Available online at www.sciencedirect.com

SciVerse ScienceDirect

journal homepage: www.JournalofSurgicalResearch.com

17β-Estradiol attenuates secondary injury through activation of Akt signaling via estrogen receptor alpha in rat brain following subarachnoid hemorrhage

Cheng-Hsing Kao, MD,[a,b] *Chih-Zen Chang, MD,*[c,d,e] *Yu-Feng Su, MD,*[c,d] *Yee-Jean Tsai, MS,*[c] *Kao-Ping Chang, MD,*[d,f] *Tzu-Kang Lin, MD,*[g] *Shiuh-Lin Hwang, MD, PhD,*[c,d] *and Chih-Lung Lin, MD, PhD*[c,d,*]

[a] *Center for General Education, Southern Taiwan University of Technology, Tainan, Taiwan*
[b] *Department of Neurosurgery, Chi Mei Medical Center, Tainan, Taiwan*
[c] *Department of Neurosurgery, Kaohsiung Medical University Hospital, Kaohsiung, Taiwan*
[d] *Faculty of Medicine, Graduate Institute of Medicine, College of Medicine, Kaohsiung Medical University, Kaohsiung, Taiwan*
[e] *Department of Surgery, Kaohsiung Municipal Ta-Tung Hospital, Kaohsiung, Taiwan, Republic of China*
[f] *Division of Plastic and Reconstructive Surgery, Department of Surgery, Kaohsiung Medical University Hospital, Kaohsiung, Taiwan*
[g] *Department of Neurosurgery, Chang Gung Memorial Hospital, Tao-Yuan, Taiwan*

ARTICLE INFO

Article history:
Received 25 July 2012
Received in revised form
12 January 2013
Accepted 17 January 2013
Available online 8 February 2013

Keywords:
Estrogen
Estrogen receptor-α
Subarachnoid hemorrhage
Cerebral vasospasm
PI3K/Akt

ABSTRACT

Background: Apoptosis is implicated in vasospasm and the long-term sequelae of subarachnoid hemorrhage (SAH). This study tested the hypothesis that attenuation of SAH-induced apoptosis after 17β-estradiol (E2) treatment is associated with an increase in phosphorylation of Akt via estrogen receptor-α (ER-α) in rats.

Materials and methods: We examined the expression of phospho-Akt, ERα and ERβ, and apoptosis in cerebral cortex, hippocampus, and dentate gyrus in a two-hemorrhage SAH model in rats. We subcutaneously implanted other rats with a silicone rubber tube containing E2; they received daily injections of nonselective estrogen receptor antagonist (ICI 182,780), selective ERα-selective antagonist (methyl-piperidino-pyrazole), or ERβ-selective antagonist (R,R-tetrahydrochrysene) after the first hemorrhage.

Results: At 7 d after the first SAH, protein levels of phospho-Akt and ERα were significantly decreased and caspase-3 was significantly increased in the dentate gyrus. The cell death assay revealed that DNA fragmentation was significantly increased in the dentate gyrus. Those actions were reversed by E2 and blocked by ICI 182,780 and methyl-piperidino-pyrazole, but not *R,R*-tetrahydrochrysene. However, there were no significant changes in the expression of the protein levels of phospho-Akt, ERα, ERβ, and caspase-3, and DNA fragmentation after SAH.

Conclusions: The present study shows that a beneficial effect of E2 in attenuating SAH-induced apoptosis is associated with activation of the expression of phospho-Akt and ERα, and alteration in caspase-3 protein expression via an ERα-dependent mechanism in the dentate gyrus. These data support further the investigation of E2 in the treatment of SAH in humans.

* *Corresponding author*. Department of Neurosurgery, Kaohsiung Medical University Hospital, No. 100 Tz-you 1st Road, Koahsiung City 807, Taiwan. Tel.: +886 7 3121101 extension 5880; fax: +886 7 3215049.
E-mail address: chihlung1@yahoo.com (C.-L. Lin).
0022-4804/$ – see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.jss.2013.01.033

1. Introduction

Although cerebral vasospasm after aneurysmal subarachnoid hemorrhage (SAH) has been recognized for more than half a century, its pathophysiologic mechanism remains illusive and no effective treatment for cerebral vasospasm exists to date [1]. Apoptosis of granule cells in the dentate gyrus, which is a frequent observation in several animal models of the disease, is detected in patients dying of SAH [2]. It has also been shown that apoptosis in the vasculature has a significant role in SAH, and that the apoptotic cascades may be responsible for vasospasm [3,4]. Furthermore, cell death after SAH has important implications not only for vasospasm, but also as the long-term sequelae of SAH [3]. Our previous studies have revealed that 17β-estradiol (E2) could attenuate experimental SAH-induced cerebral vasospasm by inhibiting endothelin-1 production [5], preventing the augmentation of inducible nitric oxide synthase expression, and preserving the normal endothelial nitric oxide synthase (eNOS) expression after SAH [6]. The prevention of increased inducible nitric oxide synthase expression is achieved by interfering with nuclear factor-κB (NF-κB) transactivation via an estrogen receptor (ER)-dependent mechanism [7]. Our other findings also demonstrated that E2 reverses the decreased expression of adenosine A_1 receptors (AR-A_1) and increases the expression of adenosine A_{2A} receptors (AR- A_{2A}) to prevent vasospasm and apoptosis induced by SAH in the dentate gyrus [8–11].

Concerning the role of female sex hormone E2 in the long-term sequelae of SAH, especially related to apoptosis, its influence has yet to be determined [12]. Recent studies have shown that estrogen offers neuroprotection against both neurodegenerative diseases and brain injury [13]. This was previously revealed in an animal study, in which estrogen was effective in attenuating neuronal death after focal and global ischemia [14]. Clinically, estrogen replacement therapy in postmenopausal women reduces the incidence of stroke, the extent of ischemic neurodegeneration [15], and the onset and severity of Alzheimer disease [16].

Akt, a serine/threonine protein kinase also known as proteinase B, has an important role in the cell death/survival pathway in multiple cell lineages by inhibiting apoptosis [17,18]. It is activated by phosphorylation at the Ser473 residue and acts downstream of the phosphoinositide 3-kinase (PI3-K) pathway [17]. To begin with, PI3-K is responsible for the recruitment of Akt to the cellular membrane and the activation of Akt [19]. Upon activation, PI3-K then induces Akt phosphorylation, which in turn phosphorylates and blocks the actions of BAD, a proapoptotic member of the Bcl-2 family [18]. Besides, PI3-K also alters the activities of a number of other proapoptotic mediators, including caspase-9, forkhead, and NF-κB [20–22]. Moreover, activated Akt also phosphorylates and inhibits downstream substrates, including glycogen synthase kinase-3β (GSK3β) [23], which leads to neuronal resistance to apoptotic stimuli through the promotion of cell survival and the suppression of apoptosis [24,25]. Currently, the Akt survival pathway in cerebral ischemia, traumatic brain injury, spinal cord injury, and SAH has been established to be involved in the mechanisms of apoptotic neuronal death [26–30]. However, the role of Akt survival pathway in the antiapoptotic effect of E2 against brain injury has not been studied.

In the present study, we aimed to clarify the following points: (1) whether SAH leads to apoptosis in rat brain in the two-hemorrhage SAH model; whether SAH down-regulates estrogen receptor α (ERα) and phospho-Akt in rat brain after SAH; whether E2 activates ERα and phospho-Akt and alternates the activity of caspase-3; and whether E2 has an antiapoptotic effect against brain injury after SAH via an ERα-dependent mechanism.

2. Materials and methods

2.1. Animal preparation

The Kaohsiung Medical University Animal Research Committee approved all experimental protocols. We used as subjects male Sprague-Dawley rats (Education Research Resource, National Laboratory Animal Center, Taiwan), weighing approximately 338–389 g. We kept these experimental rats on a 12-h light/dark cycle, with free access to food and water. They were evenly divided into seven groups. Rats in group 1 served as controls and were not subjected to SAH (control; $n = 12$). The rats in all other groups were subjected to experimental SAH, as described below. Rats in group 2 received experimental SAH without additional treatment (SAH only; $n = 12$). Rats in group 3 received experimental SAH with vehicle treatment (SAH plus vehicle; $n = 12$). Rats in group 4 received experimental SAH with E2 treatment (SAH plus E2; $n = 12$). We delivered E2 by subcutaneous implantation of a 30-mm-long silicone rubber tube with a 2-mm inner diameter and 4-mm outer diameter (Shin-Etsu Polymer Co, Ltd, Japan) containing 0.3 mg/mL 17β-estradiol benzoate in corn oil (Sigma), giving rise to the physiologic range of plasma E2 concentrations (56–92 pg/mL) [6]. Rats in group 5 received experimental SAH with E2 and ICI 182,780 (a nonselective ER antagonist) (Tocris, UK) treatment (SAH plus E2 plus ICI; $n = 12$). We gave ICI 182,780 (2 mg/kg/d, intraperitoneally) for 7 d after the first hemorrhage. We dissolved ICI 182,780 in corn oil (Sigma) to make a final 20% solution. To study the involvement of ERα or ERβ in E2-mediated antiapoptosis, we treated rats in group 6 (SAH plus E2 plus methyl-piperidino-pyrazole [MPP]; $n = 12$) and group 7 (SAH plus E2 plus R,R-tetrahydrochrysene [R,R-THC]; $n = 12$) with E2. They received daily subcutaneous injection of ERα-selective antagonist (MPP, 2.0 mg/kg) (Tocris) or ERβ-selective antagonist (R,R-THC, 0.1 mg/kg) (Tocris) for 7 d after the first hemorrhage. We modified the antagonizing dose of MPP from a mouse study by Davis *et al.* [31] and modified the relative antagonizing dose for R,R-THC from a microinjection study in rats proposed by Gingerich and Krukoff [32].

2.2. Induction of experimental SAH

We anesthetized the rats by an intraperitoneal injection of pentobarbital (50 mg/kg). We fixed the head of each animal in