Research Article

Effect of Ranitidine and Omeprazole on Serum and Gastrc Leptin in Rats with Normal or Ethanol-Injured Gastric Mucosa

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ABSTRACT

Background: Stomach is the major source of leptin in the gastrointestinal tract and the leptin has a positive role on gastric ulcer healing. Objective: The present study investigates the effect of ranitidine and omeprazole on gastric and serum leptin in rats with normal or ethanol-injured gastric mucosa. Material and methods: Six equal groups of rats were treated as: control [normal saline]; ranitidine, 50 mg.kg-[1] SC; omeprazole, 10 mg.kg-[1] orally; ethanol 75%, 4 ml.kg-[1] orally; ranitidine+Ethanol 75% and omeprazole+Ethanol 75%. After 3 h, number of lesions was counted in stomachs and lesion area was assessed by planimetry. Gastric and serum leptin was determined by ELISA method. Results: Rats of groups 4, 5 and 6 demonstrated obvious ulcers. Pretreatment only with omeprazole significantly reduced both number of lesions and lesion area as compared to group 4. Serum leptin remained statistically the same among different groups. Gastric leptin in rats treated with ethanol increased significantly as compared to control. Pretreatment with ranitidine and omeprazole decreased gastric leptin appreciably in comparison with group 4. Conclusion: Ranitidine and omeprazole do not affect serum leptin in normal condition while they significantly decrease gastric leptin in rats with acute ethanol-induced gastric damage which may not be desirable with regard to the positive role of leptin on ulcer healing.

Keywords: Leptin, Stomach Ulcer, Ranitidine, Omeprazole, Rat

INTRODUCTION

Although leptin was first found to be secreted by adipose tissue, its gene is expressed by various other tissues including salivary glands, placental trophobasts, endocrine glands such as rat pituitary and pancreas and the cardiac and skeletal muscle cells[1]. Stomach is the major source of leptin in the gastrointestinal tract. Endocrine and exocrine cells located in the gastric mucosa are able to secrete leptin either towards the blood circulation or into the gastric juice. Exocrine cells play a larger role[2,3]. Endocrine cells are present in the lamina propria of the gastric mucosa, while chief cells are responsible for exocrine secretion of leptin into the gastric juice, which is then internalized by duodenal enterocytes and delivered to the lamina propria and blood circulation quite rapidly[2,4]. Leptin affects many aspects of gastrointestinal functions including the modulation of motility, nutrient absorption, growth and inflammation[5]. On the other hand, acute gastric injury can markedly increase expression of gastric leptin at mRNA and protein level[6,7] and it has been clearly demonstrated that leptin has a positive effect on promoting gastric ulcer prevention and healing[6-11].

The dramatic success of pharmacological acid suppression in healing gastric ulcers is obvious. H2-receptor antagonists are particularly useful for on-demand symptom relief and proton pump inhibitors represent one of the most commonly prescribed classes of drugs and a major advance in the treatment of acid-related diseases[12]. As far as we know, no study has evaluated the effect of these agents on leptin levels in normal conditions as well as in acute gastric injuries.

Regarding the positive role of leptin on ulcer healing, the present study investigates the effect of ranitidine (as an H2-receptor antagonist) and omeprazole (a proton pump inhibitor) on gastric and serum leptin in rats with normal or ethanol-injured gastric mucosa.

MATERIALS AND METHODS

Animals and Experimental Design: Thirty adult male Sprague-Dawley rats with a mean body weight of 250 g were purchased from animal house of Shiraz Medical University, Shiraz, Iran. Rats were acclimatized for one week before the beginning of the experiment to the ambient conditions (temperature about 23 °C and a 12 h/12 h, light/dark cycle). Then after 18 h of fasting the animals were randomly allocated into six equal groups (five animals each) and treated as follows:

Control group (normal saline)

Ranitidine HCl (Sina Darou Pharmaceutical Co., Tehran, Iran), 50 mg.kg-[1] SC

Omeprazole (Rouz Darou Pharmaceutical Co., Tehran, Iran), 10 mg.kg-[1] by oral gavages

Ethanol 75% (Merck, Darmstadt, Germany), 4 ml.kg-[1] by oral gavages

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Table 1. Number of lesions per stomach and lesion area (mm^2) in different groups.

	Control		Ranitidine		Omeprazole		Ethanol		Ranitidine+ Ethanol		Omeprazole+ Ethanol	
	mean± SD	range	mean ±SD	range	mean ±SD	range	mean ±SD	range	mean ±SD	range	mean ±SD	range
Number of lesions/ stomach	0#	-	0#	-	0#	-	10.25 ± 2.62*	7-14	8.50 ± 3.10*	4-11	3.33 ± 1.15*, #	2-4
Lesion area (mm^2)	0#	-	0#	-	0#	-	35.81 ± 10.32 *	26.4-47	39.35 ± 18.37 *	18.8-54.1	12.08 ± 3.75#	7.8-14.9

* and # signs are used to demonstrate significant difference with control and ethanol groups respectively (p< 0.05)
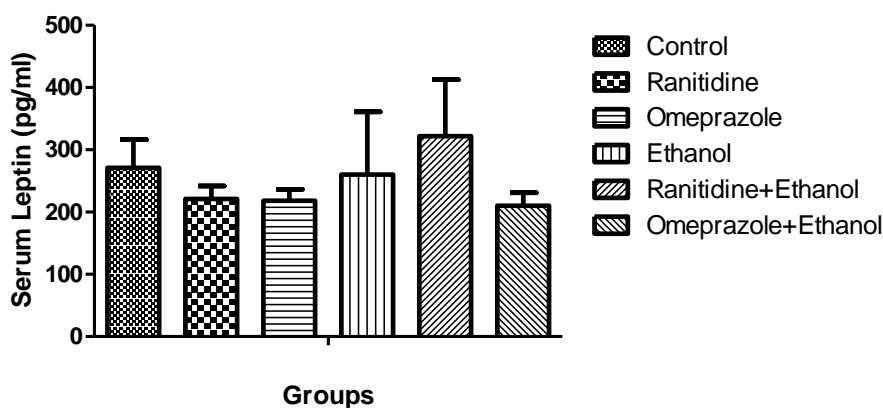

Fig 1: Serum leptin (mean±SD) in different groups. No significant difference was observed.
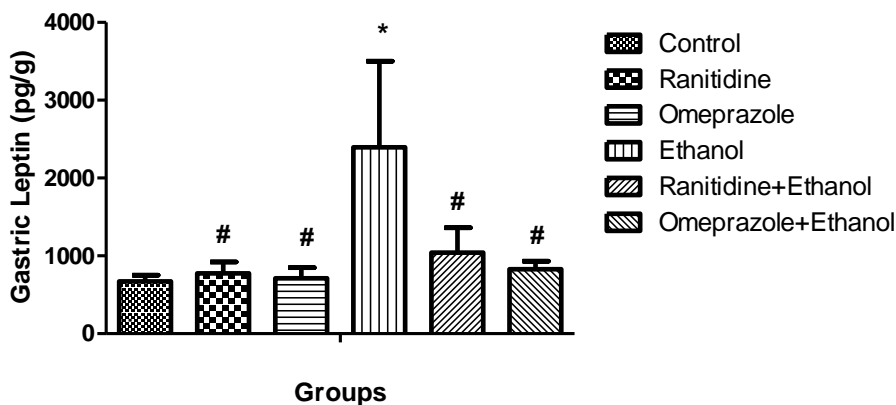

Fig 2: Gastric leptin (mean±SD) in different groups.
* and # signs are used to demonstrate significant difference with control and ethanol groups respectively (p< 0.05)

Ranitidine HCl, 50 mg.kg-1 SC+Ethanol 75%, 4 ml.kg-1 by oral gavages

Omeprazole, 10 mg.kg-1 by oral gavages+Ethanol 75%, 4 ml.kg-1 by oral gavages

It should be mentioned that ranitidine and omeprazole were administered 30 min before ethanol.

The method for induction of gastric ulcer by ethanol was selected according to Singh et al[13] and dosage regimens for ranitidine and omeprazole were chosen as described by Adeyemi et al[8].

Procedures used in the present study are in accordance with institutional ethical guidelines for care and use of laboratory animals in experiments.

Blood Collection: After 3 h, blood samples were collected from all animals under chloroform anesthesia by cardiac puncture and centrifuged at 2000 rpm for 20 min. Harvested sera were stored in -70 °C until use.

Determination of Number of Lesions and Lesion Area

All animals were killed by deepening anesthesia immediately after blood sampling. The abdomen was incised, the stomach removed and cut open along the greater curvature. Stomachs were rinsed with water to remove any adherent food particles and mucus. Gross mucosal lesions in glandular part of the stomach were recognized as hemorrhage or a linear break [erosions] with damage to the mucosal surface. Number of lesions was

counted in each stomach and lesion area was assessed by planimetry.

Quantification of Gastric and Serum Leptin Levels: For determination of gastric leptin content, stomach samples were homogenized at 4 °C in 1:5 (w:v) of phosphate-buffered saline (137 mM NaCl, 2.7 mM KCl and 10 mM phosphate buffer, pH 7.4) in a Teflon/glass homogenizer. The homogenate was centrifuged at 10000 g for 10 minutes at 4 °C and the supernatant was used for leptin quantification ([14] with little modifications). Leptin concentration in the gastric homogenates and in serum was measured with a rat/mouse leptin Enzyme-Linked Immunosorbent Assay (ELISA) kit (Mediagnost, Reutlingen, Germany).

Statistical Analysis: Data was expressed as mean±SD. The statistical significance was assessed by one-way ANOVA and Tukey's multiple comparison tests as post hoc. Threshold of significance was defined at $p < 0.05$.

RESULTS

Number of Lesions and Lesion Area: No ulcer observed in gastric mucosa of rats in groups 1, 2 and 3, while rats of groups 4, 5 and 6 demonstrated obvious ulcers. Pretreatment with omeprazole significantly reduced both number of lesions and lesion area as compared to ethanol group ($p \leq 0.001$ for both comparisons), while the number of lesions and lesion area in rats pretreated with ranitidine were the same as ethanol alone ($p > 0.05$) (Table 1).

Gastric and Serum Leptin Levels: No significant difference observed in serum leptin among different groups ($p > 0.05$) (Fig. 1).

Ranitidine and omeprazole had no appreciable effect on gastric leptin in normal rats while gastric leptin level in rats treated with ethanol increased significantly as compared to control group ($p = 0.001$). Pretreatment with ranitidine and omeprazole decreased gastric leptin level appreciably in comparison with ethanol treated rats ($p = 0.008$ and $p = 0.002$ respectively) and both reversed it to control levels ($p > 0.05$) (Fig. 2).

DISCUSSION

As the aim of the present study, evaluation of the effects of two routinely prescribed agents for the treatment of gastric ulcers on gastric and serum leptin levels seems quite important because of this fact that leptin has a gastro protective effect and can improve gastric ulcer healing in animal models with acute gastric injuries[6-11].

It has been clearly demonstrated that exposure of gastric mucosa to different noxious agents such as ethanol 75%, aspirin and acetic acid is associated with a significant increase in the expression of leptin at both mRNA and protein levels in stomach[6,7]. Consistent with previous findings, in our study ethanol administration resulted in an appreciable increase in gastric leptin content as compared to control rats, however this was not accompanied by a significant change in serum leptin level. Cammisotto et al. (2007)[4] proved that gastric leptin secreted in an exocrine way by the epithelial chief cells reaches the duodenum and crosses the intestinal barrier by transcytosis, to be released in the interstitial space and then enters the blood. These

researchers observed that this is a quite rapid process and the rise in serum leptin has been detected 30 min after stimulation of gastric leptin release by carbachol treatment in rats. Moreover, leptin was secreted bound to its soluble receptor. Although free form of leptin is rapidly removed from plasma (half-life, 3.4 min), clearance of the bound form of leptin takes a longer period (half-life, 71 min)[15]. So, regarding the interval between ethanol administration and blood sampling in our study, it seems that despite the increase in leptin content of gastric mucosa by ethanol, leptin release (both by endocrine and exocrine pathways) has not been appreciably increased.

In what concern omeprazole and ranitidine, it should be mentioned that the reduction in leptin content of injured stomach due to pretreatment with these agents was not accompanied by a change in serum leptin. Therefore the decrease in gastric leptin has not been as a result of increase in leptin release from the stomach. On the other hand, this finding that gastric leptin of normal rats pretreated with these agents was statistically the same as control group, precludes a direct inherent effect of these agents on leptin synthesis. Put it altogether, it may seem logical that the effect of ranitidine and omeprazole on reducing leptin in injured stomachs may be due to suppression of pathways by which ethanol induces leptin synthesis in injured stomachs. Another aspect may be that the reduction in gastric level of leptin by omeprazole in ethanol treated rats may be related to its ability in preventing induction of lesions by ethanol. This explanation is not applicable to ranitidine, since as stated previously, this agent did not prevent ethanol lesions although decreased gastric leptin levels as compared to ethanol group. The lack of positive effect of ranitidine on ethanol-induced gastric lesions was expected since it has been demonstrated that ranitidine at therapeutic doses inhibits gastric alcohol dehydrogenase activity in rats which reduces first-pass metabolism of alcohol[16,17]. So the absence of positive effects of ranitidine on gastric lesions due to ethanol as observed in our study may be related to this aspect of ranitidine. The mechanism behind the reduction in gastric leptin content with ranitidine may be complex and different from those responsible for its gastro protective effects.

In conclusion, ranitidine and omeprazole significantly decrease gastric leptin in acute ethanol-induced gastric damage in rats more possibly by preventing ethanol induction in leptin synthesis in injured stomachs. Regarding the positive role of leptin on ulcer healing, this may not be a desirable effect for these agents and should be more investigated in future studies.

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