginine (0.1 mM, L-NNA).

	Atorvastatin	Losartan	Combination (0.01 μM A+ 0.1 μM L)	Combination + L-NNA	Control
0.01 μM	157.2±10.4^{+++}	174.2±4.4$^{\&\&\&}$	74.1±3.9***	157.5±6.2$^{\#\#\#}$	/
0.1 μM	83.9±9.6***	165.9±2.4$^{\&\&\&}$			/
1 μM	75.9±25.9 ***	132.8±14.1$^{**,\&}$	/	/	/
K-H only	/	/	/	/	210.6±5.3

All data are means ±SEM. Each experimental group comprised five hearts. Statistical significance *vs.* control (*) or *vs.* the combination ($^{\&}$/$^{+}$/$^{\#}$) is shown.

Comparison of the area under the LDH release rate curves showed that the separate drugs significantly decreased LDH release rates compared to the control group – atorvastatin in a concentration of 0.1 μM by 150.3% (P<0.001; Table 3) or in a concentration of 1 μM by 177.5% (P<0.001; Table 3) and losartan in a concentration of 1 μM by 58.6% (P<0.01; Table 3). The low-dose combination of atorvastatin (0.01 μM) and losartan (0.1 μM) also significantly decreased LDH release rates by 184.2% compared to the controls (P<0.001; Table 3). The drugs used separately, but in the same doses as in the combination, had no influence on the LDH release rates (Table 3).

Heart rate and left ventricular pressure

Atorvastatin, losartan or their low-dose combination did not importantly influence heart rate or left ventricular developed pressure in comparison to the control group (data not shown).

Isolated rat heart experiments with NOS-inhibitor

In order to test the role of NO in the observed cardioprotective effects, the hearts were perfused with L-NNA, an NOS-inhibitor, before and after addition of the low-dose atorvastatin and losartan combination. In these experiments, the coronary flow rate significantly decreased by 1.5-fold and LDH increased by 2.1-fold in the reperfusion period, compared to the combination group (both P<0.001; Tables 2 and 3, respectively). Overall, almost all benefits that were provided by a low-dose combination were prevented by addition of L-NNA.

Discussion

In the present study we showed that a low-dose combination of atorvastatin and losartan is capable of producing substantial cardioprotective effects in a rat model of the isolated aorta and heart, but the same concentrations of atorvastatin and losartan as in the combination, when used separately, did not produce any detectable protective effects. Therefore, it seems very likely that low doses of atorvastatin and losartan, when used in combination, have an additive/synergistic mode of protective action.

Clearly, maintaining proper arterial dilatory capacity, or even improving it, is an important aim and target of cardiovascular prevention. Therefore, we chose to investigate arterial dilation-related cardiovascular protection using a rat model. We were particularly interested in investigating whether direct protective effects could be achieved by a low-dose combination of the above-mentioned drugs. Direct cardioprotective effects were indeed observed and consisted of isolated thoracic aorta endothelium-dependent relaxation improvement

and an increase in coronary flow associated with a decrease of LDH release rate in an ischaemic-reperfusion model of the isolated heart. Thus, we found that a low-dose combination of atorvastatin and losartan, but not the same doses of the drugs used separately, increased endothelium-dependent relaxation of the thoracic aorta and improved coronary flow to a significant extent (26% and 68%, respectively; both P<0.001). Even more prominent was the reduction of myocardial ischaemic-reperfusion injury, measured by the LDH release rate (–184%; P<0.001). It is important to emphasize that in order to achieve the same level of protection, approximately 10- or 100-fold higher doses of atorvastatin or losartan, respectively, were required when used separately. This comparison in efficacy clearly reveals the additive, and possibly synergistic, effects of the low-dose combination.

Although cardiovascular pleiotropic effects of statins, sartans and their combination were previously investigated in numerous studies, the effects of low doses were explored only in a small number of these. In addition, in the majority of these studies chronic and not acute effects were explored [18,19]. Overall, only scanty data exists regarding the acute/direct effects of low-dose statins, sartans and their combination [20,21]. Thus, the authors treated mice with various doses of rosuvastatin, starting from very low doses, before myocardial ischaemia and reperfusion [20], or explored the effects of administration of a low dose combination of rosuvastatin and losartan in cuff-induced neointimal arterial formation [21].

However, some studies that are relevant to our work have been published. Horiuchi et al. found in a mouse model that a low-dose combination of fluvastatin and valsartan was effective in inhibiting the proliferation of vascular smooth muscle cells and consequently vascular neointimal formation decrease, whereas the separate drugs in the same concentrations as in their combination were ineffective [22]. Similar results, but after acute administration, were reported for a low-dose combination of losartan and rosuvastatin [21]. Sohma et al showed that treatment with a low-dose combination of losartan and simvastatin improved left ventricular systolic function in a rat myocardial infarction model, but the separate drugs in the same concentrations as combined showed no effect [23]. No studies testing a low-dose combination of atorvastatin and losartan were published.

In several studies the pleiotropic efficacy of a statin and sartan combination in therapeutic doses was proved. The pravastatin and olmesartan combination acted synergistically in improving endothelium-dependent relaxation, coronary artery remodelling and increasing endothelial NOS activity in hypertensive rats [24]. In the study of Liu et al, the synergistic effects of the fluvastatin and valsartan combination were shown to be more effective than the separate drugs in reducing (postprandial) vascular inflammation and fibrinolytic activity in patients with essential hypertension [25]. The additive effects of the fluvastatin and valsartan combination in antioxidative activity were also shown in patients with arterial hypertension and hypercholesterolemia [26]. While the chronic effects of the above-mentioned combinations have been abundantly studied, acute/direct effects are still largely unknown.

To the best of our knowledge, and somewhat surprisingly, no study was performed in humans exploring the low-dose combination of a statin and a sartan. However, we have demonstrated in our previous studies the protective effects of low-dose fluvastatin and valsartan, and particularly their combination, in improving functional and structural arterial wall properties in healthy middle-aged volunteers [11,12,27]. Previous animal and human studies revealed that when a statin and an ARB are combined, additive [26] or even synergistic [22,25] beneficial effects on the cardiovascular system could be obtained. Thus, it has been shown that combined therapy improves endothelial function, decreases oxidative stress and slows down the atherosclerotic process more than the separate agents/drugs alone [28]. Statins increase NO synthesis and stimulate NOS activity/upregulation of NOS pathway [28-30] and inhibit AT1 receptor upregulation expression [28,31]. On the other hand, ARBs inhibit the binding of angiotensin II to the AT1 receptor and consequently decrease related oxidative stress. Oxygen-derived free radicals inactivate NO. Like statins, ARBs also improve endothelial function through NOS pathway stimulation [28,32]. Overall, it is very likely that the combination of a statin and an ARB has additive or synergistic effects on NO-mediated pathways.

In the present study we showed for the first time that the low-dose combination of atorvastatin and losartan is more effective in direct/acute improvement of vascular function than the separately used drugs in the same doses as in their combination. Whether additive or synergistic effects are achieved by combining the two drugs remains an incompletely answered issue. However, based on our results it seems that both effects are present (additive for thoracic aorta endothelium-dependent relaxation, and synergistic for coronary flow rate and LDH release rate).

Our results suggest that the likely mechanism (which interrelates the endothelium-dependent vasorelaxation, the increase in coronary flow rate and the decrease in LDH release rates) is probably mainly an increase in NO bioavailability. This was revealed in additional experiments on the isolated thoracic aorta and isolated heart. Namely, when aortic rings were incubated in L-NNA, an NOS inhibitor, the relaxation produced by the atorvastatin and losartan combination was completely absent. Moreover, when the isolated hearts were perfused with L-NNA, the protective action of low-dose atorvastatin and losartan in increasing coronary flow and decreasing LDH release rates was absent. Taking this into account, it could be assumed that the low-dose atorvastatin and losartan combination either increases the NO concentration in the vascular endothelium, or induces endothelial NOS activity, or employs some other NO-dependent mechanism, or any combination of these postulated mechanisms. The results found are in line with previous studies where the beneficial pleiotropic effects of statins and sartans were shown, among others, to be NO pathway-mediated [20]. The nitric oxide signalling pathway was shown to have an important role in ischemic preconditioning against ischaemic-reperfusion injury [33,34]. Our results clearly demonstrate that the protective effects of the specific low-dose drug combination (eg, atorvastatin and losartan) are mainly or exclusively NO-mediated.

Since cardiovascular diseases remain the major cause of morbidity and mortality in developed countries, a low-dose combination of a statin and a sartan (ARB), aimed to increase

vasodilatory arterial capacity and induce cardioprotection via the NO pathway, could represent an innovative and effective cardiovascular preventive approach. Such an approach in our view deserves further investigation.

Conclusions

We demonstrated the existence of protective cardiovascular pleiotropic additive or even synergistic activity of the low-dose combination of atorvastatin and losartan through isolated rat thoracic aorta vasodilation and rat myocardial ischaemic-reperfusion injury diminishment. Undoubtedly, further studies are needed to clarify this interesting issue.

Conflict of interest

The authors declare that there are no conflicts of interest.

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