Available online at www.sciencedirect.com

ScienceDirect

Nuclear Medicine and Biology 36 (2009) 247–258

NUCLEAR MEDICINE — AND — BIOLOGY

www.elsevier.com/locate/nucmedbio

Evaluation of 6-([^{18}F]fluoroacetamido)-1-hexanoicanilide for PET imaging of histone deacetylase in the baboon brain

Alicia E. Reid[a,b,*], Jacob Hooker[b], Elena Shumay[b], Jean Logan[b], Colleen Shea[b], Sung Won Kim[b], Shanika Collins[c], Youwen Xu[b], Nora Volkow[a,d], Joanna S. Fowler[b]

[a]*National Institute on Alcohol Abuse and Alcoholism, Bethesda, MD 20892, USA*
[b]*Medical Department, Brookhaven National Laboratory, Upton, NY 11973, USA*
[c]*School of Science, Health and Technology Medgar Evers College, Brooklyn, NY 11225, USA*
[d]*National Institute on Drug Abuse, Bethesda, MD 20892, USA*

Received 10 October 2008; received in revised form 24 November 2008; accepted 3 December 2008

Abstract

Introduction: Histone deacetylases (HDACs) are enzymes involved in epigenetic modifications that shift the balance toward chromatin condensation and silencing of gene expression. Here, we evaluate the utility of 6-([^{18}F]fluoroacetamido)-1-hexanoicanilide ([^{18}F]FAHA) for positron emission tomography imaging of HDAC activity in the baboon brain. For this purpose, we assessed its in vivo biodistribution, sensitivity to HDAC inhibition, metabolic stability and the distribution of the putative metabolite [^{18}F]fluoroacetate ([^{18}F]FAC).
Methods: [^{18}F]FAHA and its metabolite [^{18}F]FAC were prepared, and their in vivo biodistribution and pharmacokinetics were determined in baboons. [^{18}F]FAHA metabolism and its sensitivity to HDAC inhibition using suberanilohydroxamic acid (SAHA) were assessed in arterial plasma and by in vitro incubation studies. The chemical form of F-18 in rodent brain was assessed by ex vivo studies. Distribution volumes for [^{18}F]FAHA in the brain were derived.
Results: [^{18}F]FAHA was rapidly metabolized to [^{18}F]FAC, and both labeled compounds entered the brain. [^{18}F]FAHA exhibited regional differences in brain uptake and kinetics. In contrast, [^{18}F]FAC showed little variation in regional brain uptake and kinetics. A kinetic analysis that takes into account the uptake of peripherally produced [^{18}F]FAC indicated that SAHA inhibited binding of [^{18}F]FAHA in the baboon brain dose-dependently. In vitro studies demonstrated SAHA-sensitive metabolism of [^{18}F]FAHA to [^{18}F]FAC within the cell and diffusion of [^{18}F]FAC out of the cell. All radioactivity in brain homogenate from rodents was [^{18}F]FAC at 7 min postinjection of [^{18}F]FAHA.
Conclusion: The rapid metabolism of [^{18}F]FAHA to [^{18}F]FAC in the periphery complicates the quantitative analysis of HDAC in the brain. However, dose-dependent blocking studies with SAHA and kinetic modeling indicated that a specific interaction of [^{18}F]FAHA in the brain was observed. Validating the nature of this interaction as HDAC specific will require additional studies.
Published by Elsevier Inc.

Keywords: PET; Histone deacetylase; Pharmacokinetics; [^{18}F]FAHA

1. Introduction

The sequencing of the human genetic code (DNA base pair sequence) has led to the discovery of genes involved with various human diseases [1]. However, for most of the complex common diseases, the genes identified account for only a small effect in the vulnerability of developing them (i.e., addiction, heart disease, diabetes, depression, cancer, Parkinson's disease). This is likely to reflect the importance of the interactions between environmental and genetic factors in the development of complex human diseases.

It is now widely accepted that epigenetics (regulation of gene activity that is not dependent on DNA sequence) is just as critical as DNA to the healthy development of organisms. Epigenetic processes, such as DNA methylation and histone modifications (e.g., acetylation, methylation, phosphorylation and ubiquitination), play a crucial role in gene expression across species [2]. The epigenetic modifications of the histone proteins of chromatin can have significant impact on most major cell functions including DNA repair, cell growth, differentiation and apoptosis. Of these epige-

* Corresponding author. Tel.: +1 631 344 4393; fax: +1 631 344 5815.
E-mail addresses: areid@bnl.gov, areid_1@lycos.com (A.E. Reid).

0969-8051/$ – see front matter. Published by Elsevier Inc.
doi:10.1016/j.nucmedbio.2008.12.005

netic processes, the pattern of histone acetylation, regulated by the enzyme's histone deacetylases (HDACs) and histone acetyl transferases, has been most intensively studied [3–5]. Indeed, epigenetic research in cancer has led to new therapeutic approaches (i.e., HDAC inhibitors) and to biomarkers to predict treatment response [6]. Though current interest in HDAC as therapeutic targets in cancers, neurodegenerative diseases and inflammation is great, much is not known about the mechanisms that underlie these processes and how they are affected by aging, disease, drugs or other environmental exposures.

The 18 identified isoforms of HDAC have been broadly divided into two groups: the Zn^{2+}-dependent protease group and the NAD^+-dependent group (Class III) [7–9]. The Zn^{2+}-dependent protease group has been further divided into three classes: Class I (HDAC 1, 2, 3 and 8), Class II (HDAC 4, 5, 6, 7, 9 and 10) and Class IV (HDAC 11). The Class I and II groups are therapeutic targets for treatment of cancer and other diseases [10–14]. Class I HDACs are localized mainly in the nucleus while Class II enzymes are larger in size and shuttle between the nucleus and cytoplasm. Tissue expression of Class I and II HDACs is reported to be generally low, with significant variation within tissue types but with little differences in expression pattern between normal and malignant tissue [7]. More recently, an atlas of HDACs 1–11 throughout more than 50 regions of the rat brain was reported [15].

In addition to histone proteins, endogenous substrates for HDACs include several non-histone proteins such as transcription factors (e.g., p53, E2F1, GATA1, Re1A, YY1 and hormone receptors), α-tubulin, nuclear import protein importin-α7, signal transduction protein β-catenin, DNA repair enzymes Ku70 and WRN and heat shock protein 90 [16,17]. The deacetylation of these non-histone proteins affects their activity, suggesting that HDACs exert a much broader range of activities within the cell than control of gene transcription [18].

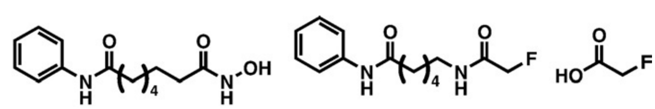
Much effort has been focused on identifying new HDAC inhibitors, but few isoform-specific ligands have been identified thus far [19–21]. Studies on several nonspecific HDAC inhibitors have advanced to clinical trials with one inhibitor, suberanilohydroxamic acid (SAHA), already approved for treatment of cutaneous T-cell lymphoma [22]. In addition to inhibiting Class I and II HDACs, SAHA was recently shown to possess no inhibitory activity to a number of kinases and phosphatases but shown to inhibit the zinc-dependent metalloproteases neprilysin and angiotensin-converting enzyme, albeit with lower potency [23].

A greater understanding of the biology and pharmacology of the different HDAC isoforms is needed to better guide preclinical and clinical investigations. In addition to insufficient pharmacological knowledge, there are also few tools for accessing the efficacy of these inhibitors in vivo. Imaging the expression/availability/activity of HDAC, using positron emission tomography (PET), may provide a complementary method for determining inhibitor action and monitoring therapeutic efficacy and may also provide insight on the pharmacokinetics and biodistribution of some of the available HDAC inhibitors. PET imaging may also provide the opportunity to track and quantify the levels of HDAC noninvasively in human tissues as a function of age, disease, drug use or other environmental exposures.

Recently, 6-([^{18}F]fluoroacetamido)-1-hexanoicanilide ([^{18}F]FAHA) was reported as a potential PET imaging agent and substrate for HDAC with utility for imaging whole-body HDAC expression and tumor activity [24]. In PET studies using rats implanted with carcinoma cells, uptake in tumor xenografts was reported to peak at 30 min at 0.43% ID/g [25]. High HDAC activity was observed in tumors as indicated by tumor-to-muscle ratios of 2.2–2.4 between 30 and 60 min postinjection. The same group also reported HDAC activity in the rat brain, using [^{18}F]FAHA, as evidenced by a peak uptake of 0.44% ID/g [26]. In both rodent studies, SAHA was shown to inhibit radiotracer uptake.

[^{18}F]FAHA is the first reported PET radiotracer with potential for monitoring an epigenetic process in vivo. The present study attempts to assess the feasibility of using [^{18}F]FAHA for imaging HDAC in the baboon brain and to determine its distribution and pharmacokinetics in peripheral organs. While [^{18}F]FAHA appears promising in rodent PET studies, no characterization of its in vitro binding and specificity has been reported. Given its similarity to the acetylated side chain of lysine, we anticipated that FAHA may be a substrate for HDACs. If so, [^{18}F]FAHA in vivo would be cleaved, resulting in a labeled metabolite, [^{18}F]fluoroacetate ([^{18}F]FAC), which has been shown to cross the blood–brain barrier (BBB) and to have selective uptake in glial cells [27,28]. The metabolism of [^{18}F]FAHA to [^{18}F]FAC in the brain and periphery could complicate interpretation of PET data depending on the rate of deacetylation of [^{18}F]FAHA and the uptake and distribution of its metabolite [^{18}F]FAC.

In order to determine the contribution of [^{18}F]FAC to [^{18}F]FAHA imaging studies, we performed the following supporting measurements: (a) the rate of peripheral metabolism of [^{18}F]FAHA was assessed by analysis of arterial plasma in baboon studies; (b) the biodistribution of [^{18}F]FAC was determined to assess the degree of brain uptake; (c) rodent/microPET studies of [^{18}F]FAHA were carried out to determine the pharmacokinetics in the rodent brain and the chemical form of F-18 in the brain evaluated by ex vivo analysis of brain tissue; (d) in vitro whole-blood and plasma incubation studies were used to investigate [^{18}F]FAHA metabolism ex vivo; and (e) in vitro distribution and stability studies were also performed in two cell lines to determine if



SAHA FAHA FAC

Fig. 1. Chemical structures of SAHA, unlabeled FAHA and FAC.