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Effects of Repeated Short versus Single Long Episodes of Focal Ischemia on Somatosensory Evoked Potentials and Development of Cerebral Infarction in Cats

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Abstract

The effects of repeated short episodes of focal ischemia at 30-minute intervals or a single equivalent long episode of focal ischemia on neuronal function and development of cerebral infarction were compared using somatosensory evoked potential (SEP) recording and 2,3,5-triphenyltetrazolium chloride staining in a cat model. Seventeen cats underwent transorbital occlusion of the middle cerebral artery (MCA), using one of three procedures: sham-operation; single 1-hour occlusion of the MCA, followed by 3 hours of recirculation; or three 20-minute occlusions of the MCA at 30-minute intervals, followed by 3 hours of recirculation. Two of six cats in the single long-term occlusion group showed recovery of SEP, whereas all six cats in the repeated short-term occlusion group showed recovery of SEP at 3 hours after recirculation. All six cats in the single long-term occlusion group had cerebral infarction of various sizes, but only one cat in the repeated short-term occlusion group developed infarction. Repeated short episodes of focal ischemia are relatively less damaging than a single equivalent long episode of focal ischemia, even if the reperfusion interval is extended to 30 minutes.

Key words: repeated occlusion, focal ischemia, somatosensory evoked potential, cerebral infarction

Introduction

Temporary occlusion of intracranial arteries is frequently used during aneurysm surgery to prevent intraoperative rupture, to control excessive bleeding from premature rupture, and to achieve clipping of aneurysms. Despite these advantages, temporary occlusion carries the risk of ischemic sequelae, depending on the duration of temporary occlusion and the presence of collateral channels. Repeated occlusions of brief duration may minimize ischemic brain damage, and are sometimes used during cerebrovascular procedures. However, experimental studies have not supported this idea. Furthermore, repeated occlusions are not known to cause less brain damage than a single occlusion of equivalent total occlusion time. Many experimental studies using global ischemic models have demonstrated that repeated occlusions at prolonged intervals for recirculation cause more brain damage than single equivalent occlusion.[1,5,10,12,13,15,18,19,21,22,25,27] Cumulative ischemic damage occurs secondary to microcirculatory impairment and secondary hypoxia, prolonged edema, protein synthesis inhibition, and progressive accumulation of cytotoxic substances such as calcium, glutamate, prostaglandins, and free radicals.[1,5,15,19,21,22,25,27] However, these global ischemia studies did not assess the effects on neuronal function, so have limited clinical implications.

The consequences of repeated focal ischemia, which has more significant clinical implications than repeated global ischemia, have been little investigated.[7,24] Recently, repeated episodes of focal ischemia were found to cause less histological injury than a single episode of focal ischemia.[7,24] This study compared the effects of repeated short-term focal ischemia with single long-term focal ischemia on somatosensory evoked potential (SEP), a reliable prognostic indicator of cortical function, and evaluated the relationship between SEP and development of cerebral infarction.

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Materials and Methods

Seventeen adult cats were anesthetized with in-

tramuscular ketamine 15 mg/kg and intravenous sodium pentobarbital 25 mg/kg. After tracheotomy the animals were immobilized with 60 mg/kg of pancuronium bromide and were ventilated mechanically (Harvard Apparatus Limited, South Natik, Mass., U.S.A.). End-tidal CO_2 was controlled at 25 to 30 mmHg. Body temperature was monitored with a rectal probe and maintained at 38° C. The animal's head was fixed in a stereotactic frame. The left internal carotid artery bifurcation and proximal middle cerebral artery (MCA) were exposed *via* the transorbital approach under the operating microscope.[9,20] A 1.0 × 1.0 cm craniectomy was performed and the dura was exposed over the left posterior sigmoid gyrus for recording of SEPs. The right sciatic nerve was exposed for stimulation.

Animals were subjected to one of three experimental procedures: sham-operation (five cats); single 1-hour clip occlusion of the MCA, followed by 3 hours of recirculation (six cats); three 20-minute clip occlusions of the MCA at 30-minute intervals, followed by 3 hours of recirculation (six cats). After recirculation, the animals were sacrificed quickly by intravenous injection of sodium pentobarbital.

I. Recording of SEP

A specially designed electrode (NE-120; David Kopt Instruments, Tujunga, Cal., U.S.A.) for selective measurement of cortical field potentials was inserted into the left posterior sigmoid gyrus at a depth of 1 mm from the cortical surface. Paired stimulation electrodes were placed on the sciatic nerve proximal to the tendon of the obturator internus. A laminectomy was performed at C-2, and two wire electrodes (Teflon-coated, 7 strand stainless steel wire, diameter 0.001 inch; A-M Systems, Inc., Everett, Wash., U.S.A.), exposed for 1–2 mm at the tip, were placed on each side of the dura for recording. Stimuli were delivered at 3 Hz with a pulse duration of 0.1 msec (A300 Pulsemaster, A360 D/R constant stimulus isolator; World Precision Instruments, Inc., New Haven, Conn., U.S.A.). A bandpass of 30 to 3000 Hz was used and a total of 150 to 200 responses were averaged. Averaging software (Experimental Workbench Version 2.2; Brain Wave Systems Corp., Broomfield, Colo., U.S.A.) was used to produce waveforms.

SEPs were recorded before exposure of the MCA, during occlusion and for 3 hours after recirculation in the occlusion groups, and for 5 hours after the procedure in the sham-operation group. The deflections of the waves were named P_1, N_1, P_2, N_2, P_3, N_3, P_4, N_4, and P_5. All components disappeared immediately after occlusion. Three cats showed preservation of SEPs after occlusion of the MCA, so were excluded to minimize the effect of collateral circulation. Recovery of the waves was judged by the following criteria: A definite peak must be present at 22 to 35 msec latency; the peak must be larger in amplitude than the noise level of the no-stimulus trace; this positive peak must continue to be present during the averaging procedure.[28]

II. Determination of cerebral infarction area

The brain was removed rapidly, placed in a freezer at −20° C for 20 minutes, and cut into four 5-mm-thick coronal sections from the frontal pole. The brain slices were immersed in a 2% solution of 2,3,5-triphenyltetrazolium chloride (TTC) in normal saline at 37° C for 30 minutes, and fixed in 10% phosphate-buffered formalin for 3 to 4 days.[2,11] The rostral surface of the TTC-stained section was photographed and the area of infarction was determined by computer imaging analysis (Yonsei Anatomy Program; Department of Anatomy, Yonsei Univ., Seoul, R.O.K.).

III. Statistical analysis

Values of mean arterial blood pressure (MABP) and PCO_2 were tested by one-way analysis of variance. The occurrences of SEP recovery and infarction development were compared by two-tailed Fisher's exact test. Differences were considered significant at $p < 0.05$.

Results

I. Changes in physiological parameters

Systemic MABP and PCO_2 were maintained at physiological values during the experiment.

II. Recovery of SEP

Five positive peaks and four negative peaks were recorded consistently in all animals before MCA occlusion. The major positive deflection (P_2) occurred at 23.8 ± 1.0 msec after stimulus. The peak-to-peak amplitude of the major negative-positive voltage complex (N_1-P_2) was 621.0 ± 76.1 μV. Prior to occlusion, there was no significant difference in the latency of the P_2 wave, or in the N_1-P_2 interpeak amplitude between the groups (Fig. 1).

The SEP did not change in the sham-operated group during the experiment. The SEP was abolished immediately after MCA occlusion and continued to be absent during occlusion. Two cats showed recovery of SEP but the other four cats showed no SEP at 3 hours after recirculation in the single long-term occlusion group (Fig. 2). All six cats showed recovery of SEP at 3 hours after recirculation in the repeated short-term occlusion group (Fig.

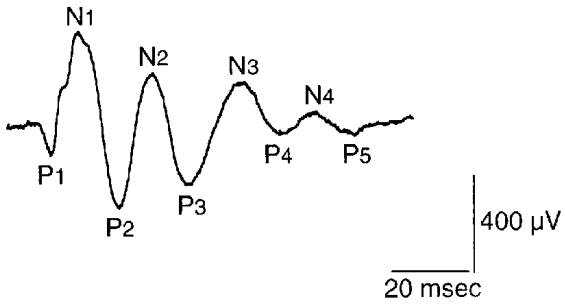

Fig. 1 Somatosensory evoked potential at the primary somatosensory cortex due to sciatic nerve stimulation in a sham-operated cat. P_1: first positive wave, P_2: second positive wave, P_3: third positive wave, P_4: fourth positive wave, P_5: fifth positive wave, N_1: first negative wave, N_2: second negative wave, N_3: third negative wave, N_4: fourth negative wave.

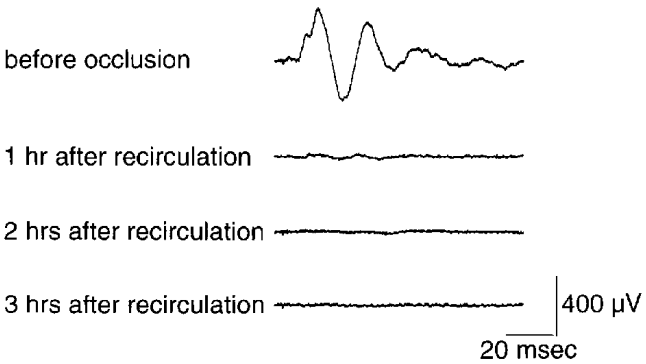

Fig. 2 Somatosensory evoked potentials in a cat which underwent single 1-hour occlusion of the middle cerebral artery. All components were abolished at 3 hours after recirculation.

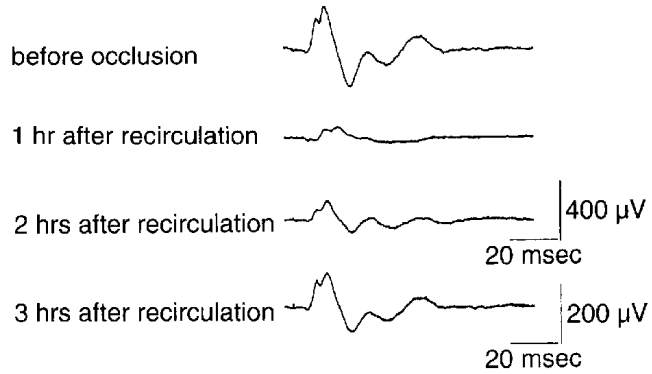

Fig. 3 Somatosensory evoked potentials in a cat which underwent three 20-minute occlusions of the middle cerebral artery at 30-minute intervals. All cortical components recovered at 3 hours after recirculation but the amplitude of all components was reduced compared to the pre-occlusion potential.

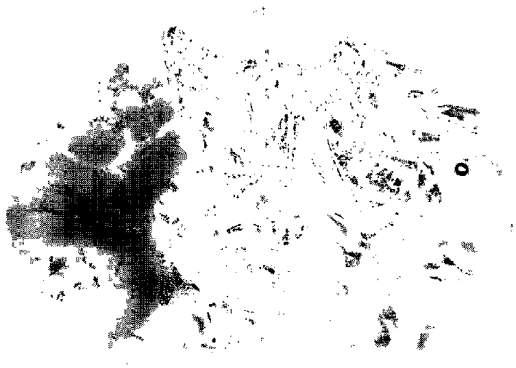

Fig. 4 Photograph of a coronal brain slice from a cat which underwent a single 1-hour occlusion of the middle cerebral artery. Note the marked reduction of cortical 2,3,5-triphenyltetrazolium chloride staining.

3). The difference in SEP recovery between the occlusion groups was statistically significant ($p = 0.030$).

III. Development of cerebral infarction

All six cats in the single long-term occlusion group had infarctions of various sizes in the posterior sigmoid, ectosylvian, suprasylvian, and sylvian gyri. In three cats, infarction extended to the basal ganglia, including the caudate head (Fig. 4). The mean infarction size in the single long-term occlusion group was 23.1 ± 4.4% of the left hemisphere. In contrast, only one cat in the repeated short-term occlusion group developed cerebral infarction in the left caudate nucleus. The infarcted area was 7.3% of the left hemisphere. The difference in infarction development was statistically significant between the two groups ($p = 0.041$). None of the sham-operated group had infarction.

Discussion

In the present study, the consequences of repeated short-term focal ischemia were compared with those of single long-term focal ischemia using SEP recording and TTC staining. Both clinical and experimental studies have shown that SEP recording is a sensi-

tive method to indicate cerebral ischemic insult and to predict functional outcome after temporary occlusion of intracranial arteries.[3,4,6,8,14,16,17,23] However, SEP recording has not previously been used to assess the effects of focal ischemia. TTC stains irreversibly damaged brain and can detect early infarction, whereas a longer maturation time of infarcted tissue is needed for light microscopic examination. Therefore, this study could provide a basis for predicting functional outcome.

Previous studies have clearly shown that duration of reperfusion is an important factor in determining whether repeated ischemia is beneficial or detrimental to the brain.[12,18,25,26] Duration of reperfusion has a biphasic protective effect in global ischemia. A short reperfusion interval of 2 to 5 minutes has a neuroprotective effect, but 1 hour causes more severe neuronal injury than a single episode of ischemia.[25,26] Further extending the reperfusion interval to more than 6 hours yields a neuroprotective effect again.[12,18] Previous focal ischemia models used a reperfusion duration of only 10 minutes, which is reported to reduce neuronal injury.[7,24] Repeated short-term occlusions, spaced at short reperfusion intervals (10 minutes) were found to cause smaller cortical infarctions than single long-term occlusion in a focal ischemia model.[7] Also, repeated short occlusions caused less neuronal damage than a single longer occlusion at 6 hours after the onset of occlusion.[24] However, the effects of prolonged reperfusion have not been investigated. Furthermore, a reperfusion interval of more than 30 minutes is not uncommon in neurosurgical procedures for occasional complex lesions. Therefore, we used a 30-minute reperfusion duration, which apparently retained the neuroprotective effect in this study. On the basis of the findings of this study, we suggest that repeated short-term focal ischemia is relatively less damaging than single long-term focal ischemia, even if the reperfusion interval is extended to 30 minutes.

The mechanism achieving the neuroprotective effect in repeated short-term focal ischemia is unknown, but there are several possibilities such as decreased release of glutamate, preserved energy store, and prevention of severe acidosis.[7,24] Another important mechanism may be related to the differences in brain edema between focal and global ischemia. In global ischemia models, repeated shorter episode of occlusion causes more severe edema than a single longer episode of occlusion, and results in microvascular compression and secondary hypoxia, raised intracranial pressure, and subsequent detrimental cascade. In focal ischemia models, repeated short-term and single long-term occlusion cause similar edema,[7,24] which may be related to the less detrimental effect of repeated episodes of occlusion. The specific mechanism responsible remains to be determined.

The clinical relevance of this study is difficult to determine because of the differences in collateral circulation and ischemic threshold of reperfusion duration between cats and humans, and even between individuals. Furthermore, there is no information about delayed sequelae of repeated ischemia. However, this study does indicate that repeated short-term occlusion with a reperfusion period of up to 30 minutes is less damaging than a single long-term occlusion. Further studies are needed to investigate the delayed consequences of repeated focal ischemia and various intervals and frequencies of reperfusion to simulate clinical situations.

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Commentary

Dr. Lee et al. have reported interesting experimental data showing that repeated short-term occlusion with a reperfusion period of up to 30 minutes is less damaging than single long-term occlusion. They also evaluated the relationship between SEP and development of cerebral infarction. Repeated temporary occlusions of intracranial arteries are frequently used during aneurysm surgery, and previous studies have shown that duration of reperfusion is an important factor in determining the benefits of this procedure. To date, however, the appropriate protocol to reduce the risk of ischemic sequelae has not been settled, and the data of prolonged reperfusion within 30 minutes are especially vague.

I believe that this study provides useful information about a therapeutic window in this clinically complex subject. However, the clinical relevance of this study is not so easy to determine in individual patients because of different conditions including development of collateral flow, threshold of ischemic tolerance, or relation to the occlusion time. The reperfusion time of

30 minutes used in this study may be too long in normal clinical practice. I hope that further studies including clinical investigations will be continued.

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This article provides an outstanding example of a laboratory approach to operative neurosurgical problems. The authors clearly demonstrate that neuronal dysfunction as well as the development of infarction caused by repeated short episodes of focal cerebral ischemia may be far less than those caused by a single equivalent long episode of focal ischemia. As mentioned in the excellent review by the authors, no one has so far been certain whether intermittent reperfusion is actually useful or not in preventing neuronal damage caused by temporary occlusion of the intracranial arteries during various neurosurgical procedures. The findings described in this article strongly indicate that such an intermittent reperfusion is indeed beneficial even if the reperfusion interval is extended to 30 minutes. The data obtained offer us hope that the most appropriate procedure for temporary occlusion can be clearly defined in the future if this problem is investigated in further detail such as with the present animal model.

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This is a very fine study, which gives data that is important in two respects; first, showing a remarkably satisfying correlation between alteration SSEP changes and pathologic evidence of infarction, and secondly by suggesting that repeated, short episodes of focal ischemia with intervals of reperfusion are relatively less damaging than a single, long episode of focal ischemia.

Although Professor Lee and his colleagues quite appropriately point out that the clinical relevance of this study is uncertain, I find the data to be quite reassuring. As one who utilizes temporary proximal occlusion frequently, I have for many years utilized the technique of short periods of occlusions interspersed with periods of reperfusion. This study adds scientific support to a commonly used neurosurgical practice.

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