Clinical applications of L-[1-11C]-tyrosine PET in laryngeal cancer
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Chapter 2

Carbon-11 Tyrosine PET for visualization and protein synthesis rate assessment of laryngeal and hypopharyngeal carcinomas

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INTRODUCTION

Advanced imaging techniques such as computed tomography (CT) and magnetic resonance imaging (MRI) have improved staging of head and neck tumors as compared to clinical examination. However, the ability of CT and MRI to delineate primary and metastatic lesions accurately is limited, due to the fact that they monitor primary tumors and lymph node metastases solely on the basis of size and structural changes. Primary tumors that do not distort tissue planes, and unenlarged lymph nodes infiltrated with tumor cells may not be detected by these modalities.

Consequently, there is room for improvement by application of modalities other than anatomical imaging techniques. Positron emission tomography (PET) is a functional imaging modality that enables in vivo determination of tissue metabolism and pathophysiology and therefore also of tumor metabolism. The potential of PET for diagnosis of cancer and monitoring the response to treatment is now widely accepted.

In PET studies in oncology, 2-[18F]-fluoro-2-deoxy-D-glucose (FDG) is the most frequently used radiopharmaceutical, its application being based on increased glucose uptake in tumor cells due to increased glycolysis. Although FDG-PET has proven to be a reliable method for the detection of head and neck tumors and a variety of other malignancies, the use of FDG has some disadvantages. Uptake of FDG is not specific to malignant tissue, and therefore the most significant drawback is the relatively low specificity, due to false positive results caused by accumulation of FDG in inflammatory tissue, reactive lymph nodes and biopsy sites.

As an alternative, radiopharmaceuticals for imaging of other metabolic processes have been developed. It has been demonstrated that amino acid uptake in tumor tissue is high as compared with that in normal tissue, owing to increased protein synthesis. Furthermore, amino acids play a minor role in the metabolism of inflammatory cells compared with FDG. The majority of the amino acid PET studies have been performed with L-[methyl-11C]-methionine (MET). However, construction of an accurate metabolic model for this tracer is almost impossible since MET is involved in several metabolic pathways. Accumulation of substantial amounts of nonprotein metabolites in tumor tissue makes correct quantification of protein synthesis rather difficult.

More appropriate compounds to determine protein synthesis activity in tumor tissue are carboxyl-labeled amino acids, such as L-[1-11C]-tyrosine (TYR). The main metabolic pathway of TYR is irreversible owing to incorporation of 11C into protein, and further metabolism generates 11CO2. This compound is rapidly cleared from tissue to plasma and expired from the lungs into air, and therefore 11CO2 does not contribute substantially to the 11C-radioactivity in tumor tissue as measured by PET. Based on the pathway of TYR, a quantitative method to determine the protein synthesis in tumors with TYR was developed by Willemsen et al. Using a dynamic scanning protocol and the determination of plasma concentrations of TYR and metabolites, the Protein Synthesis
Rate (PSR) can be calculated. Visualization of several types of malignant tumors and quantification of their PSRs has been successfully performed with TYR-PET.\textsuperscript{13-16}

The aim of the present study was to investigate the feasibility of use of TYR-PET for visualization of laryngeal and hypopharyngeal carcinomas and in vivo quantification of the Protein Synthesis Rate (PSR).

**MATERIALS AND METHODS**

**Patients**

Patients with histologically proven squamous cell laryngeal or hypopharyngeal carcinoma were eligible for this study. Patients were clinically staged according to the International Union Against Cancer primary tumor (T), regional nodes (N) and metastasis (M) classification system (UICC, 1997)\textsuperscript{17}, based on physical examination of head and neck, endoscopic examination under general anaesthesia, biopsies of all suspicious areas of the upper aerodigestive tract and CT imaging. In contrast with other tumors in the head and neck region, in which T-staging is determined by size, staging of laryngeal and hypopharyngeal carcinoma mainly depends on localization in different anatomical subsites and vocal cord fixation. The study was approved by the Medical Ethics Committee of the Groningen University Hospital and written informed consent was obtained from all patients.

**PET**

A dynamic TYR-PET study was performed in all patients at least two weeks after biopsy (median 18 days; range 15-27 days). TYR was produced via a modified microwave induced Bücherer-Strecker synthesis.\textsuperscript{18} Synthesis time was 40 minutes, including high performance liquid chromatography (HPLC) purification and testing for sterility, with a radiochemical purity of more than 99%.

The PET images were acquired using an ECAT 951/31 PET camera (Siemens/CTI, Knoxville, Tenn.). This device has a 56-cm-diameter patient aperture and acquires 31 planes simultaneously. The axial field-of-view is 10.8-cm and the resolution is 6 mm full-width of half-maximum (FWHM). The head of the patient was fixed in a position with the Frankfurter horizontal plane (line between the external meatus acusticus and the lower orbital rim) making an angle of 110° with the horizontal bed position. Patients fasted for at least 8 hr (except for water and their usual medication) before the study.

A venous canula was placed in the antecubital vein of the forearm for injection of TYR. In the radial artery of the contralateral arm an arterial canula was placed under local anaesthesia. Before injection of TYR, a 20-min transmission scan was obtained to correct for photon attenuation by body tissues in the imaged area. TYR was administered intravenously over a 1-min period. The injected dose varied from 144 to 400 MBq (median
370 MBq). Dynamic scanning with 16 time frames was performed from the time of injection to 50 min post injection at the level of the tumor. The protocol included ten 30-sec images, three 5-min images and three 10-min images. Simultaneously, arterial blood samples were taken at set time points (at 0.25, 0.5, 0.75, 1, 1.25, 1.5, 1.75, 2.25, 2.75, 3.75, 4.75, 7.5, 12.5, 17.5, 25, 35 and 45 minutes after injection) in order to assess TYR plasma time-activity curve, $^{11}$CO$_2$ concentration and other $^{11}$C-labeled metabolites by radio-HPLC.  

Data analysis

PET acquisition data were reconstructed using filtered back-projection by a 0.5 cycle/pixel Hann filter to obtain transaxial images which were displayed applying standard ECAT software.

To determine tumor PSR, a region of interest (ROI) was placed in the plane with most intense uptake at the site of the tumor as observed at visual analysis, using a 70% threshold of maximum intensity. The tissue time-activity curve obtained from this ROI and the plasma-input data (MBq/ml TYR corrected for $^{11}$CO$_2$ and $^{11}$C-labeled proteins) were used to calculate PSR in nanomoles per millilitre tumor tissue per minute (nmol/ml/min) using a modified Patlak analysis. By visually masking nontumor regions with physiologically high uptake of TYR (e.g. salivary glands), these regions were prevented from contributing to the average tumor time-activity curve. For every patient a background (B) PSR was calculated from the right trapezius muscle, because calculations of normal laryngeal or hypopharyngeal tissue were not reliable in patients with large tumors or metastatic lymph nodes located close to normal laryngeal or hypopharyngeal tissue.

Statistical analysis

To compare the PSR in tumor tissue with that in nontumor (background) tissue, the Wilcoxon test was used. A p-value < 0.05 was considered to be statistically significant.

RESULTS

Patients

Thirty-one patients (2 women, 29 men; age range 44-80 years; median age 62 years) with a clinical diagnosis of laryngeal or hypopharyngeal carcinoma participated in the study. In all 31 patients, complete clinical examination, including CT imaging and histological confirmation of squamous cell carcinoma, was performed (Table 1). The diameter of all tumors was larger than 15 mm. TYR-PET studies were performed before definitive therapy in all cases.
Table 1
Patient characteristics

<table>
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UICC = International Union Against Cancer (1997)

PET data

Visual analysis

All 31 primary malignancies were visualized as a hotspot (Fig. 1). Among the five patients with lymph node metastases (N1 in two, N2 in three) clinically staged by physical examination and CT (UICC 1997), the N2 cases were clearly identified by TYR-PET, but no hot spots were visualized in the N1 patients. The parotid, submandibular and sublingual glands showed increased uptake in all cases.
Coronal, transaxial and sagittal TYR-PET images of a patient with a T2N0 supraglottic laryngeal carcinoma. (A = tumor; B = parotid glands).

Quantification
The plasma tyrosine levels ranged from 0.031-0.089 mmol/L, which is within normal values for our laboratory and in accordance with values reported in the literature. The median PSR of normal (background) tissue was 0.51 nmol/ml/min, the values ranging from 0.22 to 0.89 nmol/ml/min. The median PSR of all tumors was 2.06 nmol/ml/min (range 0.72-6.96 nmol/ml/min), which was significantly higher (p<0.001, Wilcoxon) than the PSR of normal tissue (Fig. 2). The ratio between tumor PSR and background PSR ranged from 1.24 to 7.82 (median 4.65). No inconsistency was found between the quantitative values and visualization of the tumors.

PSR values of primary tumor tissue (median 2.06; range 0.72-6.96 nmol/ml/min) and background (nontumor) tissue (median 0.51; range 0.22-0.89 nmol/ml/min). The horizontal lines denote the median values.
DISCUSSION

This is the first study to evaluate the clinical application of L-[1-\(^{11}\)C]-tyrosine (TYR) in squamous cell carcinoma of the larynx and hypopharynx. It was demonstrated that laryngeal and hypopharyngeal tumors can be clearly visualized with TYR, thereby confirming previous results in other types of malignancy imaged with the same tracer. High uptake of TYR in squamous cell carcinoma of the larynx and hypopharynx and persistent low uptake in background tissue were found. In all patients we found TYR accumulation in the parotid, submandibular and sublingual glands. These nontumor areas with physiologically increased uptake had clear contours and were well separated from the primary tumors. Therefore, masking the salivary glands by visual interpretation could easily be performed.

2-[\(^{18}\)F]-fluoro-2-deoxy-D-glucose (FDG) is the most frequently used radiotracer in clinical oncology. FDG-PET is used for detection, staging and restaging of many types of cancer, but also for therapy evaluation and differentiation between malignant and benign lesions. In head and neck tumors, FDG has proven to be a highly sensitive tumor tracer. However, Strauss reviewed the differentiation of malignant and benign lesions with FDG, and indicated that specificity of FDG-PET is low, owing to other causes of increased glucose metabolism in tissues. This significant drawback of relatively low specificity is caused by false positive results due to accumulation of FDG in inflammatory tissue, reactive lymph nodes and biopsy sites. Collateral uptake of FDG in inflammatory tissues can cause a problem in primary tumors of the upper aerodigestive tract, which are usually associated with local inflammation around the tumor. In the head and neck region, increased uptake is also found in salivary glands, tonsil tissue, muscle tissue of the larynx and neck, benign laryngeal papilloma and the base of the tongue. Although FDG-PET has improved diagnostic accuracy for recurrent head and neck cancer as compared with conventional imaging modalities, clinical problems still arise in the differentiation between tumor recurrence and posttreatment inflammation. In FDG studies including approximately 10 to 15 patients with head and neck tumors, sensitivity for detection of residual or recurrent tumor ranged from 88% to 100%, while specificity could be as low as 43%. In more recent FDG-PET series with larger number of patients, sensitivity ranged from 50% to 100% and specificity from 83% to 95%. Only one study, by Wong et al., reported sensitivity and specificity of 100% for FDG-PET in detection of recurrent tumor. In preclinical studies, Kubota et al. investigated FDG uptake in mouse tumors with microautoradiography and found that 29% of the glucose utilization took place in nontumor macrophages which were situated in the necrotic areas of the tumor. Fischbein et al. stated that it is unlikely that a nonspecific tracer of metabolic activity such as FDG will be able to differentiate tumor from inflammation with complete accuracy.

Increased protein metabolism in cancer cells and amino acid utilization into protein is another feature of malignancy. Since the replacement of a carbon atom by \(^{11}\)C...
28, nearly all amino acids have been radiolabeled to study potential imaging properties. For reasons of ease of synthesis, biodistribution and formation of radiolabeled metabolites in vivo, mainly MET and TYR have been studied clinically. More recently, artificial amino acids such as L-3-[\textsuperscript{18}F]fluoro-alpha-methyl-tyrosine (FMT) and O-2-[\textsuperscript{18}F]fluoroethyl-L-tyrosine (FET) for PET and L-3-[\textsuperscript{123}I]iodo-alpha-methyl-tyrosine (IMT) for single-photon emission tomography (SPECT) have been studied \textsuperscript{11,29}.

The most frequently used radiolabeled amino acid in PET is L-[methyl-\textsuperscript{11}C]-methionine (MET). In the central nervous system, the clinical applications of MET are well established. The evaluation of primary brain tumors is significantly enhanced by MET in comparison to FDG \textsuperscript{30}. Other extracranial tumor types have demonstrated increased uptake of MET and correlations have been found between high MET uptake and proliferative activity \textsuperscript{31}. Autoradiography confirmed MET uptake predominantly in viable tumor cells, with low uptake in macrophages and other cells \textsuperscript{32}. The Turku University group has extensively investigated head and neck cancer with MET-PET \textsuperscript{33-35}. They found good uptake of MET, and reported a sensitivity of 91\%-97\% for detection of the primary head and neck tumors. Although several types of head and neck tumors were studied, only a few laryngeal and hypopharyngeal malignancies were included. With the increasing application of MET, the number of reports of nontumoral uptake of MET are growing, and specificity may be lower than previously suggested by in vitro studies \textsuperscript{11}. Furthermore, methyl-labeled methionine is involved in several metabolic pathways, such as transmethylation and polyamine synthesis and is converted in S-adenosyl methionine. This may lead to the accumulation of nonprotein metabolites in tumor tissue \textsuperscript{11,36}. The complicated metabolism of methionine has made it impossible to construct a precise metabolic model, and therefore quantification of protein synthesis is rather difficult.

It has been demonstrated that carboxyl-labeled amino acids, such as TYR, appear to be more appropriate compounds with which to determine protein synthesis activity in tumor tissue \textsuperscript{12}. At our institute, TYR-PET was used to develop a method to determine the protein synthesis in tumors, and this model has been successfully applied in animal and human studies to visualize different tumors and to assess the PSR \textsuperscript{13-16}. In this study, we found a sensitivity of 100\% of TYR-PET for detection of laryngeal and hypopharyngeal squamous cell carcinoma. Preclinical studies by Daemen et al. described better correlations between TYR uptake and tumor growth rate than were found for FDG \textsuperscript{37}. In our experience, TYR is less avidly metabolized by inflammatory cells and therefore TYR-PET seems useful in the evaluation of response to therapy. Recurrent brain tumors have been depicted by TYR with a sensitivity of 92\% and specificity of 67\% \textsuperscript{15}. High specificity (100\%) of TYR-PET was described by Van Ginkel et al. \textsuperscript{38} in the evaluation of therapy on soft tissue sarcomas and skin cancer. Kole et al. \textsuperscript{16} reported high uptake of TYR, and as a consequence high PSR, in various types of malignancy and low uptake in benign lesions. They also found TYR more accurate in predicting mitotic rate and proliferation, especially after treatment. Therefore, they concluded that TYR may be a more favourable tracer for monitoring
therapy than FDG\textsuperscript{39}. Because the main focus of the present study was the feasibility of using TYR-PET in laryngeal and hypopharyngeal tumors, future studies are required to address the specificity of TYR-PET and to compare these results with FDG-PET.

Visualization of tumors by PET is hampered by the lack of anatomical details, and therefore the acquired images must be compared with corresponding CT or MRI sections. Future CT-PET cameras, merging anatomical information from CT with metabolic PET images, may overcome this problem. Another drawback of TYR-PET was encountered in this present study. Detection of tumors depends on the difference between the intensity of signal from the tumor and that from the background. Consequently, false-negative results may occur when there is a low tumor signal or a high background signal. In all patients increased uptake was observed in the parotid, submandibular and sublingual salivary glands. Therefore, TYR will not be suitable for detection of salivary tumors. Uptake in salivary glands may also impair the detection of metastatic lymph nodes in head and neck tumors, as was probably the case in two patients with N1 findings in the neck. However, another explanation for failure of TYR-PET to visualize hot spots in these patients may have been technical limitations and partial volume effects; because of these factors, PET is not suited to the depiction of small lesions and therefore small lymph node metastases may not be detected.

In this study, quantification of the metabolic rates of the malignancies and normal tissue showed a significant difference between the PSR of tumor tissue and that of background tissue in all patients. Partial volume effects did not have a substantial influence on the assessment of PSR because of the size of all the tumors. However, no additional information was provided by quantification over visualization alone in the detection of primary malignancies. Therefore, in vivo quantification of tumor metabolism by assessment of PSR, requiring arterial cannulation, seems not to be necessary for the detection of previously untreated tumors. On the other hand, absolute quantification and determination of PSR with dynamic TYR-PET may be of value for the purpose of assessing (early) therapy response and detection of tumor recurrence. The use of quantification of tumor activity by dynamic TYR-PET for therapy evaluation is currently under investigation.

In conclusion, L-[1-\textsuperscript{11}C]-Tyrosine PET is potentially a clinically useful imaging modality for visualization of laryngeal and hypopharyngeal squamous cell carcinoma. Quantification of in vivo metabolic tumor activity by assessment of the Protein Synthesis Rate (PSR) was possible with a dynamic scanning procedure. The PSR of all primary tumors was significantly higher (p<0.001) than the PSR of background tissue.
REFERENCES


