[^{11}C]Vinblastine – syntheses and preliminary imaging in cancer patients

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Received April 25, 2007; Revision received May 25, 2007; Accepted May 25, 2007, Published June 18, 2007

Key words: Carbon-11, vinblastine, chemotherapy, individual-therapy-planning, PET.

ABSTRACT - Purpose: The primary aim of this work was to establish a radiolabeling procedure of vinblastine, a vinca alkaloid widely used in chemotherapy, with the positron-emitter carbon-11 for application in positron-emission-tomography (PET) studies in cancer patients. The optimized reaction conditions were transferred to an automated radiosynthesizer system for the preparation of [^{11}C]vinblastine under GMP conditions for human use. We report about the whole body activity distribution after injection of [^{11}C]vinblastine as well as the pharmacokinetic behavior in selected organs and the tumor in two patients that were investigated with [^{11}C]vinblastine PET before chemotherapy. **Methods:** For carbon-11 labeling of vinblastine the reaction conditions were determined with respect to the two possible labeling precursors (i.e. [^{11}C]methyl iodide and [^{11}C]diazomethane), solvent, reaction temperature and reaction time. Both, [^{11}C]diazomethane and [^{11}C]methyl iodide were tested as labeling precursors with the corresponding demethyl compound of vinblastine, i.e. the vinblastine acid and the potassium salt of vinblastine acid. Two patients with renal carcinoma underwent [^{11}C]vinblastine PET before chemotherapy. One patient underwent a second scan during infusion of unlabeled vinblastine at a therapeutic dose. **Results:** Best results for the labeling procedure were found when methylation was carried out at 100 °C within 20 min using 2 mg/mL of the potassium salt of vinblastine acid in DMSO and [^{11}C]methyl iodide as labeling precursor. Based on [^{11}C]methyl iodide starting activity a radiochemical yield of up 53 % [^{11}C]vinblastine was achieved. In addition, the synthesis was transferred to a remotely controlled module for routine GMP conform production for human use. In large scale production runs up to 1 GBq of [^{11}C]vinblastine was obtained ready for injection within 45 min after EOB. In one patient, whole body PET scans 40 min after injection of 112 MBq [^{11}C]vinblastine showed a focally increased [^{11}C]vinblastine uptake and [^{11}C]vinblastine metabolite uptake, respectively in the known metastases, along with a slow but continuous washout during the measurement interval (0-60 min p.i.). Another patient showed no focally increased [^{11}C]vinblastine uptake and [^{11}C]vinblastine metabolite uptake in the tumor, where radioactivity concentration was comparable to that in the blood. In this patient, a second PET scan during infusion of unlabeled vinblastine revealed similar kinetics with a trend towards delayed hepatic metabolism and higher blood and tumor concentrations. Whereas this patient showed a partial response to chemotherapy, the first patient did not, hypothetically due to the observed vinblastine washout from the tumor. **Conclusions:** The carbon-11 labeling of vinblastine using [^{11}C]methyl iodide is superior to the method using [^{11}C]diazomethane. A well working automated radiosynthesis was established for the production of [^{11}C]vinblastine for PET-investigations in cancer patients. The individual pharmacokinetic behavior of the chemo-therapeutic agent to the tumor can be assessed with PET, thus, can be considered to be a realistic approach for individualized chemotherapy.

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INTRODUCTION

The vinca alkaloid vinblastine inhibits assembly of microtubules and is therefore used as a mitosis inhibitor in chemotherapy of various malignant tumors (1-8). In chemotherapy, pharmacokinetic data cannot generally be derived for individual patients, yet it is to be assumed that considerable between-subject differences exist and optimal treatment regiments should be adjusted individually (9).

Therefore, we had to develop a procedure for the labeling of vinblastine with carbon-11 ($T_{½}$ = 20 min) as a radiotracer for positron emission tomography (PET) in order to determine the individual tumor retention and pharmacokinetics of the chemotherapeutic compound vinblastine. The use of the two different labeling precursors, i.e. [^{11}C]diazomethane and [^{11}C]methyl iodide, is described in literature (10) and was successfully applied in this work.

PET studies in two patients suffering from renal carcinoma were performed as a first attempt to determine the tumor uptake and retention of a tracer dose of ^{11}C-labeled vinblastine before and during infusion of vinblastine as therapeutic dose. The use of radiolabeled vinblastine is well known (11), but to our knowledge this is the first application of [^{11}C]vinblastine in PET-studies in carcinoma patients.

MATERIALS AND METHODS

Materials

Dimethylsulfoxide (DMSO, ≥ 99.5 %, puriss., dried over molecular sieve), dimethylformamide (DMF, ≥ 99.8 % puriss., dried over molecular sieve), 1,2-dimethoxyethane (DME, ≥ 99.5 % puriss., dried over molecular sieve) and pyridine (≥ 99.5 % puriss., dried over molecular sieve) were purchased from Fluka (Steinheim, Germany). Acetone (≥ 99.5 %, Seccosolv), acetonitrile (≥ 99.8 %, for DNA synthesis), ethanol (≥ 96 %, purity according Ph. Eur.), acetic acid (p.a., 96 %), acetic anhydride (≥ 98.5 %, purity according Ph. Eur.), 4-nitrobenzoic acid (4-NBA) (p.a., 99 %), hydrochloric acid (37 %, purity according Ph. Eur.), phosphorus pentoxide (≥ 97 %), potassium hydroxide (≥ 85%, purity according Ph. Eur.), sodium hydroxide(≥ 98 %, purity according Ph. Eur.), and triethylamine (≥ 99 %) were supplied by Merck (Darmstadt, Germany). 4-Nitrobenzoic acid methylester (99 %) was purchased from Avocado (Karlsruhe, Germany). Isotonic saline, sterile water for injection and Sterifix Paed vented sterile filters were purchased from Braun (Melsungen, Germany). Millex-GS vented sterile filters were from Millipore (Carrigtwohill, Ireland). Solid phase extraction cartridges (Sep Pak light C18, Sep Pak plus C-18) were obtained from Waters (Milford, USA). Analytical (Luna Phenyl-Hexyl 5 µm 250 x 4.6 mm) and semi preparative (Luna Phenyl-Hexyl 10 µm 150x10 mm) HPLC-columns were obtained from Phenomenex (Aschaffenburg, Germany). Other chemicals and solvents were purchased commercially and used without further purification. Vinblastine sulfate was purchased from Teva Pharma (Aesch, Switzerland).

Precursor syntheses (Figure 1)

Aqueous NaOH solution (24 mL; 5%) was added to 300 mg of vinblastine sulfate (0.33 mmol) dissolved in 12 mL water. The reaction mixture was kept stirring for four days at room temperature. The solution was filtered and the pH was adjusted to 6.3 with 1 M HCl. The product solution was evaporated to dryness by repeated evaporation with acetonitrile and stored in the desiccator over P_2O_5 over night. The crude product was dissolved under Argon in 20 mL of dry pyridine. After addition of 6 mL of acetic anhydride the solution was stirred for 6 h at room temperature. Afterwards the reaction mixture was kept at 5 °C (refrigerator) for additional 3 days. Then 10 mL H_2O were added and the mixture was stirred for 3 h at 10 °C. After repeated addition of CH_3CN the solution was evaporated to dryness. Further work up (A and B, respectively) of the intermediate as described below led to the two desired precursor molecules:

A: vinblastine acid: After dissolving the residue in 30 mL H_2O the pH was adjusted to 5.4 with 0.1 M NaOH. The product was fixed on Waters C-18 SepPak Plus cartridges washed with 2 mL H_2O followed by elution with methanol. After evaporation of the methanol the residue was dissolved in CH_3CN and evaporated to dryness.

B: potassium salt of vinblastine acid: After dissolving the residue in 30 mL H_2O the pH was adjusted to 8.5 with 0.1 M KOH. The product was fixed on Waters C-18 SepPak Plus cartridges washed with 2 mL H_2O followed by elution with methanol. After evaporation of the methanol the residue was dissolved in CH_3CN and evaporated to dryness.

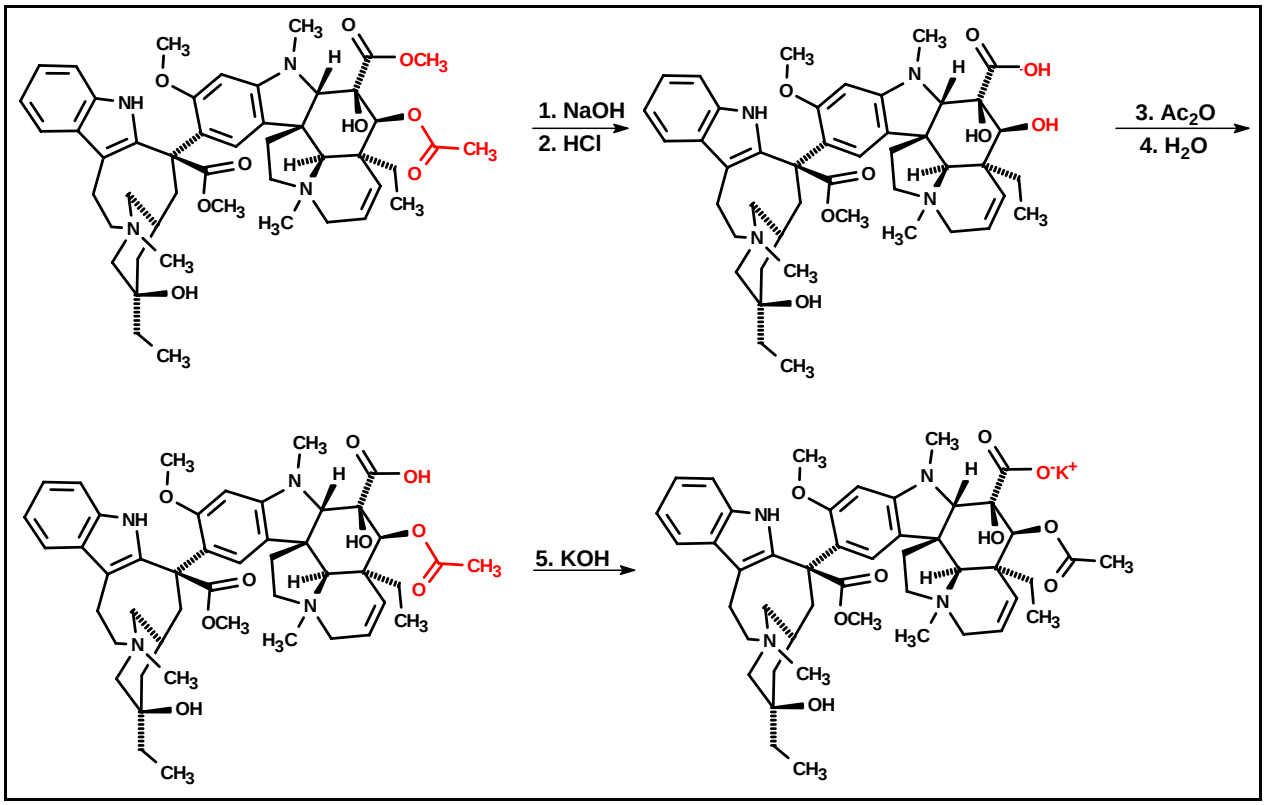

Figure 1: Preparation of the precursors.

Production of [^{11}C]CH_3I

Carbon-11 was produced as [^{11}C]CO_2 at the PETtrace cyclotron (General Electric Medical Systems, Uppsala, Sweden) by irradiation of N_2 containing 0.5 % O_2 using the $^{14}N(p,\alpha)^{11}C$ nuclear reaction with 16.5 MeV protons. [^{11}C]CH_3I was obtained by an automatic synthesis module (MeI MicroLab, General Electric Medical Systems) using the direct iodination of [^{11}C]methane as shown by Larson et al. (12) at a modified reaction temperature of 760 °C. Therein, [^{11}C]CO_2 was trapped on molecular sieve 4 Å and converted to [^{11}C]CH_4 in presence of Ni catalyst (Shimalite Ni 80/100, Shimadzu) and hydrogen at 360 °C. [^{11}C]CH_4 was reacted with elemental iodine at 760 °C to [^{11}C]CH_3I.

Production of [^{11}C]diazomethane

The production of [^{11}C]diazomethane was achieved by reaction of [^{11}C]methane with chlorine gas at 400 °C in a flow-through process, followed by an online conversion of the intermediately formed [^{11}C]chloroform with hydrazine, potassium hydroxide, 18-crown-6-crownether in an ethanolic solution as described previously by Solbach et al. (13). [^{11}C]Diazomethane thus produced was transferred online by a stream of helium into the corresponding reaction solution.

Labeling of vinblastine via [^{11}C] diazomethane

As it is impossible to determine the yield of the produced highly reactive [^{11}C]diazomethane directly (and so the radiochemical yield of [^{11}C]vinblastine) a special experimental layout (13) was necessary to allow the indirect determination using the quantitative esterification reaction of [^{11}C]diazomethane with 4-NBA as a monitor reaction (Figure 2). For this purpose the stream of He containing the online produced [^{11}C]diazomethane was bubbled simultaneously through two reaction vessels. Vessel 1 was a sealed 5 mL vial (Reactivial, Supelco, septum, screw cap) containing 1 mg (6 µmol) of 4-NBA in 1 mL DME at room temperature. Vessel 2 was a similar vial containing 2 mg vinblastine acid (2.5 µmol) in 1 mL DME at room temperature.

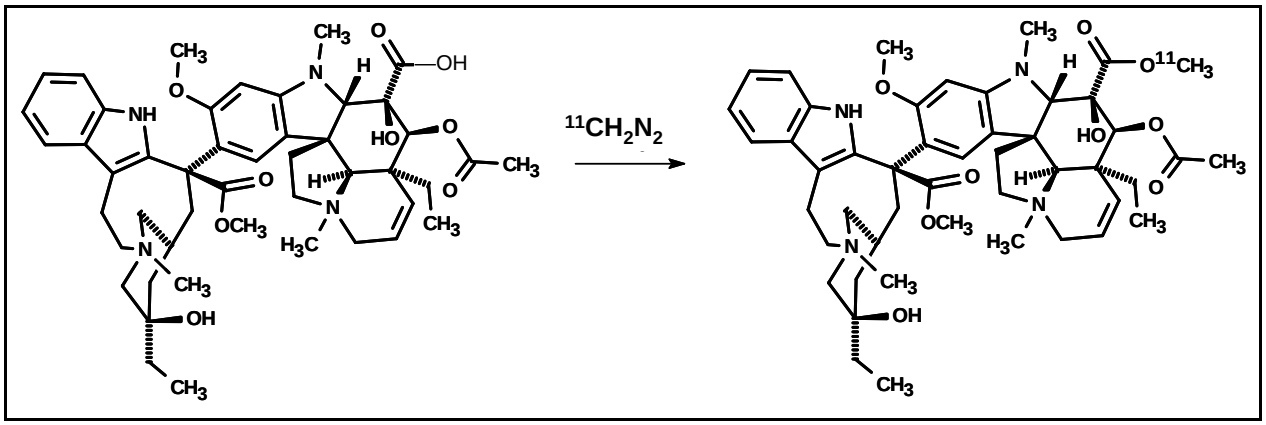

Figure 2: Synthesis scheme for the labeling with [^{11}C]diazomethane.

After completion of the reaction, both product solutions were analyzed by radio HPLC. Since it has been previously proven by Solbach et al. (13) that the reaction of [^{11}C]diazomethane with 4-NBA is quantitative, the yield for the reaction of [^{11}C]diazomethane with vinblastine acid could then be determined by the activity balance in relation to the activity assessment of the produced 4-nitrobenzoicacid[^{11}C]methylester.

Determination of reaction parameters for the synthesis of [^{11}C]vinblastine via [^{11}C]CH_3I

Stock solutions of [^{11}C]CH_3I were prepared by bubbling the produced [^{11}C]CH_3I in a helium stream of 50 mL/min into a sealed flask filled with 1 mL of solvent (acetone, acetonitrile, DME, DMF, DMSO) under adequate cooling.

In order to identify the most suited reaction solvent, 500 µL of a stock solution of the potassium salt of vinblastine acid (2 mg/mL = 2.4 mM) dissolved in acetone, acetonitrile, DME, DMF or DMSO were allowed to react with 50 µL of the [^{11}C]CH_3I stock solution in the corresponding solvent at a temperature of 100 °C. The reactions (Figure 3) were performed within 5 min in a sealed 5 ml reaction vessel (Reactivial, Supelco, septum; screw cap). After a reaction time of 5 min the reactions were interrupted by freezing the vessel in liquid nitrogen. While warming up, the reaction mixtures were diluted with 500 µL of the slightly acidic HPLC-eluent (pH 5.5). The effect of the reaction temperature on the radiochemical yield was determined using the experimental set-up described above. The reactions were carried out at 25, 50, 80, 100, 120 and 140 °C with a reaction time of 5 min using 2 mg of the potassium salt of vinblastine acid/mL in DMF (c = 2mg/mL ≡ 2.4 mM). In order to determine the optimum reaction time the reactions were performed from 1 min to 20 min at 140 °C in DMF and at 100 °C in DMSO.

Analyses

Aliquots (20 µL) of the product solutions were analyzed by radio-HPLC using a Luna Phenyl-Hexyl 10 µm, 250 x 4.6 mm (Phenomenex) as stationary phase and CH_3CN/H_2O 70/30/5 v/v/v 0.05 % NEt_3HCH_3COO (pH 5.5) as mobile phase; flow 1 mL/min; UV-detection 214 nm. The product was identified by comparison of the retention times of the isotopic non radioactive and the radioactive compound.

Automated radiosynthesis

Synthesis performance: [^{11}C]CH_3I produced by the MeI-MicroLab module was delivered into the reaction vessel of an automated synthesis module (NINA, Nuclear Interface, General Electric Medical Systems) loaded with a cooled (-40 °C) reaction mixture of 1 mg of the potassium salt of vinblastine acid in 500 µL of DMF. The methylation reaction was carried out for 5 min at 140 °C. After cooling to 25 °C the reaction mixture was diluted with 1 mL of HPLC-eluent and subsequently transferred to the semi preparative radio-HPLC system consisting of a preparative HPLC-pump (S 1021, Sykam), an automated flow detector controlled injection system with a 2 mL injection loop, a semi preparative HPLC-column (Phenomenex Luna Phenyl-Hexyl 10 µm, 150 x 10 mm) as stationary phase and CH_3CN/H_2O 65/35/5 v/v/v with 0.05 % NEt_3HCH_3COO (pH 5.0) as mobile phase (flow 6 mL/min), UV-detector (214 nm) and a NaI radioactivity detector.

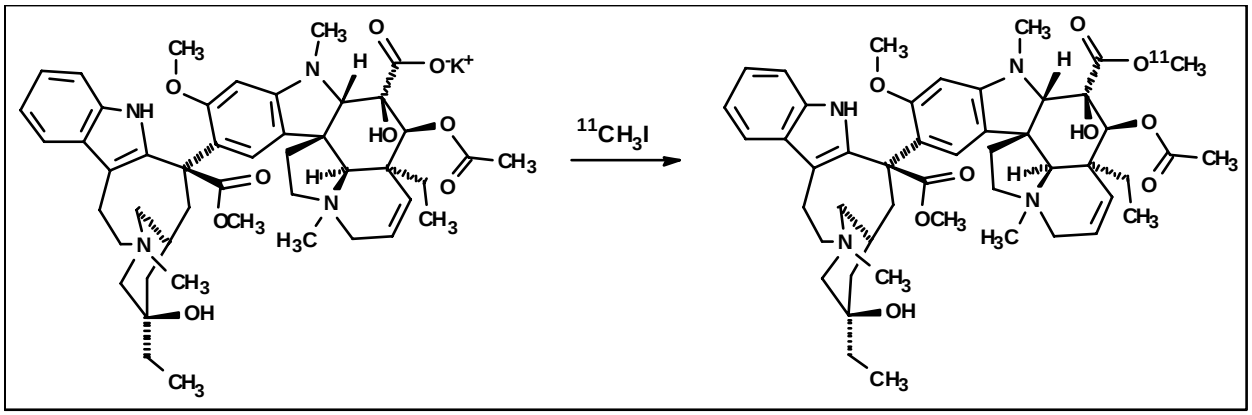

Figure 3: Synthesis scheme for the labeling with [^{11}C]methyl iodide.

The fraction containing the desired product [^{11}C]vinblastine was diluted with 30 mL of water for injection and subsequently purified by solid phase extraction using a C-18 Sep Pak light cartridge (14, 15), preconditioned with 10 mL ethanol and subsequently 10 mL water for injection. The loaded cartridge was washed with 10 mL of water for injection and the product was eluted with 0.8 mL of ethanol followed by 10 mL of isotonic saline. In the last step, sterile filtration was carried out by using a 0.22 µm vented sterile filter Millex GS.

Product analysis: Quality control of the product solution was performed by the same radio-HPLC system as described above for the investigation of the optimized reaction conditions.

PET measurements

Radioactivity distribution was measured with a GE Advance PET scanner in 2D acquisition mode. For attenuation correction a 511 keV transmission scan was used (500,000 kilocounts, no segmentation). Images were reconstructed with software developed at the PET Center Tübingen using an iterative algorithm. Metabolite analyses after injection of [^{11}C]vinblastine were not performed in this study. **Patient 1** underwent one PET scan after bolus injection of 112 MBq [^{11}C]vinblastine at tracer dose (19 µg unlabeled vinblastine) one day before chemotherapy (dynamic acquisition 0-60 min p.i. over a sternal metastasis, subsequent whole body scan). **Patient 2** underwent [^{11}C]vinblastine PET as described above (442 MBq [^{11}C] vinblastin, 60 µg) and a second scan with 395 MBq [^{11}C]vinblastine during infusion of the first therapeutic dose of vinblastine (3.75 mg) (dynamic acquisition over a parahepatic metastasis, wholebody scan 60 min after injection).

Both patients also underwent a diagnostic whole body FDG-PET on the same day after injection of 350 and 300 MBq [^{18}F]FDG, respectively.

Ethics Approval for Animal and Human Studies

Ethics approval was obtained according to German regulations, and patients were informed comprehensively to assure common consent.

RESULTS

Labeling of vinblastine via [^{11}C]diazomethane

The carbon-11 labeling of vinblastine using vinblastine acid and [^{11}C]diazomethane as labeling precursor succeeded with moderate radiochemical yields of 26 % ± 2 % (n=3).

Determination of optimized reaction parameters for the synthesis [^{11}C]vinblastine via [^{11}C]CH_3I

In order to determine the optimal reaction conditions for the methylation of the potassium salt of vinblastine acid with [^{11}C]CH_3I the effects of solvent, reaction temperature and reaction time were investigated.

Lowest amounts of [^{11}C]vinblastine were found as expected for the methylation of vinblastine acid in DME with a radiochemical yield (RCY) of 4 % ± 2 % after a reaction time of 5 minutes at 100 °C (Figure 4). The use of acetone and acetonitrile led to moderate RCY's of 16 % ± 4 % and 22 % ± 3 %, respectively. Better labeling results were found for the application of DMF with a RCY of 35 % ± 9 % and especially for DMSO which gave an increased RCY of 44 % ± 1 %.

For the reaction of the potassium salt of vinblastine acid with [^{11}C]methyl iodide the dependence on the reaction temperature was investigated within a temperature range of 25 °C and 140 °C using a reaction time of 5 min and DMF as solvent. As shown in Figure 5, the reaction proceeded with radiochemical yields of 22 % ± 5 % even at room temperature. The use of higher temperatures increased the yield of [^{11}C]vinblastine almost linearly up to a maximum of 43 % ± 5 % at 140 °C. Investigation of the time dependence (Figure 6) at the optimal reaction temperature of 140 °C showed an increase of the RCY up to 43 % ± 5 % in the first 5 min, followed by a decrease down to 18 % ± 2 % at a reaction time of 20 min. Time dependence was also determined for DMSO at 100 °C (Figure 6) and reached a plateau after 20 min with a maximum RCY of 53 % ± 3 % of [^{11}C]vinblastine.

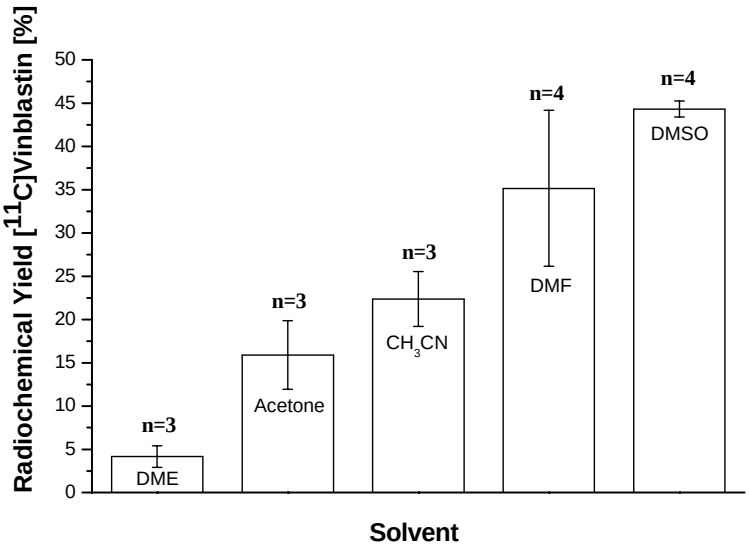

Figure 4: Solvent influence on the reaction of the potassium salt of vinblastine acid with [^{11}C]methyl iodide; 1 mg potassium salt of vinblastine acid, 500 µL solvent, 5 min, 100 °C.

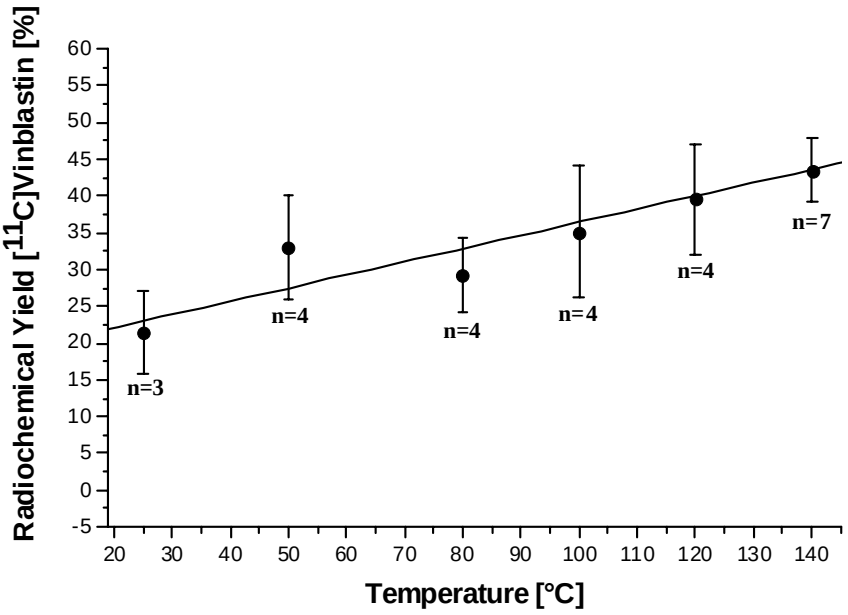

Figure 5: Temperature dependence on the reaction of the potassium salt of vinblastine acid with [^{11}C]methyl iodide; 1 mg potassium salt of vinblastine acid, 500 µL solvent, 5 min, DMF.

Automated radiosynthesis

In the radiosyntheses for patient application 1 mg of the precursor dissolved in 500 µL DMF was reacted with [^{11}C]CH_3I for 5 min at 140 °C. The preparation including semi-preparative radio-HPLC separation, solid phase extraction purification, formulation and sterile filtration was completed within 45 min. The product was obtained in a sterile solution ready for intravenous injection. Quality control by radio-HPLC always showed a radiochemical purity of more than 95 %.

In the bench experiments the labeling step was performed with a yield of 43 % ± 5 %. The high activity production runs with complete work-up including purification, solid phase extraction and sterile filtration resulted in overall radiochemical yields of about 19 %. The production process thus resulted in absolute yields up to 1 GBq [^{11}C]vinblastine starting from approximately 15 GBq [^{11}C]methyl iodide with specific activities between 9 and 44 GBq/µmol at EOS.

Application of [^{11}C]vinblastine in two patients

The purpose of the application of [^{11}C]vinblastine-PET in cancer patients was the determination of the patient's individual pharmacokinetics of [^{11}C]vinblastine. It was to be investigated whether the pharmacokinetics of [^{11}C]vinblastine correlate with the therapy success and if a prognosis for the therapy response expected for the treatment with the chemotherapeutic agent vinblastine can be derived from the data. Two patients suffering from metastasized renal cell carcinoma were measured with [^{11}C]vinblastine.

Patient 1: Figure 7 shows the activity distribution (top) 60 min after injection of 112 MBq [^{11}C]vinblastine along with the corresponding [^{18}F]FDG scans (bottom). Known bone metastases in the sternum, in the right shoulder and in the left pelvis showed focally increased [^{11}C]vinblastine uptake and [^{11}C]vinblastine metabolite uptake, respectively and increased glucose metabolism. The time-activity curve of [^{11}C]vinblastine and metabolized [^{11}C]vinblastine uptake, respectively in the sternal metastasis (Figure 8) showed an early maximum at about 2 min after injection followed by a continuous decline.

Patient 2: Figure 9 shows corresponding sections from [^{11}C]vinblastine- and [^{18}F]FDG-PET.

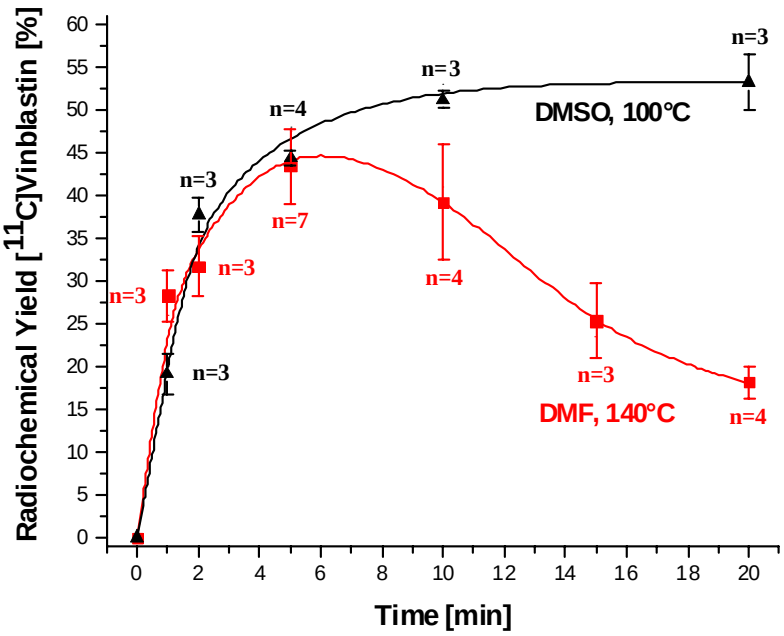

Figure 6: Time dependence on the reaction of the potassium salt of vinblastine acid with [^{11}C]methyl iodide; 1 mg potassium salt of vinblastine acid, **A:** 500 µL DMF, 140 °C. **B:** 500 µL DMSO, 100 °C.

While [^{18}F]FDG-PET revealed clearly hypermetabolic mediastinal metastases, no corresponding focal uptake of [^{11}C]vinblastine and metabolized [^{11}C]vinblastine, respectively, was observed. The same pattern was found in paraaortal lymphnode metastases. Figure 10 shows the wholebody activity distribution after injection of [^{11}C]vinblastine. After adjusting the grayscale to the maximum radioactivity concentration (Figure 10b), PET shows biliar and renal excretion of vinblastine and its metabolites. In the second PET scan during infusion of vinblastine at a therapeutic dose (3.75 mg), the liver uptake was slightly decreased while the blood concentration was increased.

The carbon-11 labeling via [^{11}C]diazomethane resulted only with moderate radiochemical yield. It can be assumed, that inter- and intramolecular interactions lead to a significant reduced acidity of the precursor molecule vinblastine acid. Since the reactions were performed in a sort of prototype synthesis apparatus that was not located in a hot cell and was therefore not sufficiently shielded for the production of [^{11}C]diazomethane in high activities and since the labeling yields were not superior to the yields obtained using [^{11}C]methyl iodide as labeling precursor, this labeling pathway was not applied for producing [^{11}C]vinblastine for patient investigation.

DISCUSSION

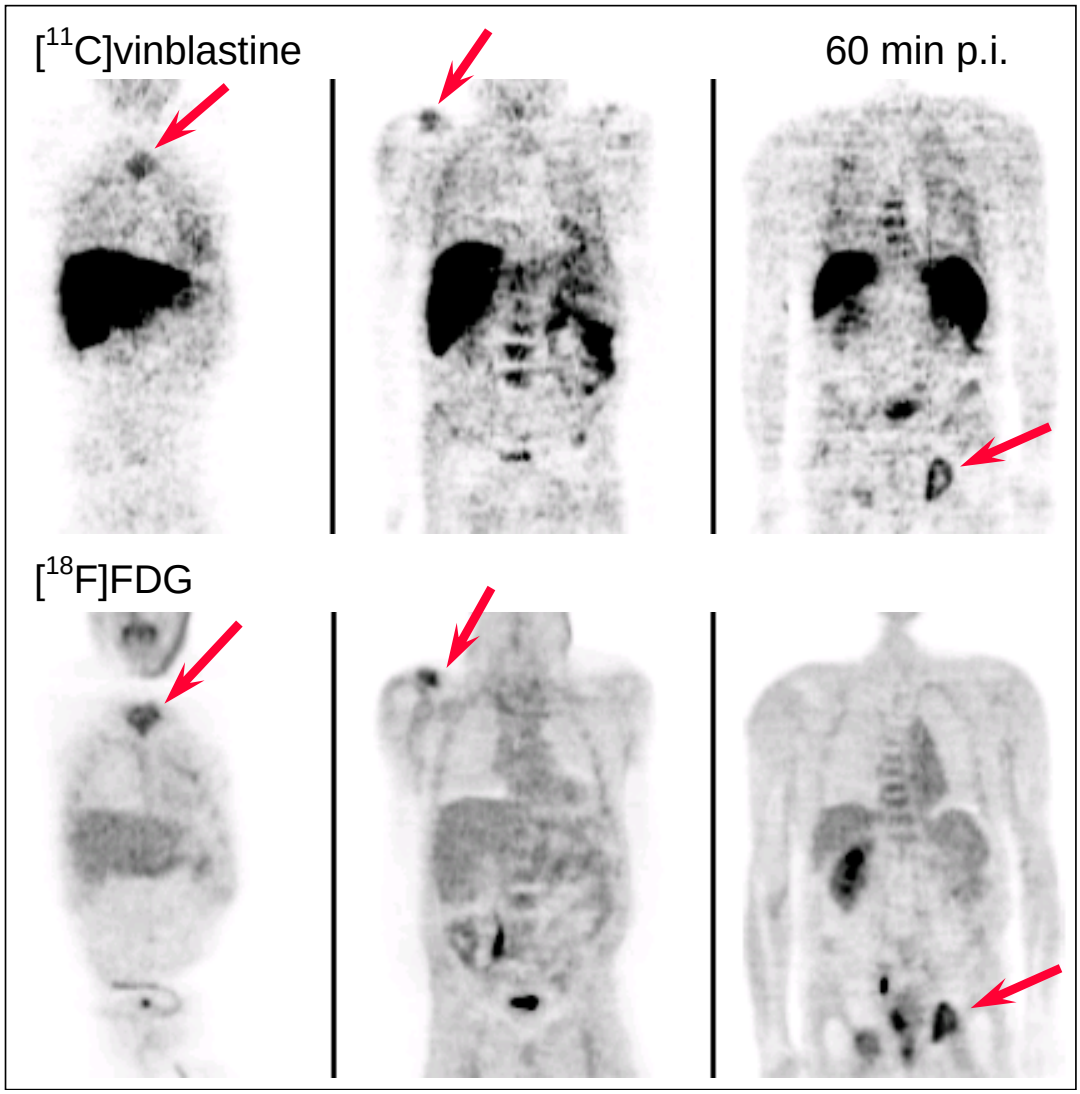

Figure 7: Patient 1: Whole body PET images 60 min after injection of [^{11}C]vinblastine show focally increased uptake of [^{11}C]vinblastine and metabolized [^{11}C]vinblastine respectively in multiple bone metastases of renal carcinoma (top). [^{18}F]FDG-PET shows increased glucose metabolism (bottom).

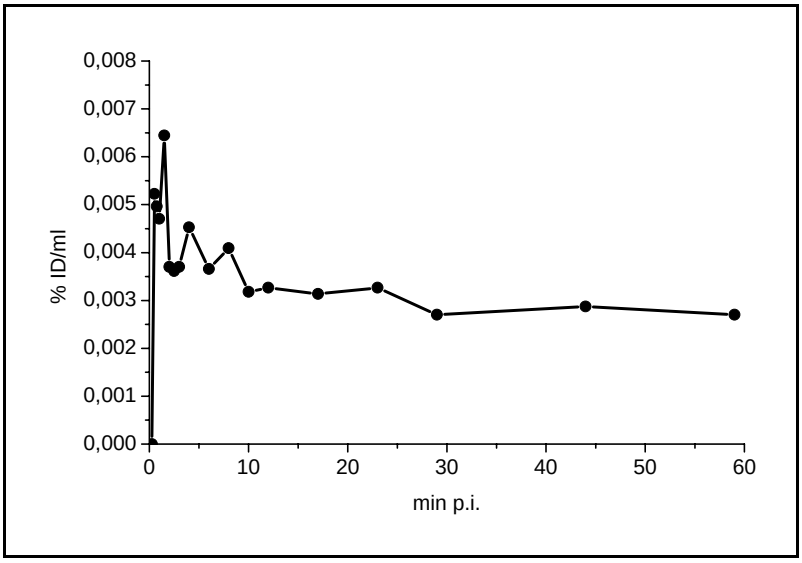

Figure 8: Patient 1: Time-activity curve in the sternal metastasis after injection of [^{11}C]vinblastine.

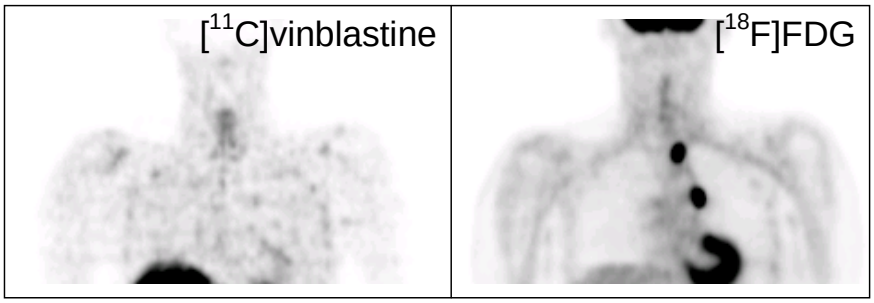

Figure 9: Patient 2: No focally increased [^{11}C]vinblastine uptake and [^{11}C]vinblastine metabolite uptake, respectively (left) was found in mediastinal and abdominal metastases (latter not shown) of a renal carcinoma, clearly identifyable by their elevated glucose metabolism (right).

The labeling of vinblastine by use of [^{11}C]methyl iodide was optimized with respect to solvent, reaction temperature and reaction time. Second order nucleophilic substitution reactions are supposed to be supported by dipolar aprotic solvents such as acetone, acetonitrile, DMF and DMSO. DME was also investigated as an additional dipolar aprotic solvent because of its low polarity. The results indeed showed a clear dependence on the type of solvent and are principally in agreement with the individual polarity of the solvents since the dissociation of the potassium salt of vinblastine acid is supported by more polar solvent such as DMSO or DMF compared to acetone, acetonitrile and DME (Figure 4). Therefore, by using DMSO, the labeling yields could be improved significantly. But with respect to the aim of an automated synthesis procedure DMF as the second best solvent was chosen for the determination of the time dependence because of its lower freezing point (- 60 °C) in contrast to DMSO (16 °C). That allowed an almost quantitative trapping of the gaseous labeling precursor [^{11}C]methyl iodide for the reaction with potassium salt of vinblastine acid. As optimal reaction temperature 140°C was obtained and applied in the investigation of the time dependence (Figure 5).

The investigation of the time dependence at the optimal reaction temperature of 140°C showed in the first 5 min an increase of the RCY up to 43 % ± 5 %. Interestingly, [^{11}C]vinblastine appeared to be unstable under the applied reaction conditions, since reaction times > 5 min resulted in a decrease of the RCY down to 18 % ± 2 % at a reaction time of 20 min (Figure 6).

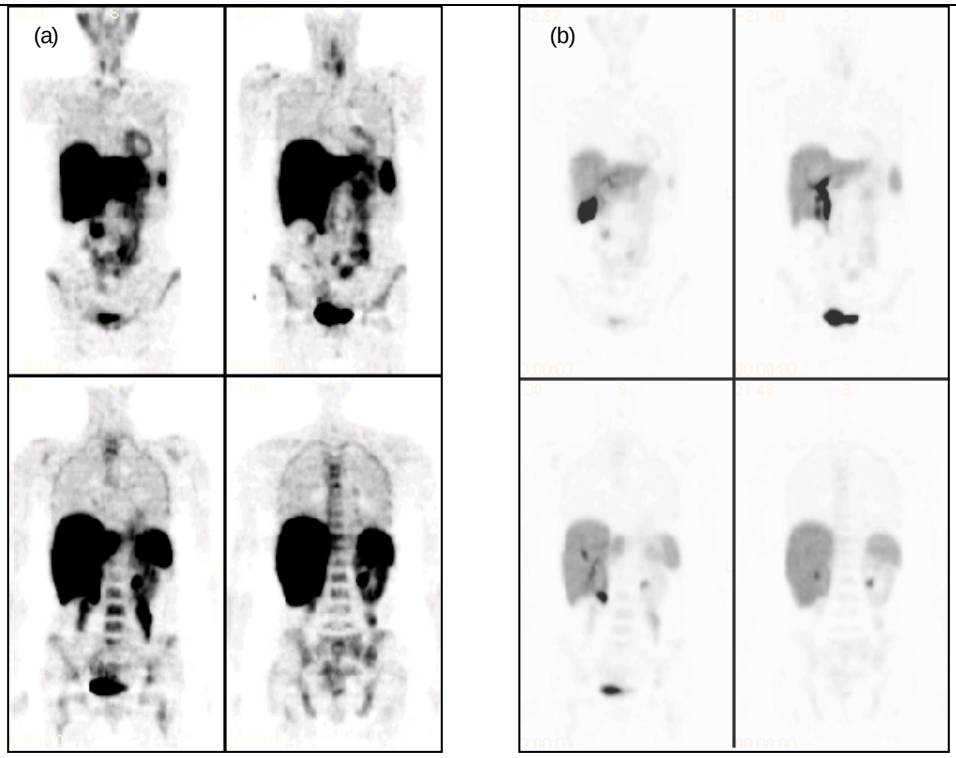

Figure 10: Patient 2: wholebody activity distribution 60 min after injection of [^{11}C]vinblastine (a). After adjusting the grayscale to the maximum radioactivity concentration (b, corresponding slices), PET shows biliar and renal excretion of vinblastine and its metabolites.

With respect to these results the time dependence was investigated additionally in DMSO at a reaction temperature of 100°C. In DMSO at 100°C the reaction kinetics proceeded in the first 5 min similar to the reaction in DMF at 140°C. Even though the reaction temperature was 40°C lower the RCY (44 % ± 1 %) was approximately the same as in DMF at 140°C. In contrast to the reaction in DMF the product remained unchanged under these conditions and a maximum RCY of 53 % ± 3 % of [^{11}C]vinblastine was reached after 20 min. Although the best radiochemical yields were obtained by use of DMSO at 100°C with a reaction time of 20 min, it was decided to conduct the automated syntheses using DMF as solvent at 140°C with a reaction time of 5 min as with respect to the short half-life of carbon-11 (20.4 min) a reaction time of 20 min was not considered to be of practical value for productions to be used in patient investigations. The achieved reaction parameters were transferred to a completely shielded remotely controlled synthesizer module and enabled the production of patient doses of [^{11}C]vinblastine within 45 min from EOB.

The reported patient investigations are to be considered to be attempts of a clinical application of [^{11}C]vinblastine for individualisation of therapeutic concepts supported by PET. Tumor uptake and washout of [^{11}C]vinblastine differed considerably between both patients, suggesting that chemotherapy efficacy and tolerability might be improved from an individualization of the treatment regimes. In contrast to patient 2 the first patient (patient 1) showed a clear washout of [^{11}C]vinblastine. This result may help to explain why the patient did not respond to chemotherapy in spite of a rather high initial uptake.

CONCLUSION

The carbon-11 labeling of vinblastine was optimized with respect to the labeling precursor, the solvent, the reaction temperature and the reaction time. The radiolabeling of vinblastine with carbon-11 succeeded with a RCY of 53 % ± 3 % when the reaction was carried out in DMSO for 20 min using the sodium salt of vinblastine acid and [^{11}C]methyl iodide as labeling precursor. The radiosynthesis was adapted to an automated radiosynthesizer system suitable for the preparation of [^{11}C]vinblastine for patient investigations under routine conditions. In production runs, absolute yields up to 1 GBq of [^{11}C]vinblastine were achieved starting from about

15 GBq of [^{11}C]methyl iodide within 45 minutes from EOB.

The conducted PET investigations in two patients with renal carcinoma demonstrate the possibility to individually assess the dosing of the chemotherapeutic agent to the tumor combined with the pharmacokinetic behaviour in organs and tumor.

REFERENCES

[1] Dodd P.M., McCaffrey J.A., Mazumdar M., Scher H., Vlamis V., Higgins G., Herr H., Bajorin,m, D.F. Phase II trial of intermediate dose methotrexate in combination with vinblastine, doxorubicin, and cisplatin in patients with unresectable of metastatic transitional cell carcinoma. Cancer 85: 1145-1150, 1999.

[2] von der Maase H., Hansen S.W., Roberts J.T., Dogliotti L., Oliver T., Moore M.J., Bodrogi I., Albers P., Knuth A., Lippert C.M., Kerbrat P., Sanchez Rovira P., Wersall P., Cleall S.P., Roychowdhury D.F., Tomlin I., Visseren-Grul C.M., Conte P.F. Gemcitabine and cisplatin versus methotrexate, vinblastine, doxorubicin, and cisplatin in advanced or metastatic bladder cancer: results of a large, randomized, multinational, multicenter, phase III study. J. Clin. Oncol., 19: 1229-1231, 2001.

[3] Dodd P.M., McCaffrey J.A., Herr H., Mazumdar M., Bacik J., Higgins G., Boyle M.G., Scher, H.I., Bajorin D.F. Outcome of postchemotherapy surgery after treatment with methotrexate, vinblastine, doxorubicin, and cisplatin in patients with unresectable or metastatic transitional cell carcinoma. J. Clin. Oncol., 17: 2546-2552, 1999.

[4] Pyrhonen S., Salminen E., Ruutu M., Lehtonen T., Nurmi M., Tammela T., Juusela H., Rintala E., Hietanen P., Kellokumpu-Lehtinen P.L.. Prospective randomized trial of interferon alfa-2a plus vinblastine versus vinblastine alone in patients with advanced renal cell cancer. J. Clin. Oncol., 17: 2859-2867, 1999.

[5] von der Maase H., Sengelov L., Roberts J.T., Ricci S., Dogliotti L., Oliver T., Moore M.J., Zimmermann A., Arning M.. Long-term survival results of a randomized trial comparing gemcitabine plus cisplatin, with methotrexate, vinblastine, doxorubicin, plus cisplatin in patients with bladder cancer. J. Clin. Oncol., 23: 4602-4608, 2005.

[6] Lehmann J., Retz M., Wiemers C., Beck J., Thuroff J., Weining C., Albers P., Frohneberg D., Becker T., Funke P.J., Walz P., Langbein S., Reiher F., Schiller M., Miller K., Roth S., Kalble T., Sternberg D., Wellek S., Stockle M. Adjuvant cisplatin plus methotrexate versus methotrexate, vinblastine, epirubicin, and cisplatin in locally advanced bladder cancer: results of a randomized, multicenter, phase III trial (AUO-AB 05/95). J. Clin. Oncol., 23: 4963-4974, 2005.

[7] Loehrer P.J. (Sr), Einhorn L.H., Elson P.J., Crawford E.D., Kuebler P., Tannock I., Raghavan D., Stuart-Harris R., Sarosdy M.F., Lowe B.A., Blumenstein B., Trump D. A randomized comparison of cisplatin alone or in combination with methotrexate, vinblastine, and doxorubicin in patients with metastatic urothelial carcinoma: a cooperative group study. J. Clin. Oncol., 10: 1066-1073, 1992.

[8] Roberts J.T. Chemotherapy for metastatic bladder cancer. Clin Oncol (R Coll Radiol),. 17: 514-523, 2005.

[9] Garrett T.J., Vahdat L.T., Kinne D.W. Systemic adjuvant therapy of breast cancer. J. Surg. Oncol., 64: 167-172, 1997 (review).

[10] Dolle F., Hinnen F., Valette H., Fuseau C., Duval R., Peglion J.L., Crouzel C. Synthesis of two optically active calcium channel antagonists labelled with carbon-11 for in vivo cardiac PET imaging. Bioorg. Med. Chem., 5: 749-764, 1997.

[11] Stewart D.J., Lu K., Benjamin R.S., Leavens M.E., Luna M., Yap H.Y. Loo T.L. Concentration of vinblastine in human intracerebral tumor and other tissues. J. Neurooncol., 1: 139-144, 1983.

[12] Larsen P., Ulin J., Dahlstroem K., Jensen M. Synthesis of [^{11}C]iodomethane by iodination of [^{11}C]methane. Appl. Radiat. Isot., 48: 153-157, 1997.

[13] Solbach, C., Machulla, H.-J. Production of [^{11}C]chloroform by direct chlorination of [^{11}C]methane without catalyst support for the synthesis of [^{11}C]diazomethane. Appl. Radiat. Isot., : 2007 (accepted).

[14] Solbach C., Reischl G., Machulla H.-J. Determination of reaction parameters for the synthesis of the serotonin transporter ligand [^{11}C]DASB: Application to a remotely controlled high yield synthesis. Radiochim. Acta 92: 341-344, 2004.

[15] Solbach C., Uebele M. Reischl G, Machulla H.J. Efficient radiosynthesis of carbon-11 labeled uncharged Thioflavin T derivatives using [^{11}C]methyl triflate for β-amyloid imaging in Azheimer's Disease with PET. Appl. Radiat. Isot., 62: 591-595, 2005