The Effect of Estradiol and Male Gender on Gastric Emptying Rate in Rats

SIÇANLARDA MİDE BOŞALMA ORANINA ERKEK CİNSİYETİ VE ÖSTRADİOLÜN ETKİSİ

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Abstract

Objective: The aim of this study was to investigate the effect of estradiol and castration on gastric emptying rate.

Material and Methods: 48 Adult male 230-250 g Wistar-Albino rats were divided equally into six groups. Control group (Group-1) did not receive any medical or surgical treatment. Sham group (Group-2) underwent scrotal skin incision and closure only. Eight rats (Group-3) were given 20 μg/kg/day 17-β ethynyl estradiol benzoate subcutaneously, for five days, while eight rats (Group-4) were receiving 25 mg/kg/day cyproterone acetate (CPA) subcutaneously for five days. 16 rats underwent bilateral orchiectomy. Nine days after the operation, eight rats (Group-5) were given oil 1ml/kg/day subcutaneously as the vehicle of the estradiol for five days. Eight rats (Group-6) were treated with 20 μg/kg/day 17-β ethynyl estradiol benzoate subcutaneously for five days. All rats in the treatment groups were subjected to methyl cellulose-phenol red gastric emptying test at the fifth day of the treatment.

Results: Gastric emptying rate was found to be decreased in estrogen treated (group-3) rats when compared to control group. CPA treatment alone has no effect on GER. Although castration has not significantly inhibited the GER, inhibition of GER in estrogen treated-castrated rats approached the level in Group-3.

Conclusion: We concluded from this study that estrogen decreases the GER. Only chemical or anatomical castration did not cause a significant delay in gastric emptying. Inhibitory effect of estrogen on GER seemed to need combined or synergistic effect of androgenic hormones.

Key Words: Gastric emptying rate, estradiol, male gender

Özet

Amaç: Bu çalışmanın amacı kastrasyon ve östradiolün mide boşalma oranına etkisini araştırmaktır.

Gereç ve Yöntemler: Erişkin erkek 230-250 g ağırlıklarındaki 48 Wistar-Albino sıçan eşit olarak altı gruba ayrıldı. Kontrol Grubu (Grup-1) herhangi bir medikal veya cerrahi tedavi almadı. Sham grubuna (Grup-2) sadece skrotal cilt insizyonu yapılıp kapatıldı. Sekiz sıçana (Grup-3) beş gün süre ile subkutan 20 μg/kg/gün 17-β ethinil östradiol benzoat uygulanırken, diğer bir sekiz sıçana da (Grup-4) beş gün süre ile subkutan 25 mg/kg/gün siproteran asetat uygulanıldı. 16 sıçana bilateral orşiektomi uygulandı. Operasyondan dokuz gün sonra, sekiz sıçana (Grup-5) östradiol taşıyıcısı olarak 1ml/kg/gün sıvı yağ subkutan olarak beş gün süreyle uygulandı. Sekiz sıçan (Grup-6) subkutan olarak beş gün süreyle 20 μg/kg/gün 17-β ethinil östradiol benzoat ile tedavi edildi. Tedavi gruplarındaki tüm sıçanlara, tedavini beşinci gününde methyl cellulose-phenol red Mide boşalma testi uygulanıldı.

Bulgular: Kontrol grubuyla kıyaslandığında estrogenle tedavi edilmiş sıçanlarda (Grup-3) Mide boşalma oranlarının (MBO) azalmış olduğu bulundu. Siproteron asetatın MBO'na anlamlı etkisi olmadığı izlendi. Kastrasyonun belirgin olarak MBO'nını inhibe etmemesine rağmen, östrojenle tedavi edilen kastre edilmiş sıçanlarda (Grup-6) MBO'nın, Grup-3 MBO seviyelerine yaklaşan oranlarda azaldığı gözlenmiştir.

Sonuç: Bu çalışma sonucunda östrojenin MBO'larını azalttığı sonucuna vardık. Sadece kimyasal veya anatomik kastrasyon mide boşalmasında anlamlı bir gecikmeye neden olmamıştır. MBO üzerinde östrojenin inhibitör etkisi androjenik hormonların ilave veya sinerjistik etkisine ihtiyaç duymaktadır.

Anahtar Kelimeler: Mide boşalma oranı, östradiol, erkek cinsiyet

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It has long been known that pregnant women often complain of heartburn, emesis, dyspepsia, abdominal distension and constipation. Coşkun et al.[1] concluded that both estrogen and progesterone exerts inhibitory effects on gastric

emptying, and this may account for the disturbances in gastrointestinal function that pregnant women frequently experience. It was promulgated that gastric emptying was influenced by the gender and menopausal state.[2] Although the Caballero[3] found no difference between men and women in gastric motility during fasting, he reported a "postprandial physiologic gastroparesis" in women.

An antiandrogen agent, cyproterone acetate (CPA) has been shown to inhibit the uterine contractility either directly or indirectly.[4] The agent competes with dihydrotestosterone for binding to the androgen receptor.[5] There has been no known effect of this drug on gastrointestinal motility. It was also shown that pre-treatment with testosterone dose-dependently aggravated cysteamine induced gastroduodenal ulcer.[6] These findings hinted us that the testosterone might have similar or stimulating effect on gastrointestinal system. We did not encounter a study of which evaluated the effect of CPA on gastric motility in the literature. Castration ceases almost all the body testosterone production. Subsequent testosterone withdrawal may have some effects on gastrointestinal system. How modulation with testosterone affects the GER, either on receptor or production level, is being expected to be investigated.

Current study was planned to investigate effect of estrogen and male gender on gastric emptying rate (GER) and try to explain the modulatory mechanism.

Material and Methods

48 Adult 230-250g. weighed male rats were divided equally into six groups. This study has been approved by the Marmara University Animal Study Ethical Committee. All rats were anesthetized with 100 mg/kg IP ketamine anesthesia. Control group (Group-1; n=8) did not receive any medical or surgical intervention and have only gastric emptying test. Sham group (Group-2; n=8) has subjected to scrotal skin incision with continuous suture skin closure.

Group-3 and group-4 have subcutaneous estradiol (Est; n=8) and cyproterone acetate (CPA; n=8) treatment, respectively, for five days. Group-5 (n=8) and group-6 (n=8) have both castrated by bilateral orchiectomy through a midline scrotal skin incision and closure with continuous suture. Group-5 has been pre-treated with oil (O) as the vehicle of estradiol. Group-6 has estradiol (Est) pre-treatment after the operation. Drug administrations have commenced 9 days after the castration operation due to the expectation of completeness wound healing process and to return to the normal body hormonal status after surgery. Treatment has carried on for five days subcutaneously for each medication. Estradiol was given as the 17-β-ethynyl estradiol benzoate 20 μg/kg/day. CPA administered as a dose of 25 mg/kg/day. Oil injection was done as a dose of 1 ml/kg/day.

Gastric Emptying Test (GET): Rats were kept from oral feeding and coprophagy 24h before GET. One ml of methyl cellulose-phenol red solution was given to the rat stomach through an orogastric polyethylene tubing. 30 minutes later, rats were decapitated and the stomach was excised subsequent to clamping the entrance and exit of the stomach. This test was performed by the application of methyl cellulose-phenol red method. Gastric emptying rate (GER) was measured as the percentage amount of the methyl cellulose-phenol red solution evacuated from the stomach. All operated groups subjected to the GET at postoperative 15^{th} day.

Statistical analysis: Results were presented as mean ± standard error of mean. Groups were compared by the analysis of variance (ANOVA) test. Post-hoc test (Tukey-Kramer) has intended to perform if P value was less then 0.05.

Results

One-way analysis of variance of all groups has not revealed a statistically significant dif-

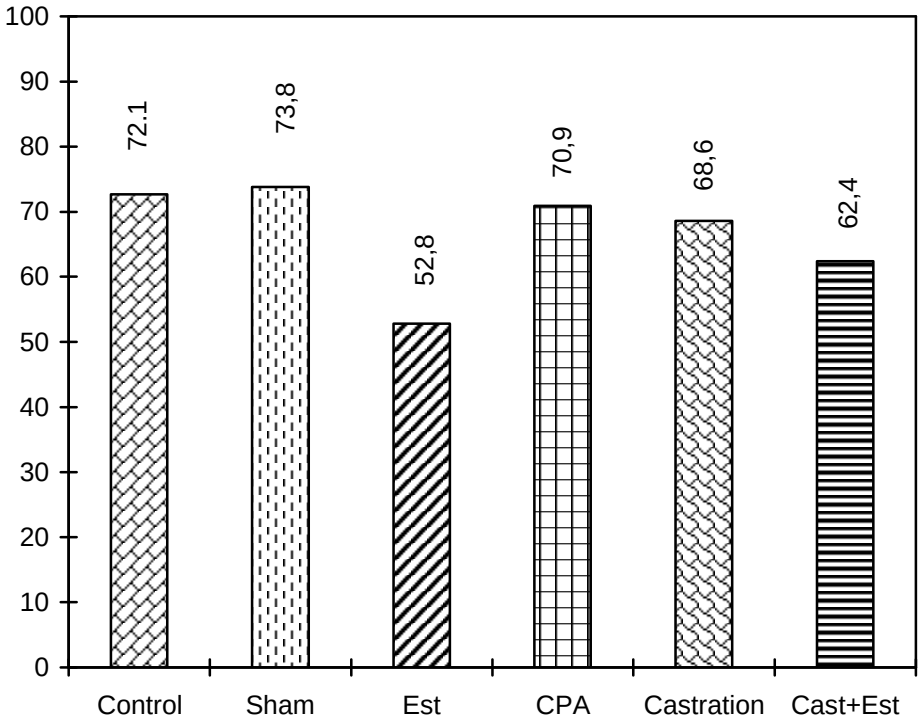

Figure 1. Gastric emptying rates of the study groups
GER %: Gastric Emptying Rate % Est: Estradiol. CPA: Cyproteron Acetate. Cast+Est: Castration and Estradiol.

Table 1. Gastric emptying rates (GER %) in study groups.

	Control	Sham	Est	CPA	Castration	Cast+Est
GER %	72.13±3.5 %	73.86±3.54%	52.8±7%	70.9±5.2%	68.6±10%	62.4±8.7%
ANOVA	p> 0.05					

Est :Estrogen treatment group
CPA : Cyproterone acetate treatment group.
Cast+Est : Castration and estrogen treatment group.
ANOVA : Analysis of variance. No significant difference between all groups by ANOVA.
* Comparison between control vs sham p>0.05 student's t test.
** Comparison between control vs est p<0.05 student's t test.
*** Comparison between control vs cast+est p>0.05 student's t test.
**** Comparison between control vs cast p>0.05 student's t test
***** Comparison between control vs CPA p>0.05 student's t test

ference among all the groups (p=0.2140; F=1.492) (Figure 1). GER in control group was 72.13±3.5%. Sham operation did not affect the GER (73.86±3.54%). GERs in Estradiol, CPA

treatment groups were 52.8±7% and 70.9±5.2% respectively. Although there was not a statistically significant difference between group-1 and group-3 by ANOVA, a significant difference was found when two groups were compared by unpaired student's t test (p=0.03; t=2.4; 14 df). GER in group-5 was 68.6±10%. Group-6 has a lower level of GER (62.4±8.7%) than group-5. This value was not statistically significant by both ANOVA and unpaired student's t test (Comparison with group-1 and group-6; p=0.3; t=1.07; 13 df). Neither CPA (Group-4), nor castration (Group-5) caused a significant decrease in GER, Est administration in castrated animals (Group-6) inhibits the GER to a deeper level (62.4±8.7%). Nevertheless, this was not found to be statiscally significant (Table 1).

Discussion

Gastric emptying is one of the most reliable indicators of the gastric function. It can be used as a measure in the assessment of the gastric motility. Several clinical observations, and limited animal experiments have led to speculations concerning the possible effects of sex steroids on gastrointestinal function.[7] It has been promulgated that the most of the symptoms of upper gastrointestinal origin in gestation was due to the alterations in gastric functions. Many researchers has suggested that Est is responsible for the gender effect on GER. In our study, the decreased GER in group-3 was possibly the result of the inhibitory effect of Est on gastric smooth muscle. It was reported that both vomiting and abdominal distension which are common pregnancy-related symptoms mainly occurring in the early phases of pregnancy, tend to occur at about the time of rapid increase in estradiol.[8] Horowitz et al.[9] suggested that the menstrual cycle has no effect on solid or liquid phase gastric emptying. Bond[10] showed that estrogen treated female rats have lower and male rats elevated levels of gastric emptying rates. In our study, Est decreased the GER in male rats (Figure 1). However this effect did not seen in castrated animals. Bruce et al have reported a progesteronic inhibitory[11] and an estrogenic excitatory[12] influence on gastrointestinal motility. The ratio between the androgenic and estrogenic hormonal activity seemed to be the important factor in the appearance of the effects of various stimulus. Coskun et al.[1] demonstrated that progesterone dose dependently inhibits the GER. Although Chen[13] showed that testosterone had no influence on gastric emptying or gastrointestinal transit, he found that progesterone-estradiol mixture decreased the gastric emptying to a degree as estradiol did. Our findings in non castrated animals support this data (Group-3). Est administration in castrated rats (Group-6) caused a decrease in GER that was not as low as in the Group-3. This implicated that Est worsens the GER when it works together with testosterone. Est administration to the castrated rats caused a more decrease in GER than in the group-5. It was reported that estrogen increases propagation velocity of canine gastrointestinal slow waves, while progesterone has the reverse effect.[14] Studies have also demonstrated that estrogen increases gastrointestinal contractile activity and decreases transit time, while progesterone or the combination of estrogen and progesterone decreases contractile activity and slows transit.[12,15]

CPA blockade of testosterone receptors seemed to dominate the Est effect on GER. That did not turned out to be so in our study. CPA did not induce a remarkable change in GER (Group-4) (Table 1). This is convenient above mentioned Chen's[13] findings. Chen[13] also suggested that progesterone at lower (10 mg/kg) doses decreases the gastric emptying and increases it at higher (20-40 mg/kg) doses. This study also showed that a mixture of estradiol (10 microgram/kg) and progesterone (20 mg/kg) inhibited the GER to a similar degree as estradiol (10 microgram/kg) did. We used 20 microgram estrogen in all study groups. Despite the lack of data about the testosterone effect on gastric emptying,

it has been reported that testosterone aggravated the cysteamine-induced gastroduodenal mucosal injury.[6] Surgical (group-5) or chemical (Group-4) castration with CPA did not significantly alter the GER while castrated animals treated with estrogen (Group-6) have more decreased GER not as much as group-3 rats. These findings implied that Est modulates the GER through the combined effect with androgenic or progestative hormones. This combination requiring effect of Est on GER, as promulgated in Chen's[13] study, may modulate in a dose dependent manner. However, current study did not aim to find out this dose interval. Our study is a preliminary trial that intended to show the Est and male gender effect on GER. As ongoing studies in the near future, we are planing to investigate if there is any difference between both sexes in GER as a response to the various noxious gastrointestinal stimuli such as gastrointestinal ulceration, distension, pain etc.

Pharmacologic ovariectomy of female inflammatory bowel syndrome patients by administration of long acting gonadotropin releasing hormone (GnRH) analogue has been reported to alleviate the symptoms.[16] Matthias[17] treated a chronic intestinal pseudoobstruction patient with GnRH analogue (leuprolide acetate). The patient had improvement of intestinal motility documented by antroduodenal manometric study. These data suggest an opposing effect of GnRH in response to elevated testosterone on GER in castrated animals (Group-5). In Group-6, elevated levels of GnRH in response to lack of testosterone could be responsible for the elevated GER levels when compared to group-3. This proposed mechanism needs to be further studied.

As a conclusion; Est, in male has an inhibitory action on GER. Testosterone as a sole has no effect on GER and male gender itself is not a determinant factor in GER. Est itself has not as effective in castrated animals as in the non-castrated animals. This finding implicated us that Est carry out its inhibitory effect on GER through the action of testosterone.

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