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Communication

Prodrug to probe solution HFA pMDI formulation and pulmonary esterase activity☆

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Abstract

A novel salbutamol prodrug was synthesised. Solubility in HFA-134a and susceptibility to rat lung homogenate, blood and plasma esterase enzymes were investigated. Whereas salbutamol had a very low solubility in HFA-134a, the prodrug was found to be miscible in all proportions. In lung homogenate, the prodrug hydrolysed with a half-life of 45 min, re-generating approximately 17% of expected salbutamol after 8 h incubation. The use of a solution pMDI for pulmonary delivery of the salbutamol prodrug is predicted to result in liberation of salbutamol in the lungs following in vivo hydrolysis by lung esterases.

Keywords: Salbutamol; Ester; Prodrug; Solution; Inhaler; Lung

1. Introduction

Chlorofluorocarbons (CFC), the traditional propellants used in the formulation of pressurised metered dose inhalers (pMDI), are being phased out under the Montreal Protocol. Hydrofluoroalkane (HFA) propellants, such as HFA-134a, have been identified as replacements for CFC. However, a number of re-formulation problems have been encountered due to the disparate solvency properties of HFA and CFC propellants. In particular, the surfactants used to stabilise CFC suspensions have insufficient solubility in HFA to effect stable suspensions.

Formulations in which the drug is soluble in the propellant offer several potential advantages over suspension pMDI formulations, including a greater lung deposition and less oropharyngeal deposition. Ashworth et al. (1991) report highly efficient lung deposition from a solution pMDI. The solubility of some commonly used anti-asthma agents is higher in HFA than CFC propellants (Byron et al., 1994) although not high enough to produce a therapeutic solution pMDI formulation.

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The use of prodrugs to modify the physicochemical characteristics of a drug, such as poor water solubility, is well-established (Nielsen and Bundgaard, 1987; Kerr and Kalman, 1992). An HFA-soluble prodrug could be produced by linking a highly HFA-soluble moiety to a functional group on the parent drug molecule. This would allow the prodrug to be formulated as a solution pMDI. The pulmonary administration of ester prodrugs is predicted to liberate the parent drug due to pulmonary esterase activity (Dickinson and Taylor, 1998).

The aims of this study were to synthesise a highly HFA-soluble salbutamol prodrug and to investigate the susceptibility of the prodrug to esterase enzymes.

2. Materials and methods

2.1. Materials

All reagents used in the synthesis of the salbutamol prodrug were of analytical grade. All reagents used for HPLC analysis were of HPLC grade.

2.2. Synthesis of salbutamol prodrug

A suspension of salbutamol (2.5 g, 10.45 mmol) and triethylamine (4.8 ml, 34.5 mmol) in dry acetonitrile (85 ml) was stirred rapidly over ice (0°C). Butyryl chloride (32.8 ml, 0.31 mol) in dry acetonitrile (40 ml) was added dropwise slowly. Once addition was complete, the reaction was brought to room temperature then refluxed with stirring at 95°C under nitrogen for 65 h. The reaction was cooled to room temperature then concentrated under reduced pressure. The residue was diluted with ethyl acetate (200 ml) then washed with water (5×50 ml). The combined aqueous layers were washed with ethyl acetate (3×80 ml). The product was found in the ethyl acetate layers, which were combined, dried over anhydrous magnesium sulphate then concentrated under reduce pressure to give an orange syrup. Column chromatography was used to purify the product (dichloromethane: methanol 100:0 v/v increasing to 94:6 v/v). Concentration of the required fraction under reduced pressure yielded a yellow syrup.

2.3. Determination of HFA-134a solubility

The solubility of salbutamol in HFA-134a was determined using the method developed by Dalby et al. (1991). The solubility of butyric acid and the salbutamol prodrug in HFA-134a was determined by visual observation, using a fibre-optic light source, of the one to two-phase change after venting propellant from the pMDI vial.

2.4. Preparation of tissues

Rat lung homogenates were prepared as reported previously (Dickinson and Taylor, 1995). The protein concentration of each homogenate was assayed using a BCA™ protein assay kit (Pierce, IL, USA). Blood was collected from anaesthetised male Wistar rats (200–300 g). Lung homogenate, blood and plasma were stored at 1–4°C and used within 4 days of preparation.

2.5. Determination of enzymatic susceptibility of salbutamol prodrug

2.5.1. Lung homogenate

The incubation media consisted of rat lung homogenate (10 mg protein) and magnesium chloride (6.25 mM) in 50 mM Tris buffer pH 7.4 at 37°C. Salbutamol prodrug (1 mg/ml) was incubated and samples (200 μl) were taken over 8 h. Acetonitrile (300 μl) was added to the samples to terminate the reaction. The degradation of the prodrug and subsequent formation of salbutamol was assayed by HPLC. Incubation media spiked with salbutamol served as a control.

2.5.2. Blood

Salbutamol prodrug (2 mg/ml) was incubated in rat blood at 37°C and samples (50 μl) were taken over 24 h. Acetonitrile (200 μl) was added and the samples centrifuged at 5000 rpm for 2 min. The supernatant was assayed by HPLC. Blood spiked with salbutamol served as a control.

2.5.3. Plasma

Salbutamol prodrug (1.8 mg/ml) was incubated in plasma at 37°C and samples (50 μl) were taken

over 20 h. Acetonitrile (200 μl) was added to the samples followed by centrifugation at 5000 rpm for 2 min. The supernatant was assayed by HPLC. Plasma spiked with salbutamol served as a control.

2.6. Assay of salbutamol and salbutamol prodrug

Salbutamol and salbutamol prodrug concentrations were determined using reverse-phase HPLC. Salbutamol was assayed using a C_{18} column at ambient temperature, with detection at 278 nm. The mobile phase (1 ml/min) consisted of methanol–water (containing heptane sulphonic acid 1.1013 g/l) 40:60 v/v, adjusted to pH 3.0 with glacial acetic acid. Salbutamol eluted with a retention time of approximately 8 min. The salbutamol prodrug was assayed using a cyano column at ambient temperature, with detection at 264 nm. Acetonitrile–0.1% trifluoroacetic acid 60:40 v/v was used as the mobile phase at a flow rate of 1 ml/min. The salbutamol prodrug eluted with a retention time of approximately 10 min.

Table 1
Solubility of salbutamol, butyric acid and salbutamol prodrug in HFA-134a

Compound	Solubility in HFA-134a
Salbutamol	<0.004 mg/g
Butyric acid	Miscible
Salbutamol prodrug	Miscible

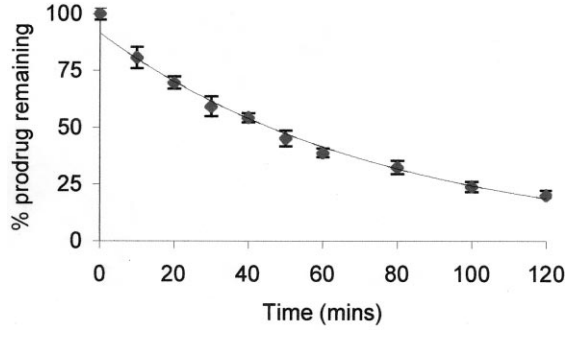

Fig. 1. Salbutamol prodrug degradation in lung homogenate (mean ± S.E.M., $n = 3$).

3. Results and discussion

3.1. Synthesis of salbutamol prodrug

The synthesis gave a yield of 1.27 g, equivalent to 27%. The synthetic product was identified by ^{1}H- and ^{13}C-NMR as a butyryl triester of salbutamol, 1-butyryloxy-1-(4-butyryloxy-3-butyryloxy-methyl)-phenyl-2-*t*-butyl aminoethane, $C_{25}H_{39}NO_6$, M_w 449.

3.2. Determination of HFA-134a solubility

The solubilities of salbutamol, butyric acid and the salbutamol prodrug are shown in Table 1. By attaching a highly HFA-soluble prodrug moiety (butyric acid) to the insoluble salbutamol molecule, a highly HFA-soluble salbutamol prodrug has been produced.

3.3. Determination of enzymatic susceptibility of salbutamol prodrug

Rat lung homogenate, blood and plasma were used as sources of esterase enzymes. In lung homogenate, the salbutamol prodrug was found to hydrolyse fairly rapidly, with a half-life of approximately 45 min (Fig. 1). Diester, monoester and finally salbutamol were produced. By the end of the 8 h incubation period, approximately 17% salbutamol had been formed (Fig. 2).

The prodrug was also found to disappear fairly rapidly in blood, with a half-life of approximately 10 min; however no salbutamol could be detected at the end of the 24 h incubation period. Analysis of the salbutamol control demonstrated the ability of the extraction technique to extract salbutamol that had been added to blood. There are two possible explanations for the inability to detect salbutamol after 24 h incubation of the prodrug. The first is that the prodrug is being hydrolysed to diester, monoester and salbutamol, with salbutamol being produced more than 24 h after the prodrug has disappeared. The second possible explanation is that the prodrug is partitioning into a lipophilic compartment within the blood from which any salbutamol formed cannot be extracted using the assay technique.

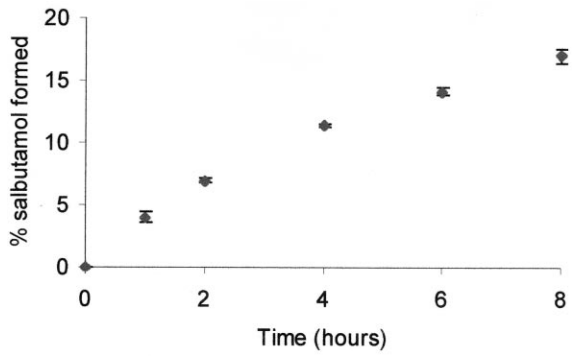

Fig. 2. Salbutamol formation in lung homogenate (mean ± S.E.M., $n = 3$).

Plasma was used as an alternative enzyme source to blood. The prodrug was found to hydrolyse fairly rapidly in plasma, with a half-life of approximately 10 min. Unlike the finding after blood incubation, salbutamol was detected, with approximately 30% salbutamol formed at the end of the 20 h incubation period.

A number of alternative techniques to extract salbutamol produced by prodrug hydrolysis in blood were investigated. Hutchings et al. (1983) and Cabral Marques et al. (1991) reported techniques for extracting salbutamol from plasma samples. When applied to blood samples, large amounts of endogenous material from the blood were also extracted, which interfered with the HPLC assay. Techniques to minimise extraction of endogenous material from the blood failed to extract any salbutamol. This, combined with the results of the plasma incubation, suggests that salbutamol was produced in the blood incubation, but the extraction techniques used were unable to extract salbutamol without extracting large amounts of endogenous material from blood.

Salbutamol has negligible solubility in HFA-134a. A highly HFA-soluble moiety, butyric acid, was selected and a butyryl triester of salbutamol synthesised. This prodrug was found to be miscible with HFA-134a in all proportions. Therefore, the possible utility of prodrugs with desirable physicochemical properties for the formulation of a solution pMDI has been demonstrated. The prodrug has been shown to be esterase-labile, hydrolysing to diester, monoester and finally salbutamol. Pulmonary delivery of the salbutamol prodrug, through the use of a solution pMDI, would result in liberation of salbutamol in the lungs following in vivo hydrolysis by lung esterases.

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