Comparison of silicon-coated nylon suture to plain nylon suture in the rat middle cerebral artery occlusion model

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Abstract

A variety of intraluminal sutures have been used in the middle cerebral artery occlusion model (MCAO) of focal ischemia. In the present study we tested commercially available silicon-coated nylon suture in the MCAO model and compared the results to traditional monofilament nylon suture occlusion. Twelve Sprague-Dawley male rats were randomly divided into two groups, MCAO with 4-0 nylon suture (Group N, \( n = 6 \)) and MCAO with silicone-coated 4-0 nylon suture (Group S, \( n = 6 \)). Rats were sacrificed 24 h after reperfusion. Assessment included mortality rates, neurological evaluation, and infarct volume. One rat died in each group from subarachnoid hemorrhage. Neurological evaluation demonstrated that Group S tended to have worse neurological outcomes than Group N, although this difference was not statistically significant. On TTC stain Group S had significantly larger infarct volumes than Group N. We conclude that the commercially available silicone-coated occlusion suture provides better occlusion of the middle cerebral artery than the traditional uncoated nylon suture. Classification: Disease-related neuroscience (Section 6).

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1. Introduction

In 1986, Koizumi et al. first described the middle cerebral artery occlusion model (MCAO) of focal ischemia in rats Koizumi et al., 1986. The MCAO protocol requires occlusion of the middle cerebral artery with an intraluminal suture. Multiple studies since then have employed this model, frequently with different types of intraluminal sutures. Monofilament nylon sutures Bhardwaj et al., 2001; Chen et al., 2001; Kuge et al., 1995; Li et al., 1998; Longa et al., 1989; Palmer et al., 2001 as well as silicon-coated nylon sutures Barber et al., 2004; Dittmar et al., 2003; Wegener et al., 2005 have been used in this model. Although both types of sutures are accepted in this model, there has been an informal trend toward use of silicone-coated sutures in published literature. Presently there is little published data to support the use of silicone-coated sutures to the exclusion of traditional nylon monofilament. In this study, we tested the commercially available silicone-coated 4-0 nylon suture in the MCAO model, and compared the results to the traditional 4-0 nylon monofilament occlusion suture.

2. Materials and methods

All experiments were approved by the Loma Linda University Animal Care Committee and performed in accordance with the guidelines provided by the American Academy of Accreditation of Laboratory Animal Care.

Twelve male Sprague-Dawley rats (290–310 g) were housed under identical conditions. Rats were fasted overnight with free access to the water before the experiment. Rats were anesthetized with a-chloralose (Fisher Scientific, Fair Lawn, NJ. 60 mg/kg ip) and urethane (Acros Organics, Morris Plains, NJ. 600 mg/kg ip). Rats were randomly divided into two groups; Nylon suture...
Fig. 1. Occlusion sutures. (A) Non-coated 4-0 nylon suture. The tip of this nylon was shaped round with flame heating. (B) Silicon-coated 4-0 monofilament. Grid width = 1 mm.

occlusion group (N, \( n = 6 \)), Silicon-coated 4-0 monofilament occlusion group (S, \( n = 6 \)).

The rounded head of the 4-0 monofilament occlusion suture was fashioned over an open flame of a Bunsen burner. Prior to manipulation, the diameter of the 4-0 monofilament suture was 0.19–0.20 mm when measured by caliper. The flame was used to melt the end of the suture. In the process of melting, the suture material naturally becomes somewhat round and solidifies into a spherical shape when allowed to cool. The diameter of the flame-rounded 4-0 nylon suture tip was 0.35–0.38 mm. The diameter of the 4-0 silicon-coated monofilament was 0.35–0.40 mm along its length (Doccol Co., Albuquerque, NM). The diameter of the internal carotid is about 0.3 mm and the diameter of the origin of the middle cerebral artery and anterior cerebral artery are about 0.2 mm Koizumi et al., 1986. Fig. 1 shows the two types of sutures used in this study.

2.1. MCAO model

The MCAO protocol has been described elsewhere in detail Koizumi et al., 1986; Longa et al., 1989.

After induction of general anesthesia rats were intubated and placed on a small animal ventilator (Harvard Apparatus, South Natick, MA). Rectal temperature of rats was maintained at 37 ± 1 °C throughout surgery, and mean arterial pressure was monitored via femoral artery. The left common carotid artery (CC), external carotid artery (EC) and internal carotid artery (IC) were exposed through a midline incision. The external carotid artery was ligated, coagulated, and cut down just proximal to the lingual and maxillary artery branches. All other branches of the EC were coagulated and transected. The IC was then isolated to avoid damage to the vagus nerve. The pterygopalatine artery was ligated, coagulated, and cut down at its origin.

In Group N, a 4-0 monofilament nylon suture (Nescosuture\textsuperscript{®}, Alfresa Pharma Co., Osaka, Japan) with a flame-rounded head (Fig. 1A) was inserted through the IC via a small incision in the external carotid artery stump, while in Group S, a 4-0 silicon-coated occlusion suture (Doccol Co., Albuquerque, NM) (Fig. 1B) was used. The distance from bifurcation of the common carotid artery to the tip of the suture was approximately 18 mm in all rats, consistent with published descriptions of the MCAO model. After 2 h of occlusion the suture was withdrawn and cerebral blood flow recovered. The skin was sutured and the rats were allowed to recover. All rats were sacrificed at 24 h after cerebral ischemia.

2.2. Neurological evaluation

We measured neurological scores 24 h after cerebral ischemia. Scoring was done blindly by the third author with a modified Garcia’s neurological scoring system (Table 1, and Garcia et al., 1995). A thin wood beam (1 m × 3 cm × 0.5 cm) was used for the beam-walking task. The beam-walking task assesses fine vestibulomotor function in the MCAO model Petullo et al., 1999.

2.3. Measurement of infarct size

Under deep anesthesia rats were perfused with cold phosphate buffered saline (PBS, Sigma–Aldrich, St. Louis, MO) via the ascending aorta. Brain tissue was cut coronally into 2-mm sections with a brain slicer (Harvard Apparatus; South Natick, MA) and immersed in 2% 2,3,5-triphenyltetrazolium chloride monohydrate (TTC, Sigma–Aldrich, St. Louis, MO) solution at 37 °C for 5 min, followed by 10% formaldehyde solution. The
infarct areas were traced and quantified by an image analysis system (Image J 1.33 u).

2.4. Statistical analysis

Data are expressed as mean ± S.E.M. Differences between Groups N and S groups were analyzed using standard post hoc t-tests supported by Sigma Stat 3.0.1. Spearman rank order correlation coefficients were calculated for correlation analysis. An F-test was used to compare the size of the error bars in Fig. 3A. \( P < 0.05 \) was considered statistically significant.

3. Results

3.1. Mortality

One rat died in each group from subarachnoid hemorrhage, for an overall mortality of 16.7% in each group.

3.2. Neurological evaluation

Using a modified Garcia’s neurological evaluation (Table 1, and Garcia et al., 1995, the neurological Score of Group N was 9.2 ± 1.5 (n = 5) and of Group S was 5.6 ± 1.4 (n = 5). There was not a statistically significant difference between the two groups due to relatively high variability.

3.3. Correlation of neurological score with infarct volume

The neurological scores correlated well with the size of infarct on a case-by-case basis, as shown in Fig. 3B. The correlation between cortical infarction volume and neurobehavioral score was slightly better than the correlation between basal ganglia infarction volume and neurobehavioral score.

3.4. Infarction volume

Fig. 2 shows representative infarction volumes from each group as assessed by TTC stain. Both cortical and basal...
structures were affected in this study. Group S demonstrated a significantly larger total infarction volume (Fig. 3A). Group S also demonstrated larger infarction volume in the basal ganglia when this area was analyzed separately. There was not a significant difference in the cortical infarction areas when analyzed separately (Fig. 3A). In Fig. 3A, the error bars are qualitatively different, although this difference is not statistically significant when analyzed by F-test.

4. Discussion

Although both flame-rounded nylon occlusion sutures and silicone-coated occlusion sutures are used in the MCAO model, there has been an informal trend toward use of silicone-coated suture in the literature. Presently, there is limited published data to support the use of one type of occlusion suture to the exclusion of the other. In this study, we demonstrated that commercially available silicone-coated occlusion sutures produce better occlusion in the MCAO model.

One of the weaknesses of the 4-0 nylon suture method of occlusion lies in the inability to obstruct blood flow through both the anterior and posterior communicating arteries because of lack of suitable thickness of the suture proximal to the rounded head (Fig. 1 and Laing et al., 1993). The silicon-coated suture prevents flow in both these communicating arteries, as well as flow in the origin of the MCA because of its continuous thickness. This difference between the two types of sutures is reflected in the differences in infarct area, demonstrated on TTC stain (Figs. 2 and 3A).

The success rate of MCAO in this study is consistent with previous reports in which 88% of animals undergo successful MCAO, even with MRI monitoring Li et al., 1998.

Garcia’s scoring system is commonly used in the MCAO model Chen et al., 2001; Garcia et al., 1995; Shohami et al., 1995. We have modified the original scoring system to include a beam-walking task. The beam-walking task assesses fine vestibulomotor function in the MCAO model Petullo et al., 1999. The extent of injury correlated well with the neurological score,
even though the two groups did not demonstrate significant differences on aggregate analysis.

We conclude that the commercially available silicon-coated 4-0 nylon suture produces predictable results in the MCAO model, and that it provides better occlusion than the traditional flame-rounded nylon filament by virtue of its thickness.

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References


