Modulation of gastric mucosal mast cell population: Role of vestibulo cerebellar lesion

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Posterior cerebellar lesion induced severe focal inflammatory ulcers at the stomach associated with extensive damage of the surface epithelial cells, leading to focal necrotic ulcers. The ulcer index increased maximally and progressively between day 7 and day 14 after lesion. The total mucosal mast cell and degranulated mucosal mast cell increased maximally on day 7 and progressively declined from day 14 to day 21. Gastric histamine content was also significantly increased on day 7 and 14. A significant reduction in mucous content (total CHO:P) was observed within 7-28 days after lesion. The results suggest that the gastric mucosal mast cells play an important role in ulcerogenesis induced by cerebellar lesion.

Keywords: Cerebellar lesion, Cerebellum, Gastric mucosal mast cell, Histamine, Ulcer

Numerous pathophysiological defects have been identified in gastric ulcer disease. The defects include improper acid secretion, decreased parietal cell mass and back diffusion in acid. Gastric ulceration is believed to be the result of unswerving confrontation between acid-pepsin aggression and defensive functions of the gastric mucosa¹.

The sight, smell, taste or thought of food result in vagal stimulation that releases acetylcholine. The released acetylcholine acts on the parietal cells to produce acid, also the vagal afferents stimulate the antral G cells to produce gastrin. The gastrin and acetylcholine act directly on the parietal cells or mast cells. The mast cells in turn release histamine, which stimulates gastric acid secretion. Central nervous system is believed to have influence on gastric ulceration. Since the classical report of Cushing⁷ that ulceration and haemorrhage is associated with CNS damage, numerous attempts have been made to clearly define the specific areas of the brain involved in such ulcer formation. Major emphasis has often been given to hypothalamus. Cushing⁷ reported acute gastrointestinal haemorrhage in patients following surgery of cerebellar tumors. However, little information is available on the cerebellar influence on gastric ulceration excepting that of Wolfe³, Brooks⁴, Maiti and Guha⁵, Sarkar and Guha⁶,⁷ and Guha et al⁸, who reported occurrence of chronic gastric ulceration following posterior cerebellar vermal lesions in cats and rats.

The interglandular space of the gastric mucosa wall is the host site of mast cells. Mast cells, which are widely distributed in the connective tissue of submucosal muscular and serosal layers of the wall of the stomach, have structural and functional linkage with ulceration. Changes in the number of mast cells in various anatomic sites, or evidence of activation of the cells for mediator release have been observed in a wide spectrum of adaptive/pathologic immune responses and in a large number of disease processes, many involving the gastrointestinal tract⁹,¹⁰. Gastric inflammation is one such disease process initiated and regulated by mast cells¹¹-¹⁴. Colonic mast cell activation has been known to mediate acute stress induced gastrointestinal function disturbance¹⁵. Mast cells contribute to the genesis of stress and ethanol induced ulceration in rat stomach¹⁶. Acetylcholine induced release of mast cell histamine¹⁷, or cholinergic mediated gastric mast cell degranulation with subsequent histamine induced severe inflammatory stress ulcers in rats have been documented¹⁸.

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There is definite evidence regarding the involvement of cerebellum with ulceration. Mast cell alterations have also been coupled with gastric ulcer pathology. However, no comprehensive study is available comparing the gastric ulceration following posterior cerebellar lesion with mast cell pathology. Therefore, in an attempt to elucidate the pathophysiological mechanisms of gastric ulcers the present investigation has been undertaken to observe whether there is any significant change in mast cell counts following cerebellar lesion in experimental ulcer model.

Materials and Methods

Animals and experimental design—Experiments were carried out on inbred Wistar rats (18) of either sex (240-260 g), maintained in a light (12L:12D) and temperature (26 ± 1°C) controlled room with constant humidity (60 %). Pellets of rat diet from Hindustan Lever Ltd., India, and tap water were provided *ad libitum*. The animals were divided into following 3 groups of 6 each: (a) control (b) sham control (c) experimental. The animals belonging to the experimental group were subjected to vestibulocerebellar lesion and were sacrificed at an interval of 7, 14, 21 and 28 days following lesion.

Electrolytic lesion of cerebellum—For stereotaxic surgery, rats were injected with chloropentanol (3ml/kg, ip), until a level of anaesthesia was achieved where the fore-paw withdrawal reflex was abolished. Electrolytic lesion of nodulo-uvular areas of posterior cerebellar vermis was produced following the horizontal skull surface technique, as per the stereotaxic coordinates-AP= 12.8 mm posterior to bregma, L=0.4 mm, D= 6.8 mm ventral to the dura. 0.2mA DC current for 40 sec was passed through conventional bipolar electrode to produce lesion. In the sham operated controls only electrode was inserted in the nodulo-uvular area of posterior cerebellar vermis. Routine antibiotic injection (im) of penicillin (10,000 IU) was given in all the animals for three consecutive days post-operation.

Collection of gastric juice—After a post operative period of 7 days all animals were implanted with stainless steel gastric cannula (10 mm in length, external diameter 5.5 mm) under anaesthesia following the method of Guha *et al*. For gastric juice collection, the animals were placed in restraining cages (No. 51339, Stoelting Co., Chicago). Prior to juice collection stomachs were lavaged with 0.9% saline until the effluent was clear of any food particles. Gastric juice was collected for 1 hr daily in the morning under fasting condition through a sialistic tube firmly attached to the cannula.

Gastric juice analysis—

(a) Acidity: Acidity of gastric juice was measured titrametrically with 0.01 N NaOH solution by using 0.5% Topfer’s reagent and 1% alcoholic phenolphthalein as indicator.

(b) Mucous content of gastric juice: Dissolved mucosubstances were estimated by determining the total carbohydrate (sum of total hexose, hexosamine, fucose and sialic acid) and protein in a 5% ethanol precipitate of gastric juice. The total carbohydrate and total protein ratio (CHO:P) has been accepted as a reliable index of mucous secretion and mucosal resistance.

Histological evaluation—After termination of the experiment, the animals were sacrificed by overdose of sodium pentothal following overnight fasting. Serial coronal brain sections (H&E) were examined to confirm the area of lesion. The stomach was cut opened along the greater curvature, and indexed accordingly. Identical portions of the glandular area from each stomach were taken for ulcer study.

Ulcer index—Briefly, after mucosal exposure, ulcers were examined with a magnifying glass. The grade of lesions was scored according to the following scale: 0—no pathology; 1— a small ulcer (1-2 mm); 2— a medium (3-4 mm); 4= a large (5-6 mm); 8= a large ulcer (>6 mm). The sum of the total severity scores in each group of rats divided by the number of animals was expressed as the mean ulcer index.

Every eighth formaldehyde (8%) fixed paraffin sections (6um) were stained alternatively.

Enumeration of mast cells—Two identical counter portions from each glandular stomach were taken to study the mast cell profile and histamine content respectively. For mast cell study, the gastric strips were rinsed with 0.9% NaCl, and then following fixation in formol-alcohol for 12 hr serial paraffin sections (8 μm thick) were stained with 0.25% alcoholic toluidine blue solution. Counting of both, total mucosal mast cell and total degranulated mucosal mast cells per 3 random microscopic fields (×400 magnification) were made on every fifth section and averaged for a total of 8 sections (24 fields) from each stomach.

Assay of histamine content—The histamine content (μ/100g of gastric tissue) was assayed fluorometrically.
Results

Lesion of cerebellum—The extent of lesion was confined to the wide area of the nodule and uvula (Fig. 1). Marked disorganization of the granular layer along with selective lysis of Purkinje cells of the nodulo-uvular area confirms the effectiveness of lesion (Fig. 2).

Ulcer histopathology—Histological observation under light microscope revealed that vestibulocerebellar lesion resulted in superficial creamy oedematous plaques mostly parallel to the long axis of the stomach (0.5-2 mm × 1-10 mm). With H & E stain intense inflammatory changes in the upper one third of the lamina propria were found around day 7 (Fig. 3 b) showing signs of acute gastritis with ulceration. PAS staining revealed focal inflammatory mass with PAS +ve materials (mucosubstances) at the superficial layers on day 7 (Fig. 3 e). Just about on day 14, a variety of surface epithelial metaplastic changes in size, shape and orientation by nuclear enlargement with marked disorganization and atrophy of the glands were invariably noted (Fig. 4 b). Around day 21 and 28 focal ulcers with superficial necrotic fibrinoid debris (Fig. 3 c) along with widespread surface erosive changes were frequently noted with PAS staining (Fig. 3 f). In the control (Fig. 3 a and d) and sham operated (Fig. 4 a) rats normal glandular arrangement and mucus lining were observed.

Ulcer index—Acute gastric ulceration in response to vestibulocerebellar lesion was observed in all the animals. Mean UI was found to be significantly enhanced between day 7 and day 14, thereby gradually diminishing from day 21 onwards (Fig. 5). The highest percentage of glandular ulceration was evidenced between day 7 and day 14, as has been shown histologically. Both in the control and sham operated animals no ulceration was evidenced except a few petacheal haemorrhages observed in the glandular part of the stomach.

Mast cell, histamine and ulcer—Vestibulocerebellar lesion induced significant changes in the mucosal mast cell as well as the total histamine level in the rat glandular stomach. The total mucosal mast cell and degranulated mucosal mast cell increased (P<0.001) maximally on day 7 (Figs. 6 and 7). After 14 days of cerebellar lesion the mast cell values still remained significantly (P<0.001) higher than the control and sham operated levels and then progressively declined closely to the corresponding control values on day 21. Gastric histamine content was also found to be significantly (P<0.001) increased on day 7 and 14 followed by a progressive decline (Fig. 8).

Gastric juice analysis—In control animals the gastric juice pH was 2.8 and it remained 1.50 to 1.52 within 7-28 days after vestibulocerebellar lesion (Table 1). The average total acidity in vestibulocerebellar lesioned group increased from 38 (in control and sham animals) to more than 60 mEq/L/hr. The gastric acidity in cerebellar lesioned animals remained at much higher level till 21st day after operation. But within 21–28 day it came down to normal pre-operated level (Table 2).
Fig. 3—Effect of nodular lesion on the histomorphology of gastric mucosa of rats. Changes are constantly similar in each study group, stained by either HE or PAS. Photomicrographs (3 a and 3 d, ×100) illustrate HE & PAS stained normal glandular arrangement and mucous lining in control rat stomach. Intense inflammatory changes with acute ulceration (3b, HE, ×150) is evident around day 7 after cerebellar lesion followed by superficial necrotic fibrinoid debris (3 c, HE, ×150) and widespread surface erosive changes (3 f, PAS, ×150) around day 21 and 28. Inset illustration (3g) shows portions of stomach (A, antrum; B, Body) studied histologically. Here 3 a -3 f represent portion B only.
Ulceration and mucus content—While analysing the mucus secretion after vestibulo-cerebellar lesion in the rats (Table 2) maximum significant reduction in mucus content (total carbohydrate – protein ratio) was observed within 7–28 days after surgery.

Discussion
The analysis of result revealed that vestibulo-cerebellar lesion caused extensive ulceration, hyperacidity and decreased mucous content in the rat stomach. Significant increase in gastric histamine, total mucosal mast cell and total degranulated mucosal mast cell was also observed following cerebellar lesion. None of the effects observed was due to surgical stress as the results were compared with sham controls.

Gastric hyperacidity situation following lesion was probably because of the involvement of cerebellum in the gastric secretory function by the modulation of the vagal system (which is the final secretory motor pathway to stomach)—influenced either through hypothalamo-limbic and/or direct pathways to vagal centers.

Disrupted vagal activity and hindered mucous synthesis also contribute to a lower mucous content of gastric juice that further aggravates ulceration as has been evidenced in the present study. Similar view has been opined by other workers, by associating dystrophic process in the gastric mucosa to post histamine induced ulceration.

Mast cells are initiators and regulators of inflammation. Both the mucosal mast cells, found in the bladder and GI tract and the mast cells of different connective tissue sites (connective tissue mast cells) like skin, tongue etc release mediators like histamine upon activation and stimulation. Through their activation and degranulation mast cells play a central role in the inflammatory process.
role in triggering immediate hypersensitivity reactions. Gut mucosal mast cells are reported to be associated with nerves in the GI tract. Newson et al.\textsuperscript{29} provided ultra structural evidence for bouton formation between mast cells and enteric nerves. Yonei\textsuperscript{30} demonstrated association of colonic mucosa in ulcerative colitis where mast cells were often degranulated and suggested that excessive cholinergic stimulation resulted in mast cell activation that may have been involved in exacerbation of the disease. In the present study, both total mucosal mast cell and total degranulated mucosal mast cell density was significantly greater in the mucosa with gastric ulceration. This observation is also in corroboration with the study of Nakajima \textit{et al.},\textsuperscript{14} who reported of increased mast cell population in ulcerated patients, with or without \textit{H. pylori} infection. In the present study, mast cell alteration was found to be maximal on day 7 post lesion. Till day 14, the level of both total mucosal mast cell and total degranulated mucosal mast cell remained significantly higher than the control and sham control level. Histamine is synthesized and stored in the mast cells. The present results showed a strong positive correlation between total mucosal mast cell, total degranulated mucosal mast cell population and histamine level of the gastric tissues suggesting that vestibulo-cerebellar lesion increased gastric histamine content by possibly increasing the mast cell population. Such changes were well associated with severe inflammation of gastric mucosa (Fig. 3b and e). A similar correlation between mast cell population and histamine content of the tissues has also been endorsed by Riley and West\textsuperscript{31}. Direct vagal stimulation or cholinergic mediated gastric mast cell degranulation with subsequent histamine induced inflammatory stress ulcers in rats have been documented\textsuperscript{17,18,32}.

Pathogenesis of ulcer disease is believed to reflect an imbalance between increased aggressive factors

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**Table 1**—Effect of cerebellar lesion on pH unit of gastric juice of rats

[Values are mean ± SE from 6 observations]

<table>
<thead>
<tr>
<th>Experimental condition</th>
<th>Days</th>
<th>7</th>
<th>14</th>
<th>21</th>
<th>28</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td>2.80±0.21</td>
<td>2.80±0.21</td>
<td>2.80±0.21</td>
<td>2.80±0.21</td>
</tr>
<tr>
<td>Sham control</td>
<td></td>
<td>2.78±0.15</td>
<td>2.77±0.02</td>
<td>2.80±0.03</td>
<td>2.78±0.02</td>
</tr>
<tr>
<td>Nodular lesion</td>
<td></td>
<td>1.50±0.06*</td>
<td>1.51±0.04*</td>
<td>1.52±0.03*</td>
<td>1.52±0.05*</td>
</tr>
</tbody>
</table>

*P<0.001, when compared to sham control group using ANOVA followed by Multiple comparison t-test.

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**Table 2**—Effect of cerebellar lesion on total acid output (mEq/L) and mucosal content (CHO:P) in gastric juice in rats

[Values are mean ± SE from 6 observations]

<table>
<thead>
<tr>
<th>Experimental condition</th>
<th>7</th>
<th>14</th>
<th>21</th>
<th>28</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acid output CHO:P</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>38.44±0.11</td>
<td>2.16±0.01</td>
<td>38.44±0.11</td>
<td>2.16±0.01</td>
</tr>
<tr>
<td>Sham control</td>
<td>38.03±0.15</td>
<td>2.18±0.15</td>
<td>39.25±0.51</td>
<td>2.17±0.02</td>
</tr>
<tr>
<td>Nodular lesion</td>
<td>64.35±0.16*</td>
<td>1.71±0.08*</td>
<td>67.49±0.80*</td>
<td>1.69±0.06*</td>
</tr>
</tbody>
</table>

*P<0.001, when compared to sham control group using ANOVA followed by Multiple comparison t-test.
and decreased protective factors. A progressive increase in ulcer index and gastric acidity from day 7 to day 14 as depicted in the present study may be due to consistent failure in gastro protective and repair mechanisms leading to disrupted ‘mucosal barrier’ as a consequence of cerebellar lesion, where histamine undoubtedly played a key initiative role in genesis of gastric ulcer.

It is evident from the present study that mucosal mast cell derived histamine in the glandular stomach played an important role in ulcerogenesis. The present results also confirm evidence of vestibulo-cerebellar interaction in mediating gastro-intestinal pathophysiology. The vagus nerve is the largest visceral nerve in the body that mediates tonic sensory inputs from a variety of thoracic and abdominal receptors to the brain stem. It is the only secretory motor pathway to the GI tract and is under the influence of cerebellum. The vagus nerve in turn modifies the functions of the GI tract through its close influence on the enteric system. The enteric system, which lies in close association to the mast cells, regulates the functional activities of these cells. The mast cell in these tissues may act as relays communicating immunological, mechanical and chemical stimuli between the outside world and the organism and thus plays an important role in the regulation of the homeostasis. Banisacchi et al. demonstrated histamine release from mast cells and diminution in mast cell granule metachromasia following an increased release of acetylcholine upon field stimulation of rat ileum. Ganguly et al. have shown that mast cells in the rat stomach increase in granularity following sectioning of the vagus nerve; the stress of pylorus ligation causes a decrease in tissue histamine (possibly mast cell derived), which can be prevented by vagotomy. This suggests that mast cells may be under tonic influence of the vagus, thus supporting a role of these cells in the maintenance of homeostasis in the gastrointestinal tract.

Hypothalamus has also been shown to be implicated in regulation of the autonomic nervous system. Gastric vagal and cerebellar fastigial nuclear afferents have been implicated in the regulation of food intake by their communication with lateral hypothalamic area (LHA); which is generally referred to as the feeding center. There are reports of possibility that activity originating in the lateral vestibular nucleus (LVN) may reach LHA through fastigioreticular or fastigiohypothalamic pathway. It has been well established that the direct cerebellohypothalamic projections arise from all three cerebellar deep nuclei and terminate in almost all hypothalamic nuclei/areas.

All these reports and the present results provide support for hypothesis that lesioning of the cerebellum results in ulceration either indirectly by affecting the vagal control and/or by disruption of the cerebellohypothalamic pathway.

Acknowledgement
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