The effect of gamma knife irradiation on functions of striatum in rats

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Object. An animal model has been developed to study the effect of gamma knife surgery (GKS) on cerebral function.

Methods. A rat was fixed in a newly developed Régis–Valliccioni frame that enables the target region to be planned directly on the magnetic resonance images. The left striatum was irradiated with 150 Gy via a 4-mm collimator of the Leksell gamma knife. Apomorphine (dopamine agonist) was administered to elicit a circling behavior (apomorphine test) after the GKS so as to examine the time course of the changes in dopaminergic functions of irradiated striatum. After a series of behavioral analyses, irradiated brains were subjected to histological examination.

Necrosis was observed in the irradiated area surrounded by hemorrhage and gliosis. The distance between the histologically estimated and planned centers of the irradiation areas was 1.0 ± 0.5 mm. The extent of the distance was due to errors along the dorsoventral axis. The distribution of the irradiation areas influenced the activity and the circling behaviors in the apomorphine test, which was suggestive of involvement of the nigrostriatal pathway.

Conclusions. Targeting by using the Régis–Valliccioni frame was very accurate compared with targeting with coordinates based on brain maps used hitherto. Although targeting improved the accuracy, further effort will still be necessary to reduce errors along the dorsoventral axis. The apomorphine test indicated a reduced dopaminergic function of the irradiated area including striatum, which accompanied histological changes after a high dose of irradiation (150 Gy).

Key Words • gamma knife • stereotactic surgery • magnetic resonance imaging • striatum • dopamine • rat

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amma knife surgery has been initiated for functional neurological diseases such as trigeminal neuralgia,14 focal epilepsy,22–24 and pain control.2 Despite the recent proliferation of GKS, the neurophysiological mechanisms of the biological effects of focal GKS remain largely undefined. There exists a need for a simple model in which the effects of focal cerebral irradiation can be evaluated in a systematic and controlled manner; that is, a study on functional changes in a temporal sequence. Our group has been engaged in developing an animal model for this purpose. The rat striatum was irradiated to examine the effects of GKS on cerebral tissue when treating functional diseases.

In our preliminary experiments 12 rats underwent 150-Gy GKS based on a standard stereotactic atlas.23,24 Behavioral changes were observed several months postprocedure. In spite of a relatively high dose of irradiation, no histological change was observed in the brains 42 to 57 weeks after the irradiation. Because some rats showed minor histological changes in the oral cavity, we speculated that the irradiated volume had deviated significantly from the intended target. Those preliminary data underlined the importance of accuracy in the targeting procedure.15

The Régis–Valliccioni frame (Neurospace, Neuilly, France) has been introduced and enables the target region to be planned directly on the MR images. The accuracy of the GKS targeting with the new frame was evaluated by comparing the planned center of the irradiated area and the actual necrotic lesion on brain slices. The biological effects of GKS on rat striatum were analyzed by the apomorphine test and histological examination of the slices of the irradiated brain.

Materials and Methods

The study was performed with the approval by the Animal Experiments Committee of the Tokyo Women’s Medical University. A total of 10 male Wister rats weighing 320 to 380 g (Sankyo Lab. Co., Tokyo) were used in this study.

Abbreviations used in this paper: GKS = gamma knife surgery; MR = magnetic resonance.
Gamma Knife Irradiation

Rats were anesthetized with pentobarbital (40 mg/kg intraperitoneally) and fixed in the Régis–Valliccioni frame by using earplugs and an incisor bar so that the head was in the flat skull position. The Régis–Valliccioni frame is a specially designed frame, which enables target planning directly on the MR images as shown in Fig. 1.

Imaging was performed using an Excelart 1.5-tesla unit (model MRT2001/P3; Toshiba Medical Systems Co., Tochigi, Japan). Coronal steady-state free precession three-dimensional images were acquired using a 1-mm slice thickness with no gap and the matrix was 224 × 256. The repetition time, echo time, and the number of acquisitions were 12 msec, 6 msec, and two, respectively.

The images were transmitted to the Leksell GammaPlan treatment planning system (Elekta Instrument AB, Stockholm, Sweden), where the three-dimensional coordinates of the target area were calculated and defined in the caudate–putamen region. A central maximum dose of 150 Gy was administered to the left striatum with the Leksell gamma knife model C (Elekta Instrument AB) unit by means of a 4-mm collimator.

The intended center of the target area was 0.2 mm caudal and 3 mm to the left of the bregma 5.6 mm deep to the dorsal surface of the cerebrum. This location should enable the irradiation volume to cover the left striatum. The GKS area was targeted either by one of two methods.

Plan A. The area to be irradiated was manually drawn on the MR images in five cases, assuming that the left striatum was covered. It should be noted that it was difficult to find the “intended” center on the MR images, so the “planned” coordinates did not necessarily fall exactly on the intended center.

Plan B. The center of the area to be irradiated was calculated with reference to the visible structures of the cerebrum on the MR images in five cases (Fig. 2). The rostral and caudal ends of the cerebrum on the MR images were identified (Fig. 2, horizontal) and the distance between them was calculated. A coronal plane 35.4% of the distance posterior from the...
rostral end was defined. 2) The median line was determined on the MR images. A sagittal plane 3 mm to the left of the median line was determined. 3) The dorsal surface of the cerebrum at the level of the coronal plane defined above was identified. A horizontal plane was defined 5.6 mm inferior from the dorsal surface at the afore-mentioned level. The intersection of those three planes was the center of the target area of GKS irradiation.

**Behavioral Analysis**

Apomorphine is a dopamine agonist that stimulates dopamine receptors directly. If dopaminergic function is impaired on the irradiated side, apomorphine administration will result in unbalanced dopaminergic activity between the right and left striatum and induce circling behavior.3,17 All rats were screened with an apomorphine test prior to the GKS and those with unbalanced dopaminergic activity were excluded from the study. The apomorphine test was performed 2, 4, 6, and 8 weeks after the GKS and changes in dopaminergic function in the striatum were evaluated based on the results of the behavioral test. The nature of the test is described as follows.

A rat was placed in an area made of clean acrylic resin (60 cm × 60 cm × 40 cm), and its behavior was recorded with a video camera. The location and the movement of the rat were tracked and analyzed by a computerized rat behavior tracking system.15 The test procedures were as follows.

Initially there was an acclimatization period: a rat was placed in the area and acclimatized for 30 minutes. Next there was the predrug period: before administration of apomorphine, tracking data were recorded for 30 minutes. Third, apomorphine was administered intraperitoneally at a dose of 2 mg/kg. After apomorphine administration, the tracking data were recorded for 60 minutes.

Parameters for “activity” and “circling behaviors” were indexed by analyzing the tracking records of apomorphine test. Activity is a sum of the distance the rat moved, which reflects the level of the movements of the rat in the arena. Circling behavior is a sum of turning angles between the adjacent vectors, which represent the circling movement of the rat.

**Histological Examination**

The histopathological effects of 150-Gy GKS were evaluated 4 to 8 weeks after irradiation by examining serial coronal sections of the brain. The rats were deeply anesthetized and transfused transcardially with normal saline for 10 to 15 minutes and then placed in a fixative solution containing 2.5% paraformaldehyde and 0.05% glutaraldehyde in 0.1 M phosphate buffer, pH 7.4. The brain was dissected out and immersed overnight in the same fixative at 4°C. After cryoprotection with 25% and 40% sucrose in phosphate-buffered saline, the brain was quick frozen. Serial 25 μm-thick cryosections were mounted on glass strips and kept frozen at −80°C until further staining procedures were performed. One section in every five slides was stained in...
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Fig. 5. Schematics showing the distribution of the irradiation centers in brain structure. Coordinates are given relative to the bregma. Views are from the top (upper) and from the left side (lower). The centers irradiated by using Plan B (circles) were located anterior, medial, and shallower than those irradiated using Plan A (triangles), and significantly closer to the intended center (p < 0.05, Wilcoxon rank-sum test).

Fig. 6. Graphs showing the effect of GKS irradiation on predrug (spontaneous) activity. Predrug (spontaneous) activity was compared before and 4 weeks after GKS. No significant difference was observed in those treated with Plan A (left). Predrug indices after GKS were significantly larger than those before GKS in those rats irradiated with Plan B (p = 0.031, sign test, right).

FIG. 6. Graphs showing the effect of GKS irradiation on predrug (spontaneous) activity. Predrug (spontaneous) activity was compared before and 4 weeks after GKS. No significant difference was observed in those treated with Plan A (left). Predrug indices after GKS were significantly larger than those before GKS in those rats irradiated with Plan B (p = 0.031, sign test, right).

Results

Accuracy of Targeting

Sections through the irradiated volume revealed a necrotic lesion, as shown in Fig. 3. The center of the radiation was determined to be the center of the necrotic volume. The mean difference between the targets planned by GammaPlan and the histologically observed centers of radiation was 1.1 ± 0.5 mm. The deviations in the different directions were as follows: rostrocaudal 0.3 ± 0.3 mm, left–right 0.4 ± 0.3 mm, and depth 0.9 ± 0.6 mm, as illustrated in Fig. 4.

With Plan A, the mean deviation of the estimated centers of the irradiation volumes from the intended anatomical targets was 2.8 ± 0.5 mm. The deviations in the different directions were as follows: rostrocaudal 1.9 ± 0.4 mm, left 0.5 ± 0.4 mm, depth 2 ± 0.4 mm deeper than intended as illustrated in Fig. 5 triangles. With Plan B the mean deviation of the irradiated volumes from the intended anatomical target locations was 1.4 ± 0.3 mm. In the different directions the deviations were: caudal 0.2 ± 0.3 mm, medial 0.5 ± 0.4 mm, medial 1.4 ± 0.3 mm deeper than intended as illustrated in Fig. 5 circles. The estimated centers irradiated in Plan B were located anterior, medial, and dorsal to those produced using Plan A. They were significantly closer to the intended target point (p = 0.014, Wilcoxon rank-sum test). One rat treated with Plan B was excluded from the analysis of the accuracy because of unsatisfactory MR images.

Reactions to GKS and the Apomorphine Tests With Plans A and B

Predrug (spontaneous) activities were compared between before and 4 weeks after GKS. No significant difference was observed in those treated with Plan A, as illustrated in Fig. 6 (left). Predrug activity after GKS was significantly greater 4 weeks later in animals in which Plan B was used (p < 0.05). This is illustrated in Fig. 6 (right).

Activities were compared between predrug and postdrug periods 4 weeks after the GKS. No significant difference was observed in animals treated with Plan A (Fig. 7 left). A significant reduction of the activity index was observed on apomorphine administration 4 weeks after GKS in animals set up according to Plan B (p < 0.05). This is shown in Fig. 7 right.
Accuracy of Targeting

The mean discrepancy between the target location planned on the images and the actual lesion found at histological examination was 1.1 mm. The error between the intended target in the brain and the center of the part of the brain which was the real target was 2.8 mm when using Plan A and 1.4 mm when using Plan B. In other words there was good agreement between the location of the chosen target on the images and the place where the treatment planning system placed the lesion. The choice of the desired target on the images was more difficult to make when chosen by eye. The use of measurements similar in principle to those used to calculate the location of a thalamotomy lesion based on the anterior commissure–posterior commissure line gave better accuracy.

Kamiryo and colleagues' argued that localization of target coordinates based on the stereotactic atlas alone is generally sufficient. Indeed, they considered that the anatomical target will be better localized using the stereotactic atlas because of the size and poor differentiation between white and gray matter of the rat brain. Some sophisticated skills are needed to fix an animal to the frame with earplugs and an incisor bar, and it would be difficult to conduct a series of experiments consistently without such skills. Our method of targeting does not require these skills and would enable researchers to irradiate the target point precisely based on the MR images.

One of the reasons why the error in the depth direction was relatively large in the present study is its referencing method. In the rostrocaudal direction, the coordinate was determined based on two reference points: the rostral and caudal ends of the cerebrum. Although the coordinate in the left–right axis was determined based on one reference (the median line), it was drawn in the middle of the bilateral hemispheres; that is, determined based on virtually two references. As for the determination of the depth, only the dorsal surface was used as the reference. A reference line had to be drawn on a gradient of signal intensity and the decision depended on the operator.

Histological Examination

Figure 3 shows a coronal section of an irradiated brain with a necrotic lesion stained with H & E. Arrows indicate the necrotic lesion by which the center of the irradiated area was estimated. Recent hemorrhage was observed around the irradiated necrotic area. Astrogliosis was observed on the irradiated side on glial fibrillary acidic proteinlike immunoreactivity staining. It surrounded the necrotic lesion. Deterioration of axons of dopaminergic cells was observed on the irradiated side near the origin of axons by means of tyrosine hydroxylase–like immunoreactivity.

Discussion

Although the clinical use of focal cerebral irradiation for functional brain disorders has grown,12 studies on the impact of GKS on basic cellular mechanisms are hard to find. Thus the development and refinement of appropriate tools for studying the brains of small experimental animals is important for the investigation of the mechanisms whereby GKS induces alterations in central nervous system function. Some data have been accumulated.1,13,15,16,15,25,30 In stereotactic radiosurgery, the visualization and accurate localization of target volumes are of the greatest importance. In the experimental context, the small size of the rat brain requires even greater precision in the targeting of irradiation treatments. In the present study, a small difference in the center of irradiated area resulted in differences in the results of the behavioral test. It is indicated that a high level of spatial accuracy in targeting GKS is required in conducting any behavioral study. It is emphasized that it is essential to establish an accurate targeting procedure in an animal model of functional brain diseases if the results of the experiments are to have significance.

Accuracy of Targeting

Circling behavior was evaluated 4 weeks after GKS. Apomorphine produced leftward circling behavior only in those rats treated according to Plan A (p < 0.031), as illustrated in Fig. 8.

Histological Examination

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Kamiryo and coworkers19,20 reported a mean spatial error of 0.5 mm with the maximum of 1 mm in a study in which a phantom was used.

We are the first to report on the use of the Régis–Valliccioni frame in directly targeting the irradiation point by using MR images of the experimental animal. Gamma knife surgery based on MR imaging has the great advantage of eliminating errors due to individual anatomical variation and differences in skill levels when fixing an animal to the frame. The mean errors in rostrocaudal and left–right axes were 0.2 and 0.5 mm, respectively, with Plan B. On the contrary, the error in the depth direction was as large as 1.4 mm, which contributed to the net error in targeting with Plan B. The net error observed in this study was larger than those previously reported.6,19,20,26

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Another factor to consider is that MR images suffer to varying degrees from spatial distortion. The two most complex sources of spatial distortion in MR imaging are gradient field nonlinearities and magnetic field inhomogeneities. The differences between computerized tomography and MR images were reported to be 1.8 to 2 mm. Using the normal head coil, the mean difference was 1.2 ± 0.4 mm. If it is possible that such spatial distortion might contribute to the error in our method of targeting.

Another technical concern associated with performing GKS would be the calibration of dosages in a small-animal brain. The error in dosage was less than 5% of the calculated dose when the target was deeper than 1 mm from the surface of the skull when using model U and less than 10% for model B. Novotny, et al., 19,20 reported similar accuracy in their studies using a phantom. From the results of those previous studies, it is reasonable to speculate that the error in dosage would be within 10% in the present study.

Histological Examination

Astrocytosis, hemorrhage, and necrosis were observed from the periphery to the center of the irradiated area. The time course and extent of cerebral damage induced by gamma knife irradiation are known to vary as a function of dose. A relatively high dose of radiation (>100 Gy) elicits necrotic changes within a few weeks, preceded by astroglialosis and vasculopathy. Lower doses (≤50 Gy) are subnecrotic but elicit changes in glial structure and physiological function in the brain.9

Behavioral Analysis

The apomorphine test was performed to detect unbalanced activity of dopaminergic neurons in the rat striatum.1,7 By administering a dopamine agonist, otherwise compensated unbalanced activity is made apparent. The results of the apomorphine test indicated a reduced dopaminergic function of the irradiated areas approximately 4 weeks after GKS, which coincided with the appearance of the histological changes. It was reported that changes in a behavioral test after irradiation to bilateral hippocampus were accompanied by necrosis and edema.10

Circling behavior was observed in those rats set up by using Plan A whereas increased predrug (spontaneous) activity was observed in those treated with Plan B. The difference in the results of apomorphine test may be attributable to the difference in the distribution of irradiated areas. With Plan A, the centers of irradiation were distributed posterior and deeper than the intended center point. If an area posterior and ventral to the intended area were irradiated, it is possible that it covered the left nigrostriatal pathway. This pathway is lesioned by 6-hydroxydopamine in an animal model of Parkinson disease. It is tempting to think that irradiation of the left nigrostriatal pathway by means of Plan A caused the loss of dopaminergic cells in the left substantia nigra through retrograde axonal transport and dopaminergic terminals in the left striatum through axonal disruption. These results should indicate the importance of the accuracy of targeting.

Future Study

Further improvement of the accuracy of targeting is the next challenge. In particular the error in the depth direction must be reduced. One possible way would be taking two reference points; that is, the dorsal and ventral surface of the cerebrum. Another way would be referencing to a point close to the target such as the anterior commissure.

Doses lower doses than 50 Gy are subnecrotic but elicit physiological changes in the brain. An animal model could be used to test the effects of GKS at these lower doses. Functional alteration would be evaluated by behavioral tests such as the apomorphine test and detailed histopathological studies could reveal morphological changes at a subnecrotic dose.

Conclusions

The accuracy of matching the target to the intended anatomical location was less in this study than in previously reported work. The Regis–Valliccioni frame was used for the first time in this kind of study. It has the advantage that it requires no skill in fixation, in contrast with experimental arrangements reported by others. If animals are set up according to Plan B or something like it adequate accuracy should be achievable. The apomorphine test was a useful indicator of correct target localization.

Meaningful results in stereotactic experiments in small animals can only be achieved if the anatomical accuracy of the method can be guaranteed.

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