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Targeted Increase in Cerebral Blood Flow by Transcranial Near-Infrared Laser Irradiation

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Background and Objective: Brain function is highly dependent on cerebral blood flow (CBF). The precise mechanisms by which blood flow is controlled by NIR laser irradiation on the central nervous system (CNS) have not been elucidated. In this study, we examined the effect of 808 nm laser diode irradiation on CBF in mice.
Study Design/Materials and Methods: We examined the effect of NIR irradiation on CBF at three different power densities (0.8, 1.6 and 3.2 W/cm^2) and directly measured nitric oxide (NO) in brain tissue during NIR laser irradiation using an amperometric NO-selective electrode. We also examined the contribution of NO and a neurotransmitter, glutamate, to the regulation of CBF by using a nitric oxide synthase (NOS) inhibitor, N^g-nitro-L-arginine methyl ester hydrochloride (L-NAME), and an *N*-methyl-D-aspartate (NMDA) receptor blocker, MK-801, respectively. We examined the change in brain tissue temperature during NIR laser irradiation. We also investigated the protection effect of NIR laser irradiation on transient cerebral ischemia using transient bilateral common carotid artery occlusion (BCCAO) in mice.
Results: We showed that NIR laser irradiation (1.6 W/cm^2 for 15–45 minutes) increased local CBF by 30% compared to that in control mice. NIR laser irradiation also induced a significant increase in cerebral NO concentration. In mice that received L-NAME, NIR laser irradiation did not induce any increase in CBF. Mice administered MK-801 showed an immediate increase but did not show a delayed additional increase in local CBF. The increase in brain tissue temperature induced by laser irradiation was estimated to be as low as 0.8°C at 1.6 W/cm^2, indicating that the heating effect is not a main mechanism of the CBF increase in this condition. Pretreatment with NIR laser irradiation improved residual CBF and reduced the numbers of apoptotic cells in the hippocampus.
Conclusion: Our data suggest that NIR laser irradiation is a promising experimental and therapeutic tool in the field of cerebral circulation research. Lasers Surg. Med. 42:566–576, 2010. © 2010 Wiley-Liss, Inc.

Key words: cerebral blood flow; near infrared laser; MK-801; forebrain ischemia; nitric oxide

INTRODUCTION

Brain function is highly dependent on cerebral blood flow (CBF). Human brain weight is about 2% of the whole body weight. However, CBF reaches about 15% of cardiac output. Improved outcome results when reduced CBF are prevented or respond to treatment not only in brain ischemia but also in traumatic brain injury, degenerative disease such as Parkinson's disease and Alzheimer's disease [1–6].

Photobiostimulation effects of near-infrared (NIR) laser irradiation have been known for almost 40 years [7]. Many studies have shown increased blood flow in various organs during and after NIR laser irradiation [7–10]. Recently, it has been reported that NIR laser irradiation is effective for controlling cerebral ischemia in vivo and clinically [11–14]. However, the precise mechanisms by which blood flow is controlled by NIR laser irradiation on the central nervous system (CNS) have not been elucidated. The vasodilatory action of NIR laser irradiation is most likely mediated by nitric oxide (NO). In this study, we examined the effect of 808 nm laser irradiation on CBF in mice and directly measured NO in brain tissue during NIR laser irradiation using an amperometric NO-selective electrode [15]. We also examined the contribution of NO and a neurotransmitter, glutamate, to the regulation of CBF by using a nitric oxide synthase (NOS) inhibitor, N^g-nitro-L-arginine methyl ester hydrochloride (L-NAME), respectively. It is currently accepted that *N*-methyl-D-aspartate (NMDA) receptor interacts with NOS in the neurotransmitter pathway

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All authors have seen and agree with the contents of the manuscript.

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[16]. To gain an insight into potential mechanisms linking neurotransmission with increased CBF, the effect of an NMDA receptor blocker, MK-801, was investigated. The thermal effect has been frequently discussed on the mechanisms of the effect of NIR laser irradiation. Therefore, we examined the change in skull surface and brain tissue temperature during NIR laser irradiation. We also investigated the protective effect of NIR laser irradiation on transient cerebral ischemia using transient bilateral common carotid artery occlusion (BCCAO) in mice [17,18]. We evaluated the CBF during BCCAO and apoptosis in the cerebral cortex and dorsal hippocampus 96 hours after the reperfusion by using in situ terminal deoxynucleotidyl transferase (TdT)-mediated dUTP-biotin nick end-labeling (TUNEL) of fragmented DNA.

MATERIALS AND METHODS

Animals

The institutional animal care committees of National Defense Medical College approved all experimental procedures. Extreme care was taken throughout the study to minimize pain and discomfort. Male C57BL/6J mice 9–11 weeks in age and weighing 23–27 g (CLEA Japan, Inc., Tokyo, Japan) were allowed free access to food and water before experimental use.

NIR Laser Irradiation and Cerebral Blood Flow Measurement

Mice were administered 20 mg/kg sodium pentobarbital intraperitoneally. Rectal temperature was maintained at approximately 37.0°C, until consciousness was regained, by using a heating pad (Digital Thermo Control Meter DT-102; Inter Medical, Nagoya, Japan) connected to a rectal thermistor. The head of each mouse was fixed with a head clamping device (Stereotaxic instrument SR-5M; Narishige, Tokyo, Japan). The skull was exposed after a midline scalp and periosteum incision with lidocaine local anesthesia. A mild dose of sodium pentobarbital was used to minimize its effects on CBF [19,20]. Therefore, local anesthesia was also used for surgery. Eight hundred eight nanometers CW diode laser (B&W Tek, Inc., Newark, DE) was applied to the left hemisphere transcranially (2 mm posterior to and 3 mm left of the bregma; Fig. 1a). The exposure field was set to 3 mm in diameter. The duration of laser irradiation was 45 minutes. To determine the appropriate power density (PD) of NIR laser irradiation, we irradiated the brain with the laser at three different PDs (0.8 W/cm^2, $n = 6$; 1.6 W/cm^2, $n = 9$; 3.2 W/cm^2, $n = 6$: NIR laser irradiation group), and laser power was checked using a photodiode-type laser power meter (PD300; Ophir Optronics Ltd, Jerusalem, Israel) before and after every irradiation. CBF in the cortex was measured semiquantitatively for both hemispheres with a non-invasive and non-contact laser Doppler blood perfusion imager (PeriScan PIM II, PeriMed, Stockholm, Sweden). By scanning the tissue with a low-power laser beam, color images of blood perfusion in the scanned area were created (Fig. 1b,c). CBF values were recorded before and at 15, 30, and 45 minutes after starting NIR laser irradiation. Indirect arterial blood pressure was monitored by using a non-preheating blood pressure monitor (MK-2000ST, Muromachi, Tokyo, Japan). Respiratory rates were also recorded. CBF and physiological parameters were also measured for sham-operated mice ($n = 9$: sham group) in the same way as described above without NIR laser irradiation.

Measurement of Brain Tissue Temperature

Information on tissue temperature is essential for understanding the mechanisms underlying the effect of laser irradiation on tissue. The temperature of tissue subjected to laser irradiation is often measured with a thermocouple directly inserted into the tissue exposed to laser light or into tissue in the vicinity of the laser irradiation area. In these cases, however, scattered laser light can directly hit the thermocouple, possibly causing an error in measurement. Thus, we estimated the temperature of brain tissue exposed to laser light by inserting a thermocouple (diameter, 300 μm; type-T;

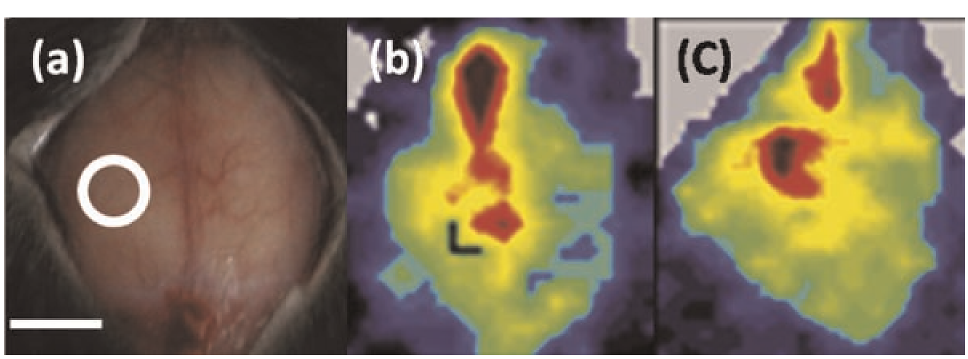

Fig. 1. Representative examples of the exposure field and perfusion images. **a**: The exposure field was set to 3 mm in diameter (2 mm posterior to and 3 mm left of the bregma). Scale bar is 5 mm in length. At the time of measurement, perfusion images were obtained (**b**: pre, **c**: 15 minutes after NIR laser irradiation). Targeted increase in cerebral blood flow of the irradiated field was observed (c).