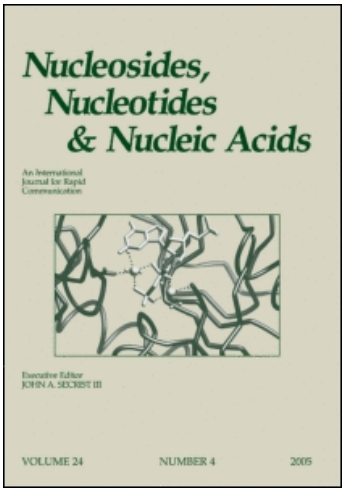

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STIMULATORY EFFECTS OF ATORVASTATIN ON EXTRACELLULAR NUCLEOTIDE DEGRADATION IN HUMAN ENDOTHELIAL CELLS

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□ *Endothelial degradation of extracellular nucleotides is known to be an important mechanism in regulation of thrombosis, inflammation and immune response. It is possible that this pathway is a target for pleiotropic drugs such as atorvastatin. We studied therefore the effect of atorvastatin on extracellular nucleotide degradation in human endothelial cells. Atorvastatin treatment of human umbilical vein endothelial cells (HUVEC) and human microvascular endothelial cells (HMEC-1) resulted in significant increase in ATP breakdown and adenosine formation both if analysed in intact cell studies and as enzyme activity in cell lysates. We conclude that one of the beneficial effects of atorvastatin may include acceleration of extracellular nucleotide breakdown. This will attenuate nucleotide mediated pro-inflammatory effect and stimulate protective mechanisms of adenosine.*

Keywords Atorvastatin; Adenosine; Endothelium; Ecto-5′-nucleotidase; Extracellular nucleotide degradation

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INTRODUCTION

Atorvastatin is an inhibitor of 3-hydroxy-3-methylglutaryl co-enzyme A (HMG-CoA) reductase and cholesterol synthesis pathway that is believed to be primary responsible for its beneficial effect in cardiovascular disease.[1,2] However, atorvastatin, as well as other related drugs, are known to exert number of other effects. Metabolism of the extracellular nucleotides is an important mechanism in maintaining the endothelial function. Adenosine, which is a breakdown product of the adenine nucleotides, exerts number of protective effects that are opposite to the actions of ATP or ADP. It is known that increased activity of the enzymes involved in degradation of nucleotides and formation of adenosine is protective during ischemia/reperfusion induced injury.[3] We analyzed here the effect of atorvastatin on extracellular ATP to adenosine degradation in human endothelial cells using intact cell experiments and by measuring activities of enzymes in cell lysates.

METHODS

Primary cultures of human umbilical vein endothelial cells (HUVEC) and human microvascular endothelial cells (HMEC-1) were treated with 1 μM, 10 μM, and 100 μM Atorvastatin for 24 hours. Intact cell assays were conducted by adding ATP or AMP to the medium of cultured cells followed by sequential collection of the medium for analysis by HPLC for nucleotide nucleoside and nucleobase content using our method described previously.[4] Enzyme assays were performed as well as intact cell studies, to evaluate capacity of cells to degrade ATP to ADP, ADP to AMP, and AMP to adenosine. All studies were conducted at 37°C.

RESULTS

Atorvastatin significantly increased the rate of ATP breakdown and adenosine formation in the medium of intact cells in a concentration-dependent manner. The rate of extracellular ATP breakdown in HUVEC's pretreated for 24 hours with 100 μM atorvastatin was increased almost three fold (Table 1), while adenosine formation rate increased by 40%. With AMP added as the substrate (Table 2) the rate of nucleotide degradation increased three times while adenosine formation rate was 8 times higher. The effect of atorvastatin on nucleotide degradation was similar with HMEC-1 cell line although the degree of changes was smaller. Analysis of the extracellular ATP metabolising enzyme activities in the HMEC-1 lysates was consistent with the results of the intact cell studies. ATPase activity was increased following pre-treatment with Atorvastatin in a dose-dependent manner. ATPase activity was increased from 0.25 ± 0.05 in control to 0.55 ± 0.07 nmol/min/mg protein in cells treated with 100 μM atorvastatin

TABLE 1 Rate of Extracellular ATP (10 μM) Breakdown and Adenosine Formation in Cultured HUVECS Following Pretreatment for 24 hours with Atorvastatin

	Rate of ATP breakdown (nmol/min/mg protein)	Rate of adenosine formation (nmol/min/mg protein)
Control	93 ± 41	51 ± 1
1 μM Atorvastatin	120 ± 24	57 ± 2
10 μM Atorvastatin	195 ± 30	55 ± 7
100 μM Atorvastatin	270 ± 35*	75 ± 6*

Values are expressed as means ± SEM, n = 5. *P < 0.05 versus control.

(P < 0.05 versus control). This was accompanied by a significant increase in ADPase activity from 0.34 ± 0.03 to 0.67 ± 0.03 nmol/min/mg protein (P < 0.05 versus to control). A significant increase in E5′N activity in cells treated for 24 hours with 100 μM atorvastatin was also observed from 6.38 ± 0.23 to 10.24 ± 1.12 nmol/min/mg protein in control (n = 8, P < 0.05 versus control). Interestingly, the activity of purine nucleoside phosphorylase (PNP) also was significantly increased following pre-treatment with Atorvastatin from 3.51 ± 0.39 to 10.29 ± 0.63 nmol/min/mg protein (n = 8, P < 0.05 versus control). There were no unspecific effects of atorvastatin. Cellular lactate dehydrogenase (LDH) activity was constant with different treatments and there was no release of LDH into the medium.

DISCUSSION

We have demonstrated in this study that increased extracellular adenine nucleotide degradation could be a new potential mechanism of the pharmacological effect of atorvastatin. This finding is highlighted by analysis of both nucleotide breakdown rate in the intact cells and activities of enzymes of nucleotide degradation in the cell homogentes. Extracellular nucleotide dephosphorylation is catalyzed by the 3 activities of the 2 enzymes: ecto-ATPase and ecto-ADPase (ecto-ATPDase) and ecto-5′-nucleotidase.[5] This process is essential for regulation of inflammation, thrombosis, cell growth and differentiation. Nucleotides such as ATP and

TABLE 2 Rate of Conversion of 10 μM Extracellular AMP to Adenosine in Cultured HUVECs Following Pretreatment for 24 hours with Atorvastatin at Different Concentrations

	Rate of AMP breakdown (nmol/min/mg protein)	Rate of adenosine formation (nmol/min/mg protein)
Control	80 ± 41	33 ± 1
1 μM Atorvastatin	149 ± 24	85 ± 2*
10 μM Atorvastatin	201 ± 30*	153 ± 7*
100 μM Atorvastatin	297 ± 42*	278 ± 6*

Values are expressed as mean ± SEM, n = 5. *P < 0.05 versus control.

especially ADP triggers adverse responses mediated by P2 receptors. The product of this pathway adenosine is known to exert therapeutic effects on all these mechanisms, predominantly via P1 receptors. An increase in the activity of purine nucleoside phosphorylase (PNP) in the endothelium following pretreatment with Atorvastatin is an interesting phenomenon. PNP is the enzyme of phosphorolytic nucleoside degradation that converts inosine into hypoxanthine. The significance of the increase in PNP activity may relate to regulation of the intracellular phosphate concentration that is one of the substrates of the enzyme. Beneficial effects of atorvastatin on the endothelium have been previously reported but the mechanisms involved remain poorly characterised. Other authors were able to show that statins upregulate endothelial NO synthase (eNOS).[6] Some previous studies indicated that lovastatin increased ecto-5′-nucleotidase activity.[7] However, this study was conducted with rat cells and no other enzymes of the extracellular nucleotide metabolism were analysed. In summary, our results provide evidence that atorvastatin accelerates extracellular nucleotide breakdown and production of adenosine which is caused by an increase in the extracellular enzyme activities degrading ATP (ATPase), ADP (ADPase) and AMP (ecto-5′-nucleotidase) in the human endothelial cells. This mechanism may be part of pleiotropic protective repertoire of atorvastatin.

REFERENCES

1. Bocan, T.M.; Mueller, S.B.; Brown, E.Q.; Lee, P.; Bocan, M.J.; Rea, T.; Pape, M.E. HMG-CoA reductase and ACAT inhibitors act synergistically to lower plasma cholesterol and limit atherosclerotic lesion development in the cholesterol-fed rabbit. *Atherosclerosis* **1998**, 139, 21–30.
2. Altieri, D.C. Statins' benefits begin to sprout. *J. Clin. Invest.* **2001**, 108, 365–366.
3. Cronstein, B.N. Adenosine, an endogenous anti-inflammatory agent. *J. Appl. Physiol.* **1994**, 76, 5–13.
4. Smolenski, R.T.; Lachno, D.R.; Ledingham, S.J.M.; Yacoub, M.H. Determination of sixteen nucleotides, nucleosides and bases using high-performance liquid chromatography and its application to the study of purine metabolism in hearts for transplantation. *J. Chromatogr.* **1990**, 527, 414–420.
5. Zimmermann, H. Extracellular purine metabolism. *Drug Dev. Res.* **1990**, 39, 337–352.
6. Laufs, U.; La Fata, V.; Plutzky, J.; Liao, J.K. Upregulation of endothelial nitric oxide synthase by HMG CoA reductase inhibitors. *Circ.* **1998**, 97, 1129–1135.
7. Ledoux, S.; Laouari, D.; Essig, M.; Runembert, I.; Trugnan, G.; Michel, J.B.; Friedlander, G. Lovastatin enhances ecto-5′-nucleotidase activity and cell surface expression in endothelial cells: implication of rho-family GTPases. *Circ. Res.* 2002, 420–427.