# Validation of an Animal FDG PET Imaging System for Study of Glioblastoma Xenografted Mouse and Rat Models

Tien-Jen Lin[1,2,3], Chi-Chang Huang[1,3], I-Jen Wang[4,5], Jia-Wei Lin[2], Kuo-Sheng Hung[2,3], Ling Fan[6], Hsin-Hsin Tsao[6], Ning-Sun Yang[1], Kun-Ju Lin[6,7]

*[1]Agricultural Biotechnology Research Center, Academia Sinica, Taipei, Taiwan*
*[2]Department of Neurosurgery, Wan Fang Hospital, Taipei Medical University, Taipei, Taiwan*
*[3]Institute of Injury Prevention and Control, Taipei Medical University, Taipei, Taiwan*
*[4]Department of Pediatrics, Taipei Hospital, Department of Health, Taipei, Taiwan*
*[5]China Medical University, Taichung, Taiwan*
*[6]Department of Nuclear Medicine and Molecular Imaging Center, Chang Gung Memorial Hospital Linkou Medical Center, Taoyuan, Taiwan*
*[7]Department of Medical Imaging and Radiological Sciences, Chang Gung University, Taoyuan, Taiwan*

**Introduction:** Because of the high incidence and poor prognosis of gliomas, the development of pre-clinically effective diagnostic tools is of great importance. The objective of this study is to validate the use of FDG PET imaging system for monitoring glioma proliferation in two rodent models.

**Methods:** Two kinds of glioblastoma cells (human DBTRG-05MG and rat RG2 tumor cells) were implanted intracerebrally to SCID mice and Wistar rats, respectively. To characterize the optimal scanning time required for effective detection of brain tumors, dynamic animal PET were acquired for 1 and 2 h immediately after intravenous injection of the FDG radiotracer to mice and rats, respectively. Test animals were then subjected to serial animal PET scans at day 7, 10, 14, and 17 after tumor cell implantation.

**Results:** Mouse and rat brain tumors were first detected by FDG microPET imaging at day 7 and 10 after tumor implantation, respectively. The smallest tumor size detectable was 2.5 mm in diameter. The peak tumor-to-background ratio was observed at 40 min post-injection in the mouse model and at 90 min post-injection in the rat study. Both the peak standard uptake value of FDG and the tumor-to-background ratios were found to increase as the tumors grew over time.

**Conclusion:** A FDG PET scan protocol was validated for detecting and monitoring glioma tumor growth in both mouse and rat models. Optimal FDG uptake period required and optimal scanning times for experimental tests were hence established for future systematic studies in relevant animal models.

**Key words:** animal PET, FDG, glioma, brain tumors

Ann Nucl Med Sci 2010;23:77-83

## Introduction

Gliomas are the most common human primary intracranial neoplasms and 50% of gliomas are glioblastomas, the most fatal primary brain neoplasm [1,2]. Glioma is one of the most challenging conditions, both diagnostically and therapeutically, for the neuro-oncologist and the prognosis for patients with malignant gliomas is often very poor [3]. In view of the high incidence (10-15 per 100,000) of malignant brain tumors and their poor prognoses when treated with con-

Received 1/6/2010; revised 2/24/2010; accepted 3/2/2010.
For correspondence and reprints contact: Kun-Ju Lin, M.D., Ph.D., Department of Nuclear Medicine, Chang Gung Memorial Hospital. 5 Fu-Hsing Street, Kuei-Shan, Taoyuan 333, Taiwan, ROC. Tel: (886)3-3281200 ext. 2625, E-mail: lin4857@adm.cgmh.org.tw, kunjulin@gmail.com

ventional therapies, such as surgery, chemotherapy, brachytherapy, and radiotherapy, research focusing on the development of pre-clinically effective diagnostic tools is of great importance for future improvement in therapy [4,5]. Although gliomas rarely metastasize outside the central nervous system, they are however, capable of spreading long distances within the brain [6]. Preventing such glioma invasion therefore has the potential to convert this highly malignant tumor into a focal disease, which hopefully could then be effectively treated with focal therapies.

Positron-emission tomography (PET) can provide specific metabolic activity data related to specific areas of tissue/tumor growth, and it has particular advantages for the visualization of glioma development [7-11]. In addition, it can evaluate treatment response and predict prognosis [7]. In previous reports, a good correlation was established between [$^{18}$F]fluorodeoxyglucose (FDG) uptake and grade of glioma [7-9]. FDG allows the detection of increased glucose uptake, which is characteristic of many malignant cells; as a result FDG PET has previously been used successfully in a variety of oncology studies. Recent studies of other organ systems have demonstrated a close correlation between FDG uptake and the proliferative activity of tumors [10-12]. The objective of this study was to establish useful malignant glioma animal models in both mice and rats by which we can develop the experimental protocols and capacity to use a noninvasive FDG PET imaging system for characterizing and monitoring intracranial tumor size and behavior, especially over a range of time and treatments.

## Methods

### *Cell lines and culture conditions*

DBTRG-05MG cells (Bioresources Collection and Research Center; BCRC, Hsinchu, Taiwan), a human brain glioblastoma cell line, and RG2 cells, a rat glioma cell line (American Type Culture Collection; ATCC), were grown in Dulbecco's modified Eagle medium (DMEM, Life Technologies, Gaitherburg, MD, USA) supplemented with GlutaMAXTM and 10% heat-inactivated fetal bovine serum, 100 U/mL penicillin, and 100 μg/mL streptomycin (Invitrogen, Carlsbad, CA), in a humidified 5% $CO_2$ incubator at 37˚C.

### *Animals*

SCID mice (n = 4, 6-8 weeks old) and Wistar rats (n = 5, 12-13 weeks old) obtained from the Laboratory Animal Center, National Taiwan University College of Medicine (Taipei, Taiwan) were implanted with test glioma cells. All procedures were performed in compliance with the standard operating procedures at approved by the IACUC of the Laboratory Animal Center, Academia Sinica, Taipei, Taiwan.

### *Establishment of orthotopic human brain tumor xenograft in SCID mice*

Intracerebral tumors were induced by xenografting human DBTRG-05MG glioma cells into SCID mice. Animals were first anesthetized with ketamine (20 mg/mL) plus xylazine (0.75 mg/mL) in saline administered intraperitoneally at 0.7 mL/10 g of body weight. A midline scalp incision (0.5-1 cm) was made, skin was reflected, and a 1-mm burr hole was made in the skull 1.5 mm to the right of midline and 0.5-1.0 mm anterior to the coronal suture. Tumor cells were loaded into a 250-μL Hamilton syringe fitted with a 30-gauge 0.5-inch needle, attached to a repeating dispenser, and mounted in a stereotactic holder. The needle was inserted vertically through the burr hole to a depth of 2.5 mm as read from the vernier scale. After injection of $5\times10^4$/10 μL of cells into the right caudate nucleus, the needle was withdrawn after 30 sec, the burr hole was plugged with gel foam, and the skin was closed with sterile Michel 9-mm wound clips.

### *Generation of othotopic brain tumor in rat*

Syngeneic animals (three rats per group) were implanted with $5\times10^4$/10 μL RG2 cells intracerebrally (i.c.) into the striatum as described above.

### *PET imaging procedure*

All rats and mice were studied using FDG microPET in a resting state. Rats underwent serial scans on day 7, 10, 14 and 17 after tumor implantation and mice underwent serial scans on day 7, 10, 14 post implantation. After intravenous (i.v.) injection of FDG (18.5 and 11.1 MBq for each rat and mouse, repectively), PET scans were acquired on a Siemens Inveon microPET using 3D mode with an axial FOV of 12.7

cm. These studies were performed with the animal positioned at the center of the observation field. Following scatter, dead time and random corrections, and PET images were reconstructed by 2D OSEM.

To optimize the scanning time for detecting glioma tumor, dynamic PET scans were performed for 1 and 2 h immediately after injection of FDG to mice and rats, respectively. The time-activity curves (TACs) obtained from brain tumor and normal brain background regions are shown in Figures 1A and 1C, respectively. The tumor-to-background ratios in rat and mouse studies are shown in Figures 1B and 1D, respectively. Of noted, the peak ratios reached at 90 and 40 min post-injection in rat and mouse studies, respectively.

To evaluate the FDG activity in the brain tumor, the static PET images were extracted from the dynamic PET study during the optimal scanning time period. In this regards, a 3D emission data of 40-60 min and 90-120 min post-injection were used for mouse and rat, respectively.

### *Tissue sampling and histology*

After the final PET measurements, animals were sacrificed and intracerebral tumors were removed rapidly. After tissue fixation (with 4% paraformaldehyde at 4˚C for 24 h), tumors were embedded in a standard tissue-freezing medium (O.C.T. compound; Tissue-Tek, Sakura, Torrance, CA, USA) and 20-μm frozen sections were made along the transaxial plane relative to the tumor position in the test animal. Standard H & E staining was used for histomorphological evaluation.

## Results

### *Tumor growth in FDG images*

Having determined that the optimal scanning time was 40 min post-injection, all static PET images were collected at

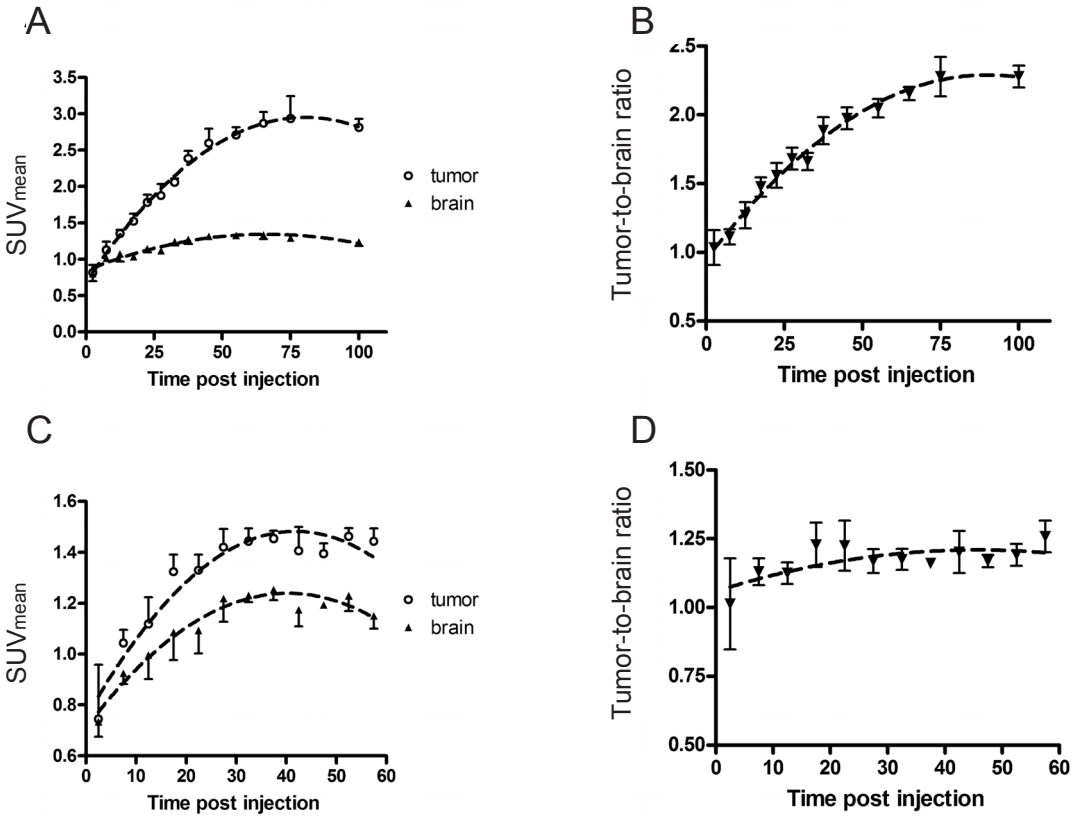


**Figure 1.** Tumor and normal brain background time-activity curve (TAC) and its ratio in rat (A and B) and mouse (C and D) studies (n = 3, mean ± SD) after intravenous injection of FDG.

that time point. Profile of the mouse tumor growth is shown in Figure 2. Active imaging activities on tumor cells were observed 7 days after tumor implantation, and the volume had increased slightly on day 10 (Figure 2). On day 14, we observed that tumor mass had reached the highest level of FDG uptake. In the rat glioma model, tumor cells were detected 10 days after tumor cell implantation (Figure 3). The effect of surgical wounding was observed on day 7 and 10, after then it became diminished. The volume of tumor cells kept expanding until day 17. Tumor masses were first detected by animal FDG PET at day 7 and day 10 after tumor implantation in mouse and rat, respectively.

***Tumor harvesting, preparation and histopathologic studies***

As shown in Figures 2 and 3, *in vivo* tumor cell growth was clearly distinguishable and their progression was effectively observed via FDG scans. After sacrificing, animals and the tumor cells were verified by H & E staining, the correspondent data between *in vivo* images and tumor necropsy were correlated. (Figures 2 and 3B, C, D, E).

## Discussion

In this study, we have successfully demonstrated the capability of a noninvasive FDG PET imaging system to effectively estimate the tumor size *in vivo*/ *in situ* and study the behavior of emerging tumors in intracranial SCID mouse and Wistar rat glioma models. Our results show that optimal FDG uptake period can be readily obtained experimentally and useful scanning time periods were detected and comparatively evaluated on day 7, 10, 14, and 17 after tumor implantation. The rat glioma in cell culture grew progressively through three phases, namely, exponential, stationary, and a variable phase, limited by space and medium. The cell-cycle time of the glioma *in vivo* was 20 h, the growth fraction was 0.35 to 0.46, the observed doubling time was 72 h, and the cell-loss factor was 0.42. Since test mice need to be terminated after 17 days in experiment, and these data, along with the chronology of the cell-cycle phases, enhance the usefulness of this system as a model for brain tumor chemotherapy [13]. Thus, we suggest the optimal time frame for post tumor cell inoculation chemotherapy treatment to be set between day 4 and 10 in a regular or standard protocol.

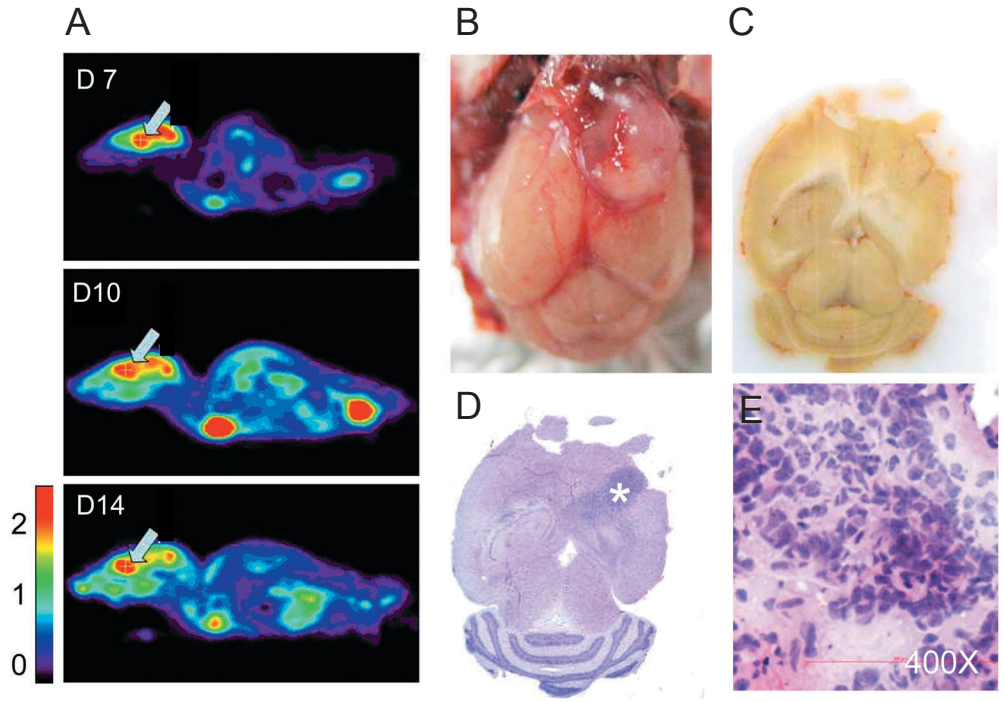


**Figure 2.** FDG PET images of mice (A, arrow: brain tumor, the color scale represents the standard uptake value of FDG). The brain tumor gross pictures (B and C) and histopathologic results (D and E) of the brain tumor were acquired immediately after the last PET scan was taken.

Traditional methods for preclinical evaluation of intracranial glioma models, including postmortem tumor volumetry and survival studies, are time-consuming and often require a large number of experimental animals due to the intrinsic biological variability of test tumors. In contrast, *in vivo* FDG-PET scan system produces good evidence-based results which allow estimation of the glioma size in small cohorts of test mice and rats. We have therefore established two animal model protocols that can be manipulated for rapid screening and effective evaluation of potential therapeutic approaches for malignant gliomas. Since the sensitivity of the assay procedure can be adjusted according to the number of tumor cells inoculated, it is therefore possible to prolong the survival of test animals by inoculating lower doses of tumor cells. As a result, this system provides an increased opportunity to investigate the tumoricidal effects of multiple treatments with a low dose of initial inoculating tumor cells. Of noted, the image protocol needs further test for the first tumor detectible time period might change accordingly. In this study, the smallest detectable tumor size was 2.5 mm in diameter. The peak tumor-to-background ratios reached at 40 min after radiotracer injection in mice and 90 min post-injection in rats. Both peak standard update value of FDG and tumor-to-background ratio were detected to increase with tumor growth over the test time course. Therefore, these results demonstrate the capacity of FDG-PET activities to provide insights into early cellular and metabolic events in regulating tumor growth.

As these studies successfully demonstrated the feasibility of FDG-PET for detection of intracranial tumor growth in both rat and mouse glioma models, we can now effectively plan to conduct systematic studies that will employ multiple time points and longer observation periods to determine the optimal imaging window for a broad range of therapeutic agents with a variety of regulatory mechanisms. Future studies may also compare metabolic PET to the *in vivo* tumor volumetric MRI studies, in order to determine whether PET analysis can provide a temporal advantage for assessing glioma cell proliferative activities as well as for potential benefit in combining the respective information to be obtained from these two different modalities. Finally, other PET tracers, such as $^{18}$F-fluorothymidine or 5-[$^{124}$I]iodo-

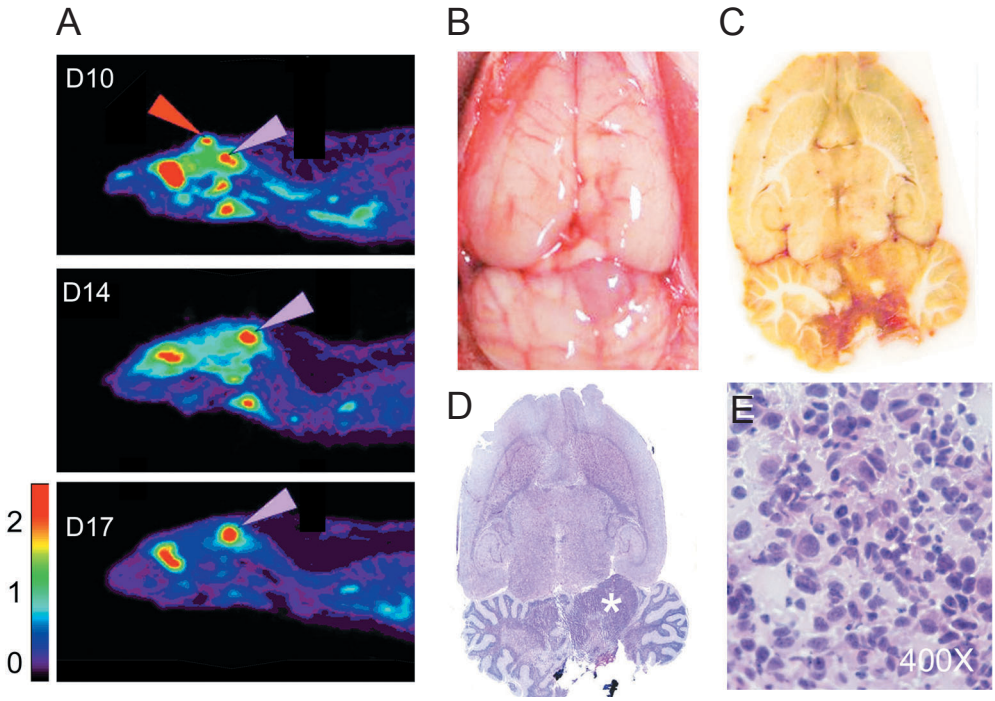


**Figure 3.** FDG PET images of rats (A, pink arrow: brain tumor; red arrow: operation wound, the color scale represents the standard uptake value of FDG). The brain tumor gross pictures (B and C) and histopathologic results (D and E) of the brain tumor were acquired immediately after the last PET scan was taken.

deoxyuridine, can now be usefully investigated for their ability to provide complementary information to FDG-PET studies.

## Conclusions

The use of FDG PET for evaluating tumor growth in rodent glioma models was validated, and deserves further exploration for the integration of multimodal, noninvasive imaging techniques into preclinical oncology studies. These PET studies can contribute to presurgical planning and aid in evaluating the timing for post-surgical adjuvant chemo-radiotherapy in therapeutic strategies for glioma. In the preclinical phase of drug development, these studies may provide rapid screening of drug efficacy and reduce the research and development costs through earlier termination of ineffective agents and expedited study of promising compounds. Future studies should be extended to the investigation of the intra-tumoral metabolic change in long-term follow-up to calculate and estimate survival rate of test animals.

## Acknowledgement

This study was partially supported by a grant from Chang Gung Memorial Hospital (CMRP # 340192).

## References

1. Kleihues P, Soylemezoglu F, Schäuble B, Scheithauer B, Burger PC. Histopathology, classification, and grading of gliomas. Glia 1995;15:211-221.
2. Chamberlain MD. Treatment options for glioblastoma. Neurosurgical Focus 2006;20:E2.
3. Wen PY, Kesari S. Malignant gliomas in adults. N Engl J Med 2008;359:492-507.
4. Berg G, Blomquist E, Cavallin-Stahl E. A systematic overview of radiation therapy effects in brain tumours. Acta Oncol 2003;42:582-588.
5. Bradbury MS, Hambardzumyan D, Zanzonico PB, et al. Dynamic small-animal PET imaging of tumor proliferation with 3'-deoxy-3'-$^{18}$F-fluorothymidine in a genetically engineered mouse model of high- grade gliomas. J Nucl Med 2008;49:422-429.
6. Burger PC, Kleihues P. Cytologic composition of the untreated glioblastoma with implications for evaluation of needle biopsies. Cancer 1989;63:2014-2023.
7. Assadian S, Aliaga A, Del Maestro RF, Evans AC Bedell BJ. FDG-PET imaging for the evaluation of antiglioma agents in a rat model. Neuro Oncol 2008;10:292-299.
8. Delbeke D, Meyerowitz C, Lapidus RL, et al. Optimal cutoff levels of F-18 fluorodeoxyglucose uptake in the differentiation of low-grade from high grade brain tumors with PET. Radiology 1995;195:47-52.
9. Di Chiro G, DeLaPaz RL, Brooks RA, et al. Glucose utilization of cerebral gliomas measured by [$^{18}$F]fluorodeoxyglucose and positron emission tomography. Neurology 1982;32:1323-1329.
10. Tateishi U, Yamaguchi U, Seki K, Terauchi T, Arai Y, Hasegawa T. Glut-1 expression and enhanced glucose metabolism are associated with tumour grade in bone and soft tissue sarcomas: a prospective evaluation by [$^{18}$F] fluorodeoxyglucose positron emission tomography. Eur J Nucl Med Mol Imaging 2006;33:683-691.
11. Buck A, Schirrmeister H, Kühn T, et al. FDG uptake in breast cancer: Correlation with biological and clinical prognostic parameters. Eur J Nucl Med 2002;29:1317-1323.
12. Vesselle H, Schmidt RA, Pugsley JM, et al. Lung cancer proliferation correlates with [F-18] fluorodeoxyglucose uptake by positron emission tomography. Clin Cancer Res 2000;6:3837-3844.
13. Barker M, Hoshino T, Gurcay O, et al. Development of an animal brain tumor model and its response to therapy with 1,3-bis(2-chloroethyl)-1-nitrosourea. Cancer Res 1973;33:976-986.

# 氟化去氧葡萄糖正子電腦斷層掃描評估神經膠質瘤小鼠及大鼠模式之研究

林天仁[1,2,3] 黃啓彰[1,3] 王怡人[4,5] 林家瑋[2] 洪國盛[2,3] 樊玲[6] 曹馨心[6] 楊寧蓀[1] 林昆儒[6,7]

[1]中央研究院 農業生物科技研究中心
[2]台北醫學大學 萬芳醫院 神經外科
[3]台北醫學大學 傷害防治研究院
[4]行政院衛生署台北醫院 小兒科
[5]中國醫藥大學
[6]林口長庚紀念醫院 核子醫學科與分子影像中心
[7]長庚大學 醫學影像暨放射科學系

**背景**：由於神經膠質瘤的高發生率與預後不易，若能發展出有效的早期診斷工具將具有重要的臨床意義。本研究目的爲建立動物用正子電腦斷層掃描 (PET) 配合氟-18-氟化去氧葡萄糖 (FDG) 作爲監測動物腦部神經膠質瘤生長的工具，研究對象分爲小鼠與大鼠兩種品系。

**方法**：使用DBTRG-05MG與RG2兩種神經膠質瘤細胞，分別植入小鼠與大鼠顱內，品系各爲SCID與Wistar。植入腫瘤的動物靜脈注射FDG後待1-2小時，進行動態 (dynamic) FDG-PET掃描以求得的腦部最佳造影時間點；其後分別於第7、10、14與17天進行腫瘤影像追蹤。

**結果**：腫瘤最小可偵測的限值爲2.5 mm。最佳造影時間即腫瘤攝取FDG與背景組織差異最大的時間點，小鼠爲藥物注射後40分鐘，大鼠爲90分鐘。腫瘤攝取FDG的程度也隨著腫瘤成長而明顯增加。

**結論**：本研究已成功探討FDG-PET用於監測大、小鼠腦部神經膠質瘤生長的最適條件，此結果將有助於未來更多相關動物研究的進行。

**關鍵詞**：動物正子電腦斷層造影，氟化去氧葡萄糖，神經膠質瘤，腦部腫瘤

**核子醫誌** 2010;23:77-83

99年1月6日受理 99年2月24日修改 99年3月2日接受刊載
聯絡人：林昆儒醫師 桃園縣333龜山鄉復興街5號 長庚紀念醫院核子醫學科 電話：(03)-3281200轉2625 電子信箱：lin4857@adm.cgmh.org.tw, kunjulin@gmail.com