Nuclear Medicine and Biology 43 (2016) 232–242

Contents lists available at ScienceDirect

Nuclear Medicine and Biology

journal homepage: www.elsevier.com/locate/nucmedbio

Development of carbon-11 labeled acryl amides for selective PET imaging of active tissue transglutaminase

Berend van der Wildt [a,b,*], Micha M.M. Wilhelmus [b], Jonne Bijkerk [a], Lizeth Y.F. Haveman [a], Esther J.M. Kooijman [a], Robert C. Schuit [a], John G.J.M. Bol [b], Cornelis A.M. Jongenelen [b], Adriaan A. Lammertsma [a], Benjamin Drukarch [b], Albert D. Windhorst [a]

[a] Departments of Radiology & Nuclear Medicine, VU University Medical Center, Amsterdam, The Netherlands
[b] Departments of Anatomy & Neurosciences, VU University Medical Center, Amsterdam, The Netherlands

ARTICLE INFO

Article history:
Received 27 October 2015
Received in revised form 4 January 2016
Accepted 16 January 2016

Keywords:
Tissue transglutaminase
TG2
Carbon-11
Carbonylation
[^{11}C]acryl amides
MDA-MB-231

ABSTRACT

Introduction: Tissue transglutaminase (TG2) is a ubiquitously expressed enzyme capable of forming metabolically and mechanically stable crosslinks between the γ-carboxamide of a glutamine acyl-acceptor substrate and the ε-amino functionality of a lysine acyl-donor substrate resulting in protein oligomers. High TG2 crosslinking activity has been implicated in the pathogenesis of various diseases including celiac disease, cancer and fibrotic and neurodegenerative diseases. Development of a PET tracer specific for active TG2 provides a novel tool to further investigate TG2 biology *in vivo* in disease states. Recently, potent irreversible active site TG2 inhibitors carrying an acrylamide warhead were synthesized and pharmacologically characterized.

Methods: Three of these inhibitors, compound **1**, **2** and **3**, were successfully radiolabeled with carbon-11 on the acrylamide carbonyl position using a palladium mediated [^{11}C]CO aminocarbonylation reaction. *Ex vivo* biodistribution and plasma stability were evaluated in healthy Wistar rats. Autoradiography was performed on MDA-MB-231 tumor sections.

Results: [^{11}C]**1**, **-2** and **-3** were obtained in decay corrected radiochemical yields of 38–55%. Biodistribution showed low uptake in peripheral tissues, with the exception of liver and kidney. Low brain uptake of <0.05% ID/g was observed. Blood plasma analysis demonstrated that [^{11}C]**1** and [^{11}C]**2** were rapidly metabolized, whereas [^{11}C]**3** was metabolized at a more moderate rate (63.2 ± 6.8 and 28.7 ± 10.8% intact tracer after 15 and 45 min, respectively). Autoradiography with [^{11}C]**3** on MDA-MB-231 tumor sections showed selective and specific binding of the radiotracer to the active state of TG2.

Conclusions: Taken together, these results identify [^{11}C]**3** as the most promising of the three compounds tested for development as PET radiotracer for the *in vivo* investigation of TG2 activity.

1. Introduction

Transglutaminases (TGs) consist of a family of nine members. Eight of them catalyze the transamidation reaction (TG1-TG7 and FXIIIa) and one catalytically inactive protein band 4.2 exists [1–2]. Catalytically active TGs use an active site cysteine residue in an Asp-His-Cys triad to catalyze the post-translational modification of specific glutamine residues. Following the cysteine's nucleophilic attack on a glutamine residue, an ammonia molecule is released and an intermediate thioester is formed. These thioesters are susceptible to nucleophilic attack and can thus be hydrolyzed, reacting with small organic amines or with lysine residues of a protein donor substrate. The net effect on the glutamine acyl-acceptor substrate is deamidation, incorporation of a small organic amine or intra- or intermolecular protein crosslinking via an isopeptide bond. The latter, formation of an isopeptide bond, is commonly referred to as the 'transamidation reaction' [1].

TG2, also known as tissue transglutaminase, is a calcium dependent TG that is expressed ubiquitously throughout the body, including the brain. Four distinct domains, two consecutive C-terminal β-barrels, a core α/β domain containing the transglutamidation site and an N-terminal domain able to bind to fibronectin, make up the enzyme. At least two structurally distinct conformations of TG2 exist [3,4], a closed conformation, favored by low calcium and high GTP/GDP concentrations, and an open conformation that is favored by high calcium and low GTP/GDP concentrations. Transamidation is hampered by steric hindrance of the active site by the bulky β-barrels in the GTP/GDP bound closed conformation. Only the calcium dependent open form is capable of executing the transamidation reaction as a result of the exposed active site [5,6]. Furthermore, the transamidation reaction

* Corresponding author at: Department of Radiology and Nuclear Medicine, VU University Medical Center, Radionuclidecenter, De Boelelaan 1085C, 1081 HV Amsterdam, The Netherlands. Tel.: +31 20 22249101.
E-mail address: b.vanderwildt@vumc.nl (B. van der Wildt).

http://dx.doi.org/10.1016/j.nucmedbio.2016.01.003
0969-8051/

only occurs when TG2 has the appropriate, reduced redox state. As shown *in vitro*, a disulfide bond between Cys-370 and Cys-371 locks the enzyme in the open conformation but renders it catalytically inactive, although the active site cysteine Cys-277 is not directly involved in the disulfide bond [7]. As a result of these activity modulators, it is postulated that intracellularly, and under physiological conditions, TG2 is catalytically inactive. Under pathological stress conditions however, where intracellular Ca^{2+} homeostasis is often disturbed and energy levels are depleted, TG2 mediated transamidation could occur. For example, during apoptosis TG2 crosslinks cellular components to prevent inflammatory and autoimmune reactions [8]. Extracellularly, despite relatively high calcium levels and low GTP/GDP concentrations, TG2 activity is thought to be controlled by the oxidating environment.

Increased expression levels and enzyme activity of TG2 have been observed and are believed to be strongly involved in the onset and progression of various diseases, e.g. celiac disease, cancer and fibrotic and neurodegenerative diseases [9–15]. In celiac disease, TG2 is responsible for deamidation or crosslinking of gluten peptides in the small intestine, ultimately resulting in T-cell activation and an autoimmune response [9]. In breast cancer, it has been shown that extracellular TG2 plays a role in increasing the motility and migration of tumor cells, thereby increasing the possibility of metastases and worsening patient prognosis [10]. Furthermore, drug resistance of tumor cells expressing high amounts of TG2 is increased. Multiple mechanisms have been proposed for this phenomenon, one of which is the TG2-mediated inactivation of the natural inhibitor of nuclear factor NF-κB, IκBα, by intermolecular crosslinking [11]. In an *in vivo* rat model of renal scarring, kidney fibrosis has been prevented by locally applying extracellularly acting TG2 inhibitors [12]. Moreover, in a mouse model of pulmonary fibrosis significant reduction of lung scarring was observed in TG2 knockout mice compared with wild type mice, suggesting a role for TG2 in fibrotic diseases [13–14]. In neurodegenerative disorders characterized by the formation of neurotoxic protein aggregates such as Alzheimer's, Huntington's and Parkinson's diseases, TG2 is associated with the formation of these protein aggregates by intra- and intermolecular crosslinking, both inside and outside nerve cells [15].

The finding that TG2 knock-out mice show no severe phenotypic deficits and are able to reproduce normally has further stimulated interest in TG2 as a therapeutic target [16]. Recently, potent irreversible TG2 inhibitors designed for treating Huntington's disease were reported [17,18]. The general motif of the most potent compounds in these series is the acrylamide warhead that is able to bind covalently to the enzyme's active site cysteine (Fig. 1A). Given the fact that this active site is only accessible in the enzyme's open conformation, these compounds should specifically target active TG2 [17,18]. Together with the interest of developing potent inhibitors, the demand for *in vivo* imaging probes targeting TG2 is increasing [19].

Positron emission tomography (PET) is an imaging technique that allows for non-invasive *in vivo* quantification of receptor expression or enzyme and transporter activity [20]. Therefore, a labeled compound that selectively binds to active TG2 would provide a means to further understand TG2 biology in normal and diseased states. Based on their high inhibitory potency and isozyme selectivity, the inhibitors **1**, **2** and **3** (compound **18d** in Ref. [17] and compounds **4l** and **8d** in Ref. [18], respectively) were selected as candidate PET tracers (Fig. 1B). These inhibitors, by virtue of their acrylamide warhead, potentially allow for radiolabeling by means of a palladium mediated [^{11}C]CO aminocarbonylation reaction between amine precursor molecules and vinyl iodide to obtain the radiolabeled acryl amides [21].

Therefore, the aim of this study was to develop carbon-11 labeled PET tracers based on these inhibitors.

2. Materials and methods

2.1. General

All reagents were obtained from commercial sources (Sigma Aldrich, St. Louis, USA; Bachem, Bubendorf, Switzerland or Fluorochem, Hadfield, UK) and used without further purification. Human recombinant TGs, assay reagents A101 (Abz-NE(CAD-DNP)EQVSPLTLLK-OH) and T26 (Biotinyl-HQSYVDPWMLDH) and inhibitor **Z006** (Z-DON-VPL-OMe) were obtained from Zedira GmbH (Darmstadt, Germany). Solvents were obtained from Biosolve (Valkenswaard, The Netherlands) and used as received unless stated otherwise. Dichloromethane (DCM) and N,N-dimethylformamide (DMF) were dried over activated 3 Å molecular sieves. Tetrahydrofuran (THF) was first distilled from $LiAlH_4$ and then stored on activated 3 Å molecular sieves. Reaction mon-

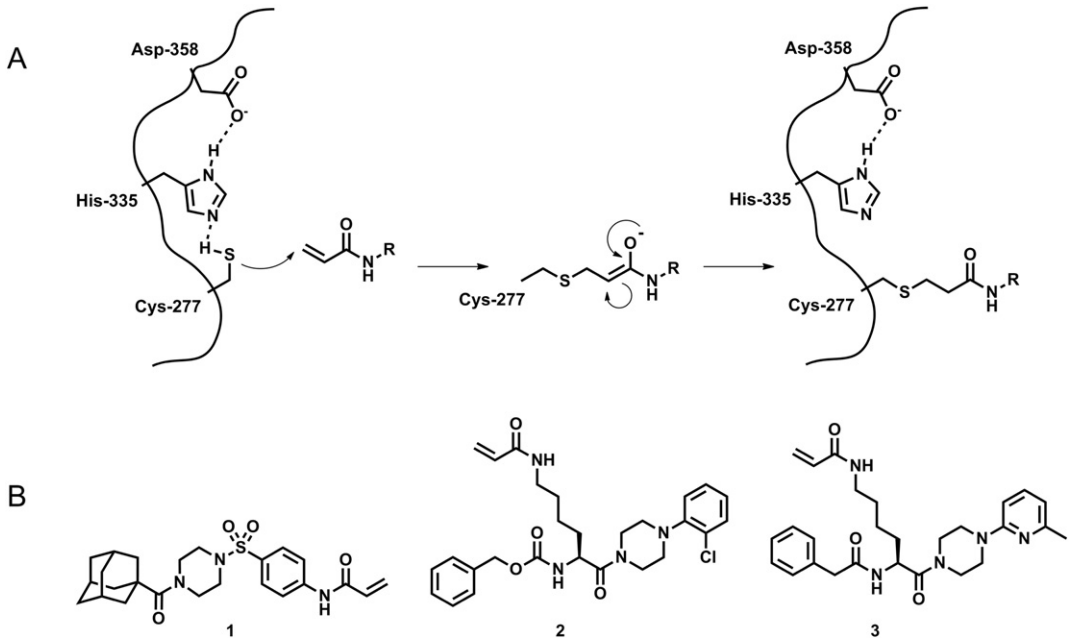

Fig. 1. A: Inhibitory mechanism of acrylamide-bearing TG2 inhibitors; B: TG2 inhibitors **1**, **2** and **3**.