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 ischemia/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,
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 adrenocorticotropin 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°C throughout the experiment. 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.

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

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 (*).

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 pCO2, 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
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 mesenteric 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


