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Improvement of physicochemical and biopharmaceutical properties of theophylline by poly(ethylene glycol) conjugates

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Abstract

In the present paper two theophylline esters with poly (ethylene glycol) (PEG) and methoxy poly (ethylene glycol) (mPEG) were prepared. Quantitative yields of the pure products were obtained. Unlike the free drug, the drug–polymer conjugates are freely water-soluble at room temperature. In vitro release experiments in aqueous buffer demonstrate that both conjugates are stable in buffer of pH 7.4 and 1.2. In vivo release studies after oral administration of theophylline conjugates demonstrate a good release of parent drug.

Keywords: Macromolecular prodrugs; Theophylline; Poly(ethylene glycol); Methoxy poly(ethylene glycol)

1. Introduction

Theophylline is used as a bronchodilator [1], but, in spite of oral theophylline good bioavailability, there are some problems associated with its use. The rather short half life of theophylline and its narrow therapeutic range (10–20 μg/ml) in plasma make it necessary to administer the drug relatively often, while the peak levels achieved shortly after administration are associated with the observed side effects.

Moreover, because of a marked inter-individual variability, connected to genetic and environmental factors, in the metabolism as well as in theophylline elimination rapidity, it is difficult to maintain plasmatic levels necessary for its bronchodilating effect induction. Besides, theophylline low solubility in water (lower than 0.9% a 23 °C) restricts highly its clinical applications.

There are three ways to improve the delivery of drugs consisting in a development of:

1) an analogue which has better physical properties;
2) a prodrug which exhibits better physical properties than its parent;
3) a better formulation.

The second approach has been chosen in this work. The preparation of polymeric drug adducts, in which active substances are linked to polymeric matrices by means of a covalent bond naturally hydrolyzable in the body fluids, is presently recognized as an effective way to prolong the pharmacological activity by a free drug gradual release from the macromolecular matrix.

Poly(ethylene glycols) (PEGs) appear to be particularly convenient as oligomeric matrices, since they are easily available in a wide range of well-definite molecular weights. PEG is well known to be non toxic, non antigenic and biocompatible, to be soluble in water and in most organic solvents and by itself to have solubilizing properties. Given these properties, it is suitable for use as drug carrier in the body. Moreover it is rapidly eliminated from the body [2].

In this paper we report:

1) The derivatization of PEG to obtain the carboxyl-PEG.

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2) The synthesis of the drug–polymer conjugates by linking the modified theophylline to carboxyl-PEG, using an ester bond.
3) The results of in vitro and in vivo studies on the drug release from the macromolecular prodrugs.

2. Experimental

2.1. Materials

Theophylline, aminophylline, 8-chloro theophylline, triethylamine (TEA) and formaldehyde 37% were obtained from Sigma Chemicals Co. (St. Louis, MO, USA). 4-Dimethylaminopyridine (DMAP), PEG of MW = 1500, methoxypoly(ethylene glycol) of MW = 1900 (mPEG), succinic anhydride, dicyclohexylcarbodiimide (DCC) were purchased from Fluka.

All the other chemicals and solvents used in this work were of the highest quality commercially available. Organic solvents were dried over molecular sieves (3 Å).

2.2. Laboratory animals

Male rabbits (Charles, River, Calco, Italy) weighing 5 kg free any signs of gross abnormality were used in this study.

2.3. Analytical methods

Infrared spectra were recorded using a Perkin–Elmer 1720 FT IR Spectrophotometer.

HPLC analyses were carried out with a Perkin–Elmer Series 4 high performance liquid chromatography, equipped with a Rheodyne Model 7125 injector with a 20 μl loop and connected to a Perkin–Elmer Model LC 75 variable-wavelength UV detector. A LiChrosorb RP18 (Perkin–Elmer) 250 × 4.6 mm ID column packed with 10 μm particle size was used.

Elemental analysis were carried out on a Carlo Erba model 1016 analyzer.

^{1}H NMR and ^{13}C NMR spectra ($CDCl_3$) were recorded on a Varian Gemini-200 MHz Spectrometer.

^{13}C NMR: 100.4 MHz. Chemical shifts are given in ppm from TMS as internal standard. The following abbreviations are used: s, singlet; br s, broad singlet; d, doublet; t, triplet; m, multiplet; respectively.

2.4. Preparation of PEG-COOH

PEG_{1500}-$(COOH)_2$ and $mPEG_{1900}$-COOH have been synthesized according to previously published procedure [3].

2.5. Preparation of 7-(hydroxymethyl) theophylline

7-(Hydroxymethyl) theophylline has been prepared according to previously published procedure [4].

2.6. Preparation of PEG–[7-(hydroxymethyl) theophylline] (I–II)

7-(Hydroxymethyl) theophylline (0.022 mol; 4.62 g), DCC (0.026 mol; 5.4 g) were added in small portions to a solution of PEG_{1500}-$(COOH)_2$ (0.01 mol; 17 g) and DMAP (0.008 mol; 0.977 g) in CH_2Cl_2 (50 ml); the mixture was stirred for 5 h at room temperature. The precipitate of dicyclohexyl urea was filtered and the filtrate evaporated to dryness. The residue was extracted by acetone, filtered from dicyclohexyl urea residue and the product precipitated by ether. The PEG_{1500}–[7-(hydroxymethyl) theophylline]$_2$ (I) was recrystallized twice from ethanol. Yield 85%.

The absence of the free drug in the adduct was confirmed by HPLC analysis.

Anal. Calc. for $C_{91.36}H_{160.72}N_8$: C, 52.63; H, 7.72; N, 5.38. MW 2083. Found: C, 52.7; H, 7.80; N, 5.4%.

^{1}H NMR ($CDCl_3$): $\delta = 2.62$ ppm (br s, 8H, CH_2COO–), 3.38 (s, 6H, N–CH_3), 3.56 (s, 6H, N–CH_3), 3.6 (br s, 130H, CH_2–O–CH_2), 4.18 (t, 4H, CH_2–O–CO), 6.21 (s, 4H, O–CH_2–N), 7.82 (s, 2H, CH = N).

^{13}C NMR ($CDCl_3$): $\delta = 26.3$ ppm (CH_3–N), 28.1–30.0 (CH_2–COO), 30.8 (CH_3–N), 64.0 (CH_2–OCO), 66.83 (O–CH_2–N), 69.0–70.6 (CH_2–O–CH_2), 106.3 (C-5), 143.2 (C-8), 148.8 (C-6), 151.6 (C-2), 154.9 (C-4), 171.7–171.9 (COO–CH_2).

The same procedure was used to prepare $mPEG_{1900}$–[7-(hydroxymethyl) theophylline] (II). In this case 10 g (0.005 mol) of $mPEG_{1900}$–COOH, 0.24 g (0.002 mol) of DMAP, 1.34 g (0.0065 mol) of DCC and 1.16 g (0.0055 mol) of 7-(hydroxymethyl)theophylline were employed. Yield 87.2%.

Anal. Calc. for $C_{98}H_{186}N_4$: C, 53.63; H, 8.48; N, 2.55, MW 2193. Found: C, 53.47; H, 8.46; N, 2.62%.

^{1}H NMR ($CDCl_3$): $\delta = 2.63$ ppm (br s, 4H, CH_2COO), 3.38 (s, 3H, N–CH_3), 3.40 (s, 3H, N–CH_3), 3.45–3.80 (br s, 84H, CH_2–O–CH_2, CH_3–O), 4.20 (t, 2H, CH_2–O–CO), 6.22 (s, 2H, O–CH_2–N), 7.82 (s, 2H, CH = N).

^{13}C NMR ($CDCl_3$): $\delta = 28.1$ ppm (CH_3–N), 28.7–28.9 (CH_2–COO), 30.0 (CH_3–N), 59.2 (CH_3–O), 64.1 (CH_2–OCO), 66.8 (O–CH_2–N), 69.0–72.0 (CH_2–O–CH_2), 106.2 (C-5), 143.2 (C-8), 148.8 (C-6), 152.0 (C-2), 155.0 (C-4), 171.8–172.0 (COO–CH_2).

2.7. In vivo studies

Three rabbit males were fasted for 12 h before the experiments. Aminophylline (19 mg/kg), PEG_{1500}–[7-

(hydroxymethyl) theophylline]$_2$ (92 mg/kg) and mPEG$_{1900}$–[7-(hydroxymethyl) theophylline] (193 mg/kg), equivalent to 15.8 mg/kg of theophylline, were solubilized in physiological solution and administered orally via a conventional gastric delivery tube. Each drug solution was prepared immediately prior to administration.

Blood (200 μl) was withdrawn immediately prior to drug administration. Then 200 μl blood samples were obtained at 15, 30 and 60 min, 2, 4, 6, 8, 10, 12, 15, and 24 h after drug administration. The plasma was separated conventionally [5] and stored in a freezer pending assay.

Theophylline concentrations in plasma were determined by HPLC method.

The sample was prepared following a method already known in literature. [6].

To 100 μl of plasma in a capped test tube were added 300 μl of chloroform/isopropanol (1:1, v/v%) containing 8-chloro theophylline as internal standard. After vortexing for 60 s, the samples were centrifuged for 5 min at 10 000 rpm. Into a glass tube, 200 μl of the organic layer were transferred and evaporated to dryness under a nitrogen stream. The residue was reconstituted with 50 μl of the mobile phase, and 20 μl of the solution were injected into the chromatograph.

2.8. HPLC analysis

Reverse phase HPLC C18 column is used. A mixture of CH_3COONa 10 mM, acetonitrile and tetrahydrofuran (94:5:1 v/v%) pH 5 was employed as mobile phase, and the detection wavelength was 270 nm. 8-Chloro theophylline was used as internal standard.

The analyses of theophylline in plasma were validated. The limit of quantification was approximately 1 μg/ml. The coefficient of variation calculated for the six samples analyzed did not exceed 5%. The precision of the assay method was calculated by determining the relative standard deviations of peak height ratios

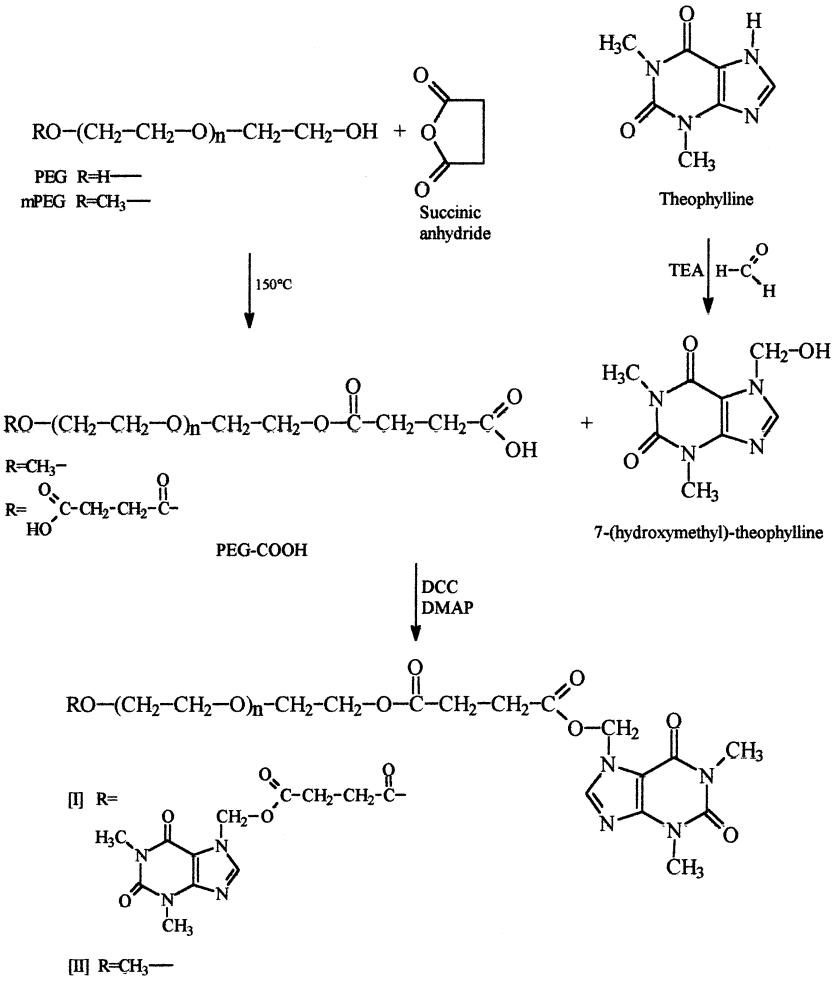

Fig. 1. Schematic synthetic procedure of PEG–[7-(hydroxymethyl)-theophylline] (I and II) conjugation.

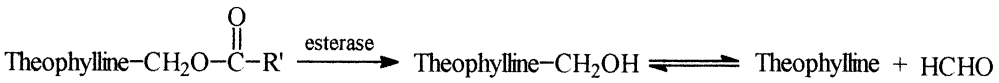

Fig. 2. Hydrolysis of α-(acyloxy)alkyl derivative of theophylline.

obtained from six replicate assays within a concentration interval of 1.5–50 μg/ml. The relative standard deviation ranged from 1 to 3.5% for intraday analysis and from 2 to 4% for interday analysis. The absolute recoveries of theophylline and the internal standard in plasma were determined by comparing the slopes of the standard curves of the processed buffer and plasma to those of the standard curves prepared in isopropanol. The recoveries of theophylline and the internal standard were 82 ± 4 and 84 ± 6%, respectively.

2.9. Hydrolysis studies

The hydrolysis of I and II were studied at pH 1.2, 0.2 M (HCl, NaCl, and glycine), and pH 7.4, 0.1 M phosphate buffer.

Equal known aliquots of polymeric conjugate, PEG_{1500}–[7-(hydroxymethyl) theophylline]$_2$: 10 mg/ml = 1.73 mg of theophylline and $mPEG_{1900}$–[7-(hydroxymethyl) theophylline]: 10 mg/ml = 0.83 mg of theophylline, each containing an appropriate quantity of internal standard, were dissolved in equal volumes of preheated buffer solutions at diverse pH values, maintained at 37 ± 0.1 °C and sampled at suitable intervals. Twenty microliter of each sample were directly injected into the chromatograph. The quantity of theophylline released by hydrolysis was quantified with the HPLC method mentioned before. Each experiment was repeated three times.

3. Results

3.1. PEG-adducts

In a preceding paper [7] a polymeric drug conjugate was prepared by linking covalently the acyclovir or valacyclovir, known antiviral drugs, to PEG, a macromolecular drug carrier. Now, the theophylline is linked to PEG. This coupling is carried out by three successive steps. First step: the 7-(hydroxymethyl) derivative of theophylline is prepared in excellent yield from drug alkylation with an excess of formaldehyde [4]. Second step: the reaction of PEG with succinic anhydride was used to introduce carboxylic end groups [3]. Third step: the attachment of the 7-(hydroxymethyl)-theophylline to the carboxylated polymer is performed by means of DCC and DMAP with a good yield Fig. 1.

All the structural assignments of the synthesized compounds were made on the basis of ^{1}H NMR and ^{13}C NMR analysis. The singlet at $\delta = 6.21$ ppm in the ^{1}H NMR spectrum and the signal at $\delta = 66.8$ ppm in the ^{13}C NMR spectrum, due to methylenic protons 2H-7, confirmed the complete ester bond formation between drug and activated polymer.

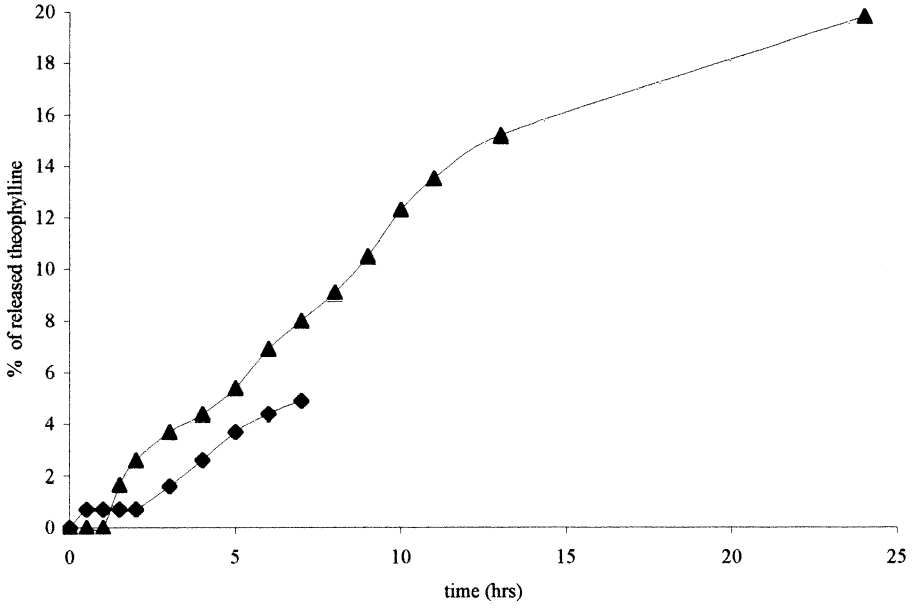

Fig. 3. Release of theophylline in buffer solutions, ◆ pH 1.2, 0.2 M (HCl, NaCl, and glycine) and ▲ pH 7.4, 0.1 M phosphate, at 37 °C from PEG_{1500}–[7-(hydroxymethyl)-theophylline]$_2$.

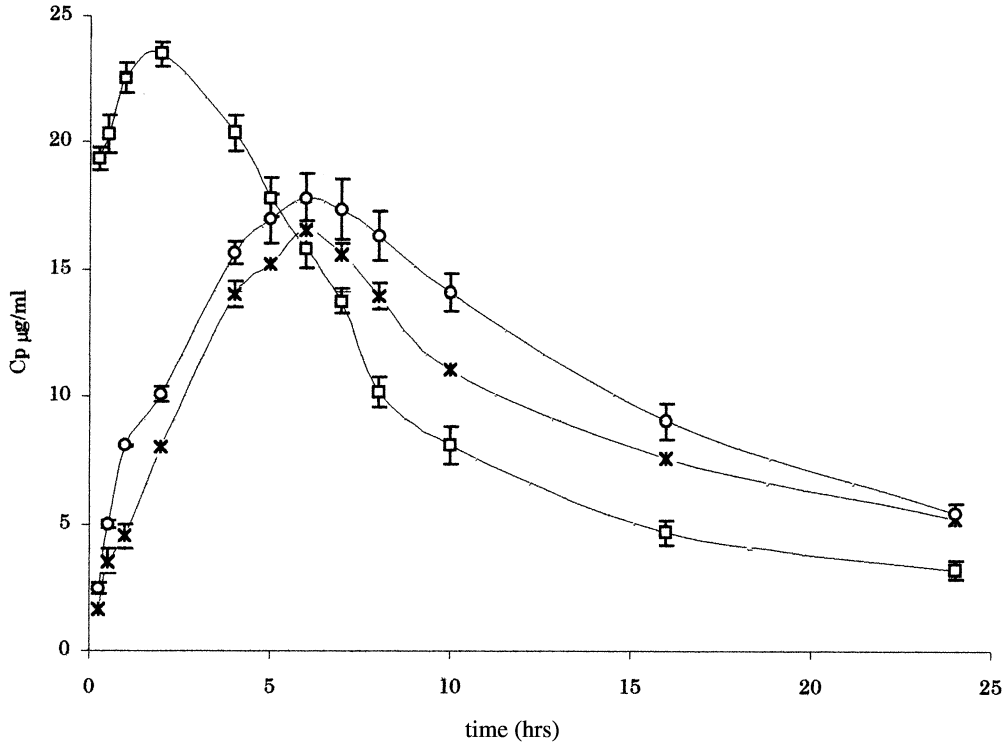

Fig. 4. Bioavailability of (□) aminophylline (19 mg/kg), (○) PEG_{1500}–[7-(hydroxymethyl) theophylline]$_2$ (92 mg/kg) and (*) $mPEG_{1900}$–[7-(hydroxymethyl) theophylline] (193 mg/kg). Each value is the mean ± SD for three animals.

The content of linked drug in the conjugates was measured by HPLC analysis, on the basis of the release of theophylline in alkaline media after 15 min at 80 °C. This was found to be 100% (w/w) in both the macromolecular prodrugs.

It is well known [8] that theophylline acyl derivative, in vivo, is able to release the parent drug by esterase action Fig. 2. First an intermediate is produced that immediately dissociates spontaneously and rapidly in theophylline and formaldehyde.

3.2. In vitro release studies

In order to gain some preliminary informations about the potential use of (I) and (II) as a drug delivery system we subject them to hydrolysis in buffer solutions at pH 1.2 (simulated gastric juice), and at pH 7.4 (extracellular fluids).

The hydrolysis rate of PEG_{1500}–[7-(hydroxymethyl)-theophylline]$_2$ in buffer solutions at three different pH Fig. 3 is the following: at pH 1.2, after 6 h, is hydrolyzed by 4.4%; at pH 7.4, after 24 h, is hydrolyzed by 19.8%.

The $mPEG_{1900}$–[7-(hydroxymethyl)-theophylline] monofunctional adduct release kinetics in buffer solutions at two different pH shows a superimposable tendency to the bifunctional product.

3.3. In vivo studies

The adducts I and II were administered orally, through a gastric tube, in three rabbit, using the aminophylline as reference drug, and taking blood samples from the right ear of the rabbit at regular time intervals.

The administrations were carried out in a week-distance in order to guarantee the complete elimination of theophylline from the body.

The theophylline present in the samples was quantified as described in the Section 2.

It is interesting to notice Fig. 4 that theophylline administration in the shape of PEG adducts allows to obtain therapeutic levels (10–20 µg/ml) that last for longer periods than in the case of aminophylline administrations. Moreover, no toxic concentrations occur.

4. Conclusion

In order to test the hypothesis that theophylline PEG-adducts should be suitable for oral admistration, aminophylline, I and II were administrated to three rabbits.

Fig. 4 shows the typical profile for the compounds in plasma. Compounds I and II show a controlled release

of free drug and theophylline pharmaceutical properties appear to be modified positively.

These adducts present an excellent solubility in water, so that they can be administered as a solution, that can be considered as an advantage over the classic formulation of theophylline.

It is therefore possible to suggest a posology regime which can contemplate the adduct administration every 12 h. Since a sustained release is, in this case, a molecular property, the some time dangerous 'dose dumping' observed in formulations based on physical sustained release would not occur. It is interesting to underline that, on the contrary to what happens with aminophylline, after the administration of these polymeric adducts no toxic plasmatic concentrations occur.

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