ORIGINAL ARTICLE

Effect of central administration of ondansetron, a 5-hydroxytryptamine-3 receptor antagonist on gastric and duodenal ulcers

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ABSTRACT

The effect of central administration of ondansetron, a 5-hydroxytryptamine-3 (5-HT$_3$) receptor antagonist on gastric secretion and gastric cytoprotection was evaluated using four different models of gastric ulcers and cysteamine induced duodenal ulcer. Ondansetron was administered at two different doses of 20 μg/kg, intracerebroventricular (i.c.v.) and 40 μg/kg, i.c.v. Both doses of ondansetron showed significant increase in healing of acetic acid induced gastric ulcers and reduced the formation of ethanol-induced and pylorus ligation-induced gastric ulcers and cysteamine-induced duodenal ulcer. High dose of ondansetron (40 μg/kg, i.c.v.) was more effective compared with the low dose (20 μg/kg, i.c.v.). However, both doses of ondansetron did not influence the development of cold restraint stress induced gastric ulcers. It was concluded that blocking of 5-HT$_3$ receptors in brain decreases gastric acid secretion and increases gastric mucus secretion.

INTRODUCTION

5-hydroxytryptamine (5-HT) is a well known ulcerogenic agent and is used to induce gastric lesions in rats [1]. Agents that release 5-HT are also reported to induce gastric lesions [2]. The role of different 5-HT receptors and antagonists on the development of gastric ulcers has also been reported. Most of the studies have been carried out on the role of 5-HT$_3$ receptors. Antagonists of 5-HT$_3$ receptor are reported to reduce development of gastric ulcers [3–5]. Cisapride, an agonist of 5-HT$_3$ and antagonist of 5-HT$_4$ receptor is also reported to reduce gastric ulcer formation [6]. However, there are several other reports that show that cisapride is an antagonist of 5-HT$_3$ receptors and agonist of 5-HT$_4$ receptors [7,8]. Despite these reports on the role of 5-HT$_3$ and 5-HT$_4$

receptors, an earlier study reported that 5-HT produces gastric lesions only through 5-HT$_{ID}$ and antagonist of other 5-HT receptors such as 5-HT$_1$, 5-HT$_2$, 5-HT$_3$ and 5-HT$_4$ receptors do not antagonize 5-HT induced gastric lesions [9]. On the contrary, Ogle and Hui [5] reported that an intracerebral injection of ondansetron, a 5-HT$_3$ antagonist into nucleus amygdaloideus reduces development of gastric lesions and injection into nucleus accumbens aggravates gastric lesions. They also concluded that ondansetron shows reduction of stress induced gastric lesions by blocking 5-HT receptor peripherally.

Apart from the nucleus amygdaloideus and nucleus accumbens, the 5-HT$_3$ receptors are present in very high density in nucleus tractus solitarii in the brain [10], a region that is involved actively in the regulation

of acid secretion [11]. As the earlier reports on the role of 5-HT receptors and effect of ondansetron on gastric lesions are confusing, this study was undertaken to evaluate the effect of intracerebroventricular (i.c.v.) administration of ondansetron, a specific 5-HT$_3$ receptor antagonist on gastric secretion, gastric and duodenal ulcer formation and gastric ulcer healing in rats.

MATERIALS AND METHODS

Experimental animals
Male albino Wistar rats weighing between 200–250 g were used. Institutional Animal Ethics Committee approved the experimental protocol; animals were maintained under standard conditions in an animal house approved by Committee for the Purpose of Control and Supervision on Experiments on Animals.

Drugs and chemicals
Ondansetron (Cadila Pharmaceutical Ltd, Gujarat, India), ketamine HCL (Prem Pharmaceutical Ltd, Indore, India), xylazine (Indian Immunological Ltd, Guntur, India), acetic acid (SD Fine Chem. Ltd, Mumbai, India), cysteamine HCL (Himedia laboratories Pvt. Ltd, Mumbai, India) and ethanol (Jebsen & Jessen, Hamburg, Germany) were the drugs and chemicals used in this study.

Surgery for intracerebroventricular injection
Following anesthesia with ketamine hydrochloride [100 mg/kg, intramuscular (i.m.)] and xylazine (16 mg/kg, i.m.) [12,13], the skull of the animal was uncovered and a steel cannula (20 G) was sterotactically fixed to the skull using acrylic dental cement. The coordinates were 1.0 mm posterior and 1.3 mm lateral to brigma. The injection needle (27 G) reached 4.4 mm below the surface of the skull, with the needle tip in the ventricle. The cannula was implanted 48 h before the start of the ulcerogenic experiment. At the end of the experiment, the placement of the cannula was confirmed by injecting 2 μL ink after fixing the brain.

Acetic acid induced chronic ulcer
The method described by Asad et al. [14] was followed. The animals were fasted for 24 h prior to the experiment. Under ether anesthesia, the abdomen was opened by midline incision below the xiphoid process and the stomach was exposed. Glacial acetic acid (0.05 mL) was added to the cylindrical mould of

6 mm diameter placed tightly over the anterior serosal surface of the stomach and this was allowed to remain there for 60 s. The acid solution was removed by rinsing the mould with normal saline twice or thrice to avoid damage to the surrounding tissues. The stomach was placed back carefully and the abdominal wall was closed. The animals were treated with two different doses of 5-HT$_3$ receptor antagonist, ondansetron (low dose – 20 μg/kg, i.c.v. and high dose – 40 μg/kg, i.c.v.), [15] dissolved in artificial cerebrospinal fluid (CSF) [16] for a period of 10 days after induction of ulcer and the control group received only artificial CSF. On the 10th day, rats were killed by using ether and the stomachs were removed. The stomach was cut opened along the greater curvature. The ulcerated and total areas were measured and the ulcer index was determined using the formula [17]:

$$\text{Ulcer index} = 10/X$$

where X = Total mucosal area/Total ulcerated area.

The ulcers were given scores based on their intensity as follows: 0, no ulcer; 1, superficial mucosal erosion; 2, deep ulcer or transmural necrosis; 3, perforated or penetrated ulcer.

Pylorus ligation induced ulcers
Animals were fasted for 36 h before pylorus ligation with water *ad libitum* by placing them individually in cages to avoid coprophagy and cannibalism [18,19]. Normal saline [1 mL/rat, per oral (p.o.)] was administered twice daily during the fasting period [20]. Under ether anesthesia, the abdomen was opened by midline incision below the xiphoid process. The pyloric portion of the stomach was slightly lifted out and ligated, avoiding damage to its blood supply. The stomach was placed back carefully and the abdominal wall was closed with sutures. Ondansetron or artificial CSF was administered immediately after pylorus ligation through i.c.v. route. The animals were deprived of food and water during the post-operative period and were killed 6 h after pylorus ligation by overdose of ether anesthesia. The stomachs were isolated and the contents of the stomach were collected and centrifuged. The gastric juice was used for estimation of free and total acidity [21], pepsin content [22], total proteins [23] and mucin content [24]. The stomachs were cut opened along the greater curvature. The ulcers were formed in the glandular portion of the stomach. The ulcer index was determined as mentioned above.

Ethanol induced ulcers

The ondansetron or artificial CSF was administered i.c.v. to 36-h fasted rats. One hour later, ethanol (90%) was administered orally to all the animals at a dose of 1 mL/200 g and after 1 h all the animals were killed. The stomach was isolated and ulcer index was determined [25].

Cold restraint stress induced ulcers

The ulcers were induced by subjecting the animals to cold restraint stress. Ondansetron or artificial CSF was administered 30 mins prior to subjection of stress. The animals were placed in a restraint cage and the cage was placed at a temperature of 2–3 °C for 2.5 h. The animals were killed and the ulcer index was determined [26].

Cysteamine induced duodenal ulcers

Duodenal ulcers were induced by administering cysteamine hydrochloride (400 mg/kg, p.o.) twice at an interval of 4 h. Ondansetron or vehicle was given 30 mins prior to each dose of cysteamine. After 24 h of first dose of cysteamine, all the animals were killed and the duodena were excised carefully and cut opened along the antimesentric side. The duodenal ulcer area, ulcer score and ulcer index were determined [27].

The ulcers were given scores based on their intensity as follows: 0, no ulcer; 1, superficial mucosal erosion; 2, deep ulcer or transmural necrosis; 3, perforated or penetrated ulcer.

The ulcer index was calculated using the following equation [28]

$$U.I. = \text{Arithmetic mean of intensity in a group}$$
$$+ \frac{\text{number of ulcer positive animals}}{\text{total number of animals}} \times 2$$

Statistical analysis

The statistical significance was assessed using one-way analysis of variance (ANOVA) followed by Bonferroni's comparison test. For comparing non-parametric ulcer scores, ANOVA followed by non-parametric Dunn post-test was used. The values are expressed as mean ± SEM and $P < 0.05$ was considered significant.

RESULTS

Effect on ulcer healing of acetic acid induced chronic gastric ulcers

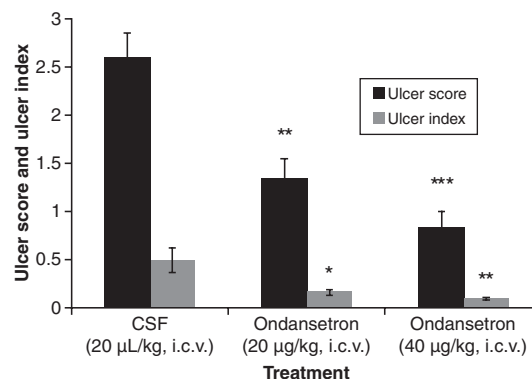The healing of acetic acid induced chronic gastric ulcer was significantly increased after central administration

Figure 1 Effect of ondansetron on ulcer score and ulcer index in acetic acid induced chronic gastric ulcer. All values are mean ± SEM, *n*=5–6, *P<0.05, **P<0.01, ***P<0.001 compared to cerebrospinal fluid (CSF) treated group.

of ondansetron in a dose dependent manner as indicated by reduction in ulcer index and ulcer score. The low dose of ondansetron produced 68.30% reduction in ulcer index while the high dose produced 82.12% reduction in ulcer index (*Figure 1*).

Effect in pylorus ligation induced gastric ulcers

Ondansetron administered immediately after pylorus ligation significantly reduced ulcer index ($P < 0.001$), free acidity, total acidity and pepsin content ($P < 0.001$). The effect observed was dose dependent with high dose (40 μg/kg, i.c.v.) showing more effect compared with the low dose (20 μg/kg, i.c.v.). The mucin content ($P < 0.001$) and total protein were significantly increased by both doses of ondansetron in dose dependent manner when compared with the control (*Table I*).

Effect on ethanol induced gastric ulcers and stress induced gastric ulcers

Animals treated orally with ethanol developed lesions in the glandular portion of the stomach. Ondansetron at both doses produced a significant reduction in ulcer index when compared with the control ($P < 0.05$). No significant reduction in stress induced gastric ulcers was observed after administration of both doses of ondansetron (*Table II*).

Effect on cysteamine induced duodenal ulcer

The central administration of ondansetron before each dose of cysteamine produced a highly significant reduction in the ulcer area ($P < 0.001$) and ulcer score ($P < 0.001$). The low dose of ondansetron (20 μg/kg,

Table I Effect of ondansetrson on free acidity, total acidity, ulcer index, mucin, pepsin content, and total protein.

Treatment	Free acidity (mEq/L)	Total acidity (mEq/L)	Ulcer index	Mucin content (μg/g)	Pepsin content (μmol/6 h)	Total proteins (mg/mL)
CSF (20 μL/kg, i.c.v.)	17.50 ± 1.784	54.50 ± 5.841	0.60 ± 0.053	0.89 ± 0.134	0.53 ± 0.060	7.35 ± 0.74
Ondansetron (20 μg/kg, i.c.v.)	10.66 ± 1.626*	27.33 ± 3.422**	0.30 ± 0.026***	2.13 ± 0.106***	0.27 ± 0.013***	10.96 ± 0.498**
Ondansetron (40 μg/kg, i.c.v.)	8.66 ± 1.406**	23.50 ± 4.072***	0.15 ± 0.019***+	3.33 ± 0.109***+++	0.18 ± 0.007***	14.38 ± 0.350***+++

CSF, cerebrospinal fluid; i.c.v., intracerebroventricular.

All values are mean ± SEM, $n = 6$, $*P < 0.05$, $**P < 0.01$, $***P < 0.001$ when compared with CSF treated group, $+++P < 0.001$, $+P < 0.05$, $++P < 0.01$ when compared with low dose.

Table II Effect of ondansetron on ulcer index in ethanol induced and stress induced gastric ulcers.

Treatment	Ethanol induced	Stress induced
CSF (20 μL/kg, i.c.v.)	1.68 ± 0.3479	2.67 ± 0.415
Ondansetron (20 μg/kg, i.c.v.)	0.712 ± 0.069*	1.51 ± 0.267[ns]
Ondansetron (40 μg/kg, i.c.v.)	0.558 ± 0.222*	1.30 ± 0.385[ns]

CSF, cerebrospinal fluid; i.c.v., intracerebroventricular.

All values are mean ± SEM, $n = 6$, $*P < 0.05$, $^{ns}P > 0.05$ compared with CSF treated group.

Table III Effect of ondansetron on ulcer area, ulcer score, and ulcer index in cysteamine induced duodenal ulcers.

Treatment	Ulcer area	Ulcer score	Ulcer index
CSF. (20 μL/kg, i.c.v.)	48.16 ± 4.729	2.16 ± 0.307	4.333
Ondansetron (20 μg/kg, i.c.v.)	6.83 ± 3.525***	0.5 ± 0.223***	1.500
Ondansetron (40 μg/kg, i.c.v.)	0.00 ± 0.00***	0.00 ± 0.00***	0.00

CSF, cerebrospinal fluid; i.c.v., intracerebroventricular.

All values are mean ± SEM, $n = 6$, $***P < 0.001$, when compared with CSF treated group.

i.c.v.) produced 65% reduction in ulcer index when compared with the control while the high dose of ondansteron (40 μg/kg, i.c.v.) produced 100% reduction in ulcer index with no animal in this group developing duodenal ulcer after cysteamine administration (*Table III*).

DISCUSSION

This study was carried out to evaluate the effect of central administration of ondansetron, a $5HT_3$ receptor antagonist, on the gastric and duodenal ulcers. Both doses of ondansetron produced a significant increase in healing of acetic acid induced chronic gastric ulcers, decreased gastric secretion in pylorus ligated rats and

prevented the development of ethanol induced gastric ulcers and cysteamine induced duodenal ulcer. However, administration of ondansetron did not influence the development of cold-restraint induced gastric ulcers.

This study was carried out using different gastric ulcer models to evaluate the effect on gastric secretion, gastric cytoprotection and gastric ulcer healing. The effect on healing of gastric ulcers was studied using acetic acid induced chronic gastric ulcer models. Application of glacial acetic acid (0.05 mL) on to the serosal surface of the stomach produces deep penetrating gastric ulcers. Acetic acid induced ulcer in rats resembles that of human peptic ulcer and the healing process of this ulcer closely resembles that of human peptic ulcer disease. Hence this model is quite useful for study of the human ulcer and for evaluating the pharmacological agents possessing gastric antisecretory and/or cytoprotective effects [29].

The ligation of the pyloric end of the stomach causes accumulation of gastric acid in the stomach. This increase in the gastric acid secretion causes ulcers in the stomach. The original Shay rat model involves fasting of rats for 72 h followed by ligation of pyloric end of the stomach. The ulcer index is determined 19 h after pylorus ligation. The lesions produced by this method are located in the rumen region of the stomach [17]. Many authors have modified the original model. In this study, the Shay rat model described by Kulkarni was followed [18]. Unlike the original model, where ulcers are produced in the rumen region of the stomach, in this model, the ulcers developed as lesion in the glandular portion of the stomach. The agents that decreases gastric acid secretion and increase mucus secretion are effective in protecting the ulcers induced by this method. Both doses of ondansetron significantly decreased the total acidity and free acidity and increased the mucus content when compared with the control.

Ethanol induced gastric ulcer was employed to study effect on gastric cytoprotection. Ethanol induced gastric lesion formation may be due to stasis in gastric blood flow that contributes to the development of the hemorrhage and necrotic aspects of tissue injury. Alcohol rapidly penetrates the gastric mucosa apparently causing cell and plasma membrane damage leading to increased intracellular membrane permeability to sodium and water. The massive intracellular accumulation of calcium represents a major step in the pathogenesis of gastric mucosal injury. This leads to cell death and exfoliation in the surface epithelium [30,31]. Agents that increase gastric mucus secretion and/or decrease gastric motility are effective in this model. Central administration of ondansetron showed significant cytoprotective effect. Peripheral (subcutaneous) administration of ondansetron reduces development of ethanol induced gastric ulcers due to an increase in gastric mucosal blood flow [5]. Further, it is known that ondansetron does not affect gastric motility [32]. As ondansetron is known to cross blood–brain barrier [33], it is speculated that the gastric cytoprotective effect observed due to peripheral administration of ondansetron may also be partly caused by its central effect and the gastric cytoprotective effect observed in this study suggests that blocking of 5-HT$_3$ receptors in the brain may increase gastric mucosal blood flow and enhanced mucosal blood flow observed after peripheral administration of ondansetron might be due to either peripheral or central action or both actions, but to elucidate this question further experiments are needed.

The pathophysiology of stress-induced ulcers is complex. Stress induced ulcers are probably mediated by histamine release with enhancement in acid secretion and a reduction in mucus production [34,35]. In stress condition, there is an increase in gastrointestinal motility (GI) motility, which causes folds in the gastrointestinal tract that comes in contact with acid secretion leading to induction of gastric ulcers and the stress also brings central nervous system into play. The lesions produced by these methods are located in the glandular region of the stomach. Agents that either decrease the GI motility or have effect on central nervous system are helpful in reducing stress induced ulcers. Ondansetron failed to influence the formation of stress induced ulcers. The results are consistent with earlier report that central administration does not reduce stress induced gastric ulcers [5]. However, it is worth mentioning here that peripheral administration of ondansetron is reported to reduce stress induced ulcer formation by decreasing release of histamine from gastric mast cells [5,36]. The exact reason why ondansetron failed to reduce the development of stress induced gastric ulcers despite reducing gastric acid secretion can not be explained.

Cysteamine induced duodenal ulcer in rat is a widely used model of peptic ulcer disease. The ulcer induced by this model resembles histopathologically and pathophysiologically that of the duodenal ulcer in humans. Cysteamine hydrochloride inhibits the alkaline mucus secretion from the Brunner's glands in the proximal duodenum and stimulates gastric acid secretion rate. Gastric emptying is also delayed and serum gastric concentration is increased [27]. Apart from this, dopamine deficiency may also contribute to the development of cysteamine induced duodenal ulcers [37]. Ondansetron prevented the formation of duodenal ulcers probably because of its antisecretory effect.

The 5-HT$_3$ receptor is a neurotransmitter-gated ion channel and is a member of the Cys-loop family of receptors. Although, drugs acting through 5-HT$_3$ receptors are used only in the management of emesis and irritable bowel syndrome, it is believed that a number of diseases can be cured using 5-HT$_3$ receptor selective compounds [38]. The 5-HT$_3$ receptor is widely distributed in the adult human brain, internal organs and extraneuronal cells [39,40]. The 5-HT$_3$ receptors are present in several regions of brain [41–43], a very high density of 5-HT$_3$ receptors is present in nucleus tractus solitarii in the brain [9], which is involved actively in the regulation of acid secretion [10]. The 5-HT$_3$ receptors are also located in the dorsal motor nucleus [44] and this region of brain might also be a potential site for action of ondansetron [45–47].

Ondansetron is a highly potent and selective antagonist at 5-HT$_3$ receptors that is used as anti-emetic. It is hypothesized that ondansetron blocks nausea and vomiting by 5-HT$_3$ receptor antagonism at two specific sites: (i) centrally, in the area postrema/NTS; and (ii) peripherally on vagus nerve terminsls [48].

Several authors have reported the role of peripheral 5-HT$_3$ receptors and the effect of ondansetron on gastric function and mucosal damage. Ondansetron, when given subcutaneously increases gastric mucosal blood flow and also basal acid and Na$^+$ secretion without affecting pepsin output. Ondansetron also attenuates the toxicities of ethanol in the stomach and this action is due an increase in gastric mucosal blood flow [49]. Ondansetron is also reported to reduce stress induced gastric ulcers and ethanol induced gastric ulcers by acting on

peripheral 5-HT$_3$ receptors and not through brain 5-HT$_3$ receptors [5].

The role of brain 5-HT receptors on the gastrointestinal function has been reported. The 5-HT$_2$ receptors present in dorsovagal complex are believed to mediate the thyrotropin releasing hormone mediated increase in gastric function [50]. The findings of this study suggest that 5-HT in the brain affects gastric function not only through its action on 5-HT$_2$ receptors but also through its action on brain 5-HT$_3$ receptors. The present results suggest that ondansetron exerts antiulcer and mucosal protective effects by central mechanism. However, other studies demonstrated that peripheral mechanism may also be involved in the gastroprotective action of ondansetron. Further studies have to be carried out to evaluate the effect of 5-HT$_3$ antagonist on different regions of the brain.

CONCLUSION

Antagonism of 5-HT$_3$ receptors in the brain leads to decreased secretion of gastric aggressive factors such as acid and pepsin and increased gastric mucus secretion producing an overall increase in healing of ulcers as well as reduction of gastric mucosal damage in other ulcer models.

ACKNOWLEDGEMENTS

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