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Protective effects of estrogen combined with sevoflurane in an experimental model of cerebral infarction and focal cerebral ischemia-reperfusion injury

J.-J. LIU, S.-Y. PAN

Department of Neurology, Nanfang Hospital, Southern Medical University, Guangzhou City, Guangdong Province, People's Republic of China

Abstract. – OBJECTIVE: Potential additive effects of estrogens and sevoflurane against cerebral infarction after transient or permanent middle cerebral artery occlusion (MCAO) have not been addressed. We evaluated these using a rat model of MCAO.

MATERIALS AND METHODS: 60 adult female Sprague-Dawley rats were used in the experiments after ovariectomy. Animals were divided into placebo/no MCAO, placebo + MCAO, and estrogen + MCAO groups. Each group was further subdivided into subgroups exposed to sevoflurane or oxygen. Animals in the placebo group received intraperitoneal injections of saline, whereas the estrogen group animals received intraperitoneal injections of estradiol (1 mg per day). MCAO was performed 1 week after the ovariectomy. Sevoflurane and oxygen subgroups breathed either sevoflurane or oxygen for 30 min during the surgery. Outcomes were the levels of serum estradiol (E2), interleukin (IL)-6, and beta-amyloid protein (β-AP) (all by ELISA), neurological deficit scores (24 hours, 7 and 28 days after the operation), spatial learning and memory (both by the Morris water maze test on days 7 and 28).

RESULTS: MCAO significantly up-regulated serum levels of IL-6 and β-AP ($p < 0.05$ for both comparisons). The animals that received the combined treatment with estrogen and sevoflurane showed less extensive up-regulation of these markers ($p < 0.05$ *vs.* placebo-treated animals). Furthermore, MCAO induced severe neurological dysfunction and disorders of spatial learning and memory. All these were attenuated by the combined treatment.

CONCLUSIONS: We demonstrate neuroprotective effects of pre-conditioning with estrogen and post-conditioning sevoflurane in experimental animal undergoing MCAO.

Key Words:
Estrogen, Sevoflurane, cerebral infarction, Reperfusion, Interleulin-6, beta amyloid protein.

Introduction

Cerebral infarction and focal cerebral ischemia-reperfusion injury are the main injuries leading to irreversible brain damage[1]. As ischemic tolerance of brain is limited, oxidative metabolic abnormalities and free radical-induced neuron damage occur. It would be beneficial to be able to attenuate the extent of cerebral infarction and focal cerebral ischemia-reperfusion injury to increase the windows of opportunity for therapeutic interventions[2].

Neuroprotective properties of estrogens and their analogues have been in the focus of research for several years now[3]. The interest arises from observations of gender differences in the incidence and outcomes of stroke, which suggests that factors related to sexual hormones may modulate the susceptibility to stroke[4]. Specifically, neuroprotective effects of estrogens have been documented in different types of neuronal cells exposed to various harmful conditions, including serum deprivation, oxidative stress, amyloid β peptide, and excitotoxicity[5,6]. These observations are applicable to both endogenous and exogenous estrogens[3].

Another potential neuroprotective agent is sevoflurane. Sevoflurane is a novel volatile anaesthetic with minimal pungency and is a preferable drug for anaesthesia[3,7]. In this regard, it has recently been shown that post-conditioning for 15 min with sevoflurane after 1-hour ischemia provides neuroprotection in experimental animals[8].

To date, no studies have been conducted on potential additive effects of estrogens and sevoflurane against cerebral infarction after transient or permanent middle cerebral artery occlusion (MCAO). To this end, we addressed this in the present study using a rat model of MCAO.

Corresponding Author: Suyue Pan, MD; e-mail: pansuyue82@yahoo.com.cn

Materials and Methods

Animals

The study was conducted in strict accordance with the National Committee for Animal Research of our Hospital. The protocol has been approved by the Hospital Ethics Committee for Animal Care and Use.

A total of 60 adult female Sprague-Dawley (SD) rats weighing between 220 and 240 g were used in the experiments. The rats were kept in cages (3 animals per cage) at a constant room temperature (23 ± 1° C) with free access to water and standard rat chow, and with 12 hours of light-dark cycle. All rats were subjected to ovariectomy and randomly divided into the following study groups: placebo/no MCAO, placebo + MCAO, and estrogen + MCAO groups. In addition, animals of each of the aforementioned group have been randomly divided into subgroups exposed to sevoflurane or oxygen. Thus, there were six subgroups in total: placebo with MCAO and sevoflurane, placebo with MCAO and oxygen, estrogen with MCAO and sevoflurane, estrogen with MCAO and oxygen, placebo without MCAO and sevoflurane, placebo without MCAO and oxygen.

Animals in the placebo group received intraperitoneal injections of saline, whereas the estrogen group animals received intraperitoneal injections of estradiol (1 mg per day). MCAO was performed 1 week after the ovariectomy. Sevoflurane subgroups breathed sevoflurane for 30 min during the surgery, whereas oxygen subgroup breathed oxygen for 30 min during the surgery.

MCAO

Animals were anesthetized by intraperitoneal injection of 10% kessodrate (350 mg/kg). For this, rats were placed with their left side facing up on a thermostatic heating pad, and rectal temperature was maintained at 37.0 ± 0.5° C. The right common carotid artery, right external carotid artery, and right internal carotid artery were exposed through the midline neck incision. The proximal part of the common carotid artery was ligated, and a monofilament nylon suture wrapped in silicone was inserted into the internal carotid artery to a point of approximately 18 mm distal of the carotid bifurcation incision. The suture was then pulled out for reperfusion after 90 min and the wound was closed. Rats that were not exposed to MCAO underwent the same procedure with the exception of the suture. Blood pressure, arterial pH, pO_2, pCO_2, heart beat rate, and temperature were closely monitored during the experiment. Two hours after the operation, cerebrospinal fluid and serum specimens were collected. After the operation, all rats recovered from anaesthesia.

As outcomes, we quantified the levels of serum estradiol (E2), interleukin (IL)-6, and beta-amyloid protein (β-AP) by respective ELISA assays. Serum was obtained as follows. Two ml of blood were collected, coagulated at room temperature, and subjected to centrifugation at 300 rpm for 5 min. The supernatants were kept at -30° C. ELISA assays were performed according to the manufacturers' instructions.

Also, neurological deficit scores, spatial learning and memory were evaluated on days 7 and 28 after the operation. Furthermore, neurological deficit scores were also evaluated at 24 hours after the operation[9]. The following scoring system was utilized: "0" – no nerve injury symptoms, "1" – the animal could not fully expend the left forepaw, "2" – the animal circled to the left, "3" – the animal dumped to the left, and "4" – the animal could not walk spontaneously. The spatial learning and memory were evaluated using the Morris water maze test. The procedure followed the previous reports [10]. In experiments, we used video image capturing which was analyzed with computer image analysis software (Biological Equipment, Zhenghua, Huaibei China).

Statistical Analysis

The data have been presented as mean ± SEM. Results of the Morris water maze tests were compared with the two-way analysis of variance (ANOVA) followed by Tukey post-hoc test. Other data were compared using the *t* or one-way ANOVA tests. The differences were considered significant when $p < 0.05$.

Results

We analysed blood pressure, arterial pH, pO_2, pCO_2, heart beat rate and rectal temperature at three-time points of the operation: suture placement, reperfusion, and 45 min after reperfusion. There were no significant differences among study subgroups in tested parameters (Table I).

The data on E2, IL-6 and β-AP levels are presented in Table II. E2 levels in the subgroups that received estrogen were significantly ($p <$

Table I. Physiological indices.

Stage of the operation and study groups	Blood pressure (mm Hg)	Arterial pH	pO_2	pCO_2	Heart beat rate (beats per min)
Suture placement					
Placebo with MCAO and sevoflurane	115 ± 4	7.39 ± 0.02	75.7 ± 0.7	48.0 ± 1.9	96 ± 4
Placebo with MCAO and oxygen	102 ± 3	7.38 ± 0.02	75.3 ± 1.7	47.2 ± 0.9	106 ± 12
Estrogen with MCAO and sevoflurane	103 ± 6	7.40 ± 0.01	76.9 ± 2.7	49.3 ± 2.1	116 ± 11
Estrogen with MCAO and oxygen	101 ± 3	7.38 ± 0.02	75.3 ± 0.3	47.6 ± 1.6	102 ± 11
Placebo without MCAO and sevoflurane	110 ± 8	7.41 ± 0.02	74.0 ± 2.2	46.4 ± 1.4	111 ± 7
Placebo without MCAO and oxygen	112 ± 9	7.37 ± 0.01	75.2 ± 1.9	45.9 ± 1.8	108 ± 9
Reperfusion					
Placebo with MCAO and sevoflurane	113 ± 6	7.42 ± 0.01	75.0 ± 1.1	46.6 ± 1.4	99 ± 7
Placebo with MCAO and oxygen	109 ± 3	7.40 ± 0.02	74.7 ± 2.2	47.3 ± 1.6	106 ± 8
Estrogen with MCAO and sevoflurane	108 ± 6	7.39 ± 0.02	74.3 ± 1.2	48.3 ± 1.5	99 ± 8
Estrogen with MCAO and oxygen	107 ± 7	7.37 ± 0.02	73.2 ± 2.3	48.6 ± 1.5	102 ± 7
Placebo without MCAO and sevoflurane	106 ± 9	7.42 ± 0.02	75.7 ± 0.7	47.2 ± 1.3	101 ± 9
Placebo without MCAO and oxygen	107 ± 8	7.38 ± 0.01	75.7 ± 0.7	48.5 ± 2.5	112 ± 4
45 min after reperfusion					
Placebo with MCAO and sevoflurane	110 ± 6	7.39 ± 0.02	75.7 ± 0.7	46.0 ± 2.1	96 ± 14
Placebo with MCAO and oxygen	105 ± 3	7.38 ± 0.01	75.7 ± 0.7	45.9 ± 2.9	104 ± 12
Estrogen with MCAO and sevoflurane	110 ± 8	7.40 ± 0.01	75.7 ± 0.7	48.3 ± 1.9	101 ± 13
Estrogen with MCAO and oxygen	104 ± 7	7.41 ± 0.02	75.7 ± 0.7	47.0 ± 1.3	102 ± 7
Placebo without MCAO and sevoflurane	107 ± 2	7.38 ± 0.02	75.7 ± 0.7	46.3 ± 2.5	104 ± 6
Placebo without MCAO and oxygen	113 ± 5	7.37 ± 0.02	75.7 ± 0.7	46.4 ± 2.2	103 ± 7

Footnote: Data are expressed as mean ± SEM.

Table II. Levels of E2, IL-6 and β-AP.

Study groups	E2 (pg/ml)	IL-6 (pg/mL)	β-AP (μg/L)
Placebo with MCAO and sevoflurane	3.55 ± 0.54	362.66 ± 55.53	25.03 ± 2.49
Placebo with MCAO and oxygen	3.67 ± 0.36	385.21 ± 43.42	27.30 ± 2.35
Estrogen with MCAO and sevoflurane	16.71 ± 3.07	200.57 ± 37.49	19.03 ± 4.02
Estrogen with MCAO and oxygen	17.07 ± 2.67	209.64 ± 39.43	21.31 ± 3.21
Placebo without MCAO and sevoflurane	3.37 ± 0.47	90.27 ± 12.92	16.30 ± 2.09
Placebo without MCAO and oxygen	3.21 ± 0.27	89.67 ± 14.32	15.02 ± 1.89

Footnote: Data are expressed as mean ± SEM.

0.05) higher than those in placebo subgroups, whereas there was no difference among subgroups that received placebo. MCAO let to up-regulation of serum IL-6 levels (Table II). The estrogen-treated animals showed significantly lower up-regulation of IL-6 levels than animals on placebo ($p < 0.05$; Table II). Comparable differences were observed with regard to the levels of β-AP in animals that received estrogen vs. placebo (Table II).

The cerebral infarction and focal cerebral ischemia-reperfusion of rats induced severe neurological dysfunction, as demonstrated by elevated neurological deficit scores (Figure 1).

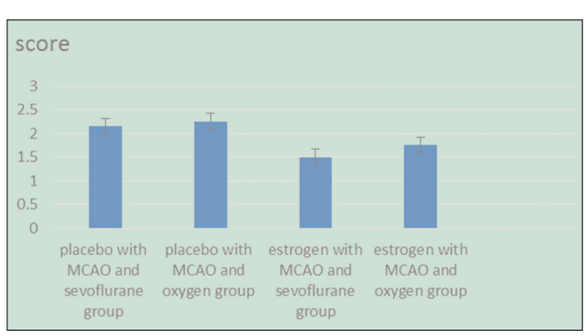

Figure 1. Neurological deficit scores in study groups. Data are expressed as mean ± SEM.

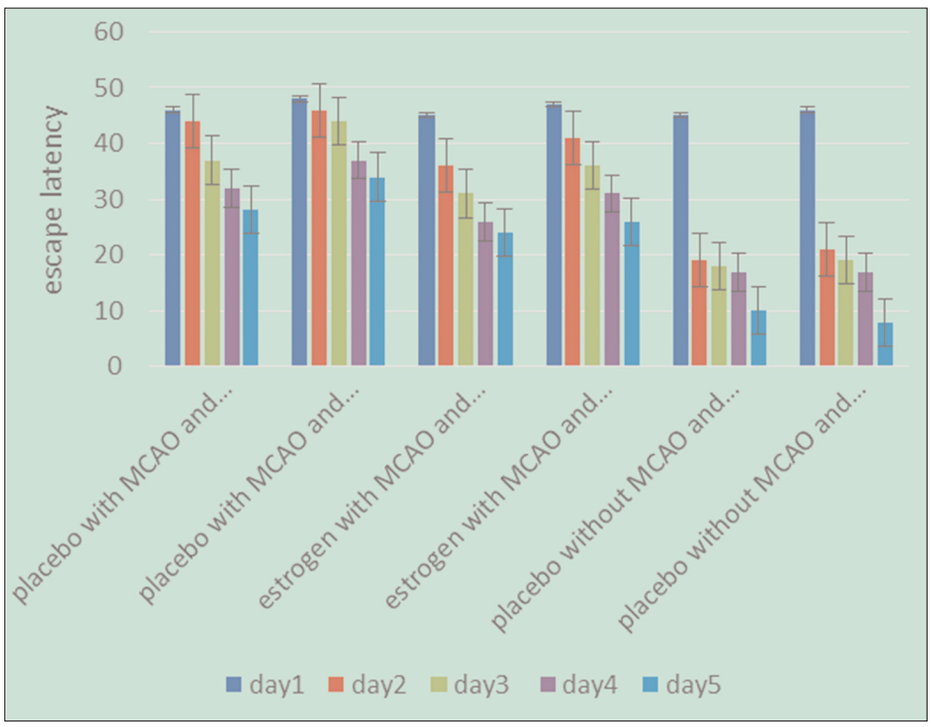

Figure 2. Escape latency in study groups. Data are expressed as mean ± SEM.

Specifically, neurological deficit scores of animals that received placebo and MCAO were higher than those of animals exposed to estrogen with MCAO ($p < 0.05$; Figure 1). In addition, animals in sevoflurane and oxygen subgroups also showed significantly different neurological deficit scores ($p < 0.05$; Figure 1). In contrast, there was no significant difference between animals in placebo/MCAO subgroups treated with sevoflurane and animals in placebo/MCAO subgroups treated with oxygen (Figure 1).

We next assessed spatial learning and memory by the Morris water maze test. Rats in all study subgroups showed a gradual decline in escape latency (Figure 2). However, on day 5 rats in the subgroup exposed to placebo/MCAO and oxygen required more time to find the platform than those that were treated estrogen and sevoflurane (Figure 2). This finding was corroborated by the number of target crosses, which was lower in the rats exposed to placebo and oxygen than in those treated with estrogen and sevoflurane (Figure 3). The swimming speed was also significantly different between the rats treated with estrogen and sevoflurane group and those treated with placebo and oxygen group (Figure 4).

Discussion

We demonstrated in this work that preconditioning of experimental animals with estrogen and post-conditioning with sevoflurane attenuated negative consequences of focal cerebral ischemia and reperfusion injury. Specifically, the intervention improved neurological deficit score and indices of learning and memory. Post-condi-

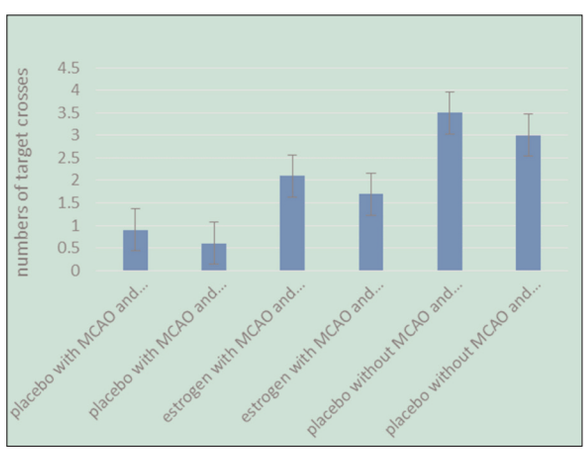

Figure 3. Number of target crosses in study groups. Data are expressed as mean ± SEM.

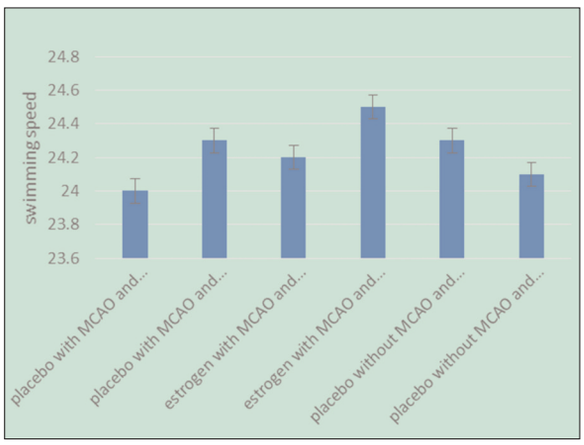

Figure 4. Swimming speed in study groups. Data are expressed as mean ± SEM.

tioning with sevoflurane, thus, seems to be a safe and easy option to diminish the neurotoxicity.

Furthermore, when estrogen and sevoflurane were combined, they decreased systemic levels of the pro-inflammatory cytokine IL-6 in MCAO animals. This implies that the combination of both compounds may reduce the inflammatory damage to nerve cells[11-13]. In addition, we documented that combination of both compounds modulated the levels of β-AP. β-AP is one of the breakdown products of the Amyloid Precursor Protein. β-AP has multiple biochemical functions[14], including regulation of cell apoptosis[15], complement system[16], and inflammation[17]. Levels of β-AP indicate the extent of the cerebral infarction. Estrogen and sevoflurane may block the P13K/Akt signalling pathway by diminishing the deposition of β-AP in cerebral vessels[18], and this could explain the neuroprotective mechanism of the combined treatment[19]. As a result, animals treated with both estrogen and sevoflurane showed better neurological deficit scores and indices of spatial learning and memory. The mechanism of estrogen and sevoflurane may be additive[20,21] or complementary[22,23].

Conclusions

We demonstrate neuroprotective effects of pre-conditioning with estrogen and post-conditioning sevoflurane in experimental animal undergoing MCAO. This suggests that this combination could be used as an intervention to prolong the therapeutic window of cerebral circulation intervention for the neurosurgical procedure.

Conflict of Interest

The Authors declare that there are no conflicts of interest.

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