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Detection of Muscarinic Receptors in the Human Lung Using PET

Ton J. Visser, Aren van Waarde, Thomas W. van der Mark, Jan Kraan, Kees Ensing, Antoon T.M. Willemsen, Philip H. Elsinga and Willem Vaalburg

PET Center, Department of Pulmonary Diseases, Groningen University Hospital, and Department of Analytical Chemistry and Toxicology, University Center for Pharmacy, Groningen, The Netherlands

The characterization of pulmonary muscarinic receptors with PET is still in its infancy. Because approximately 70% of the lungs consists of air and pulmonary muscarinic receptor densities are low, ligands with high receptor affinity are required to obtain reasonable signal-to-noise ratios on PET images. Therefore, the potent ^{11}C-labeled muscarinic antagonist N-methyl-piperidin-4-yl 2-cyclohexyl-2-hydroxy-2-phenylacetate methiodide ([R]-VC-002) was developed. We administered this radioligand to four healthy human volunteers to examine its suitability for studying pulmonary muscarinic receptors in vivo. **Methods:** [^{11}C]VC-002 (185 MBq, specific activity > 7.4 TBq/mmol) was intravenously injected on 2 separate days, with an interval of at least 1 wk. On the first day the volunteers were not pretreated, but on the second day they received the anticholinergic glycopyrronium bromide (Robinul; 2 × 0.1 mg intravenous) 25 and 30 min before the injection of the radiopharmaceutical. C[^{15}O]O scans (approximately 740 MBq [20 mCi] by inhalation) were acquired before the receptor scan to calculate pulmonary blood volume. **Results:** On PET images of the thorax, the lungs were clearly visible. After the volunteer was pretreated with glycopyrronium bromide, pulmonary uptake of the radioligand was reduced to 32% ± 12% of the control value at 60 min postinjection and the lungs could no longer be seen. (R)-[^{11}C]-VC-002 was rapidly cleared from plasma and was slowly metabolized during the time course (60 min) of the PET scan. The fraction of radioligand representing parent compound decreased from 99.9% at the time of injection to 82% at 40–60 min postinjection, both in the presence and absence of Robinul. Pulmonary tissue-to-plasma ratios, calculated on a count-per-minute-per-gram basis, reached a plateau value of 17.8 ± 1.2 at 40–50 min postinjection. **Conclusion:** [^{11}C]VC-002 appears to be suitable for in vivo studies of pulmonary cholinoceptors.

Key Words: muscarinic receptors; PET; human; lung

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The autonomic nervous system regulates the contraction and relaxation of the smooth muscle cells in the airways. Binding of noradrenaline to β-adrenergic receptors causes relaxation, whereas the binding of acetylcholine to muscarinic receptors causes contraction. The balance between the adrenergic (sympathetic) and cholinergic (parasympathetic) systems determines airway calibre. Both types of receptors are also involved in the regulation of bronchial and alveolar mucus secretion (*1–3*).

Changes in the number or the properties of these receptors may play a role in asthma and chronic obstructive pulmonary disease (COPD). Medicamentous treatment may also lead to changed characteristics of these binding sites. For example, patients suffering from asthma and COPD are often treated with β_2-adrenoceptor agonists (sympathomimetics) and muscarinic antagonists (parasympatholytics). Chronic exposure of tissues to these drugs can lead to rebound effects after cessation of therapy and to a phenomenon known as tolerance, which is a reduction in the sensitivity of the tissue to the drug, accomplished by uncoupling, internalization or downregulation of the receptors (*4–10*).

PET offers a unique possibility to study the muscarinic and β-adrenergic receptors in vivo. Consequently, it may become clear whether there are differences in receptor densities between the lungs of patients suffering from asthma or COPD and healthy volunteers. Also the effects at the receptor level after chronic treatment of these patients with long- and short-acting β-sympathomimetics and parasympatholytics can be studied.

Pulmonary β-adrenergic receptor densities have been determined with PET in asthmatics and age-matched healthy volunteers after intravenous administration of 4-(3-t-butylamino-2-hydroxypropoxy)-benzimidazol-2[^{11}C]-one ([^{11}C]CGP 12177). No differences could be found between both groups indicating the absence of a primary β-adrenergic deficit in asthma (*11*), although it should be noted that [^{11}C]CGP 12177 binds primarily to the alveolar receptors (for >90%). These receptors are not believed to be involved in the pathophysiology of asthma. Because only a fraction of the binding occurs to receptors on the smooth muscle cells in the airways (<10%), it may not be possible to detect changes in this population. Also, the effect of long-term β_2-adrenergic-agonist dosing on pulmonary receptor density was studied. After salbutamol treatment, β-adrenoceptor density decreased on average by 22% ($P < 0.05$) in human lungs (*12,13*).

Because the cholinergic nervous system is the dominant

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For correspondence or reprints contact: Ton J. Visser, PhD, PET Center, Groningen University Hospital, P.O. Box 30001, 9700 RB Groningen, The Netherlands.

bronchoconstrictor neural pathway in the human airways, it is reasonable to presume that this system may play a role in the pathophysiology of asthma and COPD. Increased muscarinic cholinergic activity may occur through an increase in receptor density or affinity or through an increase in the efficacy of receptor/signal transduction coupling. Studies of the binding of [^{3}H]quinuclidinyl benzilate ([^{3}H]QNB) to human lung tissue from healthy subjects and patients with chronic obstructive lung disease showed no differences in binding affinity (K_D) but an unexpectedly low number of muscarinic receptors in the patient group (83 and 26 fmol/mg protein for normals and patients, respectively) (*14*). In another study, no significant differences were found in either the K_D or receptor concentration between the healthy subject and patient groups (*15,16*). A possible defect in the feedback inhibitory M_2 receptor was reported in patients suffering from asthma (*17,18*).

Although the potent muscarinic antagonist [^{11}C]methylquinuclidinyl benzilate ([^{11}C]MQNB) has been used extensively for the visualization/quantification of muscarinic receptors in the heart by PET (*19–21*), to our knowledge no attention has been paid to the in vivo characterization of pulmonary muscarinic receptors by means of this imaging technique.

During animal experiments in our laboratory, it became clear that N-methyl-piperidin-4-yl 2-cyclohexyl-2-hydroxy-2-phenylacetate methiodide ([R]-VC-002) is a suitable candidate for the evaluation of pulmonary muscarinic receptors (*22*). This study reports the results of the first human studies with this ^{11}C-labeled ligand in which pulmonary binding was assessed.

MATERIALS AND METHODS

Radioligands

(R)-[^{11}C]-VC-002 was synthesized by quaternization of N-methyl-piperidin-4-yl 2-cyclohexyl-2-hydroxy-2-phenylacetate with [^{11}C]CH_3I (*22*). The product was purified by high-performance liquid chromatography (HPLC) (10 μm) (CN-RadPak; Waters, Milford, MA). Mobile phase: H_2O (1% NH_4OH [20%], acidified to pH 4.5 with acetic acid): CH_3CN = 68:932, v/v, running at 4 mL/min. The retention time of the product was 9 min, and the chemical and radiochemical purity was >99%. The total synthesis time was 40 min, and the radiochemical yield varied between 40% and 60% (corrected for decay). The specific activity was always higher than 7.4 TBq/mmol at the time of injection. After evaporation of the solvents, the radioligand was dissolved in 8 mL phosphate-buffered saline (140 mmol/L NaCl; 9.0 mmol/L Na_3PO_4; 1.3 mmol/L NaH_2PO_4). Before injection, the solution was filtered (0.22-μm Millex GP filter; Millipore, Danvers, MA) into an evacuated stervial. The solution was colorless, isotonic, sterile and apyrogenic, and the pH was 6.5–7.5. (R)-[^{11}C]-VC-002 passed the test on "acute toxicity" (European Pharmacopoeia; Dutch Pharmacopoeia, 9th ed.) at a 10,000-fold higher dose than was administered to humans per kilogram of body weight.

C[^{15}O]O was synthesized from [^{15}O]-O_2 produced by our cyclotron by the ^{14}N[d,n]^{15}O nuclear reaction. The [^{15}O]-O_2 was led through a column containing charcoal at 900°C, transforming it into C[^{15}O]O. After removal of traces of C[^{15}O]O_2 by means of a soda lime trap, the C[^{15}O]O was passed into a chemical processing box (Scanditronix, Uppsala, Sweden). Quality control consisted of radio-gas chromatography analysis of a sample of C[^{15}O]O obtained from a test run, performed immediately before the actual radiosynthesis. To avoid bacteriological contamination, the C[^{15}O]O was transferred to the mouth by a 0.22-μm filter. The total amount of nonradioactive CO administered is such (<90 μmol) that it does not produce any physiological effect and may be considered nontoxic (<0.25% of total hemoglobin occupied).

Human Volunteers

The participants were recruited according to the following criteria: age 18–50 y, prebronchodilator forced expiratory volume in one second (FEV_1) > 80% of predicted, nonsmoker or ex-smoker (smoking terminated for >1 y). Excluded were people with a positive history regarding wheezing and tightness of the chest, angina pectoris, myocardial infarct or stroke, presence of pulmonary diseases including asthma, open angle glaucoma, pyloric obstruction, obstruction of the neck of the urinary bladder, obstruction of the intestine, severe renal, hepatic or cardiovascular disease or atopy (at least one positive reaction to known allergens in intracutaneous skin tests). Also excluded were people who used β-mimetics (salbutamol) and theophylline (≤12 and 48 h, respectively) before the study, people with a diastolic blood pressure persistently >100 mm Hg and women who were pregnant.

All volunteers underwent the following screening: anamnesis, physical examination, routine blood biochemistry to assess kidney and liver function, electrocardiogram, skin tests for allergic reactions and pulmonary function tests (spirometry, bronchial hyperactivity). Airway responsiveness to methacholine was determined using the 2-min tidal breathing method (*23,24*). The provocation concentration to achieve a 20% decrease in FEV_1 had to be >8 mg/mL. The study was approved by the Medical Ethics Committee of the Groningen University Hospital. Each subject was informed both orally and in writing about the purpose and risks of the experiment and gave informed consent.

Study Protocol

At the beginning of the study, a venous cannula was placed in one of the veins of one of the forearms. After patency of the ulnary artery was proven by the Allen test (*25*), an arterial cannula was placed in the radial artery of the contralateral arm under local anesthesia with lidocain. The venous cannula was used for injection and the arterial line for blood sampling. Subsequently, the volunteer was placed into the PET camera (full width at half maximum = 6 mm, axial field of view of 10.8 cm, images reconstructed in 31 transaxial planes) (Siemens ECAT 951/31; Siemens Medical Imaging, Knoxville, TN). A rectilinear scan was obtained for proper positioning (carina at the center of the field of view). Then a transmission scan was obtained, using the internal ^{68}Ge/^{68}Ga sources, to correct for attenuation. Subsequently, approximately 740 MBq (20 mCi) C[^{15}O]O were administered as a bolus by inhalation. Camera acquisition started at complete expiration and proceeded over an interval of 10 min (10 time frames of 1 min) to acquire information on the size of the pulmonary blood pool. During the C[^{15}O]O scan, arterial blood samples (1.5 mL) were drawn at 2.5, 5 and 7.5 min after the onset of data acquisition. These blood samples were then counted in a gamma counter that was cross calibrated with the PET camera. Finally, 185 MBq (5 mCi) (R)-[^{11}C]-VC-002 were administered to the volunteer and arterial blood samples (2 mL) were drawn at 0.5-min intervals

during the initial 5 min and at 10-min intervals from 10 to 60 min postinjection. PET images were acquired over a period of 60 min. The following time frames were used: 8 frames of 15 s were followed by 4 frames of 30 s, 4 frames of 1 min, 4 frames of 2 min, 6 frames of 4 min and 2 frames of 10 min.

On the second day of the study (at least 1 wk later), an identical protocol was performed with the following modification: two 100-μg doses of glycopyrronium bromide (total = 0.2 mg) (Robinul; Wyeth laboratoria b.v., Hoofddorp, The Netherlands) were administered by intravenous injection 30 and 25 min before injection of the radioligand.

Metabolite Analysis and Plasma Protein Binding

For the determination of the concentration of (R)-[^{11}C]-VC-002 and labeled metabolites in plasma, arterial blood samples were drawn at the following time points: 1, 2, 5, 10, 40 and 60 min postinjection. Plasma and red blood cells were separated by centrifugation (2 min, Hettich centrifuge, EBA III; Tuttlingen, Germany). Subsequently, the plasma samples were treated with two volumes of CH_3CN and spiked with nonlabeled (R)-VC-002. Then the samples were subjected to HPLC (stationary phase: Waters C_{18} RadPak radial compression module column, 5 μm; mobile phase: CH_3CN:NaH_2PO_4 [50 mmol/L]:diethylamine = 50:50:1, pH = 6, flow rate 2.0 mL/min). Fractions (1 mL) of the eluate were collected during 15 min and counted in a calibrated gamma counter (Compugamma 1282 CS; LKB-Wallac, Turku, Finland). The recovery from the column exceeded 92%.

Blood samples (1 mL) for the determination of plasma clearance were drawn at time intervals of 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5, 10, 20, 30, 40, 50 and 60 min after administration of the radioligand. After centrifugation to separate plasma from blood cells, plasma samples (0.25 mL) were counted in the gamma counter.

Data Analysis

On the transmission scan, the lungs were clearly visible. Regions of interest (ROIs) were drawn on left and right lungs using the transmission scan. Hilar structures were avoided. Subsequently, the ROIs were projected on the original dynamic emission scan. Then, time-activity curves for the different ROIs were generated using ECAT software (version 6.5D) running on a SUN/SPARC workstation (Sun MicroSystems, Mountain View, CA). Because data from different studies had to be compared, values of tissue uptake were normalized to an injected radioactivity of 185 MBq (5 mCi). The time-activity data were analyzed using a nonlinear regression data analysis program (Enzfit [Elsevier Biosoft, Cambridge, UK], Multifit [Johannes Proost, University of Groningen, The Netherlands]). The fraction of radioactivity in the pulmonary vascular compartment in the ROIs was calculated with the help of the C[^{15}O]O scan and the blood samples drawn during this scan.

RESULTS

In Vivo Experiments with (R)-[^{11}C]-VC-002 in Healthy Volunteers

In these experiments, (R)-[^{11}C]-VC-002 was administered to four healthy volunteers (three men, one woman; age range 20–26 y, mean age 32 ± 13 y) to assess radioligand uptake in the lungs before and after intravenous injection of a standard dose of glycopyrronium bromide (Robinul). Each volunteer was scanned twice, first with no pretreatment and then with glycopyrronium bromide.

In Figure 1, four PET images are shown. Figures 1A and B show the uptake of (R)-[^{11}C]-VC-002 in the thorax of a female volunteer, with (B) and without glycopyrronium bromide pretreatment (A). In Figures 1C and D, the lungs of

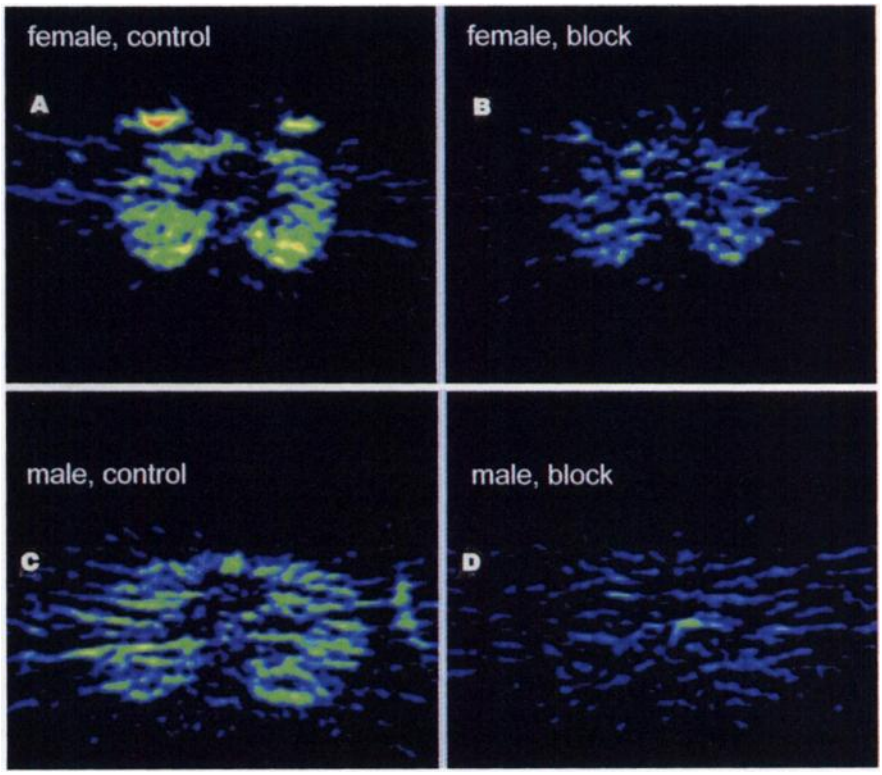

FIGURE 1. PET images of muscarinic receptors in lungs of female (A and B) and male (C and D) healthy volunteers acquired with (R)-[^{11}C]-VC-002 without (A and C) and with receptor blocking (B and D). Transaxial cross-sections in time frames 24–60 min postinjection are shown. Note high uptake in mammae of female volunteer.

a male volunteer are shown with (D) and without glycopyrronium bromide pretreatment (C). Peripheral lungs are clearly visible in the control studies (A and C) but after intravenous injection of glycopyrronium bromide, the lungs could no longer be seen (B and D). In these studies, the large airways can hardly be distinguished. In the female volunteer, remarkably high uptake was observed at the level of the mammae.

Kinetics of (R)-[^{11}C]-VC-002 in Lungs

(R)-[^{11}C]-VC-002 binds to muscarinic receptors within a few minutes, resulting in an almost constant level of radioactivity in lungs from 10 to 60 min postinjection (Fig. 2). After glycopyrronium bromide pretreatment, the level of radioactivity in the lungs of the male volunteers was significantly reduced to 32% ± 12% of the control value at 60 min (Fig. 3). Glycopyrronium bromide caused a more rapid washout of radioactivity from lung tissue. From the C[^{15}O]O scan, the fraction of radioactivity that originated from pulmonary blood was calculated. In the control study, on average 2%–4% of the radioactivity in the ROI was localized in pulmonary blood 30–60 min after injection of the radioligand (Fig. 2). In the glycopyrronium bromide study, the fraction of radioactivity in pulmonary blood corresponded to 8%–19% of total radioactivity in the ROI.

Clearance of Radioactivity from Plasma

After injection of (R)-[^{11}C]-VC-002, plasma radioactivity decreased rapidly (Fig. 4). In the control study, radioactivity decreased to 5.5% of the peak value within 10 min; thereafter, the clearance was much slower (to 1.1% of the peak value after 60 min). Although the clearance of radioactivity from plasma after pretreatment of the volunteer with glycopyrronium bromide was less rapid than that in the control study for each individual, due to large interindividual variability the difference between control and treated groups was not statistically significant.

Metabolism

Metabolism of (R)-[^{11}C]-VC-002 was slow. The fraction of unmetabolized radioligand in human plasma decreased from >99% at time zero to approximately 82% at 60 min postinjection of the radioligand. Glycopyrronium bromide did not affect metabolism; the data of the control and the glycopyrronium bromide study could therefore be averaged (Fig. 5).

Pulmonary Tissue-to-Plasma Ratios

In Figure 6, pulmonary tissue-to-plasma ratios calculated on a count-per-minute-per-gram basis are depicted. These ratios gradually rise to a plateau value of 17.8 ± 1.2, which is reached at 40–50 min postinjection. Blood-to-plasma ratios were also calculated. These were rather constant (mean 0.72 ± 0.08) during the time course of the PET study.

DISCUSSION

(R)-[^{11}C]-VC-002 meets all the criteria necessary for the determination of muscarinic receptor densities in human lungs by means of a multiple injection protocol. The radioligand is rapidly cleared from plasma (Fig. 4) and is slowly metabolized in the time period necessary for the PET scan (Fig. 5). Therefore, the input of radioligand to the tissues can be accurately determined. Moreover, the blood concentration of the radioligand can be used as an input function because whole blood-to-plasma ratios are constant (mean 0.72 ± 0.08) during the PET study. It may therefore be possible to estimate the input of (R)-[^{11}C]-VC-002 to the lungs from an ROI placed over the blood pool in the right ventricular cavity. This was not done in this study because the carina was at the center of the field of view and the heart was therefore outside the field of view of the PET camera. In future studies, arterial blood sampling may be avoided by the use of blood-pool data as an input function.

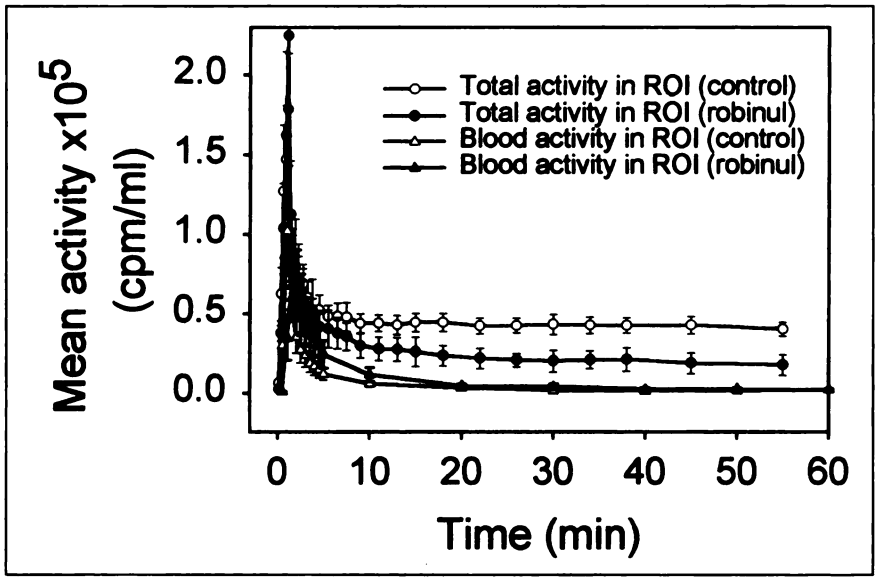

FIGURE 2. Time-activity curves generated from ROI in lungs of healthy volunteers. Blood volume normalized blood curves for control and glycopyrronium bromide studies are also shown. At 50 min, total activity in ROI is 4.29 × 10^4 cpm/mL, activity in blood in same ROI is 0.15 × 10^4 cpm/mL (control studies).

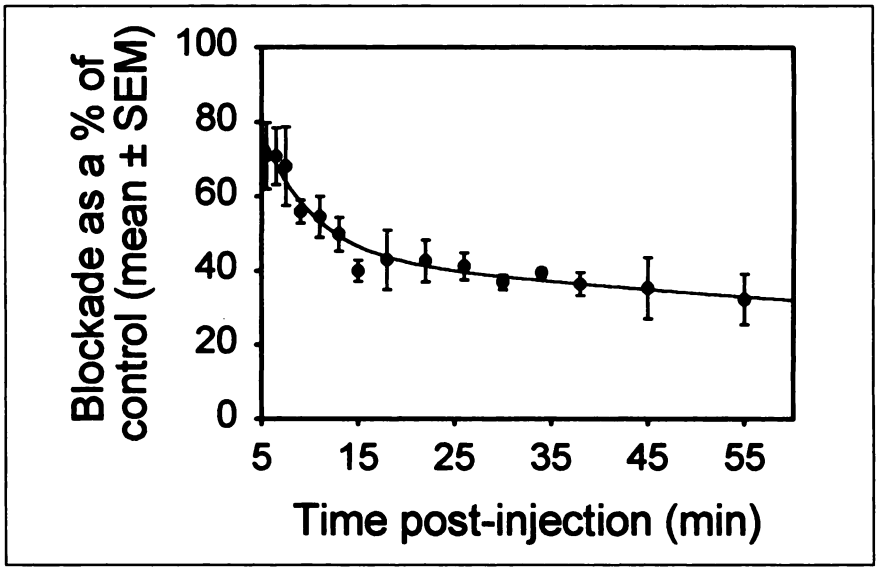

FIGURE 3. Blockade of pulmonary muscarinic receptors after glycopyrronium bromide pretreatment (data not corrected for blood contribution and expressed as percentage of control).

Because the number of muscarinic receptors decreases with the size of the airways, with 10 times higher densities in trachea than in peripheral lungs (*26,27*), the highest uptake of radioligand was expected in the large airways. Therefore, the volunteer was positioned in the PET camera with the carina in the center of the field of view. Although in PET images of the thorax the lungs were clearly visible, the trachea and the main bronchi were hard to distinguish. This may be due to partial-volume effects since these receptors are located in a small volume of smooth muscle. A camera with higher resolution/sensitivity may therefore improve the PET images. PET images of the thorax of the female volunteer showed high uptake at the level of the mammae. Because tracer uptake could be blocked by glycopyrronium bromide, the elevated uptake may be caused by the interaction with muscarinic receptors on the secretory or myoepithelial cells in the mammary gland (*28–32*).

The pulmonary uptake of radioactivity was significantly reduced (with 68% ± 12% at 60 min postinjection) by treating the volunteers with glycopyrronium bromide. This indicates that the pulmonary uptake of the radioligand is largely receptor mediated. To limit the risks to an acceptable minimum, all participants received only a low dose of glycopyrronium bromide. No significant effects of the anticholinergic drug on the heart rate could be detected during the PET study, in part because glycopyrronium bromide displays selectivity for the M_1 and M_3 muscarinic receptor subtypes (*33*). In human heart, the majority of muscarinic receptors are of the M_2 subtype (*26*). Because blockade of the muscarinic receptors by glycopyrronium bromide is not complete, the amount of nonspecific binding is hard to determine from this study. From a biexponential curve fit in Figure 3, it is clear that the percentage of blockade becomes greater at greater intervals postinjection

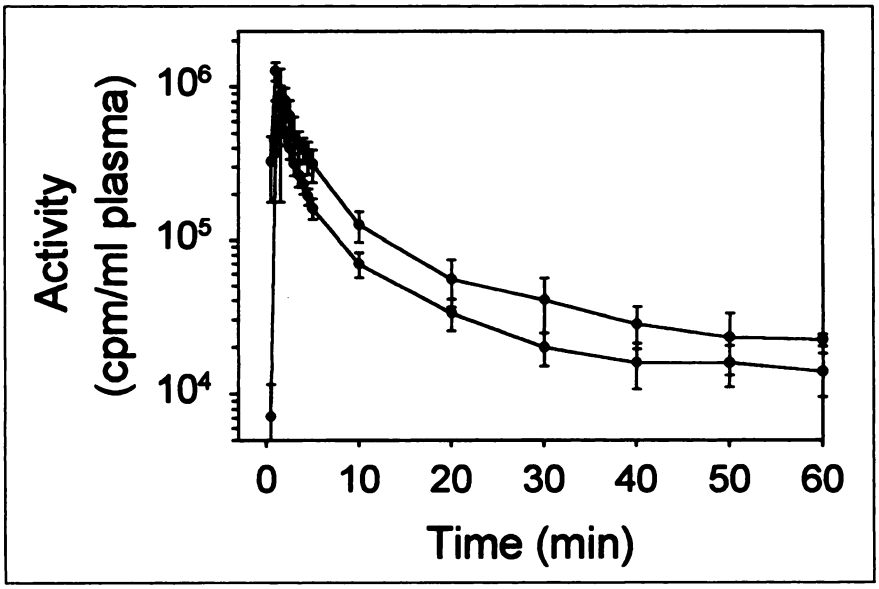

FIGURE 4. Clearance of radioactivity from plasma after injection of (R)-[^{11}C]-VC-002 in human volunteers (data plotted as mean ± SD). Note logarithmic scale on y-axis.

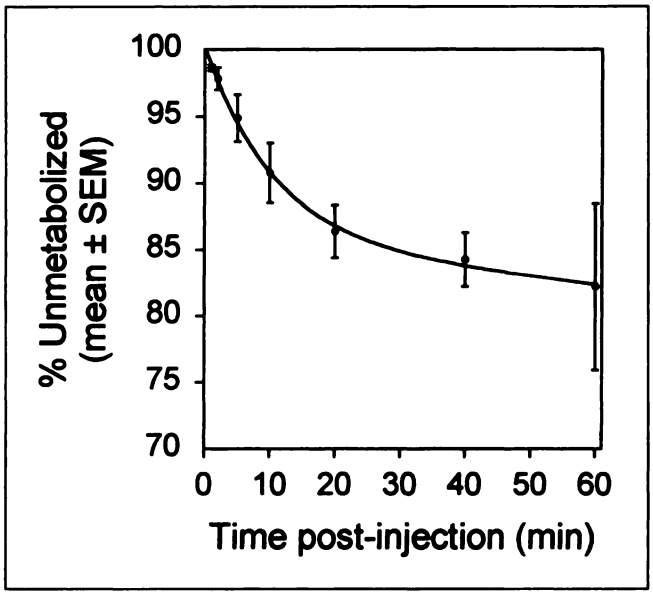

FIGURE 5. Metabolism of (R)-[^{11}C]-VC-002 in human volunteers, fraction of plasma radioactivity representing parent compound is expressed as mean ± SEM of control and glycopyrronium bromide study (solid line is biexponential curve fit).

(extrapolated to 120 min, block is 77%). Studies of rats pretreated with saturating doses of atropine before the injection of the radioligand (*22*) showed that nonspecific binding in the target organs such as lungs and heart is low (i.e., 10.7% and 0.9% of total tissue uptake, respectively). That complete blockade was not reached under our experimental conditions can also be deduced from Figure 6. In the glycopyrronium bromide-blocked situation, the increase of the tissue plasma ratio to a plateau level of 4.7 ± 1.0, which is reached at 45–55 min postinjection, suggests that only approximately 74% of all muscarinic receptors in human lung were occupied by glycopyrronium bromide.

In lungs the apparent off-rate of the radioligand from the receptor was slow (control study). Although this could be interpreted as irreversible binding of the radioligand to the receptors, for [^{11}C]MQNB it was calculated that after dissociation from cardiac muscarinic receptors, the probability of rebinding is much higher than the probability of diffusing into capillary blood (*21*). Thus, the apparent off-rate in a PET study is much slower than the real dissociation of the ligand/receptor complex.

By performing a C[^{15}O]O scan before the injection of the radioligand, pulmonary blood volume as well as the fraction of radioactivity in the pulmonary vascular compartment in the ROIs could be calculated. In the time frames 30–60 min, only 2%–4% of the radioactivity in the ROI originated from pulmonary blood, indicating that nearly all the radioactivity is located in pulmonary tissue. After glycopyrronium bromide pretreatment, the fraction of radioactivity in the vascular compartment corresponded to 8%–19% of total radioactivity in the ROI.

CONCLUSION

(R)-[^{11}C]-VC-002 is the first radioligand to visualize pulmonary muscarinic receptors in human volunteers. (R)-[^{11}C]-VC-002 has favorable in vivo properties and the synthesis of this radioligand is relatively simple and very reliable. Because this radiopharmaceutical can be produced in high yield and with high specific activity, it has considerable potential for the quantification of pulmonary muscarinic receptors in humans to detect changes induced by disease, age or treatment.

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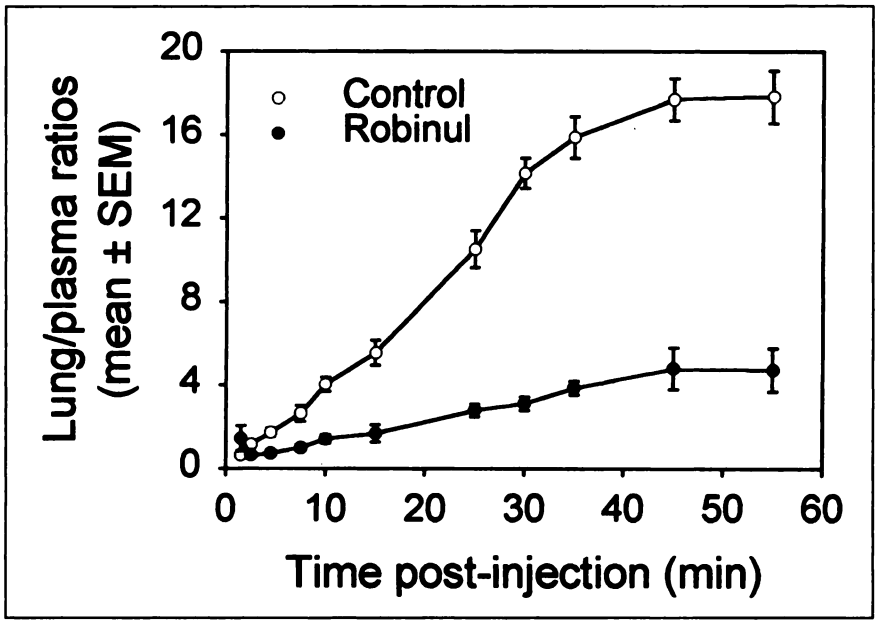

FIGURE 6. Pulmonary tissue-to-plasma ratios after injection of (R)-[^{11}C]-VC-002, with and without glycopyrronium bromide pretreatment (mean ± SEM), calculated on count-per-minute-per-gram basis (not count per minute per milliliter).

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