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[^{11}C]diclofenac sodium: synthesis and PET assessment of transdermal penetration

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Abstract

The aim of this work was to study the feasibility of using Positron Emission Tomography (PET) imaging as a new tool to detect transdermal penetration of topical drugs in human subjects. The compound used in the study is sodium 2-[(2,6-dichlorophenyl)amino]phenyl]acetate, better known as diclofenac sodium. This molecule belongs to the family of non-steroidal anti-inflammatory drugs and is considered one of the first choices among non-steroidal anti-inflammatory drugs for the treatment of inflammatory diseases; it is widely used and commercially present in a large number of pharmaceutical forms and formulations. ^{11}C-labeled diclofenac has been synthesized and coformulated, as an internal indicator, with a proprietary preparation based on the use of a sprayer. The radiolabeled preparation was topically administered to healthy volunteers, and PET imaging was used to evaluate transdermal penetration. Results obtained have demonstrated the efficacy of PET and radiolabeled tracers for the evaluation of transdermal penetration of active pharmaceutical ingredients as topical formulations.

Keywords: Diclofenac; Carbon-11; PET; Transdermal penetration

1. Introduction

Positron Emission Tomography (PET) is a molecular imaging modality widely used in medicine for clinical and medical research and is based on the in vivo detection of radiolabeled tracers. The availability of the active molecule labeled with a suitable positron emitter in such a way that the biological properties of the molecule itself are not changed makes it possible to (i) evaluate the distribution of the drug in organs and tissues in good spatial resolution (5–7 mm), (ii) monitor the time course of the radioactivity distribution over time and (iii) quantify the tissue concentration of labeled compounds.

These features have triggered considerable attention on the perspective applications of PET imaging in the field of drug discovery and development, where molecular imaging is regarded as a potentially valuable tool for the reduction of the attrition affecting efficient access to new medicines. Indeed, nuclear imaging has been used to help and support drug development in not only assessing the pharmacokinetic and/or pharmacodynamic features of active molecules but also supporting and ameliorating the formulation and the pharmaceutical technology aspects. In this view, topical formulations [1,2] represent a very important segment of the pharmaceutical market for the use of many classes of drugs and the evaluation of transdermal penetration is of interest to many companies as a possible "killing" reason for the product.

The ability of the skin to absorb compounds has been extensively used to develop topically administered drugs able to deliver the active pharmaceutical ingredient (API) both locally and systemically. The nature of the molecule and its formulation strongly influence the passage across the external keratinized layer of the skin and the eventual rate and depth of adsorption. Topically administered drugs have to cross the stratum corneum barrier, acting as a protective shield against the penetration of exogenous products toward the underlying tissues. Special formulations are often used to

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facilitate this passage. To this purpose, excipients, such as lipids and DMSO, are coformulated with the API. However, this upgraded formulation is not a warranty per se that the API will follow the same penetration pattern of the vehicle.

The present study focused on the feasibility of using PET imaging as a tool to detect transdermal penetration of a topical drug, based on a well-known medicine such as sodium 2-[(2,6-dichlorophenyl)amino]phenyl]acetate (diclofenac sodium), in human subjects. Diclofenac was discovered in the 1950s [3,4] and belongs to the family of non-steroidal anti-inflammatory drugs (NSAIDs) (i.e., phenylbutazone, ibuprofen, indomethacin). NSAIDs mainly act as inhibitors of cyclooxygenase-2, the enzyme that converts arachidonic acid to prostaglandins, mediators of inflammatory processes [5,6]. Diclofenac is still considered one of the first-choice NSAIDs for the treatment of inflammatory diseases [6] and is commercially available in a large number of pharmaceutical forms and formulations. Thus, ^{11}C-labeled diclofenac has been synthesized and coformulated, as an internal indicator, with a proprietary formulation to evaluate with PET the transdermal penetration of such preparation in healthy volunteers.

2. Methods

2.1. Radiolabeling method

Diclofenac has been labeled with stable or long half-life isotopes, such as deuterium [7] and carbon-14 [8], to assess its pharmacokinetics and biodistribution ex vivo in humans. The radionuclide carbon-11 (half-life of 20 min) was selected to keep the intact chemical structure of the active molecule and use the labeled compounds to assess whether PET could be used to evaluate in vivo transdermal penetration of a topical utilization.

The methods of preparation of diclofenac reported so far [3–6,9,10] were not compatible with the radionuclide half-life and the ^{11}C-labeled precursors that are available at a cyclotron; therefore, a new method had to be developed.

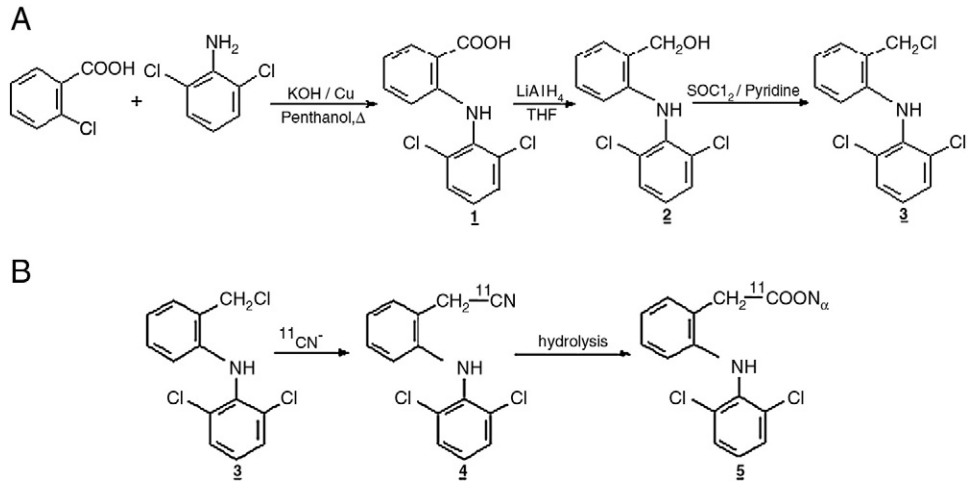
The method is based on the synthesis of a chlorinated precursor, namely the 2-[(2,6-dichlorophenyl)amino]benzyl chloride (**3**), which, by reaction with sodium [^{11}C]cyanide and successive hydrolysis, can be converted into [^{11}C] diclofenac. The chlorinated precursor was not commercially available: it was prepared optimizing the synthesis described in the literature [3]. The sequence of reactions is reported in Scheme 1A. Reaction between 2-chlorobenzoic acid and 2,6-dichloroaniline produced 2-[(2,6-dichlorophenyl)amino] benzoic acid (**1**), which was then reduced with $LiAlH_4$ to 2-[(2,6-dichlorophenyl)amino]benzyl alcohol (**2**). The alcohol (**2**) was chlorinated with thionyl chloride in pyridine to produce the precursor (**3**).

[^{11}C]cyanide was prepared starting from cyclotron-produced [^{11}C]CO_2 by using a home-made gas-processing system (Fig. 1) based on the catalytic reduction on nickel catalyst of [^{11}C]CO_2 to [^{11}C]methane and its conversion to hydrogen [^{11}C]cyanide on platinum in the presence of anhydrous ammonia.

Labeling was performed by reacting compound **3** with sodium [^{11}C]cyanide and hydrolysis of the corresponding nitrile (**4**) (Scheme 1B), followed by purification by preparative HPLC.

2.2. Chemistry

2-Chlorobenzoic acid and 2,6-dichloroaniline were purchased from Sigma-Aldrich and used without further purification. Nickel kieselguhr was obtained from Sigma-Aldrich as well. Solvents were dried with conventional methods prior to use. NMR spectra were obtained with a 200-MHz Gemini 200BB spectrometer (Varian, USA) using $SiMe_4$ as internal standard. $^{11}CO_2$ was produced at a PETtrace cyclotron (GE Medical System, USA) by bombardment of a nitrogen–oxygen (0.5%) gas target with protons (16.5 MeV, 30 μA). HPLC analyses were performed



Scheme 1. Synthesis of [^{11}C]diclofenac sodium. (A) Synthesis of the precursor. (B) Radiochemistry.