ACUTE VASOACTIVE EFFECTS OF FATTY ACIDS OXIDATION INHIBITORS ON ISOLATED VASCULATURE

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ABSTRACT

This study aimed to investigate whether systemic administration of the HMG-CoA reductase inhibitor atorvastatin to healthy male mice could lower intraocular pressure while increasing retinal blood flow. Adult healthy males mice were given atorvastatin orally or intraocularly. Atorvastatin, on its own, does not appear to cause dilation of retinal microvessels. The effects of atorvastatin on retinal arterioles were investigated in order to better understand how it works. To conduct an in vitro study, adult male mice retinal arterioles were isolated, cannulated, and pressurized without flow. Adult male retinal arterioles were isolated, cannulated, and pressurized without flow for in vitro study. Atorvastatin dose-dependently caused dilation of the retinal arterioles in this study (1 nM to 10 mM). The endothelium removal significantly reduced the amount of vasodilation. L-NAME (NG-nitro-L-arginine methyl ester) was found to inhibit vasodilation, and adding L-NAME to the cyclooxygenase inhibitor in combination increased vasodilation in the laboratory. Sulphonamide had no effect on the dilation that was caused by the cytochrome P450 epoxygenase inhibitor sulphonamide, as was the case with sulphonamide. Metabolite of HMG-CoA reductase caused an inhibition of vasodilation in vessels that had been incubated with it. This is why atorvastatin causes increased production of NO in the retina, resulting in increased dilation of retinal arterioles to be endothelium-dependent and NO-mediated. It has been found that atorvastatin induces vasodilation in endothelial cells by suppressing the mevalonate-Rho kinase signaling pathway. It is possible that a better understanding of statins' effects on the retinal vasculature could lead to new treatments for retinal vascular disease.

Key words: Atorvastatin, Vasodilitation, Retinal vasculature

I. INTRODUCTION

In patients with normal cholesterol levels, statins have been shown to improve endothelium-dependent relaxation, and they may even help lower the risk of cardiovascular disease. These findings suggest that statins may have additional effects besides just cholesterol reduction and cholesterol lowering, such as vascular protection. Studies in rats with streptozotocin-induced diabetes suggest that atorvastatin inhibits the interaction between leukocytes and endothelial cells. Aside from that, long-term statin use has been shown to lower the risk of various eye diseases, including age-related macular degeneration, diabetic retinopathy, and glaucoma. It is essential to study the direct role of statins in regulating ocular vascular function in order to learn additional therapeutic insights, especially given the relationship between these disorders and circulatory system problems. Many statins fall into the first two categories: Lovastatin and Pravastatin are both naturally derived, while atorvastatin is synthetic (atorvastatin, fluvastatin, cerivastatin, and others). Lactone structure statins (in particular, atorvastatin and lovastatin) demonstrate potent vasodilatory effects in the intact heart and in vessels isolated from various tissues, respectively. Sixteenth, seventeenth, eighteenth, nineteenth, and twentieth. The disagreement on whether or not this vasomotor effect can be observed in the retinal arterioles continues. It is reported that the systemic administration of atorvastatin may increase the flow of blood in the retinal arteries and veins in healthy human subjects. Thus, it is unclear whether the increased flow is attributable to microvascular dilation or to other causes. Pravastatin, another type of statin, also increases the amount of NO which elevates pulsatile choroidal blood flow in people with hypercholesterolemia. These results were also published in the journal Hypercholesterolemia. According to animal and clinical studies, cholesterol-lowering medications may increase ocular blood flow and improve vascular function. The question of whether statins have a vasodilatory effect on
retinal microvessels is currently unresolved. Secondary changes in perfusion pressure, intraocular pressure, blood flow, and tissue metabolism could have an impact on vasomotor activity in vivo. Further, no human studies have directly examined the acute effect of statins on the retinal microvascular system. We used isolated vessels to investigate the direct effects of atrovastatin on the microvascular diameter as well as the signaling mechanisms that are involved in this vasomotor activity.

II. METHODS

Experimental Protocols

All animal experiments were approved by the University of Mosul's College of Veterinary Medicine's Animal Experimentation Ethics Committee. In order to obtain basal tone, the arterioles were bathed in a temperature range from 36°C to 37°C. After developing a steady basal tone in the vessels, atrovastatin (1 nM to 10 mM) can elicit a dose-dependent vasodilation response (dependent on concentration) from 15th to 30th day of the month. A 30-minute follow-up study was conducted to ensure that the atrovastatin-induced vasodilation was stable after the controls were completed. To restart basal tone, the vessels were flushed with PSS after the control responses were finished. Three to five minutes was allowed for the vessels to stabilise following each addition of agonists.

Prior to and after denudation, we performed a dose-dependent dilation of the vessel to ensure that vascular smooth muscle function was not impeded as a result of CHAPS administration. Only vessels with normal basal tone and no vasodilation reaction to the endothelium-dependent vasodilator bradykinin (10 nM) were used to investigate the role of endothelium-derived vasodilators. Prostaglandins, indio-methacin (10 mM), and L-nitro-L-arginine methyl ester (L-NAME, 10 mM) were used as specific enzyme inhibitors.

The SEM is the standard error of the mean, and n is the number of vessels studied. When it comes to interventions, the vessels control their own actions. In comparison experiments, researchers examined the effects of antagonists on vasodilation. The subjects' resting tone was studied using a student's t-test. Changes in diameter were examined under the one-way analysis of variance (ANOVA). The Bonferroni test was utilized to find out if there was a difference between the control and experimental interventions. The significance level was set at $P<0.05$.

III. RESULTS

Atrovastatin causes retinal arteriolar dilation.

The vessels in this study showed a similar basal tone (measured at 68% of their maximal diameter) at 36 to 37 degrees Celsius with 55 cmH2O intraluminal pressure. The vessel resting and maximal diameters were 74 and 109 mm on average. After removing the PSS bath, the vessel diameter was at its normal state. Dose-dependent widening of the retinal arterioles occurred with atrovastatin, and the arteriolar dilation process took two to three minutes. A 50% increase in maximal dilation was realized at a concentration of 0.1 mM of concentration. Use of concentrations higher than 10 mM of solvent (ethanol) was avoided due to the high concentration of solvent (ethanol). When atrovastatin was applied repeatedly, the dilation was reproducible and caused no harm to the mice (Figure 2).
Endothelium-Derived Factors: The cyclooxygenase-derived prostaglandins, and cytochrome P450-derived EDHF were all used to study atrovastatin-induced vasodilation. Atrovastatin vasodilation was unaffected by inhibition of cytochrome P450 epoxygenase by sulfonamides. Indomethacin reduced vasodilation from 55% to 38% when the concentration of atrovastatin was the highest (P < 0.01, two-way ANOVA with Bonferroni post hoc test) (P < 0.01)(Figure.3). Data are expressed as mean percentage of maximum dilation SEM. Based on two-way ANOVA, responses to sodium nitroprusside were not affected by any perturbations compared with the control.

**Sodium Nitroprusside**

Table 1 shows the various interventions' effects on arteriolar dilation to sodium nitroprusside. There was no change in the dilations induced by sodium nitroprusside because of L-NAME, indomethacin, and intraluminal mevalonate.

**Table 1: Diameter Responses of Retinal Arterioles to Sodium Nitroprusside**

<table>
<thead>
<tr>
<th>Sodium Nitroprusside (μM)</th>
<th>0.1</th>
<th>1</th>
<th>10</th>
<th>100</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>6.3 ± 1.4</td>
<td>28.1 ± 5.1</td>
<td>61.3 ± 3.5</td>
<td>82.0 ± 7.7</td>
</tr>
<tr>
<td>L-NAME+indomethacin</td>
<td>6.6 ± 3.6</td>
<td>30.3 ± 4.2</td>
<td>64.1 ± 4.8</td>
<td>83.0 ± 6.2</td>
</tr>
<tr>
<td>Mevalonate</td>
<td>5.8 ± 2.6</td>
<td>32.0 ± 9.7</td>
<td>56.6 ± 8.6</td>
<td>81.5 ± 9.1</td>
</tr>
<tr>
<td>L-NAME</td>
<td>4.3 ± 1.8</td>
<td>22.9 ± 4.1</td>
<td>62.8 ± 6.5</td>
<td>83.0 ± 5.6</td>
</tr>
<tr>
<td>Indomethacin</td>
<td>2.8 ± 1.7</td>
<td>27.0 ± 5.0</td>
<td>63.5 ± 5.9</td>
<td>82.2 ± 5.3</td>
</tr>
<tr>
<td>Denudation</td>
<td>9.1 ± 4.6</td>
<td>33.1 ± 7.5</td>
<td>64.8 ± 5.8</td>
<td>76.7 ± 2.7</td>
</tr>
</tbody>
</table>

**Figure 2:** Retinal arterioles' response to atrovastatin representative tracing shows a atrovastatin (10 μM)-induced arteriolar dilation that persisted for 5 minutes. After the washout, the diameter was back to normal. In a dose-dependent manner, the atrovastatin induced vasodilation response was examined and then repeated after a 30-minute washout period. number of vessels By one-way ANOVA and the Dunnett post hoc test, P < 0.05 vs. resting values.
IV. DISCUSSION

It has been shown in mice and humans that the drug atrovastatin increases the circulation of the retina. This phenomenon is associated with a drop in intraocular pressure and LDL cholesterol levels in the blood. A reduction in plasma total cholesterol in hypercholesterolemic patients is associated with a greater improvement in choroidal vascular response to a NOS inhibitor. Studies have shown that statins are beneficial for retinal circulation, but the reasons for this remain unclear because of their influence on LDL cholesterol levels, which can influence circulation. Thus, whether statins impact the vascular structure of the retina is unknown. In this study, we discovered for the first time that Atrovastatin causes small retinal arterioles to dilate when the drug is present at a concentration of 0.1 mM. Vasodilatory response was consistent, and no tachyphylaxis was observed. Our previous human study showed that plasma concentrations of atrovastatin ranging from 10 nM to 1 mM result in an increase in retinal blood flow.

Our results proved that the endothelial barrier was destroyed at the highest tested concentration of atrovastatin, ruling out any vasodilation as a result. This partial endothelium-dependent response has been observed in the aorta and mesenteric arteries of mice before. Notably, the endothelial removal had a greater impact on arteriolar dilation (as evidenced by a 65% reduction) than it did on the mesenteric artery (which was reduced by 25%) (i.e., 25-30 percent reduction). It appears that atorvastatin affects vasodilation in small resistance arterioles more than large ones. When micetion concentrations increase, smooth muscle activation occurs directly. The exact signaling pathway that induces endothelin-independent vasodilation in retinal arterioles remains unknown. Multiple studies have demonstrated that atorvastatin decreases extracellular calcium entry and releases intracellular calcium in denuded aortic rings. Atrovastatin decreases cytosolic calcium levels in smooth muscle cells, causing vasodilation.

Atrovastatin causes vasodilation by releasing vasodilators produced by the endothelium, such as nitric oxide (NO), prostaglandins (PGs), and endothelium-derived heparin (EDHF). NOS is clearly needed for vasodilation, as the atrovastatin-induced response was reduced when NOS was blocked. Furthermore, in animal models of the disease, increased levels of nitrite has been demonstrated in mice to increase retinal blood flow. Atorvastatin appears to cause the dilation of the retinal arteriolar capillaries in minutes. Reports show that statin use can increase endothelial NOS (eNOS) expression and activity within seconds or minutes of starting the medication. Because phosphorylation of the eNOS protein decreases eNOS activity, it is possible that the rapid increase in eNOS activity was responsible for the initial surge in NO. Small arterioles opened within a minute or two after receiving an intraarterial infusion of atorvastatin (10 mM). Atorvastatin infusions (3–30 mM) increased coronary blood flow by 15% in an isolated heart preparation, according to the findings. This suggests that NO is involved in the vasoconstrictor response to atorvastatin. Although no study tested the concentration of atorvastatin in the vasculature, the findings suggest that microvascular beds, including the retinal arteries, can release NO following exposure to atorvastatin in a controlled environment.
Several recent in vitro studies have shown that the COX-2 inhibitor blocks the vasodilation response to atorvastatin, which is beneficial.\textsuperscript{34}

Prostaglandins are likely involved in the vasodilation response to atorvastatin, but more research is needed.\textsuperscript{35} However, atorvastatin's effect on cyclooxygenase activity has yet to be fully elucidated. Following administration of atorvastatin, endothelial calcium increases transiently, and calcium-activated phospholipase A2 produces arachidonic acid, which produces the vasodilator prostacyclin, which is then excreted in the urine.\textsuperscript{36} Epsilonprostacilin synthase is susceptible to tyrosine nitric oxide, and atorvastatin can inhibit this in multiple tissues, according to recent research. Despite these discoveries being made in various cells, tissues, and species in various biological environments, it appears that these discoveries contribute to the production of prostacyclin\textsuperscript{37}. Prostacyclin levels could be increased by fluvastatin (i.e., 0.1 mM). Reports suggest that prosta-cyclin was allegedly behind the dilation of retinal arterioles. Atorvastatin's antithrombotic effect occurs immediately in vivo. This results from the eNOS prostacyclin release. It appears that the release of endothelial factors causes microvascular dilatation while also protecting the vascular wall from potentially thrombogenic insults.\textsuperscript{38,39}

Studies have found that NO activates potassium channels, which increases calcium uptake by the sarcoplasmic reticulum, which inhibits the formation of 20-HETE of inositol, as well as other as yet unidentified mechanisms. The molecules responsible for the atorvastatin-induced arteriolar dilation are still unknown\textsuperscript{40,41}. The selective soluble guanylyl cyclase inhibitor ODQ was shown for the first time to lower retinal arterial dilation in response to atorvastatin\textsuperscript{42}. This discovery illustrates that soluble guanylyl cyclase/cGMP is essential for the NO-dependent dilation response to atorvastatin in vivo.\textsuperscript{43}

EDHF compounds have been shown to be derived from the enzyme cytochrome P450 epoxy-genase in laboratory mice.\textsuperscript{44}

Research on EDHF and statin-induced vasodilation has not been completed at this time. Our findings suggest that dilation of retinal arterioles occurs even when a cytochrome P450 enzyme inhibitor, such as sulfaphenazole, is administered.\textsuperscript{45} Fluvastatin causes relaxation of the mesenteric artery in hypertensive male mice, but not EDHF-mediated relaxation. Lovastatin inhibited both endothelium-dependent and endothelium-independent relaxation in hypercholesterolemic rabbit carotid arteries.\textsuperscript{46}

V. CONCLUSION

Atorvastatin primarily induces an endothelium-dependent, NO-mediated dilation of retinal arterioles via activation of guanylyl cyclase; cyclooxygenase has a minor effect. Inhibition of the mevalonate-Rho kinase pathway in endothelial cells appears to contribute to atorvastatin-induced vasodilation in part. An even more complete understanding of statins' action on the retinal vasculature may shed light on their therapeutic potential in retinal vascular disease.

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Conflicts of interest

No potential conflicts exist. We had full access to all the information in the study and take full responsibility for the integrity of the information and the accuracy of the data analysis.

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