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The role of ECA transection in the development of masticatory lesions in the MCAO filament model

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Abstract

In the intraluminal suture model of middle cerebral artery occlusion (MCAO) in the rat, lesions of the masticator muscles associated with impaired functional outcome occur. We evaluated the role of external carotid artery (ECA) transection. We assessed whether isolated interruption of an arterial or a venous connection to the ECA territory was sufficient to induce masticatory hypoperfusion and lesions. We also evaluated a direct access to the common carotid artery (CCA) with subsequent vascular closure with regard to its feasibility, frequency of masticatory lesions, complications, and cerebral ischemia. Cerebral and masticatory lesions and perfusion deficits were assessed by in vivo magnetic resonance imaging (MRI). Vessel patency was evaluated using computerized tomography angiography and histology. An interruption of arterial blood flow led to masticatory hypoperfusion. Masticatory lesions occurred in 6% of the rats. Access to and closure of the CCA were feasible in all animals, leading to moderate or severe vessel stenosis in 20%, and intraarterial thrombosis in 25% of the rats. Reproducible cerebral infarctions were obtained in all animals. In 24% of the rats, hyperintense MRI signal changes were observed in the ipsilateral temporal muscle. Thus, the induction of masticatory hypoperfusion and lesions by arterial transection supports the role of the ECA in this context. Direct access to the CCA with subsequent vessel closure led to stenosis in most animals. Preservation of ECA continuity was not suitable to fully prevent masticatory lesions.

Keywords: Brain ischemia; Disease models; Animal; Rats; Adverse effects; External carotid artery; Masticatory muscles; Magnetic resonance imaging; Computed tomography angiography; Vascular surgery

Introduction

The intraluminal filament model of middle cerebral artery occlusion (MCAO) is a commonly used and widely accepted model of ischemic stroke first described by Koizumi et al. (1986). In the modification of this model that is most frequently applied (Longa et al., 1989), the filament is introduced via the stump of the transected external carotid artery (ECA) and advanced into the circle of Willis until it occludes the origin of the middle cerebral artery (MCA). Several complications have been associated with this model: insufficient occlusion, inadvertent premature reperfusion, and subarachnoid hemorrhage (Schmid Elsaesser et al., 1998). Recently, ischemic damage to the ipsilateral ECA supply territory caused by ECA transection has also been reported (Dittmar et al., 2003). Early in vivo magnetic resonance imaging (MRI) has revealed hyperintense areas in the ipsilateral temporal muscle in approxi-

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mately half of all rats subjected to the Longa method. ECA ischemia alone impairs body weight development and motor performance, and, thus, potentially interferes with the results gained during experimental settings (Dittmar et al., 2003). In addition, a significant disturbance of the animals' well-being associated with the damage of the mastication muscles has to be assumed.

Several modifications of the filament model, in which access to the vessel is made through the common carotid artery (CCA) (Ardehali and Rondouin, 2003; Koizumi et al., 1986; Lei et al., 2001) or the internal carotid artery (ICA) (Kent et al., 1999; Quast et al., 1995), have been described earlier in rats, but reperfusion through the carotid arteries (CAs) has not been established so far when using these techniques. In the current study, we investigated the introduction of a filament via a direct incision in the CCA with subsequent closure of the cut and reperfusion of the artery in an attempt to avoid masticatory lesions. In this respect, the following issues are addressed: (a) is the interruption of arterial supply to or venous drainage from the ECA territory sufficient to create masticatory lesions; (b) is hypoperfusion of the ECA supply territory detectable after arterial ligation and is it coincidental with masticatory lesions; (c) is interruption of the ECA suitable to serve as a model for further studies on masticatory lesions in the filament model; (d) is introduction of the filament via a direct incision in the CCA with subsequent closure and reperfusion feasible; and (e) does preservation of the ECA, obtained by following a surgical approach via the CCA, prevent masticatory damage.

To examine these aims, two groups of experiments were performed. Aims (a) to (c) were addressed by isolated arterial interruption of the supplying arteries of the extracranial tissue, while for the remaining topics, a modification of the filament model with vessel access via the CCA with subsequent microsurgical closure was evaluated.

Materials and methods

Animal care and all experimental procedures were conducted in accordance with German laws governing animal care and with the European Communities Council Directive (86/609/EEC). Protocols were approved by the Ethics Committee for Animal Research of the local authorities. All invasive procedures were performed by experienced investigators.

Animals and housing conditions

Male Wistar (n = 41) and Sprague–Dawley (n = 6) rats, each weighing between 256 and 349 g, were purchased from Charles River (Sulzfeld, Germany). The animals were housed under conventional conditions with free access to food and water for at least 3 days prior to surgery.

Isolated arterial or venous interruption

Anesthesia and surgery

In 24 Wistar rats, anesthesia was induced by using an inhalation mixture of 5% isoflurane in N_2O/O_2 (70%/30%). Anesthesia was maintained with a mixture of 2% isoflurane in N_2O/O_2 after the animals had been given endotracheal intubation and mechanical ventilation by a small animal respirator (RS Biomedtech, Sinzing, Germany). A midline incision was made in the scalp in all animals as an analog to the cut needed for installation of laser Doppler flowmetry (LDF). Subsequently, the right CA bifurcation was exposed. For this experiment, the rats were divided into four groups with six animals in each group. In Group A, the right ECA, occipital artery, and cranial thyroid artery were transected. In Group B, the same arteries were transected and in addition the right pterygopalatine artery was ligated. In Group C, the arteries mentioned for Group B were transected and the ipsilateral CCA was ligated. In Group D, the right jugular veins and their collateral vessels were electrocoagulated without any arterial occlusion. MCAO was not performed in Groups A to D.

MRI protocols

Before all imaging procedures, anesthesia was induced in the animals by using a subcutaneous injection of xylazine (4–8 mg/kg body weight) and ketamine (60–120 mg/kg) as described elsewhere (Dittmar et al., 2004). Twenty-four hours after the procedure, the animals in Groups A through D underwent in vivo MRI performed using a 1.5 T clinical MRI system (Magnetom Sonata, Siemens, Germany). First, a coronal T_2-weighted turbo-spin echo (TSE) sequence was performed and later a T_1-weighted contrast-enhanced perfusion sequence (one measurement every 0.91 s). As a contrast agent, 0.2 ml Magnevist (Schering, Germany), containing 15.7 mg gadolinium, was applied intravenously. To assess perfusion deficits, differences between both temporal muscles with regard to the start of the signal rise and the steepness of the ascending slope over 10 and 25 consecutive images (counted from the beginning of the signal rise to be independent of any perfusion delay) were examined. On both sides, two independent regions of interest were measured.

Histological analysis

Three Group C rats and three Group D rats were killed 4 days after surgery. Their temporal muscles were fixed in paraformaldehyde and stained with hematoxylin-eosin (HE) and Elastica-van-Gieson (EvG).

Filament MCAO via the CCA with anterograde reperfusion

Anesthesia and surgery

In 17 Wistar rats, occlusion of the MCA (n = 10) or sham surgery (n = 7) was performed according to the method reported by Koizumi et al. (1986) with modifications herein