The Gamma Knife is a radiosurgical device that is routinely used to treat primary TN; however, its mechanism of action remains unknown. We established a rhesus monkey model and used the Gamma Knife device to deliver different doses of radiation to the trigeminal nerve root. We targeted a single point on one side and two points on the contralateral side. Six months after the radiosurgical treatment, the histopathological changes in the nerve tissue were examined using light microscopy and TEM to investigate the nerve length–dosage effect in the irradiated nerve, and the radiobiological mechanism of GKS for primary TN.

Methods

Experimental Animals

Five healthy male rhesus monkeys (Macaca mulatta) between 8 and 10 years of age and weighing between 7.4 and 7.8 kg were provided by the Laboratory Animal Centre, Academy of Military Medical Sciences, Beijing, China, which is fully accredited by the Institutional Animal
Care and Use Committee. One of the 5 monkeys randomly served as an untreated control, and the other 4 received GKS with irradiation to a single target in the trigeminal nerve root on one side and to two target points on the contralateral side, at a different dose in each animal.

The GKS Protocol

After the animals were anesthetized with a mixture of ketamine hydrochloride (10 mg/kg) and Sumianxin (0.05 ml/kg), a Leksell Model G stereotactic frame was placed at the maxilla and the occipital bone on either side. Initial MR imaging was performed for target localization (T1-weighted images, 1- to 2-mm-thick axial and coronal slices). The MR imaging positioning data were processed and transferred to the Gamma Knife computer station. Conformal dose planning was established using version 2.1 Betato software to localize the target point and the radiation dose. Through a 4-mm collimator, the trigeminal nerve roots were stereotactically irradiated with 60, 70, 80, or 100 Gy (a different dose in each animal) to a single target on one side and two targets 5 mm apart on the contralateral side. The treatment dose in the 4 experimental monkeys was administered in a blinded fashion.

Histopathological Investigation

Six months after irradiation, the animals were anesthetized deeply, and the trigeminal nerve roots were removed by craniotomy. The hearts were punctured, and the animals died of hemorrhagic shock. Samples were divided into 3 parts. One part was fixed in 10% buffered formalin and stained with H & E. The second part was fixed in 1% formaldehyde in calcium chloride, and the myelin was stained using multiacid 2R–brilliant green. The final part was fixed in 3% glutaraldehyde and examined using TEM.

Immunohistochemical Studies

A mixture of biotinylated secondary antibody, peroxidase linked with the streptavidin-biotin complexes, and the chromogenic substrate was used to detect the trigeminal nerve structure. The mouse anti–human neurofilament 68-kD monoclonal antibody was used to stain the filamin of nerve fibers. The color of positively stained nerve fibers in the trigeminal nerve axons ranged from tan to light brown.

Results

Examination With Light Microscopy

Routine H & E Staining. The structure of the nerve tissue was normal in the control monkey (Fig. 1A). At the same target dose, the damage to the nerve tissue by single-target-point radiation was identical to that induced by double-target-point radiation. There was no significant difference when the trigeminal nerve tissues of the monkeys irradiated with 60 and 70 Gy were compared with those of the control monkey (data not shown). In the trigeminal nerve tissue of the monkey irradiated with 80 Gy, histological examination revealed focal and patchy interruptions and vacuolation in some nerve fibers (Fig. 1B). In the trigeminal nerve tissue of the monkey irradiated with 100 Gy, there were interruptions, necrosis, and vacuolation in most nerve fibers, which was accompanied by Schwann cell hyperplasia (Fig. 1C).

Multiacid 2R–Brilliant Green Staining of Myelin. In the trigeminal nerve tissue of the control monkey, the structure of the tissue was normal, with an orderly arrangement of the nerve fibers, and the myelin sheaths and axons were normally stained (Fig. 2A and B). At the same target dose, the radiation-induced damage in the nerve tissue by single-target-point radiation was the same as that created by double-target-point radiation. In the trigeminal nerve tissues of the monkeys irradiated with 60 and 70 Gy, there was limited demyelination, fragmentation, and dissolution of axons (Fig. 2C). In the trigeminal nerve tissue of the monkey irradiated with 80 Gy, there was patchy demyelination, fragmentation, and dissolution of axons in some of the nerve fibers (Fig. 2D). In the trigeminal nerve tissue of the monkey irradiated with 100 Gy, there was extensive demyelination, fragmentation, and dissolution of axons. Necrosis in some parts of the nerve fibers was also observed, with scarce myelin sheaths and axons in the region surrounding the lesions (Fig. 2E).
Immunohistochemical Findings. In the trigeminal nerve tissue of the control monkey, the neurofilament protein of the nerve fibers was stained light brown, and the fibers exhibited an orderly arrangement (Fig. 3A). In the trigeminal nerve tissues of the monkeys irradiated with 60 and 70 Gy, there was patchy staining of the nerve fibers, indicating the presence of mild degeneration, fragmentation, and dissolution of axons (Fig. 3B). In the trigeminal nerve tissue of the monkey irradiated with 80 Gy, there were extensive patches of negatively stained nerve fibers, suggesting the degeneration, fragmentation, or dissolution of axons in some nerve fibers (Fig. 3C). In the trigeminal nerve tissue of the monkey irradiated with 100 Gy, there was extensive NF-L–negative staining, indicating degeneration, fragmentation, or dissolution of axons in most nerve fibers. Only scarce nerve fibers remained in the region surrounding the lesions (Fig. 3D).

Examination with TEM

In the trigeminal nerve tissue of the control monkey, TEM revealed a normal structure of the nerve tissue, including compact lamellar structure of the myelin sheaths, intact axon membranes, regular patterns of neurofilament, normal mitochondrial structure, normal Schwann cells, and normal vascular structures (Fig. 4A). In the trigeminal nerve tissues of the monkeys irradiated with 60 and 70 Gy, the structure of the myelin sheaths and axons was still intact. However, a loose lamellar structure of the myelin sheaths, focal collapse, membrane shrinking, and occasional dissolution of axons were observed (Fig. 4B). In the trigeminal nerve tissue of the monkey irradiated with 80 Gy, the nerve tissue showed a disordered structure, with partial collapse of the myelin sheaths, dissolution of axons, disappearance of neurofilaments, vacuolation in mitochondria, and degranulation in rough endoplasmic reticulum (Fig. 4C). In the trigeminal nerve tissue of the monkey irradiated with 100 Gy, the structural derangement was more noticeable, with diffuse plaques in myelin sheaths and dissolution of axons; however, the myelin sheaths remained. Schwann cells shrank in volume, and there were prominent lipid droplets (Fig. 4D).

Discussion

Gamma Knife surgery has been used as a routine treatment method for primary TN. Many studies support GKS as the primary treatment for TN. However, the radiobiological mechanism of Gamma Knife radiation remains unknown; there have been very few studies on this mechanism. Kondziolka et al. studied the trigeminal nerves in 2 adult baboons. These authors used a 4-mm collimator and single-target-point radiation on the trigeminal nerve roots, with a target dose of 80 Gy in one animal and 100 Gy in the other. Six months after irradiation, light microscopy and TEM studies revealed vacuole-like demyelination in the myelin sheaths of the
irradiated nerve root, and swelling, degeneration, and even decomposition and dissolution of the axons in the lower-dose (80-Gy) group. In addition to these changes, there were hardly any normal myelinated nerve fibers near the center of the target in the higher-dose (100-Gy) group. One of the trigeminal nerves was totally necrotic. The authors suggested that the radiobiological mechanism of GKS for TN might be related to axonal degeneration and decomposition of a large number of neurons, which helped to relieve the pain. In the meantime, the normal neural function was maintained by a sufficient number of intact axons. There was a dose-dependent relationship between pain relief and the sensory function that was retained.

We established a new animal model in which the Gamma Knife was used to irradiate the trigeminal nerve root. Different doses of radiation were applied, with a single target point on one side and two target points on the contralateral side. After 6 months, light microscopy, TEM, and immunohistochemical examination revealed pathological changes similar to the results detailed by Kondziolka et al. We found that the target doses of 60 and 70 Gy can induce very mild damage in the trigeminal nerve, such as degeneration, fragmentation, and dissolution of axons in a few nerve fibers, suggesting that the irradiation-induced biological effect was not marked. At a target dose of 80 Gy, the neuropathy in the trigeminal nerve became worse, with partial demyelination and degeneration, and fragmentation or dissolution of the axons, suggesting a marked radiobiological effect. At a target dose of 100 Gy, the neuropathy in the trigeminal nerve became even more severe. The nerve tissue showed a disordered structure, with extensive demyelination and loss of axons. The nerve roots were necrotic, with scarce myelin sheaths and axons in the peripheral tissue.

Our study suggests that the radiobiological mechanism of GKS in treating the trigeminal nerve may be related to the degeneration and dissolution of axons, which block the afferent component of the trigeminal nerve and interfere with the electrophysiological changes, thus reducing or relieving TN. The extent of damage is related to the target dose of radiation. With lower doses of radiation, such as 60 and 70 Gy, pain relief is not produced because the number of axons damaged is not sufficient. When 80 Gy is administered, more axons are degenerated and dissolved, but there is a sufficient number of intact axons to maintain normal neural function. Clinically, the efficacy of pain relief is significantly increased and the incidence of complications is very low. The relationship between pain relief and sensory function is dose dependent and is kept in balance. This therefore suggests that the target dose of 80 Gy may produce the best radiobi-
Gamma Knife irradiation-induced histopathological changes in TN

**Fig. 4.** Photomicrographs showing TEM studies of trigeminal nerve. A: Control trigeminal nerve; TEM revealed a normal structure of the nerve tissue, including compact lamellar structure of the myelin sheaths, intact axon membranes, regular patterns of neurofilament, and normal mitochondrial structure, Schwann cells, and vascular structures. B: The trigeminal nerve irradiated with 60 Gy. The structure of the myelin sheaths and axons was still intact. However, loose lamellar structure of the myelin sheaths, focal collapse, membrane shrinking, and occasional dissolution of axons were observed. C: The trigeminal nerve irradiated with 80 Gy. The nerve tissue showed a disordered structure, with the myelin sheaths partially collapsed, dissolution of axons, disappearance of neurofilaments, vacuolation in mitochondria, and degranulation in rough endoplasmic reticulum. D: The trigeminal nerve irradiated with 100 Gy. The structural derangement was more noticeable, with diffuse plaques in myelin sheaths and dissolution of axons; however, the myelin sheaths remained. Schwann cells shrank in volume, and there were prominent lipid droplets. Original magnification × 6000 (A and D), × 10000 (B), × 2500 (C).

**Conclusions**

The advantage of double-target-point treatment is more obvious than that of single-target-point treatment. Our study also confirmed that at the same dose of radiation, the damage in the trigeminal nerve resulting from single-target-point treatment was similar to that from double-target-point treatment. There was no significant difference in the nerve length–dosage effect in the exposed nerve, but an increase in the irradiated target area can compensate for the lack of exposure of the trigeminal nerve (especially when the trigeminal nerve root is close to the pons) to single-target-point radiation. This provides a preliminary theoretical basis for the further application of double-target-point treatment in clinical practice.

**Disclosure**

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