JACC: CARDIOVASCULAR IMAGING
VOL. 5, NO. 3, 2012

ISSN 1936-878X/$36.00
PUBLISHED BY ELSEVIER INC.
DOI:10.1016/j.jcmg.2011.11.017

4-[^{18}F]-Tetraphenylphosphonium as a PET Tracer for Myocardial Mitochondrial Membrane Potential

Gagandeep S. Gurm, MD,* Stephan B. Danik, MD,* Timothy M. Shoup, PhD,† Steven Weise,† Kazue Takahashi, PhD,‡ Shawna Laferrier, BS,* David R. Elmaleh, PhD,† Henry Gewirtz, MD*

Boston, Massachusetts

OBJECTIVES This study tested the hypothesis that 4-[^{18}F]fluorophenyltriphenylphosphonium (^{18}F-TPP) is useful for in vivo positron emission tomography (PET) measurement of mitochondrial membrane potential ($\Delta\Psi$m). Its utility as a blood flow tracer also was evaluated.

BACKGROUND Tetraphenylphosphonium is useful for in vitro measurement of $\Delta\Psi$m. In vivo measurement of $\Delta\Psi$m has potential value in the assessment of heart failure pathophysiology and therapy as well as assessment of myocardial viability and so may be a very useful clinical tool.

METHODS Anesthetized swine (N = 6) with a balloon catheter in the left anterior descending coronary artery were studied. Microsphere measurements of myocardial blood flow (MBF) were made after balloon inflation (baseline) and ~10 min after intravenous administration of adenosine and phenylephrine after which ~10 mCi ^{18}F-TPP was injected intravenously and dynamic PET data acquisition obtained for 30 min. After the swine were killed, the hearts were sectioned for microsphere measurement of MBF and ^{18}F-TPP measured by well counter in these same samples. PET images provided whole blood and myocardial ^{18}F-TPP concentration for determination of $\Delta\Psi$m by the Nernst equation, corrected for nonspecific ^{18}F-TPP binding. Microsphere MBF, absolute (ml/min/g) and relative, was compared with PET data (standard uptake value and K1).

RESULTS Nonspecific binding of ^{18}F-TPP overestimated $\Delta\Psi$m measured by -37 ± 4 mV (mean $\pm$ SD). Normal zone $\Delta\Psi$m of ex vivo samples (-91 ± 11 mV; N = 52; sample weight, 1.07 ± 0.18 g) correlated strongly ($R^2 = 0.93$) with normal zone by PET (-81 ± 13 mV). Both ex vivo and PET normal zone $\Delta\Psi$m, although somewhat lower, compared well with that reported for tritium labeled triphenylphosphonium in normal working Langendorff rat heart (−100 mV). Although the relative MBF by ^{18}F-TPP correlated strongly with relative microsphere MBF ($R^2 = 0.83$), there was no correlation between absolute MBF by ^{18}F-TPP and microsphere MBF.

CONCLUSIONS ^{18}F-TPP is a promising tracer for noninvasive PET measurement of $\Delta\Psi$m in living subjects. It is useful as well for assessment of relative but not absolute MBF. (J Am Coll Cardiol Img 2012;5:285–92) © 2012 by the American College of Cardiology Foundation

From the *Cardiology Division, Department of Medicine, Massachusetts General Hospital, Harvard Medical School, Boston, Massachusetts; †Department of Radiology–Nuclear Medicine Molecular Imaging, Massachusetts General Hospital, Harvard Medical School, Boston, Massachusetts; ‡Department of Pediatrics, Massachusetts General Hospital, Harvard Medical School, Boston, Massachusetts. Dr. Elmaleh is the chairman and CSO of FluoroPharma, Inc. and has received a salary from them. Dr. Gewirtz has received financial support from the Wild Family Foundation and FluoroPharma, Inc. All other authors have reported that they have no relationships relevant to the contents of this paper to disclose.

Manuscript received September 7, 2011; revised manuscript received November 9, 2011, accepted November 10, 2011.

Tetraphenylphosphonium was shown to be a useful chemical indicator of the mitochondrial membrane potential ($\Delta\Psi$m) in 1969 (1) and has been used for in vitro studies of myocardial metabolism (2–7) and tumor imaging in which differences between normal and malignant cell $\Delta\Psi$m are exploited to facilitate tumor identification (8). Its specificity for mitochondrial accumulation has been established (2). Labeling of tetraphenylphosphonium with ^{18}F (^{18}F-TPP) for purposes of positron emission tomography (PET) imaging was recently reported (9). This study tests the hypothesis that ^{18}F-TPP is useful for quantitative PET measurement of myocardial $\Delta\Psi$m. Its utility for quantitative measurement of absolute myocardial blood flow (MBF) also was evaluated.

See page 293

ABBREVIATIONS AND ACRONYMS

BZ = border zone
^{18}F-TPP = 4-[^{18}F]fluorophenyltriphenylphosphonium
LAD = left anterior descending coronary artery
LV = left ventricular
MBF = myocardial blood flow
MBFado = myocardial blood flow during adenosine infusion
NZ = normal zone
PET = positron emission tomography
SAP = systolic arterial pressure
SUV = standard uptake value

METHODS

In vitro experiment: human blood. A 15-ml sample of venous whole blood (WB) was obtained in heparinized tubes from a human volunteer and pipetted into 1.5-ml microcentrifuge tubes to which ^{18}F-TPP was added in several concentrations. The WB was mixed at 37°C for 30 min after which [^{18}F-TPP] (nCi/ml) was measured in a well counter. Tubes then were centrifuged for 10 min at 5,000 *g* after which plasma was transferred into a 1.5-ml microcentrifuge tube and [^{18}F-TPP] measured. The tube of packed red cells was reweighed and [^{18}F-TPP] measured. All measurements were corrected for physical decay.

In vivo experiment: domestic swine. After overnight fast, the swine (~40 kg) were sedated with Telazol (tiletamine) (4.4 mg/kg) and xylazine (2.2 mg/kg) intramuscularly, intubated, and then maintained on isoflurane anesthesia (1% to 3%) and oxygen for the entire experiment (N = 6).

Groin sites were used to insert catheters in right and left femoral arteries and veins. The right femoral vein was used to place a catheter by transseptal technique in the left atrium. This catheter was used to administer neutron-activated, radiolabeled microspheres (15 μm diameter, 9×10^6 spheres/injection). Catheters in the right and left femoral arteries were used to monitor arterial pressure and for reference withdrawal of blood (for 2 min at 10 ml/min) for microsphere measurements of MBF. Catheters placed in the left femoral vein and right external jugular vein were used for administration of drugs and ^{18}F-TPP.

The right internal carotid artery was accessed by cut down and used to insert a 6-F "hockey stick" catheter under fluoroscopic control to the left anterior descending coronary artery (LAD). Next, an angioplasty catheter with a 2.75 × 15-mm balloon was passed through the hockey stick catheter and positioned in the mid to distal third of the LAD. The angioplasty balloon was partially inflated sufficient to produce an ~1- to 2-mm ST-segment elevation in anterior precordial electrocardiography leads. An amiodarone drip (1 mg/min) was begun before balloon inflation after an intravenous loading dose of 150 mg. A lidocaine drip at 2 mg/min also was begun after the loading dose of 50 to 100 mg intravenously. Phenylephrine was given intravenously to maintain systolic arterial pressure (SAP) at ~110 to 120 mm Hg.

Experimental protocol. In the PET laboratory, baseline hemodynamics and pulse oxymetry (O_2 and CO_2) were recorded and microspheres injected in the left atrium. Next, adenosine was begun at 140 μg/kg/min intravenously and phenylephrine increased to increase the SAP to 150 to 170 mm Hg. Once a stable, a second injection of microspheres was given. Immediately after completion of the reference blood withdrawal, dynamic PET imaging acquisition was begun, and approximately 5 to 10 s later, 10 mCi ^{18}F-TPP was injected intravenously. The phenylephrine infusion was adjusted to maintain a constant SAP. Adenosine infusion continued throughout the 30-min dynamic PET acquisition (8 frames @ 15 s/frame, 8 frames @ 1 min/frame and 10 frames @ 2 min/frame). Hemodynamic and pulse oxymetry data were recorded at ~2- to 5-min intervals throughout the study.

At the end of the PET imaging acquisition, the angioplasty balloon was fully inflated and blue dye injected to assist in marking the LAD territory distal to the balloon. The animals were killed by pentobarbital overdose (200 mg/kg intravenously). Subsequently, the chest of each swine was opened and the position of the balloon documented with sutures and photographed in situ. Next, the heart was removed, photographed, and sectioned for counting of ^{18}F-TPP in a region distal to the LAD balloon (distal zone), adjacent to the balloon (border zone) and proximal to the balloon on the high anterolateral wall and in the posterobasal segment (normal zones). These same tissue samples were