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学位論文名	Retinal Changes After Macular Translocation With 360-Degree Retinotomy in Monkey Eyes （猿眼における360度網膜切開による黄斑移動術後の網膜変化）
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論文内容の要旨

[Perpose]

Macular translocation surgery with 360-degree retinotomy is a new surgical approach to treat subfoveal diseases and has generated widespread interest among ophthalmologists. During macular translocation surgery with 360-degree retinotomy, the retina undergoes considerable mechanical and ischemic stress, and thus there is great potential for retinal morphologic and physiologic alterations even though the central visual function has been reported to be improved in the short term in many clinical case series.

The purpose of this study was to determine whether macular translocation surgery with 360-degree retinotomy alters the morphology and physiology of the retina and fovea in monkey eyes by histologic, immunohistochemical, and electrophysiologic methods.

[Methods]

One eye each from eight female *Macaca fascicularis* monkeys weighing 2.5 to 3.5 kg whose ages were 2 to3 years was used.

Surgical procedures: The monkey eyes underwent macular translocation surgery with 360-degree retinotomy similar to those used in human eyes. The monkeys were general anesthetized and the pupil was fully dilated. Surgical procedures involves lensectomy, vitrectomy, detaching the entire neurosensory retina from the retinal pigment epithelium (RPE) by injecting irrigating BSS Plus solution into the subretinal space, freeing the retina by a

360-degree peripheral retinotomy, rotating retina to relocate the fovea onto healthier RPE, laser photocoagulation applied near the edges of the retinotomy to fix the retina, and silicone oil intraocular temponade. Silicone oil was removed in a second operation 4 weeks after the first surgery.

Fundus examination, fundus photography, and fluorescein angiography were performed before, and 1, 2, and 3 months after the surgery.

Eyes without marked postoperative complications were enucleated for further histologic investigation. (one eye at 1 month, one eye at 2 months, four eyes at 3 months after surgery). In addition, two unoperated eyes were examined as controls.

For histochemical study: Epoxy sections were prepared by a light microscope. Ultrathin sections were prepared and examined by a transmission electron microscope. Cryosections were processed for immunohistochemical study using fluorescein isothionate (FITC)-conjugated peanut agglutinin (PNA) lectin or antihuman glial fibrillary acidic protein (GFAP) antibody, and terminal deoxynucleotidyl transferase (TdT)-dNTP terminal nick-end labeling (TUNEL) assay.

For electroretinography: Retinal function was assessed by full-field, dark-adapted (scotopic) and light-adapted (photopic) electroretinograms [ERGs] that were recorded before, and at 1, 2, and 3 months (four eyes each) after the surgery. Three responses were averaged for both the scotopic ERGs (at 1-minute intervals) and the photopic ERGs (at 30-second intervals) for each eye. The amplitudes and implicit times of the b-waves were expressed as the percentages of the preoperative values.

Statistical analysis: Paired-sample *t* tests were used to compare the data. A *P* value of less than .05 was considered statistically significant.

[Results]

Surgical outcomes: Macular translocation surgery was performed successfully on the eight eyes. The retina remained reattached after removal of the silicone oil and the fovea was successfully relocated superiorly approximately 30 to 40 degrees in six of eight eyes.

Pigmentation and depigmentation of RPE were observed by ophthalmoscopy in association with hypofluorescence and hyperfluorescence during fluorescein angiography postoperatively in all six eyes. No other specific fluorescein angiography changes were detected in all six eyes.

Light microscopy: The sensory retina in the macular area was relatively well preserved with no marked misalignment of the outer segments of the photoreceptors. The RPE was juxtaposed against the outer segments. A mild retinal edema was observed in the outer plexiform layer in the perifoveal area. The fovea had a concave shape but was mildly distorted.

Transmission electron microscopy: Examination of the macula of the four eyes at 3 months showed that the inner and outer segments of the photoreceptors were essentially intact. Some swollen mitochondria were seen in the inner segments of photoreceptors and in the RPE. Some debris of outer segments was observed between outer segments and RPE microvilli. Phagosomes were present in RPE. The outer limiting membrane was intact. The RPE cells were preserved and their apical microvilli were interdigitated with the outer segments.

TUNEL assay: Positive controls showed reddish-orange, TUNEL-positive staining in all nuclei of cells in the retina. No TUNEL-positive nuclei were seen in retinal sections from negative controls. TUNEL-positive cells were not detected in the retinas of all six eyes after macular translocation surgery.

Immunohistochemistry: In unoperated retinas, the PNA-stained cone matrix sheath extended over the entire inner and outer segments of the photoreceptors. Peanut agglutinin-positive staining of the cone matrix sheaths was present in the operated retinas at 1, 2, and 3 months after surgery. The alignment of the outer segments was slightly distorted in the operated retinas after surgery. In unoperated eyes, GFAP staining was observed in the Mueller cell endfeet and the processes of astrocytes in the nerve fiber layer. At 1 month after surgery, the Mueller cells were strongly positive to GFAP and the staining extended into the inner nuclear layer. Similar positive staining for GFAP was observed at 2 and 3 months after surgery.

Electroretinography: The mean amplitudes of the b-wave at 1 month were significantly reduced to 18.2% ± 3.5% (mean ±SD) for the scotopic rod ERGs, to 45.1% ±3.0% for the rod-cone ERGs, to 23.2% ±5.2% for the cone ERGs, and to 30.2% ±3.1% for the 30-Hz flicker ERGs of the preoperative amplitudes ($P < .05$ for all). The amplitudes recovered gradually except the rod-cone ERGs, but at 3 months, they were still significantly reduced at 47.3% ±4.5% for the scotopic rod ERGs, 38.1 ±4.2% for the scotopic rod-cone ERGs, 36.1% ± 5.8% for the cone ERGs, and 40.1% ± 6.9% for the 30-Hz flicker ERGs of the preoperative amplitudes ($P < .05$, for all).

The mean implicit times of the b-wave were delayed by 28.1%± 4.1% for the scotopic rod ERGs, by 30.1% ±3.5% for the scotopic rod-cone ERGs, by 13.1% ±4.9% for the cone ERGs, and by 1.2% ± 3.1% for the 30-Hz flicker ERGs of the preoperative implicit times at 1 month after the surgery. However, these delays were not statistically significant ($P > .05$ for all). The implicit time of the ERGs recovered almost to the preoperative values at 2 months after surgery.

[Conclusions]

Macular translocation surgery with 360-degree retinotomy is feasible in terms of postoperative morphologic changes. However, there is considerable depression of physiologic function, and methods to reduce damage to the entire retina and retinal pigment epithelium cells during macular translocation surgery should be developed to make this surgery less traumatic.

論文審査の結果の要旨

本研究者はサル眼で360度網膜切開による黄斑移動術を施行し、術後1 、 2 、 3ヵ月に、移動した中心窩網膜形態を光学と透過型電子顕微鏡で検討した。視細胞のapoptosisをTUNEL分析、およびpeanut agglutinin (PNA) lectin とGlial fibrillary acidic protein (GFAP)抗体を用いて免疫組織化学的に検討した。網膜の生理学な変化を網膜電位図 (ERG)で評価した。研究結果で、360度網膜切開による黄斑移動術後中心窩網膜の形態的変化は軽微だが、有意な電気生理学的障害が残ることが示唆された。又、黄斑移動術中Ca^{2+}とMg^{2+}freeの眼内灌流液を用い網膜剥離を作成すれば、網膜色素上皮と網膜の間の粘着性を減弱させ、より侵襲の低い手術手技が可能となることが実験的に示唆された。

新生血管黄斑症は本邦の主要な失明原因のひとつである。360度網膜切開による黄斑移動術は新生血管黄斑症を治療する1つの新しい外科のアプローチで、最近多くの注目を集めている。研究目的は明確、研究方法は正確で、研究の結果は信用できるものである。本研究は世界のこの領域の研究者にきわめて貴重な実験資料を提供して、多くの患者さんを救う基礎となりうるものである。

したがって、本研究は学位に値するものと認める。