17β-Estradiol Inhibits Endothelin-1 Production and Attenuates Cerebral Vasospasm After Experimental Subarachnoid Hemorrhage

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Though cerebral vasospasm after aneurysmal subarachnoid hemorrhage (SAH) has been recognized for over half a century, it remains a major complication in patients with SAH. Clinical studies have shown that elevated levels of endothelin-1 (ET-1) are present in the cerebrospinal fluid of patients with SAH, suggesting that ET-1–mediated vasoconstriction contributes to vascular constriction after SAH. Administration of estrogen promotes vasodilation in humans and in experimental animals, in part by decreasing the production of ET-1. This study evaluated the influence of 17β-estradiol (E2) on the production of ET-1 and cerebrovasospasm in an experimental SAH 2-hemorrhage model in rat. A 30-mm Silastic tube filled with E2 in corn oil (0.3 mg/ml) was subcutaneously implanted in male rats just before SAH induction. The degree of vasospasm was determined by averaging the cross-sectional areas of basilar artery 7 days after first SAH. Plasma samples collected before death were assayed for ET-1. The protective effect of E2 in attenuating vasospasm achieved statistical significance when compared with the SAH only or SAH plus vehicle groups ($P < 0.01$). Concentrations of ET-1 were higher in the SAH only and SAH plus vehicle groups than in controls ($P < 0.001$). Serum levels of ET-1 in the SAH plus E2 and E2 only groups were significantly lower than those in the SAH only and SAH plus vehicle groups ($P < 0.001$). There was no significant difference between ET-1 levels in the healthy control and SAH plus E2 groups. A significant correlation was found between the cross-sectional areas of basilar artery and ET-1 levels ($P < 0.001$). The beneficial effect of E2 in attenuating SAH-induced vasospasm may be due in part to decreasing ET-1 production after SAH. The role of E2 in the treatment of cerebral vasospasm after SAH is promising and is worthy of further investigation. Exp Biol Med 231:1054–1057, 2006

Key words: estrogen; subarachnoid hemorrhage; vasospasm; endothelin-1

Introduction

Delayed cerebral vasospasm remains an unpredictable and inadequately treated complication of aneurysmal subarachnoid hemorrhage (SAH) (1). Despite intensive research, its pathogenesis is still a matter of debate, and adequate pharmacotherapy has been elusive. In recent years, increasing evidence has implicated endothelins (ETs) in the pathophysiology of cerebral vasospasm after SAH (2). Endothelin-1 (ET-1) is the most potent endogenous vasoconstrictors yet identified. Various studies have demonstrated that the increased ET levels in the cerebrospinal fluid (CSF), plasma and the basilar artery after SAH (3, 4), suggesting that ET-1–mediated vasoconstriction contributes to vascular constriction after SAH. The potential involvement of ETs in SAH-induced vasospasm has triggered considerable interest in therapeutic strategies that inhibit the biological effects of ET. Such strategies include (i) blocking the biosynthesis of ET-1, (ii) reducing extracellular ET-1 levels by specific anti–ET-1 antibodies, and (iii) antagonizing ET receptors.

Extensive epidemiologic observations, clinical mechanistic studies, and basic laboratory research have suggested that estrogen therapy has a cardioprotective effect in postmenopausal women (5). By stimulating prostacyclin and nitric oxide synthesis, and by decreasing the production

This work was supported by The National Science Council, ROC, under grant NSC91-2314-B-037-309.

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Received September 25, 2005.
Accepted November 10, 2005.

1535-3702/06/2316-1054$15.00

of vasoconstrictor agents such as cyclooxygenase-derived products, reactive oxygen species, angiotensin II, and ET-1, estrogens elicit vasodilatory and antiatherogenic actions (5). *In vitro*, estrogens inhibit angiotensin-II–induced cell proliferation and ET-1 gene expression, partially by interfering with the extracellular signal-regulated kinase pathway *via* attenuation of reactive oxygen species generation (6). Several clinical studies on the effect of oral or transdermal hormone therapy have demonstrated a beneficial reduction of ET blood levels (7). This study evaluated the influence of the female sex steroid 17β-estradiol (E2), which is the most active natural estrogen, on SAH-induce vasospasm and the production of ET-1 in an experimental rat SAH model.

Materials and Methods

Animal Preparation and General Procedures. All procedures were approved by the Kaohsiung Medical University Animal Care and Use Committee. Forty Sprague-Dawley male rats, each weighting 380–435 g, were divided evenly into the following five groups: control (no SAH, no E2), SAH only, SAH plus vehicle, SAH plus E2, and control plus E2. 17β-Estradiol was given by sc implantation of a 30-mm–long Silastic tube E2 pellet (2-mm i.d., 4-mm o.d.) containing 0.3 mg/ml corn oil 1 hr after the first induction of SAH.

In our previous study, implantation of an E2 Silastic tube maintained stable serum E2 concentration for at least 7 days. The E2 concentration of the E2-treated group (56–92 pg/ml) increased significantly when compared with the levels of the control group (26–40 pg/ml) and SAH plus vehicle group (28–36 pg/ml; $P < 0.01$ at Days 1, 2, and 3; $P < 0.05$ at Days 4 and 7).

Experimental SAH. Rats were anesthetized by an ip injection of pentobarbital (50 mg/kg). Each animal's head was fixed in stereotactic frame and the cisterna magna was punctured percutaneously with a 25-gauge butterfly needle. About 0.1–0.15 ml CSF was slowly withdrawn and the junction of butterfly needle and tube was clamped. Freshly autologous nonheparinized blood (0.3 ml) was withdrawn from the tail artery. Blood was injected slowly into the cisterna magna. The same procedure was repeated 48 hrs later. Thus, in this study, the term SAH refers to an experimental model of SAH produced by double injection of blood into the cisterna magna (2-hemorrhage SAH model). Seven days after the first SAH, animals were killed by perfusion and fixation. Then the brain was removed, placed in a fixative solution, and stored at 4°C overnight.

Tissue Morphometry. Basilar arterial tissues were removed from the brain stems and the middle third of each artery was dissected for analysis. Cross sections of basilar arteries were cut at a thickness of 0.5 μm with an ultramicrotome, mounted on glass slides, and stained with toluidine blue for morphometric analysis. Five random arterial cross sections from each animal were analyzed, and cross-sectional areas were measured using computer-assisted morphometry (Image 1; Universal Imaging Corp., West Chester, PA). For group comparisons, ANOVA with the Bonferroni post hoc test was performed. Differences were considered to be significant at $P < 0.05$.

Measurement of ET-1. Blood samples (1 ml) were collected from each rat before death through the tail artery, and then perfused into a plastic tube containing EDTA and aprotinin. Blood samples were centrifuged at 1600 *g* for 10 mins at 4°C and assayed using commercially available ET-1 radioimmunoassay kit (Phoenix Pharmaceuticals, Belmont, CA).

Results

General observations. Prior to perfusion-fixation, there were no significant differences among the treatment groups in the physiological parameters recorded, including body weight, pH, $PaCO_2$, PaO_2, and mean arterial blood pressure. A thick subarachnoid clot was observed over the basal surface of the brain stem in each animal subjected to SAH.

Cross-Sectional Luminal Area Measurements. The average cross-sectional areas of basilar arteries were reduced by 42% and 41% in the SAH only and SAH plus vehicle groups, respectively, when compared with control areas. The cross-sectional area of the E2-treated group differed significantly from that of the SAH only and SAH plus vehicle group ($P < 0.01$ and $P < 0.005$, respectively). The average cross-sectional areas of the healthy animals with the administration of E2 also differed significantly from that of the SAH only and SAH plus vehicle group ($P < 0.01$ and $P < 0.005$, respectively). There was no significant difference between areas in the treatment group and the healthy animals (Fig. 1).

ET-1 Plasma Level. ET-1 concentrations were higher in SAH only and SAH plus vehicle groups than the healthy control ($P < 0.001$). Serum levels of ET-1 in SAH plus E2 and E2 only groups were significantly lower than those in the SAH only and SAH plus vehicle groups ($P < 0.001$; Fig. 2). There is no significant difference between the ET-1 levels in the control healthy and SAH plus E2 groups. Significant correlation was found between the cross-sectional areas of basilar artery and ET-1 levels ($P < 0.01$).

Discussion

Substantial evidence has accrued in recent years implicating the potent vasoconstrictor peptide, ET-1, in the pathophysiology of cerebral vasospasm after aneurysmal SAH (2–4). First, various studies have demonstrated increased ET levels in the CSF, plasma, and basilar artery after SAH (3, 4). Increased serum ECE-1 activity during the second week after aneurysmal SAH may reflect the severity of endothelial injury to cerebral arteries (8). Menon *et al.* (10) demonstrated that larger arteriojugular gradient differences may predict vasospasm in patients with aneurysmal

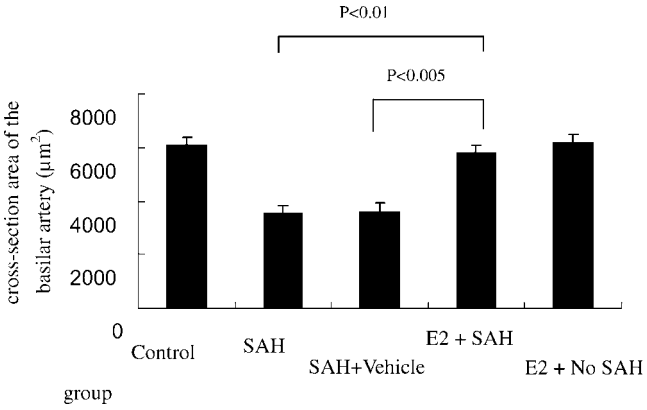

Figure 1. Bar graph showing the effects of E2 on cerebral vasospasm in cross-sectional areas. The average luminal area (mean ± SEM) of cross sections of basilar arteries is demonstrated for each group of animals. The cross-sectional areas of E2-treated animals did not differ significantly from those of controls. The protective effect of E2 was significant when compared with SAH only or SAH plus vehicle ($P < 0.01$ and $P < 0.005$, respectively).

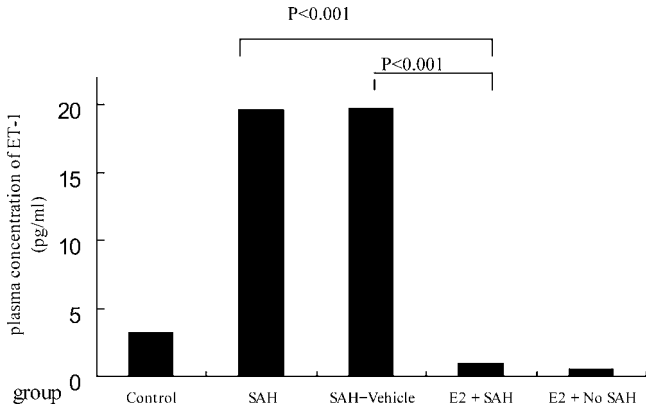

Figure 2. Concentrations of ET-1 were higher in the SAH only and SAH plus vehicle groups than in controls ($P < 0.001$). Serum levels of ET-1 were significantly lower in the SAH plus E2 and E2 only groups than in the SAH only and SAH plus vehicle groups ($P < 0.001$). No significant difference in ET-1 levels was found between the healthy control and SAH plus E2 groups.

SAH (9). Second, delayed vasospasm can be experimentally evoked by the administration of ET-1. Third, increased expression of ET_A and ET_B receptors after experimental SAH was found in dogs and in monkeys and rats, respectively (2). Fourth, antagonists of ET-1 attenuate vasospasm in experimental models of SAH (11). However, some studies have found no correlation between the increased levels of ETs and SAH (12, 13).

Unlike other kinds of stroke, aneurysmal SAH occurs more frequently in women than in men (14). Gender differences in the outcome of SAH are controversial, and the influence of the female sex hormone is unclear. However, estrogens have been found to exert neuroprotective effects in models of ischemic stroke both *in vitro* and *in vivo* (15). Estradiol induces vasodilation *via* both genomic and nongenomic mechanisms that cause generation of vasodilatory agents, such as nitric oxide, cGMP, cAMP, adenosine, and prostacyclin, and alterations in ion channel activity (16). Estradiol also induces vasodilatory effects on the vasculature by influencing membrane fluidity and ion channel activity (16).

Estradiol also has been implicated in cardiovascular protection in postmenopausal women and inhibits experimental atherosclerosis (17). It also causes downregulation of prepro–ET-1 (the precursor of ET-1) mRNA and protein both *in vitro* and *in vivo* (18, 19). In addition, increased expression of prepro–ET-1 mRNA has been observed in porcine aortic endothelial cells in the absence of female ovarian hormones (5). It has been reported that E2 attenuates ET-1–induced coronary artery constriction both *in vitro* and *in vivo*, and inhibits angiotensin II–induced ET-1 gene expression in rat cardiac fibroblasts (20). Studies conducted on healthy postmenopausal women who received continuous hormone therapy report increased plasma nitrite/nitrate levels and decreased ET-1 levels (7, 21). Taken together, these results suggest that E2 may be a good candidate for the treatment of SAH-induced vasospasm. After reviewing the literatures, we found only 1 study reporting the effect of E2 in an experimental SAH animal model (22). Yang *et al.* (22) induced experimental SAH by an endovascular technique in ovariectomized female rats with a physiologic dose of E2, and demonstrated that E2 can reduce mortality and secondary ischemic damage without affecting the clot volume. However, the mechanism of E2 in the attenuating vasospasm after SAH was not surveyed.

In this study, we present the first evidence that SAH causes an increase in ET-1 serum levels, and that E2 treatment attenuates ET-1 levels and vasospasm. Our findings further highlight that, in a 2-hemorrhage SAH rat model, the continuous systemic administration of physiologic doses of E2 is effective in the treatment of vasospasm without producing cardiovascular complications. The protective effects of estrogen may be partially due to downregulation of plasma ET-1 levels after SAH. Estradiol may provide an alternative therapeutic modality in the treatment of patients with delayed-onset vasospasm.

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