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ORIGINAL ARTICLE

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Establishment of a swine experimental model of non-occlusive mesenteric ischemia: Combining induced hemorrhagic shock and vasopressor administration

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Abstract

Aim: Non-occlusive mesenteric ischemia (NOMI) is associated with high mortality rates, but definitive treatments have not yet been established. Although experimental animal models are worthwhile, reproducible models that reflect the pathophysiology of NOMI have not been developed.

Methods: We combined risk factors for NOMI, comprising hemorrhagic shock, systemic vasopressor infusion, and local vasopressor infusion from the superior mesenteric artery (SMA) in swine under maintained anesthesia. Experiment 1 involved full-intensity (40%) phlebotomy and systemic vasopressor (norepinephrine and epinephrine). Experiment 2 involved full-intensity (40%) phlebotomy, systemic norepinephrine, and local vasopressor infusion into the SMA. Experiment 3 involved moderate (27%) phlebotomy, systemic norepinephrine infusion, and local epinephrine infusion. We evaluated serum lactate levels, intestinal serosa color, computed tomography (CT) angiography, and pathological findings.

Results: After inducing hemorrhage, systemic vasopressor alone and in combination with local vasopressin or norepinephrine infusion did not induce ischemic color changes in the intestine. The combination of systemic norepinephrine and local epinephrine (0.5 μg/kg/min) after moderate (27% blood loss) hemorrhage induced gross color change, pathological destruction, and elevation of serum lactate. Patent flow in the SMA was confirmed on CT angiography.

Conclusion: We established a swine NOMI model with systemic norepinephrine infusion and local epinephrine with moderate hemorrhagic shock.

KEYWORDS
acute abdomen, experimental animal, mesenteric vascular insufficiency

INTRODUCTION

Non-occlusive mesenteric ischemia (NOMI) develops with septic shock and multiorgan failure, resulting in a mortality rate of 70%–90%, representing a notably high value among the various types of AMI.[1–3] Various risk factors have been reported, including hypovolemia, cardiac dysfunction, and mesenteric hypoperfusion due to vasopressors.[1,4] The definitive treatment of NOMI requires improving the underlying conditions and eliminating risk factors.[1] Interventional local vasodilatory therapy, such as papaverine infusion or prostaglandin E1, has shown possible potential as a rescue therapy.[1,5–7] Laparotomy should be performed for patients presenting with intestinal necrosis

or subsequent peritonitis.[4] However, current outcomes for NOMI patients after diagnosis and treatment strategy remain insufficient.

Animal models are expected to allow the establishment of novel treatment strategies, as NOMI remains associated with high mortality rates despite the numerous treatment options proposed. No reproducible experimental models simulating the pathophysiology of NOMI have yet been developed. This study investigated a swine experimental model to understand NOMI better.

METHODS AND ANALYSIS

Animal preparation

Experimental swine were 3–4 months old and weighed 30–40 kg (Figure 1). All swine were female Landrace. In Experiments 1–3, one swine was used for each experiment to avoid any effect of the previous experiment. Swine were isolated for at least 7 days, fasted for 24 h, and allowed to drink water ad libitum before experiments. Prior to the experiments, we premedicated swine intramuscularly with medetomidine (0.06 mg/kg; Nippon Zenyaku Kogyo, Fukushima, Japan), midazolam (0.3 mg/kg; Astellas Pharma Inc., Tokyo, Japan), and atropine (0.08 mg/kg; Mitsubishi Tanabe Pharma Corporation, Osaka, Japan). Swine were then transported to the animal operating room. We started and maintained anesthesia with sevoflurane and then performed tracheal intubation. Ventilation was maintained with a tidal volume of 7–10 mL/kg and a respiratory rate of 10–15 breaths/min. End-tidal CO_2 was maintained at 40 ± 5 mmHg and peripheral oxygen saturation at 95%–100%. A heated blanket (39°C) was used to prevent hypothermia of the swine during the experiment. After inducing general anesthesia, a central venous catheter was placed in the right jugular vein. An arterial line was placed in the right carotid artery for blood pressure monitoring and sampling. We placed a 10-Fr sheath in the right femoral artery. Additionally, in Experiments 2 and 3, we inserted an angiographic catheter to administer vasopressor into the SMA before phlebotomy. To prevent thrombosis, 5000 units of unfractionated heparin were injected intravenously and then infused at 1000 units/h.

We defined the beginning of phlebotomy as $T = 0$. Swine were phlebotomized through the femoral artery sheath for 20 min ($T = 0$ to $T = 20$). Based on a previous report, we performed phlebotomy to achieve blood loss of 30 mL/kg, representing approximately 40%.[8] Swine were phlebotomized at 2.15 mL/kg/min for 7 min, followed by 1.15 mL/kg/min for 13 min, defined as "full intensity." We also titrated moderate hemorrhage using two thirds of that full-intensity phlebotomy rate, defined as "moderate intensity." In moderate-intensity hemorrhage, swine were phlebotomized at 1.43 mL/kg/min for 7 min, followed by 0.77 mL/kg/min for 13 min.

Systemic vasopressor was started at $T = 0$ to maintain hemodynamics during phlebotomy. Local vasopressor administration was added at $T = 30$. We performed blood gas analysis and observed the gross appearance of the intestinal serosa, then obtained pathological intestine specimens every 60 min until $T = 270$. We also performed computed tomography (CT) angiography to clarify the relationship between radiological and surgical findings.

Evaluation modality of mesenteric ischemia

Blood gas analysis (lactate)

We assessed the systemic ischemic burden with blood sampling. Using the blood samples, we employed point-of-care blood gas analysis (Epoc; Siemens Healthineers, Erlangen, Germany) every 60 min and measured lactate levels (Lactate Pro; AKRAY, Kyoto, Japan) every 30 min.

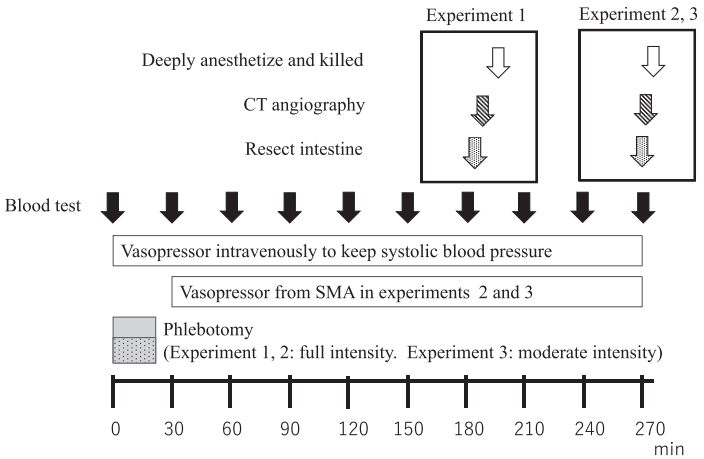

FIGURE 1 Experimental methods. Experiment 1 was performed from $T = 0$ to $T = 180$. At $T = 180$, CT angiography was performed, and the swine was killed in Experiment 1. In Experiments 2 and 3, intestinal resection, CT angiography, and killing were performed at $T = 270$.

Gross findings at laparotomy

The intestinal serosa became pale due to ischemia, and surface veins became dark red due to venous congestion. All members, including surgeons, confirmed color changes in the intestinal serosa every 30 min and recorded those findings as photographs.

Pathological evaluation

Before killing, we resected small pieces of intestine ($T = 270$). Resected specimens were fixed in formalin and stained with hematoxylin and eosin. We evaluated the intestinal damage pathologically.

CT angiography

Swine were transferred to a CT system (SOMATOM® Definition AS+ [128-slice]; Siemens Healthcare GmbH). Before killing, CT angiography was performed ($T = 270$). CT angiography confirmed SMA patency and mesenteric ischemia.

Finally, swine were deeply anesthetized and killed without awakening from anesthesia.

Experimental procedures

Experiment 1

Full-intensity hemorrhagic shock and systemic vasopressor infusion

After inducing full-intensity hemorrhagic shock, an intravenous infusion of norepinephrine 0.5 μg/kg/min was started simultaneously with phlebotomy [$T = 0$]. We then also examined systemic epinephrine infusion.

Experiment 2

Full-intensity hemorrhage in combination with both systemic and local vasopressors

After full-intensity hemorrhage and systemic norepinephrine infusion, we added local vasopressor infusion at $T = 30$. A local vasopressor was administered through an angiographic catheter in the superior mesenteric artery. We validated three local vasopressors: norepinephrine, vasopressin, and epinephrine. We chose norepinephrine as the systemic vasopressor in all experiments.

Local vasopressin or norepinephrine infusion. With the titration of systemic norepinephrine, we infused vasopressin or norepinephrine locally and increased the dose of each vasopressor.

Local epinephrine infusion. After the two vasopressors, we validated epinephrine and investigated the vasopressor dose and intestinal ischemia.

Experiment 3

Moderate-intensity hemorrhage in combination with both systemic and local vasopressors

Full-intensity hemorrhagic shock induces severe hemodynamic collapse that requires a high-dose systemic vasopressor. We attempted moderate-intensity hemorrhagic shock at two thirds of the blood loss (approximately 27% blood loss).

RESULTS

Experiment 1

We increased the dose of norepinephrine from 0.5 to 3.0 μg/kg/min, and serosa color remained unchanged. Lactate levels were not significantly elevated (Figure 2). Following the examination with norepinephrine, we changed the vasopressor to epinephrine and increased the dose from 4.0 to 7.5 μg/kg/min, showing a slight change in mesenteric color. Lactate levels were markedly elevated at >25 mmol/L (beyond the upper limit of the Lactate Pro "High >25") (Figure 2). CT of the abdomen did not demonstrate bowel non-enhancement or other radiological findings of NOMI.

Experiment 2

Local vasopressin or norepinephrine infusion

After phlebotomy began, we titrated systemic norepinephrine. We then recorded the color changes of the intestinal serosa during gross observation of the abdomen with local vasopressin infusion. Serum lactate levels were elevated (Figure 3), but the change in intestinal serosa color was minimal (Figure 4C). We then changed the vasopressor to norepinephrine up to 2.0 μg/kg/min. The serum lactate level elevated to 11.1 mmol/L ($T = 210$), but the color of the intestinal serosa changed only minimally (Figure 4D).

Local epinephrine infusion

Following norepinephrine infusion, we examined the local infusion of epinephrine at 2.0 μg/kg/min (Figure 3). Unlike norepinephrine and vasopressin, local epinephrine infusion significantly changed the serosa color (Figure 4E). Serum lactate level also increased markedly (Figure 3). CT angiography revealed poor bowel enhancement with patent flow in the SMA (Figure S1). Histopathological examination

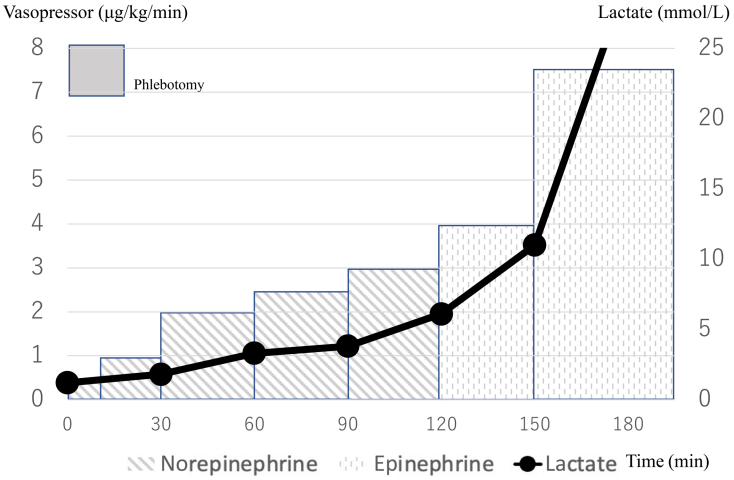

FIGURE 2 High-dose intravenous infusion of norepinephrine and epinephrine. In Experiment 1, lactate levels were markedly elevated when epinephrine rather than norepinephrine was administered to swine. However, only a slight change in the color of the intestinal serosa was observed.

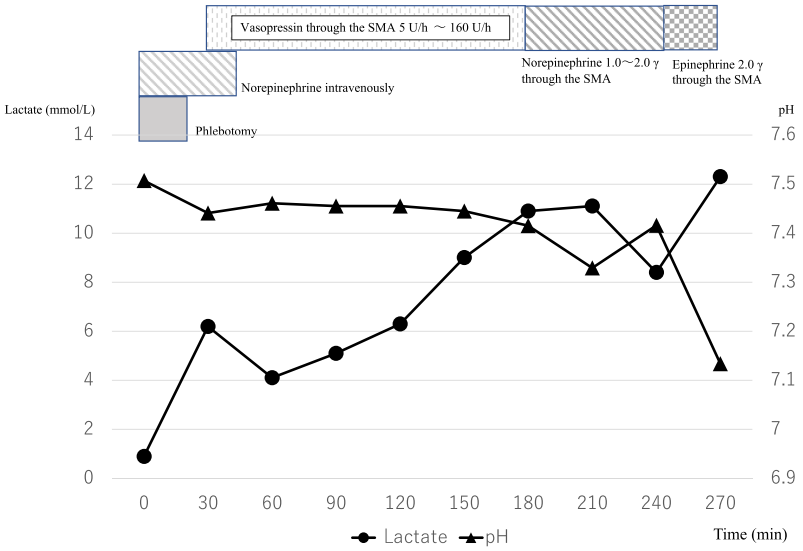

FIGURE 3 Comparison of types of vasopressors with intra-arterial infusion. In Experiment 2, intra-arterial infusion of vasopressin resulted in elevated lactate levels and decreased pH, but no change in the color of the intestinal serosa. Lactate levels improved when norepinephrine was used instead of vasopressin, and lactate levels increased when epinephrine was administered selectively into the SMA.

revealed damage to the mucosal epithelium at $T = 270$ (Figure S2).

In Experiments 1 and 2, swine were alive until killing. However, full-intensity hemorrhage significantly reduced blood pressure and circulatory stability.

Experiment 3

After applying two thirds phlebotomy and a maintenance dose of norepinephrine, we evaluated intestinal ischemia with gross observation and lactate levels during local epinephrine infusion. The moderate-intensity hemorrhage model appears more hemodynamically stable. The model with moderate hemorrhage and local epinephrine showed progressive elevation of lactate levels (Figure 5) and intestinal mucosal damage at $T = 270$ (Figure 6). CT angiography confirmed a patent SMA (Figure 7).

DISCUSSION

Non-occlusive mesenteric ischemia is a life-threatening sequela resulting from the combination of multiple risk factors.[1,4] In creating mesenteric ischemia in the experimental animal, direct SMA occlusion is the most straightforward option.[9,10] However, the NOMI model must be induced without arterial occlusion. Pericardial tamponade and partial aortic occlusion have been reported as possible methods for creating NOMI models.[11,12] Previous reports have described NOMI models induced using only a single risk factor.[11,12] Those models may not reflect the pathogenesis of NOMI.

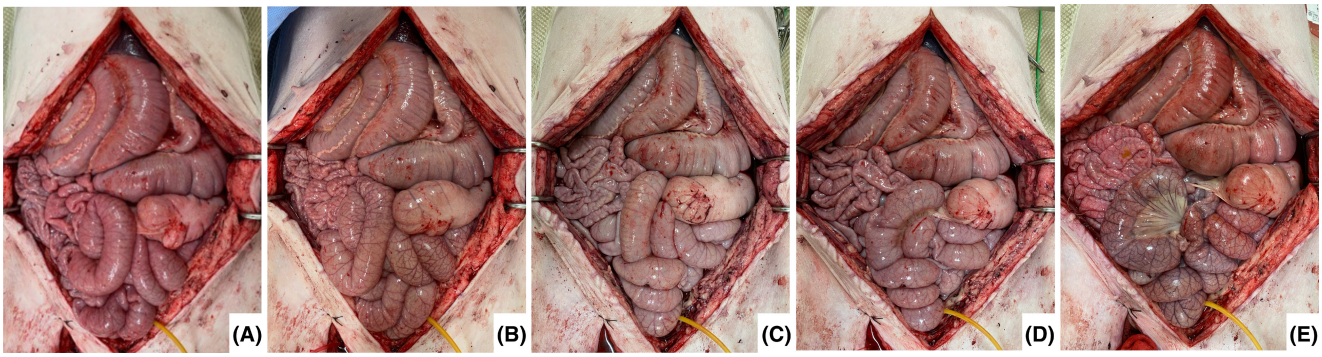

FIGURE 4 Changes in color of the intestinal serosa. (A) Before phlebotomy, (B) after phlebotomy, (C) intra-arterial vasopressin, (D) intra-arterial norepinephrine, and (E) intra-arterial epinephrine. Venous vasopressor infusion and intra-arterial infusion of norepinephrine or vasopressin induced slight ischemic changes as reflected by intestinal coloration. Intra-arterial epinephrine infusion at 2 μg/kg/min markedly changed the intestinal serosa.

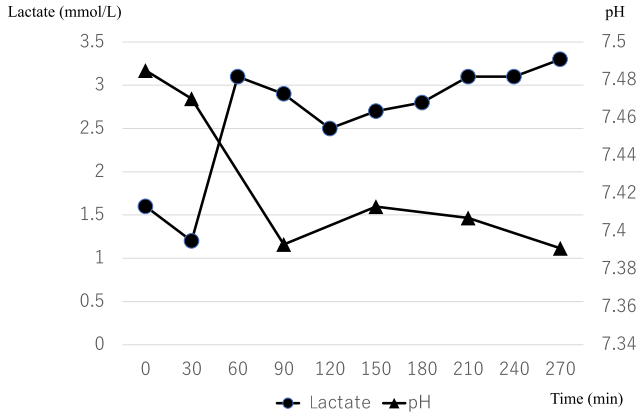

FIGURE 5 Combination of hemorrhagic shock and intra-arterial epinephrine infusion. We employed moderate-intensity hemorrhage, systemic norepinephrine, and local epinephrine infusion at 0.5 μg/kg/min into the SMA. With the administration of epinephrine, lactate levels gradually increased, and pH gradually decreased.

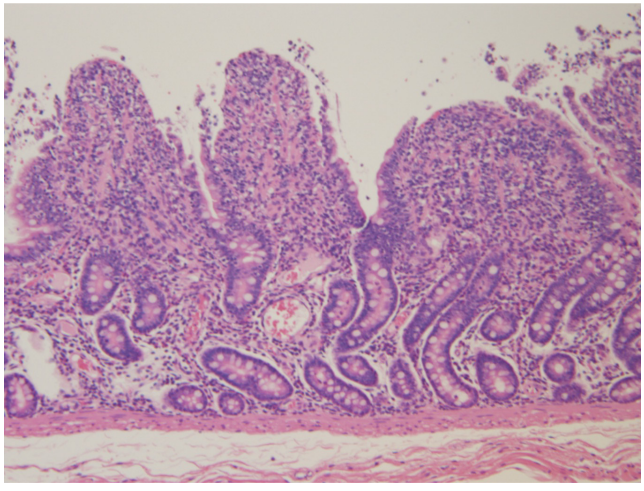

FIGURE 6 Intestinal mucosal changes at $T = 270$ in Experiment 3. Hematoxylin and eosin staining revealed inflammatory cell infiltration, epithelial shedding, exposed and dilated capillaries, and denuded villi. No hemorrhage or ulceration is evident.

Our study is the first to describe a multifactor-induced NOMI animal model that combines hypovolemic shock and intravenous and intra-arterial infusion of vasopressors. Clinicians employ various modalities to diagnose NOMI, including CT angiography and biomarker measurement. However, previous animal models of NOMI did not validate pathological changes in the intestine or on CT angiography. Our study evaluated the NOMI animal model with multiple modalities to adapt to clinical settings.

We assumed that if we could create NOMI by systemic vasopressor administration and phlebotomy, that model would be simple and highly reproducible. Therefore, we attempted to create NOMI up to very high doses. However, changes in intestinal coloration were insufficient. The vasopressor was changed from norepinephrine to epinephrine to obtain more potent vasoconstriction and bowel ischemia via high-dose epinephrine-induced systemic lactic acidosis. We attributed a slight change in bowel coloration to impaired bowel circulation as a part of multiorgan failure. Systemic vasopressor administration mainly affects systemic ischemia. Since we judged that such administration for a few hours showed insufficient local ischemia for a NOMI animal model, we decided to administer vasopressor from SMA to obtain bowel ischemia. In Experiment 2, systemic vasopressor combined with local vasopressin or norepinephrine infusion did not induce gross color changes. Combination with local epinephrine infusion to produce a more potent ischemic reaction induced significant ischemic changes on gross observation within 30 min of infusing epinephrine at 2.0 μg/kg/min. Compared to the other two drugs, local infusion of epinephrine induced intestinal ischemia. Findings from CT angiography and histological evaluations revealed irreversible necrotic changes with this dose.

Since we administered epinephrine for only 30 min, we considered this dose excessive, with less chance of therapeutic intervention. Moreover, a previously reported animal model of hemorrhagic shock with 30 mL/kg of blood loss (approximately 40% blood loss)[8] induced excessively unstable hemodynamics. In order to establish models that can survive until the end of an experiment, we employed phlebotomy at 20 mL/kg (approximately 27% blood loss). Finally,

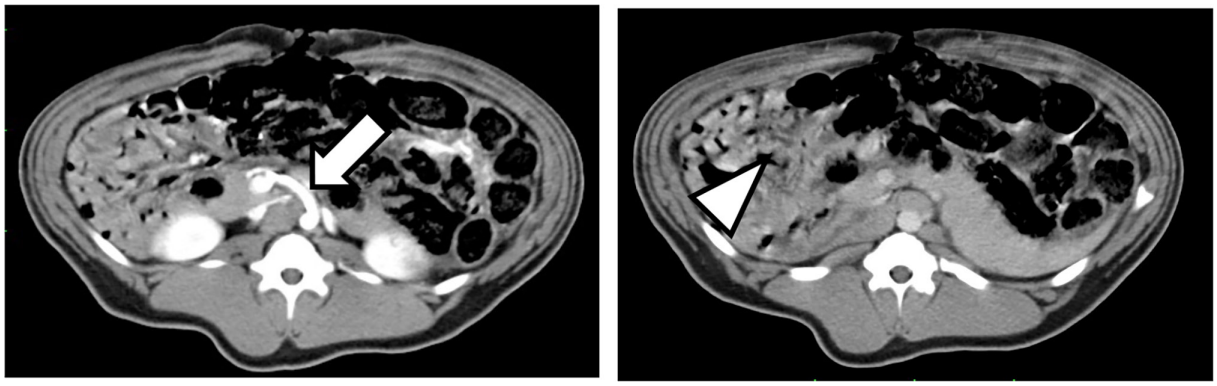

FIGURE 7 CT angiography at $T = 270$ in Experiment 3. CT angiography reveals patent flow in the SMA (arrow). The effect of insufficient contrast enhancement of the bowel was extremely slight (arrow head).

we applied local epinephrine infusion at 0.5 μg/kg/min in the SMA and employed 27% blood loss with supportive systematic norepinephrine infusion as a feasible NOMI model with acceptable hemodynamics. Experiment 3 tested whether the NOMI model was valid, even if the phlebotomy volume was reduced to 27% blood loss. Compared to moderate-intensity hemorrhagic shock, lactate levels were higher, and ischemia was more evident on CT angiography and pathological findings in full-intensity hemorrhagic shock. However, necrosis of the intestinal mucosa also appeared in moderate-intensity hemorrhagic shock, which could be interpreted as a NOMI model with more stable circulatory dynamics. This large animal model of NOMI that we developed might prove helpful in developing new therapeutic strategies.

Several limitations to this study need to be considered. First, we employed hypovolemic shock and vasoconstriction to create mesenteric ischemia but did not incorporate cardiac dysfunction to simulate NOMI. We could not incorporate cardiac dysfunction into this experiment because we had experienced in other studies that swine can quickly die from myocardial ischemia. Second, our observation period was only 4 h, which could be shorter than seen with clinical deterioration in cases of NOMI. Third, the systemic ischemic burden with blood sampling was evaluated using only blood gas analysis. The results of blood gas analysis can be obtained in minutes, unlike such biomarkers as CK, LDH, and transaminase. Further studies are needed to assess other biomarker trends. Finally, as shown in Figures 3 and 5, lactate levels decreased markedly. The cause of this reduction is not yet understood. However, in contrast to situations in which blood flow is physically obstructed, blood flow is maintained in the NOMI animal model. This suggests that ischemic intensity may vary with the degree of vasoconstriction. Despite these limitations, our NOMI model appears reproducible and feasible in swine and could facilitate the development of therapies for NOMI.

CONCLUSION

We developed an NOMI animal model with phlebotomy comprising 27% blood loss, systemic administration of norepinephrine to maintain blood pressure, and local epinephrine infusion at 0.5 μg/kg/min into the SMA.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

Research data are not shared.

ETHICS STATEMENT

Approval of the research protocol: The experimental protocol received approval from the Jichi Medical University Animal Ethics Committee (Animal Ethics Approval Number: 21054-01).
Informed consent: N/A.
Registry and the registration no. of the study/trial: N/A.
Animal studies: All animal experiments were conducted in accordance with the national guidelines and the relevant national laws on the protection of animals.

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REFERENCES

1. Gnanapandithan K, Feuerstadt P. Review article: mesenteric ischemia. Curr Gastroenterol Rep. 2020;22:17.
2. Ruchkin DV, Okonskaya DE, Karmazanovsky GG, Kozlov VA, Rymar OA, Gorin DS. Non-occlusive mesenteric ischemia in reconstructive digestive tract surgery. Khirurgiia (Mosk). 2022;5:18–24.
3. Mitsuyoshi A, Obama K, Shinkura N, Ito T, Zaima M. Survival in nonocclusive mesenteric ischemia: early diagnosis by multidetector row computed tomography and early treatment with continuous intravenous high-dose prostaglandin E(1). Ann Surg. 2007;246:229–35.

4. Bala M, Kashuk J, Moore EE, Kluger Y, Biffl W, Gomes CA, et al. Acute mesenteric ischemia: guidelines of the World Society of Emergency Surgery. World J Emerg Surg. 2017;12:38.
5. Stahl K, Rittgerodt N, Busch M, Maschke SK, Schneider A, Manns MP, et al. Nonocclusive mesenteric ischemia and interventional local vasodilatory therapy: a meta-analysis and systematic review of the literature. J Intensive Care Med. 2020;35:128–39.
6. Habboushe F, Wallace HW, Nusbaum M, Baum S, Dratch P, Blakemore WS. Nonocclusive mesenteric vascular insufficiency. Ann Surg. 1974;180:819–22.
7. Miyazawa R, Kamo M. What affects the prognosis of NOMI patients? Analysis of clinical data and CT findings. Surg Endosc. 2020;34:5327–30.
8. Frankel DAZ, Acosta JA, Anjaria DJ, Porcides RD, Wolf PL, Coimbra R, et al. Physiologic response to hemorrhagic shock depends on rate and means of hemorrhage. J Surg Res. 2007;143:276–80.
9. Blikslager AT, Roberts MC, Rhoads JM, Argenzio RA. Is reperfusion injury an important cause of mucosal damage after porcine intestinal ischemia? Surgery. 1997;121:526–34.
10. Walensi M, de Groot H, Schulz R, Hartmann M, Petrat F. Mesenteric ischemia-reperfusion injury: clearly improved hemodynamics but only minor protection of the rat small intestine by (sub)therapeutic heparin sodium and enoxaparin doses. J Surg Res. 2013;179:e57–e69.
11. Szűcs S, Bari G, Ugocsai M, Lashkarivand RA, Lajkó N, Mohácsi Á, et al. Detection of intestinal tissue perfusion by real-time breath methane analysis in rat and pig models of mesenteric circulatory distress. Crit Care Med. 2019;47:e403–e411.
12. Nógrády M, Varga G, Szűcs S, Kaszaki J, Boros M, Érces D. Effects of complement C5a inhibitor therapy in animal models of non-occlusive mesenteric ischemia. Magy Seb. 2017;70:221–31.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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