Available online at www.sciencedirect.com
ScienceDirect
Nuclear Medicine and Biology 36 (2009) 833–843
NUCLEAR MEDICINE AND BIOLOGY
www.elsevier.com/locate/nucmedbio

Validation of ^{99m}Tc-labeled "4+1" fatty acids for myocardial metabolism and flow imaging
Part 1: myocardial extraction and biodistribution

Peter Mirtschink[a,*,1], Sebastian N. Stehr[b,1], Martin Walther[c], Jens Pietzsch[c], Ralf Bergmann[c], Hans-Jürgen Pietzsch[c], Johannes Weichsel[a], Annette Pexa[a], Peter Dieterich[a], Gerd Wunderlich[d], Bert Binas[e], Joachim Kropp[f], Andreas Deussen[a]

[a]*Institute of Physiology, Technical University Dresden, 01307 Dresden, Germany*
[b]*Department of Anesthesiology, Technical University Dresden, 01307 Dresden, Germany*
[c]*Institute of Radiopharmacy, Forschungszentrum Dresden-Rossendorf, 01314 Dresden, Germany*
[d]*Department of Nuclear Medicine, Technical University Dresden, 01307 Dresden, Germany*
[e]*Department of Veterinary Pathobiology, Texas A&M University, College Station, TX 77843, USA*
[f]*Department of Nuclear Medicine Carl-Thiem Hospital Cottbus, 03048 Cottbus, Germany*

Received 31 March 2009; received in revised form 12 June 2009; accepted 27 June 2009

Abstract

Introduction: ^{13}C, ^{18}F and ^{123}I fatty acids (FA) are used for myocardial imaging. Recently, our group showed that [^{99m}Tc]-labeled "4+1" FA are extracted into the rat and guinea pig myocardium. The present study evaluates determinants of myocardial uptake and whole body biodistribution of these FA derivatives.

Methods: Studies were performed with isolated perfused hearts of Wistar-Kyoto (WKY) and spontaneously hypertensive rats (SHR) with a FAT/CD36 deficiency, as well as with heart type FA binding protein knockout mice (H-FABP)$^{-/-}$ and H-FABP$^{+/+}$. Eight 4+1-^{99m}Tc-FA were applied for 3 min followed by 1-min washout. A mathematical model was used to analyze FA dynamics and binding to proteins. Whole-body distribution was studied in rats with and without Tween 80. In vitro fractionation studies with [^{99m}Tc]-FA assessed red blood cell uptake as well as association with plasma lipoproteins very low-density lipoprotein (VLDL), low-density lipoprotein (LDL) and high-density lipoprotein (HDL).

Results: Myocardial extraction was 19.0–33.0% of the infused dose in isolated WKY and 15.2–26.4% in SHR hearts. However, H-FABP$^{-/-}$ showed a marked reduction of tracer extraction [2.8±0.6%ID (percent injected dose) vs. 17±2%ID $P<.001$]. Uptake in red blood cells (<1.2% ID) and incorporation into lipoproteins were negligible. Incubation of ^{99m}Tc-FA with albumin reduced ventricular extraction ($P<.001$) into the range of established iodinated FA tracers. polyoxyethylene(20) sorbitan monooleate improved the heart-to-liver ratio in the biodistribution studies.

Conclusions: Myocardial uptake of [^{99m}Tc]-FA 4+1 derivatives is dependent on H-FABP. These substances may therefore provide a new tool to specifically assess regional myocardial changes of H-FABP.

Keywords: Technetium fatty acid; isolated heart; H-FABP; CD36; myocardial imaging

1. Introduction

In ischaemia, oxidation of fatty acids (FAs) is suppressed, and glycolysis and glycogen breakdown is used as an energy source [1]. Therefore, radiolabeled FA allows the assessment of viable myocardium by using single photon emission computed tomography offering a valuable diagnostic tool for ischemic heart disease [2]. In addition to global perfusion

* Corresponding author. Institute of Physiology, Medical Faculty Carl Gustav Carus, Technical University of Dresden, 01307 Dresden, Germany. Tel.: +49 351 458 6006; fax: +49 351 458 6030.
E-mail address: peter_mirtschink@web.de (P. Mirtschink).
[1] Both authors contributed equally to this work.

0969-8051/$ – see front matter © 2009 Elsevier Inc. All rights reserved.
doi:10.1016/j.nucmedbio.2009.06.009

characteristics, FA transport and metabolism are reflected by tracer kinetics on a regional level [3-5]. The first labeled FA such as [^{123}Iod]-15-(*p*-iodophenyl)-pentadecanoic acid [^{123}I]-IPPA or [^{11}C]-palmitate was straight-chain FA mimicking native long chain FA regarding uptake and metabolism [6]. These compounds show a fast myocardial clearance and myocardial metabolism [7,8]. Therefore, methyl-branched FA, e.g., [^{123}I]-BMIPP with prolonged myocardial retention and partial β-oxidation were developed [9] to slow the rate of metabolism and improve cardiac residence time.

Our group has concentrated on the development of [^{99m}Tc]-labeled FA [1,10,11]. The ventricular extraction of some of these radiolabeled FA derivatives clearly exceeded those for [^{123}I]-BMIPP and ^{123}I-IPPA in preliminary studies with isolated perfused hearts of rats and guinea pigs, therefore combining the advantages of the ^{99m}Tc-nuclide with an adequate myocardial retention [1,10,11]. In addition to high myocardial uptake as an important characteristic of long chain FA, in vivo β-oxidation products were detected in the intestine after being processed in liver [10].

With the present study, we sought to gain more detailed information about myocardial transport of these FA derivatives. It was shown that alterations in FA metabolism are already reflected in early stages of FA-uptake and intracellular transport [12-14]. Two important steps in the transport of native long chain FA are membrane transport, which occurs largely via FAT/CD36 protein [15], and protein binding in the cytosolic region via H-FABP protein [16]. Thus, we determined whether a decrease in FAT/CD36 function (as found in spontaneously hypertensive rats) or lack of H-FABP affect myocardial uptake and retention of [^{99m}Tc]-labeled FA. Here, we additionally used a mathematical model to quantitatively analyze the dynamics of FA and its binding to H-FABP (or even to unspecific binding proteins) used in the experimental setups.

In vivo biodistribution of lipophilic [^{99m}Tc] complexes is affected by a high liver uptake. Besides measuring liver uptake, possible factors that may influence biodistribution were investigated, including albumin binding, association with lipoprotein fractions and partitioning between blood plasma and red blood cells (RBC). As described recently, polyoxyethylene(20) sorbitan monooleate (Tween 80) is a useful tool to improve the heart-to-liver ratio of lipophilic [^{99m}Tc] complexes [17,18]. Therefore, we also addressed the effect of Tween 80 on the biodistribution of [^{99m}Tc]-labeled FA.

2. Material and methods

2.1. Synthesis of ^{99m}Tc "4+1" FA derivatives

The synthesis of [^{99m}Tc] 4+1 FA derivatives (Fig. 1), as well as the control of their purity, lipophilicity and radiochemical purity (RCP) was performed as described recently [10,11]. For better clarity, [^{99m}Tc]-FA derivatives were organized into groups (denoted by **1–5**) according to their physicochemical properties determined mainly by the ^{99m}Tc-chelate position and the monodentate ligands (Fig. 1).

2.2. Animals

Biodistribution experiments and experiments using the isolated perfused Langendorff rat heart were carried out in Wistar rats of either gender (body weight 200–250 g) as well as 10-week-old spontaneously hypertensive rats (SHR) (NCrlBR) and age- and sex-matched Wistar-Kyoto rats (WKY) purchased from Charles River (Sulzfeld, Germany). For experiments using isolated perfused mouse hearts, mice with a targeted deletion of the entire H-FABP locus (H-FABP$^{-/-}$, body weight 29.3±3.4g) and corresponding control mice (H-FABP$^{+/+}$, body weight 24.3±2.4 g, $P<.05$) bred on C57Bl6 background were used. The investigations conformed to the Guide for the Care and Use of Laboratory Animals issued by the US National Institutes of Health and were approved by the local government authority (AZ 24-9168.24-1-2002-14). Animals were kept under a 12-h light-dark cycle and fed with commercial animal diet and water ad libitum.

2.3. Biodistribution

Biodistribution experiments were performed as described recently [11]. In brief, a 200-µl (0.5 MBq) bolus of a solution containing the [^{99m}Tc] 4+1 FA compound and human serum albumin (HSA) (6%) in a 1:2 ratio were injected into a tail vein. In experiments evaluating the impact of Tween 80, the [^{99m}Tc] 4+1 FA was solved in Tween 80 (1%). The animals were decapitated at 5 or 60 min after injection. The accumulated radioactivity in organs and tissues was calculated as the percentage of the injected dose (%ID) per gram (%ID/g) of tissue.

2.4. Experiments with isolated perfused hearts

2.4.1. Experimental solutions

Isolated rat and mouse hearts were perfused with modified, nonrecirculating Krebs–Henseleit buffer as used in Ref. [11]. For experiments with isolated mouse hearts, 0.5 mM EDTA-sodium was added. The buffer was equilibrated with a 95% O_2/5% CO_2 gas mixture (resulting pH 7.38) FA-free bovine serum albumin (BSA) (0.1%/14.9 µM/L) and maintained at 37°C. Perfusate was filtered (0.45 µm) to remove particulate matter before use.

Radionuclide infusion was prepared by solubilizing 5–15 MBq of the ^{99m}Tc-labeled FA in 200 µl propylene glycol and 0.9% NaCl and incubating this mixture with 6% BSA diluted in a ratio of 1:2 with Krebs–Henseleit buffer at room temperature for 30 min. In some experiments, no BSA was added to the Krebs–Henseleit perfusate, and the 30-min incubation period of [^{99m}Tc]-FA with BSA was omitted. To avoid a precipitation of the radiolabeled FA, the amount of propylene glycol (serving as solvent) was increased three-fold. Furthermore, all plastic tubes with contact to the FA were kept as short as possible to minimize adhesion.