Abstract: Many factors are known to play a role in flap necrosis, such as inadequate blood flow and disturbed venous drainage, which lead to decreased flap nutrition and necrosis. The aim of this study was to determine whether adrenomedullin (ADM) and glucagon-like Peptide-1 (GLP-1) administered at various doses directly to the superficial inferior epigastric artery (SIEA) had an effect on the normal healing process of flap tissue. Methods. Under 3% isoflurane anesthesia, the rats were put in the dorsal decubitus position before the surgery. A cutaneous flap 8 cm x 3 cm in size was marked on the abdominal wall, divided into four equal sections, and marked from 1 to 4 (proximal to distal). A laser Doppler flowmeter was used to measure the blood supply of each area in the flap tissue. On the seventh postoperative day, an image of the final condition of the flap was obtained with a 5-megapixel camera; the rats were sacrificed afterward. Results. Groups treated with ADM or GLP-1 showed a statistically significant increase in the blood flow of the four separate regions compared to the saline group. The percent necrosis area decreased in a statistically significant manner in the groups treated with ADM and GLP-I. Conclusion. The authors believe that both peptides play an important role in the normal flap recovery process.
and expression have been found in many tissues of the body. ADM expression is stimulated by cytokines and in hypoxic conditions. There are many reports indicating an important role of ADM on angiogenesis. It has been shown that adrenomedullin stimulates autocrine growth factors and angiogenesis in the endometrium.

Glucagon-like peptide-1 (GLP-1), a regulatory peptide, is a hormone with various activities in the body and is secreted by the enteroendocrine L-cells in the small intestines. Both GLP-1 and its receptors have been found to be widespread in the nervous system, heart, lungs, and vascular smooth muscles besides the gastrointestinal system. GLP-1 has been shown to have an effect on the blood glucose level, gastrointestinal motility and secretion, food intake, and appetite, in addition to its hemodynamic effects. Studies of GLP-1 in an organ bath have shown a direct vasodilation effect on vascular beds. This effect of GLP-1 has been directly blocked by exendin, a specific GLP-1 receptor antagonist, while L-NAME administration and elimination of endothelial cells have not prevented this effect of GLP-1, indicating that the direct vascular effect is independent of NO and the endothelium. GLP has caused vasodilation in the pulmonary vessels in rats. The authors have previously reported that peripherally injected GLP-1 decreases gastric lesions caused by ethanol and increases gastric blood flow.

Many studies have found important effects of peptides, such as GLP-1 and ADM, on the physiological and pathological states of normal cell life. However, there are no studies on the role of the peptides' local effects on flap vitality. The aim of the present study was to determine whether ADM and GLP-1 administered at various doses directly to the superficial inferior epigastric artery (SIEA) had an effect on the normal healing process of flap tissue.

**Materials and Methods**

The study was started after consent was obtained from the Uludag University Animal Experiments Local Ethic Committee. A total of 48 Wistar rats weighing 250 g–300 g each were used. The rats were put into acclimatization in separate cages for 1 week before the study on a 12-h light/dark cycle with access to feed and water ad libitum. The rats were placed in separate cages following the experiment to prevent damage to the flap tissue during normal activity.

The rats were put in an aerochamber and anesthesia was started with 5% oxygen and 4% isoflurane. Anesthesia was maintained by administering 2%–3% isoflurane via a mask. The rats were put in the dorsal decubitus position before the surgery and the surgical area was manually depilated. A cutaneous flap area 8 cm x 3 cm in size was marked on the abdominal wall, divided into four equal sections, and marked from 1 to 4 from proximal to distal (Figure 1).

Antisepsis was provided with iodine before starting the surgical procedure and the surgical area periphery was covered with sterile drapes. The flap was lifted based on the superficial inferior epigastric artery (SIEA) and the edges were re-sutured into place. A laser Doppler flow-meter (LDF) was used to measure the blood supply of each area in the flap tissue. The measurements were re-
corded into the computer as tissue perfusion units (mL/min/100 g tissue) via the “MP30 Data Acquisition System.” The flap tissue was marked so that blood flow measurements could be made from the same point every time. The blood flow values measured once the flap was lifted were accepted as the baseline values. The femoral artery and branches were then dissected, again from the left side. A vascular clamp was placed to the proximal and distal of the femoral artery to leave SIEA as the only vessel feeding the flap tissue, and injections were made into the femoral artery with a 30-gauge needle for intra-arterial injections. The clamps were opened 10 minutes after the injection to facilitate reperfusion. The flap pedicle was sutured at the conclusion of the procedure, and blood flow of the four sections was measured again with LDF after the injection. The difference between the values before (basal) and after the injection was shown as percent change. All rats received a buprenorphine injection for postoperative analgesia. The rats were kept in separate cages with access to feed and water ad libitum for 7 days. On the seventh postoperative day, an image of the last condition of the flap was obtained with a 5-megapixel camera from a distance of 15 cm; the rats were sacrificed afterward.

Analysis of the digital photographs was performed in a blinded manner. The total size of each flap tissue and the necrotic area that developed were measured separately to determine the ratio of necrotic area to the total flap tissue area and the results were presented as a percentage of the total flap area (area % = [necrosis area/flap area] x 100).

The rats were divided into four groups (sham, saline, ADM, and GLP-1). The flap tissue was prepared and re-sutured in place in the sham group, and no injection was made into the SIEA. The flap tissue was prepared and 1 mL of saline was injected into the SIEA after the flap was re-sutured in the saline group. ADM or GLP-1 was injected into the SIEA in the other two groups at doses of 166, 332, and 500 pmol in 1 mL of saline to determine the effect of intra-arterial (IA) ADM on flap blood flow. The blood flow values were recorded every 5 minutes after the injection and any changes from the basal value were determined.

GLP-1 was injected at doses of 1.5, 3, and 30 nmol in 1 mL of saline to determine the effect of IA ADM on the flap blood flow. The blood flow values were recorded every 5 minutes after the injection, and any changes from the basal values were determined.

The ADM and GLP-1 used in the study were obtained from Sigma (Sigma-Aldrich Co, St. Louis, MO). The drugs were prepared in saline (0.9% NaCl) for a final dose within 1 mL.

**Statistical Analysis**

The results were presented as mean blood flow change (percentage ± SEM) and mean necrosis (area ± SEM). The non-parametric Mann-Whitney test was used for statistical analysis. P < 0.05 was considered significant.

**Results**

Groups treated with ADM or GLP-1 showed a statistically significant increase in the blood flow of the 4 separate regions compared to the saline group (Figures 2–5). There was no statistically significant difference between the doses of ADM or GLP-1 used and the blood flow percent change (P > 0.1).

The black necrotic area in the flap tissue was measured using ImageJ software for the digital photographic analysis of the amount of necrosis that developed in the flap tissue. Evaluation of the results showed no statistically significant difference between the sham and saline group (Figures 6, 7A, 7B). The percent necrosis area decreased in a statistically significant manner in the groups treated with 166 pmol and 332 pmol ADM compared to the saline group (P < 0.001; Figures 6, 8A). Similarly, the percent necrosis area decreased in a statistically significant manner in the groups treated with 1.5 pmol and 3 pmol GLP-1 compared to the saline group (P < 0.001; Figures 6, 8B). A statistically significant difference was not found between the high-dose ADM and GLP-1 groups, although the amount of necrosis was less than that in the saline group.

**Keypoints**

- Groups treated with ADM or GLP-1 showed a statistically significant increase in the blood flow of the 4 separate regions compared to the saline group (Figures 2–5). There was no statistically significant difference between the doses of ADM or GLP-1 used and the blood flow percent change (P > 0.1).

**Discussion**

The present study found that ADM and GLP-1 prevented flap necrosis and increased blood flow in all areas of the lifted flap tissue. The necrosis that develops in flap tissue is known to appear more commonly in the distal region where vascularization is the lowest.1 It was observed that both peptides used in the present study prevented necrosis in this area. The most common reasons for flap necrosis are said to be inadequate blood flow and venous drainage.2 Research has therefore been carried out on the relation between...
the effects of various substances that increase blood flow and accelerate the development of new vessels in the flap tissue and necrosis development. A previous study has reported that intraperitoneal sildenafil administration decreases flap necrosis.\(^3\) Another study has shown that intraarterial VEGF and L-arginine increase flap perfusion and decrease flap necrosis when administered to rats, taking their vasodilating activity into account.\(^4\) The vasodilator agent minoxidil has been shown to decrease flap necrosis when administered orally.\(^24\)

ADM is a very potent vasodilator peptide and shows its blood flow-increasing effect in various vascular beds.\(^25\) It has also been shown to increase VEGF expression in vitro and in vivo in experimental models,\(^9\) and to pos-

Figure 2. Blood flow % change in flap tissue area 1. The difference between the basal measurement after the flap was lifted and those after the injections were made have been shown as % change. ADM was injected at doses of 166, 332, and 500 pmol and GLP-1 at 1.5, 3, and 30 pmol doses via the IA route. *** P < 0.001 shows the difference with the saline group. There were 6 rats in each group.

Figure 3. Blood flow percentage change in flap tissue area 2. The difference between the basal measurement after the flap was lifted and those after the injections were made have been shown as % change. ADM was injected at doses of 166, 332, and 500 pmol and GLP-1 at 1.5, 3, and 30 pmol doses via the IA route. *** P < 0.001 shows the difference with the saline group. There were 6 rats in each group.

Figure 4. Blood flow % change in flap tissue area 3. The difference between the basal measurement after the flap was lifted and those after the injections were made have been shown as % change. ADM was injected at doses of 166, 332, and 500 pmol and GLP-1 at 1.5, 3, and 30 pmol doses via the IA route. *** P < 0.001 shows the difference with the saline group. There were 6 rats in each group.

Figure 5. Blood flow % change in flap tissue area 4. The difference between the basal measurement after the flap was lifted and those after the injections were made have been shown as % change. ADM was injected at doses of 166, 332, and 500 pmol and GLP-1 at 1.5, 3, and 30 pmol doses via the IA route. *** P < 0.001 shows the difference with the saline group. There were 6 rats in each group.
sess an angiogenic activity in endothelial cells through Akt, MAPK, CRLR/RAMP2-CRLR/RAMP3 receptors and focal adhesion kinases.26–28 Besides vasodilator and angiogenic effects, ADM is also said to have anti-apoptotic effects through the cAMP-PKA pathway.29,30 The authors did not find any studies on vasodilator efficacy and ADM’s effects on flap necrosis (flap tissue-blood flow). The authors believed that these results are consistent with the ADM effects reported in the literature.

Previously, research has focused on the hemodynamic effects on GLP-1, but the focus has turned to ADM more recently. Studies have reported that GLP-1 mildly increases blood pressure and heart rate,17,20 but has an anti-hypertensive effect in salt-sensitive rats.31 GLP-1 has been found to be effective in the correction of left ventricular heart failure in humans.32 Both exendin-4 and GLP-1 have been found to have beneficial effects in cardiac ischemia-reperfusion damage.33 Dose-dependent relaxation has been observed in rat ileal artery isolated preparations perfused with GLP-1 and the peptide has been postulated to be a quite potent vasorelaxant in rats.34 GLP-1 has been shown to have a direct relaxant effect in vascular beds in a rat organ bath model. This effect of GLP-1 has been said to be dose-dependent on the femoral artery rings and to be inhibited by the GLP-1 receptor antagonist exendin.9–39 but to also be NO-independent.35 GLP-1 is also reported to have a beneficial effect on endothelium-related vasodilation in humans.34 The authors have shown previously that GLP-1 has a gastric mucosal blood flow-increasing effect, and that it increases gastric mucosal blood flow when administered both by itself and with ethanol.22 The present results show that GLP-1 increases blood flow in flap tissue, which is consistent with the results of other studies showing vasodilation effects of GLP-1.34,35 GLP-1 also has anti-apoptotic effects, and it has been postulated that GLP-1 modulates pro-apoptotic and anti-apoptotic proteins with signal pathways, such as phosphoinositol-3 kinase, Akt, and MAPK.36,37 The success of GLP-1 in preventing flap necrosis may have been due to its anti-apoptotic effect, in addition to its vasodilating features.

Although the blood flow-increasing effect of both peptides at the low, medium, and high doses used in the present study were dose-dependent, there were no statistically significant differences between the three doses. The bell-shaped, dose-response curve seen regarding the
effect on flap necrosis for both peptides is also seen with some of their other effects.\textsuperscript{36-41} This may be due to the two or more receptors that mediate opposite effects of the peptides influencing each other or the interactions of various intracellular signal mechanisms. It is also possible that the decrease in peptide mediators following the tissue ischemia that is created in the tissue following the lifting of the flap may have decreased the response expected at high doses. Previous studies have postulated that the decrease in ADM’s vascular response is due to a decrease in ADMBP-1,\textsuperscript{42-46} as ADMBP-1 is the agent that increases the vasodilator effect of ADM.\textsuperscript{47,48} The decreased blood flow after the flap is lifted, and the resultant decreased ADMBP-1 level in the tissue in the present study, may have led to the lack of more effective vasodilation from ADM at medium and high doses.

### Conclusion

Both ADM and GLP-1 increase flap blood flow and decrease necrosis when administered directly to the superficial inferior epigastric artery. The authors, therefore, conclude that both peptides play an important role in the normal flap recovery process.

### References


