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Research Report
Does Somatostatin Decrease Hemorrhage From Injured Liver in Rats?

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ABSTRACT

In portal hypertensive patients, somatostatin (SMT) and octreotide have been widely used to decrease variceal bleeding because of its splanchnic hypoperfusion effect. The aim of this study was to explore the effects of somatostatin treatment for decreasing blood loss of uncontrolled liver hemorrhage model in rats.Twenty-one male rats were divided into 3 groups including group 1; non-treatment, group 2; isotonic saline infusion and group 3; isotonic saline plus SMT infusion. Intra-abdominal bleeding was induced by transection of median lobe of liver. Mean arterial pressures (MAP), amount of intra-peritoneal blood collection and hematocrit (Hct) changes were evaluated for 60 minutes.There was no difference in the MAP changes between the groups until 25th minute. Thereafter, MAP remained similar in the group 1 while gradually increased ($P< 0.05$) in the group 2 and 3. There was no statistically significant difference between the groups 2 and 3. End of study, the highest Hct value was determined in the nontreatment group (41.0 ± 3.26 %) and it significantly different from other two groups. We found increase of Htc value in the group 3 (32.3 ± 2.75 %) when compared with group 2 (29.7 ± 4.19 %), but it was not statistically significant. The highest intra-peritoneal blood volume was determined in group 2. We found decrease of the hemorrhage in the group 3 when compared with the group 2, but it was not statistically significant. Somatostatin using has a tendency, although not statistically significant, to decrease of intraperitoneal hemorrhage from liver in the rat model.

1. Introduction

Non-compressible hemorrhages such as intra-abdominal and intra-thoracic are among the most important causes of death before trauma victims reach hospital (Holcomb et al., 2000; Hoyt et al., 1991). Traditional approach to the hemorrhagic shock is early and aggressive isotonic fluid infusion because blood products are generally not available in the pre-hospital phase. However, early aggressive fluid resuscitation usually causes further blood loss and increased mortality rate due to the hemodilution of coagulation factors and increased arterial blood pressure in animal studies (Bickell et al., 1991; Gross et al., 1998; Krausz et al., 2001). On the other hand, some authors suggest that delayed fluid resuscitation may be detrimental by way of cytokine production (Lee et al., 2001).

We attempt to produce an alternative method to decrease blood loss from injured liver by administration of a splanchnic vasoactive drug. Somatostatin (SMT) has been proposed as a treatment for variceal gastrointestinal bleeding because of its ability to decrease portal pressure without significant adverse systemic effects. The effect of somatostatin is possibly mediated through its ability to cause a splanchnic vasoconstriction and reduction of splanchnic blood flow (Carsson et al., 1994; Cerini et al., 1998; Cirera et al., 1995; Jenkins et al., 1998; Saruc et al., 2003; Sonnenberg et al., 1981). To our knowledge there are no data on the use of SMT in traumatic intra-abdominal hemorrhages. Using a modified uncontrolled massive liver hemorrhage model, we evaluated whether SMT decreases blood loss or not during early resuscitation phase.

2. Experimental Procedure

2.1 Animals

The Ethical Committee of experimental animals at the Faculty of Medicine, Inonu University, Turkey, approved the study protocol. Twenty-one adult (6-8 month old) male Sprague-Dawley rats, weighing 310 to 350 g, were allowed access to food and water ad libitum until the day of surgery. The animals were randomly divided into three groups each contains 7 rats.

The animals were anesthetized by intra-peritoneal injection of ketamine (80 mg/kg) and xylasine HCl (8 mg/kg) and, anesthesia was maintained by small additional doses as necessary. The animals were kept supine during the experiments. Body temperature was monitored with a rectal thermometer and maintained by heating pad.

The right carotid artery was cannulated with 22-gauge angiocath via a neck incision for monitoring of mean arterial pressure (MAP) and blood sampling. The left femoral vein was cannulated with 24-gauge angiocath via groin incision for fluid and drug administration. Blood samples were taken by microcapillar tube to measure basal hematocrit (Hct I) values. During these procedures, loss of blood was less than 0.5 ml.

2.2 Uncontrolled hemorrhage

Massive liver injury model was modified from that of Matsuoka et al. (1995). Briefly, a midline laparotomy was performed five minutes after anesthesia and cannulations. Liver trauma was performed by sharp resection of the median lobe 1 cm distally from suprahepatic vena cava (Fig. 1).

The cut edges of the liver were remained in abdominal cavity for simulation of traumatic injury. Abdominal incision was closed with a running suture. SMT and/or fluid infusion were started 3 minutes after liver injury. MAP was recorded at 5 min intervals for 60 minutes throughout the study using a monitor (Petaş KMA 800 Ankara, Turkey) connected to the arterial line. End of the study, all rats were sacrificed with intravenous ketamine (150 mg/kg) after blood samples were taken by microcapillar tube for Hct II. The laparotomy incision was reopened, and intra-peritoneal free blood and coagulum were collected by previously weighed dry cotton. The intra-peritoneal blood accumulation was determined by the difference in wet and dry weights of cotton. Blood Hct values were measured by centrifugation.

Fig. 1- Specimen of rat liver and seperated lower segments of the median lobe

2.3 Treatment protocols

Group 1 rats did not receive any treatment. Group 2 rats were treated by warm isotonic saline infusion (40 ml. kg-1. h-1) 3 min after liver injury. Group 3 rats received 3.5 µgr. kg-1. h-1 continuous infusion of SMT in isotonic saline (40 ml. kg-1. h-1) for 60 minutes after 3.5 µgr/kg bolus injection of SMT.

2.4 Statistical Analysis

Statistical analyses were accomplished by using SPSS computing program (version 13.0). All results were reported as means ± S.D. The comparison of the results from the various experimental groups and their corresponding controls was carried out using a one-way analysis of variance (ANOVA) followed by pairwise multiple comparison procedures (Tukey test). The differences were considered significant when $P<0.05$.

3. Results

All of the rats survived throughout study. Fig. 2 demonstrates the time course of the MAP in the three experimental groups. The liver injury induced a marked decrease of the MAP in all the groups during the first 5 minutes. There was no difference in the MAP values between the groups until 25th minute. Thereafter, MAP remained similar in the group 1 while gradually increased ($P< 0.05$) in the other two groups. The group 1 was significantly different from the group 2 and 3 after 35th and

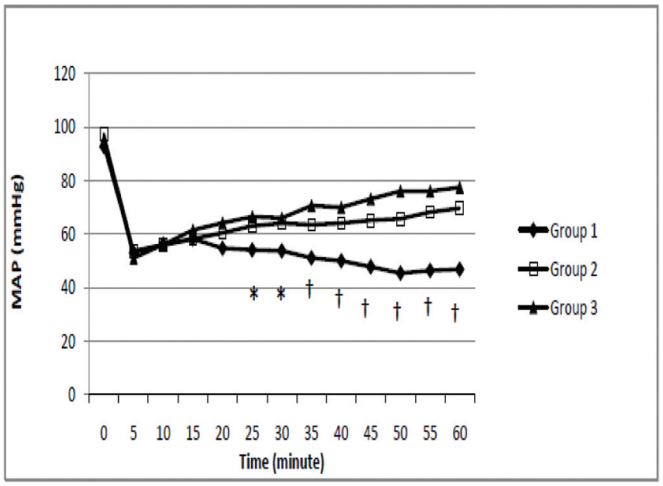

Fig. 2- P<0.05 the group 1 compared with the group 3. P<0.05 the group 1 compared with the group 2 and 3.

25th minutes respectively (P<0.05). There was no statistically significant difference between the group 2 and 3.

Table 1 demonstrates the amounts of intra-peritoneal hemorrhage and the hematocrit values of all the goups. There was no difference in the Hct I values among three groups. The highest Hct II value was determined in the group 1 (41.0 ± 3.26 %) and it significantly different from other two groups. We found increase of Hct II value in the SMT treatment group (32.3 ± 2.75 %) when compared with the group 2 (29.7 ± 4.19 %), but it was not statistically significant (P=0.36).

The highest intra-peritoneal bleeding volume was determined in group 2. We found decrease of the hemorrhage in the SMT treatment group when compared with the group 2, but it was not statistically significant (P=0.56).

Table 1- The amounts of intra-peritoneal hemorrhage and the hematocrit values (mean±SD).

	Hct I (%)	Hct II (%)	Blood Loss (gr)
Group 1 (no treatment)	46 ± 3,0	41,0 ± 3,26*	4,39 ± 1,17*
Group 2 (saline)	48 ± 3,1	29,7 ± 4,19	8,31 ± 1,94
Group 3 (saline + SMT)	47 ±1,8	32,3 ±2,75	7,11 ±1,70

P<0.05 compared with the group 2 and 3.

4. Discussion

Fluid resuscitation in hemorrhagic shock is one of the most challenging aspects of trauma care. For the past 4 decades, the standard approach to the hypotensive trauma victims suffering from hemorrhagic shock to maintain adequate tissue perfusion and oxygenation is massive infusion of crystalloid solutions such as Ringer's lactate because blood products are not readily available in the pre-hospital settings (Stadlbauer et al., 2003, Stern 2001). However, rapid intravascular volume replacement with isotonic crystalloids according to the recommendations of the Advanced Trauma Life Support Protocol may be harmful (American Collage of Surgeons 1991, Stadlbauer et al., 2003).

Animal studies have shown that try to enhance normal blood pressure by early aggressive fluid resuscitation during uncontrolled hemorrhage of liver or spleen causes increased mortality (Rafie et al., 2004; Solomonov et al., 2000). Suggested mechanisms for increased mortality include diluted coagulating factors, increased blood pressure and disruption of an effective clot followed by secondary hemorrhage (Kowalenko et al., 1992). Conversely, Lee et al. (2007) demonstrated that delayed resuscitation also may be detrimental in terms of cytokine production profile and histopathological changes. Hence, some authors suggest that "limited" or "hypotensive" resuscitation may be preferable in the trauma patients to avoid detrimental effects of early aggressive or delayed fluid infusion (Kowalenko et al., 1992; Lee et al., 2007; Rafie et al., 2004; Stern, 2001).

Decreasing blood supply of bleeding organs using a drug can be another alternative method to reduce blood loss in uncontrolled hemorrhage. For example, authors suggested that using vasopressin, which mediates transient hypoperfusion of abdominal organs, enhances survival in a liver trauma model with hemorrhagic shock (Stadlbauer et al., 2003). Similarly, we aimed to explore the effect of SMT, which has splanchnic vasoactive effects, for decreasing blood loss of uncontrolled liver hemorrhage model in rats.

Somatostatin and its long-acting analogue, octreotide (OCT), have been widely used in the treatment of acute variceal bleeding with portal hypertensive patients due to its decreasing effect of portal pressure without systemic adverse effects (Palazon et al., 2006). The observed hemodynamic effects of OCT are essentially a reduction in hepatic, portal and collateral blood flows (Cerini et al., 1998; Eriksson et al., 1987; Lin et al., 1992). However, exact mechanism of the action remains unknown. In a double-blind, placebo-controlled hemodynamic study, Cirera et al. (1995) demonstrated that a bolus injection of SMT caused a rapid decrease in portal pressure gradient (52%) and azygos blood flow (45%). Vanheule et al. (2008) suggested that the very rapid onset effect of SMT is indicative of a direct action involving the vascular smooth muscle cell. In addition, these effects are seen in non portal hypertensive subjects (Sonnenberg et al., 1981). Intravenous administration of SMT or OCT decreases gastric, pyloric, duodenal, pancreatic and colonic blood flow in rats (Carlsson and Jansson, 1994). It has also been suggested that these drugs may be useful for the control of non-variceal upper gastrointestinal bleeding due to peptic ulcers (Jenkins et al., 1998; Saruc et al., 2003).

We hypothesized that if SMT lowers the pressure and volume of blood in the vessels of splanchnic and portal system, it might be useful to decrease blood loss caused by traumatic liver injury. Our study was resembled the clinical condition of rapid blood loss due to liver trauma without availability of blood for transfusion in pre-hospital phase. In many cases, the approximate arrival time to the emergency room is 30 to 60 minutes after major trauma. For this reason, we chose 60 minutes of experiment duration to simulate pre-hospital phase.

Splanchnic vasoconstriction is a known physiologic compensatory reaction of organism aimed blood flow shift to vital organs such as brain and heart during hypovolemic shock (Scannell et al., 1992). However, prolonged splanchnic flow reduction may lead to mortal complications such as

bacterial translocation, absorption of endotoxins from the gut lumen, and multiple organ failure (Deitch, 1990). For this reason, we chose SMT which has a very short half-life time (1-3 min) instead of OCT (90-120 min) not to cause prolonged hypo-perfusion of splanchnic vessels (Sheppard et al., 1979).

In this study, the blood loss and MAP in the group 1, which did not receive any treatment, were significantly less than the other two groups. This result was in accordance with preview studies which suggest that fluid infusion increases hemorrhage from injured organ by increasing MAP and diluting coagulation factors (Kowalenko et al., 1992; Rafie et al., 2004; Solomonov et al., 2000). We found that intra-peritoneal blood accumulation in the group 3 less than the group 2, but it was not statistically significant. The highest Hct II value was determined in the group 1 (41.0 ± 3.26 %) and it was significantly different from other two groups. This result was in accordance with earlear studies which suggest that early fluid infusion decrease hematocrit level in systemic sirculation by increasing hemorrhage and diluting blood (Kowalenko et al., 1992; Solomonov et al., 2000). However, the idea that use of splanchnic vasoconstructive drugs to decrease liver hemorrhage is seen an advisable method we did not find any result statistically significant for favor of SMT, unfortunately. This condition may be related to some reasons. First, this drug could not perform its optimal effect, because splanchnic vasoconstructive response to the hypovolemic state might have already been started physiologically. Second, very effective coagulative features of rat blood may be responsible. Garcia-Manzano et al. (2001) demonstrated that clotting and bleeding times are shorter in rats (125±3.8 sec and 88±4 sec) than humans (508±5 sec and 270±3 sec). Truly, in our study, the MAP values of untreated rats continued unchanged throughout experiment after dropped abruptly in first 5 minutes. This condition suggests that liver hemorrhage stopped spontaneously in first 5 minutes of the study.

In addition, there are some limitations in this study. The liver hemorrhage model mimics more a penetrating than blunt trauma because of sharp transection of liver and opened abdomen.

In the light of our findings, we can say that somatostatin using has a tendency, although not statistically significant, to decrease of intraperitoneal hemorrhage from liver in the rat model. This pharmacologic treatment modality may yield better results when used in other animal species which have similar coagulation properties with humans.

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