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Cyclooxygenase inhibition and rosuvastatin-induced vascular protection in the setting of ischemia–reperfusion: A human in vivo study

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ABSTRACT

The 3-hydroxy-3-methylglutaryl coenzyme A (HMG Co-A) reductase inhibitors have preconditioning effects involving up-regulation of cyclooxygenase (COX)-2. We investigated the effect of selective and non-selective COX inhibition on rosuvastatin-mediated protection against ischemia–reperfusion (IR)-induced endothelial dysfunction in the human forearm. Healthy volunteers ($n = 66$) were allocated to placebo, acetylsalicylic acid (ASA) 81 mg daily, ASA 325 mg daily, celecoxib 200 mg twice daily or 400 mg ibuprofen four times daily, each administered for 5 to 7 days. On the last day of study drug therapy, subjects received a single dose of 40 mg rosuvastatin. Twenty-four hours later flow-mediated dilation (FMD) of the radial artery was evaluated before and after IR. In the placebo group, FMD was similar before and after IR (8.1 ± 1.0 vs $7.2 \pm 0.8\%$; $P =$ NS) indicating a significant protective effect of rosuvastatin. There was also no effect of IR on FMD in the ASA 81 mg group (6.7 ± 0.6 vs $6.1 \pm 0.7\%$; $P =$ NS). In contrast, following IR there was a significant decrease in FMD in the ASA 325 mg group (7.2 ± 0.8 vs $3.3 \pm 0.7\%$, $P < 0.001$), the celecoxib group (7.3 ± 1.5 vs $2.6 \pm 1.5\%$, $P < 0.01$) as well as the ibuprofen group (6.8 ± 0.7 vs $2.6 \pm 0.8\%$; $P < 0.01$). Therefore, nonselective COX inhibition with ASA 325 mg and ibuprofen completely inhibit the protective effects of rosuvastatin in the setting of IR injury, as does therapy with the specific COX-2 antagonist celecoxib. In contrast, therapy with low dose ASA (81 mg daily) does not have such inhibitory effects.

1. Introduction

Ischemia–reperfusion (IR) injury has been documented in a number of organs, and is particularly damaging in the heart [1]. A large body of evidence exists that brief periods of ischemia or exposure to certain pharmacologic agents can protect the heart from the effects of infarction and IR injury. To date, a number of pharmacologic agents, including (but not limited to) angiotensin converting enzyme inhibitors, angiotensin II receptor blockers, nitroglycerin and sildenafil have been demonstrated to have preconditioning effects, reducing the adverse effects of IR-injury [2–6]. Furthermore, drugs commonly used in cardiovascular disease may have adverse effects in that they either inhibit the development of the preconditioning phenotype and/or prevent pharmacologic preconditioning effects of other drugs. Indeed, given the increasingly large spectrum of pharmacotherapy available for patients with cardiovascular disease, a better understanding of the impact of drug therapy on preconditioning responses is of clear importance [7,8]. The 3-hydroxy-3-methylglutaryl coenzyme A (HMG Co-A) reductase inhibitors are effective as preconditioning agents, protecting against IR-injury and reducing infarct size in models of myocardial infarction [5,9,10]. The mechanism(s) behind this protection are complex, however recent animal and clinical studies suggest an important role for cyclooxygenase (COX)-2 and inducible nitric oxide synthase as mediators [5,11–13].

Recently, we documented that a single dose of rosuvastatin was a potent pharmacologic preconditioning agent, providing late window protection in a human forearm model of IR injury. We also demonstrated this preconditioning effect was completely abolished by the concurrent administration of the COX-2 inhibitor celecoxib, a finding consistent with a number of observations made in animal models [12]. Nonselective cyclooxygenase inhibitors are widely used in the general population, both in patients with and without overt coronary artery disease, including many who take HMG Co-A reductase inhibitors. Such agents, acetylsalicylic acid (ASA) and other nonsteroidal anti-inflammatory agents, could, theoretically, inhibit preconditioning induced by HMG Co-A reductase inhibitors, as well as other preconditioning stimuli. Here we report the result of an investigation examining the impact of ASA, the selective COX-2 inhibitor celecoxib and the nonselective COX inhibitor ibuprofen, as compared to placebo, on the protective effects of rosuvastatin in the setting of IR injury.

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2. Methods

The Mount Sinai Hospital Research Ethics Board approved the study and written informed consent was obtained from all subjects.

2.1. Subjects

Healthy, male, non-smoking volunteers (18 to 35 years old) were recruited for this study. Each subject's state of health was evaluated using a brief questionnaire that was completed during a consultation visit prior to enrolment. Exclusion criteria included any current disease or illness or the presence of risk factors for cardiovascular disease such as hypercholesterolemia, hypertension, atherosclerosis and smoking. All subjects were also requested to abstain from caffeine on the day of each study visit and from any drugs, including supplemental vitamins, for at least 2 weeks prior to and for the duration of the study.

2.2. Materials

All study drugs – including rosuvastatin (Crestor, AstraZeneca), ASA (Aspirin, Bayer), ibuprofen (Advil, Wyeth), celecoxib (Celebrex, Pfizer) and placebo (Lactose Tablets, Odan) – were purchased from the pharmacy department at Mount Sinai Hospital (Toronto, Ontario).

2.3. Study protocol (Fig. 1)

A summary of the protocol design can be seen in Fig. 1.

Visit 1 After screening for admission into the study, subjects returned to the laboratory at 8 AM following a 12-hour fast. Standing blood pressure and heart rate measurements were obtained, and 10 mL of venous blood was drawn for the assessment of plasma lipids. Subsequently, subjects were allocated to one of the five treatment arms: ASA 81 mg once daily, ASA 325 mg once daily, celecoxib 200 mg twice daily, ibuprofen 400 mg four times daily or matching placebo for the next 5–7 days.

Visit 2 Five to seven days after visit 1, subjects returned to the laboratory at 8 AM for the administration of a single dose of 40 mg rosuvastatin.

Visit 3 Twenty-four hours after rosuvastatin administration, subjects returned to the laboratory after a 12-hour fast and underwent repeated measures of standing blood pressure and heart rate. This was followed by the assessment of flow-mediated dilatation (FMD) of the radial artery before and after the induction of IR in the forearm vasculature. 10 mL of venous blood was again drawn at the end of this visit by a registered nurse for the assessment of plasma lipids.

2.4. Measurement of flow-mediated dilatation

Radial artery FMD was measured using a protocol that has been described and validated by our laboratory in previous studies [12,17]. Briefly, subjects rested in the supine position on a partially inclined bed for a minimum of 20 min to allow for proper acclimatization to the study environment. The subject's left arm was then positioned on a pillow and a pneumatic pressure cuff was placed around the wrist. Using an ultrasound probe (Vivid 7, General Electric), an image of the radial artery was captured and recorded onto an external computer for a period of 10 min. The 10-minute period was initiated by a 60 s of baseline imaging, followed by inflation of the pneumatic wrist cuff to 220 mmHg for 4.5 min to inhibit antegrade blood flow, causing temporary forearm ischemia. Following cuff deflation, the radial artery was continually imaged for an additional 4.5 min and FMD measured as the maximum radial diameter observed during this period. This procedure was performed before and after inducing IR in the forearm to evaluate changes in endothelial function afforded by IR-injury. A three lead ECG was recorded simultaneously with ultrasound images. Imaging of the radial artery and the ECG was recorded onto an external computer at ~60 frames/s, with the image capture triggered by the R-wave of each cardiac cycle. Edge detection software program (SpLiNeS — HeartWorks, Italy) was used to measure arterial diameters (from intima to intima). Radial artery FMD was calculated as follows: percent FMD = (peak diameter − baseline diameter) / baseline diameter.

2.5. Induction of IR in the forearm

Forearm ischemia was induced by inflating a sphygmomanometer (Welch Allyn) placed on the upper arm to a pressure of 220 mmHg for 20 min. At the end of this period of ischemia, the cuff was deflated to allow for 15 min of reperfusion. FMD was assessed before and after this process.

2.6. Measurement of forearm blood flow

Measurements of blood flow were made using a pulsed-wave Doppler system (Vivid 7, General Electric) at 3 different time points

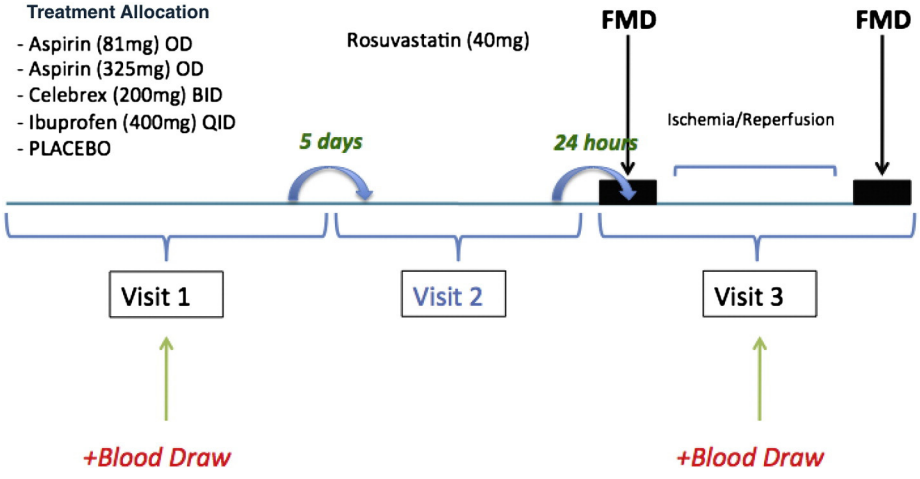

Fig. 1. Protocol design and sequence of study procedures. FMD, flow-mediated dilatation.

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