International Journal of Cardiology xxx (2017) xxx–xxx

Contents lists available at ScienceDirect

International Journal of Cardiology

journal homepage: www.elsevier.com/locate/ijcard

Atorvastatin combined with ticagrelor prevent ischemia-reperfusion induced vascular endothelial dysfunction in healthy young males – A randomized, placebo-controlled, double-blinded study

Stefan Weisshaar *[,1], Brigitte Litschauer [1], Tillmann Kerbel [1], Michael Wolzt [1]

Department of Clinical Pharmacology, Medical University of Vienna, Austria

ARTICLE INFO

Article history:
Received 19 September 2017
Received in revised form 28 November 2017
Accepted 16 December 2017
Available online xxxx

Keywords:
Atorvastatin
Ticagrelor
Ischemia-reperfusion injury
Forearm blood flow

ABSTRACT

Background: Atorvastatin and ticagrelor have been shown to prevent against tissue injury in animals. It is unclear if these beneficial effects are also detectable in humans. We studied the effect of high-dose atorvastatin combined with ticagrelor loading on endothelial dysfunction in a model of forearm vascular ischemia-reperfusion (IR) injury.
Methods: 32 healthy subjects (n = 16 per group) were enrolled in this randomized, placebo-controlled, double-blinded trial. Forearm blood flow (FBF) measurements in response to increasing intra-arterial doses of the vasodilator acetylcholine (ACh; endothelium-dependent agonist) and glyceryltrinitrate (GTN; endothelium-independent) were performed before and after a cuff-induced 20 min forearm ischemia, respectively. FBF reactivity was assessed prior to any pharmacological intervention and after 14 days intake of 80 mg atorvastatin once daily or placebo, followed by an oral loading dose of 180 mg ticagrelor. In addition, lipoprotein parameters and platelet aggregation were evaluated.
Results: Ticagrelor loading mitigated ischemia-induced endothelial dysfunction and in combination with repeated atorvastatin dosing the response to ACh during reperfusion was completely normalized (FBF ACh_{AUC} ratio post- vs. pre-ischemia: 0.81 [ticagrelor] vs. 1.04 [atorvastatin + ticagrelor]; P = 0.001). As expected, GTN-induced vasodilation was not affected by IR injury. Atorvastatin significantly reduced total and low density lipoprotein cholesterol concentrations, while high density lipoprotein cholesterol and triglyceride levels remained unchanged.
Conclusion: Chronic atorvastatin treatment combined with ticagrelor loading prevents against endothelial dysfunction after acute forearm ischemia. Ticagrelor alone mitigated the impaired endothelium-dependent FBF response as compared to no pharmacological intervention.
Clinical trial registration: URL: https://clinicaltrials.gov. Unique identifier: NCT02910778.

1. Introduction

Rapid reestablishment of perfusion to ischemic tissue is vital to prevent cell death but may adversely affect organ function due to paradoxical ischemia-reperfusion (IR) injury [1,2]. The vasculature has also been shown to be vulnerable to IR resulting in endothelial dysfunction with markedly suppressed endothelial nitric oxide (NO) synthase activity. Endothelial dysfunction itself with impaired NO-bioactivity and accumulation of reactive oxygen species (ROS), which have been proposed to further mediate tissue damage, is considered to contribute substantially to tissue damage in IR [3–7]. Further, it has been shown that incapacitated physiologic antioxidant mechanisms mediate a pro-inflammatory state with augmented platelet aggregation [8–13].

Activated thrombocytes are potent sources of ROS and further increase oxidative stress [14]. Previous studies have shown that antiplatelet medicines improve endothelial function in populations with a risk for ischemic events [15,16]. Preclinical animal data has suggested that ticagrelor, that has become a mainstay in the treatment of acute coronary syndrome (ACS), attenuates IR injury [17–19]. We have confirmed recently the finding that ticagrelor has greater salutary effects after a brief period of local ischemia on endothelial function during reperfusion than clopidogrel [20]. Although ticagrelor prevented vascular IR injury after acute occlusion more efficiently than clopidogrel, the microvascular pharmacological reactivity was not completely normalized. However, ticagrelor in combination with statins have been shown to exert additive protective effects on IR-induced injury in an animal model [21], where both medicines increased local adenosine levels following downstream activation of the endothelial NO synthase. It is unknown if the observed pleiotropic effects of ticagrelor and lipid lowering

* Corresponding author at: Medical University of Vienna, Department of Clinical Pharmacology, Währinger Gürtel 18-20, A-1090 Vienna, Austria.
E-mail address: stefan.weisshaar@meduniwien.ac.at (S. Weisshaar).
[1] This author takes responsibility for all aspects of the reliability and freedom from bias of the data presented and their discussed interpretation.

https://doi.org/10.1016/j.ijcard.2017.12.067
0167-5273/

drugs can be detected in humans either and, based on our previous own data, further ameliorate endothelial dysfunction during reperfusion after local ischemia.

The aim of this healthy volunteer study was to investigate if chronic treatment with atorvastatin for 14 days in combination with a ticagrelor loading dose may exert additive salutary effects on ischemia-induced impaired vasomotor response after a brief period of vascular occlusion as compared to ticagrelor treatment alone. In addition, the impact of local ischemia on the transient loss of endothelium-dependent vasodilation without pharmacological treatment was assessed. A previously described experimental approach of ischemia-induced endothelial dysfunction of the forearm resistance vasculature was employed, as IR injury studies in patient with acute ischemia are difficult to perform [22,23]. Available data suggests that the endothelium-dependent response of the forearm vasculature correlates with coronary artery reactivity and may serve as surrogate for coronary endothelial function [24].

2. Methods

Approval for the study was provided from the Ethics Committee of the Medical University of Vienna, Austria (EK 1591/2016), and the national competent authority. The trial protocol complies with the principles outlined in the Declaration of Helsinki, including current revisions, and the ICH Good Clinical Practice Guidelines. It is registered at ClinicalTrials.gov (NCT02910778) and at the European Clinical Trials database (EudraCT 2016-002378-11). All study participants gave written informed consent before enrolment.

2.1. Study population

43 healthy, non-smoking male volunteers aged 18 to 40 years with a body mass index (BMI = weight [kg] ∗ height [m] $^{-2}$) of 18 to 27 kg ∗ m^{-2} were evaluated for eligibility to randomize 32 subjects (Fig. 1). Exclusion criteria included any significant laboratory or physical finding; a history of cardio-vascular disease, renal, hepatic or gastrointestinal impairment; arterial hypertension, hypercholesterolemia or bleeding disorders; use of any medication within 2 weeks prior inclusion. The screening visit was scheduled 3–21 days before the first study day. Subjects abstained from caffeine or xanthine-containing beverages 24 h before the study days and from alcohol during the period of drug intake. Oscillometric blood pressure measurement at the screening visit was performed with an automatic device (Infinity® Delta monitoring system; Drägerwerk AG & Co. KGaA, Lübeck, Germany). This study was conducted in healthy subjects rather than patients with cardiovascular disease due to ethical concerns of discontinuation of pre-existing pharmacological treatment that may affect endothelial function [25–27]. Concomitant medication was not allowed, as previous own data suggest that medicines, which improve endothelium-dependent vasodilation, only exert salutary effects during reperfusion and not in the absence of vascular occlusion [20].

2.2. Study design & study drugs

This prospective, randomized, placebo-controlled, double-blinded trial was conducted between October 2016 and March 2017 at the Department of Clinical Pharmacology, Medical University of Vienna, Austria. Subjects were randomly allocated according to a predefined randomization code obtained from a web-based application to treatment with atorvastatin or placebo. Investigators directly involved in the study were not aware of the randomization sequence. Unblinded study staff dispensed the medicines under investigation and supervised oral intake.

Subjects received between the two study days that were 14 days apart 80 mg atorvastatin (Atorvastatin 80 mg Krka, Krka d.d., Novo mesto, Slovenia) once daily (qd) or placebo (Fagron GmbH & Co. KG, Barsbüttel, Germany) qd in combination with a loading dose of 180 mg ticagrelor (Brilique®, Astra Zeneca AB, Södertälje, Sweden) on the last treatment day (= day 15; Fig. 1). All study medicines were administered at approved doses for hypercholesterolemia or acute coronary syndrome. On the first study day atorvastatin or placebo was dispensed after FBF measurements and subjects were instructed to follow regular daily intake of atorvastatin or placebo in the evening from day 2–14. On the second assessment day (day 15) the study drugs (atorvastatin or placebo combined with ticagrelor) were administered 2 h prior forearm ischemia (Fig. 2). An end-of-study (EOS) visit was scheduled one week after the last treatment day.

2.3. Forearm blood flow measurements

Forearm blood flow (FBF) was assessed in both arms by strain gauge plethysmography as described previously [20,28,29]. Briefly, strain gauges, placed on both forearms, were connected to plethysmographs (EC-6; D.E. Hokanson Inc., Bellevue, WA, USA) for measurement of changes in forearm volume in response to inflation of congesting cuffs on the upper arms to a supravenous pressure (45 mm Hg). The distances from the elbows to the strain gauges were measured to ensure a comparable setting between study days. Measurements were recorded for 9 s at 30 s intervals during repeated inflation of the upper arm cuffs. The early linear increases of the curves were used for FBF analysis using the NIVP3 software (version 5.27, D.E. Hokanson Inc., Bellevue, WA, USA) and are expressed as ml ∗ min^{-1} ∗ 100 mL^{-1} forearm volume.

2.4. Experimental protocol

After an overnight fast FBF measurements were performed in a quiet room with an ambient constant temperature of 23 °C (±1 °C). FBF was assessed on both study days before and after the non-dominant forearm was made ischemic (Fig. 2).

Subjects were in a supine position and a 27 gauge fine-bore needle (Sol Care, Sol-Millennium Medical Inc., Lawrenceville, USA) was inserted into the brachial artery of the non-dominant arm for intra-arterial administration of vasoactive agents. Control (0.9% sodium chloride solution; Fresenius Kabi, Graz, Austria) FBF measurements were recorded over 5 min followed by assessing the response to the endothelium-dependent vasodilator

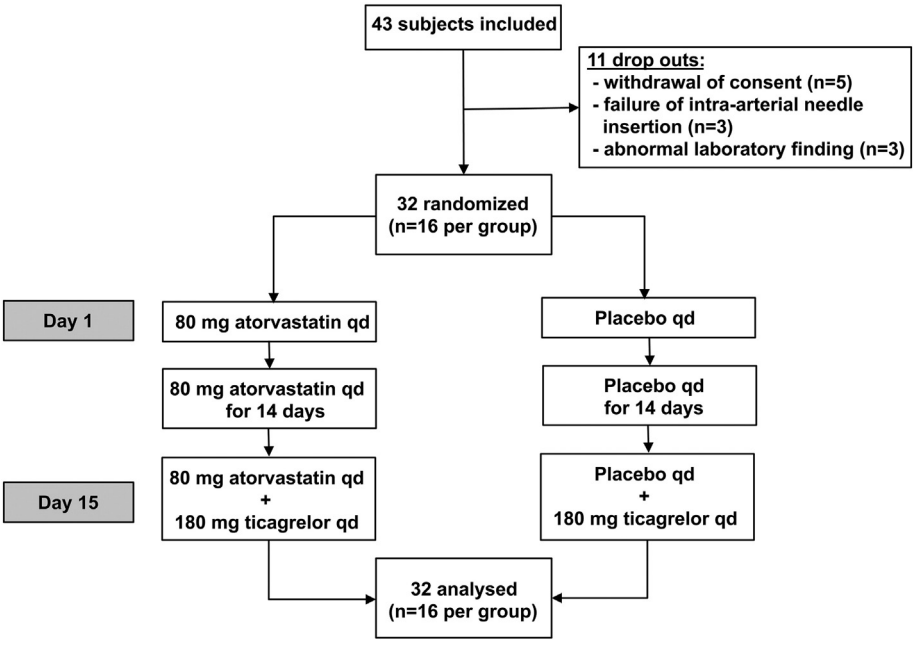

Fig. 1. Study flow diagram. qd = once daily.