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The brain cytokine levels are modulated by estrogen following traumatic brain injury: Which estrogen receptor serves as modulator?

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ABSTRACT

The present study was designed to explore whether administration of estrogen affects brain cytokine levels in TBI. We also sought determine which one of type of classical estrogen receptors (ERs) is involved. Ovariectomized female rats were divided in to eight groups. Estrogen or vehicle was administered following TBI (E2 and oil groups). Antagonist of ER(ICI 182, 780) or vehicle was also administered following TBI (ICI and DMSO groups). The ICI or vehicle was administered either before induction of TBI and administration of estrogen (ICI+E2 and DMSO+E2 groups). TBI was induced by Marmarou's method. In addition to brain water content, the levels of brain proinflammatory and anti-inflammatory cytokines were measured 24 hours post- TBI. Present results demonstrated that, estrogen reduced TBI- induced brain edema. The antiedema effect of estrogen was attenuated by ICI. The brain measures of IL-1β, IL-6 and TNF-α in TBI were also reduced by estrogen. The anti-inflammatory effect of estrogen was attenuated by ICI. The inhibition level of estrogen by ICI was 53.2%, 12.09% and 48.45% for IL-1β, IL-6 and TNF-α, respectively. Estrogen also elevated IL-10 in TBI. ICI inversely controlled the effect of estrogen on IL-10, by 33.84%. This effect was not observed once ICI was used alone. The estrogen administration following TBI probably results in proinflammatory cytokines reduction, and inversely enhancement of anti-inflammatory cytokines. In our study, the neuroprotective effect of estrogen is proposed to be mediated by both ERα and ERα, and accordingly the inhibition of neuroprotective effect of estrogen by ICI.

1. Introduction

Traumatic brain injury (TBI) is among the most prevalent causes of both mortality and disability, worldwide. Despite enormous efforts have been performed for introducing a neuroprotective method of treatment to either reduce or eliminate the complications of TBI, a proper treatment with adequate consequences has not been defined, to date [1].

There is an inflammation in many brain pathologies, such as multiple sclerosis, stroke and TBI. The accompanied inflammation with TBI is a key mechanism for the emergence of secondary injury following TBI, and the blood–brain barrier (BBB) is a mediator for neuroinflammatory processes. An inflammation in TBI patients begins at almost immediate early hours following injury [2]. The injury is rapid in the early phase of TBI, therefore BBB permeability reaches peak in the early hours and leads to brain edema [3].

The diverse endogenous mediators are in study for controlling of the brain edema following TBI, since these mediators function in the development of brain edema following TBI via affecting integrity of tight junctions in BBB [4]. Some of the side effects related to TBI are most often due to brain edema. The brain injury induces neuroinflammatory processes, including the production and secretion of inflammatory cytokines involved in the development of inflammation and neurodegenerative processes [5] Therefore, proinflammatory cytokines, may be considered as one of the key factors involved in the progression and development of brain edema.

In addition to, the fact that brain injury leads to the infiltration of immune cells to the site of injury through damage to the BBB, it also causes activation of microglia which plays an important role in the response to brain injury. The activation of microglia results in neurotoxication via release of cytotoxic substances, such as proinflammatory cytokines, including interleukin (IL) -1β, tumor necrosis factor (TNFα) and IL-6 [6].

It is now well established that IL-1β plays an important role in the inflammatory responses in the brain and is responsible of a number of the brain responses to injury. The IL-1β functions are various, dependent on its expression and target tissue [7]. The IL-6 increased measures in response to TBI [8] have multiple effects, some beneficial and some detrimental in the central nervous system. The IL-6 is low in physiological conditions, but increases in many diseases leading to increased production of inflammatory factors such as cytokines (IL-1β, TNFα and TGF-β) and prostaglandins [9]. The elevation of the level of TNF-α following brain

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ischemia [10] causes the increase of IL-1β and IL-6 expression. The initial increase in cytokines following brain injury leads to infiltration of other inflammatory mediators to the sites of injury [9].

The anti-inflammatory cytokines are also altered following TBI [11]. Studies have shown that IL-10 acts in favor of, following trauma by reducing pro-inflammatory cytokines (e.g. IL-1β and TNF-α) [12]. The prevalence of stroke is lesser in women than men, and there is evidence for the neuroprotective role played by gonadal hormones, particularly estrogen (E2), and estrogen functions as an anti-inflammatory agent [13], therefore, research programs have been focused on reducing brain injuries using estrogen. Previous studies in our research group revealed the neuroprotective effect of estrogen in TBI via reducing brain edema [14] and BBB permeability [15] by estrogen. It is well established that estrogen prevents the invasion of proinflammatory cytokines to the damaged tissue following TBI [16].

The effects of estrogen in brain are exerted via both genomic and non-genomic functions [17] mediated by both alpha (ERα) and beta (ERβ) estrogen receptors [18]. It has been demonstrated that estrogen modulates the reduction of brain edema and BBB permeability, and the neurological involvement following TBI via ERs [19].

ICI is an estrogen compound and analog of 17β-estradiol, in which 89% of its structure is closely similar to estradiol [20] ICI; as a pure estrogen receptor antagonist completely blocks the binding of estradiol to ER [21]. ICI has similar affinity for both receptors ERα and ERβ [11]. This antagonist eliminates the neuroprotective effects of E2 on the pre-apoptotic and anti-apoptotic factors in cultured neurons and cerebral ischemia [22] via ERs. In another study it has been shown that the trophic effects of estrogen on the uterus are inhibited by ICI [23] Although the above studies indicate that ICI acts as a pure antagonist, it has recently been reported that ICI may act as a partial agonist [24].

Previous attempts in our laboratory showed that acute and intraperitoneal administration of estrogen plays a neuroprotective role in TBI by reducing brain edema, intracranial pressure (ICP) and damage to BBB [14,25]. In the present study we sought to investigate that firstly, whether the neuroprotective effect of estrogen is applied through changes in the brain levels of cytokines and Secondly, whether the action of estrogen is mediated via the classic receptors (α and β), using 182,780 ICI, and thirdly, partial agonist action of ICI will be tested as well.

2. Materials and methods

2.1. Animals

The study was designed in accordance with the protocol approved by the ethic committee (No: K/92/314) of Kerman University of Medical Sciences, in accordance with internationally approved principles for animal use and care, as found in the European Community guidelines (EU Directive of 2010; 2010/63/EU) or US guidelines (NIH publication #85-23, revised in 1985). Animals (mature female Albino N Mary rats, weighing 200–250 g) were housed in an air-conditioned room at 22–25 °C, with a 12 h light/dark cycle and had free access to food and water.

2.2. Method of bilateral ovariectomy

All of the animals were ovariectomized (OVX) 2 weeks before the experiments as previously described [25] Briefly, following anesthesia, an incision was created in the sub-abdominal part of the animals. Thereafter, the tube of uterus and vascular base of ovaries in proximal area were cut off from distal area in each side. Finally the muscles and skin were replaced back and stitched.

2.3. Animal groups and drugs

The animals were randomly put in 8 different groups (n = 7 in each group), as follows:

(i) Sham group: OVX rats underwent an incision of skull skin after anesthesia (with thiopental, 50 mg/kg, i.p.), but brain trauma was not induced for them.
(ii) TBI group: OVX rats were exposed to brain trauma after anesthesia.
(iii) Oil group: OVX rats received an injection of equal volume of vehicle (sesame oil, which were used as estrogen solvent), 30 min following TBI [14].
(iv) E2 group: OVX rats received an injection of estrogen (33.3 μg/kg), 30 min following TBI [26].
(v) ICI + E2 group: OVX rats received an injection of ICI 182,780 (4.0 mg/kg), two times, 24 h apart from and before TBI and then received an injection of estrogen, 30 min following TBI [27].
(vi) DMSO + E2 group: OVX rats received an injection of equal volume of vehicle (DMSO, which was used as ICI182, 780 solvent) two times, 24 h apart from and before TBI, and then received an injection of estrogen, 30 min following TBI.
(vii) ICI group: OVX rats received an injection of ICI 182, 780, 30 min following TBI.
(viii) DMSO group: OVX rats received an injection of equal volume of vehicle (DMSO) 30 min following TBI.

The 17-ß estradiol and sesame oil were purchased from Aburaihan Pharmaceutical Company (Tehran, Iran). ICI182, 780 was purchased from Sigma. It should be mentioned that drugs were injected intraperitoneal (i.p).

2.4. Model of diffuse TBI

After tracheal intubation in anesthetized rats, diffuse TBI in animals was induced using a device made in the Physiology Department of Kerman University of Medical Sciences with Marmarou's method. For induction of TBI, a 250 g weight was dropped from a 2-m height through a free-falling tube onto a steel disc attached to the skull of anesthetized animal (by halothane in mixture of 70% N_2O and 30% O_2). After induction of TBI, the animal was immediately connected to the animal respiratory pump (TSA compact, Germany) and as soon as normal breathing, was isolated from ventilator and located in the cage. Intubation was performed in anesthetized (with thiopental, 50 mg/kg, and i.p.) animals before TBI [25].

2.5. Determination the brain water content

Brain edema was determined by measuring brain water content, 24 h post TBI. The animal's brain was removed under anesthesia and weighed (wet tissue weight). Then, it was placed inside an oven (Memmert, Germany) at 60 °C for 72 h and again weighed (dry tissue weight). Then the percentage of water content in each brain sample was calculated using an equation published previously: 100 percent × [(wet weight − dry weight) / wet weight] [25].

2.6. Measurement the levels of brain cytokines

The brains of rats anesthetized with thiopental (50 mg/kg, i.p.) were quickly removed and immediately frozen in liquid nitrogen, 24 h following TBI [28] Each brain sample was weighed and then homogenized using tissue protein extraction reagent (T-PER) with 50 mmol/L Tris, 150 mmol/L NaCl, protease inhibitor cocktail, and 0.5% Triton X-100 (500 mg tissue per 1 mL of the reagent). After homogenization, the samples were shaken for 90 min, and centrifuged (4°, 4000 *g* and 15 min). The supernatant was isolated from each sample, and protein of supernatant was estimated using a BCA Protein Assay Reagent Kit. The concentration of each cytokine in the supernatant was measured via respective enzyme-linked immune-sorbent assay (ELISA) kit (BMS, Austria). The