ELSEVIER

European Journal of Pharmacology 433 (2001) 173–176

ejp

www.elsevier.com/locate/ejphar

Imaging of β-adrenoceptors in the human thorax using (*S*)-[^{11}C]CGP12388 and positron emission tomography

Philip H. Elsinga [a,*], Petra Doze [a], Aren van Waarde [a], Remge M. Pieterman [a], Paul K. Blanksma [b], Antoon T.M. Willemsen [a], Willem Vaalburg [a]

[a] *PET-center, Groningen University Hospital, P.O. Box 30001, 9700 RB Groningen, The Netherlands*
[b] *Department of Cardiology, Groningen University Hospital, P.O. Box 30001, 9700 RB Groningen, The Netherlands*

Received 16 August 2001; accepted 26 October 2001

Abstract

We report positron emission tomography studies of β-adrenoceptors in the human thorax with (*S*)-[^{11}C]CGP12388 (4-(3-(2′-[^{11}C]-isopropylamino)-2-hydroxypropoxy)-2*H*-benzimidazol-2-one). β-Adrenoceptors have previously been quantified using (*S*)-[^{11}C]CGP12177 (4-(3-*tert*-butylamino-2-hydroxypropoxy)-2*H*-benzimidazol-2[^{11}C]-one), but (*S*)-[^{11}C]CGP12388 is more easily prepared and therefore more suitable in a clinical setting. (*S*)-[^{11}C]CGP12388 was administered to five healthy volunteers on two separate days (control and pindolol block study). Arterial plasma samples were used to determine clearance, metabolites, and protein binding of the radioligand. Heart, lung and spleen showed high uptake of radioactivity, which was strongly suppressed (68–77%) by pindolol. Plasma clearance of (*S*)-[^{11}C]CGP12388 was rapid, binding to plasma proteins was low (53 ± 4%), and the radioligand was slowly metabolized. (*S*)-[^{11}C]CGP12388 produces high-quality images of the human thorax. Uptake of (*S*)-[^{11}C]CGP12388 in heart, lung and spleen represents binding to β-adrenoceptors. (*S*)-[^{11}C]CGP12388 seems useful for imaging of β-adrenoceptors in a clinical setting.

Keywords: β-Adrenoceptor; (*S*)-[^{11}C]CGP12388; PET (positron emission tomography)

1. Introduction

Quantification of myocardial and pulmonary β-adrenoceptors by positron emission tomography (PET) is of clinical interest. β-Adrenoceptors density is altered in various pathophysiological conditions, such as heart failure, hypertension, ischemia, hypertrophic and dilated cardiomyopathy (HCM, DCM), asthma and chronic obstructive pulmonary disease (Pike et al., 2000).

The hydrophilic β-adrenoceptor antagonist (*S*)-[^{11}C]CGP12177 (4-(3-*tert*-butylamino-2-hydroxypropoxy)-2*H*-benzimidazol-2[^{11}C]-one) has been used to determine cardiac and pulmonary β-adrenoceptor density in healthy volunteers and patients with hypertrophic and dilated cardiomyopathy or asthma (Elsinga et al., 1998). Using a two-injection protocol (high and low specific activity), the B_{max} was calculated with a graphical method.

(*S*)-[^{11}C]CGP12177 is produced by reaction of [^{11}C]phosgene with the appropriate (*S*)-diamine precursor in high radiochemical yield (Aigbirhio et al., 1992). Unfortunately, several PET centers have found the regular preparation to be too demanding for clinical experiments, since the production of [^{11}C]phosgene requires highly controlled conditions. This is an important drawback to apply [^{11}C]CGP12177 for clinical studies and prevents widespread use of this radioligand. This has led to a search for simpler radiosyntheses to equally effective radioligands (Pike et al., 2000).

(*S*)-CGP12388 (4-(3-isopropylamino-2-hydroxypropoxy)-2*H*-benzimidazol-2-one), the isopropyl analog of (*S*)-CGP12177, can easily be labeled via a one-pot procedure using 2-[^{11}C]acetone (Elsinga et al., 1997). Because of an in vivo behaviour in rats comparable to CGP12177, (*S*)-[^{11}C]CGP12388 seems a promising tracer for in vivo studies of β-adrenoceptors in humans (Van Waarde et al., 1998). Here we present preliminary imaging studies in healthy volunteers to determine the utility of (*S*)-[^{11}C]CGP12388 for clinical PET.

* Corresponding author. Tel.: +31-50-3613247; fax: +31-50-3611687.
E-mail address: p.h.elsinga@pet.azg.nl (P.H. Elsinga).

0014-2999/01/$ - see front matter
PII: S0014-2999(01)01499-6

2. Methods

2.1. Chemistry

(*S*)-[^{11}C]CGP12388 was prepared as described previously (Elsinga et al., 1997). In brief, (*S*)-[^{11}C]CGP12388 was prepared by reductive amination of the corresponding (*S*)-desisopropyl precursor and [^{11}C]acetone in the presence of $NaCNBH_3$ and methanol followed by reversed phase High Performance Liquid Chromatography using 20 mM NaH_2PO_4 in saline/ethanol (9/1) as mobile phase. [^{11}C]Acetone was prepared by trapping [^{11}C]CO_2 in a solution of ethereal methyllithium. The overall radiochemical yield and specific activity were 10–20% and 22,000–37,000 GBq/mmol, respectively.

2.2. Healthy volunteers

The study was approved by the Medical Ethics Committee of the Groningen University Hospital. All five subjects (21–65 years old, mean 32 years; 3 males, 2 females; body weight 76 ± 7 kg) gave informed consent. Excluded were people with a positive history regarding myocardial ischemia, hypertension, heart failure, angina, wheezing or tightness of the chest related to asthma or chronic obstructive pulmonary disease, infections of the upper respiratory tract in a period shorter than 4 weeks prior to the study, use of β-mimetics or theophylline, and pregnant females.

2.3. PET-experiments

A cannula was placed in a vein of one of the lower forearms. Another cannula was placed in the radial artery of the contralateral arm. The venous cannula was used for injection and the arterial line for blood sampling.

The volunteers were placed into the PET-camera (Siemens ECAT 951/31, FWHM = 6 mm), a rectilinear scan was made for proper positioning and a transmission scan for attenuation correction.

To determine blood volume in the regions of interest, C[^{15}O]O (600–750 MBq) was inhaled using a Gas Delivery System (Victoreen, Cleveland, OH). A frame rate of 8×1 min was applied. Three blood samples were drawn at 0, 4 and 8 min.

Twenty minutes later, (*S*)-[^{11}C]CGP12388 (100–201 MBq) was injected over a period of 1 min using a remote-controlled pump (Medrad OP-100, Indianola, PA). Data acquisition was started with a frame rate of 8×15 s, followed by 4×30 s, 4×1 min, 4×2 min, 6×4 min, 2×10 min. Arterial blood samples (2 ml) were drawn at 0.5 min intervals during the first 5 min and at 10 min intervals from 10 to 60 min post-injection. The radioactivity in plasma and in whole blood was determined using a gamma counter (LKB Wallac CompuGamma 1282 CS, Turku, Finland) which was cross-calibrated with the PET-camera. Additional samples of 3 ml were drawn at 1, 2, 5, 10 and 20 min for HPLC-analysis of metabolites.

After an interval of at least 1 week, the volunteer ingested pindolol: 5 mg at the evening before the second PET-study, 5 mg early in the morning and 5 mg 1 h before injection of (*S*)-[^{11}C]CGP12388. Cannulas were placed in the veins of each of the lower forearms. No arterial catheter was used. One cannula was used for injection and the other for blood sampling.

2.4. Metabolite analysis and protein binding

Plasma was deproteinized by addition of 0.1 vol 70% perchloric acid and centrifugation ($13{,}000 \times g$; 2 min). The supernatant was directly applied to the HPLC column

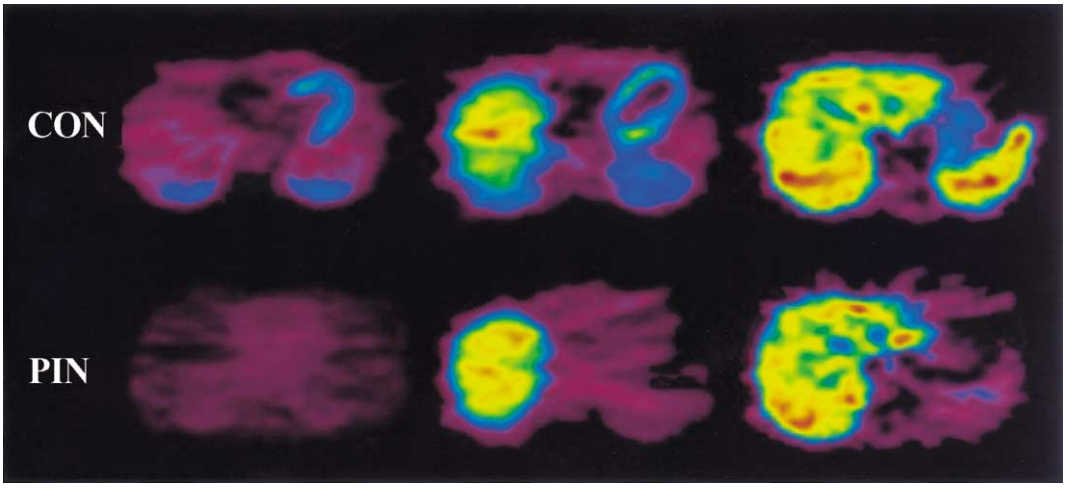

Fig. 1. PET images of the human thorax (radiological view) obtained after iv injection of (*S*)-[^{11}C]CGP 12388. Transaxial cross-sections of the thorax are displayed, containing the data summed over the last 14 frames. Upper row: control, lower row: pindolol study. In the control study, the left-hand image shows the left ventricle and parts of the lungs; the middle image also shows the left ventricle, part of the left lung as well as the liver; the right-hand image displays liver and spleen. In the pindolol blocked study, the uptake of radioactivity in heart, lung and spleen has decreased to background, whereas the uptake in liver was not affected. Data are not corrected for injected dose or bodyweight.

(Waters Rad-Pak C18, 5 μm). The mobile phase was 1% triethylamine-acetate pH 4:acetonitrile 95:5% v/v. Retention time of the parent compound was 11 min, while the major metabolite eluted at about 5 min. Protein-bound radioactivity was assessed by ultrafiltration (MPS-1 reusable micropartition system with YMT-30 membrane (Amicon, Beverly MA)). Samples of plasma were dispensed into MPS-1 units and centrifuged ($2000 \times g$, 30 min). Radioactivity in the clear ultrafiltrate and on the filter were then determined.

2.5. Data analysis

Time-activity curves for all regions of interest were calculated using ECAT software (CAPP7.1, CTI/Siemens, Knoxville, TN). Values were converted to Bq/ml and normalized to an injected radioactivity of 185 MBq (5 mCi) and a body weight of 70 kg. Curve fitting was performed using a nonlinear data analysis program (Multifit, copyright J.H. Proost, PhD, Dept. of Pharmacology, University of Groningen, The Netherlands). Differences between the control and the pindolol group were tested with a paired samples *t*-test (SPSS, Microsoft). Total/non-specific binding ratios in tissue, defined as the ratio of control and pindolol values, were also analyzed by nonlinear regression (EnzFitter, Elsevier Biosoft).

3. Results

After injection of (*S*)-[^{11}C]CGP12388 in human volunteers, high quality PET-images of the thorax were obtained (Fig. 1). In control studies, the myocardial left ventricle, peripheral lung, liver and spleen were clearly visible. After administration of pindolol, heart, lung and spleen were no longer visible, whereas the uptake in liver was unchanged. After injection, tissue levels of radioactivity rose to a maximum followed by a relatively slow decline (Fig. 2). In control studies, the injected amounts of (*S*)-CGP12388 ranged from 0.12 to 0.27 (mean 0.19 ± 0.08) nmol/kg body weight resulting in estimated receptor occupancies of $11.5 \pm 2.8\%$ for heart, $3.3 \pm 0.8\%$ for lung and $8.2 \pm 1.3\%$ for spleen. Neither injection of (*S*)-[^{11}C]CGP12388 nor pindolol treatment caused any change in blood pressure, heart rate or electrocardiogram of the volunteers.

The time–activity curves were best fitted by multiexponentials. The parameters of the slow kinetic phase of the washout of (*S*)-[^{11}C]CGP12388 were expressed as maximal tissue uptake (kBq/ml) and a time constant (min^{-1}). The values were 14.7 ± 3.7 kBq/ml and 0.0056 ± 0.0013 min^{-1} for heart, 5.5 ± 1.9 kBq/ml and 0.0033 ± 0.0010 min^{-1} for lung and 22.9 ± 8.0 kBq/ml and 0.015 ± 0.0001

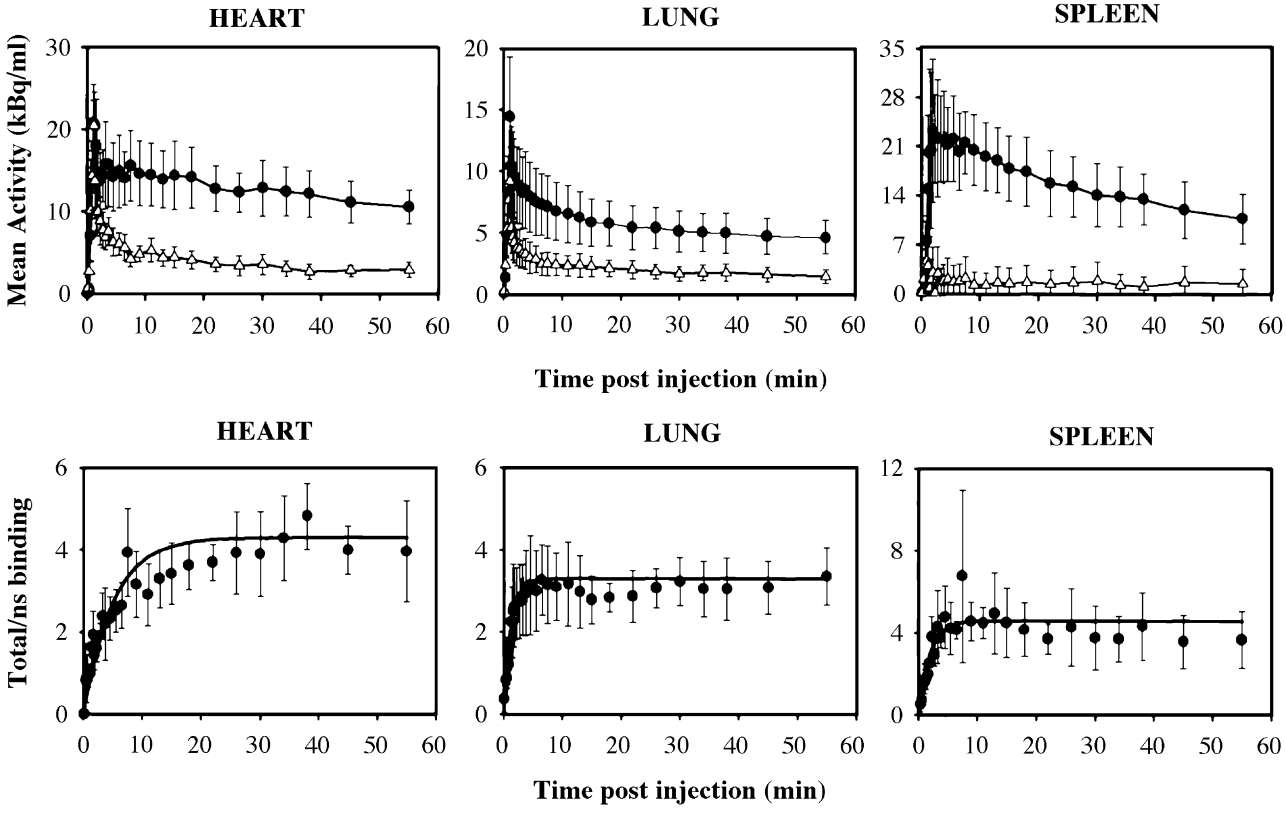

Fig. 2. Time-activity curves (not corrected for blood volume) for heart, lung and spleen and ratios of total/non-specific binding, i.e. tissue uptake of radioactivity in the absence or presence of pindolol. The lines in the lower panel represent monoexponential curve fits.

min^{-1} for spleen, respectively. After ingestion of pindolol, tissue uptake of radioactivity decreased, whereas the washout rate was increased by a factor 3–4. Pindolol reduced the uptake of radioactivity by 76 ± 6 (heart), $68 \pm 9\%$ (lung) and $77 \pm 7\%$ (spleen) at 60 min post-injection ($p < 0.05$). If uptake of carbon-11 in the presence of pindolol is considered to represent non-specific binding, ratios of total over non-specific binding can be calculated. The ratio increased rapidly and reached a maximum in the heart within 15 min, and in lung and spleen within 3 min (Fig. 2). Curves fitted to these data suggest that ratios of 4.3 ± 0.9 (heart), 3.3 ± 0.7 (lung) and 4.6 ± 1.5 (spleen) are obtained with times to reach half maximum of 3.40 ± 1.10, 0.85 ± 0.25 and 1.30 ± 0.54 min, respectively.

From the C[^{15}O]O scans it was calculated that in the control studies, 4.4–7.0% of radioactivity in heart, lung and spleen was present in blood 15 min after injection of the radioligand. In the pindolol-blocking studies 30–40% of radioactivity was present in blood at 15 min post-injection.

(*S*)-[^{11}C]CGP12388 was rapidly cleared from plasma. The clearance was best fitted by a tri-exponential with half lifes of 0.22, 2.51 and 101.53 min^{-1}. Similar results have been reported with (*S*)-[^{11}C]CGP12177.

Like (*S*)-[^{11}C]CGP12177, (*S*)-[^{11}C]CGP12388 was slowly metabolized. At 10 min after injection, $87 \pm 8\%$ of the radioactivity in plasma was still unchanged radioligand. Metabolite analysis at later time points was not possible, because of the very low radioactivity levels in plasma due to rapid clearance and radioactive decay. Analysis of blood samples showed that (*S*)-[^{11}C]CGP12388 rapidly associated to red blood cells (RBC). After an initial rapid association phase, the RBC/plasma ratio slowly increased to a value of 3.5 (at 60 min post-injection). Analysis of blood samples showed that $53 \pm 4\%$ of plasma radioactivity was bound to proteins. No significant change of this fraction was observed over time.

4. Discussion

We evaluated (*S*)-[^{11}C]CGP12388, a hydrophilic non-subtype-selective β-adrenoceptor antagonist, in humans (five healthy volunteers) to investigate its suitability for β-adrenoceptor imaging and as a prelude to estimate B_{max} values using multi-injection protocols. The quality of the obtained PET-images as well as its pharmacokinetics are similar to that obtained with (*S*)-[^{11}C]CGP12177 (Elsinga et al., 1998).

The production of (*S*)-[^{11}C]CGP12388 does not require a complicated setup. The only critical step is the preparation of MeLi in diethyl ether under argon atmosphere. Commercially available solutions of MeLi as LiBr-complex however are very stable. Advantages of the production method of (*S*)-[^{11}C]CGP12388 over (*S*)-[^{11}C]CGP12177 are the use of a stable precursor (also when used as stock solution) and no laborious manipulations to prepare [^{11}C]phosgene.

The pharmacokinetic properties, i.e. high quality PET-images, low non-specific binding and blood activity, rapid blood clearance and slow metabolism allow the determination of β-adrenoceptor densities with PET in the myocardium. With a spatial resolution of 6 mm FWHM, differences in densities of 10% from baseline might be detected.

In conclusion, with (*S*)-[^{11}C]CGP12388 excellent PET-images of heart, lung and spleen have been produced. Because of its favourable pharmacokinetic properties which are similar to (*S*)-[^{11}C]CGP12177, (*S*)-[^{11}C]CGP12388 appears suitable to quantify the β-adrenoceptor density with PET using a multi-injection protocol in a clinical setting.

References

Aigbirhio, F., Pike, V.W., Francotte, E., Jaeggi, K.A., Waters, S.L., 1992. Simplified asymmetric synthesis and chiral analysis for S-[carbonyl-11C] CGP12177 production. J. Labelled Compd. Radiopharm. 31, 159–161.

Elsinga, P.H., Van Waarde, A., Jaeggi, K.A., Schreiber, G., Heldoorn, M., Vaalburg, W., 1997. Synthesis and evaluation of (*S*)-4-(3-(2′-[11C]isopropylamino)-2-hydroxypropoxy)-2*H*-benzimidazol-2-one ((*S*)-[11C]CGP 12388) and (*S*)-4-(3-((1′-[18F]-fluoroisopropyl)amino)-2-hydroxypropoxy)-2*H*-benzimidazol-2-one ((*S*)-[18F]fluoro-CGP 12388) for visualization of beta-adrenoceptors with positron emission tomography. J. Med. Chem. 40, 3829–3835.

Elsinga, P.H., Van Waarde, A., Visser, T.J., Vaalburg, W., 1998. Visualization of β-adrenoceptors using PET. Clin. Pos. Imaging 1, 81–94.

Pike, V.W., Law, M.P., Osman, S., Davenport, R.J., Rimoldi, O., Giardina, D., Camici, P.G., 2000. Selection, design and evaluation of new radioligands for PET studies of cardiac adrenoceptors. Pharm. Acta Helv. 74, 191–200.

Van Waarde, A., Elsinga, P.H., Doze, P., Heldoorn, M., Jaeggi, K.A., Vaalburg, W., 1998. A novel beta-adrenoceptor ligand for positron emission tomography: evaluation in experimental animals. Eur. J. Pharmacol. 343, 289–296.