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The selective estrogen receptor modulator, bazedoxifene, reduces ischemic brain damage in male rat

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HIGHLIGHTS

- Acute 17β-estradiol reduces total infarct volume after transient focal cerebral ischemia in male rats.
- Bazedoxifene mimics the neuroprotective action of 17β-estradiol.
- A regional selectivity seems to exist for both 17β-estradiol and bazedoxifene.
- Bazedoxifene could be a candidate in stroke treatment deserving further research.

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ABSTRACT

While the estrogen treatment of stroke is under debate, selective estrogen receptor modulators (SERMs) arise as a promising alternative. We hypothesize that bazedoxifene (acetate, BZA), a third generation SERM approved for the treatment of postmenopausal osteoporosis, reduces ischemic brain damage in a rat model of transient focal cerebral ischemia. For comparative purposes, the neuroprotective effect of 17β-estradiol (E2) has also been assessed. Male Wistar rats underwent 60 min middle cerebral artery occlusion (intraluminal thread technique), and grouped according to treatment: vehicle-, E2- and BZA-treated rats. Optimal plasma concentrations of E2 (45.6 ± 7.8 pg/ml) and BZA (20.7 ± 2.1 ng/ml) were achieved 4 h after onset of ischemia, and maintained until the end of the procedure (24 h). Neurofunctional score and volume of the damaged brain regions were the main end points. At 24 h after ischemia–reperfusion, neurofunctional examination of the animals did not show significant differences among the three experimental groups. By contrast, both E2- and BZA-treated groups showed significantly lower total infarct volumes, BZA acting mainly in the cortical region and E2 acting mainly at the subcortical level. Our results demonstrate that: (1) E2 at physiological plasma levels in female rats is neuroprotective in male rats when given at the acute stage of the ischemic challenge and (2) BZA at clinically relevant plasma levels mimics the neuroprotective action of E2 and could be, therefore, a candidate in stroke treatment.

1. Introduction

While the neuroprotective role of 17β-estradiol (E2) in a variety of CNS diseases including stroke has repeatedly been shown in a variety of experimental setups [1,2], in the clinical setting E2 has been reported to increase or, at best, do not modify a woman's risk of stroke [3]. While the reasons for such translational failure are under debate, the enormous advance in the understanding of the estrogenic system in the brain has raised a variety of potential interventions for the prevention and/or treatment of stroke. Of particular interest are some natural and synthetic compounds

Abbreviations: SERMs, selective estrogen receptor modulators; BZA, bazedoxifene; E2, 17β-estradiol; MCAO, middle cerebral artery occlusion; CP, cerebrocortical laser-Doppler flow (cortical perfusion); ABP, arterial blood pressure; T, core temperature; LC–ESI-MS, liquid chromatography–electrospray ionization-mass spectrometry; TTC, 2,3,5-triphenyltetrazolium chloride; ER, estrogen receptors.

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Table 1
Distribution of animals excluded from the study in accordance with the criteria established.

	MCAO + vehicle	MCAO + 17β-estradiol	MCAO + bazedoxifene
Criterion 1 (no ischemia)	5	3	1
Criterion 2 (no reperfusion)	5	1	2
Criterion 3 (no infarction)	2	0	3
Criterion 4 (death)	4	5	0

aimed to mimic the beneficial role of E2, while being devoid of its detrimental effects. On the whole, these compounds are currently named "selective estrogen receptor modulators" (SERMs). Ideally, a SERM should demonstrate agonistic (protective) effects in bone (osteoporosis), brain (cognitive status) and cardiovascular system (vasomotor symptoms, coronary heart disease), and neutral or antagonistic (safe) effects in breast and uterine tissues (cancer) [4].

Among natural SERMs, soy-derived isoflavones (e.g. genistein) have received particular attention. We and others have demonstrated that soy-based high-isoflavone diets as well as genistein, reduced cerebral infarct size in rat models of ischemic stroke [5–9]. On the other hand, synthetic SERMs are an ever-growing family of compounds aimed at preventing/treating diseases derived from estrogen deprivation (natural or hysterectomized postmenopausal women), including impaired brain function [10]. Some "classical" SERMs such as raloxifene and tamoxifen have shown a neuroprotective action in several paradigms of neural damage [11]. Bazedoxifene (acetate, BZA) is the first third-generation SERM approved for the treatment of postmenopausal osteoporosis in the EU and Japan [12]. Since BZA has been reported to prevent neuronal loss in the hippocampus of rats exposed to kainic acid [13], and to decrease the inflammatory response of astrocytes exposed to lipopolysaccharide [14], we hypothesize that such neuroprotective role of BZA can be extended to ischemic stroke as well. To test such a hypothesis, the ability of BZA to minimize the impact of an ischemic challenge has been assessed in parallel with that of E2, by using a rat model of transient focal cerebral ischemia. Both treatments were administered at the acute stage of the ischemic challenge, what may have strong clinical implications if non-reproductive estrogen-like compounds can be designed (e.g. SERMs), and used as the treatment as opposed to the preventive method [15].

2. Materials and methods

Experimental procedures were conducted in compliance with the Spanish legislation on "Protection of Animals used for Experimental and other Scientific Purposes" (RD 53/2013), and in accordance with the EC Directive 86/609/EEC.

2.1. Animals

Sixty-nine male Wistar rats weighing 300–350 g (Charles River, Barcelona, Spain) were housed under standard environmental conditions, and fed an isoflavone-free chow (TD96155 diet, Harlan Teklad, Barcelona, Spain) with water ad libitum.

2.2. Surgical procedure

Transient occlusion of the middle cerebral artery (MCAO) was performed by following the intraluminal suture procedure as originally described [16], and adapted to our experimental setup [5]. This includes continuous monitoring under anesthesia of cerebrocortical laser-Doppler flow (cortical perfusion, CP), arterial blood pressure (ABP) and core temperature (T), and discontinuous measurement of blood pH, PaO_2, $PaCO_2$ and glucose at the three stages during surgery: pre-ischemia (basal), ischemia and reperfusion. MCAO was maintained for 60 min, after which the filament was carefully retracted to allow reperfusion which was monitored for 30 min.

2.3. Experimental groups: exclusion criteria and drug dosages

Thirty-one rats were excluded from the study according to the following criteria: (1) CP did not drop during filament insertion (no ischemia); (2) CP did not recover after filament withdrawal (no reperfusion); (3) no brain infarction in spite of a right ischemia–reperfusion pattern; and (4) death before the 24 h time limit. Distribution of the animals excluded in each group for each criterion is presented in Table 1. Therefore, three MCAO groups were established: vehicle- (dimethyl sulfoxide, $n = 12$, controls), E2- ($n = 8$), and BZA- ($n = 11$) treated rats. Treatments were applied 1 h before ischemia in such a way that optimal plasma concentrations were reached 4 h later, and were sustained until the end of the procedure (24 h). The E2-treated rats received a single dose (s.c.) of 30 μg/kg E2, followed immediately afterwards by implantation (i.p.) of an osmotic pump (Alzet model 2ML1 with a pumping rate of 10 μl/h, Durect Corp., Cupertino, CA, USA) delivering 100 μg/kg/day E2. The BZA-treated rats received a single dose (s.c.) of 1 mg/kg BZA, followed immediately afterwards by implantation (i.p.) of the same osmotic pump model delivering 3 mg/kg/day BZA. Such dosages were established from two additional groups of non-operated rats in which the plasma concentration-versus-time curves for E2 ($n = 3$) and BZA ($n = 4$) were obtained. Blood samples were obtained at 0, 30 min, 2, 4, 8 and 24 h. Plasma concentrations of E2 (Sigma–Aldrich, Madrid, Spain) were determined by the IMMULITE 1000 Estradiol procedure (Siemens Healthcare España, Getafe, Madrid, Spain). Plasma concentrations of BZA (Axon Medchem, Groningen, The Netherlands) were determined by the liquid chromatography–electrospray ionization-mass spectrometry (LC–ESI-MS) technique (Waters, Barcelona, Spain). Plasma concentrations of E2 and BZA at 4 h were 45.6 ± 7.8 pg/ml and 20.7 ± 2.1 ng/ml, respectively (Fig. 1). Since both E2 and BZA were dissolved in 100% dimethyl sulfoxide (1 mg/ml stock), animals in all three experimental groups received the same amount of dimethyl sulfoxide: 1 ml/kg as the initial bolus, plus 10 μl/h during 24 h (osmotic pump). This represents a total amount of 500–600 μl.

2.4. Assessment of ischemic brain damage

At 24 h after ischemia–reperfusion, neurofunctional condition was examined just before euthanization based on four tests: (a) spontaneous activity; (b) circling to the left; (c) parachute reflex; and (d) resistance to left forepaw stretching. Total score could range from 0 (no neurological deficits) to 9 (highest neurological deficits) [5]. To measure cerebral infarct volume rats were euthanized by intracardiac 5 mEq KCl under anesthesia. The whole brain was removed and infarct volume was determined by the 2,3,5-triphenyltetrazolium chloride (TTC) vital staining method [17], followed by morphometric analysis. In order to correct the influence of edema, infarcted volume was calculated as follows: corrected infarct volume = infarct volume × [contralateral hemisphere volume/ipsilateral hemisphere volume]. The operational sequence was applied separately to cortex and subcortical regions.