European Journal of Pharmacology 651 (2011) 234–239

Contents lists available at ScienceDirect

European Journal of Pharmacology

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

Endocrine Pharmacology

The statins fluvastatin and pravastatin exert anti-flushing effects by improving vasomotor dysfunction through nitric oxide-mediated mechanisms in ovariectomized animals

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ARTICLE INFO

Article history:
Received 18 June 2010
Received in revised form 6 October 2010
Accepted 29 October 2010
Available online 27 November 2010

Keywords:
Estrogen
Fluvastatin
Hot flush
Nitric oxide
Pravastatin
Vascular reactivity

ABSTRACT

Statins have pleiotropic vascular protective effects that are independent of their cholesterol-lowering effects. The aim of the present study was to determine if statins have anti-flushing actions in an animal model of forced exercise-induced temperature dysregulation in menopausal hot flushes, and to clarify the critical role of statins in regulating vascular reactivity in the tail arteries of ovariectomized rats. Administration of fluvastatin or pravastatin (3 mg/kg/day for 7 days, p.o.) significantly ameliorated the flushing of tail skin in ovariectomized mice, and the effect of each statin was comparable with that of estrogen replacement (1 mg/kg/week for 3 weeks, i.m.). In phenylephrine-pre-contracted rat-tail arteries, ovariectomy inhibited acetylcholine-induced relaxation, but augmented sodium nitroprusside-induced relaxation. These ovariectomy-altered vasodilator responses were restored by fluvastatin treatment as well as by estrogen replacement. Nitrite/nitrate levels in the plasma of ovariectomized animals showed significantly lower values than those in sham-operated animals; this ovariectomy-reduced production of nitric oxide was improved by fluvastatin treatment. These data provide the first experimental evidence that statins such as fluvastatin and pravastatin exert anti-flushing effects by improving vasomotor dysfunction through nitric oxide-mediated mechanisms in ovariectomized animals. Thus, therapeutic methods that target improvement of vasomotor dysfunction could be novel strategies for reducing menopausal hot flushes.

1. Introduction

Hot flushes are the commonest symptoms occurring in women after age-related or surgery-induced menopause. This symptom manifests as a transient increase in skin temperature that is associated with objective signs of cutaneous vasodilation and a subsequent drop in core temperature. Accumulating evidence regarding the etiology of hot flushes suggests that vasomotor instability associated with a significant loss of estrogen underlies this pathophysiology (Freedman, 2001; Shanafelt et al., 2002; Stearns et al., 2002). It is well known that estrogen has anti-inflammatory and vasoprotective effects, and modulates vascular physiology and function in cellular, animal, and human models (Mendelsohn and Karas, 1999; Chambliss and Shaul, 2002; Florian et al., 2004; Orshal and Khalil, 2004; Guo et al., 2005, 2006; Xing et al., 2009). In the endothelium, estrogen enhances the production and release of nitric oxide (NO) by increasing the activity of endothelial nitric oxide synthase (eNOS) and/or expression of the NO-related gene. This suggests that NO-mediated mechanisms are involved in vasomotor dysfunction underlying hot flushes in menopausal females.

For many years, estrogen-based hormone replacement therapy has been the standard treatment for women experiencing menopausal hot flushes. However, results obtained from large clinical trials have demonstrated increased risks of stroke, thromboembolic events, heart disease, and breast cancer in women receiving long-term hormone replacement therapy (Women's Health Initiative Investigators, 2002). As alternatives to hormone replacement therapy, selective serotonin reuptake inhibitors and venlafaxine have been considered to be first-line therapy for hot flushes (Kalantaridou et al., 2008). However, selective serotonin reuptake inhibitors could interfere with the metabolism of tamoxifen in patients with breast cancer (Stearns et al., 2003). Therefore, the development of effective and safe non-hormonal treatments for patients with menopausal disorders (including hot flushes) is required.

Recent experimental and clinical evidence suggests that statins (i.e., 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase

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doi:10.1016/j.ejphar.2010.10.084

inhibitors) have cholesterol-independent ("pleiotropic") effects. The pleiotropic effects of statins include improving endothelial function, attenuating vascular and myocardial remodeling, and inhibiting inflammation and oxidation in vascular tissue (Laufs and Liao, 2000; Endres and Laufs, 2004; Zhou and Liao, 2010). Statins increase expression of eNOS and enhance NO production by endothelial cells (Laufs et al., 1998, 2000; Feron et al., 2001; Lefer et al., 2001). This suggests that statins may improve endothelial dysfunction and exert beneficial effects on menopausal hot flushes.

We recently demonstrated that serotonin reuptake inhibitors have anti-flushing effects using our animal model of temperature dysregulation in menopausal hot flushes (Shuto et al., 2005; Ikeda et al., 2008). The aim of the present study was to test whether the statins fluvastatin and pravastatin have anti-flushing actions using our animal model of menopausal hot flushes, and to clarify the critical role of statins in regulating vascular reactivity in the isolated tail artery of ovariectomized rats. We present the first experimental evidence that fluvastatin and pravastatin exert anti-flushing effects by improving vasomotor dysfunction through NO-mediated mechanisms in ovariectomized animals.

2. Materials and methods

All procedures involving experimental animals adhered to the law (number 105) and notification (number 6) of the Japanese Government. The study protocol was approved by the Laboratory Animal Care and Use Committee of Fukuoka University (Fukuoka, Japan).

2.1. Animals

Female ICR mice (25–30 g) and female Wister rats (180–200 g) were purchased from Kyudo (Kumamoto, Japan). Animals were maintained on a 12-h light–dark cycle (lights on at 7 am) at 24±1 °C with free access to food and water.

2.2. Drugs and reagents

The agents used for *in-vivo* study were fluvastatin and pravastatin (LKT Laboratories, Saint Paul, MN, USA). Reagents used for *ex-vivo* study were acetylcholine (Ach; Daiichi Pharmaceutical, Tokyo, Japan), phenylephrine (Wako Pure Chemical, Osaka, Japan) and sodium nitroprusside (SNP; Wako Pure Chemical). Other reagents were the highest purity available commercially. All drugs and reagents were dissolved in distilled water on the day of use.

2.3. Ovariectomy and estrogen replacement treatment

Mice or rats underwent bilateral ovariectomy or sham operation under anesthesia using sodium pentobarbital (50 mg/kg, i.p.). Vehicle (sesame oil) or estradiol valerate (1.0 mg/kg, i.m.; Pelanin Depot; Mochida Pharmaceutical, Tokyo, Japan) was injected into the thigh muscle at 0.01 ml/10 g body weight once a week for 3 weeks starting 7 days after surgery. The experiments were carried out 28 days after surgery. As previously described (Ikeda et al., 2008), the effects of ovariectomy and estrogen replacement were confirmed by measuring body weights and uterine weights at the time of the experiment.

2.4. Measurement and analyses of the temperature of tail skin in mice

Tail-skin temperature in mice was measured as described previously (Shuto et al., 2005; Ikeda et al., 2008). Conscious mice were restrained, and tail-skin temperature measured at the dorsal surface of the tail, ~1 cm from its base, with a thermo tracer (TH5108ME; NEC San-ei, Tokyo, Japan). Data were stored in 1-min blocks and analyzed with a Thermal Image Processing Program (TH51-701; NEC San-ei) and a Remote Control Program (TH51-723; NEC San-ei). Tail-skin temperature in each 1-min block was measured for 15 min just before forced exercise, and the mean temperature of the tail skin during the first 6-min period defined as basal tail-skin temperature. Mice were then forced to run (15 m/min) on a motor-driven treadmill (MK-680S; Muromachi Kikai, Tokyo, Japan) for 10 min. Tail-skin temperature in each 1-min block was measured for 15 min after forced exercise. Changes in the tail-skin temperature (Δtail-skin temperature) 1–6 min after forced exercise were assessed. Δtail-skin temperature was calculated using the following equation:

$$\Delta\text{tail-skin temperature} = (\text{taill-skin temperature in each 1-min block after forced exercise}) - (\text{mean basal temperature of the taill-skin at 1-6 min})$$

As shown in our previous study (Shuto et al., 2005; Ikeda et al., 2008), the tail-skin temperature in ovariectomized mice subjected to forced exercise shows a rapid and marked increase compared with that in sham-operated mice. The elevated tail-skin temperature in ovariectomized mice recovers to the sham-operated level within 7–8 min. Based on these time-courses of Δtail-skin temperature in sham-operated and ovariectomized mice, we evaluated the changes in tail-skin temperature by measuring Δtail-skin temperature 1–6 min after forced exercise (total Δtail-skin temperature). All measurements of tail-skin temperature were conducted between 11 am and 4 pm, and room temperature was maintained at 24±1 °C throughout the recording period.

2.5. Drug administration

For *in-vivo* study, fluvastatin (0.5, 1.0 and 3.0 mg/kg), pravastatin (0.5, 1.0 and 3.0 mg/kg), or distilled water as vehicle was orally administered to mice at 0.05 ml/10 g body weight once a day for 7 days, starting 21 days after surgery. For *ex-vivo* study, fluvastatin (3.0 mg/kg) was orally administered to rats at 0.05 ml/10 g body weight once a day for 7 days starting 21 days after surgery.

2.6. Preparation of rat-tail arterial rings

Rats were anesthetized with ether and decapitated. Arterial blood was collected in tubes containing ethylenediamine tetra-acetic acid (EDTA) and the tail artery quickly removed. Tail arteries were placed in chilled Krebs buffer solution (118.7 mM NaCl, 4.7 mM KCl, 1.8 mM $CaCl_2$, 1.2 mM KH_2PO_4, 1.2 mM $MgSO_4$, 24.8 mM $NaHCO_3$ and 10.1 mM glucose; pH 7.4). Arteries were freed from adipose tissue and connective tissue under a microscope and cut into rings (outer diameter, 0.8–1.2 mm; length, 2 mm). Each arterial ring was mounted on an L-shaped wire attached to a force–displacement transducer (T7-8-240; NEC Medical Systems, Tokyo, Japan) in an organ bath (US-5; UFER, Kyoto, Japan) containing 5 ml of Krebs buffer. The organ bath was maintained at 37 °C and bubbled with a mixture of 95% O_2 and 5% CO_2.

2.7. Vasoconstrictor and vasodilator responses in isolated rat-tail arterial rings

As previously described (Mizuta et al., 1995), arterial rings were adjusted to a resting tension of 0.3 g and allowed to stabilize for 2 h during which time the medium was replaced every 15 min. Before the start of experiments, equilibration was preliminary confirmed by the contraction response to 50 mM KCl. After the equilibration period, vasoconstrictor responses induced by phenylephrine (10^{-9}–10^{-4} M) in rat-tail arterial rings were assessed. Vasoconstrictor responses were expressed as a percentage of the tone generated by 100 mM KCl. We then evaluated the vasodilator responses induced by Ach (10^{-8}–10^{-4} M) and SNP (10^{-8}–10^{-4} M) in rat-tail arterial rings pre-contracted by