Drug Metabolism and Pharmacokinetics 31 (2016) 146–155

Contents lists available at ScienceDirect

Drug Metabolism and Pharmacokinetics

journal homepage: http://www.journals.elsevier.com/drug-metabolism-and-pharmacokinetics

Regular article

Biodistribution and pharmacokinetics of the ^{99m}Tc labeled human elastase inhibitor, elafin, in rats

Mark Kaschwich [a, 1], Ulf Lützen [b, 1], Yi Zhao [b], Angelina Tjiong [b], Marlies Marx [b], Sierk Haenisch [c], Oliver Wiedow [d], Stefanie Preuss [e], Juraj Culman [c], Maaz Zuhayra [b, *]

[a] Department of Cardiovascular Surgery, University Hospital of Schleswig-Holstein (UK-SH), Campus Kiel, Arnold-Heller-Strasse 3, D-24105 Kiel, Germany
[b] Department of Nuclear Medicine, Molecular Imaging, Diagnostics and Therapy, University Hospital of Schleswig-Holstein (UK-SH), Campus Kiel, Karl Lennert Cancer Center North, Feldstrasse 21, D-24105, Germany
[c] Institute of Experimental and Clinical Pharmacology, University Hospital of Schleswig-Holstein (UK-SH), Campus Kiel, Arnold-Heller-Strasse 3, D-24105 Kiel, Germany
[d] Department of Dermatology, Venereology and Allergology, University Hospital of Schleswig-Holstein (UK-SH), Campus Kiel, Schittenhelmstrasse 7, D-24105 Kiel, Germany
[e] Department of General Pediatrics, University Hospital of Schleswig-Holstein (UK-SH), Campus Kiel, Arnold-Heller-Strasse 9, D-24105 Kiel, Germany

ARTICLE INFO

Article history:
Received 24 August 2015
Received in revised form
25 January 2016
Accepted 27 January 2016
Available online 5 February 2016

Keywords:
Elafin
Radiolabeling
Tc-^{99m}
Pharmacokinetic
SPECT

ABSTRACT

Elafin is a potent reversible inhibitor of the pro-inflammatory proteases leukocyte elastase and protease 3. It is currently in clinical development for the use in postoperative inflammatory diseases. We investigated the pharmacokinetics of ^{99m}Tc-labeled elafin (^{99m}Tc-Elafin) in blood and individual organs in rat after bolus intravenous injection using the single photon emission tomography (SPECT). ^{99m}Tc-Elafin predominantly accumulated in the kidney reaching a maximum of 8.5% ± 0.1% of the injected dose per gram (ID/g) at 5 min post injection (p.i) and decreased only slowly during 24 h. In contrast, the initially high radio activity recorded in the other organs rapidly decreased parallel to the radioactivity detected in blood. The blood kinetics fits to a two compartment kinetics model. The radio activity in the dissected kidney was 4.98 ± 1.24%ID/g 24 h p.i, while in other organs, including the brain, no accumulation of ^{99m}Tc-Elafin was detected. At this time point 30% of the detected radioactivity in the kidney was identified to be not metabolized ^{99m}Tc-Elafin. In conclusion, the blood and organ-specific kinetic data provide a basis for planning of adequate dosing regimens and the high accumulation of intact elafin in the kidney favors clinical developments targeting inflammatory kidney diseases, such as chronic allograft nephropathy after kidney transplantation.

1. Introduction

Elafin, also known as the peptidase inhibitor 3 or skin derived antileukoproteinase (SKALP), is a soluble, 6 kDa endogenous protein containing 57 amino acids composed of an N-terminal whey acidic protein (WAP)-domain, which includes anti proteolytic properties [1]. Elafin is generated from pre-elafin, also known as trappin-2, which is a longer molecule containing 117 amino acids (10 kDa). Trappin-2 undergoes proteolytic cleavage, possibly by tryptase, releasing the elafin molecule [2]. Both proteins, elafin and trappin-2, are inhibitors of neutrophil serine proteases [3,4]. Elafin and its precursor, trappin-2, possess anti-inflammatory properties and therapeutic potential for the treatment of inflammatory diseases of the lung [5].

Besides the presence in the human skin [6,7], elafin can be found in a variety of organs and tissues including the oral mucosa, esophagus, vagina [8], uterus, ovary tubes [9], lung [10] and ovarial carcinoma [11]. Furthermore, elafin was detected in epithelial cells including keratinocytes as well as in neutrophils and macrophages [9].

In addition to the protease inhibiting function, elafin has immunomodulatory functions which depend on the environment. Elafin can either reduce or stimulate immunological reactions [12]. Furthermore, elafin has antimicrobial properties [13,14]. A number of animal studies have indicated that elafin may also be effective in treatment of myocardial ischemia, viral infections and proliferative inflammatory vasculopathies [1]. Beneficial effects of serine elastase inhibitors in experimentally induced myocarditis, myocardial infarction and reperfusion injury associated with ischemia, have

* Corresponding author.
E-mail address: mzuhayra@nuc-med.uni-kiel.de (M. Zuhayra).
[1] Both authors contributed equally to this work.

http://dx.doi.org/10.1016/j.dmpk.2016.01.004
1347-4367/

also been reported [15,16]. Moreover, elafin minimized the post-cardiac transplant coronary arteriopathy after heart transplantation [16].

Elafin is currently in clinical development for the use in post-operative inflammatory diseases. For the establishment of elafin as a therapeutic drug, it is necessary, among others, to study its biodistribution and pharmacokinetics in order to analyze whether sufficient concentrations of elafin can be reached in the target organs and tissues. Therefore, in the present study we have analyzed the biodistribution and pharmacokinetics of intravenously applied human elafin labeled with the radionuclide ^{99m}Tc in rats. The present study provides a good framework for the design of further pharmacological studies assessing the optimal dosage and side effects.

2. Material and methods

All animal experiments have been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki), and were approved by the "Ministry of Agriculture, Environment and Rural Areas of Schleswig-Holstein-V 31-animal welfare according to § 8 Section 1" of animal protection act as amended on Mai 18th 2006 (BGBL. I S. 1206, 1313), modified by Section 2 of the law from December 13th 2007 (BGBL. I S. 2936).

Recombinant human elafin (molecular weight = 6 kDa) was provided by Proteo Biotech AG (Germany) and stored at − 40 °C. All other chemicals were obtained commercially in the highest available purity.

For the labeling experiments ^{99m}Tc, obtained in physiological saline as $Na^{99m}TcO_4$, was eluted from a commercial ^{99m}o/^{99m}Tc-generator (Mallinckrodt Medical, Petten, Netherlands). The radionuclide purity was >99.99%.

2.1. Radioactivity measurements

The radioactivity of injected ^{99m}Tc-Elafin to the rats was measured using a dose calibrator (Isomed 1010, MED Germany). The radioactivity in dissected organs and blood samples was detected with a gamma counter (γ-counter) (Berthold LB 5310 Multi logger; Berthold, Bad Wildbad, Germany). The daily quality control was carried out with a ^{137}Cs-source (A = 37 kBq, 1.10.1998, Amersham Buchler GmbH & Co KG, Braunschweig, Germany). A calibration row with $Na^{99m}TcO_4$ was also established.

2.2. HPLC and LC-MS analysis

Two analysis systems (System A and B) were used in order to perform the chromatographic purification and the quality control of the used and synthesized radioactive and non-radioactive substances. The systems included reversed phase high performance liquid chromatography (RP-HPLC), electro spray ionization mass spectrometry (ESI-MS) and gel filtration chromatography.

System A was a combination of HPLC and ESI-MS (LC-MS) and included a quaternary HPLC pump, a diode array detector, an auto sampler (Agilent HPLC system 1100; Santa Clara, CA, USA), a NaI scintillation crystal detector (Ginastar, Raytest, Germany) and a Quattro Premier XE mass spectrometer (Waters, Milford, MA, USA). The analysis using system A were performed on a Nucleosil C18 column (250 mm × 4.6 mm ID, Macherey–Nagel, Dueren, Germany) with water 0.1% formic acid (solvent A) and acetonitrile 0.1% formic acid (solvent B); solvent flow: 1 ml/min; gradient: 0% B to 65% B in 25 min. The solutions were injected automatically with a maximum volume of 100 μl.

System B consisted of a Beckmann programmable Solvent Module 126 (software 32 Karat), a Beckmann 166 UV detector (260 nm) and a NaI-crystal scintillation detector (Berthold, Germany). In this system, chromatographic purification of ^{99m}Tc-Elafin was carried out using a Superdex® 75 HR 10/300 GL gel filtration column (GE Healthcare Life Sciences, Germany). The mobile phase was phosphate buffered saline (PBS) and the flow rate was set at 0.7 ml/min. The solutions were injected manually with a maximum volume of 500 μl (Rheodyne® 500 μl loop).

To determine the radiochemical purity, the isolated ^{99m}Tc-Elafin fraction from HPLC system B was analyzed by means of the described HPLC system A. For this purpose the peak areas of the radioactive compounds in the radio-HPLC chromatogram were set in relation to each other. To determine the specific radioactivity, the radioactivity of the purified radiotracer was measured immediately after the chromatographic purification and set in relation to the amount of precursor used. The radiochemical yield is expressed as the measured radioactivity of the purified ^{99m}Tc-Elafin solution relative to the radioactivity of the unpurified solution measured directly before chromatographic purification. The radiochemical yields were non decay corrected.

2.3. Synthesis of HYNIC-Elafin

The synthesis of HYNIC-Elafin was carried out using N-hydroxysuccinimide hydrochloride in dimethylformamide: 16.7 nmol elafin were dissolved in 100 μl buffer (consisted of 1 ml PBS + 70 μl of 1 M $NaHCO_3$, pH = 8.5). A solution of N-hydroxysuccinimide hydrochloride salt (HS-HCl) (consisted of 76.8 nmol HS-HCl in 20 μl dimethyformamide) was added drop-wise to the elafin solution. The mixture was protected with aluminum foil from light and stirred for 140 min at room temperature. After 140 min the entire reaction solution was transferred to the HPLC system A. The peak from 15.5 to 17.0 min containing HYNIC-Elafin was collected and freed from the solvent overnight. The HYNIC-Elafin was characterized by ESI-MS analysis.

2.4. Synthesis of ^{99m}Tc-Elafin

The HNYC-Elafin was dissolved in 100 μL of 200 mM Tricine solution consisting of 35.8 mg Tricine in 1 ml H_2O. 1.0–1.5 GBq freshly eluted $Na^{99m}TcO_4$ in 400 μl physiological saline solution was added to the HYNIC-Elafin solution. Thereafter, 5 μl of $SnCl_2$ solution (prepared from 50 mg $SnCl_2$, 0.975 ml water and 25 μl 4 M HCl) was added. The solution was allowed to stand for 10 min at room temperature. Then the whole solution was injected to the HPLC system b. The ^{99m}Tc-Elafin fraction with the retention time (Rt) = 15.8 min was drawn into 1 ml plastic syringe and measured in a dose calibrator.

2.5. Metabolic stability of ^{99m}Tc-Elafin

The metabolic stability of the labeled ^{99m}Tc-Elafin was studied in vitro in PBS and in rat plasma. Blood obtained from normal Wistar rats was centrifuged at 6000 g (4 °C) in heparinized polypropylene tubes for 30 min and the supernatant (plasma) was collected. 100 μl (15.7 MBq) of ^{99m}Tc-Elafin was incubated with 400 μl plasma at 37 °C. After 24 h, the plasma proteins were precipitated with ethanol (2:1 v/v) [17], and samples were centrifuged at 15,000 × g at 4 °C for 10 min. The supernatants was then filtered through a filter (0.22 m, Millipore GV) and analyzed with RP-HPLC as described above (system B). The recovery of radioactivity in the supernatant was 65–85%.