Effect Of Overiectomy and of Estrogen Administration Upon Duodenal Ulceration Induced By Cysteamine

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Abstract

Duodenal ulcers were induced in ovariectomized, intact and old mice using cysteamine hydrochloride. Under these experimental conditions ovariectomy and old age strikingly increased sensitivity to ulcer induction while estrogen administration showed a decrease in sensitivity to ulcer induction. Nevertheless, the administration of estrogen in old mice showed no effects in either intact or overiectomized mice. This change in ulcer sensitivity reflected from histological, histochemical and biochemical studies. The histological study was performed by using haematoxylin-eosin and haematoxyline staining technique. The histochemistry of the duodenal region was studied by using periodic acid Schiff reaction (PAS) for glycoproteins. The biochemical study was performed to study various constituents of glycoproteins like hexose, fucose, sialic acid and the protein. The result showed that ulcer severity was more in overiectonized cysteamine treated mice and old mice treated with cysteamine. The histological studies showed that ovariectomy decreased or did not show any change in the ulcer sensitivity considering cryptus Lieburkuhn and Brunner's glands. The same result reflected by differential intensity in the staining property of the Brunner's gland. The biochemical study showed that the glycoprotein contents were reduced many times in overiectomized cysteamine treated mice and their reversal in estrogen administered ovariectomized cysteamine injected mice. These findings prove that estrogen protects the duodenal ulcer from cysteamine administration.

Keywords: Brunner's gland, Cysteamine, Duodenal ulcer, Estradiol-17β, Ovariectomy, Ulcer index.
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Introduction

Duodenal ulcer is a mucosal erosion of duodenum, due to multiple causes, including bacteria (Marshall and Warren 1984; Lykoudes 1958), chewing gum, tobacco smoking, not eating properly, blood group, spice (NADDIC-National Alcohol and Drug Dependence Industry Committee), chronic stress (Kim et al. 2007) and gender differences (Andes et al. 2008). Specific protection of estrogen against gastric –acid induced duodenal injury was reported (Andes et al. 2008). Peptic ulcer occurs more frequently in men than in women (Grey 1929). The sex differences are less marked after 45 years of age probably because the incidence of ulcer increases in post menopausal women (Crean 1963; Watkinson 1960). The general assumption is that the ulcer differences between sexes are related in some way to sex hormones and that the female sex hormones protect against ulceration (Crean 1963; Kyle et al. 1963). In contrast to this Ovariectomy (2 weeks before cysteamine) decreased plasma 17β-estradiol level as assessed by radioimmunoassay, gastroduodenal macroscopic injury and albumin extravasation and increased mucus levels following cysteamine challenge (Drago et al. 1999). The actions of the female sex steroid, estradiol on

cysteamine-induced mucosal ulceration have been reported in female Wistar rats (Szepes et al. 1999). The immunity against duodenal ulcer increases in females during pregnancy (Dey and Dey 1974). It was shown that the chance of men developing duodenal ulcer remains remarkably constant between the ages of 20 years and 65 years. In contrast the chances of women developing duodenal ulcer remain relatively low throughout the whole life of reproductivity (Truelove 1960). The role of estrogen treatment on the healing of acetic acid-induced gastric or colonic injury was reported in rats (Günal et al. 2003) and liver and intestine injury in rats (Sener et al. 2005). Nevertheless, female sex hormones have been reported to promote ulceration (Antonsen 1955; Guerrine et al. 1967) or at best to have no effect on the disease. Increased susceptibility to gastric ulceration during the late pregnancy was also reported (Kelly and Robert 1969). Duodenal ulcer sensitivity increases with age (Christensen et al. 2006). Therefore the present study was undertaken to investigate the effect of ovariectomy in mice on the sensitivity of duodenal ulceration induced by using cysteamine –HCl and to determine whether estrogen administration reverses the effects of ovariectomy and aging. The study was also carried out using histological, histochemical and biochemical methods in the duodenal region of the normal, ovariectomized, ovariectomized-estradiol, ovariectomized—cysteamine-estrdiol, cysteamine injected, ovariectomized– cysteamine injected, old female mice, cysteamine injected old female mice and cysteamine-estradiol injected old female mice.

Materials and methods

Experimental animals

There were 60 mice (Swiss albino mice (*mus musculus*) in the present study. The mice were divided into two groups- 2 months old females (40 members) n=10 (Control animals), n=10(mice treated with cysteamine-HCl), n=5(ovariectomized mice), n=5(ovariectomized mice treated with cysteamine-HCl),n=5 (ovariectomized treated with estrdiol), n=5 (ovariectomized mice treated with cysteamine-HCl and estradiol) and 3 years old females (20 members) n=10 (control animal), n=5(mice treated with cysteamine-HCl), n=5 (mice treated with cysteamine-HCl and estradiol).

Ovariectomy and administration of estrogen

The ovariectomy was done under mild ether anesthesia. The operated mice were maintained for 15 days in separate cages with optimum care of light, temperature, humidity, food and water. On the 16th day of ovariectomy 10 ovariectomized mice were treated with cysteamine (40mg/100g body weight) (i.p) and five mice were treated with estradiol-17β (2 mg/100g body weight dissolved in olive oil) (Sigma, Batch no. E9505) (i.p) consecutively for 3 days. The five ovariectomized cysteamine treated mice were injected estradiol in olive oil. On the 4th day of the 1st injection, cysteamine treated ovariectomized and cysteamine treated ovariectomized estrogen administered mice and the ovariectomized mice were used for duodenal ulcer index calculation (Szabo 1978). Ten 3 years old mice were used for administration of cysteamine (40mg/100 body weight) (i.p) consecutively for 3 days. Out of ten, cysteamine treated 3 years old mice, five mice received estradiol (2 mg/100g body weight dissolved in olive oil). After three days, cysteamine treated, cysteamine treated - estradiole administered and control, three years old mice were subjected for ulcer index calculation.

Duodenal ulcer index

The duodenal ulcer index were critically evaluated with respect to percentage incidence, number of ulcers per mice and severity of ulcers and were graded according to scale from 0 to 3 based on microscopic observations and later confirmed by sectioning and staining of the ulcer regions. Zero for no ulcers, one for superficial mucosal ulcers, two for deep ulcers and three for perforating ulcers. The ulcer index was calculated by the formula:-

Ulcer index = Mean severity + Incidence (i.e. positive/ total) × 2

Histology

The tissues were fixed in 10% neutral buffered formaline, washed and routinely processed for histological technique. The sections were stained with haematoxyline-eosine (Gurr1962). Histology of pyloric glands, duodenal villi, crypts of Lieberkuhn and Brunner's glands in all groups were studied.

Histochemistry

To study the changes in the duodenal mucosa glycoproteins of crypts of Lieberkuhn, goblet cells, pyloric gland cells and Brunner's glands in all groups, PAS techniques (McManus 1946) were used.

Biochemistry

The glycoprotein from Brunner's gland was isolated by the method of Satakopan and Kurup (1977). To study various constituents of glycoproteins biochemical estimations of fucose (Dische and Shettles 1977), hexose (Dubois et al. 1956), sialic acid (Warren 1959) and protein (Lowry et al. 1951) were used.

Data analysis

Statistical analyses were performed using the Statistical Package for Social Science (version 13.0, SPSS, Inc) software. Results were expressed as means ± SE (standard Error). All reported p-values were made on the basis of 2-sided tests and compared to a significance level of 5%, differences were considered statistically significant at $p < 0.05$.

Results

The ovariectomized mice showed little ulceration (Ulcer index 2.6) or no ulceration. The overiectomozed + estradiol- 17β injected mice showed very low ulceration compared to the ovariectomized mice (ulcer index 1.64) (Table 1) The ovariectomized + Cysteamine administered mice showed higher ulceration (Ulcer index 6.2) (Table 1).The administration of estradiol- 17β to cysteamine injected ovariectopmized mice showed little recovery of ulceration (Ulcer index 4.2) (Table No.1). The old mice administered with cysteamine showed ulceration as high as ovariectomized + cysteamine administrated mice (Ulcer index 5.8) (Table 2).

Table 1: Ulcer severity in normal mice with and without administration of estradiol- 17β

Group	Experimental mice	Ulcer (In %)					
		Percentage incidence	Superficial	Deep	Perforating	Mean severity	Ulcer index
Two months old female mice	Ovariectomized	100	93	05	2	0.12±0.10	2.6
	Ovariectomized + estrogen	100	97	2	1	0.82±0.12	1.64
	Ovariectomized + cysteamine	100	10	70	20	3.1±0.10	6.2
	Ovariectomized + Cysteamine + Estrogen	100	30	60	10	2.20±0.06	4.2

Table 2: Ulcer severity in ovariectomized mice with and without cysteamine and estrogen administration

Group	Experimental mice	Ulcer (In %)					
		Percentage incidence	Superficial	Deep	Perforating	Mean severity	Ulcer index
Three years old female mice	Old mice + Cysteamine	100	10	70	20	2.90 ±0.12	5.8
	Old mice + Cysteamine + estrogen	100	14	68	18	2.85±0.11	5.7
	Old mice (Control)	00	00	00	00	00	00

Table 3: Carbohydrates and protein contents of soluble glycoprotein isolated from Brunner's glands of female mice

Group	Hexose	Fucose	Sialic acid	protein	P values	
Normal	73.916 ±0.83 **1**	4.07+0.12 **10**	0.31+0.09 **19**	20.17+0.09 **28**	**1:2** p<0.005	**19:20** P<0.001
Normal + Cysteamine	20.5±0.25 **2**	1.49+0.12 **11**	0.06+0.01 **20**	14.89+0.33 **29**	**3:4** p<0.005	**21:22** P<0.001
Ovariectomized	63.00±0.74 **3**	3.68+0.04 **12**	0.43+0.01 **21**	19.23+0.04 **30**	**5:6** p<0.005	**23:24** P<0.001
Ovariectomized + Cysteamine	11.20±0.29 **4**	0.55+0.07 **13**	0.04+0.01 **22**	12.45+0.18 **31**	**7:8** p< 0.005	**25:26** p< 0.001
Ovariectomized + Estrogen	81.25±0.24 **5**	3.97+0.07 **14**	0.32+0.01 **23**	21.98+0.18 **32**	**8:10** p<0.005	**27:28** P< 0.005
Ovariectomized + Cysteamine + Estrogen	35.66±0.97 **6**	2.18+0.03 **15**	0.43+0.01 **24**	14.44+0.33 **33**	**11:12** p<0.001	**29:30** p< 0.005
Old	61.12±0.74 **7**	21.68+0.0 **16**	0.30+0.00 **25**	17.13+0.12 **34**	**13:14** p<0.05	**31:32** p< 0.001
Old + Cysteamine	21.50±0.31 **8**	1.90+0.02 **17**	0.08+0.01 **26**	15.81+0.22 **35**	**15:16** P<0.005	**33:34** P<0.005
Old + Cysteamine + Estrogen	20.9.23± 0.25 **9**	1.85± 0.1 **18**	0.09± 0.01 **27**	14.80±0.12 **36**	**17:18** P<0.5	**35:36** P<0.5

- Values are mean ± Standard error, p< 0.05 is significant

Administration of estradiol- 17β to cysteamine treated old mice failed to recover the severity of ulceration (Table 2). The histology of duodenum of normal, cysteamine treated and ovariectomized mice are shown (Fig. 1 to 3). The histology of ovariectomized and cysteamine treated mice showed that the pyloric glands were simple tubular and situated deeply in the sub mucosa. The duodenal villi were tall, leaf like and uniformly arranged with desquamation intermittently. The crypts of Lieburkuhn and Brunner's glands were unaffected. The cysteamine administration to

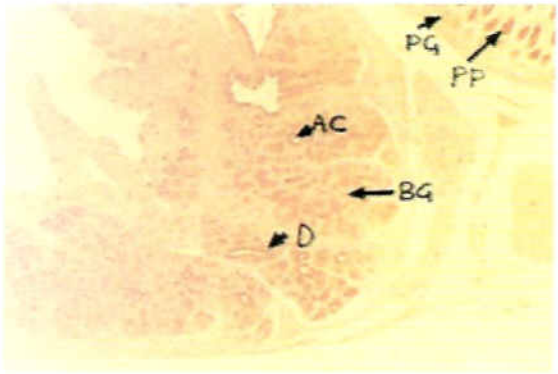

Figure 1. Micrograph of duodenum of Normal mice
PP-pyloric pit, D-Duct, PG-Pyloric gland
BG- Brunner's gland

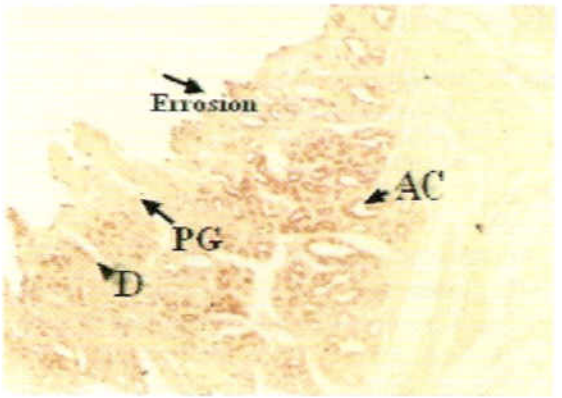

Figure 2. Micrograph of duodenum of cysteamine Treated mice
AC-Acihi R-Duct, PG-Pyloric Gland

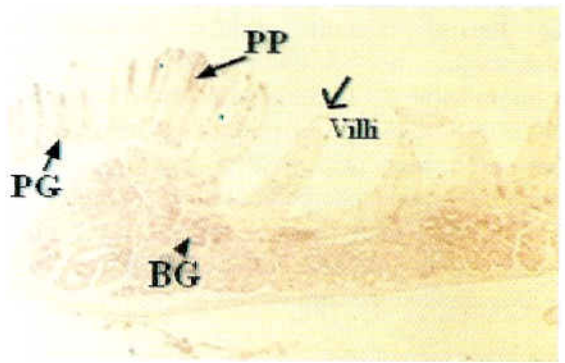

Figure 3. Micrograph of duodenum of Ovariectomized mice
PP-Pyloric Pit, PP-Pyloric gland
BG-Brunner's gland

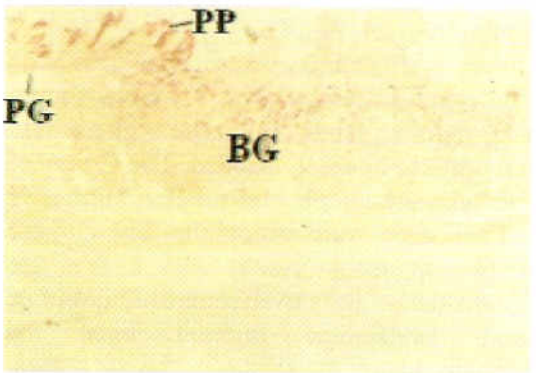

Figure 4. Micrograph of duodenum of Ovariectomized-cysteamine Injected Mice
PP-Pyloric Pit, PP-Pyloric gland
BG-Brunner's gland

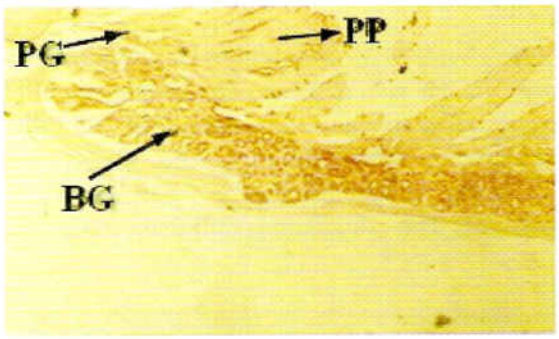

Figure 5. Micrograph of duodenum of Ovariectomized Hormone treated Mice
PG-Pyloric gland, PP-Pyloric pit, BG-Brunners gland

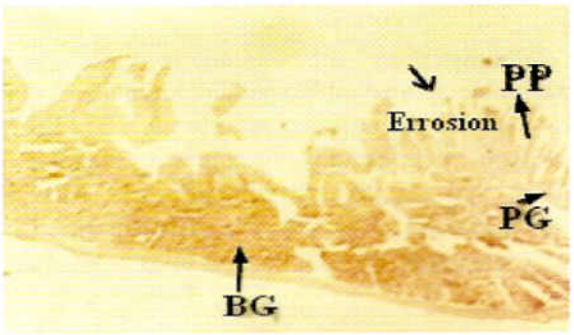

Figure 6. Micrograph of duodenum of Ovariectomized-Hormone injected - Cysteamine treated Mice
PP-Pyloric pit, BG-Brunners gland, PG-Pyloric gland

the ovariectomized mice (Fig. 4) causes pyloric glands with dilated lumen and picnotic nuclei and increased eosinophilia. The pyloric villi showed fissures and ramifications, and ulcer formation. The goblets cells were less in number. The Brunner's gland acini showed reduced height, dilated lumen and nuclei with abnormal size and shape. The histochemistry showed strong PAS reaction in pyloric glands, Brunner's glands in ovariectomized mice but less in pyloric pit (Fig. 4).

In the ovariectomized-estrogen treated mice the duodenal histology (Fig. 5) shows simple tubular pyloric glands. The pyloric glands and pyloric pit are intensely stained. The Crypts of Lieberkuhn are simple and tubular. Brunner's gland and ducts were esinophilic. The villi are leaf like at many places and a few are desquamated. In ovariectomized-hormone treated- cysteamine injected mice the duodenum histology (Fig. 6) shows pyloric glands, acinar cells and ducts reduced in PAS staining. Goblets cells reduced in number. The erosion was more and pyloric pit was diffused. The histology of duodenum of old mice, old mice treated with cysteamine and old mice treated with cysteamine and estrogen (Fig. 7, 8 and 9 respectively) showed that after cystemaine treatment the villi reduced in height and shows erosion. The Crypts of Lieberkuhn were narrower and deep. The Brunners gland, acinar cells and ducts stain less intensely. On the treatment of estrogen in cysteamine treated old mice the histological changes are similar to the cysteamine treated old mice (Fig. 9).

The duodenal villi showed PAS activity but other cells were PAS negative. The cysteamine treated ovariectomized mice showed reduction in PAS reactivity in all cells of pyloric glands, pyloric pits, goblet cells and Brunner's glands (Fig. 4). The biochemical studies showed that the hexose contents reduce 3 fold in cysteamine injected mice compared to the normal (Table 3). In the ovariectomized group, it was less than that of normal but more than that of cysteamine treated mice. But in ovariectomized cysteamine treated mice it was 6 times less than that of normal mice. The fucose, sialic acid and protein contents (Table 3) also showed the same trend as hexose.

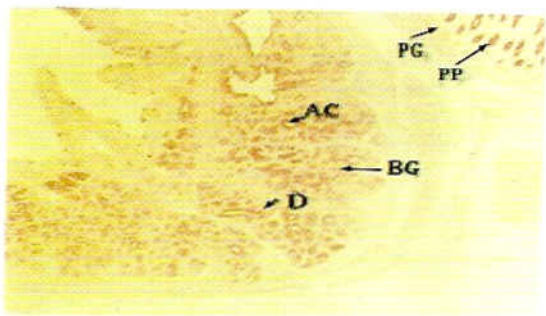

Figure 7. Micrograph of duodenum of Old Mice
PG-Pyloric gland, PP-Pyloric pit

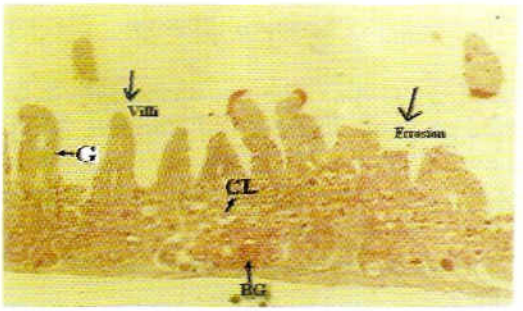

Figure 8. Micrograph of duodenum of Old Mice treated with cysteamine
G-Goblet cell, CL-Crypts of Leiberkuhn,

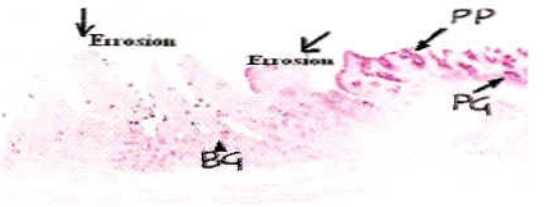

Figure 9. Micrograph of duodenum of Old Mice treated with cysteamine and Hormone
PP-Pyloric pit, PG-Pyloric gland
BG-Brunner's gland

Discussion

Peptic duodenal and gastric ulcers raise serious health problems and significant global economic cost. There are approximately 500,000 new cases and 4.5 million people suffering from these diseases each year in USA (Valle et al. 2003). Duodenal ulcer is three times more common than gastric ulcer. Available evidence suggests that duodenal ulcer most likely results from an imbalance between "aggressive" factors, such as infection of *Helicobacter pylori (H pylori),* gastric acid and pepsin, and "defensive" factors, such as duodenal mucosal bicarbonate (HCO^-_3) secretion (DMBS) (Valle et al. 2003; Hogan et al. 1996) and sex hormones . It has long been observed that the ratio between men and women who develop duodenal ulcer is 1.9:1 in the US, whereas in Europe and in Asia this ratio is 2.2: 1 (Kurata et al. 1985; Rosenstock and Jorgensen 1995; Ostensen et al. 1985; Bonnevie 1975) and 3.1: 1 (Wu et al. 2008), respectively. These clinical observations suggest a sexual difference in the incidence and severity of duodenal ulcer; however, the underlying mechanism(s) are currently unknown. As far as sexual differences are concerned, sex hormones have been often evaluated as the causative factors. For example, numerous studies have suggested a protective role of estrogen in the development of various diseases including cardiovascular diseases (Gerhard and Ganz 1995; Grodstein et al. 1996; Orshal and Khalil 2004), cerebral damage and mortality (Zhang et al. 2004; Hurn and Macrae 2000), and osteoporosis (Gerhard and Ganz 1995; Grodstein et al. 1996; Orshal et al. 2004; Popp et al. 2006). In contrast, information regarding the protective effects of sex hormone in the gastrointestinal tract is very limited (Furner et al. 1989). In contrast sex hormone protection by duodenal ulcer is also challenged (Günal et al. 1999). In rats it is also reported that estrogen can protect liver and intestines against sepsisi-induced injury (Sener et al. 2005). Thus the effect of estrogen as a protective or as a cause of duodenal ulcer still remains a controversial subject. The present investigation showed that cysteamine induced duodenal ulcer severity decreased on the administration of estrogen. But estrogen failed to recover the ulcer incidence in old females. This indicates that estrogen itself is not the reason for the low incidence of duodenal ulcer in females during their reproductively active period. The high incidence of duodenal ulcer in ovariectomized – cysteamine injected mice may be due to the lack of estrogen in association with other factors. Anders et al. (2008) revealed the gender specific duodenal protection by estrogen in terms of HCO_3 secretion and the underlying molecular mechanisms of estrogen stimulation of DMBS that is linked to ER-Ca_2+-CFTR (cystic fibrosis transmembrane conductance regulator) and Cl-/HCO_3 exchanger pathways. We observed that the incidence of duodenal

ulcer is more or less identical in ovariectomized –cysteamine injected females and old – cysteamine injected females. The result was reflected in histological, histochemical, and biochemical studies. Histological changes that took place in goblet cells, pyloric gland cells and Brunner's gland cells in ovariectomized cysteamine treated mice were not observed in ovariectomized estrogen injected cysteamine treated mice. Sugars from glycoprotein of Brunner's gland were depleted in cysteamine treated females. These findings indicate that the estrogen is able to protect the duodenal mucosa from damaging effects of cysteamine. Manekar and Namaji (1977) suggested that female sex hormones have protective effects against ulcer formation. It has been established that glycoprotein containing bicarbonate is mainly responsible for the protection of duodenal mucosa. The glycoprotein and bicarbonate are mainly secreted by Brunner's glands. Thus it can be concluded that estrogen may be assisting in the secretion of glycoprotein from Brunner's glands. Increase in the secretion of glycoprotein from Brunner's gland acini and duct cells have been shown in the present investigation in estrogen treated mice. Secretion of glycoprotein of other exocrine cells of the duodenum is also influenced by the estrogen. This substantiates the role of estrogen in protecting the duodenal mucosa, but the exact mechanism is to be explored further.

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