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# Polyethylene glycol-drug ester conjugates for prolonged retention of small inhaled drugs in the lung ☆

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ABSTRACT

Typically, inhaled drugs are rapidly absorbed into the bloodstream, which results in systemic side effects and a brief residence time in the lungs. PEGylation was evaluated as a novel strategy for prolonging the retention of small inhaled molecules in the pulmonary tissue. Hydrolysable ester conjugates of $PEG_{1000}$, $PEG_{2000}$, $mPEG_{2000}$, $PEG_{3400}$ and prednisolone, a model drug cleared from the lungs within a few minutes, were synthesised and thoroughly characterised. The conjugates were stable in buffers with hydrolysis half-lives ranging from 1 h to 70 h, depending on the pH and level of substitution. With the exception of $PEG_{3400}$-prednisolone, conjugates did not induce a significant lactate dehydrogenase (LDH) release from Calu-3 cells after a 20 h exposure. Following nebulisation to isolated perfused rat lungs (IPRL), the $PEG_{2000}$ and $mPEG_{2000}$ conjugates reduced the maximum prednisolone concentration in the perfusate ($C_{max}$) by 3.0 and 2.2 fold, respectively. Moreover, while prednisolone was undetectable in the perfusion solution beyond 20 min when the free drug was administered, prednisolone concentrations were still quantifiable after 40 min following delivery of the conjugates. This study is the first to demonstrate hydrolysable PEG drug ester conjugates are a promising approach for optimising the pharmacokinetic profile of small drugs delivered by inhalation.



## 1. Introduction

Although drug inhalation is being explored as a non-invasive route of delivery to the systemic circulation [1], it is currently still mainly exploited to obtain a therapeutic effect in the lungs as part of the management of respiratory diseases such as asthma and chronic obstructive pulmonary disorders (COPD). A major limitation of local pulmonary delivery is that typical inhaled drugs are small hydrophobic molecules which exhibit a sub-optimal pharmacokinetic profile characterised by a high maximum blood concentration (high $C_{max}$) reached within a few minutes (short $t_{max}$), coupled with a rapid elimination from the pulmonary tissue [2]. This often results in systemic side effects and a short duration of action in the lungs. Enhancing their lung residence time would improve the overall therapeutic index of inhaled drugs as well as decrease their administration frequency [3–6].

Due to the high permeability of the lungs and the presence of mucociliary clearance in the upper airways, achieving prolonged drug retention in the pulmonary tissue after administration by inhalation remains a major challenge [2]. The most common strategy involves decreasing the solubility of the drug in the lung fluid so that the absorption rate is controlled by the slow dissolution of the particles [2,7]. This is not always a safe approach as poorly soluble particles might cause lung irritancy or stimulate phagocytosis by alveolar macrophages with the risk of inducing a foamy phenotype [2,8]. Alternatively, sustained release formulations such as liposomes [9] and biodegradable polymer-based particles [10,11] have been successfully developed for pulmonary drug delivery. Amongst these, nanoparticles appear the most promising for substantially extending drug residence time in the lungs due to their ability to escape clearance mechanisms in the respiratory tract [12,13]. However, inhaled nanoparticulate delivery systems raise important



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safety concerns, precisely because of their persistence in the pulmonary tissue [13]. A third strategy to reduce or slow down pulmonary absorption consists in altering the permeation rate of the therapeutic compound, for instance, by modifying its physico-chemical properties [2]. This might not however be largely applicable through conventional drug design since the lung distribution and efficacy of the parent compound are likely to be affected. Considering pulmonary absorption decreases dramatically with an increase in molecular weight and hydrophilicity [14,15], a large hydrophilic pro-drug which slowly releases the active molecule in the lung fluid has the potential to improve its pharmacokinetic profile after inhalation while minimising the impact on the therapeutic response.

For the first time, we describe a drug conjugation strategy between polyethylene glycol (PEG), and a low molecular weight molecule aiming at optimising the pharmacokinetics of inhaled drugs. PEG being biocompatible and non toxic [16], PEGylation of both macromolecules and small drugs is extensively used to enhance their circulating half-life and/or safety profile after parenteral administration [17]. PEG conjugates have recently been developed to increase the stability of salmon calcitonin in the lung fluid [18] as well as to improve the biocompatibility of the biphosphonate derivative alendronate [19] and antimicrobial peptides [20] following pulmonary administration. In contrast, PEGylation of small inhaled drugs has not yet been attempted with the objective of prolonging their residence time in the lungs.

This study evaluates the potential of such an approach using hydrolysable ester conjugates of PEG and the corticosteroid prednisolone, herein used as a model inhaled drug rapidly absorbed from the lungs. Conjugates of various molecular weights were synthesised and characterised before their rate of hydrolysis was measured in buffers over a range of relevant pH. The innocuousness of the molecular constructs was verified *in vitro* and the ability of selected conjugates to increase pulmonary retention after inhalation was finally assessed in an isolated perfused rat lung (IPRL) model.

## 2. Material and methods

### 2.1. Materials

TEMPO (2,2,6,6-Tetramethylpiperidine-1-oxyl) was purchased from Alfa Aesar (Karlsruhe, Germany). Laboratory grade solvents, concentrated hydrochloric acid and sodium hydroxide were obtained from Fisher scientific (Loughborough, UK) while dry solvents were purchased from Acros Organics (Geel, Belgium). Unless stated otherwise, all other chemicals were purchased form Sigma-Aldrich (Saint Louis, MO, USA).

### 2.2. Synthesis of PEG-prednisolone conjugates

PEG with a molecular weight of 1000, 2000 or 3400 Da and monomethoxyPEG (mPEG) of 2000 Da were oxidised by sodium hypochlorite in water, at pH 10 using TEMPO and sodium bromide as catalysts [21]. The reaction was carried out on ice to maintain the temperature between 2 and 7 °C. Oxidised products were then protonated in 2 M HCl, extracted with dichloromethane (DCM) and precipitated in diethyl ether. Oxidised PEG were reacted with prednisolone and the Mukaiyama reagent (2-chloro-1-methylpyridinium iodide) under dry conditions to give esters of PEG and prednisolone. Conjugates of $PEG_{3400}$ and $mPEG_{2000}$ were purified by crystallisation in isopropanol. $PEG_{1000}$ and $PEG_{2000}$ conjugates were purified by silica gel chromatography using acetonitrile for washing and tetrahydrofuran (THF) as the eluant. After purification, all conjugates were precipitated in diethyl ether. The purified products were analysed by Fourier Transform Infrared (FTIR) spectroscopy using a Nicolet 380 spectrometer with data processed by OMINIC software (Thermo) as well as by elemental microanalysis, using an in-house analytical service (CE-440 Elemental Analyzer from Exeter Analytical). One Dimensional Nuclear Magnetic Resonance (1D NMR) spectra were acquired using a Bruker AV(III)400 NMR spectrometer. The spectra were then analysed using ACDlab software v12.

### 2.3. Hydrolysis in buffers

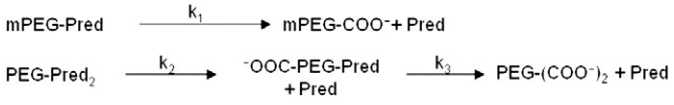
Hydrolysis profiles were obtained in McIlvaine buffers at pH 6.2 and 7.4 or phosphate buffer at pH 8.0. The ionic strength of each buffer was adjusted to 0.5 with KCl. Stock solutions of PEG-prednisolone were prepared in methanol at a concentration of 30 mM prednisolone. At time 0, 14.3 μL of stock solution was added to 1 mL of pre-heated buffer. The resulting solution was thoroughly mixed and maintained at 37 °C throughout the course of the hydrolysis study. Twenty microlitre samples were collected at pre-determined time intervals and directly analysed by High Performance Liquid Chromatography (HPLC-UV). The HPLC system consisted of an Agilent 1050, controlled by the Chemstation software (rev A 08.03, Agilent). The stationary phase was a Gemini (Phenomenex, Macclesfield, UK) 3 μm, $C_{18}$ column, 100 × 3 mm and the mobile phase was a gradient of methanol in water as follows: 0–0.5 min: 60% methanol; 0.5–1 min: gradient from 60% to 90% methanol; 1–2.5 min: 90% methanol; 2.5–5 min: 60% methanol. Prednisolone and the conjugates were monitored by UV absorption at 242 nm. Prednisolone eluted at 2.1 min, PEG-$Pred_2$ (disubstituted PEG, i.e., with a prednisolone molecule attached to each chain end) at 4.0 min and mPEG-Pred (monosubstituted) at 4.4 min. Quantification of free prednisolone and conjugates was performed by measuring the chromatogram peak areas. The limit of quantification was 0.06 μM for the drug and <10 μM for the conjugates, depending on their composition.

The hydrolysis pathways of the mono and disubstituted conjugates are illustrated by Scheme 1.

The hydrolysis constant $k_1$ and $k_2$ were determined by the slope of the plots showing the log of the concentration in conjugates with time (first order kinetics). Although a low trace signal of the intermediate $^{-}$OOC-PEG-Pred was visible on the chromatograms at a retention time similar to that of mPEG-Pred, this was not quantifiable by the HPLC-UV method. The Berkeley–Madonna software (v.8.3.18, the University of California, Berkeley, CA, USA) was used to calculate the hydrolysis rate constant $k_3$. The software solved the system of differential Eq. (1), using the degradation profiles of PEG-$Pred_2$ along with the release profiles of prednisolone in buffers.

$$\begin{cases} \dfrac{d[PEG-Pred_2]}{dt} = -k_2[PEG-Pred_2] \\ \dfrac{d[Pred_2]}{dt} = k_3[^{-}OOC-PEG-Pred] + k_2[PEG-Pred_2] \\ \dfrac{d[^{-}OOC-PEG-Pred]}{dt} = k_2[PEG-Pred_2] - k_3[^{-}OOC-PEG-Pred] \\ \dfrac{d[PEG-(COO^{-})_2]}{dt} = k_3[^{-}OOC-PEG-Pred] \end{cases} \quad (1)$$

The values calculated for the rate constants $k_3$ were consistently much higher than $k_2$, likely due to the participation of the free carboxylate of the intermediate compound in the hydrolysis via an intramolecular acid catalysed or an intramolecular nucleophilic process. This




**Scheme 1.** Hydrolysis pathways of PEG-prednisolone conjugates.