Abstract. Background: Finding new therapeutic agents is of great clinical interest in neuroblastoma research because prognosis of children with disseminated stages of disease is still poor. As xenograft mouse models are frequently used for studying anticancer drugs in vivo, small animal imaging is an important method of monitoring in anticancer research. Materials and Methods: SCID mice inoculated with human neuroblastoma SK-N-SH cells were examined with positron-emission tomography-computed tomography (PET-CT) using [18F]fluorodeoxyglucose (FDG) or [18F]fluoro-L-thymidine (FLT) and with magnetic resonance imaging (MRI). Results: All neuroblastomas were detected by MRI. In PET-CT imaging, no tumour was visualized with [18F]FDG, but 13 out of 14 (93%) were found with [18F]FLT. Uptake of [18F]FLT was significantly associated with tumour weight. Necrotic areas could not be identified either by MR imaging or on PET-CT scans. Conclusion: Both MR and PET-CT imaging with [18F]FLT are highly qualified for the detection of neuroblastomas grown in SCID mice. However, [18F]FDG, which is the standard tracer in clinical PET-CT imaging, is not suited for PET-CT imaging in the neuroblastoma model.

The use of positron-emission tomography (PET) with the glucose analogue [18F] fluorodeoxyglucose (FDG) has been applied for initial staging and follow-up of several types of cancer and in particular for monitoring the efficacy of anticancer therapy. [18F]FDG is internalised into the cells, where it is subsequently phosphorylated by the enzyme hexokinase, but not further metabolised in the glycolytic pathway. Thus, the accumulation of [18F]FDG-6-phosphate observed in PET scans is correlated to cellular glucose metabolism (1). Neoplastic cells usually exhibit increased anaerobic metabolism, which is associated with trapping of [18F]FDG within the cells (2). Furthermore, the therapeutic response of tumour cells is normally correlated with their metabolic changes and these appear earlier than conventional morphological changes. Thus, functional PET images can document therapeutic success earlier than morphological techniques such as computed tomography (CT) and magnetic resonance imaging (MRI) (3, 4). However, [18F]FDG is not specific for neoplastic processes as it is also taken up by various normal organs such as the brain, heart and urinary bladder, and especially by inflammatory cells irrespective of their organ localisation (2, 5-7). This lack of specificity can lead to false-positive results of [18F]FDG PET scans, so that [18F]FDG PET is not perfectly suited to studying the effect of anticancer agents (2).

Uncontrolled cell proliferation is a major characteristic of malignant growth and the anticancer effect of most drugs is based on the inhibition of tumour cell proliferation (8). Hence [18F]FLT has been introduced as a marker for the noninvasive detection of cell proliferation by PET (9). [18F]FLT uptake is correlated to the activity of the enzyme thymidine kinase 1 (TK1), which is expressed during the S-phase of the cell cycle (10). TK1 catalyses phosphorylation of [18F]FLT to [18F]FLT-phosphate which is intracellularly trapped resulting in increased radioactivity (11, 12). Previous studies have demonstrated that [18F]FLT uptake is in fact lower than [18F]FDG uptake in several types of tumours, but is more specific (13, 14). However, in a neuroblastoma SCID mouse xenograft model, the use of [18F]FLT was shown to be more sensitive in tumour detection than [18F]FDG PET (15). Furthermore, [18F]FLT PET seems to be a potent imaging method for monitoring anticancer treatment, even though changes in cellular proliferation cannot always be detected by [18F]FLT uptake (16, 17).
PET is an imaging method reflecting only metabolic information, not anatomical landmarks which would allow determination of the precise anatomical localization of tracer uptake. This lack of morphological information may also produce false-positive or false-negative results (2). Recently, combined PET and CT (PET-CT) scanners have been developed which allow the acquisition of functional PET images and morphological CT images of the same individual. Thus the areas of tracer uptake can be better localized to the corresponding anatomical structure, resulting in improved sensitivity and specificity in tumour imaging (2, 18).

In this study, we evaluated whether PET-CT imaging with the tracers $[^{18}F]$FDG and $[^{18}F]$FLT in a clinical scanner is suitable for tumour monitoring of SCID mice bearing human neuroblastoma. Furthermore, eight mice were additionally examined by MR imaging using a clinical scanner equipped with a small animal solenoid receiver coil. The MR images and the PET scans were fused in order to allow spatial resolution of the molecular images.

**Materials and Methods**

**Cell line.** SK-N-SH neuroblastoma cells were obtained from Professor R. Erttmann (Department of Pediatric Hematology and Oncology, University Medical Center Hamburg-Eppendorf, Germany). They were maintained under standard cell culture conditions in RPMI-1640 medium (GIBCO, Karlsruhe, Germany) supplemented with 10% fetal calf serum, penicillin and streptomycin at 37°C in a humidified incubator with 5% CO2 and were free of mycoplasma infection as monitored by polymerase chain reaction (PCR) (MP Biomedical, Eschwege, Germany). For injection, SK-N-SH neuroblastoma cells were harvested by trypsinization and 5×10⁶ viable cells were suspended in 1 ml cell culture medium. A volume of 200 μl of this cell suspension was injected subcutaneously between the scapulae of each SCID (severe combined immunodeficiency) mouse.

**SCID mice.** In this study, 18 male and 18 female pathogen-free 15-week-old SCID mice were used. The mice were obtained from Charles River Laboratories (Sulzfeld, Germany) and weighted on average 25 g (SD±3.2 g) at the beginning of our experiments. They were housed in filter-top cages and provided with sterile water and food ad libitum. All manipulations were carried out aseptically inside a laminar flow hood. The animal experiment was approved by the local Animal Experiment Approval Committee (Behörde für Gesundheit und Verbraucherschutz, Aulendorf, Germany) and assigned the project no. 64/05.

**MRI.** Magnetic resonance imaging of eight mice with neuroblastomas weighing between 0.3 and 0.7 g was performed four weeks after the inoculation of SK-N-SH neuroblastoma cells. High resolution MR data sets were acquired on a clinical 3Tesla whole-body MR system (Intera; Philips Medical Systems, Best, The Netherlands). A dedicated small animal solenoid receiver coil (Philips Research, Hamburg, Germany) was used for signal detection. During MR examination, the animals were anaesthetized by intraperitoneal injection of 0.1 ml/10 g body weight Rompun/Ketanest [0.8 ml Rompun 2% (Bayer, Leverkusen, Germany), 1.2 ml Ketamin Gräub (100 mg/ml; Albrecht, Aulendorf, Germany) in 8 ml 0.9% NaCl].

The MR protocol consisted of a short survey scan and two-dimensional high-resolution T1-weighted gradient echo and T2-weighted turbo spin-echo sequences in sagittal, coronal and axial planes. Imaging parameters were as follows: T1-weighted: time of repetition (TR), 424 ms; time of echo (TE), 4.9 ms; flip angle (FA), 45°; number of acquisitions (NA), 3; time of acquisition (TA) 5:58 minutes; T2-weighted: TR, 3438 ms; TE, 90 ms; FA, 90°; echo train, 10; NA, 3; TA, 6:04 minutes. Both sequences were acquired with the same spatial parameters: field of view (FOV), 90 mm; matrix, 400×400 pixels; slice thickness, 0.6 mm. The effective spatial resolution corresponded to a voxel size of 0.225×0.225×0.6 mm³. The overall time of examination was ~30 minutes for each mouse.

MR data sets were analysed using dedicated software for three-dimensional visualization and volume assessment of medical image data (Voxel-Man Group, University Medical Center Hamburg-Eppendorf, Hamburg, Germany). Determination of the tumour volume was carried out on the high-resolution T2-weighted data sets using a semi-automated segmentation method with a threshold-dependent region-growing algorithm. Starting from manually placed seed points within the tumour, all voxels with signal intensities in a predefined threshold interval were automatically included into the segmentation. The tumour volume was calculated from the total number of segmented voxels and the spatial resolution (19).

**Radiopharmaceutical preparation.** $[^{18}F]$FDG was synthesized in a nuclear interface synthesis module using a modification of a technique described by Hamacher et al. (20). $[^{18}F]$FLT was synthesized using 3-N-boc-5’-O-dimethoxytrityl-3’-O-nosyl-thymidine (ABX Advanced Biochemical Compounds, Radeberg, Germany) as precursor in a synthesis module developed in-house. The precursor reacted with the $[^{18}F]$ fluoride ions in the presence of Kryptofix 2.2.2, phase transfer catalyst in acetonitrile at 130°C. After a subsequent hydrolysis with 1 M hydrochloric acid, $[^{18}F]$FLT was purified on a semi-preparative NUCLEODUR® Pyramid C18 HPLC column (Macherey-Nagel, Düren, Germany). The column was eluted with a mobile phase of 7.5% ethanol in 0.015 M phosphate buffer (pH 5.5).

$[^{18}F]$FDG and $[^{18}F]$FLT imaging. Data acquisition for this study was performed on a Gemini GXL 10 integrated PET-CT scanner (Philips Medical Systems, Germany) on day 31 after inoculation of human neuroblastoma cells. The 36 animals of the study were randomly divided into two groups: one group of 18 (nine male and nine female) mice received $[^{18}F]$FDG as tracer. $[^{18}F]$FLT was administered to the other group of 18 (nine male and nine female) mice. A dose of 13 MBq of either $[^{18}F]$FDG or $[^{18}F]$FLT were injected via tail vein into each mouse, respectively. Sixty minutes after tracer application, the animals were anesthetized by intraperitoneal injection with a mixture of ketamin-rompun-sodium chloride (1% of the body weight). The SCID mice were placed in a rack in the PET-CT scanner which allows 18 mice to be examined in parallel (21). Low-dose CT acquisition was performed first with 90 kV, 30 mAs and a FOV of 600 mm. Images were reconstructed on a 512x512 pixel matrix with a...
voxel size of 1.17×1.17×3.00 mm³. Immediately after the CT scan, dynamic PET scans were carried out for 45 min using conventional full-ring, whole-body PET. Images were reconstructed with an iterative reconstruction algorithm, 3D RAMLA with a spatial resolution of 6-8 mm.

Animals were killed by cervical dislocation on day 32. Tumours were excised, fixed in formalin, embedded in paraffin and cut into 5.0 μm thick sections.

**Visual analysis.** Image analysis (PET-CT) of tracer uptake was performed by two readers. The last frame of the dynamic scan on a computer screen was inspected using a display with three orthogonal planes. The following score was used: no uptake=0, tracer uptake of tumour greater than background=1, tracer uptake of tumour greater than reference organ=2. The liver acted as reference organ for [18 F]FLT uptake and the brain for [18 F]FDG uptake. The tumours scored with 0 were classified as non-visualised, those scored with 1 or 2 as visualised.

**Statistical analysis.** The correlation between tumour volume determined by MRI and postmortem tumour weight was analysed with Pearson’s product-moment correlation coefficient.

The number of tumours visualised in the PET-CT by [18 F]FLT uptake or [18 F]FDG uptake, respectively, were compared with Fisher’s exact test. Tumour weight between the [18 F]FLT and [18 F]FDG group was analysed by unpaired t-test. Tumour weights of the three visually evaluated groups 0, 1 and 2 within the [18 F]FLT group were compared with an analysis of variance (one-way ANOVA) and Bonferroni post test.

All statistical tests were carried out using GraphPad Prism™ software (GraphPad™, San Diego, USA). The values of $p<0.05$ were considered as a statistically significant result.

**Results**

**MRI.** The MR images were acquired in T1 as well as in T2 weighting. The T1-weighted images adequately illustrated the anatomical structures. However, primary tumours and perifocal oedema could be better evaluated in T2-weighted images (Figure 1). All neuroblastomas could be visualized by MR imaging, but necrotic areas within the primary tumours could not be delineated.

Tumour volume was evaluated in MR images by using a semi-automated segmentation (Voxel-Man Group). T2-weighted images in sagittal or coronal slice direction were used for this analysis. The corresponding tumour volumes were calculated from the total number of voxels and the spatial resolution (Figure 2).

The tumour volumes measured by semi-automated analysis of the MR images were correlated with the primary tumour weights determined after surgical excision of the neuroblastomas. Pearson’s product-moment correlation coefficient was $r=0.6$, however, correlation was not statistically significant (Figure 3).

The primary tumour of one SCID mouse was virtually reconstructed by editing the MR images with dedicated three-dimensional visualization software (Voxel-Man Group). The reconstructed tumour and the corresponding native tumour are demonstrated in Figure 4. The two images showed a good match.

**PET-CT.** The PET-CT images of one mouse of the [18 F]FLT group could not be evaluated because of artefacts. Two mice of the [18 F]FDG group (n=18) and three mice of the [18 F]FLT group (n=18) did not bear visible, palpable neuroblastomas and showed no tumour signal in the PET-CT images. Thus, no false-positive results were recorded. These mice were not included in the following image analysis. In the PET-CT images, none of 16 neuroblastomas could be detected with [18 F]FDG, whereas [18 F]FLT allowed the visualization of 13 out of 14 neuroblastomas (93%) ($p<0.001$; Figure 5). In these cases, primary tumours could be clearly delineated in the subcutaneous retrocervical region of the SCID mice. The signal intensity of five of these 13 tumours were classified as 2, the remaining eight tumours as 1 (see Material and Methods). The tumour weights in the [18 F]FDG and [18 F]FLT group showed no statistically significant difference ([18 F]FDG: $0.30±0.18$ g, [18 F]FLT: $0.33±0.23$ g; n.s.), Uptake of [18 F]FLT by the neuroblastomas increased with growing tumour weight (Figure 6). In this analysis, the SCID mice which did not develop neuroblastomas were also considered. Neuroblastomas weighing below 0.1 g did not show [18 F]FLT uptake, tumours weighing 0.1 g to 0.3 g exhibited a signal which was stronger than the background signal, and tumour weights of 0.4 g and more were associated with an [18 F]FLT uptake greater than the reference organ. The tumour weights in the three visually evaluated neuroblastoma groups were statistically significant different (F=25.02, $p<0.001$; Figure 6). The direct comparison of the groups with Bonferroni post test showed a significant difference between group 0 and 2 and 1 and 2.
The fusion of CT and PET images allowed the high uptake areas of the PET image to be matched with the corresponding anatomical structure (Figure 7).

For a better association of the anatomical structures and the positive areas in the PET scans, PET and MR images were merged as the resolution of the MR images was considerably higher (Department of Nuclear Medicine, University Medical Center Hamburg-Eppendorf, Germany). This is exemplarily shown in Figure 8.

Figure 2. A. T2-weighted MR image of SCID mouse in coronal slice direction. B. Tumour is coloured red after processing of the MR image with VOXEL MAN.

Figure 3. Correlation between tumour volume as measured by MRI and tumour weight. Pearson’s product-moment correlation coefficient was $r=0.6$ (n.s.).

Figure 4. Comparison between the three-dimensional tumour reconstruction made by VOXEL Man (A) and a photograph of the primary tumour after surgical excision at day 32 (B). The shape of the reconstructed tumour corresponds well to the actual tumour. Thus, editing of MR images by dedicated visualization software is well suited to the reconstruction of tumours in situ.
Discussion

Xenograft models of human cancer cells grown in immunodeficient mice are particularly useful in evaluating the therapeutic effect of anticancer drugs in vivo and are widely used in cancer research. Thus, small animal imaging, which is a non-invasive and repeatable method for monitoring the course of disease under therapy has become more and more important in recent years. In the present study, human neuroblastomas grown in SCID mice were imaged by MRI in a clinical MR scanner equipped with a small animal solenoid receiver coil and by [18F]FDG and [18F]FLT otherwise unaltered PET-CT in clinical scanners, respectively.

All eight neuroblastomas were detected by MRI and weighed between 0.3 g and 0.7 g. MR images were converted with dedicated volume analysis and visualization software (Figures 2 and 4) and volume assessment of the primary tumours ranged from 175 to 439 mm³. The comparison of the tumour volumes with the respective post-mortem tumour
weight did not show a significant correlation \( r = 0.6 \) (Figure 3). However, the three-dimensional reconstructed tumour corresponded well with the image of the native tumour (Figure 4) and the editing of MR images by dedicated software applications seems to be well suited to the reconstruction of tumours in situ.

A recent imaging study in mice demonstrated that MR volumetry of the liver correlates well with post-mortem liver weights (22). The statistically insignificant correlation of MRI-based neuroblastoma volumetry and post-mortem weight in our study might be attributed to the small number and range of the values. Furthermore, the reliability of calculating the tumour volume by MR images was highly dependent on the tumour form. The neuroblastomas grown in SCID mice showed various forms including spheroid, terete, compact or lobate, so that the quality of our segmentation differed. The MR images of lobate neuroblastomas were more difficult to evaluate than MR images of spheroid tumours, possibly resulting in inaccurate volume determination.

Several novel anticancer drugs are cytostatic and do not necessarily lead to a reduction of tumor volume, but of viable tumour tissue (16). As MRI-based volumetry can account for necrotic areas, it is described as a sophisticated method of therapeutic monitoring of cancer disease (23). However, in order to analyse necrotic tumor areas, the use of dynamic contrast-enhanced MRI is a more effective approach (24). In our study necrotic areas of the primary tumours could not be determined in the MR images, although they contained 50% to 80% necrotic areas in the histological analysis (data not shown). This is not surprising as the neuroblastomas were very small and the contrast between viable and necrotic areas without using a contrast agent was not noticeable in the MR images.

Although MRI is a sensitive method to calculate neuroblastoma growth in SCID mice, functional aspects concerning changes of the cell metabolism are normally not detected by noninvasive MR imaging. In this regard PET-CT, which combines morphological and functional imaging, is of considerable interest. However, one problem of using clinical PET-CT scanners for small animal imaging as used in this study is the limited resolution compared to high resolution PET-CT scanners for small animal imaging as used in this study. A disadvantage of PET with \([^{18}\text{F}]\)FDG, which is the PET tracer than \([^{18}\text{F}]\)FLT (14, 32-36). However, in gliomas and breast cancer, PET with \([^{18}\text{F}]\)FLT seems to be superior to \([^{18}\text{F}]\)FDG as in the present neuroblastoma xenograft study (3, 7). \([^{18}\text{F}]\)FLT was assessed to be a reasonable marker for tumour cell proliferation because it is phosphorylated by TK1, whose activity correlates with the level of cellular proliferation (9, 16). However, some previous studies showed that the Ki-67 labelling index, the histopathological standard for cell proliferation analysis, and the uptake of \([^{18}\text{F}]\)FLT in the PET do not always correlate (28). Thus, in oesophageal, pancreatic and breast cancer, as well as in neuroblastomas grown in SCID mice, \([^{18}\text{F}]\)FLT uptake did not reflect the Ki-67 index and consequently Ki-67 was not investigated in the present study (15, 32, 37, 38).

The signal intensity in the PET images with \([^{18}\text{F}]\)FLT was related to the tumour weight, as the tumour weight in the three visually evaluated groups was significantly different. In contrast to the report of Krieger-Hinck et al. (15), in this study, neuroblastomas weighing less than 1.0 g were detected by accumulation of \([^{18}\text{F}]\)FLT in the PET. The use of a different PET-CT scanner with a higher resolution in this study explains this discrepancy and confirms the importance of high resolution for small animal imaging studies (25, 39). As in the MR images, the necrotic areas in the neuroblastomas could not be detected by PET-CT imaging with the clinical scanner in our study. Tatsumi et al. (25) detected necrotic portions in tumours by \([^{18}\text{F}]\)FDG PET-CT only in rabbits and rats using a small animal-specific protocol with high-quality CT mapping images. However, in agreement with our results the heterogeneity of tracer uptake within the primary tumours could not be demonstrated in mice, probably because of small animal and tumour size (25). Thus PET-CT with \([^{18}\text{F}]\)FLT in a clinical scanner is suited for the diagnosis of neuroblastomas grown in mice, but not for specific functional analysis.

Fusion of the PET scans and the MR images, which were of higher resolution, improved the differentiation of primary neuroblastomas and surrounding tissue in the PET scans, though the fusion was made retrospectively. Changes...
of the PET-CT protocol leading to higher contrast in the CT scans would certainly upgrade quality of CT images in future studies. However, an optimal resolution of PET-CT images in mice can be only achieved by using a small animal PET-CT (25).

Conclusion

Both MRI and PET-CT with $^{18}$FFLT, but not with $^{18}$FDG, are qualified for the detection of neuroblastomas grown in SCID mice. In particular, MRI using the small animal solenoid receiver coil provided excellent morphological information of high resolution. Furthermore, MRI data can be used for noninvasive volumetry, which is important for therapy monitoring, even though the correlation with the tumour weight post mortem was not significant in our study. The use of small animal scanners with higher resolution and future development of more specific tracers and/or contrast agents will be important for functional analysis and monitoring of tumour growth at the cellular level in xenograft models.

Acknowledgements

This study was supported by grants of the Behörde für Wissenschaft und Forschung (BWI) der Hansestadt Hamburg (Germany) within the research project “Molecular Imaging North” (MOIN).

References


Received Apr 18, 2008
Revised June 24, 2008
Accepted July 1, 2008