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Importance of Hydrostatic Pressure and Irrigation for Hemostasis in Neuroendoscopic Surgery

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Abstract

Recently neurosurgical operations have been carried out with water irrigation such as endoscopic third ventriculostomy and tumor resections in ventricles. Water irrigation is one of several published methods that promote hemostasis; however, not enough experimental evidence exists on its efficacy. In this study, we investigate whether hydrostatic pressure and persistent irrigation promote hemostasis in neuroendoscopic surgery. We dissected tails of 12–16-week-old C57BL/6 male mice at 5 mm proximal from the tip and checked for bleeding times under dry and wet conditions at pressures of 0 cmH_2O, 10 cmH_2O, 15 H_2O, and 20 cmH_2O without persistent irrigation to bleeding point and 10 cmH_2O with persistent irrigation. We then examined the dissected edge with hematoxylin–eosin staining and measured the size of vessels. The average bleeding time of each group is as follows: dry: 203.4 sec, wet: 164.4 sec, 5 cmH_2O: 138.6 sec, 10 cmH_2O: 104.6 sec ($P < 0.001$), 20 cmH_2O: 56 sec ($P < 0.001$), and 10 cmH_2O with persistent irrigation: 72.8 sec ($P < 0.01$ compared to 10 cmH_2O without persistent irrigation). The maximum caliber of mice's tail artery was 50–60 μm. Hydrostatic pressure and irrigation are important factors contributing to hemostasis.

Keywords: hydrostatic pressure, irrigation, hemostasis, wet field operation

Introduction

Endoscopy has recently gained considerable prominence in the field of neurosurgery. Its biggest advantage is that it allows a surgeon to get a much wider and closer view of the object. A wet field operation is an endoscopic procedure performed with abundant water or saline buffer irrigation. Wet field operations have been successfully carried out for intraventricular and large cystic tumors.[1,2] Irrigation fluids can enlarge, without a need for a brain retractor, the ventricles or excretory cavities that often shrink during the later stages of an operation owing to normal brain pressure. Under wet conditions, surgeons can easily identify the residual tumors behind the ventricle walls or within a cyst; such conditions have been commonly utilized in urology and orthopedic surgeries such as cystoscopy and arthroscopy, and studies report increased efficiency,[3] better visualization,[4,5] and minimal blood loss[6] with the use of wet conditions.

Hemostasis is a major concern in surgery, and several coagulation systems have been developed. Some endoscopic experts recommend that bleeding during a wet field operation should only be stopped using water irrigation.[7,8] However, certain chemicals in the irrigation water may facilitate hemostasis. Kozuma et al. reported that sodium bicarbonate facilitates hemostasis in the presence of cerebrospinal fluid (CSF) through the amplification of platelet aggregation.[9] Calcium and magnesium ions are thought to further contribute to hemostasis.[10,11] While in agreement with these studies, we believe that an additional important mechanism, physiological pressure, may contribute to hemostasis in surgery. As demonstrated by Wang et al., intraoperative bleeding can be controlled by the application of water pressure at 40–50 cmH_2O for 2 min.[12] This pressure appears to be quite high compared to the normal intracranial pressure and increases the risk to the brain during surgery. We therefore think it is necessary to consider lower hydrostatic pressure and its effect on

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hemostasis. We examined whether persistent irrigation to the bleeding point promotes or inhibits hemostasis, especially because we often irrigate the bleeding point to get a clearer view under wet conditions. To date, very few studies have reported the relationship between hydrostatic pressure and irrigation and their effect on hemostasis efficiency.

Methods

There has been a report about the effect of irrigation contents to hemostasis on the mice's brain.[11] However, in that model, it is impossible to apply hydrostatic pressure to the bleeding point. Besides, to date, there have been no experimental model to verify the hemostatic effect to any tissues by hydrostatic pressure and irrigation, and we constructed a novel following experiment model.

Surgical conditions and procedure

We measured bleeding times under dry and wet conditions at water pressures of 0 cmH_2O, 5 cmH_2O, 10 cmH_2O, and 20 cmH_2O without persistent irrigation to bleeding point and at a water pressure of 10 cmH_2O with persistent irrigation. By comparing bleeding times without irrigation groups (dry, wet, 10 cmH_2O, and 20 cmH_2O), we tried to clarify the effect of hydrostatic pressure on hemostasis. Besides by comparing bleeding times of 10 cmH_2O without persistent irrigation groups, we intended to clarify the effect of persistent irrigation on hemostasis. We chose 10 cmH_2O groups for irritation experiment, because the lengths of tubular retractors, we usually use, are 9 cm (Neuroport mini; Olympus, Tokyo, Japan) and 12 cm (Neuroport) and then the pressure of 10 cmH_2O is easily available. Besides, we intended to minimize the number of sacrifice mice and then we focused on 10 cmH_2O for persistent irrigation experiment.

We used 12–16-week-old C57BL/6 male mice in this study. Each experiment group included seven mice, and the mice with the longest and shortest bleeding times from each group were excluded from analysis, in order to avoid excessive influence due to possible outliers.

Mice were anesthetized with a mixture of medetomidine hydrochloride, midazolam, and butorphanol tartrate at 0.3 mg/kg, 4 mg/kg, and 5 mg/kg, respectively. Animals were placed in a prone position and kept at 38°C. Blood pressure and heart rate were measured using a sphygmomanometer BP-98A-L (Softron, Tokyo, Japan), before cutting the tail and after the hemostasis. To avoid any possible effects of deep anesthesia on hemostasis, we eliminated mice with low blood pressure (mean blood pressure <60 mmHg) from our study. The tails were cut at a distal point 5 mm from the tip with Mayo scissors. Previous reports on hemostasis and bleeding time of mice's tail involved cutting points at 5 mm[9] or 10 mm[13] from the tip of the tail. In our preliminary experiment, some mice whose tails were cut at 10 mm from the tip suffered from hypovolemic shock; therefore, we only included mice with tails cut at 5 mm from the tip. None of the mice reported in this study suffered from shock.

Bleeding time of the dry group was checked by placing blood on KimWipes (Kimberly Clark, Neenah, WI, USA). The tails from the other groups, i.e., wet conditions at a pressure of 0 cmH_2O, 5 cmH_2O, 10 cmH_2O (with and without persistent irrigation), or 20 cmH_2O, were immediately immersed into a tube containing artificial cerebrospinal fluid (aCSF; Artcereb; Otsuka Pharmaceutical, Tokyo, Japan), which was warmed to 37°C. The tail was inserted horizontally into the lowest port of the tube, which was placed 20 cm below the top of the vertical tube. Blood flowed down to the bottom (Fig. 1). In the 5, 10, and 20 cmH_2O groups, after the insertion of tail into the port, aCSF was poured into the tube to the level above the port in which the tail was inserted. Especially in the persistent irrigation group, the tip of irrigation tube (18 gauge) was fixed 1 cm above the mice tail and irrigation was kept at 60 ml/min.

Bleeding time was determined using a stopwatch. Inserting the tail into the port took about 10 sec and pouring up to 5–20 cm of aCSF took another 10 sec on average. The resulting times reported account for this manipulation time.

Histological analysis

The dissected edges of the mouse tails were evaluated using hematoxylin and eosin staining for two groups: the dry and 10 cmH_2O with persistent irrigation groups. The maximum diameter of blood vessels at 5 mm proximal to the tail tip was also measured.

Statistical analysis

Statistical analysis was performed by using Excel software (Microsoft, Seattle, WA, USA). The data were expressed as mean ± standard deviation values. Probability values for differences between two measured responses were obtained using the *t* test, and those below 0.05 were considered as significant.

Result

Bleeding time and blood pressure data are depicted in Tables 1 and 2 and Figs. 2 and 3. Significant

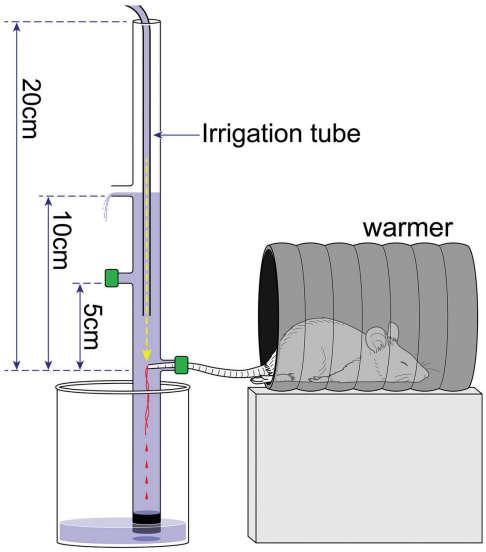

Fig. 1 A schema of experimental condition. A mouse under anesthesia was placed in a prone position and kept warmer (37°C), and then, its tail was cut at 5 mm from the tip. The tail was immediately immersed in the lowest port, and then, aCSF was poured into the tube to the target level. A tail of mouse was not completely wedged to the port. Instead, the tail was partially coved the soft cray, which prevented water leaking from the gap as shown in the figure. Bleeding from the tail flew downward according to gravity. Besides, in the case of a group with persistent irrigation, an irrigation tube was inserted at 1 cm above mouse's tail as shown in the figure, and then, irrigation was started. Excess water was discharged from the port 10 cm above the lowest port. In cases of group without persistent irrigation, this manipulation was not done. aCSF: artificial cerebrospinal fluid

differences were observed between the 5, 10, and 20 cmH_2O and dry condition groups (P = 0.019, 0.0013, and 0.000066, respectively). Diastolic blood pressure and mean blood pressure in the 5 cmH_2O group were significantly higher than those in the dry group, while the other groups showed comparatively similar blood pressures (Table 1). The persistent irrigation group showed a significantly shorter bleeding time than the no persistent irrigation group (P = 0.0096), and no significance was observed in blood pressure between these groups (Table 2).

Maximum arterial diameter in the dry and 10 cmH_2O with persistent irrigation groups ranged 39.64–55.5 μm and 50–66.16 μm, respectively. Maximum venous caliber in the dry and 10 cmH_2O groups ranged 50–100 μm and 38.1–81.5 μm, respectively (Fig. 2).

Discussion

Hemostasis is an important factor to consider in all surgeries. Traditional and reliable methods of hemostasis include pressing, ligation, and coagulation. For endoscopic surgeries, many such methods have been developed and are utilized. In neurosurgery, however, we sometimes encounter bleeding from a point where pressing or using coagulating factors is not possible, especially in areas such as the tissue around the fornix, hypothalamus, and pyramidal tract.

In this study, we show that high hydrostatic pressure decreases bleeding time; even the 5 cmH_2O group had a much shorter bleeding time than the dry conditions group. In an actual operation, this hemostatic effect increases with the hydrostatic pressure. The mechanism behind it is remarkably simple: bleeding does not occur when the pressure around the injured vessels is higher than the blood pressure of the injured vessels themselves. Even when the hydrostatic pressure was much lower than the blood pressure, decreasing the pressure difference is assumed to reduce the bleeding flow rate of bleeding, which makes it easier to stop the bleeding.

Cerebral tissue is especially vulnerable, and excess pressure might damage the surrounding brain tissue. We believe that hydrostatic pressure should be equal or similar to the intracranial pressure (30–180 mmH_2O) for efficient hemostasis. Besides, a pressure of 30–180 mmH_2O can be reached under wet field operation conditions by using tubular retractors currently available for brain surgery. These retractors come in several sizes, ranging from 3 to 12 cm in length, and allow for variable hydrostatic pressure owing to the differing amounts of water (or aCSF during the operation) in them. In intraventricular tumor resections, there is an increased distance between the edge of the tube retractors and the resection site, which allows for a higher hydrostatic pressure.

Blood pressure in veins and capillaries is sufficiently low (0–10 cmH_2O and 15 cmH_2O, respectively), which is to be stopped by hydrostatic pressure alone using tubular retractors. This of course excludes the unusual cases of high venous pressure brought on by lesions having an arteriovenous shunt or a high congestive state brought on by severe sinus thrombosis. In addition, actual venous pressure in the operative site depends on the head position, and most neurosurgeons position the patient's head to be slightly upright in order to reduce the venous and intracranial pressure.

Table 1 Difference of bleeding time by hydrostatic pressure

	Dry	Wet (0 cmH_2O)	5 cmH_2O	10 cmH_2O	20 cmH_2O
Bleeding time					
Mean (sec)	203.4 (43.3)	164.4 (59.5)	138.6 (24)	104.6 (14.6)	55.2 (6.9)
Median (sec)	190	175	130	100	56
P value (compare to dry)		0.27	0.019	0.0013	0.000066
P value (compare to wet)			0.39	0.06	0.0036
Pre-cut blood pressure					
Systolic (mmHg)	110.8 (13.2)	99.6 (11.0)	118.2 (5.7)	110.2 (15.6)	104.8 (16.9)
Diastolic (mmHg)	66.6 (6.4)	58 (11.6)	80* (7.2)	67.6 (5.5)	66.2 (12.9)
Mean (mmHg)	81.4 (8.0)	71.4 (7.8)	92.8* (6.8)	81.8 (7.9)	79.2 (13.8)
Post-cut blood pressure					
Systolic (mmHg)	115.4 (9.4)	103.2 (14.7)	124.4 (10.1)	104 (12.4)	97.6 (22.7)
Diastolic (mmHg)	63.4 (12.5)	61 (7.5)	78 (8.1)	66.5 (4.2)	65.2 (12.8)
Mean (mmHg)	81.2 (10.2)	79.2 (12.5)	93.8 (7.0)	81.2 (10.1)	75.2 (15.8)

*P <0.05 (comparing to dry condition), (): standard deviation.

Table 2 Comparison between bleeding times without irrigation

	Without irrigation (10 cmH_2O)	With irrigation (10 cmH_2O)
Bleeding time		
Mean (sec)	104.6 (14.6)	72.8 (15)
Median (sec)	100	70
P value		0.0096
Pre-cut BP		
Systolic (mmHg)	110.2 (15.6)	123.8 (8.5)
Diastolic (mmHg)	67.6 (5.5)	70 (10.4)
Mean (mmHg)	81.8 (7.9)	88 (8.2)
Post-cut BP		
Systolic (mmHg)	104 (12.4)	120.6 (24.1)
Diastolic (mmHg)	66.5 (4.2)	71.6 (15.7)
Mean (mmHg)	81.2 (10.1)	90 (14.7)

BP: blood pressure, (): standard deviation.

Another major consideration is that massive bleeding from main trunks of the cerebral arteries cannot be stopped by hemostatic pressure supplied by water in tubular retractors. However, in this study, we verified that hemostatic pressure supplied by water in tubular retractors did indeed stop bleeding from 60-μm diameter arteries of mouse tails. This arterial diameter is similar to the diameters of the smallest cerebral perforators or most peripheral cerebral arteries. Marinkovic et al. have further reported precise data on cerebral perforators (Table 3).[14–21] We believe that hydrostatic pressure can stop bleeding from small perforators. Moreover, hydrostatic pressure may be able to induce hemostasis of slightly larger perforators in humans, since humans have greater blood volume than mice. Although some mice suffered shock when their tail was cut at 1 cm from the tip; the equivalent amount of blood loss is trivial for human beings. The whole blood volume in mice is thought to be only several milliliters, which accounts for 1/1000 of the human blood volume. Therefore, if bleeding increases by several milliliters, hemostasis may be possible without shock.

On the other hand, it is unknown whether similar 60-μm arteries have a similar blood pressure in different species and different tissues. In this study, blood pressures at the root of the tail in mice under anesthesia were similar to those of humans under general anesthesia. The correct blood pressure at the bleeding point, however, was not measured in this study, and the blood pressure of individual peripheral cerebral arteries or perforators is currently unreported in the literature. These two points may be a topic of research in future.

A biochemical mechanism may also underlie hemostasis under wet conditions. In this study, we did not conduct any biochemical analyses; however, some researchers have reported on this subject. Amelot showed that dilution of plasma, especially of antithrombin, is important for hemostasis.[22] *In vitro*, although thrombin generation is strongly affected by plasma dilution, anticoagulant pathways are the most affected by dilution.[23] On

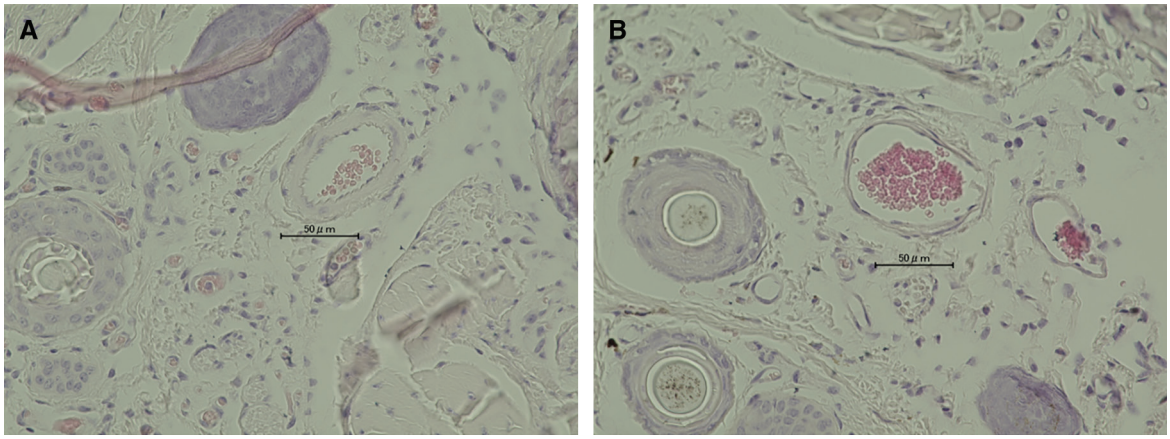

Fig. 2 Specimen of mouse tail after hemostasis. Hematoxylin and eosin staining.

Table 3 Diameters of cerebral perforators in literature

Author	Ref	Location of small branches	Diameter Minimum (μm)	Diameter Maximum (μm)	Diameter Mean (μm)
Marinković et al.	19	Anterior communicating artery	70	270	151 ± 49
		ACA small perforating branch	80	200	122
		ACA large perforating branch	210	710	325
	16	Heubner terminal stem	180	850	462
		Collateral branches of intracerebral segment of Heubner artery			
		Small branch	50	120	86
		Large branch	130	290	199
	18	M1 perforators proximal stem			
		Lateral branch	120	840	510
		Medial branch	90	480	280
	18	M1 perforators distal stem			
		Lateral branch	115	800	470
		Medial branch	80	470	270
	15	ICA perforators	70	470	243
	14	Vertebral artery	210	520	338
Djulejić et al.	21	Lenticulostriate artery	100	1280	480
Milisavlijević et al.	17	Thalamogeniculate artery	70	580	345.8 ± 92
Pedroza et al.	20	Premamillary artery branches			
		Cerebral peduncles			200 ± 30
		Sulcus			200 ± 100
		Paramedian perforated substance			200 ± 40

ACA: anterior cerebral artery, ICA: internal carotid artery, ref: reference number.

the other hand, Kozuma et al. demonstrated that sodium bicarbonate in CSF (and aCSF) facilitates hemostasis through the amplification of platelet aggregation.[9]

The effect of direct irrigation on the bleeding point was also shown to be an effective method for hemostasis possibly in this study. Although the mechanism has not been revealed, we assume it is

because of the amplification of aforementioned biological mechanism, i.e., increase of pressure to the bleeding vessels, dilution of antithrombin, and constant supply of sodium bicarbonate.

Limitations

As mentioned above, the blood pressure at the bleeding point was unknown. Ideally, the arterial, capillary, or venous pressures at the dissected edges would be measured directly and their relationship to the hydrostatic pressure would be evaluated. However, such measurement of capillaries is quite difficult and requires special devices that need direct puncture by a needle. Moreover, accurate measurement of blood pressure of normal human cerebral perforators, arterioles, or major arteries is impossible using such a method.

The next limitation is that the coagulation ability of each mouse is unknown. In this study, we did not measure coagulation ability and amount of hemorrhage. It is also considered that the mice with a long bleeding time were in a situation where the bleeding was more likely to be prolonged due to disseminated intravascular coagulation (DIC) accompanied by an excessive bleeding amount. These are challenges that could possibly be addressed in the future.

Conclusion

Under wet conditions, hydrostatic pressure is an important factor for hemostasis, and direct irrigation to the bleeding point can shorten bleeding time.

Ethical Approval

All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. All procedures performed in studies involving animals were in accordance with the ethical standards of Nagoya University Animal Experiment Committee. The approval number was Nagoya University Animal Experiment Committee 30137.

Conflict of Interest Disclosure

All authors certify that they have no affiliations with or involvement in any organization or entity with any financial interest (such as honoraria; educational grants; participation in speakers' bureaus; membership, employment, consultancies, stock ownership, or other equity interest; and expert testimony or patent-licensing arrangements) or non-financial interest (such as personal or professional relationships, affiliations, knowledge or beliefs) in the subject matter or materials discussed in this manuscript.

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