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EFFECT OF IRRIGATION FLUID COMPOSITION ON HEMOSTASIS IN MOUSE BLEEDING MODELS

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ABSTRACT—**Introduction:** Intraoperative irrigation, usually with normal saline (NS), aids in bleeding identification and management. We investigated the effect of different irrigation fluids, with additives, on hemostasis using two bleeding models. **Methods:** C57BL/6 J mice were subjected to a tail bleed model or uncontrolled abdominal hemorrhage *via* liver laceration followed by abdominal cavity irrigation. We compared NS, lactated Ringer's (LR), and PlasmaLyte. We examined NS and LR at different temperatures. Normal saline or LR with calcium (Ca^{2+}) or tranexamic acid (TXA) was studied. **Results:** Compared with room temperature (RT), increasing the temperature of the irrigation fluid to 37°C and 42°C reduced tail vein bleeding times substantially in both NS and LR (all $P < 0.001$), with no significant differences between the two fluids. At RT, LR, but not PlasmaLyte, substantially reduced bleeding times in comparison to NS ($P < 0.0001$). Liver injury blood loss was lower with LR ($P < 0.01$). Normal saline supplemented with 2.7 mEq/L of Ca^{2+} decreased bleeding time and blood loss volume ($P < 0.001$ and $P < 0.01$, respectively) to similar levels as LR. Normal saline with 150 mg/mL of TXA markedly reduced bleeding time ($P < 0.0001$), and NS with 62.5 mg/mL TXA decreased blood loss ($P < 0.01$). **Conclusion:** Whereas Ca^{2+}- and TXA-supplemented NS reduced bleeding, LR remained superior to all irrigation fluid compositions. As LR contains Ca^{2+}, and Ca^{2+}-supplemented NS mirrored LR in response, Ca^{2+} presence in the irrigation fluid seems key to improving solution's hemostatic ability. Because warming the fluids normalized the choice of agents, the data also suggest that Ca^{2+}-containing fluids such as LR may be more suitable for hemostasis when used at RT.

KEYWORDS—Bleeding, fluid, hemostasis, intraoperative, irrigation, surgery

ABBREVIATIONS:—Ca^{2+}: calcium, HWI: hot water irrigation, LR: lactated Ringer's, NS: normal saline, RT: room temperature, SSI: surgical site infections, TXA: tranexamic acid

INTRODUCTION

Approximately 313 million surgeries are carried out around the world annually (1). Intracavitary irrigation is a standard step used in many surgeries to wash away debris and contaminants, thus reducing surgical site infections (SSIs). The default fluid of choice for irrigation, as confirmed by a survey of perioperative nurses (2), is normal saline (NS). However, little quality evidence exists as to why this is the case (2).

Irrigation is also used to control surgical bleeding. Hot water irrigation (HWI) was first used in the late 19th century by obstetricians to achieve hemostasis in prepartum and postpartum hemorrhage (3). In 1878, Guice and Fayette were the first to use HWI in the treatment of epistaxis (3). However, there were two important caveats: HWI uses pure water, which causes severe cellular damage and rupture of human nasal epithelial cells (4), and water heated to 52°C or higher results in epithelial necrosis of animal nasal mucosal cells (5). Although several studies have looked into the effect of different irrigation fluids and additives on surgical outcomes, such as SSIs (6,7), perioperative hypothermia (8), wound closure (9,10), fracture healing (11), and tumor recurrence and metastasis (12–14), their influence on bleeding control has not been adequately explored.

Intraoperative bleeding can complicate any procedure, challenging visualization and hindering surgical progress (15). Continuous surgical bleeding increases the chance of tissue damage, prolongs surgery, reduces success rates, and increases the risk of major complications (3,16,17). Intraoperative irrigation improves visualization, helps clear the surgical field from intraoperative bleeding, decreases injury to obscured structures, and aids in identification and management of sources of bleeding. Nonetheless, it remains as an underexplored area, with insufficient literature and data on the properties of the ideal hemostatic irrigation solution. In this study, we hypothesized that lactated Ringer's (LR) was a superior irrigation fluid

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compared with NS due to, at least in part, the presence of calcium (Ca^{2+}). We investigated the effect of different irrigation fluids, with and without additives, on physiologic hemostasis using two mouse models of bleeding.

METHODS

Animals

C57BL/6 J mice, 8 to 12 weeks old, were purchased from Jackson Laboratories. Mice were housed in accordance with the University of Pittsburgh (Pittsburgh, PA) and National Institutes of Health (Bethesda, MD) animal care guidelines. All animal experiments were approved and conducted in accordance with the guidelines set forth by the Institutional Animal Care and Use Committee of the University of Pittsburgh.

Sample size

Following a preliminary pilot set of experiments, we found that a minimum n = 5 in the tail vein bleeding model would provide a confidence level of 80%. In the liver laceration model, our calculations showed that an n = 3 would be sufficient to provide a confidence level of 80%. In addition, both male and female mice were utilized while testing LR at different temperatures to account for sex differences (Supplemental Digital Content, http://links.lww.com/SHK/B531).

Murine bleeding models

Mice were anesthetized with an i.p. injection of sodium pentobarbital (Nembutal; 70 mg/kg) and subjected to one of two validated models of bleeding (18,19). In the tail bleed model (19), 1 cm of the tail tip was cut with a scalpel followed by submerging the tail in 20 mL of the irrigation solution of choice (Supplemental Digital Content Fig. 1, http://links.lww.com/SHK/B531). Time to the cessation of bleeding was measured in seconds and recorded as the bleeding time to quantify bleeding. We decided to halt measurement at 1,200 s as differences between groups were uniformly observed well before this timepoint. The mice were then immediately killed. Bleeding time was quantified as opposed to volume of shed blood or hemoglobin concentration in the irrigation fluid. We used this model to screen for solutions that improve hemostasis and then confirmed our results using a model of uncontrolled abdominal hemorrhage.

For uncontrolled abdominal hemorrhage, our validated liver laceration model (18) was adapted to include a midline laparotomy followed by resection of the left middle lobe. The abdominal cavity was then irrigated with 3 mL of the selected irrigation solution, which was left in the abdomen for 15 min. Subsequently, the fluid and any resultant clots were aspirated, and the volume was measured in milliliters to quantify blood loss (Supplemental Digital Content Fig. 2, http://links.lww.com/SHK/B531). The aspirated volume was measured directly and is the absolute volume, not determined by or adjusted to the weight of the animal. Based on the results from the tail vein bleeding model, we used the liver laceration model to further validate and test irrigation fluids that could improve or outperform LR. In both models, a heating pad was used to prevent hypothermia.

Irrigation solutions

We assessed bleeding with three commonly used crystalloid solutions: NS, LR, and PlasmaLyte (Baxter, Deerfield, IL). The composition of each is displayed in Table 1. We next investigated the effect of the temperature of NS and LR when used for irrigation at room temperature (RT, 24°C), physiologic body temperature (37°C), and 42°C, the recommended temperature to which i.v. fluids are warmed to treat hypothermia (20,21). An incubator was used to reach the desired temperature, which was maintained constant at all times and confirmed using a thermometer. Modification of the pH of the fluids was done by adding sodium bicarbonate where indicated in Table 2 and verified using a pH meter. To evaluate the impact of different additives on hemostasis and to elucidate differences between solutions, we examined the supplementation of NS or LR with either 50 to 150 mg/mL of tranexamic acid (TXA; USP, Frederick, MD), an inhibitor of fibrinolysis, or Ca^{2+} in the form of $CaCl_2$. We tested the augmentation of NS with 2.7 mEq/L of Ca^{2+} (the same concentration of Ca^{2+} as that of LR) and Ca^{2+} in excess at a concentration of 1,000 mEq/L (1 mEq/mL) in both fluids. All experiments were conducted at RT, except where otherwise stated. pH was not intentionally altered except where otherwise stated.

Statistical analysis

All data are reported as mean ± SEM and presented as mean ± SD unless stated otherwise in the figure legends. Statistical significance was determined using one-way and two-way ANOVA with Bonferroni and Tukey *post hoc* testing, Brown-Forsythe test, or Kruskal-Wallis test using GraphPad Prism software (GraphPad Software Inc, San Diego, CA). $P < 0.05$ was considered significant.

Table 1. **Composition of NS, LR, and PlasmaLyte**

	NS	LR	PlasmaLyte
Osmolarity (mOsm/L)	308	273	296
Na^+ (mEq/L)	154	130	140
Cl^- (mEq/L)	154	109	98
K^+ (mEq/L)	—	4	5
Ca^{2+} (mEq/L)	—	2.7	—
Mg^{2+} (mEq/L)	—	—	3
SID	0	28	50
Buffer (mEq/L):			
Lactate	—	28	—
Gluconate	—	—	23
Acetate	—	—	27
pH at RT	5	6.5	7.4
Price/L*	$4.834	$4.715	$4.834

*Prices are obtained from Baxter, USA.

Ca^{2+}, calcium; Cl^-, chloride; K^+, potassium; LR, lactated Ringer's; Mg^{2+}, magnesium; Na^+, sodium; NS, normal saline; RT, room temperature; SID; strong ion difference.

RESULTS

Effect of different irrigation fluids on physiologic hemostasis

First, we investigated the effect of NS, LR, and PlasmaLyte at RT. Using the tail vein bleeding model, LR substantially reduced bleeding times in comparison to NS ($P < 0.0001$), whereas PlasmaLyte showed no significant difference from NS (Fig. 1). PlasmaLyte was not tested in additional assays given the results of the primary comparison of NS, LR, and PlasmaLyte. Similarly, blood loss in the liver injury model was lower in LR versus NS ($P < 0.01$) (Fig. 2A). All values are presented in Table 2 (n ≥ 5 for all groups).

Effect of different additives on physiologic hemostasis

We next sought to assess whether the Ca^{2+} concentration of LR, an important difference between the composition of this fluid and NS (Table 1), could explain the benefit of LR versus NS. To evaluate whether we could improve upon the results of these common fluids, we tested the addition of escalating doses of Ca^{2+} to NS or LR as well as the impact of adding the antifibrinolytic TXA. Table 2 displays the values (n ≥ 5 for all tail vein bleeding groups and n ≥ 3 for all liver laceration groups). The effects were as follows:

Ca^{2+}

NS supplemented with 2.7 mEq/L of Ca^{2+} decreased both bleeding time and blood loss volume ($P < 0.001$ and $P < 0.01$, respectively) to similar levels as LR (Fig. 2, A and B). However, further augmenting NS or LR with 1,000 mEq/L (1 mEq/mL) of Ca^{2+} resulted in bleeding times comparable to both those of LR alone and those of NS + 2.7 mEq/L of Ca^{2+}.

TXA

Supplementation of NS with 50 mg/mL of TXA did not result in reduced bleeding times. However, NS with both 62.5 mg/mL of TXA in the liver laceration model ($P < 0.01$) and 150 mg/mL of TXA in the tail vein bleeding model ($P < 0.0001$) markedly reduced bleeding. Despite that, the addition of TXA to LR did not

Table 2. **Bleeding time and blood loss volume values with different irrigation fluids**

	Tail vein bleeding model	Liver laceration model
Fluid	Bleeding time (s)	Blood loss volume (mL)
NS at 24°C	893.4 ± 187.8	
NS at 37°C	83.20 ± 10.02	
NS at 42°C	89.40 ± 10.20	
LR at 24°C	422.3 ± 83.75	
LR at 37°C	136.5 ± 22.36	
LR at 42°C	78.50 ± 17.18	
PlasmaLyte	739.3 ± 175.3	
NS	884.6 ± 193.3	
LR	347.5 ± 24.54	
NS (pH 7.36)		2.98 ± 0.07348
LR (pH 7.32)		2.675 ± 0.04787
NS	1,059 ± 89.29	3.22 ± 0.06633
LR	426.8 ± 57.06	2.74 ± 0.1166
NS + Ca^{2+} (2.7 mEq/L)	420 ± 27.76	2.70 ± 0.04082
NS + Ca^{2+} (1 mEq/mL)	510.9 ± 131.9	2.400 ± 0.07303
LR + Ca^{2+} (1 mEq/mL)	394.0 ± 60.60	
Saline + TXA (50 mg/mL)	672.3 ± 129.4	
Saline + TXA (150 mg/mL)	342.3 ± 62.88	
LR + TXA (50 mg/mL)	397.8 ± 53.37	
LR + TXA (150 mg/mL)	433.6 ± 108.1	
Saline + TXA (62.5 mg/mL)		2.700 ± 0.09129
LR + TXA (62.5 mg/mL)		3.238 ± 0.04605

The values displayed were obtained at room temperature where temperature is not stated. Cell shading indicates groups in the experiment that were done on the same day; repetition of groups (e.g., NS or LR) on these days was carried out to minimize any extraneous variables. Values are displayed as mean ± SEM.

Ca^{2+}, calcium; LR, lactated Ringer's; NS, normal saline; TXA, tranexamic acid.

decrease bleeding time in comparison to LR or blood loss in comparison to the usage of NS (Fig. 2, A and B).

Effect of pH on physiologic hemostasis

We decided to study the effect of pH of the solution at RT on hemostasis as the commercially available NS and LR have different pH values (shown in Table 1; NS has a pH of 5, and LR has a pH of 6.5). To do that, we increased the pH of both solutions to a more neutral value similar to that of the human blood and compared the blood loss volume in NS at a pH of 5.0 with LR at a pH of 6.5, and NS at a pH of 7.36 with LR at a pH of 7.32 in our liver laceration model (Table 2). At both acidic and neutral pH levels, when compared with NS, LR resulted in a significantly lower blood loss volume (both $P < 0.001$, $n \geq 3$ for all groups) (Fig. 3). The difference in the hemostatic ability between the two fluids persisted regardless of the pH. In addition, there were no substantial differences in blood loss volume when comparing NS with NS, or LR with LR at the different pH levels used (data not shown).

Effect of temperature on physiologic hemostasis

To assess the effect of temperature on hemostasis, we examined the effect of NS and LR at 24°C, 37°C, and 42°C on tail vein bleeding times (Fig. 4, A and B, respectively). Tail vein bleeding times with NS at 37°C and 42°C were significantly shorter than with NS at RT (both $P < 0.001$). No significant differences were seen between NS at 37°C and 42°C. Bleeding times were also markedly shorter in LR at 37°C and 42°C as compared with LR at RT (both $P < 0.001$). Noticeably, there were no significant differences in hemostasis between NS and LR at 37°C and 42°C. Most importantly, the differences in values between LR 24°C and NS at 37°C or 42°C were insignificant, suggesting that LR at ambient temperatures performed as well as heated NS (Fig. 4C). All values are shown in Table 2 ($n \geq 5$ for all groups). A confirmatory subset of experiments was also carried out in female mice (Supplemental Digital Content Table 1 and Fig. 3, http://links.lww.com/SHK/B531) that recapitulated the results seen in male mice.

DISCUSSION

Intraoperative bleeding is common and can have negative consequences. Irrigation is one method that is often used to achieve hemostasis, and NS is most frequently used for that purpose, with limited data to support its usage (2). In this study, we sought to determine the effect of different irrigation fluids on bleeding using murine bleeding models and tested the hypothesis that LR is superior to NS. Here, we present evidence that the composition and temperature of the irrigation fluid influence hemostasis.

We opted to use the tail bleeding model as it is easy to apply, does not require advanced equipment or skills, and is one of the most commonly utilized animal bleeding models (22). This model is not without its limitations, and so we used this model to screen for solutions that improve hemostasis and then confirmed our results using a model of uncontrolled abdominal hemorrhage. We adjusted our liver laceration model, which as we have previously shown is sensitive to positive and negative controls (18), to evaluate the hemostatic abilities of different irrigation fluids. Following the resection, with the exception of irrigation, no intervention was made to aid in bleeding cessation. We postulated that 15 min would be long enough of a timepoint to allow us to detect differences in the hemostatic ability of different fluids.

As compared with NS, LR was able to decrease bleeding times by 60% (tail vein model; Fig. 1) and blood loss volume by 15% (liver laceration model, Fig. 2A), in agreement with the findings of Fujita et al. (17) in their mouse brain surface bleeding model.

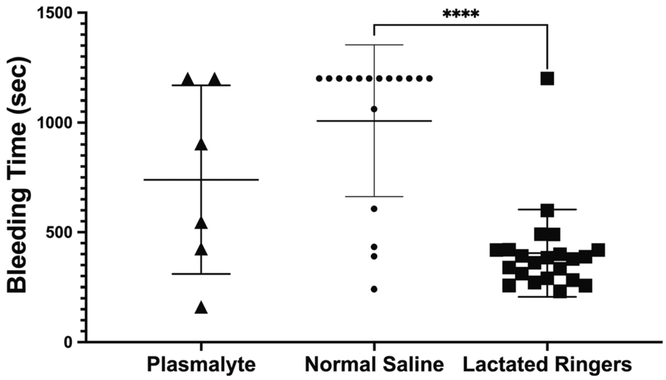

Fig. 1. **Bleeding time in a tail vein transection model with times using three commonly used crystalloids at room temperature.** Tail vein transection was done by a tail tip cut. The tail was then submerged in the irrigation fluid of choice, and the bleeding time was recorded. In comparison to NS, LR significantly shortened bleeding time, whereas PlasmaLyte did not. Values are displayed as mean ± SD. $P > 0.5$ (i.e., not significant) is not shown, ****$P < 0.0001$. Ca^{2+}, calcium; LR, lactated Ringer's; NS, normal saline; TXA, tranexamic acid.

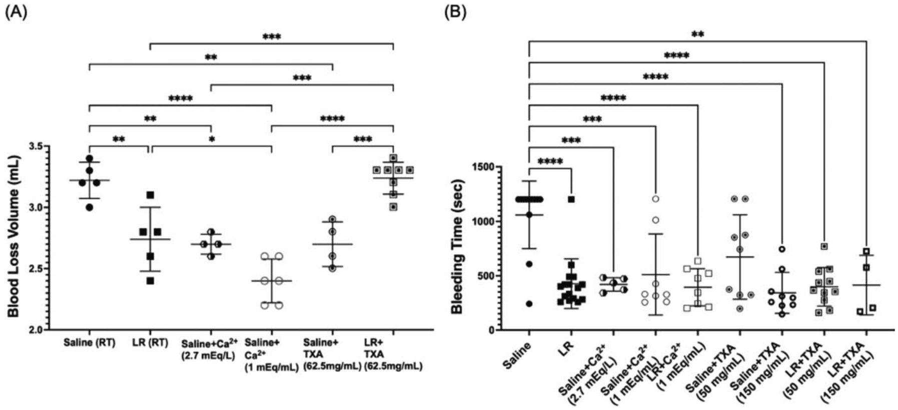

Fig. 2. **Bleeding assessment with different irrigation fluids using two bleeding models.** A, Blood loss volume in the liver laceration with NS or LR supplemented with either Ca^{2+} or TXA. In our model of uncontrolled abdominal hemorrhage, LR notably reduced blood loss volumes when compared with NS. Comparably, NS augmented with Ca^2 or TXA decreased blood loss to values similar to, but not less than, those of LR. However, LR supplementation with TXA did not show any benefit and resulted in blood loss volumes similar to those of NS. Overall, no combination outperformed LR. The experiments were conducted at room temperature. B, Bleeding time in a tail vein transection model with NS or LR supplemented with either Ca^{2+} or TXA at room temperature. Tail vein transection was done by a tail tip cut. The tail was then submerged in the irrigation fluid of choice, and the bleeding time was recorded. Supplementation of NS with Ca^{2+}—at any concentration—or 150 mg/mL of TXA considerably reduced bleeding times to values similar to, but not less than, those of LR. Interestingly, LR supplementation with Ca^{2+} or TXA did not show any added benefit and resulted in bleeding times similar to those of LR but clearly shorter than those of NS. Overall, no combination outperformed LR in terms of bleeding time in our model of tail vein transection *via* a tail tip cut.Values are displayed as mean ± SD. $P > 0.5$ (i.e., not significant) is not shown, $^*P < 0.0332$, $^{**}P < 0.0021$, $^{****}P < 0.0001$. Ca^{2+}, calcium; LR, lactated Ringer's; NS, normal saline; TXA, tranexamic acid.

In our liver laceration model, we tested NS and LR at the pH that they are commercially available at (shown in Table 1). Next, we increased the pH of both solutions to a more neutral value similar to that of the human blood (NS to a pH of 7.36 and LR to a pH of 7.32) in an attempt to ascertain whether the acidic nature of NS contributes to the difference in hemostatic abilities between the two fluids. It has been shown that acidosis impedes coagulation in an increasing manner (23). It is hypothesized that the coagulation cascade optimal enzyme pH is equal to or higher than 7.4, a hypothesis supported by the measured enzyme reaction optimal pH of 7.5 of thromboxane synthase isolated from porcine lungs (23,24). We found that blood loss volume decreased when LR was used (Fig. 3), regardless of pH, offering a seemingly insufficient explanation to the magnitude of the decrease in bleeding time with LR compared with NS.

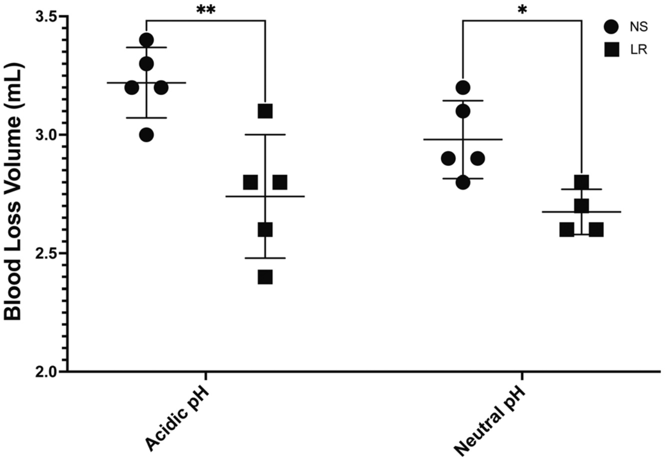

Fig. 3. **Blood loss volume in the liver laceration model with NS versus LR at different pH levels.** We compared NS and LR at an acidic pH and a neutral pH. Modification of the pH of the fluids was done by adding sodium bicarbonate. At an acidic pH, LR (pH 6.5) resulted in a markedly lower blood loss volume than NS (pH 5.0). At a neutral pH, LR (pH 7.32) significantly decreased blood loss volume in comparison to NS (pH 7.36). The experiments were conducted at room temperature. Values are displayed as mean ± SD. $P > 0.5$ (i.e., not significant) is not shown, $^*P < 0.0332$, $^{**}P < 0.0021$. LR, lactated Ringer's; NS, normal saline.

We sought an explanation for the differences and turned to the presence of Ca^{2+} in LR (Table 1). Ca^{2+} is an essential component of the intrinsic, extrinsic, and common coagulation pathways (25). Released from platelets, among other sources, it attaches to phospholipids, providing a surface for the binding of clotting factors (26). It also plays a major part in fibrin polymerization sites formation and stabilization, thus influencing all platelet-dependent functions (27). We studied the effect of NS augmentation with Ca^{2+}in both models. Supplementing NS with 2.7 mEq/L of Ca^{2+} was sufficient to decrease both bleeding time by 60.3% and blood loss volume by 16.1% to values similar to those of LR. Nonetheless, trying to elicit a dose-response relationship by supplementing NS and LR with additional Ca^{2+} in excess resulted in no difference, indicating a saturable process at least in these models (Fig. 2B).

Tranexamic acid, an antifibrinolytic that decreases bleeding by stabilizing the fibrin clot, is frequently utilized in the management of hemorrhagic emergencies (15,28,29). Some evidence suggests that topical TXA enhances bleeding control, reduces rebleeding, and ameliorates pain when used in the treatment of epistaxis (28). A systematic review and meta-analysis by Teoh et al. (30) found that surgical patients who received topical TXA had lower total blood loss, intraoperative bleeding, total drain output, and postoperative hemoglobin drop. Here, NS supplementation with TXA resulted in both a 67% decrease in bleeding time and a 16% decrease in blood loss volume, to values as low as those of LR. However, in a similar pattern to the studies above with Ca^{2+}, the addition of TXA to LR did not improve on the overall efficacy of LR on hemostasis in the tail vein bleeding model. Surprisingly, in the abdominal irrigation model, LR supplementation with TXA consistently overturned the positive hemostatic effect of LR, resulting in blood loss volumes similar to those of NS. This could be due to multiple untested variables, including chemical content changes resulting from mixing TXA with LR with unknown impacts on TXA action and effects, warranting further investigation.

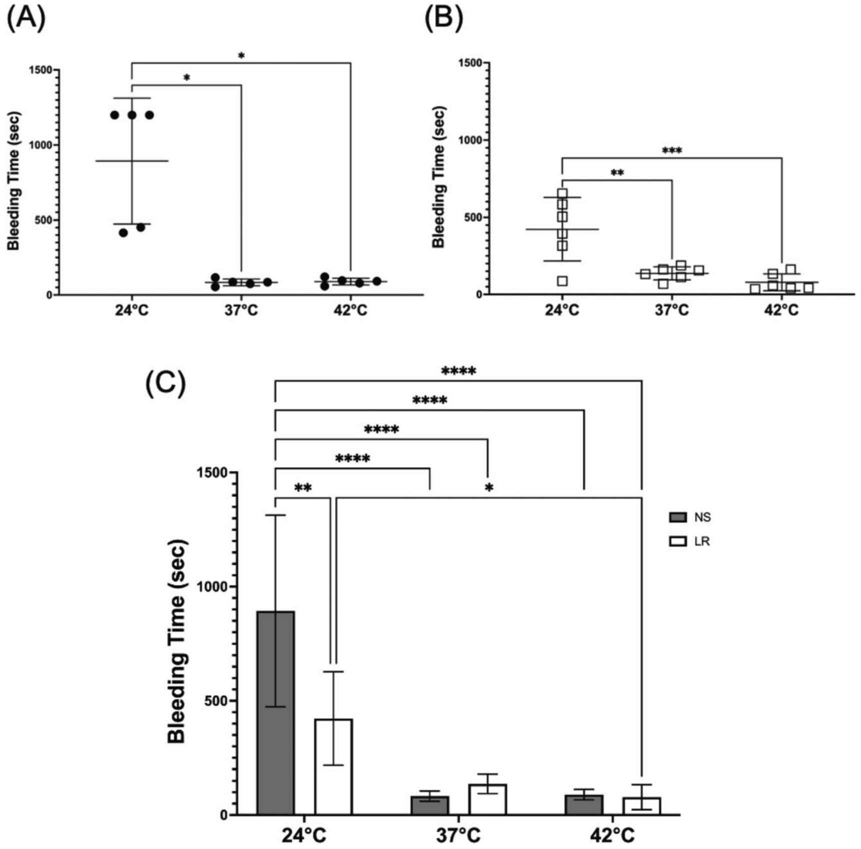

FIG. 4. **Bleeding time in a tail vein transection model with NS and LR at different temperatures.** Tail vein transection was done by a tail tip cut. The tail was then submerged in the irrigation fluid of choice, and the bleeding time was recorded. A, Bleeding times in NS at different temperatures. Increasing the temperature of NS to 37°C or 42°C resulted in a markedly reduced bleeding time than that of NS at 24°C. No significant differences were seen between NS at 37°C and 42°C. B, Bleeding times in LR at different temperatures. Similarly, bleeding times were also remarkably decreased in LR at 37°C and 42°C as compared with LR at 24°C, with no difference between LR at 37°C versus LR at 42°C. C, Bleeding times in NS and LR at different temperatures. There were no significant differences in hemostasis between NS and LR at 37°C and 42°C. Strikingly, the differences in values between LR 24°C and NS at 37°C or 42°C were insignificant. Values are displayed as mean ± SD. $P > 0.5$ (i.e., not significant) is not shown, $^{*}P < 0.0332$, $^{**}P < 0.0021$, $^{***}P < 0.0002$, $^{****}P < 0.0001$. LR, lactated Ringer's; NS, normal saline.

Administration of RT irrigation fluids can lead to hypothermia and its many adverse effects, and thus warming of fluids is common. Accordingly, we compared NS and LR at 24°C, 37°C, and 42°C. The results of our study were in congruence with findings of Javer et al. (3) that showed a lower blood loss rate when hot NS irrigation was used instead of RT NS during functional endoscopic sinus surgery. We demonstrated that increasing the temperature of NS or LR from 24°C to 37°C results in a significantly reduced tail vein bleeding time in mice, but no significant difference was detected when the temperature is increased from 37°C to 42°C (Fig. 4, A and B). The difference seen between NS and LR at RT is eliminated when these fluids are warmed (Fig. 4C). Warming the fluids normalized the choice of agents as there were no significant differences in hemostasis between NS and LR at 37°C and 42°C. The data support the established practice of using warmed fluids. However, it can be difficult to maintain the temperature of irrigation fluid in an operating room. Although NS and LR performed similarly at higher temperatures, the improved efficacy of LR at lower temperatures may support its use over NS. There is a decrease in the efficacy of both fluids as heat is lost to the surroundings. However, the magnitude of this change is different between fluid and important to consider. There is a 10.7-fold increase in bleeding time when NS cools from 42°C back to RT, whereas there is only a 5.4-fold increase in bleeding time when LR similarly cools. In most institutions, including our own, warmed NS is the standard of care for irrigation in the care of patients. However, heated fluids may rapidly cool in the environment of the operating room, and any delay in administration of fluids for irrigation likely results in some cooling. There are also situations in which access to resources is limited, and warming fluids might not be a feasible option, such as in combat or natural disasters. Furthermore, there are no standardized regulations, and each institution has its own standard of care for the temperature to which the irrigation fluid is warmed. Given the possibility of cooling with ambient exposure and the lack of an identified safety concern, LR irrigation could be considered because it results in lower bleeding times at RT and performs just as well as NS does when warmed. Clinical studies of the impact of NS versus LR irrigation in patients are warranted, and at least one study is underway in the endoscopic surgery (NCT05161780).

The present work has several limitations. Although results are validated for most groups in two models, the inherent limitations in sensitivity in each model may influence the ability to detect differences between treatment arms. Although both murine models have been previously validated with clinically relevant controls (18,31,32), the present results are unproven in humans. The majority of the experiments were performed in male mice to reduce animal usage; however, we did perform a confirmatory subset of experiments in female mice without major differences in the results (Supplemental Digital Content Fig. 3, http://links.lww.com/SHK/B531). In an attempt to reduce unnecessary animal use, only conditions that seemed to have potential to improve upon outcomes observed for LR in the tail vein bleeding model were further confirmed in a liver laceration model. Lactated Ringer's also differs from NS in its potassium content, and although seemingly unlikely to contribute to hemostasis in low concentrations, we did not explore the independent effects of potassium. A review of the literature before the conduction of the liver laceration experiments prompted us to change the TXA dose used to supplement fluids to 62.5 mg/mL (33). We did not repeat the tail vein transection experiments at that TXA concentration as the difference between 50 and 62.5 mg/mL is small, and we had already tested TXA at a higher dose of 150 mg/mL. Using RT irrigation fluids affects not only the temperature of the area of thrombus formation, but also the core body temperature. Intraoperative hypothermia is a common complication during surgery that is associated with higher morbidity and mortality (34) and increased rates of blood transfusion and coagulation disturbances (35). Although the core body temperature of the mice was not measured, we attempted to prevent hypothermia by using a heating pad in both of our models. Temperature and additives affect pH; however, we did not document the pH of fluids at the studied temperatures or after supplementation with additives. Lastly, as previously shown, irrigation fluids affect not only bleeding but also other surgical outcomes such as wound infection and healing. In this study, we did not investigate the effect that the irrigation fluids have on outcomes other than bleeding time. Further studies are needed to show how these fluids compare with the standard of care, and this may be relevant in fields, such as orthopedics, where the association of irrigation fluid with wound infection and delayed healing has been well investigated (6,7,9–11).

In summary, the lack of research on the properties of irrigation fluids used during surgery has hindered regulatory bodies from providing standardized regulations for intraoperative hemostatic irrigation, which is a common practice. In this study, we illustrate that, at RT, LR is superior to NS in terms of bleeding control, likely owing to its Ca^{2+} content. Although supplementing NS with Ca^{2+} or TXA reduces bleeding to values similar to those seen with LR, but not superior to those reported with warmed fluids, the preparation of a modified irrigation fluid requires added time and effort that cannot be properly justified in this case. Irrigation fluids are routinely warmed to decrease the risk of perioperative hypothermia, a step that also enhances their hemostatic ability. However, fluids often cool before use, decreasing their hemostatic efficiency as the temperature decreases. Taken together, our findings support the need for a prospective clinical trial comparing NS and LR, to further elucidate whether the same findings can be extended to humans.

Author Contribution

Conceptualization and study design: M.D.N., P.A.G., C.H.S.

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