African Journal of Pharmacy and Pharmacology Vol. 6(13), pp. 1017-1021, 8 April, 2012
Available online at http://www.academicjournals.org/AJPP
DOI: 10.5897/AJPP12.077
ISSN 1996-0816 © 2012 Academic Journals

Full Length Research Paper

Time dependent response in ghrelin levels in mesenteric ischemia in rat

Akgun Oral[1], Zekai Halici[2]*, Yasin Bayir[3], Emre Karakus[4], Sabri Selcuk Atamanalp[5] and Yavuz Albayrak[6]

[1]Department of Pediatric Surgery, Ataturk University Faculty of Medicine 25240 Erzurum-Turkey.
[2]Department of Pharmacology, Ataturk University Faculty of Medicine 25240 Erzurum-Turkey.
[3]Department of Biochemistry, Ataturk University Faculty of Pharmacy, 25240 Erzurum-Turkey.
[4]Department of Pharmacology and Toxicology, Ataturk University Faculty of Veterinary Medicine 25240 Erzurum-Turkey.
[5]Department of General Surgery, Ataturk University Faculty of Medicine 25240 Erzurum-Turkey.
[6]Department of General Surgery, Erzurum Regional Educational Hospital, Erzurum-Turkey.

Accepted 24 February, 2012

The objective of this study was to determine the value of time-dependent ischemia-modified ghrelin in mesenteric ischemia. The authors investigated whether or not there is changes in plasma ghrelin levels in the acute period in a rat model of mesenteric ischemia were time-related. 42 mature male rats were divided into seven groups: one control and six ischemia groups. In the ischemia groups, following laparotomy the superior mesenteric artery (SMA) was ligated using a 2/0 silk suture, and blood samples were taken at 30, 60, 90, 120, 150 and 180 min. Plasma ghrelin levels in all the ischemia groups were significantly higher compared to the control group. In addition, the highest level of ghrelin was observed at 180 min. In our current study we have shown a direct increase in ghrelin in cases of acute mesenteric ischemia (AMI). This is a result of ghrelin's protective effect against ischema/reperfusion (I/R) damage on the intestines, as in most of the other organs. With these characteristics, ghrelin can be easily applied in AMI and appears to be a prognosis parameter that can be used in clinics and needs no technological equipment or experience.

Key words: Ischemia, superior mesenteric arteria, ghrelin, rat.

INTRODUCTION

Acute mesenteric ischemia (AMI) is one of many abdominal emergencies. It refers to a sudden onset of intestinal hypoperfusion, which can be due to an occlusive or a nonocclusive obstruction of arterial or venous blood flow (Stamatakos et al., 2008). AMI is caused by mesenteric occlusive vascular disease and is more common in adult patients who have additional cardiovascular problems, with a mortality rate of 30 to 90%. The causes of AMI in the pediatric age group include mesenteric vascular occlusion due to invasive aortic monitoring devices, neonatal aortic thrombosis, and cardiogenic emboli (Grosfeld et al., 2006). Reduced perfusion is a secondary condition of cardiogenic, hypovolemic, or septic shock. The use of vasoconstrictive inotropic agents may also result in ischemic or necrotic bowel in children. A midgut volvulus may occur in newborns with malrotation in the first week or first month of age; meconium ileus or plugging may promote the development of a volvulus with intestinal necrosis (Grosfeld et al., 2006). Compartment syndrome, gastrointestinal bleeding, and intussusception are the other causes of nonocclusive AMI. When infarction occurs in adult patients, they develop increasing pain associated with vomiting (Stamatakos et al. 2008).

Therefore, in the early stages, a diagnosis is not easy even for an experienced surgeon; further, the mortality rates correlate with diagnosis time (Brandt and Boley,

*Corresponding author. E-mail: hzekai@atauni.edu.tr. Tel: +904422316561. Fax: +904422360968.

2000). Even hospitals equipped with diagnostic tools may be not enough for a diagnosis. The most useful diagnostic tools are catheter angiography and biphasic computed tomography (CT) with mesenteric computed tomography angiography (CTA) (Akyildiz et al., 2009). A plain abdominal graphics and gastrointestinal contrast study are the first steps for diagnosing nonocclusive AMI in the neonatal period. Ghrelin, a newly discovered bioactive peptide, is a natural endogenous ligand of the growth hormone (GHS) and a secretagogue receptor that is one of the molecules that stimulates protective effects on multiple organs. Ghrelin is predominantly produced in the stomach (Kojima et al., 1999), and the number of ghrelin cells decreases from the stomach to the colon (Zhao and Sakai, 2008).

In addition to the effects of stimulating GH secretion, ghrelin and many synthetic GHS exhibit hypothalamic activities that stimulate secretion of prolactin and adrenocorticotropic hormones; negatively influence the pituitary-gonadal axis at both the central and peripheral level; stimulate appetite; provide positive energy balance; influence sleep and behavior; control gastric motility, acid secretion, and modulation of the pancreatic exocrine and endocrine functions; and affect glucose levels (Van der Lely, 2009). AMI is a mortal disease due to its difficult diagnosis, high magnitude of bowel loss during an operation as a result of a late diagnosis, as well as the patient being directly affected by the magnitude of bowel loss. The current diagnosis methods are interventional and require advanced technology and experience. For this reason, an inexpensive diagnosis method is necessary that can be easily applied by all facilities and can bring to light this condition in its early stages. The aim of this study was to investigate serum ghrelin levels and whether or not they rise over time, using an experimental ischemia model of rats by establishing SMA occlusion.

MATERIALS AND METHODS

Study design

This was a prospective, evaluator blinded, randomized laboratory investigation. This was a randomized, controlled animal study. This project was approved by the Institutional Animal Care and Use Committee of the Atatürk University Institute for Medical Research.

Animal's subjects

In the present study, a total of 42 male Sprague Dawley rats (220 to 250 g) were used for the experiments. Rats were obtained from Atatürk University's Experimental Animal Laboratory of Medicinal and Experimental Application and Research Center. Animal experiments and procedures were performed in accordance with national guidelines for the use and care of laboratory animals and were approved by Atatürk University's Local Animal Care Committee. Rats were housed in a temperature-controlled room on a 12 h light/dark cycle and were fed with a standard rat chow diet.

Prior to the induction of ischemia, rats fasted overnight but allowed water *ad libitum*.

Chemicals

Ketamine (Ketalar 500 mg vial) was obtained from Pfizer, Istanbul, Turkey. Xylazine (Rompun 50 ml vial) was obtained from Roche, Istanbul, Turkey.

Study protocol

Seven groups were made up of six rats in each cage. Sham group. Group I: 30 min after superior mesenteric artery (SMA) ligation, blood sample was taken. Group II: 60 min after SMA ligation, blood sample was taken. Group III: 90 min after SMA ligation, blood sample was taken. Group IV: 120 min after SMA ligation, blood sample was taken. Group V: 150 min after SMA ligation, blood sample was taken. Group VI: 180 min after SMA ligation, blood sample was taken.

Intestinal ischemia model

An intestinal ischemia model was applied to the rats as follows. The rats fasted overnight but allowed to drink water *ad libitum*. They were anesthetized by subcutaneous administration of ketamine (30 mg/kg) and xylazine (5 mg/kg) with atropine sulfate (0.15 mg/kg), and 0.9% NaCl was administered at the rate of 10 ml/kg/h until the end of the experiment to prevent dehydration. If necessary, the rats received additional ketamine intravenously (5 to 10 mg/kg). The abdomens of the rats were shaved and washed with 10% povidone-iodine, and a 2 cm midline abdominal incision was performed. The animals were laid on their right side so that the mesenteries of the intestines were removed from the abdomen on the level of the aorta. The SMA was separated from the aorta and ligated with a 2/0 silk suture. Next, the midline incision was sutured. The rats were placed on heating pads at 37℃ throughout the experim ent. Once the SMA was ligated, blood samples were taken at 30 (Group I), 60 (Group II), 90 (Group III), 120 (Group IV), 150 (Group V), and 180 (Group VI) minutes from each group and the animals were sacrificed. Blood samples were collected immediately and transferred to the laboratory to facilitate the findings of the biochemical analysis.

Biochemical evaluations

A 2 ml blood sample was taken in each tube. Blood was collected for ghrelin testing and was taken in a shaded tube, as it is sensitive to light Ghrelin (Atatürk University Faculty of Medicine Laboratories, A05117) was measured from one sample with SPI-Bio rat acylated ghrelin highly-sensitive enzyme-linked immunoassay (ELISA) kits, in accordance with the manufacturer's instructions. Assays for each animal and the matched control were run in the same lot.

Statistical analysis

The data was entered and analyzed using SPSS 18.0 computer statistics program. The data was given as an average and standard deviation. The analysis was performed using the Friedman variance test, and the comparisons of the two groups were performed using the Wilcoxon signed-rank test. The difference was accepted as statistically significant when $p<0.05$. The significance level in the comparison of both groups was determined according to the Bonferroni correction as $p<0.01$.

Table 1. Level of plasma ghrelin in mesenteric ischemia rats.

Group	N	Ghrelin (pg/ml)		
Sham	6	37.2	±	14.47
Group I (ischemia 30)	6	70.35	±	6.82
Group II (ischemia 60)	6	81.02	±	13.63*
Group III (ischemia 90)	6	109.98	±	18.44*
Group IV (ischemia 120)	6	99.31	±	18.20*
Group V (ischemia 150)	6	103.88	±	26.07*
Group VI (ischemia 180)	6	114.55	±	33.83*

Each value is mean ± SE for 6 rats in each group and all statistical analysis was done by one-way analysis of variance followed by Tukey's. Sham group was compared with other groups and showed significant at $p<0.05$ (*).

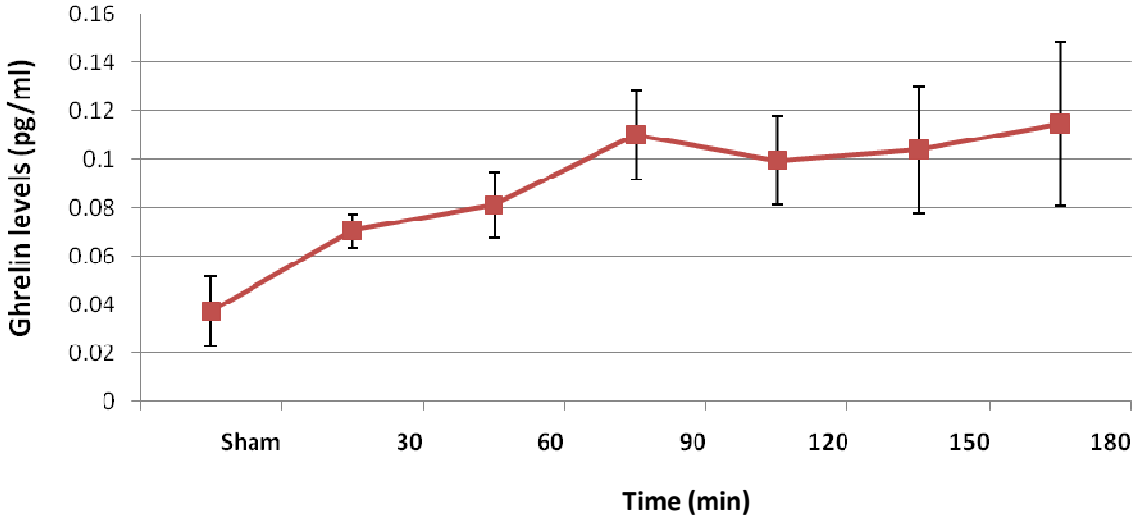

Figure 1. Level of plasma ghrelin in mesenteric ischemia rats.

RESULTS

The measurement of ghrelin levels in the plasma of rats was defined as a mean of 37.20±14.47 pg/ml in the sham group; 70.35±6.82 pg/ml in group I; 81.02±13.63 pg/ml in group II; 109.98±58.44 pg/ml in group III; 99.31±18.2 pg/ml in group IV; 103.88±26.07 pg/ml in group V; and 114.55±33.83 pg/ml in group VI (Table 1). It was found that the ghrelin averages rose gradually over time ($p=0.000$) (Figure 1). However, significance level under $p<0.01$, which is the accepted significance level after a Bonferroni correction, was not found in the comparisons carried out on both groups. This situation is completely due to the low number of rats.

DISCUSSION

Mortality rates of AMI still range between 70 and 90% (Corke and Glenister, 2001). Unfortunately, retrospective series have not shown any significant improvement in mortality in the past decades (Lock, 2002).The need for a reliable, specific test for intestinal ischemia has been recognized for many years. Numerous potential monitors have been evaluated, including intraluminal pCO_2, abdominal CT, abdominal MRI, angiography, diagnostic surgery, and specific plasma enzymes, but few have shown the potential to be clinically useful. At present there is no specific test for intestinal ischemia and/or infarction in routine clinical use (Corke and Glenister, 2001). A Doppler ultrasound is another possible option for diagnosis, as it is easy to carry out in any medical center; however, it is impossible to do in neonates, and an investigator's experience is an important factor for success. Angiography has high sensitivity for diagnosis, and it is not only a diagnostic tool; performing therapeutic intervention at the same time is also possible.

However, angiography is an invasive technique using

contrast agents, and radiation and the need for experience are other disadvantages of this method. The new concept for AMI diagnosis is contrast-enhanced biphasic multidetector-row helical CT. Over 90% sensitivity has been reported (Aschoff et al. 2009), and this is a non-invasive technique with respect to angiography, but it requires a high amount of experience. Many agents have been tested for reducing ischemia injury damage of the intestines in an effort to reduce the high mortality rate of AMI patients; but, to date, there have not been a challenge experienced on the diagnostic tools of AMI diagnosis. The most useful diagnostic tools are catheter angiography and biphasic CT with mesenteric CTA, but these techniques are expensive and require technical experience. Ghrelin and its various receptors were found to be ubiquitous in many organs and tissues.

After researching ghrelin in the literature review, it was found that in recent years there have been a lot of published journals about the protective effects of ghrelin. Zhang et al. (2010) reported that ghrelin may have cardiovascular protective effects, including lowering of blood pressure, regulation of atherosclerosis, and protection from I/R injury, as well as improving the prognosis of myocardial infarction and heart failure.

There have also been many journals published on ghrelin's gastro-protective effect (Konturek et al., 2004; Sibilia et al., 2008; Tritos and Kokkotou, 2006; Wiley and Davenport, 2002; Wu et al., 2007, 2008; Yakabi et al., 2006; Zhao and Sakai, 2008). Brzozowski et al. (2006) reported, in I/R-produced typical gastric erosions, a significant fall in the gastric blood flow (GBF), an increase in gastric myeloperoxidase (MPO) activity and malonyldialdehyde (MDA) content, and the up-regulation of mucosal ghrelin messenger ribonucleic acid (mRNA). Ghrelin dose-dependently increased gastric acid secretion and significantly reduced I/R-induced gastric erosions, while producing a significant rise in the GBF and mucosal PGE2 generation and a significant fall in MPO activity and MDA content (Brzozowski et al., 2006). Wu et al. (2007) reported that to determine whether ghrelin's beneficial effects after gut I/R requires the intact vagus nerve, in their study avagotomy was performed in sham and gut I/R animals immediately prior to the induction of gut ischemia as we described recently (Wu et al., 2007).

Ghrelin-induced protection against I/R injury could be explained, at least in part, by the suppressive effects exhibited by this peptide on neutrophil activation and generation of free oxygen metabolites under I/R conditions (Brzozowski et al., 2006). Wu et al. (2008) reported that the survival rate after gut I/R with vehicle administration was 50% on day 1 and decreased to 41.6% on days 2 to 10 during treatment with ghrelin; however, the survival rate improved to 83.3%, which was significantly higher than that in the gut I/R vehicle treated animals (Wu et al., 2008). These findings are true in light of the other studies on the protective effects of ghrelin in I/R injury. However, in the same journal Wu et al. (2008) reported that their study showed that plasma levels of ghrelin were significantly reduced after gut I/R (Wu et al., 2008), and at the end of a 90 min ischemia, they reported plasma levels of ghrelin decreased by 73%.

This finding is impossible, our results showed a serious increase after I/R and we also found that at the end of the 90 min ischemia the increase rate of ghrelin was 294%. In our current study the average ghrelin in groups was found to have gradually risen with time ($p=0.000$). A significance level under $p<0.01$, which is the accepted significance level after a Bonferroni correction, was not found in the comparisons carried out on both groups. This is because the number of rats was kept to a minimum, due to ethical reasons. An obvious statistical significance would have occurred if the number of rats in the groups were larger. Ischemia-modified ghrelin is a new biomarker. Ghrelin levels are increase in cases of AMI. We did not compare other biochemical marker or histopathological analyses. Ghrelin can be easily applied in AMI and appears to be prognosis parameter, which is possible to use in clinics and needs no technological equipment or experience.

Conclusion

Even if treatment under ideal conditions for mesentery ischemia were to be carried out, it is a high-mortality incident, an emergency clinical situation. In our current study we have shown a direct increase in ghrelin in cases of AMI. This situation is a result of Ghrelin's protective effect against I/R damage in the intestines, as in most of the other organs.

REFERENCES

Akyildiz H, Akcan A, Ozturk A, Sozuer E, Kucuk C, Karahan I (2009). The correlation of the D-dimer test and biphasic computed tomography with mesenteric computed tomography angiography in the diagnosis of acute mesenteric ischemia. Am. J. Surg., 197: 429-433.

Aschoff AJ, Stuber G, Becker BW, Hoffmann MH, Schmitz BL, Schelzig H, Jaeckle T (2009). Evaluation of acute mesenteric ischemia: accuracy of biphasic mesenteric multi-detector CT angiography. Abdom. Imaging, 34: 345-357.

Brandt LJ, Boley SJ (2000). AGA technical review on intestinal ischemia. American Gastrointestinal Association. Gastroenterol., 118: 954-968.

Brzozowski T, Konturek PC, Sliwowski Z, Pajdo R, Drozdowicz D, Kwiecien S, Burnat G, Konturek SJ, Pawlik WW (2006). Prostaglandin/cyclooxygenase pathway in ghrelin-induced gastroprotection against ischemia-reperfusion injury. J. Pharmacol. Exp. Ther., 319: 477-487.

Corke C, Glenister K. (2001). Monitoring intestinal ischaemia. Crit. Care Resusc., 3: 176-180.

Grosfeld J, Oneil J, Coran G, Fonkalsurd E (2006). Pediatric Surgery. Pages 1369 in Grosfeld J, ed. Pediatric Surgery, Philedelphia: Mosby Elsevier. vol. 2: 1369.

Kojima M, Hosoda H, Date Y, Nakazato M, Matsuo H, Kangawa K (1999). Ghrelin is a growth-hormone-releasing acylated peptide

from stomach. Nature, 402: 656-660.
Konturek PC, Brzozowski T, Pajdo R, Nikiforuk A, Kwiecien S, Harsch I, Drozdowicz D, Hahn EG, Konturek SJ (2004). Ghrelin-a new gastroprotective factor in gastric mucosa. J. Physiol. Pharmacol., 55: 325-336.
Lock G (2002). Acute mesenteric ischemia: classification, evaluation and therapy. Acta Gastroenterol. Belg., 65: 220-225.
Sibilia V, Pagani F, Rindi G, Lattuada N, Rapetti D, De Luca V, Campanini N, Bulgarelli I, Locatelli V, Guidobono F, Netti C (2008). Central ghrelin gastroprotection involves nitric oxide/prostaglandin cross-talk. Br. J. Pharmacol., 154: 688-697.
Stamatakos M, Stefanaki C, Mastrokalos D, Arampatzi H, Safioleas P, Chatziconstantinou C, Xiromeritis C, Safioleas M (2008). Mesenteric ischemia: still a deadly puzzle for the medical community. Tohoku J. Exp. Med., 216: 197-204.
Tritos NA, Kokkotou EG (2006). The physiology and potential clinical applications of ghrelin, a novel peptide hormone. Mayo Clin. Proc., 81: 653-660.
Van der Lely AJ (2009). Ghrelin and new metabolic frontiers. Horm Res., 71 Suppl 1: 129-133.
Wiley KE, Davenport AP (2002). Comparison of vasodilators in human internal mammary artery: ghrelin is a potent physiological antagonist of endothelin-1. Br J Pharmacol., 136: 1146-1152.
Wu R, Dong W, Cui X, Zhou M, Simms HH, Ravikumar TS, Wang P (2007). Ghrelin down-regulates proinflammatory cytokines in sepsis through activation of the vagus nerve. Ann Surg., 245: 480-486.
Wu R, Dong W, Ji Y, Zhou M, Marini CP, Ravikumar TS, Wang P (2008). Orexigenic hormone ghrelin attenuates local and remote organ injury after intestinal ischemia-reperfusion. PLoS One 3: e2026.
Yakabi K, Ro S, Onouhi T, Tanaka T, Ohno S, Miura S, Johno Y, Takayama K (2006). Histamine mediates the stimulatory action of ghrelin on acid secretion in rat stomach. Dig Dis Sci 51: 1313-1321.
Zhang G, Yin X, Qi Y, Pendyala L, Chen J, Hou D, Tang C (2010). Ghrelin and cardiovascular diseases. Curr. Cardiol. Rev., 6: 62-70.
Zhao Z, Sakai T (2008). Characteristic features of ghrelin cells in the gastrointestinal tract and the regulation of stomach ghrelin expression and production. World J. Gastroenterol., 14: 6306-6311.