Atorvastatin does not Affect Ischaemia-Induced Phosphatidylserine Exposition in Humans in-vivo

Constantijn W. Wouters1, 2, Patrick Meijer1, 3, Carola I.F. Janssen1, Geert W.J. Frederix1, Wim J. Oyen4, Otto C. Boerman4, Paul Smits1, 5 and Gerard A. Rongen1, 5

1 Department of Pharmacology-Toxicology, Radboud University Nijmegen Medical Centre, Nijmegen, The Netherlands
2 Department of Cardiology, Radboud University Nijmegen Medical Centre, Nijmegen, The Netherlands
3 Department of Anaesthesiology, Radboud University Nijmegen Medical Centre, Nijmegen, The Netherlands
4 Department of Nuclear Medicine, Radboud University Nijmegen Medical Centre, Nijmegen, The Netherlands
5 Department of General Internal Medicine, Radboud University Nijmegen Medical Centre, Nijmegen, The Netherlands

Aim: Statins can induce pharmacologic preconditioning and thereby reduce infarct size. Cellular phosphatidylserine (PS) exposition occurs in the course of ischaemia and reperfusion and has been associated with injury. In this experiment we studied the effect of atorvastatin on PS exposition after a standardised ischaemia and reperfusion challenge.

Methods: In a double-blind randomised cross-over trial 30 healthy volunteers were allocated to 3 day treatment with atorvastatin (80 mg/day) and placebo (n=24), or placebo treatment twice (n=6). At the end of each treatment period, volunteers underwent 10 minutes of forearm ischaemic exercise. At reperfusion radiolabeled annexin A5 was administered intravenously and Gamma camera imaging of both hands was performed 1 and 4 hours after reperfusion.

Results: Annexin A5 targeting was not different between atorvastatin treatment (26.1 ± 9.8% and 24.0 ± 9.5% respectively at 1 and 4 hours after reperfusion) and placebo treatment (25.6 ± 11.0% and 24.5 ± 10.7%) (p = 0.99). Our time control experiment did not reveal a carry-over effect.

Conclusions: Our results show that treatment with atorvastatin 80 mg does not reduce forearm PS exposition after ischaemic exercise. This suggests that the role of PS exposure in the prevention of ischemia and reperfusion injury by short term treatment with atorvastatin is limited.


Key words: Atorvastatin, Ischaemia-reperfusion injury, Phosphatidylserines, Annexin A5 scintigraphy, Pharmacologic preconditioning

Introduction

The cholesterol lowering action of statins (HMG-coenzyme A reductase inhibitors) and its associated reduction in cardiovascular events have been well documented in numerous clinical trials1-4. Traditionally, this beneficial effect of statins has been explained by lowering of plasma cholesterol and subsequent reduced progression of atherosclerosis. Apart from its cholesterol lowering properties, statins have other (“pleiotropic”) actions, such as up-regulation of ecto-5’-nucleotidase5, 7, activation of the phosphatidylinositol 3-kinase-Akt pathway8 and up-regulation of NO synthase9, 10 which all may increase tolerance against ischaemia and reperfusion (IR)11-13. Several animal studies have shown reduction of IR-injury as a result of statin treatment in both the heart and the kidney14-17. This putative protection may explain the clinical observation that early initiation of statin treatment in patients with an acute cardiovascular event improves long-term prognosis as compared with a delayed start of this treatment18-20. Furthermore in two randomised clinical trials one day treatment with...
atorvastatin prior to percutaneous coronary intervention reduced myocardial injury and improved clinical outcomes in patients with acute coronary syndromes\(^{21,22}\).

Ischaemia induced phosphatidylserine (PS) exposure on cardiomyocytes has been associated with reversible cellular damage and apoptosis\(^{23,24}\). Treatment to shield exposed PS with diannexin, a homodimer of human annexin A5 (with high affinity for exposed phosphatidylserines) significantly reduces IR-injury in the liver and kidney\(^{25,26}\). Likewise, diannexin prevents no-reflow in a rabbit cardiac ischaemia-reperfusion model\(^{27}\). These observations suggest a critical role for exposed PS in the development of IR-injury. We have developed an experimental model to study PS exposure after IR in healthy volunteers. Our model uses radiolabeled recombinant annexin A5 to visualise PS-exposition that occurs after 10 minutes of ischaemia combined with isometric exercise\(^{28}\). Interventions that have been shown in preclinical and clinical research to modulate myocardial infarct size (ischaemic preconditioning, and administration of adenosine, dipyridamole, and caffeine) similarly modulate annexin A5 targeting in our forearm model, further supporting a role for PS-exposure in IR-injury\(^{29,30}\).

Using annexin A5 scintigraphy after voluntary ischaemic exercise in healthy males, we have recently shown that annexin A5 targeting after a short pretreatment with rosuvastatin 20 mg once daily was significantly reduced (16±1% and 18±2% respectively at 1 and 4 hours after reperfusion) compared to placebo treatment (21±3% and 25±3%) \(p<0.05\)\(^{31}\). As previously reported for other statins in preclinical research with infarct size as endpoint, this effect of rosuvastatin was inhibited by caffeine, an adenosine receptor antagonist\(^{31}\).

There is ample evidence from animal and clinical research that treatment for three days with high dose atorvastatin protects against IR-injury\(^{15,21,32-34}\). However, we do not know whether PS exposure relates to this clinical benefit of atorvastatin, as differences have been observed in the efficacy of various statins to inhibit HMG-CoA-reductase\(^{35}\) and to induce pleiotropic effects\(^{36}\).

Aim

We aim to assess the role of PS-modulation in the beneficial effect of atorvastatin on IR-injury. We tested the hypothesis that a short pretreatment with atorvastatin 80 mg reduces annexin A5 targeting in our forearm IR-model.
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Statistical Analysis

Values are expressed as mean ± standard error (SE) unless indicated otherwise.

This study is the first to use our forearm IR-model in a paired set-up in healthy volunteers. Based on previous studies, a between subject SD of 10% was predicted. Conservatively assuming a correlation coefficient of 0.5 between first and second paired observation, we calculated that with an alpha of 0.05 and a power of 90%, using 24 subjects allows us to find a difference in annexin A5 targeting of 7%. This is well in the range of our previous observation after treatment with rosuvastatin. Furthermore, 6 additional participants were randomised (in a double-blind fashion) to receive placebo treatment twice to serve as time control experiment. This time-control allows us to verify our assumptions regarding this power analysis afterwards.

The effect of atorvastatin treatment on annexin A5 targeting and lipid profile was tested using ANOVA for repeated measurements. All statistical analyses were performed using SPSS 16.0 for Windows.

Results

Baseline characteristics of all participants are presented in Table 1. Caffeine levels were all under 0.85.
The effect of atorvastatin on annexin A5 targeting after voluntary ischaemic exercise. This indicates that in our forearm model treatment with atorvastatin, in a dose and duration as previously used in clinical trials with patients who present with coronary syndromes, does not modulate PS exposition after IR, in contrast to treatment with rosuvastatin, as reported recently. As the protective action of atorvastatin in a setting of ischemia and reperfusion has been convincingly shown in multiple animal experiments and even in several clinical trials, we conclude that PS modulation is not likely involved in the protective mechanism of atorvastatin.

During reperfusion after an ischaemic event, multiple detrimental processes take place simultaneously including formation of radical oxygen species (ROS), cellular and mitochondrial calcium overload, and a rapid increase (restoration) in pH. These actions result in mitochondrial damage caused by opening of the mitochondrial permeability transition pore (MPTP), activation of caspases and subsequent cell damage and possible cell death (by either necrosis or apoptosis).

PS exposition is recognised to be part of the signalling cascade in the course of apoptosis. It attracts phagocytes which remove the dying cell before its remnants evoke an inflammatory response. The importance of PS exposition in developing tissue damage after IR injury was clearly demonstrated in several experiments where IR-injury could be reduced by...
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ment duration could account for the difference in annexin A5 targeting in these experiments. We have previously shown in humans that activation of ecto-5’-nucleotidase mediates prevention of PS exposure by rosvastatin after IR. The effect of statins on ecto-5’-nucleotidase appears to occur rapidly6, 7). For example Sanada et al. found a significant increase of ecto-5’-nucleotidase activity in dogs for different statins (pravastatin, pitavastatin and cerivastatin) already 10 minutes after treatment7). Thus, our discrepant results between rosvastatin and atorvastatin are not likely explained by differences in treatment duration but rather reflect a difference in pharmacodynamics between these two statins. There are significant differences between several statins in their efficacy to lower LDL cholesterol48). Moreover, pleiotropic effects have been shown to differ between lipophilic statins (e.g. atorvastatin) and hydrophilic statins (e.g. rosvastatin). Lipophilic statins have been reported to render endothelial and vascular smooth muscle cells more susceptible to apoptosis than hydrophilic statins49). In humans rosvastatin inhibits Rho/Rho kinase activity to a greater extent than atorvastatin at a dose that equally reduced LDL cholesterol36). Moreover hydrophilic statins have been suggested to be superior in reducing event rate after a primary acute coronary syndrome in normcholesterolemic patients50).

Conclusion

Our results show that treatment with atorvastatin 80 mg does not reduce forearm PS exposition after ischaemic exercise. This suggests that the role of PS exposure in the prevention of ischemia and reperfusion injury by short term treatment with atorvastatin is limited.

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