The Effects of Female Sex Steroids on Gastric Secretory Responses of Rat Following Traumatic Brain Injury

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Abstract

Objective(s)
Gastric ulceration is induced by various forms of stress like surgery, ischemia and trauma. The female sex has more resistance to stress and the gastrointestinal lesions happen fewer than male sex. The purpose of this study was to evaluate the role of estradiol and progesterone on the gastric acid and pepsin levels following traumatic brain injury (TBI) induction.

Materials and Methods
Diffuse TBI was induced by Marmarou method in female rats. Rats randomly assigned into 9 groups: intact, OVX (ovarectomized rat), Sham+OVX, TBI (intact rats under TBI), TBI+OVX (ovarectomized rats under TBI) and treated OVX rats with vehicle (sesame oil), E2 (estradiol), P4 (progesterone) or E2+P4 combination. The acid content and pepsin levels of each gastric washout sample were measured 5 days after the TBI induction.

Results
There was no significant difference in gastric acid output between groups either after TBI induction or after treatment with E2 or P4 or E2+P4. Gastric pepsin levels were increased in Sham+OVX, TBI (P< 0.001) and TBI+OVX (P< 0.05) compared to intact group. Gastric pepsin levels were significantly lower in E2 and E2+P4 treated rats than vehicle treated group (P< 0.01). P4 treatment increased gastric pepsin level compared to TBI+OVX group (P< 0.05) and this increment was higher than rats that were treated with the E2 and E2+P4 (P< 0.01).

Conclusion
These results suggest that protective effect of estradiol and E2+P4 combination against mucosal damage after TBI, might be mediated by inhibition of pepsin secretion.

Keywords: Brain injury, Estrogen, Gastric acid, Pepsin, Progesterone

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Introduction
Traumatic injury, often accompanied by hemorrhagic shock, continues to be the most common cause of death for young people and constitutes a significant source of morbidity and mortality for all ages (1). Traumatic brain injury is the leading cause of death in the USA and Western Europe (2, 3) and a budding epidemic throughout Asia and the Middle East (4). Therefore, various forms of traumatic injury represent a pandemic disease that affects every nation in the world regardless of economic development, racial or religious predominance, or political ideology. This disease is acute in onset and often results in chronic, debilitating health problems affecting far beyond the individual victims (1).

Gastrointestinal dysfunction occurs frequently in patients with traumatic brain injury (TBI) (5, 6). More than 50% of patients with severe head injuries do not tolerate enteral feedings (7). This intolerance is manifested by vomiting, abdominal distention, delayed gastric emptying (8), esophageal reflux (9), and decreased intestinal peristalsis (10) indicating that gastrointestinal dysfunction is a common phenomenon following TBI. The association between severity of brain injury and the intolerance of enteral feeding suggests a strong link between the central nervous system and the nonfunctional gut.

Multiple organ dysfunction syndrome (MODS) is the most common cause of late deaths post-injury, accounting for substantial morbidity and mortality (11, 12). MODS is considered to be due, in part, to excessive or maladaptive activation of inflammatory pathways (13). Organs such as the liver and the gut not only become damaged or dysfunctional from trauma induced inflammation, but in turn further perpetuate this inflammatory vicious cycle (14). Furthermore, patients admitted to the intensive care unit following trauma and hemorrhage often become susceptible to infection “second hit” further complicating attempts at immunomodulation early in the clinical course (15).

It is now clear that biological sex alters the incidence of, and outcome from, ischemic and traumatic brain injury. For example, male sex is a recognized risk factor for stroke, and in most epidemiological series, stroke occurs more frequently in men than women. This sexually dimorphic disease pattern remains apparent until age well beyond the menopausal years (16). Uses of estrogen or progesterone alone or a combination of these two hormones reduce brain edema following traumatic brain injury. This determines the anti-inflammatory role of female sexual steroids (17, 18). It has been reported that steroid hormones, especially progesterone and estrogen, have effects on the gastric motility and secretions (19) and are also involved in the pathogenesis of some functional disorders in the gut (20).

With regard to usefulness of the sex female hormones consummation in reducing of injury after trauma in our previous results of our research group (17, 18), and on the other hand, to being resistant of female animals against male animals to gastrointestinal lesions following trauma induction (16), the purpose of this study was to evaluate the role of estradiol and progesterone on the gastric acid and pepsin levels following traumatic brain injury (TBI) induction.

Materials and Methods
Animals
Female Wistar rats (200 to 250 g) were purchased from Animal Center of Ahwaz University of Medical Sciences, Iran. The rats were housed in temperature and humidity controlled animal quarters with a 12-hour light/dark cycle. All procedures were approved by the Institutional Animal Care Committee of Kerman University (No 183.88.k) and were in accordance with the guidelines of the National Institutes of Health on the care and use of animals surgery.

Ovariectomy
The animals were anesthetized by injection of 60 mg/kg thiopental (i.p.). The sub-abdominal part was shaved and an incision with a length of 2 centimeter was made. The skin, fascia and abdominal muscles were opened, and the fats and intestine were sheered off until the uterus and its tubes were apparent. Then the tube of uterus and vascular base of ovaries were twisted by Cat coot 4 thread around proximal area and were cut from distal area. Finally, 1-2
ml of saline solution was poured into the abdomen and the muscles and skin were replaced back and were stitched by 0-2 silk thread and the wound was washed by Betadin solution. In order to avoid the interference of estrus cycle, all experimental animals were ovariectomized (OVX) two weeks before the experiments (21).

**Experimental groups**
The rats were randomly divided into nine groups (n= 7 in each group): 1- intact group: the animals that were neither ovariectomized nor given any drugs; 2- OVX group: the animals had a bilateral ovariectomy; 3- sham group: OVX rats were sham surgically, but without actual induction of TBI; 4- TBI group: intact rats were injured using the traumatic brain injury device; 5- OVX+TBI group: OVX rats were injured using the traumatic brain injury device; 6- vehicle group: OVX rats were injected with 0.33 ml of sesame oil; 7– Estrogen (E2) group (Aboreyhan, Iran): OVX rats were injected with 17β-estradiol (1mg/kg) (22); 8– progesterone (P4) group (Aboreyhan, Iran): OVX rats were injected with progesterone (16 mg/kg) (22); 9- estrogen+progesterone (E2+P4) group: OVX rats were injected with a combination of both 17β-estradiol (1 mg/kg) and progesterone (16 mg/kg). Rats in the treatment groups received injections at 1 and 6 hr and 1, 2, 3, 4, and 5 days after the surgery (intraperitoneally for the first and subcutaneously for the remaining six) (22).

**Induction of TBI**
All animals before TBI were intubated. The TBI was moderate and diffuse using the Marmarou method. The TBI induction device (made by Department of Physiology, Kerman University of Medical Sciences) was implemented in a process which is described as follows: a 250 g weight was dropped from a 2 meter height on the head of the anesthetized rat [halothane in an mixture of 70% N₂O/30% O₂ gas] when a metal disc (stainless steel), 10 mm in diameter and 3 mm thick is attached on the animals skull. After induction of the trauma, the rats were immediately connected to the respiratory pump (TSA animal respiratory compact, Germany) and following restoration of spontaneous breathing, the endotracheal tube was removed (23). After recovery, the rats were placed in individual cages for 5 days and the gastric acid and pepsin levels were measured on the fifth day (22).

**Evaluation of gastric acid secretion**
After surgery, a period of 30 min was allowed for stabilization; once the gastric acid secretion (gastric acid output) had been stable for 30 min, it was considered as basal acid secretion. Throughout the experiment, the gastric secretions were collected in consecutive 15-min samples. The acid content of each gastric washout sample was measured with an autotitrator pH meter (PHM 85, Radiometer, Copenhagen, Denmark) to an endpoint pH of 7 with 0.01 N NaOH and expressed as µmol H⁺/15 min (24).

**Evaluation of pepsin levels**
Pepsin levels (pepsin output) in the gastric effluent were determined as previously reported. Briefly, 2 ml of 2.5% bovine hemoglobin plus 0.5 ml of 0.3 N HCl and 0.5 ml of gastric effluent were maintained in separate tubes at 37 °C for 15 min and then they were mixed. Mixtures were incubated for 10 min at 37 °C, and the reaction was stopped by adding 5 ml of 0.3 N trichloroacetic acid. After agitation and filtration, optical density was measured at 280 nm by using a spectrophotometer (UV/VIS, PG Instrumental, America). The results were compared to a standard curve, which was generated in an identical manner using known amounts of porcine pepsin (1 µg = 3 peptic units), and were expressed as micrograms of pepsin per 15 min (25).

**Statistical analysis**
SPSS 11.5 was used for statistical analysis. Each parameter was expressed as mean ± SEM, and compared using one-way ANOVA analysis of variance, followed by LSD test. The level of significance was $P<0.05$.

**Results**
**Gastric acid secretion**
Figures 1 and 2 show the gastric acid secretory response in normal, ovariectomized female rats,
and groups that were treated with E2 and P4. Acid output in intact group was 15.25±1.74 µmol/15 min, it was 20.03±2.22 µmol/15 min and 19±2.96 µmol/15 min in TBI and TBI+OVX groups respectively, but had no difference with intact group (Figure 1). There was no significant difference in gastric acid secretion (acid output) between different groups either after TBI induction or after treatment with E2 or P4 or E2+P4 (Figure 2).

**Gastric pepsin secretion**

Changes in gastric pepsin levels (pepsin output) for different groups are shown in Figures 3 and 4. Gastric pepsin levels (pepsin output) were increased in Sham+OVX (3.6±0.28 µg/15 min), TBI (3.3±0.2 µg/15 min) groups (P<0.001) and TBI+OVX group (2.8±0.16 µg/15 min) (P<0.05) when compared to intact group (2±0.29 µg/15 min), but there are no differences in gastric pepsin levels between OVX rats with intact rats (Figure 3). Gastric pepsin levels were significantly lower in E2 (2.5±0.3 µg/15 min) and E2+P4 (2.1±0.28 µg/15 min) treated rats compared to vehicle (3.4±0.19 µg/15 min) treated group (P<0.01). P4 treatment increased gastric pepsin level (3.5±0.3 µg/15 min) when compared to TBI-OVX group (P<0.05). There is also significant difference between P4 treated rats and E2 and E2+P4 treated rats (P<0.01) (Figure 4).

![Figure 1](image1.png)

**Figure 1.** Acid output (µmol/15 min) in different groups (n=7 in each group) after traumatic brain injury. Data are presented as mean±SEM. There was no significant difference between different groups. Abbreviations: OVX: ovariectomized female rats, TBI: Traumatic brain injury.

![Figure 2](image2.png)

**Figure 2.** Acid output (µmol/15 min) in ovariectomized rats (n=7 in each group) after traumatic brain injury. Data are presented as mean±SEM. There was no significant difference between different groups. Abbreviations: E2 (estradiol), P4 (progesterone).
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Figure 3. Pepsin output (µg/15 min) in different groups (n= 7 in each group) after traumatic brain injury. Data are presented as mean±SEM. *P< 0.05, TBI+OVX vs intact group ***; **P< 0.001, Sham+OVX, TBI vs intact group. Abbreviations: OVX: ovariectomized female rats, TBI: Traumatic brain injury.

Figure 4. Pepsin output (µg/15 min) in ovariectomized rats (n= 7 in each group) after traumatic brain injury. Data are presented as mean±SEM. **P< 0.01, E2 and E2+P4 groups vs vehicle group. #: P< 0.01, E2 and E2+P4 groups vs P4 group. +: P< 0.05, P4 group vs TBI+OVX group. Abbreviations: E2: estradiol, P4: progesterone.

Discussion
Cerebro-vascular disorders are often associated with damage to gastrointestinal tract, and some gastrointestinal tract disorders in acute phase of stroke are usually considered as stress-induced injuries (26). As peptic ulcer is more common in men than women (27), in the present research, we studied the effects of estrogen and progesterone on the acid and pepsin secretion following traumatic brain injury.

The results of study showed that administration of estrogen or progesterone has no effect on the acid output in TBI-OVX rats. The results were also consistent with results of this hormones on the gastric ulceration following traumatic injury (28), acid secretion due to stress (29), antiulcer effect of sexual hormones without affecting on the gastric acidity (30), ineffectiveness of progesterone on the acid secretion in ovariectomized rats (31),
no change in acid secretion after ovariectomy in female rats (32) and reduction of acid secretion following stress (33).

In this study we didn’t notice any change in acid secretion by ovarian hormones. Some of the possible mechanisms for action of these hormones against ulcerative effects of traumatic brain injuries are: maintaining calcitonin gen related peptide (CGRP) level, that has the protective role in mucosal injuries (34), exaggeration of mucosal secretion from gastric mucosal (35), the increment of bicarbonate secretion from gastric mucosal similar to the increased secretion of bicarbonate from duodenum mucosal by estrogen (36), change of hypothalamus-pituitary axis (HPA) response to stress (37), the reduction of epidermal growth factor (EGF) secretion in stomach (38), inhibition of oxidative stress (39), modulation of nitric oxide (NO) production (40), adjustment of autonomic nervous system activity (41), interaction with melatonin in gastric mucosal level (42), upgrading of angiogenesis (43), the reduction of gastric inflammation by decreasing of tumor necrosis factor (TNF) release (44), and inhibition of apoptosis due to ischemia (45). However, there are some studies that their results do not match our results. Among these studies we can mention: inhibition of gastric injury due to ischemia-reperfusion by pharmacologic doses of estrogen (46), the inhibition of gastric acid secretion in human and animals (47), increase of ulceration in ovarectomized rats (48), and the reduction of stimulated gastric acid secretion by beta- estradiol (49). Possible reasons for the difference between the present study and above-mentioned results are: the difference in administration doses, the kind of vehicle, the kind of animal, and the time of administration.

Another possible mechanism for anti-ulcerative action of ovarian steroid hormones during stress and trauma condition is changing in pepsin secretion. There is significant relation between gastric injuries and pepsin output and consequently the pepsin output increases after ischemia (50). Recently, pepsinogen measurement is used as an effective biochemical method for assessing and monitoring gastrointestinal diseases (particularly stomach) and monitoring the therapeutic effects of drugs (51). Pepsinogen increases in gastritis and its activation in gastric lumen may be one of the reasons for mucosal injury in peptic ulcer disease (52). It also has an important role in the development of gastric lesions because the severity of these lesions was remarkably reduced by peri administration of peptatin, a specific pepsin inhibitor. Pepsin is more important than excessive acid production in gastric mucosal lesion (50). Therefore, in the other part of this study, pepsin output after trauma and in the presence of ovarian hormones was measured. The results showed that pepsin output is much higher among TBI and TBI+OVX rats compared to healthy ones. The animals in the sham group also showed an obvious increase in pepsin output, which was similar to that of the trauma animals. The reason for the increase in pepsin output in sham group is not clearly understood, although it may be attributed to the implantation of the plate for TBI induction and trachea intubation. Administration of beta estradiol led to reduction of pepsin output and this reduction was less than progesterone- treated rats. Furthermore, the similar results were seen by co-administration of estradiol and progesterone. The results of this study is consistent with other studies such as: reduction of pepsin output in pregnancy when the concentration of ovarian hormones is high (53), reduction of pepsin output by ovarian hormones in rats (32), inhibition of pepsin secretion in ovarectomized rats by estradiol (54), increment of pepsin secretion followed by gastric mucosal inflammation after stress (55).

Possible mechanisms for the reduction of pepsin secretion following administration of ovarian hormones are as follows: modulation of NO production (56), modulation of gastrin and CCK production (57), modulation of inflammatory cytokines release such as IL-1 and TNF-α (37, 58), the change in HPA axis response to stress (37), modulation of autonomic nervous system (41), cross reaction between steroid hormones, and enteric system of GI tract (57). Disruption of mucosal barrier
by enhancement of luminal pepsin causes the acid intrusion into to underlying epithelium (55). Pepsin increases the release of inflammatory cytokines which leads to inflammation and damage to gastric mucosa (59). Pepsin contributes to mucosal injury by inhibiting expression of protective mucosal proteins such as mucins and carbonic anhydrase and induces expression of stress-response genes independent of acid (60). It also causes gastric mucosal injury by damaging the mucosal barrier in patients with severe stress (61).

A number of studies have expressed a contradictory result regarding the effects of ovarian hormones on the pepsin secretion: oral consummation of estrogen don’t have any effect on the pepsin secretion (35), estrogen or progesterone doesn’t have any reduced effect on the pepsin output in patients with peptic ulcer (62) despite the control of experimental ulceration by ovarian hormones in female rats. Possible reasons for the differences with previous studies are mentioned in the previous paragraphs.

Conclusion
In the present study, authors confirmed that administration of estrogen alone or co-administration of estrogen and progesterone has an inhibitory effect on the pepsin output. These results suggest that the possible protective effect of ovarian hormones against mucosal damage induced by TBI is by inhibition of pepsin secretion and not acid secretion. Since the possible mechanism(s) of inhibitory action of hormones on the pepsin secretion and the estrogen receptors involved in this action have not been identified, therefore more research is needed.

Acknowledgment
The present study was financially supported by the Neuroscience and Physiology Research Centers of Kerman University of Medical Sciences, Kerman, Iran. The authors declare that they have no conflict of interests.

References