Technical Notes & Surgical Techniques

Evacuation of intracerebral hemorrhages by neuroendoscopy with transparent sheath. Experimental study

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A R T I C L E  I N F O

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A B S T R A C T

Objectives: Endoscopic evacuation of intracerebral hemorrhage (ICH) has been developed in order to reduce the tissue injury that conventional craniotomy could generate. Experimental studies are important to assess the effectiveness of the technique and its modifications. The objectives of this study are to develop in pig an experimental model of endoscopic evacuation of ICHs, to assess effectiveness of surgical evacuation, and to evaluate a new transparent sheath as complement to the endoscopy. Methods: Autologous blood was infused into the frontal lobe white matter in 16 pigs. In the problem group, endoscopic evacuation was performed with the aid of a new transparent sheath, which has outer and inner sheaths with blunt and closed fields. Pigs were sacrificed at 4 h, 24 h and 5 days. The volumes of hematoma and histopathological features were determined. Results: Residual volume of the problem group was significantly 70.09% lower than in control group, without significant differences in injected volumes, in percentage of subarachnoid hemorrhage, and in time interval from hematoma induction to pig’s death. The vital reaction after hemorrhage was similar in both groups. Conclusions: The experimental model developed is useful to assess endoscopic evacuation of ICHs. The endoscopy is an effective technique in the treatment of ICHs, without increasing the vital reaction secondary to hematoma. The new transparent sheath increases the visualization of surgical field and allows a continuous visual control since the beginning of the procedure. Its closed final prevents unwanted injury of the brain by the instruments used to remove the hematoma.

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Introduction

The annual incidence of spontaneous intracerebral hemorrhage (ICH) is between 10 and 23 cases per 100,000 person-years [1]. ICH is characterized by high mortality and disability [2].

The main benefits of surgical intervention are to decrease the toxic effects of blood and plasma products, limit the mechanical compression of brain, decrease the intracranial hypertension, and prevent hematoma expansion [3–5]. However, surgery has not demonstrated significant clinical benefit along several prospective randomized controlled trials [4,6–11].

Conventional craniotomy is frequently associated with additional brain tissue injury. In order to reduce this tissue damage, minimally invasive techniques, such as endoscopic evacuation and stereotactic aspiration with fibrinolysis, have been developed [11].

Experimental studies are very important to assess the effectiveness of the technique and its modifications. Although the pig has been chosen as an experimental model to generate ICHs, we have not found in the literature studies about endoscopic evacuation of ICHs using the pig as experimental animal.

The objectives of this study were to develop in the pig an experimental model of endoscopic evacuation of ICHs; assess effectiveness of evacuation surgical of ICHs; and evaluate a new transparent sheath manufactured by our team as complement to the endoscopy.

Methods

Animal preparation

This study was approved by the Committee for Animal Research of the University of Salamanca, Spain. Sixteen pigs weighing 17 and 55 kg were used. Twelve hours before the start of the experiments the animals were fasted, with only water provided ad libitum.

The pigs were sedated using intramuscular diazepam (1 mg/kg), atropine (0.05 mg/kg) and ketamine (20 mg/kg). Following this sedation, a dorsal ear vein was cannulated, through which intravenous anesthetic
drugs were administrated. For anesthetic induction, an intravenous bolus of propofol (2 mg/kg) was employed. The trachea of each animal was intubated. Then, the pigs were connected to a ventilator (K-Takaoka 1.04; K Takaoka Ind e Com, Sao Paulo, Brazil) and the following parameters were established: tidal volume, 8 ml/kg; inspiration–expiration rate 1:2; respiratory rate, 12 per minute; maximum pressure, 20 cm H2O; and inspiration pressure, 1 cm H2O. Maintenance of anesthesia was provided by intravenous propofol (10 ml/kg/h). Furthermore, experimental animals received intravenous fentanyl (2 g/kg/h) and intravenous mivacurium chloride (1 mg/kg/h). Thereafter, in the inguinal region, a femoral artery was cannulated to draw blood to induce ICH.

ICH model

All surgical procedures were performed under aseptic conditions. A cranial burr hole (14 mm) was drilled 15 mm to the right of midline and in projection line of lateral eye epicanthus (Fig. 1). Then, between 7 and 15 cc of blood was extracted from femoral artery and was injected through 20-gauge plastic catheter into the right frontal lobe, to a depth of two centimeters.

Two experimental groups were established: control and problem. Six pigs, in which evacuation was not performed, constituted the control group, while ten pigs, in which endoscopic evacuation was performed, constituted the problem group. Pigs were sacrificed at 4 h (6 animals), 24 h (n = 6) and five days (n = 4) after ICH induction by increasing the dose of propofol and administering potassium chloride.

Endoscopic evacuation. Instrumentation and surgical technique

In pigs that were sacrificed at 4 h, evacuation was performed at 2 h after blood infusion, while in the other pigs evacuation was performed at 12 h. To endoscopic evacuation, we used a 30° rigid endoscope with an outer diameter of 4 mm and 180 mm in length (Hopkins II, Karl Storz GmbH & Co, Tuttingen, Germany). An 18-gauge metal catheter attached to a vacuum system was used to aspirate the hematoma.

We developed a transparent glass sheath (outer sheath) that has 100 mm in length and outer and inner diameters of 10 mm and 8 mm, respectively. The end of this sheath is blunt and closed. Furthermore, the sheath has a lateral perforation to allow passage of aspirated hematoma (Fig. 2).

Other sheath (inner sheath) was developed to serve as a corridor for the endoscope. Its length is 110 mm and its outer and inner diameters are 6 mm and 5 mm, respectively. The end of the sheath is blunt closed, too (Fig. 2). Inner sheath (along with endoscope) is introduced into outer sheath. The corridor between these sheaths is used to introduce suction catheter (Fig. 3).

Through the burr hole, outer sheath was inserted. Then, inner sheath and endoscope were introduced into outer sheath. The entire system was advanced, seeing the border between normal brain and hematoma. Rotating outer sheath, lateral perforation was settled in the chosen suction area, and the hematoma was removed from depth to surface with suction catheter (Fig. 4). The direction and depth of the endoscope were changed many times to inspect all angles of the hematoma cavity, searching residues of hematoma. Hemostasis methods were not necessary.

Histopathological examination

Pigs were sacrificed by administration of intravenous potassium chloride. Intact brains were removed and were fixed in 2% formalin for 7 days (Fig. 5). The formalin-fixed brains were cut into 5-mm-thick coronal slices using a bandsaw (Fig. 6). For estimate of the approximate residual hematoma volume, the following formula was used: long diameter (A) × short diameter (B) × number of coronal brain slices with hemorrhage × slice thickness (C); this product was divided by 2 [12]. The three diameters were measured using the millimeter scale and the residual volume was measured in cubic millimeters.

Brain slices containing hematoma were embedded in paraffin, cut into 5-μm slices, and stained with hematoxylin and eosin (H & E). Histopathological changes and cell morphology and typology were studied.

Statistical analysis

The continuous variables were presented as means, medians, and range, with minimum and maximum. Furthermore, residual volumes
were expressed with quartiles. Discrete variables were expressed as percentage. Group comparisons for continuous variables were performed using Mann–Whitney U test, and for discrete variables Fisher exact test was used. A value of $p < 0.05$ was considered as statistically significant. SPSS 19.0 statistical software (SPSS Institute Inc., Chicago, IL, USA) was used for statistical analyses.

### Results

General data of each pig are presented in Table 1. In two pigs (011 and 018), hemorrhage was not detected, although intracerebral cavity was observed. In other animals, hemorrhage was localized in the white matter in 100% of the cases. Furthermore, in seven pigs (001, 005, 007, 009, 010, 012, and 017), hemorrhage was extended to the subarachnoid space (50% of the cases); in four pigs (001, 007, 014, and 017), to the caudate nucleus (28.57%); in two (007, and 017), to ventricular cavity (14.28%); in two (012, and 017), to cortex; and one (019), to the thalamus (7.14%).

### Hematoma volume

There were no significant differences in injected volume between both groups were significant ($p = 0.14$). Furthermore, respect to time interval from injection of hematoma to sacrifice of the animals, there were also no significant differences between groups ($p = 0.91$) (Table 2).

In pigs with subarachnoid hemorrhage, the mean and median of injected hematoma volume were 11.42 cc and 10 cc, respectively. In animals without subarachnoid extension, the mean and median were 11.64 cc and 10 cc. Also, differences were not significant ($p = 0.95$). 66.67% of control group and 37.50% of problem group had subarachnoid hemorrhage. There were no significant differences ($p = 0.59$).

With respect to residual volume in both groups, differences were statistically significant ($p = 0.008$) (Table 2). Residual volume of the problem group was 70.09% lower than in control group (Fig. 7).

Furthermore, comparing pigs whose endoscopic evacuation was performed at 2 h and 12 h with the corresponding group control (ie, on the one hand, pigs were sacrificed at 4 h, and the other hand, at 24 h and 5 days), residual volumes were lower at both times of endoscopic evacuation (Fig. 8).

A pig of the problem group (#007) had elevated residual volume. In this animal, we endoscopically checked if evacuation was adequate, so we think a hemorrhage was presented as a possible complication of procedure.
Histopathological findings

Control group

In pigs whose deaths were induced at 4 h, we observed scarce leukocytes in perihematoma area (Fig. 9a). At 24 h, more vital reaction appeared, with hemorrhage interspersed with neuropil and ballooning of astrocytes (Fig. 9b). At 5 days, an intense vital reaction was observed. A well-defined separation between hematoma and neuropil was identified. In perihematoma area, a lot of inflammatory cells, edema and “granule-adipose corpuscles” (cells with basophil nuclei and with neuron and myelin detritus in their cytoplasm) were observed (Fig. 9c).

Fig. 4. Photographs showing different times of the endoscopic evacuation in the #011 pig. In a), the outer sheath is passing through the burr-hole. In b), the border between the hematoma (asterisk) and white matter is shown. In c), the suction catheter is aspirating the hematoma. In d), white matter without hematoma is shown.

Fig. 5. Photographs showing the anterior–posterior (left) and lateral (right) views of intact and formalin-fixed brains, with the hole (arrows) generated by the outer sheath.
Problem group

At 4 h, we observed minimal cell reaction, with microglial cells in perihematomal area (Fig. 10a). At 24 h, ill-defined edge between hematoma and neuropil was identified. In this interface, reactive tissue with microglial cells, lymphocytes, neutrophils and necrotic tissue was observed (Fig. 10b). At 5 days, a well-defined edge between hematoma and neuropil was evident, with necrotic cells near to hematoma and intense vital reaction (edema, “granule-adipose corpuscle”, inflammatory cells) close to neuropil (Fig. 10c).

Discussion

The pig is a useful experimental model to study surgical evacuation of ICHs [13,14]. This model has been used to assess clot stereotactic aspiration after instillation of fibrinolytic agent [15–17]. However, we have not found experimental models of endoscopic evacuation of ICHs in previous studies.

Considerations about experimental model

One advantage of this model over others, such as rodents, is their large brain volume, enabling to generate large hematomas to assess surgical evacuation [13,14,18]. In five experimental studies about fibrinolysis and subsequent stereotactic clot evacuation, hematoma volumes ranged from 2.5 cc to 9 cc [16,17,19–21]. In this study, injected volumes were larger. We consider these volumes not lethal, because there were no spontaneous deaths from induction of hematoma. Hematoma volumes so large are advantageous in our model, because the diameter of the largest instrument (outer sheath) is 10 mm and, therefore, larger hematomas are required to assess efficacy of endoscopic evacuation.

Table 1

Summary of the animals used in the study.

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<th>WEIGHT (kg)</th>
<th>INJECTED VOLUME (cc)</th>
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<th>RESIDUAL VOLUME (mm³)</th>
<th>DEATH (h)</th>
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kg, kilograms. cc, cubic centimeters. mm³, cubic millimeters.

Table 2

Summary of data in control and problem groups.

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</tr>
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<tr>
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<td>(min-max)</td>
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<tr>
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<td>(min-max)</td>
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<td></td>
<td>Mean</td>
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<tr>
<td>Residual volume (mm³)</td>
<td>Median</td>
<td>(min-max)</td>
<td>565 (250–1750)</td>
</tr>
<tr>
<td></td>
<td>Mean</td>
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</tr>
</tbody>
</table>

kg, kilograms. cc, cubic centimeters. mm³, cubic millimeters.

* Mann–Whitney U test.

Fig. 6. Photograph showing brain coronal slices of #002 pig, containing the hematoma.

Table 2

Summary of data in control and problem groups.

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kg, kilograms. cc, cubic centimeters. mm³, cubic millimeters.

* Mann–Whitney U test.

Fig. 7. Box graphs depicting the residual volumes of control group and problem group. Values indicated by vertical line are expressed in cubic millimeters.
In previous experimental models, cranial burr hole was located taking as references coronal [14,16,18,19,21 – 24] and sagittal sutures [14,16,18,19,21 – 24]. In our model, locating both sutures was not necessary and selected references were very easy: 15 mm lateral to midline and in projection line of lateral eye epicanthus. The depth of blood injection into brain to induce ICH in this study was established to be 20 mm from cortical surface. Hematoma was located in the white matter of right hemisphere in 100% of pigs. Introducing blood away from cortical surface diminishes the likelihood of blood diffusing into subarachnoid space and causing loss of volume injected, which would interfere with the results. In fact, there was only hematoma extension into cerebral cortex in two pigs.

Although in clinical practice, ICHs may be associated with subarachnoid hemorrhage, in experimental models subarachnoid extension may challenge its reproducibility, because different hematoma volumes may be generated with the same injected hematoma volume [25,26]. In this study, subarachnoid extension was detected in 50% of pigs with residual hematoma volume. However, it did not influence the results, because the percentages of subarachnoid hemorrhage between both groups were not statistically significant. The high percentage of subarachnoid hemorrhage may be due to: 1) injected hematoma volume. In this study, there were no significant differences in injected hematoma volume between pigs with subarachnoid hemorrhage and pigs without subarachnoid hemorrhage. 2) Blood injection at cortical level. We consider that blood injection to 2 cm of cortical surface is appropriate, since only two pigs had cortex hemorrhage. And 3) a rapid blood injection rate that results in a variable reflux along the needle track [13,22,26]. This problem had been largely resolved by employing the double-injection technique [13,15,25–27]. We think used injected hematoma volume and the rapid injection rate to generate hematoma were responsible for the high percentage of subarachnoid hemorrhage in this study. We pretended to simulate a real clinical situation, in which a hematoma is rapidly generated by rupture of blood vessel, and produced hematoma volumes of sufficient size to allow evacuated by endoscopic techniques.

After ICH, inflammatory reaction is induced in and around of the hematoma, with leucocyte infiltration [3,16,21,28–36]. This infiltrate begins in the first 24 h, shows a peak between 48 and 72 h and decreases progressively from 3rd day to 7th day [28,30,32–36]. The temporal sequence observed in our study was similar to the previously described, because the vital reaction was barely present at 4 h, evident at 24 h, and maximum at 5 days after induction of hematoma. Endoscopic evacuation might accelerate vital reaction, as this was more evident in problem group than in control group at 24 h, but was similar at 5 days.

Macrophages had also been detected in hemorrhagic brains [3,32,36]. We consider that “granule-adipose corpuscles” are phagocytic cells, because neuron and myelin detritus were observed in their cytoplasm.

Finally, at 24 h in both groups, we observed hemorrhage interspersed with neuropil. It is considered that blood dissects white matter, so that this is arranged in and around the hematoma, suffering minimal destruction [37,38].

![Fig. 8. Bar graph depicting the medians of residual volumes at 4 h and at 24 h and 5 days. When pigs were sacrificed at 4 h in problem group, the endoscopic evacuation was performed at 2 h. When deaths were induced at 24 h or 5 days, hematomas were removed at 12 h. In both moments of endoscopic evacuation, residual volumes are lesser than in the respective control groups. Values indicated by vertical line and on the bars are expressed in cubic millimeters.](image)

![Fig. 9. Photomicrographs showing sections of brains with hematomas in pigs of the control group. a) #017 pig, which was sacrificed at 4 h. The hematoma is observed in the upper portion and the brain parenchyma in the lower portion. No vital reaction is observed. H & E, original magnification ×2. b) #002 pig, which was sacrificed at 24 h. The hemorrhage is interspersed with brain parenchyma. H & E, original magnification ×4. c) #012 pig, which was sacrificed at 5 days. The hematoma (left lower portion) is separated from the parenchyma, which has a spongyform appearance due to edema. H & E, original magnification ×10.](image)
The endoscopic evacuation of ICHs has some disadvantages with respect to other surgical techniques: 1) Visualization is not occasionally adequate, especially when there is a hemorrhage during the procedure [40,41,46–50]. 2) The restricted working space limits the maneuverability of the instruments and the control of a possible bleeding [48]. The inner diameter of external sheath used in this study was 8 mm, allowing only being able to work with one instrument (suction catheter) adjacent to the endoscope. 3) After removing a significant amount of hematoma, the cavity becomes collapsed. This increases the possibility that there is residual hematoma [48]. Removing hematoma from depth to surface is a method used to overcome this drawback [42]. And 4) the ICHs more than 24 h of evolution begin to harden and they could be difficult to evacuate by endoscopic technique [44]. In our study, the residual volumes of pigs whose hematomas were evacuated at 12 h were similar to those that were evacuated at 2 h. Therefore, we think endoscopic surgery is effective in these time ranges.

**Development of a new transparent sheath as complement of endoscope**

The transparent sheath developed by our team provides several technical modifications with respect to the previous ones. Its outer sheath has a blunt and closed final, and a lateral perforation for the aspiration. This development provides several advantages. First, when it is introduced into the brain, a stylet is not necessary, allowing the endoscope inside the sheath before contacting with the brain, and so, the surgeon has a continuous visual control of all areas since the beginning of the surgical intervention. Second, its blunt final is less traumatic to the brain when the surgeon performs movements of the sheath. Third, its closed final prevents the endoscope and other instruments (ie, the suck) from causing injuries due to unwanted maneuvers.

The main disadvantage of this sheath is the difficulty for hemostasis, because it is technically complicated passing coagulators through the lateral perforation and accurately placing hemostatic substances. In this study, hemostasis was not necessary because hematoma was generated by injection of autologous blood. However, this is an aspect to improve in the new settings of the system design.

Another innovation is the inner sheath, through which endoscope is introduced. In this way, as the endoscope is independent, the camera is not stained by the passage of sucked clots and the movements of the used instruments, and therefore the surgeon does not need to remove it for cleaning. The main drawback of this sheath is the decrease of working space in the outer sheath, as it adds 2 mm to 4 mm of diameter of the endoscope. We have corrected this disadvantage by using very small suction catheters. The design of this system would be improved by decreasing diameters as the inner sheath as the endoscope.

Unlike other studies, a 30° endoscope has been used, which provides a wider lateral vision of the surgical field than the 0° endoscope.

This study has some limitations. First is the high percentage of pigs with subarachnoid hemorrhage, as we have previously discussed. Second, we could not assess the neurologic affection that hematoma has generated and the clinical course after endoscopic evacuation [18]. To assess them, a larger number of pigs and long-term follow-up would be needed. Third, we did not estimate the evacuation rate of ICH in each pig because we did not use imaging techniques on it.

**Conclusion**

We have developed a new pig model for endoscopic evacuation of ICHs. Endoscopic evacuation with our new transparent sheath is efficient without increasing the vital reaction. The blunt and closed final of the sheath prevents unwanted injury of the brain by the instruments used to remove the hematoma.

**Considerations about results of endoscopic evacuation**

In 1985, Auer first described the endoscopic evacuation of ICHs [39]. Recent reports have showed that the hematoma evacuation rate of endoscopic surgery ranged from 83.4% to 99% [40–45]. Although in this study we could not estimate the rate of evacuation in each pig from diagnostic imaging tests (computed tomography or magnetic resonance), the residual volume in problem group was 70.09% lower than in control group, with no significant differences in injected volumes, in percentage of subarachnoid hemorrhage, and in time interval from hematoma induction to pig’s death. This implies that the technique used in the described model is efficient to evacuate ICHs.
Conflict of interest

All authors certify that they have NO affiliations with or involvement in any organization or entity with any financial interest, or non-financial interest in the subject matter or materials discussed in this manuscript.

Acknowledgments

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References


