Loss of the preconditioning effect of rosuvastatin during sustained therapy: a human in vivo study

Andrew Liuni,1,2 Mary Clare Luca,1,2 Tommaso Gori,3 and John D. Parker1,2

1Division of Cardiology, Mount Sinai and University Health Network Hospitals; 2Department of Pharmacology and Toxicology, University of Toronto, Toronto, Canada; and 3Department of Cardiology, University of Mainz, Mainz, Germany

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Liuni A, Luca MC, Gori T, Parker JD. Loss of the preconditioning effect of rosuvastatin during sustained therapy: a human in vivo study. Am J Physiol Heart Circ Physiol 302: H153–H158, 2012. First published October 14, 2011; doi:10.1152/ajpheart.00083.2011.—Studies have demonstrated that the acute administration of 3-hydroxy-3 methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors has protective effects in the setting of ischemia-reperfusion (IR). Previously, we demonstrated that a single dose of rosuvastatin prevented IR-induced endothelial dysfunction in humans through a cyclooxygenase-2-dependent mechanism. Whether the chronic administration of HMG-CoA reductase inhibitors provides similar protection remains controversial and is unknown in humans. Eighteen male volunteers were randomized to receive a single dose of rosuvastatin (20 mg) or placebo. Twenty-four hours later, endothelium-dependent, radial artery flow-mediated dilation (FMD) was measured before and after IR (15 min of upper arm ischemia followed by 15 min of reperfusion). In a separate protocol, 30 healthy volunteers were randomized in a double-blind fashion to receive oral rosuvastatin (20 mg/day) and placebo, rosuvastatin, and celecoxib (100 mg bid) or placebo alone, all for 21 days. Twenty-four hours after the final administration of study medication, FMD was measured before and after IR. Pre-IR FMD was similar between groups in both protocols. In the acute administration protocol, rosuvastatin significantly prevented the blunting of FMD associated with IR (FMD pre-IR: 8.4 ± 1.3%; post-IR: 6.2 ± 1.3%; P = 0.01 ANOVA, treatment group interaction). In the daily administration protocol, IR significantly blunted FMD in the placebo group (FMD pre-IR: 7.5 ± 0.9%; post-IR: 3.3 ± 0.7%; P < 0.001). Chronic treatment with rosuvastatin did not modify this ischemic injury (FMD pre-IR: 6.9 ± 0.4%; post-IR: 1.6 ± 1.0%; P < 0.001; P = NS ANOVA, treatment group interaction). Similarly, FMD responses post-IR in volunteers receiving rosuvastatin and celecoxib did not significantly differ from placebo (FMD pre-IR: 8.3 ± 0.9%; post-IR: 2.1 ± 0.8%; P < 0.001; P = NS ANOVA, treatment group interaction). In contrast to acute administration, chronic rosuvastatin does not prevent the development of IR-induced endothelial dysfunction in normal humans.

endothelium; ischemia; reperfusion; 3-hydroxy-3 methylglutaryl coenzyme A reductase inhibitor

THE 3-HYDROXY-3 METHYLGLUTARYL coenzyme A (HMG-CoA) reductase inhibitors are potent cholesterol-lowering agents and are among the most widely used medications in the treatment of hypercholesterolemia and prevention of coronary artery disease. HMG-CoA reductase inhibitors have been clearly established as effective strategies in the primary and secondary prevention of cardiovascular events (1, 2, 16, 35). Importantly, results of many studies have suggested that the vascular benefits associated with HMG-CoA reductase inhibitor admin-

istration may extend beyond cholesterol reduction, as they are hypothesized to have cholesterol-independent or “pleiotropic” effects (22, 32). One such effect is the preconditioning-like phenotype conferred by the HMG-CoA reductase inhibitors in the setting of ischemia-reperfusion (IR) injury. Animal models have consistently demonstrated that the acute administration of HMG-CoA reductase inhibitors provides protection from IR injury in the cerebral, mesenteric, and cardiac circulation (20, 26, 28, 30). Similar to the setting of ischemic preconditioning, this protective phenotype has been shown to be highly nitric oxide (NO) dependent and associated with upregulation of the cyclooxygenase-2 (COX-2) enzyme (3, 5, 26, 28, 36). Our laboratory (24) has recently confirmed these observations in a human model of IR injury.

While there appears to be no doubt concerning the protection afforded by their acute administration, it remains unclear whether the pharmacologic preconditioning effect of HMG-CoA reductase inhibitors in the setting of IR injury is maintained during sustained, daily administration, with animal models producing mixed results to date (8, 18, 21, 27, 31). With this in mind, we sought to determine whether the protective effects of acute rosuvastatin administration on endothelial function are maintained with daily administration in humans and further to determine whether such protection is COX-2 dependent.

METHODS

The Mount Sinai Research Ethics Board approved this study, and all subjects gave informed consent before beginning the study. Studies were conducted in a quiet, temperature- and humidity-controlled environment. All subjects were required to fast and abstain from caffeine for 14 h before the study. Exclusion criteria included any active disease, the use of medications (including supplemental vitamins), as well as risk factors for cardiovascular disease such as hypertension, smoking, hypercholesterolemia, and a family history of premature cardiovascular disease.

Effect of Acute and Chronic Rosuvastatin on IR-Induced Endothelial Dysfunction

Acute administration protocol. Eighteen healthy volunteers were recruited in a double blind, randomized, placebo-controlled parallel trial. After study admission, standing blood pressure measurements were obtained followed by venous blood sampling for baseline lipid analysis. Subjects were then randomized to receive a single dose of placebo or 20 mg of rosuvastatin. Twenty-four hours after randomization, standing blood pressure and plasma lipid measurements were repeated. Subsequently, radial artery flow-mediated dilation (FMD) was measured as described below. After this measurement was completed, a pneumatic cuff placed above the elbow was inflated to 250 mmHg for 15 min to induce local ischemia. The cuff was then deflated, 15 min of reperfusion were allowed, and FMD was measured again. Our laboratory (14, 15, 25) has had significant experience in the...
use of this experimental protocol that allows studying, in humans in vivo, IR-induced endothelial dysfunction and the therapeutic impact of preconditioning. We elected not to test endothelium-independent vasodilators because previous studies (19) have demonstrated that this cycle of IR specifically impairs endothelium-dependent responses while leaving nonendothelium-dependent smooth muscle responsiveness unaltered.

**Daily administration protocol.** In a separate protocol, 30 healthy nonsmoking volunteers (18 to 29 yr old) were enrolled in a double blind, randomized, placebo-controlled trial of parallel design. After study admission, blood pressure measurements and venous sampling for baseline lipid analysis were obtained as above. Subjects were randomized to receive rosvastatin (20 mg/day) and placebo, rosvastatin (20 mg/day), and celecoxib (100 mg/bid), or matching placebo. After administration of study medications, subjects were discharged from the laboratory. Twenty-one days later, and 24 h placebo. After administration of study medications, subjects were then given a 20-day supply of the study medications and were discharged from the laboratory. Twenty-one days later, and 24 h after the final doses of study medication, standing blood pressure and plasma lipid measurements were repeated. Subjects then underwent the same protocol of FMD measurements before and after IR, as described above.

**Measurement of Arterial Diameter and FMD**

The methods for assessment of FMD and blood flow in our laboratory (12, 14, 15, 25), as well as the repeatability of FMD measurements, have been previously described in detail. Briefly, end-diastolic, ECG-gated, longitudinal, B-mode images of the artery 10–15 cm below the antecubital fossa were digitally acquired and stored for offline analysis. Arterial diameter was recorded continuously for 1 min before cuff inflation (resting diameter), during the period of distal cuff inflation (4 min 30 s), and for another 4 min and 30 s after wrist cuff deflation. Analysis was performed in a blinded fashion using automatic custom-designed vascular edge detection software (4). FMD was calculated as the maximum percent increase in arterial diameter following cuff deflation compared with resting diameter.

**Statistical Analysis**

Data are presented as means ± SE. For our model of ischemic injury, sample size estimates were performed based on data from our recently published study (24) using a two-sided α of 0.05 and 1-β of 0.8. IR decreased FMD responses from 8.1 ± 0.6 to 2.2 ± 0.9%. Prevention of 50% of this impairment via rosvastatin-induced preconditioning requires a sample size of 10 subjects per group. Between group comparisons were performed with a paired t-test. Between group differences and the interaction of IR and randomization group were analyzed with a two-way ANOVA. A value of P < 0.05 was set as the threshold for significance. SAS 9.2 (SAS Institute, Cary, NC) was employed for all statistical analyses.

**RESULTS**

**Effect of Rosuvastatin on Baseline Parameters**

There were no significant differences in resting blood pressure (data not shown), baseline radial artery diameter and blood flow, reactive hyperemia, or FMD before IR between groups in either protocol (Tables 1 and 2).

**Acute Administration Protocol**

**Effect of acute rosvastatin on IR-induced endothelial dysfunction.** Radial artery diameter and radial artery blood flow returned to baseline values 15 min after local IR (Table 1; P =

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**Table 1. Arterial diameter and blood flow data for acute administration protocol**

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<tr>
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<th>Before IR</th>
<th>After IR</th>
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<tbody>
<tr>
<td></td>
<td>Baseline diameter</td>
<td>Change after wrist cuff deflation</td>
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<tr>
<td>Radial artery diameter, mm</td>
<td></td>
<td></td>
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<tr>
<td>Placebo</td>
<td>2.28 ± 0.07</td>
<td>0.20 ± 0.02</td>
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<tr>
<td>Rosuvastatin</td>
<td>2.37 ± 0.11</td>
<td>0.20 ± 0.03</td>
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<tr>
<td>Blood flow, ml/min</td>
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<tr>
<td>Placebo</td>
<td>9.6 ± 1.2</td>
<td>116.9 ± 18.1</td>
</tr>
<tr>
<td>Rosuvastatin</td>
<td>12.9 ± 2.7</td>
<td>161.9 ± 17.4</td>
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Values are means ± SE. *P < 0.001 vs. corresponding value before IR. †P < 0.05 vs. corresponding value before ischemia-reperfusion (IR).

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**Table 2. Arterial diameter and blood flow data for daily administration protocol**

<table>
<thead>
<tr>
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<th>Before IR</th>
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<tr>
<td></td>
<td>Baseline diameter</td>
<td>Change after wrist cuff deflation</td>
</tr>
<tr>
<td>Radial artery diameter, mm</td>
<td></td>
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<tr>
<td>Placebo</td>
<td>2.26 ± 0.14</td>
<td>0.16 ± 0.01</td>
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<tr>
<td>Rosuvastatin + placebo</td>
<td>2.41 ± 0.10</td>
<td>0.17 ± 0.01</td>
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<tr>
<td>Rosuvastatin + celecoxib</td>
<td>2.48 ± 0.09</td>
<td>0.20 ± 0.02</td>
</tr>
<tr>
<td>Blood flow, ml/min</td>
<td></td>
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</tr>
<tr>
<td>Placebo</td>
<td>11.1 ± 2.3</td>
<td>116.6 ± 11.9</td>
</tr>
<tr>
<td>Rosuvastatin + placebo</td>
<td>13.5 ± 2.9</td>
<td>103.3 ± 8.7</td>
</tr>
<tr>
<td>Rosuvastatin + celecoxib</td>
<td>10.0 ± 1.8</td>
<td>106.5 ± 9.1</td>
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</table>

Values are means ± SE. *P < 0.001 vs. corresponding value before IR.
NS, before vs. after IR). Similarly, peak reactive hyperemia was not significantly different after IR. IR significantly blunted FMD in the placebo group (Fig. 1; pre-IR: 8.7 ± 0.7%; post-IR: 2.3 ± 1.3%; 95% confidence interval for change in FMD after IR: −8.372 to −4.433%; P = 0.002), confirming previous data of endothelial dysfunction induced by IR in this experimental protocol (10, 12, 14, 15). Reproducing previous findings from our laboratory, rosuvastatin administration prevented the impairment in FMD associated with IR (Fig. 1; pre-IR: 8.4 ± 0.7%; post-IR: 6.2 ± 3.9%; 95% confidence interval for change in FMD after IR: −4.171 to −0.2323%; P < 0.05 vs. rosuvastatin pre-IR; P = 0.01 for the interaction of IR and group).

Sustained Administration Protocol

Effect of daily rosuvastatin on IR-induced endothelial dysfunction. As with the acute administration protocol, resting radial artery diameter and radial artery blood flow and peak reactive hyperemia were not different from baseline 15 min after local IR (Table 2; P = NS, before vs. after IR). Similarly, IR significantly blunted FMD in the placebo group (Fig. 2; before IR: 7.5 ± 0.9%; after IR: 3.3 ± 0.7%; 95% confidence interval for change in FMD after IR: −6.515 to −2.029%; P < 0.001). Importantly, chronic rosuvastatin administration did not prevent the impairment in FMD associated with IR (Fig. 2; before IR: 6.9 ± 0.4%; after IR: 1.6 ± 1.0%; P < 0.001 compared with rosuvastatin before IR; 95% confidence interval for change in FMD after IR: −7.564 to −3.078%; P = NS for ANOVA effect of group and for the interaction of IR and group). In subjects receiving rosuvastatin + celecoxib, IR blunted FMD to values similar to those observed in the placebo and rosuvastatin + placebo groups (Fig. 2, before IR: 8.3 ± 0.9%; after IR: 2.1 ± 0.8%; P < 0.001 compared with FMD before IR; 95% confidence interval for change in FMD after IR: −8.363 to −3.877%; P = NS for ANOVA effect of group and for the interaction of IR and group).

**Effect of Rosuvastatin and Celecoxib on Lipid Parameters**

Results are summarized in Table 3. No significant differences were noted in any lipid parameters after the 24-h period of treatment in the acute administration protocol. In the daily administration protocol, significant decreases were observed in total cholesterol, LDL cholesterol, and triglyceride levels in rosuvastatin + placebo and rosuvastatin + celecoxib groups. There were no significant differences in lipid profiles between visits in the placebo group.

**DISCUSSION**

HMG-CoA reductase inhibitors are among the most widely used agents in the prevention and management of patients with coronary artery disease. Their long-term treatment benefits are unquestioned, although the spectrum of the mechanism(s) of their beneficial effects remains controversial. Although originally discovered and developed based on their lipid-lowering characteristics, it is now documented that these agents possess cholesterol-independent effects that may play an important role in their beneficial effects in patients with risk factors or overt cardiovascular disease. These effects include increased activity of NO synthase (NOS) through phosphatidylinositol-3-kinase (PI3K)/protein kinase Akt-mediated phosphorylation and an increase in the production of the essential NOS cofactor tetrahydrobiopterin, as well as improved NOS mRNA stability, thus prolonging mRNA half-life for this important enzyme (17, 33). Overall there is strong evidence that therapy with HMG-CoA reductase inhibitors is associated with an increase in NO bioavailability that is independent of reductions in plasma cholesterol (6). Such effects may play an important role in the improvement of outcomes in primary and secondary prevention studies with the HMG-CoA reductase inhibitors (1, 2, 16, 32, 35).

There is also considerable evidence that HMG-CoA reductase inhibitors are cardioprotective in the setting of ischemic injury. Animal models of IR injury in the brain, cardiac, and...
mesenteric circulation have consistently demonstrated the ability of HMG-CoA reductase inhibitors, when administered acutely, to decrease infarct size, maintain vascular function, and improve functional recovery after IR in a pharmacologically induced response similar to that of ischemic preconditioning (20, 26, 28, 30). This response has been shown to be largely dependent upon NO and activation of downstream mediators, such as COX-2 (26, 28). We (24) recently confirmed the presence of a similar protective phenomenon following acute rosuvastatin administration in humans. Whether this benefit is maintained during chronic HMG-CoA reductase inhibitor administration, however, remains controversial. Animal models of chronic therapy with these agents have produced mixed results with respect to their effect on IR (8, 18, 21, 27, 31); other studies have reported a loss of protection during sustained therapy (18, 27, 31).

In the present study, we observed that sustained therapy with rosuvastatin did not provide protection from the adverse effects of IR on endothelial function. These data are in contrast to our current and previous data (23, 24) showing that acute rosuvastatin administration prevents the impairment in endothelium-dependent vasodilation associated with IR by a mechanism involving the COX-2. Although prospective and retrospective studies (26, 28) in patients undergoing percutaneous coronary interventions, coronary artery bypass grafting, or experiencing acute coronary syndromes have consistently shown that acute HMG-CoA reductase inhibitor treatment can reduce evidence of myocardial injury as well as cardiovascular morbidity and/or mortality, the fact remains that most, if not all, patients continue with chronic statin therapy. The results of the current study suggest that the direct pharmacologic preconditioning, cardioprotective benefits of the HMG-CoA reductase inhibitors are lost with chronic therapy (9). Although there is no doubt that HMG-CoA reductase inhibitors are potent in both the treatment and prevention of coronary artery disease, the findings reported here suggest that a preconditioning effect does not play a role in their beneficial effects on long-term outcome.

The mechanisms behind the loss of direct cardioprotection with sustained HMG-CoA reductase inhibitor therapy are not well understood. It is possible that sustained exposure to an intervention (either pharmacologic or ischemic) with preconditioning effects could trigger counter-regulatory responses that lead to loss of protection from IR. Although there are many possible mechanisms through which this counter-regulation could occur, some important examples have already been defined. As mentioned above, the PI3K/Akt signaling cascade is a biochemical pathway that is of essential importance in the development of the preconditioning protective phenotype induced by acute treatment with HMG-CoA reductase inhibitors. Previous studies (27) in animal models of IR injury have demonstrated an increase in the levels of the PI3K inhibitor phosphatase and tensin homolog deleted on chromosome ten (PTEN) is associated with chronic treatment with HMG-CoA reductase inhibitors, leading to a downregulation of the PI3K/Akt pathway. The response of PTEN to preconditioning interventions is time dependent and provides a conceptually important explanation concerning differences between responses to acute and sustained preconditioning interventions. During acute exposure to HMG-CoA reductase inhibitors, PTEN is downregulated with resulting disinhibition of cell survival pathways, while during chronic exposure to the same stimulus, it is upregulated, leading to loss of preconditioning effects (27). Despite the loss of the cardioprotective effect, the response is biologically appropriate since continued loss of PTEN’s tonic inhibitory effects can lead to inappropriate cellular proliferation (29).

Interestingly, we (13) have recently reported a similar phenomenon (loss of pharmacologic preconditioning protection during prolonged administration) with daily short-term (2 h) administration of nitroglycerin in humans. This loss of preconditioning effects contrasts with the effective preconditioning effects of a single short exposure to nitroglycerin (11–13). These lines of evidence serve to emphasize that the response to acute vs. repeat pharmacologic stimuli can be very different and that an understanding of their underlying mechanism may be helpful in the development of effective sustained preconditioning treatment strategies.

Of relevance, despite intense interest concerning the efficacy and mechanism of preconditioning interventions, few investigations have explored whether preconditioning protection is sustained with repeated exposure. Two reports (7, 34) in a porcine myocardial infarction model have demonstrated that repeated ischemic preconditioning stimuli are associated with sustained protection from ischemia over 96 h. Interestingly, these studies provided evidence to suggest that mechanism(s) responsible for the preserved protection with sustained ischemic preconditioning are different from those involved with acute ischemic preconditioning (7, 34). Our current observations, although negative, emphasize the need to develop a better understanding of the mechanistic differences between acute and sustained preconditioning strategies to develop effective approaches that maintain a sustained preconditioned phenotype. The fact that the present data were acquired in healthy volunteers and in the conduit circulation of the forearm, a
vascularity different from the coronary circulation, is acknowledged as a limitation. However, the model employed in the present study has been previously shown to provide reliable and relevant information of the effect of IR injury on the vasculature. Study of the endothelium has particular relevance, since this tissue is the most sensitive to IR, and damage of the (macro- and microvascular) endothelium can prevent effective reperfusion in spite of timely intervention. For these reasons, we believe that the study of potential therapies and treatment regimens aimed at protecting the endothelium in the setting of IR are clinically relevant. Further studies should be conducted in individuals with dyslipidemia and patients with cardiovascular disease to determine whether a similar phenomenon exists in the presence of risk factors and/or disease. The small sample size in the present study needs to be acknowledged as a limitation. However, the possibility of a type II statistical error appears unlikely since absolutely no trend towards a protective effect of repeated rosuvastatin administration was shown, and the blunting in FMD in the rosuvastatin groups was actually slightly more pronounced than in the placebo group. Finally, we cannot discount the possibility that a lack of compliance may have played a role in our observed responses. Although lipid parameters were significantly lowered in subjects receiving rosuvastatin, we acknowledge this as a limitation.

In conclusion, although it is clear that HMG-CoA reductase inhibitors are effective in the settings of both primary and secondary prevention, the current study does not suggest that these favorable outcomes are, in part, due to a sustained preconditioning effect. The present data are in contrast to our previous report (24) demonstrating the potent protective effects of acute rosuvastatin administration. The findings emphasize that an effective pharmacologic preconditioning stimulus induced by acute administration of a drug cannot be assumed to be associated with protection during repeated exposure.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

A.L., T.G., and J.D.P. conception and design of research; A.L. and M.C.L. performed experiments; A.L. and M.C.L. analyzed data; A.L., M.C.L., T.G., and J.D.P. interpreted results of experiments; A.L. and M.C.L. prepared figures; A.L. drafted manuscript; A.L., M.C.L., T.G., and J.D.P. edited and revised manuscript; A.L., M.C.L., T.G., and J.D.P. approved final version of manuscript.

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