Oral contraceptive administration attenuates endothelium-dependent relaxation in response to histamine but not to acetylcholine in aortic rings of female rats

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Abstract

The incidence of vascular disorders is markedly lower in cycling, pre-menopausal women and post-menopausal women receiving estrogen-progestogen therapy than in men or untreated postmenopausal women. Clinical studies demonstrate that estrogen-progestogen therapy in pre-menopausal women is associated with increased arterial vascular risk. However, the mechanism of estrogen-progestogen therapy in arterial vascular complications remain unclear. Therefore, the present study aimed at investigating whether chronic administration of combined oral contraceptive (OC) containing progestogen with androgenic property alters endothelium-dependent relaxation responses of the aorta to histamine and acetylcholine. The experiments have been studied on aortic rings obtained from vehicle-treated and OC-treated female Sprague-Dawley rats. Isometric reactivity of aortic rings was recorded with a strain gauge transducer. The maximum contractile response of the aortic rings to noradrenaline in the OC-treated group was not significantly different from that in the vehicle-treated group. Both acetylcholine and histamine caused relaxation of the noradrenaline pre-contracted aortic rings. The relaxation response to acetylcholine in rings from vehicle-treated and OC-treated rats did not differ significantly, while relaxation response to histamine was significantly attenuated in aortic rings from OC-treated rats. In conclusion, the results from the present study suggest that the increased arterial vascular risk associated with OC administration might involve altered endothelium-dependent relaxation via histaminergic-mediated mechanism, but not via muscarinergic-mediated mechanism.

Key words: acetylcholine, endothelium-dependent relaxation, histamine, oral contraceptive

Introduction

The incidence of cardiovascular disease is considerably lower in cycling, pre-menopausal women and post-menopausal women receiving estrogen-progestogen therapy than in men or untreated postmenopausal women (Gerhard and Ganz, 1995; Reckelhoff, 2001; Orshal and...
Khalil, 2004). Studies have suggested that beneficial effects of estrogen-progestogen therapy in reducing the incidence of arterial cardiovascular diseases in postmenopausal women have putative vascular protective effects of female sex steroids hormones (Rosano et al., 1993; Gerhard and Ganz, 1995; Grodstein et al., 1996). Although, reports from the Heart and Estrogen-progestogen Replacement Study (HERS) and Women’s Health Initiative (WHI) studies do not support beneficial vascular effects of estrogen-progestogen therapy (Simon et al., 2001; Enstrom et al., 2002; Grimes and Lobo, 2002; Khan and Malhotra, 2003; Mills et al., 2003).

Increasing population puts strain on the world’s finite resources such as land, water and clean air. In the last decade, the prevalence of contraceptive usage has increased worldwide. Without contraception the individual is unable to choose when and how many children he/she has (Baird, 2000). At a global level, contraception is important in helping reduce overcrowding, pressures on resources, pollution and global warming, and a loss of animal species due to loss of habitat (ESHRE, 2005). It has been estimated that over 100 million women worldwide are on oral contraceptive (OC) pills (WHO, 1998). OC preparations are preferred method of contraception because of their ease of use and proven efficacy. Since the introduction some 50 years ago, studies have linked an increased risk of venous and arterial cardiovascular complications to OC use. The risk of venous complications is well documented (Burkman et al., 2001; Kemmeran et al., 2001), but the question of whether OC use increase the risk of arterial events in premenopausal women remains unanswered (Baillargeon et al., 2005).

The ability of estrogens and progesterone to modulate vascular smooth muscle (VSM) and endothelium in response to a variety of vasoactive substances has been reported (Wild and Reis, 2001). There is a compelling evidence to indicate that the ability of the endothelium to mediate vascular relaxation is impaired in large conduit arteries from patients (Ebeigbe et al., 1999) and from laboratory animals (Sim and Chua, 1985; Adegunloye and Sofola, 1997) with hypertension. Conversely, enhanced acetylcholine-induced relaxation has been reported in some forms of hypertension (Konishi and Su, 1983). Endothelium dysfunction is characterized by impaired endothelium-dependent relaxation, an early functional sign of atherosclerosis and is associated with increased future cardiac events (Ross, 1991; Schachinger et al., 2000). Impaired endothelial function occured within a year after suppression of ovarian production of estrogen by bilateral oophorectomy in premenopausal women and is improved following estrogen therapy (Pinto et al., 1997). Orally administered synthetic progestogens with androgenic properties, may counteract the beneficial effect of estrogen on vascular functions (Wild and Reis, 2001; Torgrimson et al., 2007.)

However, the effect of combined OC preparation containing progestogens with androgenic properties on endothelium-dependent relaxation is inconsistent and elusive. Furthermore, the interpretations of the data from human studies seem to be complicated by the differences in dose and/or type of preparations that have been used over the years as well as variation in terms of time of initiation and/or duration of treatment.

The present vehicle-controlled experimental study was therefore undertaken to determine the effect of endothelium-dependent relaxation in response to acetylcholine and histamine in aortic preparations of rats treated with a combination of OC steroids, ethinyl oestradiol and norgestrel.
Materials and Methods

Six week-old female Sprague-Dawley rats obtained from the animal House of the College of Health Sciences, University of Ilorin, (Ilorin, Nigeria), and housed in a well-ventilated room under a 12:12-h light-dark cycle; food (Bendel Feeds and Flourmills Ltd. Benin city, Nigeria) and water were available ad libitum. At eight weeks, rats were randomly divided into two groups (n=8 per group) of equal mean body weight and food intake namely; vehicle-treated and OC-treated. Vehicle-treated rats received 0.2 ml of olive oil (p.o.) daily whereas OC-treated rats received 0.2 ml of olive oil containing a combination of 10 µg of norgestrel and 1 µg of ethinyl estradiol (Wyeth-Ayerst, Inc, Montreal, Canada; p.o.) daily for 10 weeks. All experimental procedures involving animals and their care were conducted in accordance with the institutional and National Institutes of Health (NIH) guidelines on the care and use of laboratory animals.

Isolated vascular reactivity

At the end of the treatment period, the rats were weighed and anaesthetized with 5 ml/kg of a mixture 25% urethane and 1% α-chloralose (w/v; BDH Chemicals Ltd., Poole, England; i.p.) and euthanized by decapitation. A medial laparotomy was performed immediately to excise the thoracic aorta. The thoracic aorta was quickly and carefully excised and immediately placed in a petri dish filled with cold physiological salt solution (PSS) containing (in mmol/l) NaCl, 119; KCl, 4.7; NaHCO₃, 14.9; KH₂PO₄, 1.2; MgSO₄, 1.2; CaCl₂, 1.6 and glucose, 11.5. The vessel was cleaned of excess connective tissue and fat, and cut into 2-mm rings under the careful protection of the endothelium. The isolated rings were mounted on two parallel and horizontal stainless steel wires inserted into the lumen of the rings in a 20-ml organ bath containing PSS which was bubbled continuously with 95% O₂-5% CO₂ gas mixture. The PSS was maintained thermostatically at 37°C and adjusted to pH 7.4, respectively. One hook was fixed, and the other was connected to a strain gauges (FT O3 Grass Instrument Co., Quincy, MA, USA) which was coupled to a polygraph (7D Grass Instrument Co., Quincy, MA, USA) for the recording of isometric tension.

The arterial ring preparations were loaded to a tension of 2 g and allowed to equilibrate for 90 min in PSS maintained at 37 ± 0.5°C, pH 7.4; subsequently, specimens were bubbled with 95% O₂-5% CO₂. During the equilibration, the ring preparations were washed with PSS every 10–15 min and the tension was re-adjusted. During this period, each ring was stimulated with 10⁻⁷ M noradrenaline (NA) 3 times at 30 min interval with each stimulation lasting for 5 min. The endothelium was considered to be intact when the addition of 10⁻⁶ M acetylcholine (ACh) produced 100% relaxation of the pre-contraction caused by NA (10⁻⁸ M).

To evaluate endothelium-dependent relaxation responses to ACh and histamine, endothelium-intact rings were pre-contracted with a submaximal concentration of NA (10⁻⁷ M). As the plateaux of the contraction is obtained, ACh (10⁻⁹ to 10⁻⁵ M) was added cumulatively. ACh was added such that the effect of the previous concentration had become maximal before the next one was added. The same procedure was followed for histamine as with relaxation-response to ACh except that histamine was added at concentrations of 10⁻⁶ to 10⁻³ M. The sensitivity to the agonists was evaluated as EC₅₀.
Drugs

The drugs used were noradrenaline bitartrate, acetylcholine chloride and histamine dihydrochloride. All these drugs were supplied by Sigma Chemicals (St Louis, MO, USA). OC steroids (norgestrel and ethinyl estradiol) were supplied by Wyeth-Ayerst, Inc. (Montreal, Canada). All other chemicals and materials were used at the highest analytical grade commercially available.

Statistical analyses

Analysis of data was conducted with SPSS statistical analysis software (Version 13.0; SPSS Inc, IL., USA). Results were expressed as the mean ± SEM. Group means were compared for two independent samples using Student’s t-test. A P value of less than 0.05 was taken as statistically significant. EC_{50} (the negative logarithm of the concentration of ACh and histamine required to produce 50% of the maximal response) was determined using a program for logic transformation of concentration-response curves. Relaxation responses for ACh and histamine were expressed as percentage decrease of the maximum contractile response induced by noradrenaline.

Results

The maximum tension produced by NA and contractile sensitivity in rings from OC-treated rats were not significantly different from that obtained from the vehicle-treated rats (Table 1). The relaxation response induced by histamine was significantly attenuated in aortic rings obtained from OC-treated rats when compared with that from vehicle-treated rats (Table 1, Fig. 1). However, the relaxation response induced by ACh in rings from OC-treated was not significantly different from that of the vehicle-treated rats (Table 1, Fig. 2).

Discussion

In this study we found that OC administration attenuated endothelium-dependent relaxation in response to histamine in aortic rings. The endothelium-dependent relaxation to ACh was preserved. To the best of our knowledge, this is the first investigation of the effects of co-administration of oral contraceptive steroids, norgestrel and ethinyl oestradiol on endothelium-dependent vascular relaxation evoked by histamine. Our results on endothelium-dependent relaxation by ACh is consistent with earlier studies in humans (John et al., 2000; Virdis et al., 2003) that demonstrated that OC use in healthy premenopausal women could not alter endothelium-dependent vasodilation. However, recent studied by Torgrimson et al. (2007) reported that flow-mediated, endothelium-dependent vasodilation of the brachial artery was significantly attenuated among premenopausal women using OC containing levonorgestrel and ethinyl oestradiol while women using a relatively higher dose of the preparation had unaltered flow-mediated, endothelium-dependent vasodilation. Furthermore, OC use has been reported to improve flow-mediated, endothelium-dependent vasodilation in amenorrheic athletes, but not in regularly cycling athletes (Rickenlund et al., 2005).
Premenopausal women using OC are at increased risk of arterial vascular disorders such as arterial hypertension, myocardial infarction, ischemic brain infarction (Jick et al., 1996, Tanis et al., 2001). All these conditions are known to be associated with impaired endothelial function (Ross, 1993). Although the mechanism for the increased arterial vascular disorders associated with OC use have not been fully established. Impaired endothelial function could be a possible factor. The role of endothelium-dependent relaxation in high blood pressure associated with OC administration has not been well defined in OC-induced high blood pressure.

Treating female Sprague-Dawley rats with OC steroids is known to result in increased blood pressure (Fowler et al., 1985; Olatunji and Soladoye, 2006; 2008). This study employed female Sprague-Dawley rats treated with a combination of norgestrel and ethinyl estradiol at the dose that resulted in increased arterial blood pressure of magnitude comparable with those observed among OC users (Kang et al., 2001; Ahmed et al., 2004). To our knowledge, there are no previous data on the influence of OC administration on endothelium-dependent vasorelaxation in female Sprague-Dawley rat model with OC-induced high blood pressure.

The vascular endothelium plays an important role in the regulation of vascular reactivity through synthesis and release of various vasodilating and vasoconstricting substances (Luscher

|                     | Histamine | ACh |
|---------------------|-----------|-----|
|                     | Vehicle-Treated | OC-Treated | Vehicle-Treated | OC-Treated |
| EC₅₀ (M)            | 2.5 ± 1.0 (× 10⁻⁴) | 6.2 ± 0.8 (× 10⁻⁴)* | 2.0 ± 0.9 (× 10⁻⁴) | 1.7 ± 1.0 (× 10⁻⁴) |
| Maximum relaxation (%) | 93.8 ± 6.2 | 54.4 ± 4.1* | 75.8 ± 4.9 | 69.3 ± 4.3 |
| Tension (mN)        | 13.9 ± 1.7 | 11.2 ± 1.6 | 14.5 ± 1.5 | 11.5 ± 1.3 |

Results are expressed as means ± SEM of 8 observations per group. *, P<0.05 vs. vehicle-treated.
Studies indicate that impaired endothelium-dependent vascular relaxation in conduit arteries is thought to contribute to the etiology of arterial vascular disorders in humans (Egbeigbe et al., 1999; Cohen, 1995) and in experimental animals (Sim and Chua, 1985; Lockette et al., 1986; Adegunloye and Sofola, 1997). However, there are other studies that have shown that endothelial function is not impaired in some forms of hypertension (Konishi and Su, 1983; Aloamaka et al., 1995). Impaired endothelium-dependent relaxation response to ACh classically reflects endothelium dysfunction, an early functional sign of atherosclerosis and is associated with increased future cardiac event (Ross, 1993; Schachinger et al., 2000). Therefore, the lack of a significant difference in ACh-induced endothelium-dependent relaxation response of aortic rings from both groups of rats suggests that OC administration would preserve endothelial function.

Histamine can invoke endothelium-dependent relaxation in several vascular beds.
Relaxant effect in aorta of OC-treated rats

(Furchgott and Vanhoutte, 1999). The attenuated endothelium-dependent relaxation response of aortic rings from OC-treated rats to histamine may thus not reflect impaired endothelium-dependent relaxing factor (EDRF). Histamine stimulates the release of EDRF (Martin et al., 1985; Yang et al., 1985), as well as the contraction of VSM (Sim and Singh, 1987; Yang et al., 1989). Hence, the impaired relaxation of aortic rings from OC-treated rats to histamine may be due to hypersensitivity of underlying VSM. This finding is in consonance with reported observations in pregnancy-induced hypertension in humans (Ebeigbe and Cabanie, 1992) and in various animal hypertensive models (Sim and Chua, 1984; Lockette, et al., 1986; Adegunloye and Sofola, 1997).

There was no significant difference in the maximum tension induced by NA in the aortic rings from both rats (Table 1), thus, the vascular responses to ACh or histamine between the two groups seem to rule out the influence of variable contractile/structural property of the VSM. Both ACh and histamine evoke endothelium-dependent vasorelaxation via different receptor-mediated mechanisms (Obiefuna et al., 1991; Adegunloye and Sofola, 1997). Thus, these findings imply that the arterial vascular risk associated with OC administration might involve altered endothelium-dependent relaxation via histaminergic-mediated mechanism but not via muscarinergic mechanism. The deviant effects of endothelium-dependent relaxations of aortic preparations from OC-treated rats by ACh and histamine has been demonstrated in some forms of experimental hypertension (Sim and Chua, 1985; Obiefuna et al., 1991; Adegunloye and Sofola, 1997).

The explanation for impaired histamine-induced endothelium relaxation response may be attributed to the progestogenic component of the OC used because progestogen with androgenic properties such as norgestrel employed in the present study might counteract the favourable effect of estrogen on vascular functions (Wild and Ries, 2001; Wakatuski et al., 2003; Torgrimson et al., 2007). Furthermore, reports suggest that progestogens with androgenic properties have some direct effect on endothelial function that might include inhibition of endothelia cell migration and VSM proliferation (Schnaper et al., 2000). Moreover, long-term use of progestogen-only OC in premenopausal women has been linked to impaired flow-mediated endothelium-dependent relaxation (Sorensen et al., 2002).

In conclusion, the results of the present study in female Sprague-Dawley rats demonstrated that OC (norgestrel/ethinyl estradiol) is associated with attenuated endothelium-dependent relaxation in response to histamine but not to ACh. The results suggest that the OC-related arterial vascular risks might involve altered endothelium-dependent relaxation via histaminergic mediated mechanism but not via muscarinergic-mediated mechanism.

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