Modulation of physiological hemostasis by irrigation solution: comparison of various irrigation solutions using a mouse brain surface bleeding model

Laboratory investigation

Yasutaka Fujita, M.S.,1 Kazuhisa Doi, M.S.,1 Daisuke Harada, Ph.D.,1 and Shuji Kamikawa, M.D., Ph.D.2

1Research and Development Center, Otsuka Pharmaceutical Factory, Inc., Naruto, Tokushima; and 2Kamikawa Clinic, Tokyo, Japan

Object: Intraoperative bleeding often obscures the surgical field and may cause neurological damage. The irrigation fluids used during surgery might affect physiological hemostasis because they modulate the extracellular fluid composition of the bleeding area directly. The authors therefore investigated the influence of irrigation fluid on hemostasis in a mouse brain surface bleeding model.

Methods: The cerebral cortices of ddY strain mice were exposed under irrigation with normal saline, lactated Ringer (LR) solution, or artificial CSF (ACF-95). To investigate the influence of electrolytes, calcium, potassium, or both were also added to the saline. After 10 minutes of irrigation at 100 ml/hour, sequential photographs of the surgical area were taken with a microscope, and the number of bleeding points was counted visually. Irrigation and counting were performed in a masked manner.

Results: There were significantly more bleeding points after irrigation with normal saline than with ACF-95; LR solution had a similar effect on physiological hemostasis as ACF-95. Saline augmented with calcium or potassium and calcium was superior to normal saline in terms of hemostasis.

Conclusions: The authors demonstrated that the irrigation fluid used in neurosurgery affects bleeding at the surgical site. To avoid surgical site bleeding, ACF-95 and LR solution should be used as irrigation fluids instead of normal saline. The calcium and potassium content of irrigation solutions seems to be important in hemostasis. (DOI: 10.3171/2009.7.JNS09561)

Key Words: • irrigation solution • intraoperative bleeding • hemostasis • normal saline • artificial cerebrospinal fluid • lactated Ringer solution

When bleeding occurs during surgical operations such as dissection and tumorectomy, it is arrested physiologically by blood coagulation and vasoconstriction of the bleeding vessel. Microneurosurgery requires clear fields of view, so continuous bleeding may obscure the target structures and risk tissue damage. Moreover, even a small amount of blood within the brain parenchyma may lead to permanent neurological damage or even death. Methods of hemostasis commonly used in neurosurgery include direct compression, ligation, and electric cauterization. Although hemostasis agents such as oxidized cellulose, gelatin sponge, and fibrin glue are available, there is a need for a faster and safer hemostatic method in brain surgery. Thus, it is clinically meaningful to achieve faster and more efficient physiological hemostasis.

Various irrigation agents, such as normal saline, LR solution, and artificial CSF, have been used in neurosurgery to prevent the brain surface from drying and to wash out blood clots. These fluids can change the composition of extracellular fluid, with possible effects on physiological hemostasis. In particular, the calcium concentration might be important in physiological hemostasis because it plays a key role in several steps of the coagulation cascade. However, the influence of irrigation solution on physiological hemostasis has not been clearly elucidated. We investigated the effects on hemostasis of various irrigation fluids in a mouse brain surface bleeding model.

Methods

This study was approved by the Otsuka Pharmaceutical Factory Committee on the Care and Use of Laboratory Animals and was conducted in accordance with...
Modulation of physiological hemostasis by irrigation

in-house guidelines that follow the Guide for the Care and Use of Laboratory Animals (US National Research Council).

Materials Used

We used commercially available forms of normal saline and LR (Otsuka Normal Saline and Lactec Injection, Otsuka Pharmaceutical Factory, Inc.). For artificial CSF, we used ACF-95 (Otsuka Pharmaceutical Factory, Inc.), which has a composition similar to that of physiological CSF (as reported by Davson and Milhorat) and similar to an artificial CSF reported by Elliott and Jasper. The ACF-95 is packaged in a dual-chamber bag: 1 chamber contains a glucose electrolyte solution incorporating sodium chloride, calcium chloride, and magnesium chloride; and the other contains an electrolyte solution incorporating sodium hydrogen carbonate, sodium chloride, potassium chloride, and potassium dihydrogen phosphate. The contents of the 2 chambers are mixed before use by opening the septum between the chambers. The Japanese Ministry of Health, Labor, and Welfare approved ACF-95 in 2007 as the medicinal product “ARTCEREB irrigation and perfusion solution for cerebrospinal surgery.” To investigate the influence of electrolytes, calcium-, potassium-, and calcium/potassium-augmented saline solutions were prepared. These were prepared by mixing 1.15 ml of 1 mEq/ml calcium chloride injection, 1.4 ml of 1 mEq/ml potassium chloride injection, or both with 500 ml of normal saline. The composition and pH levels of the solutions used in this study are shown in Table 1. These solutions were used for irrigation at room temperature (23.1–23.8°C, actual measurement).

Animal Preparation and Care

Male 9–10 week-old ddY strain mice (35.1–40.2 g, Japan SLC, Inc.) were used. The mice were housed in a light-controlled room (12-hour light-dark cycle, lights off at 19:00), with temperature (23 ± 3°C) and humidity (55 ± 15%) also controlled. The animals were allowed free access to food and water before the experiments.

Experimental Parts and Groups

This study had 4 parts. In Parts 1 and 2, we used a crossover experimental design to investigate the difference among test fluids. In Part 1, normal saline and ACF-95 were compared. Six animals were randomly allocated to each of 2 groups: 1 group underwent irrigation with ACF-95 and then with normal saline (AS group), while mice in the other group received irrigation solutions in the opposite order (SA group). In Part 2 of the experiment, LR solution and ACF-95 were compared. One group of 6 mice underwent irrigation first with ACF-95 and then with LR (AL group), while another group of 6 received the irrigation solutions in the opposite order (LA group). In Part 3, the influence of electrolytes was investigated. The animals were randomly allocated to 4 groups of 6 mice each, and irrigation with normal saline or augmented saline was performed. In Part 4, we observed the animals over the course of 60 minutes of irrigation with normal saline and with ACF-95 to assess longer-term effects.

Surgical Procedures

The mice were anesthetized via intraperitoneal injection of urethane (2 g/kg; Aldrich Chemical Company, Inc.), which is known to provide long periods of stable anesthesia and is often used in animal studies. Each animal was positioned in a stereotactic frame (SR-6N, Narishige Scientific Instrument Laboratory), and a scalpel was used to make an incision along the midsagittal line, exposing the parietal bone. A 2-mm-long silicone tube with a 6 mm internal diameter was attached to the left parietal bone to isolate a surgical area that could be adequately bathed in irrigation fluid. An ~ 2 × 4–mm opening was then made in the left parietal bone through the silicon tube. Irrigation via this opening using syringes containing test solution (predefined as indicated above) was started with a syringe pump at a rate of 150 ml/hour, which was found to be effective for removing most of the blood from the injured area. The dura and arachnoid mater were then removed from the opening, resulting in many bleeding points on the brain surface. The syringe pumping rate was then changed to 100 ml/hour, and this time point was des-

<table>
<thead>
<tr>
<th>Component or pH</th>
<th>ACF-95</th>
<th>LR</th>
<th>Normal Saline</th>
<th>Normal Saline w/ CaCl₂</th>
<th>Normal Saline w/ KCl</th>
<th>Normal Saline w/ CaCl₂ &amp; KCl</th>
</tr>
</thead>
<tbody>
<tr>
<td>Na⁺ (mEq/L)</td>
<td>145.4</td>
<td>130</td>
<td>154</td>
<td>154</td>
<td>154</td>
<td>154</td>
</tr>
<tr>
<td>K⁺ (mEq/L)</td>
<td>2.8</td>
<td>4</td>
<td>—</td>
<td>—</td>
<td>2.8</td>
<td>2.8</td>
</tr>
<tr>
<td>Mg²⁺ (mEq/L)</td>
<td>2.2</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Ca²⁺ (mEq/L)</td>
<td>2.3</td>
<td>3</td>
<td>—</td>
<td>2.3</td>
<td>—</td>
<td>2.3</td>
</tr>
<tr>
<td>Cl⁻ (mEq/L)</td>
<td>128.5</td>
<td>109</td>
<td>154</td>
<td>156</td>
<td>156</td>
<td>158</td>
</tr>
<tr>
<td>HCO₃⁻ (mEq/L)</td>
<td>23.1</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>lactate (mEq/L)</td>
<td>—</td>
<td>28</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>phosphorus (mmol/L)</td>
<td>1.1</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>glucose (g/L)</td>
<td>0.61</td>
<td></td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>pH</td>
<td>7.3</td>
<td>6.7</td>
<td>6.3</td>
<td>6.3</td>
<td>6.3</td>
<td>6.3</td>
</tr>
</tbody>
</table>
ignited the start of irrigation (0 minutes). During irrigation, the rats were kept warm with a feedback-controlled lamp and warming pad (ATB-110, Nihon Kohden Co.) to maintain a rectal temperature of ~ 37°C. The investigators were blinded to the solutions used for irrigation.

In Part 4, the right femoral artery was cannulated with a polyethylene tube filled with saline containing heparin (10 U/ml; Otsuka Pharmaceutical Factory Inc.) to monitor MABP.

**Measurement on Sequential Photographs**

In Parts 1 and 2, sequential photographs (28 frames in 10 seconds) were taken with a CCD camera (ORCA-ER, Hamamatsu Photonics K.K.) through a microscope 10 minutes after starting irrigation (Time Point 1). The sequential photographs were repeated 10 minutes after changing the irrigation solution (Time Point 2). In Part 3, sequential photographs were obtained 10 minutes after starting the irrigation, and in Part 4, sequential photographs were taken and MABP measured at 0, 10, 20, and 60 minutes after starting the irrigation.

The number of bleeding points was counted on the sequential photographs, with the investigator blinded to the solution used. All areas of bleeding noted on the brain surface during 10 seconds were counted as bleeding points.

**Statistical Analysis**

In Parts 1 and 2, statistical analysis was performed using the paired t-test to evaluate the differences within groups (Time Point 1 vs Time Point 2), and using the unpaired t-test to evaluate intergroup differences at each time point. For Part 3, statistical analysis was performed using the Dunnett multiple comparison test to evaluate differences with respect to the plain saline group. Differences between the calcium-augmented saline group and the calcium and potassium–augmented saline group were evaluated with the unpaired t-test. In Part 4, the difference between the normal saline group and the ACF-95 group was evaluated using the unpaired t-test. Differences were considered statistically significant at p < 0.05.

**Results**

**Part 1: Normal Saline Versus ACF-95**

To compare the effects on physiological hemostasis of normal saline and ACF-95, we observed the effects of these solutions on the number of bleeding points on the brain surface using a crossover design (Table 2, Fig. 1, and Videos 1–4).

**Table 2: Number of bleeding points at each time point according to solution group**

<table>
<thead>
<tr>
<th></th>
<th>Time Point 1</th>
<th>Time Point 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Test Solution</td>
<td>No. of Bleeding Points</td>
</tr>
<tr>
<td>AS†</td>
<td>ACF-95</td>
<td>2.2 ± 1.7</td>
</tr>
<tr>
<td>SA</td>
<td>saline</td>
<td>15.7 ± 3.4</td>
</tr>
<tr>
<td>AL‡</td>
<td>ACF-95</td>
<td>3.2 ± 2.4</td>
</tr>
<tr>
<td>LA</td>
<td>LR</td>
<td>2.7 ± 1.6</td>
</tr>
</tbody>
</table>

* Values are given as means ± SDs. Each group contained 6 mice.
† There were significant differences between saline and ACF-95 at Time Point 1 (p < 0.05, unpaired t-test), and between saline and ACF-95 in the AS or SA group (p < 0.05, paired t-test). There were no significant differences between ACF-95 and saline at Time Point 2 (unpaired t-test).
‡ There were no significant differences between LR and ACF-95 at the first or second time point (unpaired t-test). There were no significant differences between LR and ACF-95 in the AL or LA group (paired t-test).

**Video 1.** Video of an AS group mouse at Time Point 1 (irrigation with ACF-95). Click here to view with Windows Media Player.

**Video 2.** Video of an AS group mouse at Time Point 2 (normal saline). Click here to view with Windows Media Player.

**Video 3.** Video of an SA group mouse at Time Point 1 (irrigation with normal saline). Click here to view with Windows Media Player.

**Video 4.** Video of an SA group mouse at Time Point 2 (irrigation with ACF-95). Click here to view with Windows Media Player.

At Time Point 1, the number of bleeding points was significantly lower in the AS group than the SA group; that is, bleeding was better suppressed by irrigation with ACF-95 than with normal saline. Moreover, the number of bleeding points was significantly higher at Time Point 2 than Time Point 1 in the AS group, while the opposite pattern was evident in the SA group. This also indicated that ACF-95 suppressed bleeding more than normal sa-
Modulation of physiological hemostasis by irrigation

line. No significant differences were observed in the number of bleeding points between the AS and SA groups at Time Point 2.

Part 2: LR Solution Versus ACF-95

We compared ACF-95 and LR solution in the same manner as in Part 1 (Table 2). There was no significant difference in the number of bleeding points between the AL group and the LA group at each time point, or between Time Point 1 and Time Point 2 in each group. This indicates that LR solution and ACF-95 do not differ in their effect on bleeding.

Part 3: The Effects of Calcium and Potassium on Physiological Hemostasis

To determine the effects of calcium and potassium in irrigation fluid on physiological hemostasis, we evaluated the number of bleeding points after irrigation with normal saline, and with saline augmented with calcium, potassium, or both. There were significantly fewer bleeding points in the mice that received calcium-augmented saline (mean ± SD, 4.3 ± 1.6) and those that received saline augmented with both calcium and potassium (2.2 ± 1.5) than in mice that received only normal saline (14.0 ± 2.8; p < 0.05, Dunnett test). Mice that received saline augmented only with potassium had a mean 13.5 ± 3.9 bleeding points. There were significantly fewer bleeding points in the group irrigated with calcium and potassium—augmented saline than in those that received only calcium-augmented saline (p < 0.05, unpaired t-test).

Part 4: Comparison Between Normal Saline and ACF-95 Regarding Effects of Longer Irrigation

To test the effects of a longer irrigation period with ACF-95 and normal saline, the number of bleeding points was observed during 60 minutes of irrigation (Table 3). There were significantly fewer bleeding points in the ACF-95 group than in the normal saline group at each time point during the period.

Discussion

We developed a mouse model of brain surface bleeding to examine the effect of irrigation solution on intraoperative bleeding. We were able to induce bleeding easily from the brain surface by removing the arachnoid mater in the surgical area. Moreover, our methods of counting bleeding points on sequential photographs provided reproducible results. We therefore consider the animal model we present to be useful for investigating the influence of the irrigation solution on extent of intraoperative bleeding.

Our data demonstrated that the irrigation fluid used in neurosurgical procedures affects the extent of intraoperative bleeding at the injured site. There were significantly more bleeding points after irrigation with normal saline than when ACF-95 was used. On the other hand, no significant difference was seen in the number of bleeding points between mice that underwent irrigation with ACF-95 and LR solution. Unlike normal saline, both ACF-95 and LR solution contain calcium and potassium. As we expected, the electrolytes contained in the irrigation solution appear to affect physiological hemostasis. Adding calcium and potassium to normal saline reduced the number of bleeding points, with the addition of calcium being more effective than adding potassium.

Hemostasis is completed physiologically by the coagulation of blood and vasoconstriction. Calcium is essential for blood coagulation, as illustrated by the fact that EDTA, a calcium chelator, is used as an anticoagulant. It is also well known that for vasoconstriction to occur, calcium must act as a second messenger to cause contractions of vascular smooth muscle. Our findings therefore seem reasonable: calcium ions should be included in irrigation fluids to accelerate physiological hemostasis. In addition, there were significantly fewer bleeding points in the mice that received saline with potassium and calcium than in the mice that received saline augmented with calcium only. A low potassium concentration in the extracellular fluid increases membrane potential gradient, possibly weakening the vasoconstriction response to hemorrhage.

In the prolonged irrigation test, there were significantly fewer bleeding points in the ACF-95 group than in the normal saline group at each time point up to 60 minutes. Because we used irrigation solution while removing arachnoid mater, a difference between normal saline and ACF-95 was observed even at Time Point 0 (Table 3). It was impossible to count the number of bleeding points without irrigation solution because visualization of the site was impaired by hemorrhage. Therefore, we do not have data on the baseline number of bleeding points that would occur in the absence of any irrigation solution. However, to maintain objectivity, the surgeon and investigator were blinded to which irrigation solution they were using.
dition, particularly MABP, might cause a difference in bleeding between tested groups. However, we confirmed that the MABP in the saline group was similar to that in the ACF-95 group for the 60-minute observation period. It therefore appears that the test solution did not affect general physical condition. Our findings suggest that irrigation with normal saline could lead to continuous bleeding during the irrigation period, and that an irrigation solution containing calcium and potassium should be used instead during neurosurgical procedures.

Problematic bleeding occasionally occurs during surgery. Continuous bleeding obscures the surgical field, impeding the clear field of vision required in microneurosurgery. Moreover, blood within the brain parenchyma can cause neurological damage. Because bleeding is usually controlled with irrigation in endoscopic neurosurgical procedures, it is clinically meaningful to use an irrigation solution that contributes to physiological hemostasis. Our results in the present study suggest that the use of ACF-95 or LR solution is more effective in achieving physiological hemostasis than is normal saline; however, this probably also applies to other irrigation fluids containing calcium or potassium. Although bleeding from other arterial or venous sources may behave in the same manner as bleeding from the cortical surface of the mouse brain, this remains to be determined. The authors of clinical and animal studies have shown the importance of using artificial CSF with a physiological composition in neurosurgical irrigation. Oka et al. reported that after endoscopic neurosurgery for symptomatic aqueductal stenosis, patients who received normal saline as a perfusion fluid developed headaches, high fever, and neck stiffness, but that those who received artificial CSF experienced only a slight fever. Elliot and Jasper suggested that the composition of irrigation fluid is a factor related to the development of brain edema when the cortex is exposed to various fluids. Enomoto and colleagues reported that incubation of cultured human astrocytes with growth medium for 24 hours after exposure to saline solution for 30 minutes induced apoptotic cell death; in contrast, exposure to ACF-95 was associated with little induction of apoptotic cell death. Uchida et al. compared the influence of several solutions on mitochondrial activity (assessed using rhodamine 123 uptake) on primary cultured rat neurons and astrocytes. They reported a decrease in mitochondrial activity in the LR group and normal saline group for both cell types, and a dramatic deterioration induced by normal saline in the cultured astrocytes. In that study, no significant differences were seen in mitochondrial activity between a control (culture medium) group and an artificial CSF group for both cell cultures. In addition, we previously reported that the use of ACF-95 reduced postoperative brain edema, cerebrovascular permeability, and cellular damage compared with normal saline and LR in sites injured by experimental neurosurgery in rats. Artificial CSF with physiological composition is therefore considered a more useful irrigation fluid in neurosurgery than normal saline or LR in maintaining brain cell function and reducing the incidence of adverse events postoperatively.

**Conclusions**

Our findings demonstrated that the irrigation fluid used in neurosurgery affects intraoperative bleeding at the injured site. We suggest that normal saline should not be used as an irrigation fluid in the brain, as it may prevent physiological hemostasis. Irrigation with ACF-95 and LR solution seems to be more useful in the prevention of intraoperative bleeding, and the calcium and potassium contained in these solutions seem to play key roles.

**Disclosure**

Yasutaka Fujita, Kazuhisa Doi, and Daisuke Harada are employees of Otsuka Pharmaceutical Factory, Inc. This work was supported by Otsuka Pharmaceutical Factory, Inc.

**Acknowledgment**

The authors thank Mr. Yoshishide Tokuda for technical support.

**References**


Supplemental online information:


**Address correspondence to:** Kazuhisa Doi, M.S., Research and Development Center, Otsuka Pharmaceutical Factory, Inc., 115 Tateiwa Naruto, Tokushima 7728601, Japan. email: doi@otsuka.kj.co.jp


Accepted July 14, 2009.

Please include this information when citing this paper: published online August 7, 2009; DOI: 10.3171/2009.7.JNS09561.