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ORIGINAL PAPER

Effect of estrogen and/or progesterone administration on traumatic brain injury-caused brain edema: the changes of aquaporin-4 and interleukin-6

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Abstract The role of aquaporin-4 (AQP4) and interleukin-6 (IL-6) in the development of brain edema post-traumatic brain injury (TBI) has been indicated. The present study was designed to investigate the effect(s) of administration of progesterone (P) and/or estrogen (E) on brain water content, AQP4 expression, and IL-6 levels post-TBI. The ovariectomized rats were divided into 11 groups: sham, one vehicle, two vehicles, E1, E2, P1, P2, E1 + P1, E1 + P2, E2 + P1, and E2 + P2. The brain AQP4 expression, IL-6 levels, and water content were evaluated 24 h after TBI induced by Marmarou's method. The low (E1 and P1) and high (E2 and P2) doses of estrogen and progesterone were administered 30 min post-TBI. The results showed that brain water content and AQP4 expression decreased in the E1, E2, P1, and P2-treated groups. The administration of E1 decreased IL-6 levels. Addition of progesterone decreased the inhibitory effect of E1 and E2 on the accumulation of water in the brain. Administration of E1 + P1 and E1 + P2 decreased the inhibitory effect of E1 on the IL-6 levels and AQP4 protein expression. Our findings suggest that estrogen or progesterone by itself has more effective roles in decrease of brain edema than combination of both. Possible mechanism may be mediated by the alteration of AQP4 and IL-6 expression. However, further studies are required to verify the exact mechanism.

Keywords Interleukin-6 · Aquaporin-4 · Traumatic brain injury · Estrogen · Progesterone

Abbreviations

AQP4	Aquaporin-4
IL-6	Interleukin-6
TBI	Traumatic brain injury
E1	Low dose of estrogen
P1	Low dose of progesterone
E2	High dose of estrogen
P2	High dose of progesterone
AQPs	Aquaporins
IL-1β	Interleukin-1β
NFκB	Nuclear factor kappa b
OVX	Ovariectomized
T-PER	Tissue protein extraction reagent
ELISA	Enzyme-linked immunosorbent assay
RT-PCR	Reverse transcription-polymerase chain reaction

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β-actin	Beta-actin
ANOVA	Analysis of variance
BBB	Blood-brain barrier
mRNA	Messenger RNA
HIF-1α	Hypoxia-inducible factor 1-alpha
MMP-9	Matrix metallopeptidase 9
RT	Room temperature

Introduction

In definition, the brain edema is an increase of fluid within the brain tissue; it is regarded with types of vasogenic and cytotoxic. Vasogenic edema results from blood-brain barrier destruction that leads to fluid accumulation in brain. Cytotoxic edema arises from fluid increase within cell cytoplasm as a result of injury [20]. In moderate to severe brain injury, the untreated cerebral edema leads to high morbidity and mortality [8]. Current therapeutic methods do not control cerebral edema efficiently. These limitations emphasize the need for novel approaches to prevent or decrease cerebral edema.

Recently, it has been shown that the aquaporins (AQPs) participate in the pathophysiology of the brain edema [9, 18, 23]. The AQPs are water channels that provide a key path for transcellular water movement in many cell types [1]. It has been proved that aquaporin-4 (AQP4) is the most predominant form of AQP family expressed in the brain and plays an important role in the development of edema [34]. A gross agreement exists among the experts that AQP4 is involved both in brain edema formation and in its clearance, although the exact mechanisms remain unclear. Recent studies have demonstrated that the expression of AQP4 markedly enhanced in cerebral edema, suggesting a crucial role for this channel in the exacerbation of edema [9, 32]. AQP4-deficient mice have exhibited less brain edema and better survival following cerebral ischemia or acute water intoxication, than wild-type mice [3].

Traumatic brain injury (TBI) is also associated with increasing levels of pro-inflammatory cytokines that have a role in the development of cerebral edema [4]. The inflammation contributes in post-traumatic neurological deterioration [17]. Brain interleukin-6 (IL-6) increases in TBI [10]. Our previous studies have also demonstrated increasing IL-6 after experimental TBI [15, 25]. Several investigators have suggested that increasing IL-6 may be involved in the development of other post-trauma responses, including the brain edema [37, 39]. Interestingly, some of the cytokines have the ability to upregulate the AQP4 expression [2, 17]. Intracerebral administration of interleukin-1β (IL-1β) has led to increasing AQP4 expression via activation of nuclear factor kappa b (NFκB) [13].

Several clinical researchers have shown that the menopausal women are vulnerable to different insults which are characterized by the development of brain edema including ischemic brain injury, compared to young women [19, 36]. Multiple studies have clearly shown that estradiol (E2) may be responsible for these differences [15, 42]. It has been demonstrated that the female brain is resistant to ischemic injury during the reproductive period compared to that of male and that estrogen plays an important role in this neuroprotection [33]. It has been suggested that progesterone decreases the brain edema and increases the functional recovery after TBI in animal models of the injury [7, 26]. Recent experiments have also demonstrated that estrogen and progesterone lower the levels of inflammatory cytokines and cell death after TBI [5, 25]. The lesion volume decreases in TBI females compared to males. The administration of estrogen and progesterone decreases brain edema in males and ovariectomized rodents [8, 26]. The changes of AQP4 have been demonstrated following the administration of estrogen and progesterone in experimental brain injury [8, 29].

Despite demonstrated benefits in the decrease of brain edema after TBI, it is not exactly known how progesterone and estrogen specifically produce these beneficial effects at the cellular level. Also, few studies have evaluated estrogen and progesterone effects on the brain edema associated with the AQP expression and the IL-6 levels post-TBI. In the mind, the present study was designed to analyze the development of brain edema, the expression of brain AQP4, and the intracerebral concentration of IL-6 in response to the administration of progesterone and estrogen (at low and high levels), alone and in combination, following diffuse traumatic brain injury in the ovariectomized rats. Our goal in this study was to determine the neuroprotective mechanism of ovarian hormones in experimental TBI.

Materials and methods

Animals

The study was conducted in accordance with protocol approved by the ethics committee (No. EC/KNRC/86-30) of Kerman University of Medical Sciences, in accordance with internationally approved principles for laboratory animal use and care, as found in the European Community Guidelines (EEC Directive of 1986; 86/609/EEC) or US guidelines (NIH publication #85-23, revised in 1985). Animals (mature female Wistar rats, weighing 200–250 g) were housed in an air-conditioned room at 22–25 °C, with a 12 h light/12 h dark cycle and free access to food and water.

Method of bilateral ovariectomy

All study animals were ovariectomized (OVX) to avoid interferences due to the estrus cycle, 2 weeks before the experiments, as previously described [15]. Briefly, an incision with a length of 2 cm was created in the subabdominal part of the anesthetized animals. Then, the tube of uterus and vascular base of ovaries were twisted around proximal area and were cut from distal area. Finally, the muscles and skin were replaced back and stitched.

Animal groups

The OVX animals were randomly divided into 11 groups (n=12 in each group): (i) sham group; (ii) one-vehicle group: OVX rats received an injection of an equal volume of vehicle (sesame oil which used as estrogen or progesterone solvent); (iii) two-vehicle group: OVX rats received an injection of two equal volumes of vehicle (sesame oil which was used as estrogen and progesterone solvent); (iv) E1 group: Animals received an injection of a low dose of estrogen (33.3 μg/kg); (v) E2 group: Rats received an injection of a high dose of estrogen (1 mg/kg); (vi) P1 group: Rats received an injection of a low dose of progesterone (1.7 mg/kg); (vii) P2 group: Rats received an injection of a high dose of progesterone (8 mg/kg); (viii) E1 + P1 group: Rats received a combined injection of low doses of estrogen and progesterone; (ix) E1 + P2 group: Rats received a combined injection of a low dose of estrogen and a high dose of progesterone; (x) E2 + P1 group: Rats received a combined injection of a high dose of estrogen and a low dose of progesterone; and (xi) E2 + P2 group: Rats received a combined injection of high doses of estrogen and progesterone. It should be mentioned that the treatment was performed as an intraperitoneal injection (i.p.), a half of hour post-TBI [22].

Model of diffuse TBI

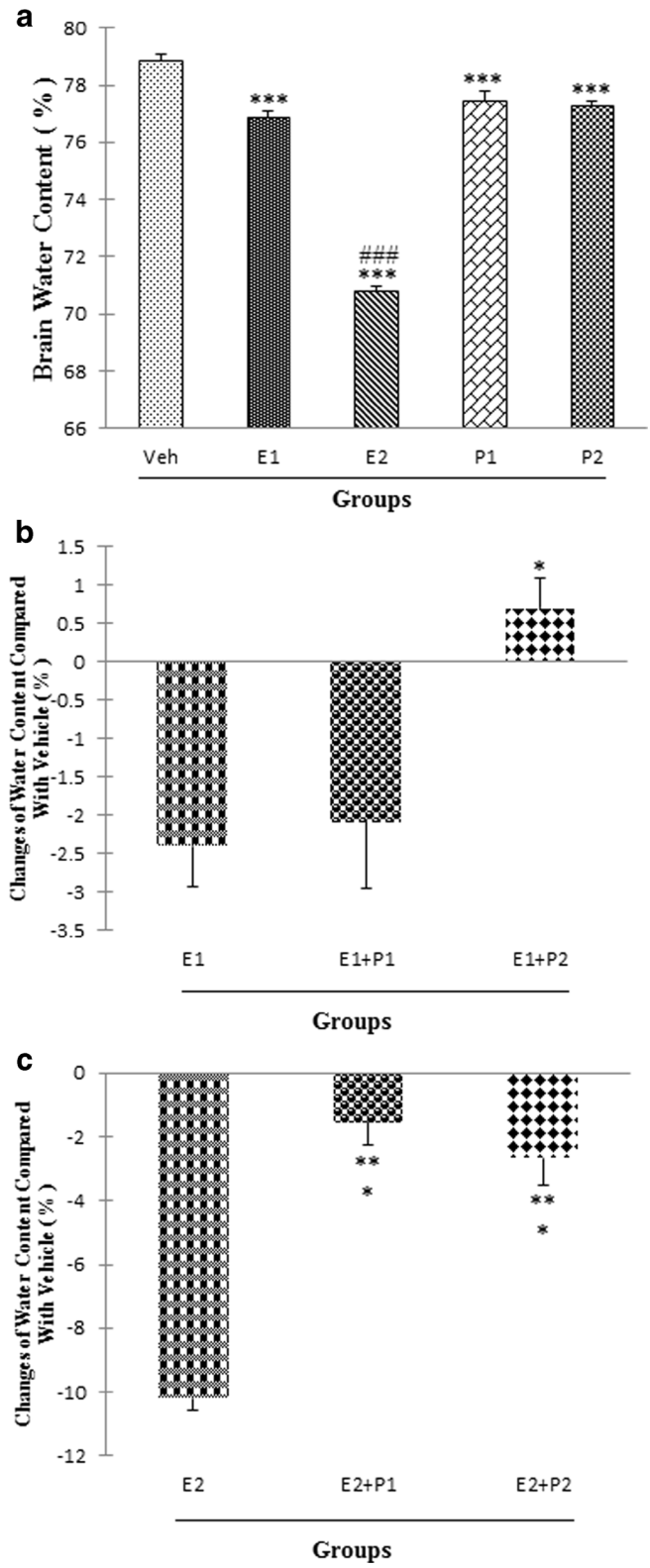
The anesthetized animals (with thiopental, 50 mg/kg, i.p.) were intubated before injury. TBI was induced as we have previously described in our articles [15]. Briefly, TBI was induced by the Marmarou method using a TBI induction device made at home (Department of Physiology, Kerman University of Medical Sciences). TBI was moderate and diffuse type. The rats were connected to respiratory pump, post-trauma (TSA animal respiratory compact, Germany). The intratracheal tube was removed after restoration of spontaneous breathing, and the rat was placed in an individual cage, following recovery.

Determination of brain water content

The brain edema was assessed by measuring the brain water content in each animal as previously described [15]. Briefly, brain wet and dry weights were acquired. Then, percentage of water was calculated in each sample using an equation: *100 %× [(wet weight− dry weight)/wet weight]*.

Measurement of brain IL-6 levels

The brains in rats anesthetized with thiopental (50 mg/kg, i.p.) were quickly removed and immediately frozen in liquid nitrogen, 24 h after TBI [11]. The brain samples were weighed and homogenized in tissue protein extraction reagent (T-PER) with 0.5 % Triton X-100, 150 mmol/L NaCl, 50 mmol/L Tris, and protease inhibitor cocktail (Pierce) (500 mg tissue per 1 mL of the reagent). Following homogenization, the samples were shaken for 90 min and then centrifuged for 15 min (4 °C and 4000 g). The supernatant was collected, and the protein content of supernatant was estimated to ensure that an equal amount of protein from each sample was used for the assay, using BCA Protein Assay Reagent kit [35]. IL-6 was measured by enzyme-linked immunosorbent assay (ELISA) kit (BMS, Austria) according to the protocol provided by the manufacturer. The


a
Brain Water Content (%)
80
78
76
74
72
70
68
66
***
***
###
***
***
Veh
E1
E2
P1
P2
Groups
b
Changes of Water Content Compared With Vehicle (%)
1.5
1
0.5
0
-0.5
-1
-1.5
-2
-2.5
-3
-3.5
*
E1
E1+P1
E1+P2
Groups
c
Changes of Water Content Compared With Vehicle (%)
0
-2
-4
-6
-8
-10
-12
**
*
**
*
E2
E2+P1
E2+P2
Groups

◀ **Fig. 1** **a** Effect of estrogen or progesterone on brain water content (%) following traumatic brain injury (TBI) in the ovariectomized (OVX) rats (n=6 in each group). ***p<0.001, all groups vs. vehicle group. ###p<0.001, E2 group vs. all groups. **b** Effect of low dose of estrogen (alone and combined administration) on the percentage changes of brain water content compared with that in vehicle, following TBI in the OVX rats (n=6 in each group). *p<0.05, E1 group vs. E1 + P2 group. **c** Effect of high dose of estrogen (alone and combined administration) on the percentage changes of brain water content compared with that in vehicle, following TBI in the OVX rats (n=6 in each group). ***p<0.001, E2 group vs. E2 + P1 or E2 + P2 groups. *Veh* vehicle, *E1* low dose of estrogen, *E2* high dose of estrogen, *P1* low dose of progesterone, *P2* high dose of progesterone, *E1 + P1* low dose of estrogen + low dose of progesterone, *E1 + P2* low dose of estrogen + high dose of progesterone, *E2 + P1* high dose of estrogen + low dose of progesterone, *E2 + P2* high dose of estrogen + high dose of progesterone. Data are presented as mean ±SEM

concentration of IL-6 was quantified as picogram of antigen per milliliter of supernatant for each sample.

Reverse transcription-polymerase chain reaction of AQP4

AQP4 gene analysis in the brain was performed by reverse transcription-polymerase chain reaction (*RT-PCR*). Total RNA was extracted from each brain sample and reverse transcribed into the cDNA. Beta-actin (β-actin) was used as the internal control. The sequences of the upstream and downstream primers for β-actin were 5′-TCG-TGG-GCC-GCC-CTA-GGC-AC-3′ and 5′-GGC-CTT-AGG-GTT-CAG-AGG-GGC-3′, respectively, and those for AQP4 were 5′-CCT-CTA-CCT-GGT-CAC-ACC-C-3′ and 5′-GTC-CTT-CCC-CTT-CTT-CTC-G-3′. After 2 % agarose gel electrophoresis, the gray scales of PCR amplification products (PCR kit, Germany Roche) were analyzed using Band Leader 3.0 Gel Image Manipulation software (http://www.bio-soft.net/draw/BandLeader.htm, accessed December 20, 2012). The relative messenger RNA (mRNA) of gene of interest was presented as ratio of the band gray scale of AQP4 to that of the β-actin for each sample [41].

Western blotting of AQP4

The AQP4 protein expression in the brain was quantified by Western blot. The homogenized brain sample was centrifuged for 30 min (12,000×g at 4 °C). Protein concentration of the supernatant was measured, and equal amounts of protein in each sample were subjected to 12 % sodium dodecyl sulfate polyacrylamide gel electrophoresis (*SDS-PAGE*) before being transferred to a nitrocellulose membrane. The membrane was blocked with 5 % fat-free milk at room temperature (RT) for 2.5 h and then incubated with rabbit polyclonal anti-AQP4 (1:500, Sigma, USA; cat. # A5971) at 4 °C overnight. The membrane was incubated with HRP-linked goat anti-rabbit IgG (1:1000, Sigma, USA; cat. # A9169) at RT for 2 h, following three washes. ECL kit (Roche, Germany) was used to visualize chemiluminescent signal bands. The membrane was exposed to the radiography film to reveal the AQP4 band (30 kDa). A

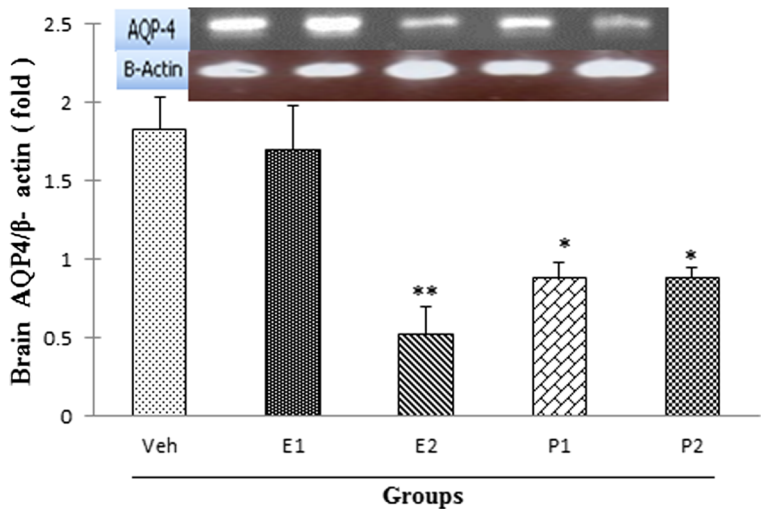
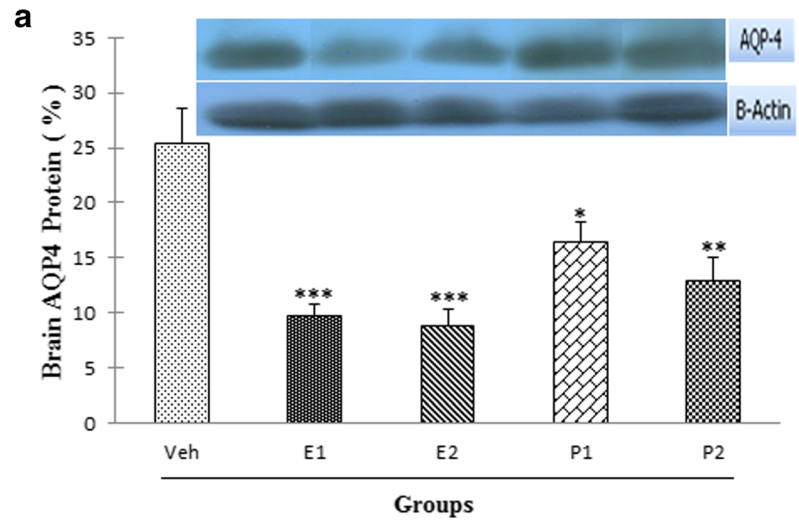
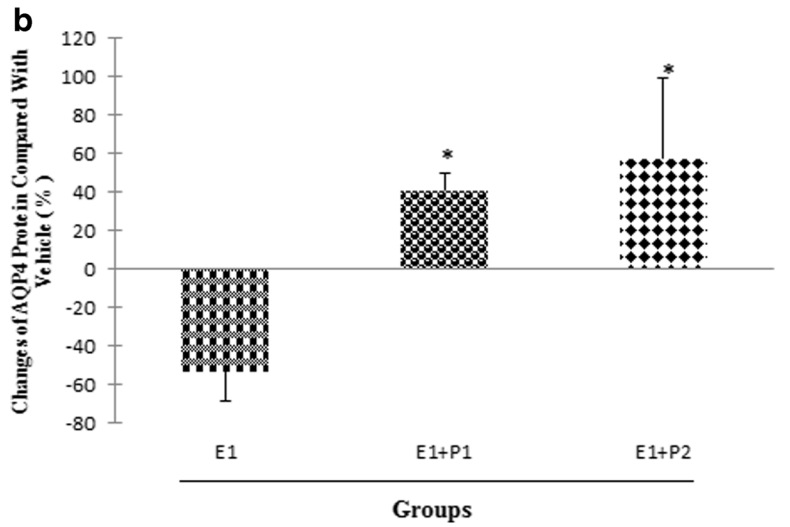
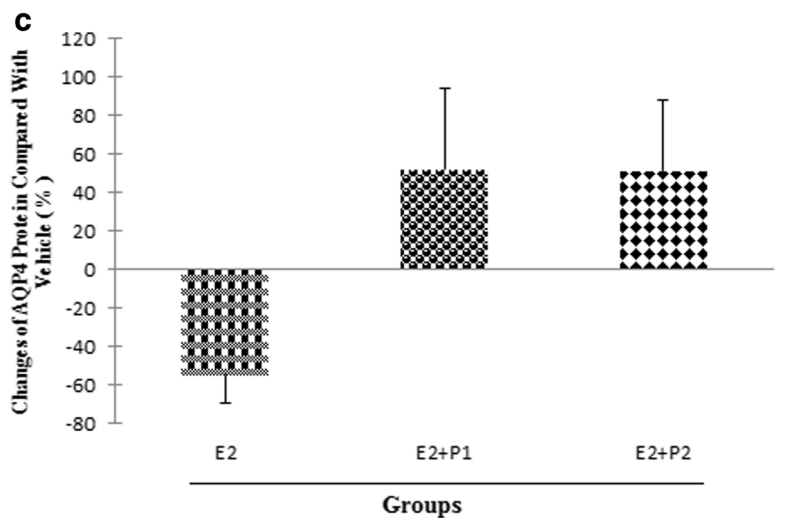


Fig. 2 Effect of estrogen or progesterone on the brain AQP mRNA (fold) following traumatic brain injury (TBI) in the ovariectomized (OVX) rats (n=6 in each group). **p<0.01, E2 group vs. vehicle group. *p<0.05, P1 and P2 groups vs. vehicle group. *Veh* vehicle, *E1* low dose of estrogen, *E2* high dose of estrogen, *P1* low dose of progesterone, *P2* high dose of progesterone. Data are presented as mean±SEM. Reverse transcription-polymerase chain reaction was the evaluation method of AQP4 mRNA


a
AQP-4
B-Actin
Brain AQP4 Protein (%)
35
30
25
20
15
10
5
0
***
***
*
**
Veh
E1
E2
P1
P2
Groups


b
Changes of AQP4 Protein Compared With Vehicle (%)
120
100
80
60
40
20
0
-20
-40
-60
-80
*
*
E1
E1+P1
E1+P2
Groups


c
Changes of AQP4 Protein Compared With Vehicle (%)
120
100
80
60
40
20
0
-20
-40
-60
-80
E2
E2+P1
E2+P2
Groups

◀ **Fig. 3** **a** Effect of estrogen or progesterone on the brain AQP protein (%) following traumatic brain injury (TBI) in the ovariectomized (OVX) rats (n=6 in each group). ***p<0.001, E1 and E1 groups vs. vehicle group. **p<0.01, P2 group vs. vehicle group. *p<0.05, P1 group vs. vehicle group. **b** Effect of low dose of estrogen (alone and combined administration) on the percentage changes of AQP protein compared with that in vehicle, following TBI in the OVX rats (n=6 in each group). *p<0.05, E1 group vs. E1 + P1 or E1 + P2 groups. **c** Effect of high dose of estrogen (alone and combined administration) on the percentage changes of AQP protein compared with that in vehicle, following TBI in the OVX rats (n=6 in each group). *Veh* vehicle, *E1* low dose of estrogen, *E2* high dose of estrogen, *P1* low dose of progesterone, *P2* high dose of progesterone, *E1 + P1* low dose of estrogen + low dose of progesterone, *E1 + P2* low dose of estrogen + high dose of progesterone, *E2 + P1* high dose of estrogen + low dose of progesterone, *E2 + P2* high dose of estrogen + high dose of progesterone. Data are presented as mean±SEM. The AQP protein in the brain was quantified by Western blot analysis

computer-assisted image analysis system (Gel Pro Analyzer 4.0, Media Cybernetics, USA) was used to analyze the integrated densities. The β-actin band was used to normalize the AQP4 expression [4] using anti-β-actin (Sigma, USA; cat. # A2668).

Statistical analysis

All data were analyzed for normality by Shapiro-Wilk's W test. The percentage change in each sample was calculated by using an equation: 100 %×[(amount vehicle group−amount of hormone group)/amount of vehicle group]. The quantitative data are expressed as mean±SEM. The data were analyzed by parametric analysis of variance (ANOVA). ANOVA post hoc analysis of the variables was performed using Tukey test; if homogeneity in variances existed otherwise, Games-Howell test was used. ANOVA post hoc analysis of changes was performed using Dunnett test. The criterion for statistical significance was set at p<0.05.

Results

Brain water content following treatment in TBI

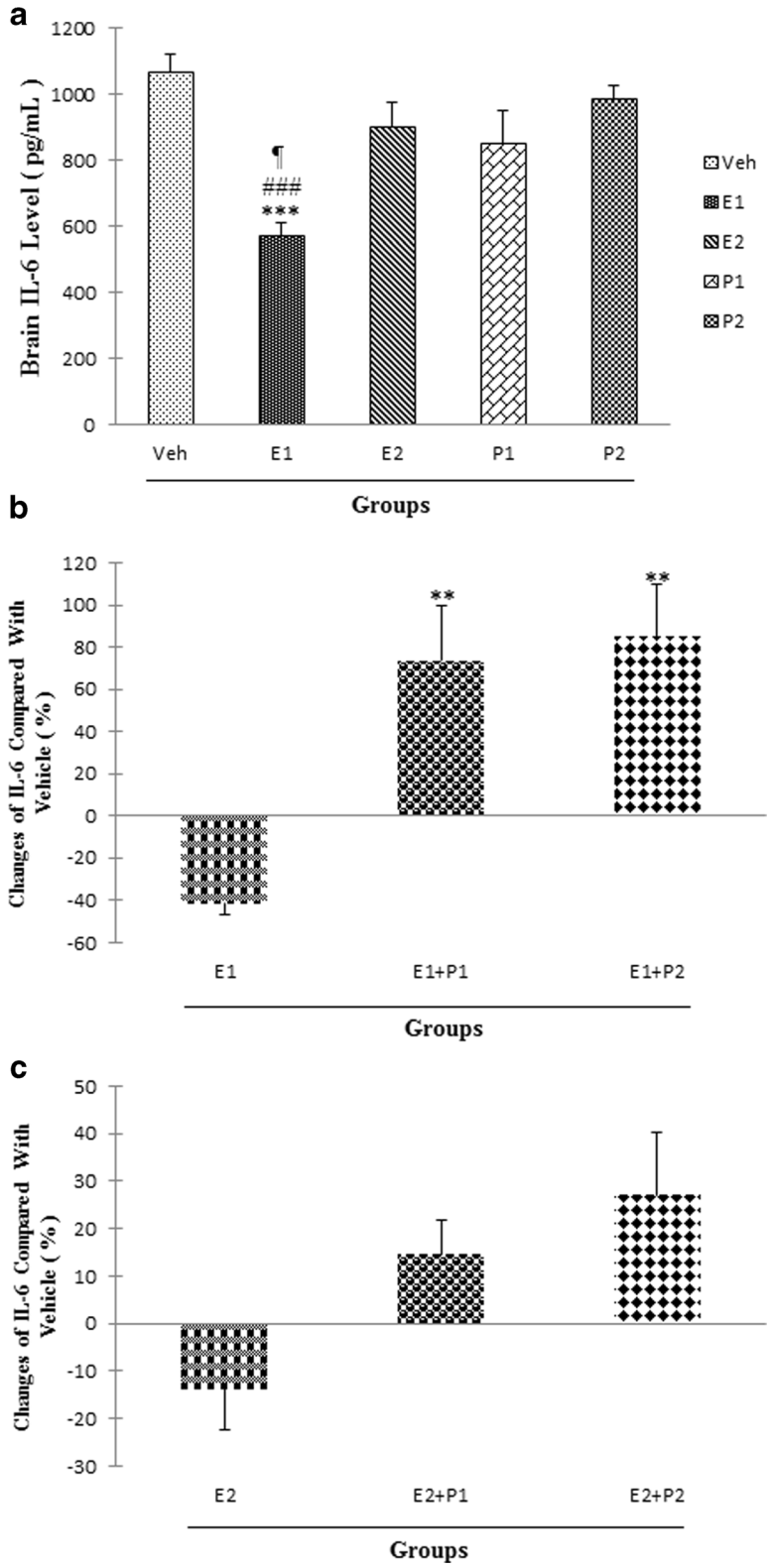
The effect of different doses of ovarian steroid hormones on the brain water content post-TBI in the ovariectomized rats is shown in Fig. 1. The brain water content significantly decreased in E1, E2, P1, and P2 groups compared with the vehicle group (78.84± 0.22 %, p<0.001). In addition, the brain water content was statistically different between the E2 group (70.8± 0.15 %) and the other groups (p<0.001) (Fig. 1a). The effect of low or high dose of estrogen (alone and combined administration) on the percentage changes of brain water content compared with that in the vehicle group is shown in Fig. 1b, c. A low dose of estrogen decreased brain water content (−2.41±0.52 %) compared with that in the E1 + P2 group (0.69±0.4 %, p<0.05). The brain water content (−2.1±0.87 %) was not statistically different between the E1 + P1 group and the E1 group (Fig. 1b). The brain water content significantly decreased in the E2 group (−10.19±0.37 %) compared with that in the E2 + P1 (−1.45±0.73 %) and E2 + P2 (−2.67±0.87 %) groups (p<0.001) (Fig. 1c).

Brain AQP4 mRNA following treatment in TBI

The effect of different doses of ovarian steroid hormones on the brain AQP4 mRNA post-TBI in the ovariectomized rats is shown in Fig. 2. The AQP4 mRNA was significantly decreased in the E2 (0.52± 0.17, p<0.01), P1 (0.88±0.1, p<0.05), and P2 (0.88 ±0.06, p<0.05) groups compared with that in the vehicle group (1.83±0.2), while the AQP4 mRNA was not significantly different between the E1 group (1.69±0.29) and the vehicle group. The effect of low or high dose of estrogen (alone and combined administration) on the percentage changes of AQP4 mRNA compared with that in the vehicle-treated group was analyzed. This analysis was not significant (data not shown).

Brain AQP4 protein following treatment in TBI

The effect of different doses of ovarian steroid hormones on the brain AQP4 protein post-TBI in the ovariectomized rats is shown in Fig. 3. The AQP4 protein significantly decreased in the E1 (9.7±1.07, p<0.001), E2 (8.85±1.55, p<0.001), P1 (16.45± 1.77, p<0.05), and P2 (12.99±2.09, p<0.01) groups compared with that in the vehicle group (25.38± 3.25) (Fig. 3a). The percentage changes of AQP4 protein in the group treated with low or high dose of estrogen (alone and combined administration) compared with that in the vehicle group is shown in


a
Brain IL-6 Level (pg/mL)
1200
1000
800
600
400
200
0
¶
###
***
Veh
E1
E2
P1
P2
Groups
b
Changes of IL-6 Compared With Vehicle (%)
120
100
80
60
40
20
0
-20
-40
-60
**
**
E1
E1+P1
E1+P2
Groups
c
Changes of IL-6 Compared With Vehicle (%)
50
40
30
20
10
0
-10
-20
-30
E2
E2+P1
E2+P2
Groups

◀ **Fig. 4** **a** Effect of estrogen or progesterone on the brain levels of IL-6 (pg/mL) following traumatic brain injury (TBI) in the ovariectomized (OVX) rats (n=6 in each group). ***p<0.001, E1 group vs. vehicle group. ###p<0.001, E1 group vs. P2 group. ¶p<0.05, E1 group vs. E2 group. **b** Effect of low dose of estrogen (alone and combined administration) on the percent changes of IL-6 levels compared with that in vehicle, following TBI in the OVX rats (n=6 in each group). **p<0.01, E1 group vs. E1 + P1 and E1 + P2 groups. **c** Effect of high dose of estrogen (alone and combined administration) on the percent changes of IL-6 levels compared with that in vehicle, following TBI in the OVX rats (n=6 in each group). *Veh* vehicle, *E1* low dose of estrogen, *E2* high dose of estrogen, *P1* low dose of progesterone, *P2* high dose of progesterone, *E1 + P1* low dose of estrogen + low dose of progesterone, *E1 + P2* low dose of estrogen + high dose of progesterone, *E2 + P1* high dose of estrogen + low dose of progesterone, *E2 + P2* high dose of estrogen + high dose of progesterone. Data are presented as mean±SEM

Fig. 3b, c. The AQP4 protein significantly decreased in the E1 group (−53.47±15.62 %) compared with that in the E1 + P1 (40.67±8.85 %) and E1 + P2 (57.05±41.7 %) groups (p<0.05) (Fig. 3b). The change in the AQP4 protein content was not significantly different between the E2 group (−55.14± 14.48 %) and the E2 + P1 (52.03±42.08 %) and E2 + P2 (50.89±36.81 %) groups (Fig. 3c).

Brain IL-6 levels following treatment in TBI

The effect of different doses of ovarian steroid hormones on the brain IL-6 levels post-TBI in the ovariectomized rats is shown in Fig. 4. The injured animals treated with E1 (573.13±37.15 pg/mL) had lower levels of IL-6 than that in the vehicle (1068± 54.18 pg/mL, p<0.001), E2 (900.13±77.17 pg/mL, p<0.05), and P2 (984.86±43.5 pg/mL, p<0.001)-treated animals. The amount of IL-6 was not statistically different between the E1 group and the P1 group (852.46±99.14 pg/mL) (Fig. 4a). The effect of low or high dose of estrogen (alone and combined administration) on the percentage changes of IL-6 levels compared with that in the vehicle group is shown in Fig. 4b, c. The deceasing IL-6 was statistically stronger in E1 (−41.53±5.66 %) group compared with that in the E1 + P1 (73.58±25.78 %) and E1 + P2 (85±24.54 %) groups (p<0.01) (Fig. 4b). The amount of IL-6 (−13.79±8.57 %) was not statistically different between the E2 group and the E2 + P1 (14.52±7.28 %) and E2 + P2 (27.16±13.25 %) groups (Fig. 4c).

Discussion

We reported for the first time the effect of low and high doses of progesterone and estrogen administered alone and in combination, on the brain edema, the levels of brain IL-6, and the expression of brain AQP4 in TBI. In this study, the decrease of brain water content was found in response to the low and high doses of progesterone and estrogen administered post-TBI. This decrease was attenuated when progesterone and estrogen were administered in combination. We suggest that these changes in the brain water content may be mediated by changing the levels of brain IL-6 and AQP4.

Brain edema plays a main role in the pathophysiology of TBI and contributes to the mortality found in these patients [16]. To date, no specific pharmaceutical approach exists for prevention or treatment of the brain edema.

A number of reports have suggested that female gonadal hormones may be responsible for the decrease of edema formation post-TBI in the female animals [24, 31]. It has been shown that the administration of low [6] and high [30] doses of estrogen dramatically causes the decrease of ischemic volume following cerebral ischemia. On the other hand, it has been shown that the exogenous progesterone has a dose- and time-dependent neuroprotective effect in the experimental stroke [21]. Therefore, in this study, the effect of progesterone and estrogen (low and high doses), alone and in combination, was evaluated in ovariectomized female rats following TBI.

Results of the present study showed that brain water content significantly increased following TBI compared with the sham group about 3 % (data not shown). Our results also showed that both the low dose (physiological) and the high dose (pharmacological) of estrogen and progesterone decreased the brain water content. The inhibitory effect of estrogen on the brain water content was attenuated as a result of estrogen and progesterone administration. The beneficial effect of steroid administration alone on brain edema has been reported in other studies [22, 31]. A study has shown that progesterone causes the decrease of estrogen receptor expression in the brain [11].

In this study, it was supposed that the probable neuroprotective mechanisms of the steroids on the brain edema are affecting the AQP4 expression and the IL-6 levels in the injured brain. AQP4 mRNA and protein and IL-6 levels in the brain significantly increased

following TBI compared with the sham group, 21.04, 28.62, and 70.1 %, respectively (data not shown) which are consistent to the results from other studies [12, 40].

Our results showed that both high and low doses of estrogen and progesterone decrease the brain expression of AQP4 mRNA and protein. Several studies have demonstrated that the decrease in the brain edema is associated with a decrease in the AQP4 levels, following estrogen and progesterone administration in an injured brain [8, 29]. The AQP4 deficiency ameliorates the acute lesions (24 h), while it worsens the delayed lesions in the later phase (7 and 14 days) of the brain injury in mice [28]. Therefore, AQP4 overexpression may be important in post-ischemic recovery processes in the chronic phase, and early inhibition of the AQP4 expression in the brain tissue may provide a new treatment approach for the cerebral edema [27]. These results are in agreement with ours. However, downregulation of AQP4 protein is reversed when estrogen and progesterone are administered in combination, especially in the E1 + P1 and E1 + P2-treated groups. Therefore, these steroids affected both transcriptional and post-transcriptional processes when administered alone, while their combination only affected post-transcriptional processes in agreement with other investigations [38]. When progesterone was administered with estrogen in, it decreased effect of E1 on water content, compatible with the decrease of estrogen effect on AQP4 protein.

The present study showed that only low dose of estrogen significantly decreased the brain levels of IL-6. The inhibitory effect of estrogen on the IL-6 expression has been demonstrated in the monocytes [14]. However, this downregulation is inverted, when a combination of low doses of estrogen and progesterone is administered. The changes of brain edema, AQP4 expression, and IL-6 levels may be a result of the inhibition of expression of the estrogen receptor by progesterone [11] in the combined administration groups. Several investigators have suggested that increasing IL-6 expression may contribute to the development of post-trauma responses including brain edema [37, 39].

Based on the results of the present study, two plausible mechanisms are needed to be considered for the anti-edema effect of steroids: (1) an inhibitory effect on the brain AQP4 expression and (2) an anti-inflammatory effect (decreasing brain IL-6). Three neuroprotective mechanisms are suggested to the inhibitory effect of steroids on the expression of AQP4. Firstly, since cytokines promote the AQP4 overexpression [4], steroids probably attenuate the brain edema by inhibiting IL-6-induced AQP-4 expression. Secondly, increasing expression of hypoxia-inducible factor 1-alpha (HIF-1α) may play a central role in brain edema and BBB disruption by increasing both AQP4 and MMP-9 in TBI [9]. Therefore, steroids may decrease the brain edema by inhibiting HIF-1α-induced AQP4 expression. Finally, steroids may decrease the brain edema by a direct effect on AQP4 expression via the genomic and nongenomic mechanisms.

Overall, the present study showed that the administration of different doses of estrogen or progesterone led to decreasing brain edema following TBI. In addition to other neuroprotective mechanisms employed by estrogen and progesterone, a decrease in the brain AQP4 expression and IL-6 levels should also be considered. It was also shown that co-administration of estrogen and progesterone may decrease the neuroprotective effect of individual administration of estrogen or progesterone on the brain edema. The latter is consistent with the decrease of inhibitory effect of estrogen on the brain levels of IL-6 and the brain expression of AQP4. Further studies are necessary to find the mechanism(s) and receptors involved in the interaction of sex steroids, AQP4 expression, and IL-6 levels in the brain.

Conclusions

Individual administration of estrogen or progesterone attenuated the development of brain edema post-TBI, probably by decreasing the brain AQP4 expression and IL-6 levels. The co-administration of estrogen and progesterone suggested decreases of inhibitory effects probably by increasing the brain expression of AQP4 and the brain levels of IL-6. Further studies are required to verify these mechanisms.

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