Effect Of Ovariectomy And Of Estrogen Administration Upon Duodenal Ulceration Induced By Cysteamine
K Ashokan, M Kurane, M Pillai

Citation

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 showed no effects in either intact or ovariectomized mice. This change in ulcer sensitivity reflected from histological, histochemistry and biochemical studies. The histological study was performed by using haematoxylin-eosin 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 ovariectomized cysteamine treated mice and old mice treated with cysteamine. The histological studies showed the ovariectomy decreased or not shown any change in the ulcer sensitivity considering cryptus Lieburkuhn and bruner’ glands. The same result reflected in histochemistry study by differential intensity in the staining property of the bruners gland. The biochemical study showed that the glycoprotein contents were reduced many folds in ovariectomized cysteamine treated mice and their reversal in estrogen administered ovariectomized cysteamine injected mice. These findings prove that estrogen protect the duodenal ulcer form cysteamine administration.

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), 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). The immunity against duodenal ulcer is increase in females during pregnancy (Dey and Dey 1974). It was shown that the chance of men developing duodenal ulcer remain remarkably constant between the age of 20 years and 65 years. In contrast the chances of women developing duodenal ulcer remains relatively low throughout the whole life of her active reproductive life (Truelove 1960). Nevertheless, female sex hormones have been reported to promote ulceration (Antonsen 1955; Guerrine et al. 1967) or at best to have no effect 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). The present study was undertaken therefore, to investigate in mice the effect of ovariectomy on sensitivity of duodenal ulceration induced by using cysteamine –HCl and to determine whether estrogen administration reverse the effects of ovariectomy and aging. The study was also carried out by histological, histochemical and biochemical methods in the duodenal region of the normal, ovariectomized, cysteamine injected, ovariectomized – cysteamine injected, old female mice and cysteamine injected old female mice.

MATERIALS AND METHODS
EXPERIMENTAL ANIMALS
There were 60 mice in the present study. The mice were divided into two groups- 2 months old females (40 numbers) and 3 years old females (20 numbers). Ten mice were used as control in both groups. The 2 months old female mice were treated with cysteamine-HCl divided into tow groups.
The group one contain 10 female mice, were used as cysteamine –HCl treated one, the second group contain 20 female mice were used for ovariectomy.

**OVARIECTOMY AND ADMINISTRATION OF ESTROGEN**

The ovariectomy was done under mild ether anesthesia. The operated mice were maintained fro 15 days in separate age with optimum care of light, temperature, humidity, food and water. On the 16th day half the number of ovariectomized cysteamine treated mice were injected (i.p) consecutively for 3 days with 2 mg/body weight estradiol- 17β (Sigma, Batch no. E9505) in olive oil. The remaining ovariectomized mice were injected with olive oil only. On the 4th day of the 1st injection the ovariectomized, ovariectomized cyteamine injected and ovariectomized cysteamine injected and estrogen administered mice were used for duodenal ulcer induction (Szabo 1978). Ten 3 years old mice were used for administration of cysteamine, after three days it was subjected for ulcer induction.

**HISTOLOGY**

The pyloro-duodenal junctions were fixed in 10% neutral buffered fromaline, 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 was studied.

**HISTOCHEMISTRY**

To study the changes in the duodenal mucosa glycoproteins of crypts of Lieberkuhn, goblet cells, pyloric gland cells and Brunner’s glands of cytteamine treated and control mice 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 ulceration very low compared to the overiectomized mice (ulcer index 1.64) (Table No.1) The ovariectomized + Cysteamine administered mice showed higher ulceration (Ulcer index 5.1) (Table No.1).The administration of estradiol- 17β to cytteamine injected overiectompimized mice showed little recovery of ulceration (Ulcer index 4.2) (Table No.1). The old mice administered with cytteamine showed ulceration as high as ovariectomized + cytteamine administrated mice (Ulcer index 4.8) (Table No.2).

![Figure 1](image1.jpg)
**Table No.1: Ulcer severity in normal with and without Administration of estradiol**

<table>
<thead>
<tr>
<th>Group</th>
<th>Experimental mice</th>
<th>Percentage incidence</th>
<th>Superficial</th>
<th>Deep</th>
<th>Perforating</th>
<th>Mean severity</th>
<th>Ulcer index</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ovariectomized</td>
<td>100</td>
<td>93</td>
<td>95</td>
<td>2</td>
<td>0.12±0.12</td>
<td>2.6</td>
<td></td>
</tr>
<tr>
<td>Ovariectomized + estrogen</td>
<td>100</td>
<td>97</td>
<td>2</td>
<td>1</td>
<td>0.82±0.12</td>
<td>1.84</td>
<td></td>
</tr>
<tr>
<td>Ovariectomized + Cysteamine</td>
<td>100</td>
<td>10</td>
<td>70</td>
<td>20</td>
<td>3.1±0.10</td>
<td>6.02</td>
<td></td>
</tr>
<tr>
<td>Ovariectomized + Cysteamine + Estrogen</td>
<td>100</td>
<td>10</td>
<td>60</td>
<td>10</td>
<td>2.29±0.02</td>
<td>4.1</td>
<td></td>
</tr>
</tbody>
</table>

![Figure 2](image2.jpg)
**Table No.2. Ulcer severity in Ovariectomized with and without cytteamine and estrogen administration**

<table>
<thead>
<tr>
<th>Group</th>
<th>Experimental mice</th>
<th>Percentage incidence</th>
<th>Superficial</th>
<th>Deep</th>
<th>Perforating</th>
<th>Mean severity</th>
<th>Ulcer index</th>
</tr>
</thead>
<tbody>
<tr>
<td>Old mice</td>
<td>100</td>
<td>10</td>
<td>68</td>
<td>18</td>
<td>2.85±0.11</td>
<td>5.7</td>
<td></td>
</tr>
<tr>
<td>Old mice + Cysteamine</td>
<td>100</td>
<td>10</td>
<td>68</td>
<td>18</td>
<td>2.85±0.11</td>
<td>5.7</td>
<td></td>
</tr>
<tr>
<td>Old mice + Cysteamine + estrogen</td>
<td>100</td>
<td>10</td>
<td>60</td>
<td>10</td>
<td>2.85±0.11</td>
<td>5.7</td>
<td></td>
</tr>
</tbody>
</table>
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Table 3. Carbohydrates and protein contents of soluble glycoprotein isolated from Brunner’s glands of female mice

<table>
<thead>
<tr>
<th>Group</th>
<th>Hecate</th>
<th>Fucose</th>
<th>Sulfate acid</th>
<th>protein</th>
<th>P values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>2.03±0.25 (2)</td>
<td>1.49±0.12 (9)</td>
<td>0.21±0.09 (17)</td>
<td>2.17±0.09 (25)</td>
<td>1.2 p&lt;0.005 17.18 0.001</td>
</tr>
<tr>
<td>Normal + Cysteamine</td>
<td>6.30±0.74 (3)</td>
<td>3.08±0.04 (11)</td>
<td>0.43±0.01 (15)</td>
<td>19.23±0.04 (27)</td>
<td>5.6 p&lt;0.005 21.22 0.001</td>
</tr>
<tr>
<td>Ovariectomized</td>
<td>1.12±0.29 (4)</td>
<td>0.55±0.07 (17)</td>
<td>0.64±0.01 (20)</td>
<td>12.45±0.18 (23)</td>
<td>7.8 p&lt;0.005 23.24 0.001</td>
</tr>
<tr>
<td>Ovariectomized + Cysteamine</td>
<td>2.03±0.25 (2)</td>
<td>1.49±0.12 (9)</td>
<td>0.21±0.09 (17)</td>
<td>2.17±0.09 (25)</td>
<td>1.2 p&lt;0.005 17.18 0.001</td>
</tr>
<tr>
<td>Ovariectomized + Estrogen</td>
<td>6.30±0.74 (3)</td>
<td>3.08±0.04 (11)</td>
<td>0.43±0.01 (15)</td>
<td>19.23±0.04 (27)</td>
<td>5.6 p&lt;0.005 21.22 0.001</td>
</tr>
<tr>
<td>Old</td>
<td>6.11±0.74 (7)</td>
<td>2.18±0.02 (14)</td>
<td>0.43±0.01 (22)</td>
<td>14.44±0.32 (29)</td>
<td>11.02 p&lt;0.001 27.28 0.001</td>
</tr>
<tr>
<td>Old + Cysteamine</td>
<td>2.10±0.31 (8)</td>
<td>1.04±0.02 (16)</td>
<td>0.68±0.04 (24)</td>
<td>15.81±0.22 (51)</td>
<td>18.16 p&lt;0.005 31.02 0.005</td>
</tr>
</tbody>
</table>

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

Administration of estradiol-17β to cysteamine treated old mice fails to recover the severity of ulceration (Table No.2). The histology of ovariectomized mice (Fig.1) showed that the pyloric glands were simple tubular and situated deep in the sub mucosa. The duodenal villi were tall, leaf-like and uniformly arranged with desquamation intermittently. The crypts of Lieberkuhn and Brunner’s glands are unaffected. The cysteamine administration to the ovariectomized mice (Fig.2) cause pyloric glands with dilated lumen and picnotic nuclei and increased eosinophilia. The pyloric villi showed fissures and ramifications, and ulcer formation. The goblet cells are less in it. 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, crypts of Lieberkuhn, Brunner’s glands in ovariectomized mice but less in pyloric pit (Fig.3).
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Figure 6
Fig 3: Micrograph of duodenum of Ovariectomized mice PAS stain

Figure 7
Fig 4: Micrograph of duodenum of Ovariectomized-Cysteamine Injected Mice stained with PAS

The duodenal villi showed PAS activity but other cells are 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 folds in cysteamine injected mice comparing to the normal (Table 3). In ovariectomized 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 contents, sialic acid contents and protein contents (Table 3) also showed the same trend as hexose.

DISCUSSION
Peptic duodenal and gastric ulcers raise serious health problems and significant economic cost worldwide. 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 (HCO3-) secretion8 (DMBS) (Valle et al. 2003; Hogan et al. 1996) a 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 HC et al. 2008), respectively. These clinical observations suggest a gender difference in the incidence and severity of duodenal ulcer; however, the underlying mechanism(s) are currently unknown. As far as gender 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). 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 female during her 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 HCO3 secretion and the underlying molecular mechanisms of estrogen stimulation of DMBS that is linked to ER-Ca2+-CFTR and Cl-/HCO3exchanger 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 ovariecctomized cysteamine treated mice were not observed in ovariecctomized 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 has 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.

References


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