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Water Soluble Cyclosporine Monomethoxy Poly(ethyleneglycol) Conjugates as Potential Prodrugs

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The highly water-soluble monomethoxypoly(ethyleneglycol) (mPEG) prodrugs of cyclosporin A (CsA) were synthesized. These prodrugs were prepared by initially preparing intermediate in the form of carbonate at the 3'-positions of CsA with chloromethyl chloroformate, in the presence of a base to provide a 3'-carbonated CsA intermediate. Reaction of the CsA intermediate with mPEG derivative in the presence of a base provides the desired water-soluble prodrugs. As a model, we chose molecular weight 5 kDa mPEG in the reaction with CsA to give water soluble prodrugs. To prove that the prodrug is decomposed in the body to produce CsA, the enzymatic hydrolysis test was conducted using human liver homogenate at 37°C. The prodrug was decomposed in human liver homogenate to produce the active material, CsA, and the hydrolysis half-life ($t_{1/2}$) of the prodrug, KI-306 was 2.2 minutes at 37°C. However, a demonstration of non-enzymatic conversion in pH 7.4 phosphate buffer was provided by the fact that the half-life ($t_{1/2}$) is 21 hours at 37°C. The hydrolysis test in rat whole blood was also conducted. The hydrolysis was seen with half-life ($t_{1/2}$) of about 9.9, 65.0, 14.2, 3.4, 2.1 9.5, and 1.6 minutes for KI-306, 309, 312, 313, 315, 316, and 317, respectively. This is the ideal for CsA prodrug. The pharmacokinetic study of the prodrug, KI-306, in comparison to the commercial product (Sandimmune Neoral Solution) was also carried out after single oral dose. Each rat received 7 mg/kg of CsA equivalent dose. Especially, the prodrug KI-306 exhibits higher AUC and C_{max} than the conventional Neoral. The AUC and C_{max} were increased nearly 1.5 fold. The kinetic value was also seen with T_{max} of about 1.43 and 2.44 hours for KI-306 and Neoral, respectively.

Key words: Cyclosporine, PEG, Prodrug, Polymer conjugate

INTRODUCTION

Cyclosporine A (CsA) is a peptide compound having a unique structure consisting of 11 poly-*N*-methylated amino acids and has been known to have useful pharmacological activities. Especially, CsA is an important immunosuppressive agent used in organ transplants and immunoregulatory disorders. After introduction of CsA in the early 1980s, cadaveric kidney graft survival improved by 15% to 20% (Strom, 1993). A particular advantage of CsA therapy is that, unlike cytotoxic immunosuppressants (e.g., azathioprine), CsA lacks clinically important myelosuppressive activity (McEvoy, 1998; Diasio, *et al.*, 1996). The clinical advantages of CsA resulted in widespread use after kidney, pancreas, liver, lung, and heart transplantation (Noble, 1995; Faulds, *et al.*, 1993; Abella, 1996).

It is also applicable to the treatment of broad range of autoimmune diseases of inflammatory etiology and also to the antiparasitic treatment. CsA is used, for example, for the treatment of rheumatic diseases (rheumatoid polyarthritis), hematological disease (aplastic anemia, idiopathic thrombocytopenia), gastric disorders (ulcerating colitis, crons disease), dermatic disease (psoriasis, sclerodermia) and eye diseases (uveitis). Also topical applications have been tested, for example, in treatment of psoriasis, uveitis and alopecia.

CsA is highly lipophilic, poorly water-soluble and, therefore, typically supplied as an olive oil or peanut oil solution for clinical use. However, the bioavailability of CsA from such oily solution is very low and gives rise to great intersubject variation with systemic availability ranging from

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4 to 25% (Shibata, *et al.*, 1988). The bioavailability of CsA has been reported to depend on food, bile and other interacting factors (Fahr, 1993). In a recent study in which a microemulsion preparation of CsA was administered locally to different parts of the small and large intestine (duodenum, jejunum, ileum and colon descendents), CsA was found to be absorbed predominantly in the small intestine (Drewe, *et al.*, 1992).

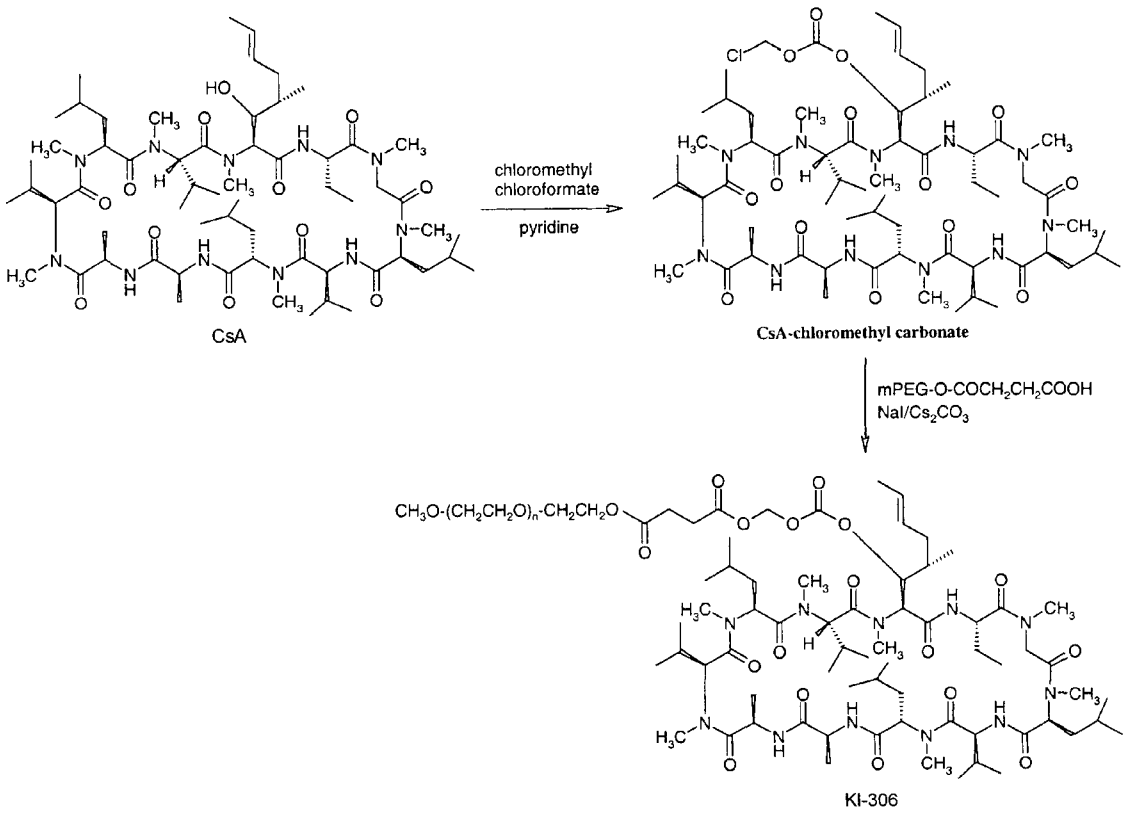
CsA has a low bioavailability and tissue-availability and thus, should be administered in an excessive amount. Therefore, the administeration of excess CsA may frequently has undesirable side effects such as nephrotoxicity, hypertension, hyperkalemia, hyperurikemia, hepatotoxicity, anemia, gastrointestinal intolerance, tremor and parestesia. The most frequent side effect is usually renal dysfunction. Acute CsA nephrotoxicity is dose-dependent. There is a correlation with the blood level and a decrease in the dose or discontinuation of CsA therapy leads to an improvement. However, progressive and irreversible damage of kidneys was reported in patients with transplants.

The pharmaceutical preparation of CsA (Sandimmune) which is used clinically is prepared in the form of solution, used for injection or oral administration, or a soft capsule filled with the solution. Formulations for oral and intravenous administrations of CsA are prepared in the form of microemulsion.

A liquid microemulsion formulation is prepared by combining CsA with a surfactant, an oil and a cosurfactant. The microemulsion of CsA is consisted of ethanol as a cosurfactant, a vegetable oil and a transesterified product of a natural vegetable oil triglyceride and a poly(alkylene glycol) as a surfactant to form the liquid formulation. Injection preparation containing a nonionic surfactant such as a cremophor EL can develop the analphylaxis reaction to a few cases. Also, the addition of nonaqueous solvents such as ethanol, propylene glycol or poly(ethylene glycol) 400 needs to be considered for parenteral administration. Using such organic solvent has a problem such as hemolysis and local irritation at injection site.

In preparing oral formulation, a soft capsule filled with the microemulsion solution as a main component has a few drawbacks during the absorption process. When the oily components are contacted with an aqueous solution in mouth or intestine, the drug component may be often separated as a solid, thereby reducing its bioavailability to a level of below 30%. Moreover, in case of a long period storage, CsA tends to be crystallized as the ethanol content decreases by evaporation of ethanol; and patients suffer from the unpleasant odor of the ethoxylated castor oil.

Even though the other processes may have achieved with some success in improving the stability of the formulation by minimizing the ethanol content therein, there still



Scheme 1. Synthesis of KI-306 from CsA

remain various deficiencies. For example, the use of surfactants having a complicated composition is not practically easy or suitable for the development of the formulation; in case of liposome the whole process is complicated and the reproducibility of particle size or inclusion rate is hard to control; and the use of polymeric polysaccharide has disadvantage that the total volume of the formulation may be too bulky for administration. Further, the prior art methods still fail to produce CsA containing composition which have a satisfactory dissolution rate in an aqueous solution.

In order to avoid the problems such as the toxicity of surfactant and solvent in preparing the formulation, if such insoluble drugs is attached to water-soluble macromolecules to act as carriers, it will greatly reduce these problems and will be suitable for parenteral and oral administrations. The delivery of CsA attached to a polymeric water-soluble carrier such as poly(ethylene glycol) (PEG) has never been considered up to now.

PEG is a linear or branched, neutral polymer having various molecular weight range and is soluble in water. One of the interests in the biomedical areas is the fact that PEG is nontoxic and was approved by Food and Drug Administration (FDA) for internal consumption. PEG is widely used for the synthesis of drug and for a wide variety of cosmetic and personal care products. One of the most extensively studied drug-delivery technologies involves the covalent linkage of the polymer mPEG to the surface of proteins. Therefore, we have developed a novel water-soluble polymer-CsA conjugated compound which is formed by chemically combining CsA to a water-soluble carrier. These compounds are water soluble prodrugs of CsA which are useful as immunosuppressant, antiinflammatory, antifungal and antiproliferative agents.

MATERIALS AND METHODS

Materials

Unless stated otherwise, all reagents and solvents were used without further purification. Analytical HPLCs were performed using Inertsil ODS-2 (4.6×150 mm) reverse-phase column under gradient conditions with a mixture of solution A (95% water-5% acetonitrile) and solution B (95% acetonitrile-5% water) as the mobile phase. Peak elutions were monitored at 214 nm using UV detector. NMR spectra were obtained using a 300 MHz spectrometer. CsA was purchased from Fluka (St. Louis, MO). Monomethoxypoly(ethylene glycol) (mPEG) was purchased from Aldrich Chemical Co. (Milwaukee, WI) and all other mPEG derivatives were synthesized from mPEG. All other chemicals not indicated were purchased from Sigma Chemical Co. (St. Louis, MO). All PEG compounds were dried under vacuum prior to use.

Synthesis of CsA-chloromethyl carbonate

Chloromethyl chloroformate (2.0 mL, 20.8 mmol) was added dropwise to a stirred mixture of CsA (1.0 g, 0.83 mmol), pyridine (2 mL) in dichloromethane or tetrahydrofuran (50 mL) and the reaction mixture was stirred for 20 h at room temperature. After stirring for 20 h, ether (50 mL) was added to the mixture. The resulting precipitate was filtered off and the filtrate was evaporated. The crude product was triturated from ether/petroleum ether (5 : 1) to yield (970 mg, Yield: 90%) of CsA-chloromethyl carbonate. IR (KBr, cm^{-1}) 1760 (ester); FAB-MS (m/z) 1294 (M^+).

Synthesis of CsA-mPEG Conjugate (KI-306)

Method 1

A mixture of CsA-chloromethyl carbonate (100 mg, 0.08 mmol), mPEG-succinate (385 mg, 0.08 mmol), and Cs_2CO_3 (50 mg, 0.15 mmol) in anhydrous acetonitrile (10 mL) was stirred at 85°C for 24 h. After diluting with acetonitrile (5 mL), the insoluble material was filtered off. The filtrate was evaporated. The crude product was triturated from CH_2Cl_2 /ether (1 : 5) to yield 350 mg of CsA-mPEG conjugate (purity = 30%, Prep. HPLC). The unreacted mPEG was removed by a preparative HPLC system. MS (MALDI/TOF) shows a mean molecular weight of 6377 for the product and 5117 for the starting mPEG-succinate. The difference in mass (1260) matched the CsA-chloromethyl carbonate. MS (MALDI/TOF) m/z 6377 (mean MW).

Method 2

A mixture of CsA-chloromethyl carbonate (143 mg, 0.11 mmol), mPEG-succinate (500 mg, 0.1 mmol), NaI (33 mg, 0.22 mmol), Cs_2CO_3 (50 mg, 0.15 mmol), and crown ether (26 mg, 0.1 mmol) in benzene (50 mL) was stirred at 80°C for 24 hours. After diluting with ethylacetate (20 mL), the insoluble material was filtered off. The filtrate was evaporated. The crude product was crystallized from CH_2Cl_2/ether (1 : 5) to yield 480 mg of CsA-mPEG conjugate (purity = 75%, Prep. HPLC)

Method 3

A mixture of CsA-chloromethyl carbonate (143 mg, 0.11 mmol), mPEG-succinate (500 mg, 0.1 mmol), NaI (33 mg, 0.22 mmol), K_2CO_3 (21 mg, 0.15 mmol), and crown ether (26 mg, 0.1 mmol) in benzene (50 mL) was stirred at 80°C for 24 h. After dilution with ethylacetate (20 mL), the insoluble material was filtered off. The filtrate was evaporated. The crude product was triturated from CH_2Cl_2/ether (1 : 5) to yield 465 mg of CsA-mPEG conjugate. (purity = 73%, Prep. HPLC).

Synthesis of CsA-mPEG conjugate (KI-309)

A mixture of CsA-chloromethyl carbonate (143 mg, 0.11 mmol), mPEG-propionic acid (500 mg, 0.1 mmol), NaI (33

mg, 0.22 mmol), Cs_2CO_3 (50 mg, 0.15 mmol), and crown ether (26 mg, 0.1 mmol) in benzene (50 mL) was stirred at 80°C for 24 h. After dilution with ethylacetate (20 mL), the insoluble material was filtered off. The filtrate was evaporated. The crude product was triturated from CH_2Cl_2 /ether (1 : 5) to yield 465 mg of CsA-mPEG conjugate (purity = 80%, Prep. HPLC). The unreacted mPEG was removed by a preparative HPLC system. MS (MALDI/TOF) shows a mean MW of 6610 for the product and 5350 for the starting mPEG-propionic acid. The difference in mass (1260) matched the CsA-chloromethyl carbonate. MS (MALDI/TOF) m/z 6610 (mean MW).

Synthesis of CsA-mPEG conjugate (KI-312)

A mixture of CsA-chloromethyl carbonate (143 mg, 0.11 mmol), mPEG-glutamate (500 mg, 0.1 mmol), NaI (33 mg, 0.22 mmol), Cs_2CO_3 (50 mg, 0.15 mmol), and crown ether (26 mg, 0.1 mmol) in benzene (50 mL) was stirred at 80°C for 24 h. After dilution with ethylacetate (20 mL), the insoluble material was filtered off. The filtrate was evaporated. The crude product was triturated from CH_2Cl_2 /ether (1 : 5) to yield 473 mg of CsA-mPEG conjugate (purity = 70%, Prep. HPLC). The unreacted mPEG was removed by a preparative HPLC system. MS (MALDI/TOF) shows a mean MW of 6388 for the product and 5128 for the starting mPEG-glutamate. The difference in mass (1260) matched the CsA-chloromethyl carbonate. MS (MALDI/TOF) m/z 6388 (mean MW).

Synthesis of CsA-mPEG conjugate (KI-313)

A mixture of CsA-chloromethyl carbonate (143 mg, 0.11 mmol), mPEG-malonate (500 mg, 0.1 mmol), NaI (33 mg, 0.22 mmol), Cs_2CO_3 (50 mg, 0.15 mmol), and crown ether (26 mg, 0.1 mmol) in benzene (50 mL) was stirred at 80°C for 24 h. After dilution with ethylacetate (20 mL), the insoluble material was filtered off. The filtrate was evaporated. The crude product was triturated from CH_2Cl_2/ether (1 : 5) to yield 478 mg of CsA-mPEG conjugate (purity = 80%, Prep. HPLC). The unreacted mPEG was removed by a preparative HPLC system. MS (MALDI/TOF) shows a mean MW of 6404 for the product and 5144 for the starting mPEG-malonate. The difference in mass (1260) matched the CsA-chloromethyl carbonate. MS (MALDI/TOF) m/z 6404 (mean MW).

Synthesis of CsA-mPEG conjugate (KI-315)

A mixture of CsA-chloromethyl carbonate (143 mg, 0.11 mmol), mPEG-diglycolic acid (500 mg, 0.1 mmol), NaI (33 mg, 0.22 mmol), Cs_2CO_3 (50 mg, 0.15 mmol), and crown ether (26 mg, 0.1 mmol) in benzene (50 mL) was stirred at 80°C for 24 h. After dilution with ethylacetate (20 mL), the insoluble material was filtered off. The filtrate was evaporated. The crude product was triturated from CH_2Cl_2 /ether (1 : 5) to yield 485 mg of CsA-mPEG conjugate (purity = 85%, Prep. HPLC). The unreacted mPEG was removed by a preparative HPLC system. MS (MALDI/TOF) shows a mean MW of 6303 for the product and 5043 for the starting mPEG-diglycolic acid. The difference in mass (1260) matched the CsA-chloromethyl carbonate. MS (MALDI/TOF) m/z 6303 (mean MW).

Synthesis of CsA-mPEG Conjugate (KI-316)

A mixture of CsA-chloromethyl carbonate (143 mg, 0.11 mmol), mPEG-methylsuccinate (500 mg, 0.1 mmol), NaI (33 mg, 0.22 mmol), Cs_2CO_3 (50 mg, 0.15 mmol), and crown ether (26 mg, 0.1 mmol) in benzene (50 mL) was stirred at 80°C for 24 h. After dilution with ethylacetate (20 mL), the insoluble material was filtered off. The filtrate was evaporated. The crude product was triturated from CH_2Cl_2 /ether (1 : 5) to yield 478 mg of CsA-mPEG conjugate (purity = 83%, Prep. HPLC). The unreacted mPEG was removed by a preparative HPLC system. MS (MALDI/TOF) m/z 6391 (mean MW).

Synthesis of CsA-mPEG conjugate (KI-317)

A mixture of CsA-chloromethyl carbonate (143 mg, 0.11 mmol), mPEG-thiodiglycolic acid (500 mg, 0.1 mmol), NaI (33 mg, 0.22 mmol), Cs_2CO_3 (50 mg, 0.15 mmol), and crown ether (26 mg, 0.1 mmol) in benzene (50 mL) was stirred at 80°C for 24 h. After dilution with ethylacetate (20 mL), the insoluble material was filtered off. The filtrate was evaporated. The crude product was triturated from CH_2Cl_2 /ether (1 : 5) to yield 475 mg of CsA-mPEG conjugate (purity = 86%, Prep. HPLC). The unreacted mPEG was removed by a preparative HPLC system. MS (MALDI/TOF) m/z 6319 (mean MW).

Hydrolysis of the CsA-prodrug (KI-306) in human liver homogenate

To prove that the conjugated compound as synthesized according to the above method is decomposed in the body to produce CsA, the enzymatic hydrolysis test was conducted using human liver homogenate at 37°C. Specifically, 3.0 g of human liver was introduced into 3.0 mL of 0.1 M phosphate buffer (pH 7.4), homogenized on ice and then centrifuged for 10 minutes. The supernatant was transferred to another tube. The test solution was prepared by dissolving 51.6 mg (10 mg CsA equivalent/mL) of the conjugated compound (KI-306) in 1.0 mL of 0.1 M phosphate buffer (pH 7.4).

A 90 μL of the supernatant was introduced into each Eppendorf tube and maintained at 37°C. Then, 10 μL of the test solution which was previously warmed to 30°C was added thereto. The reaction mixture in each tube was stirred for 5 seconds and 300 μL of acetonitrile was added at the given interval (0, 1, 3, 5, 7, 10, 15, 30, 45, 60, 90,

120 minutes) and then the mixture in the tube was stirred for one minute. The tube was centrifuged at 13,000 rpm for 10 minutes and then stored on ice. In the tube, the final theroetical concentration of the conjugate was 129 μg/mL (250 μg/mL CsA equivalent).

Hydrolysis of the CsA-prodrugs in rat whole blood

Conjugated compounds were hydrolyzed with incubation time at 37°C in rat whole blood. Each incubation tubes containing whole blood was pre-equilibrated at 37°C in water bath before test. Incubation was started by addition of compounds and then stopped by addition of 3X volume acetonitrile solution. After vortex for 1 minute and centrifugation, acetonitrile layer was applied for HPLC analysis.

Hydrolysis of the CsA-prodrugs in buffer system

In 0.1 M HCl solution, pH 1.0 and PBS buffer pH 7.4 at 37°C carried out stability test of each compounds. Buffer solutions were pre-equilibrated as a above test at 37°C in water bath. After with set point time, pippeted 150 μL from incubating tube and mixed with 150 μL acetonitrile solution. The diluted solution was used for HPLC analysis.

Each 20 μL of the sample solution was analyzed by means of HPLC. For HPLC analysis, a reverse-phase column shiseido CN, 5 μ (4.6×250 mm), was used. The mixture of 65% water-35% acetonitrile solution was used for from 0-5 minutes and 20% water-80% acetonitrile to 30 minutes as the gradient mobile phase. Contaminants were washed out using 90% acetonitrile in water for 10 minutes. The flow rate was 1.0 mL/min and the effluent was monitored at 214 nm and at 65°C.

Pharmacokinetic study

The pharmacokinetic study of KI-306 in comparison to the commercial product (Sandimmune Neoral Solution) was carried out after single oral dose. Sprague-Dawley rats weighing 220±30 g were used in this study. The rats were fasted overnight but were allowed free access to water. Each rat received 7 mg/kg of CsA equivalent dose in one of the following dosage forms:

(1) CsA commercial drug (Sandimmune Neoral, Novatis Pharm. Ag, Basel, Switzerland),
(2) The prodrug (KI-306) dissolved in saline solution immediately prior to dosing.

The oral solutions were administered using oral zonde while the marginal tail vein was used for the i.v. dosing with the aid of implanted cannula for collecting blood samples. The blood sample (200~250 μL) were collected in Eppendrof tube treated with heparine and taken at designed time intervals. The blood sample was pretreated with acetonitrile and the supernatant organic layer was subjected to HPLC analysis. It was noted in case of orally administered KI-306 that only CsA was detected, not for KI-306. The disappearance of KI-306 in rat blood by i.v. injection was found to be a half-life of 2.5 minutes. This data is good agreement with that in human liver homogenate with 2.2 minutes.

RESULTS AND DISCUSSION

Water-soluble polymers such as PEG, and mPEG are utilized to bind to poorly aqueous-soluble drugs and increase a water solubility of the drugs. PEG is a linear or branched, neutral polymer having various molecular weight range and is soluble in water and methylene chloride. PEG having a molecular weight of less than 1000 is viscous and colorless liquid. Higher molecular weight of PEG is a waxy and white solid. The melting point of the solid is proportional to the molecular weight, approaching a plateau at 67°C. The molecular weight from a few hundred to about 20,000 are commonly used in biological and biotechnological applications.

PEG as the carrier for polymer therapeutics has many good points. PEG is essentially non-toxic; PEG can be obtained with low polydispersity, is easily activated for conjugation, and PEG is relatively inexpensive for large scale processes; The FDA has approved PEG for use as a vehicle or base in foods, cosmetics and pharmaceuticals, including injectable, topical, rectal and nasal formulations. PEG is eliminated from the body intact by either the kidneys (for PEGs < 30 kDa) or in the faeces (for PEGs > 20 kDa) (Ymaoka, *et al.*, 1994).

Prodrug design comprises an area of drug research that is concerned with the optimization of drug delivery. A prodrug is a biologically inactive derivative of a parent drug molecule that usually requires an enzymatic transformation within the body in order to release the active drug, and has improved delivery properties over the parent molecule (Matthew, *et al.*, 1992; Maeda, 1991; Maeda, *et al.*, 1992; Noguchi, *et al.*, 1998). PEG prodrugs of highly insoluble agents should be especially advantageous since the solubility of the prodrug will exceed that of the original drug, increasing the possibility of more effective drug delivery. Accordingly, we prepared prodrugs based on ester formation. Esters with PEG as an electron-withdrawing substituent (alkoxy) in the α-position proved to be especially effective linking groups in the design of prodrugs since they aid in the rapid hydrolysis of the ester carbonyl bond, and are thus able to release alcohols (CsA, 2° alcohol required for activity) in a continuous and effective manner.

The basic chemical structure of PEG is $HO\text{-}(CH_2CH_2O)_n\text{-}CH_2CH_2OH$. For drug modification, one -OH group of the PEG polymer is used to covalently attach the PEG moiety to a chemical linker. The composition of the chemical linker is highly variable and can be designed to target

specific substrates. Among the most commonly used chemical linkers are cyanuric chloride, succinimidyl propionate, benzotriazolylcarbonate and tresylchloride. In this study, we used the linkers such as succinate, propionic acid, glutamate, malonate, diglycolic acid, methylsuccinate, and thiodiglycolic acid. Because a terminal hydroxyl group can confer some residual chemical reactivity to the PEG polymer, the second terminal -OH residue is typically replaced by a $-CH_3$ moiety, to produce mPEG. Because of its lower potential reactivity, mPEG is most commonly used for drug modification. Therefore, CsA, sparingly soluble drug, is utilized for covalent linking with the mPEG to be dissolved in water as potential prodrug.

The prodrugs used in this study (KI306, KI309, KI312, KI313, KI315, KI316, and KI317) are in the form of a carbonate wherein the 3'-position of CsA is conjugated in the form of carbonate (Fig. 1). These prodrugs were prepared by initially preparing intermediate in the form of carbonate at the 3'-positions of CsA with chloromethyl chloroformate, in the presence of a base, such as pyridine to provide a 3'-carbonated CsA. Reaction of the carbonated CsA with mPEG derivative in the presence of a base such as Cs_2CO_3 provides the desired CsA-mPEG conjugates as potential prodrugs. As a model, we chose molecular weight 5 kDa mPEG in the reaction with CsA to give water soluble prodrugs.

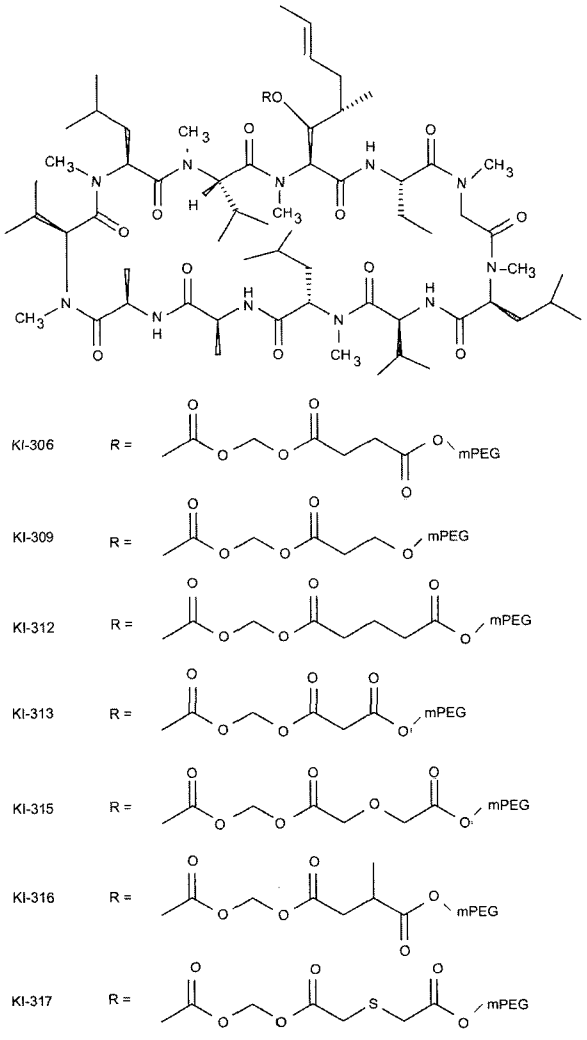


Fig. 1. Structures of CsA and prodrugs

The predicted molecular weights of prodrugs were observed in the MS (MALDI/TOF). MS (MALDI/TOF) showed a mean molecular weights of 6377 (KI-306), 6610 (KI-309), 6388 (KI-312), 6404 (KI-313), 6303 (KI-315), 6610 (KI-316), and 6319 (KI-317), respectively. The molecular weights of 5117, 5350, 5128, 5144, 5043, 5131, and 5059 for polymer derivative mPEG-succinate, mPEG-propionic acid, mPEG-glutamate, mPEG-malonate, mPEG-diglycolic acid, mPEG-methylsuccinate, and mPEG-thiodiglycolic acd were also observed. The difference in mass of 1260 between the prodrugs and the polymer derivatives matched the starting material CsA-intermediates. In addition to all the predicted peaks could clearly be observed in the ^{1}H-NMR displays resonances at 5.77 (2H, q, J = 5.67 Hz, $OCO_2\underline{CH_2}OCO$) for prodrug KI-306, 5.69 (2H, q, J = 5.65 Hz, $OCO_2\underline{CH_2}OCO$) for prodrug KI-309, 5.75 (2H, q, J = 5.67 Hz, $OCO_2\underline{CH_2}OCO$) for prodrug KI-312, 5.73 (2H, q, J = 5.61 Hz, $OCO_2\underline{CH_2}OCO$) for prodrug KI-313, 5.74 (2H, q, J = 5.69 Hz, $OCO_2\underline{CH_2}OCO$) for prodrug KI-315, 5.84 (2H, q, J = 5.58 Hz, $OCO_2\underline{CH_2}OCO$) for prodrug KI-316, and 5.70 (2H, q, J = 5.77 Hz, $OCO_2\underline{CH_2}OCO$) for prodrug KI-317 but not as major peaks.

The prodrugs were shown to function as prodrugs, i.e., breakdown occurred in a predictable fashion *in vitro*: the half-life ($t_{1/2}$) of KI-306 in pH 7.4 PBS buffer was 26.4 h

Table I. Rates of hydrolysis[a] of prodrugs in rat whole blood at 37°C

Compound	$t_{1/2}$ (min)
KI-306	9.9
KI-309	65.0
KI-312	14.2
KI-313	3.4
KI-315	2.1
KI-316	9.5
KI-317	1.6

[a] All runs done in duplicate.

Table II. Stability of prodrugs in pH 1.0 and pH 7.4 media at 37°C

Compound	$t_{1/2}$ (h)	
	PH 1.0	PH 7.4
KI-306	18.2	26.4
KI-309	32.0	16.6
KI-312	10.0	28.0
KI-313	20.0	5.5
KI-315	5.5	2.8
KI-316	43.4	-
KI-317	10.4	4.6

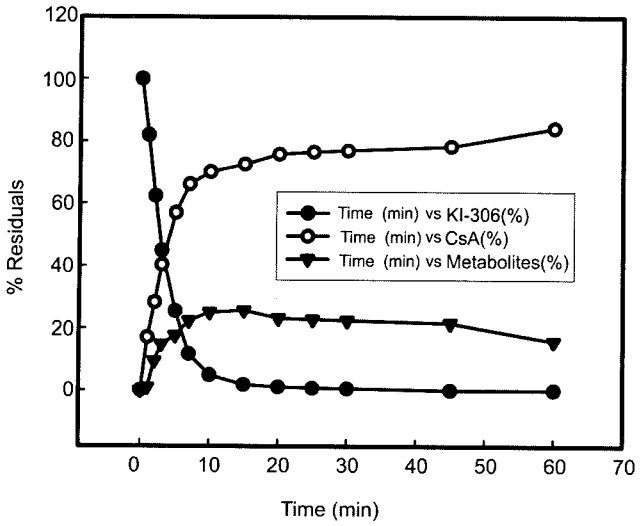


Fig. 2. Concentration-time profile of the hydrolysis of prodrug [KI-306, CsA 3'-methoxypoly(ethylene glycol) succinyloxymethyloxy carbonate ester] to CsA, as the parent drug, in human liver at 37°C. ●, prodrug [KI-306, CsA 3'-methoxypoly(ethylene glycol) succinyloxymethyloxy carbonate ester]; ○, CsA; ▼, metabolites.

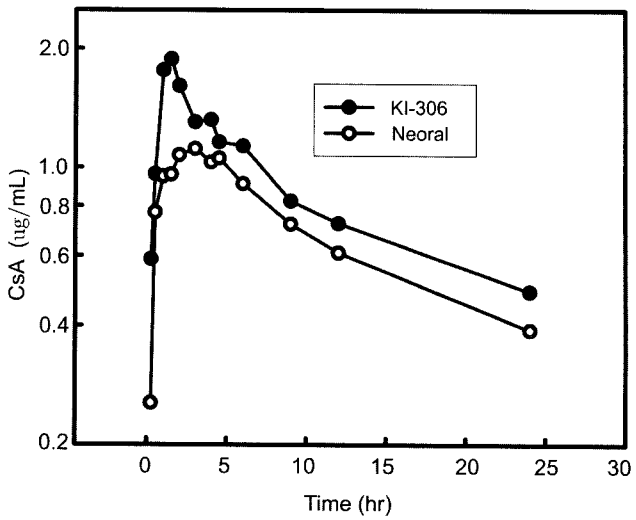


Fig. 3. Plasma concentration-time profile in rat whole blood concentration over time after CsA prodrug (KI-306) in saline solution and Sandimmune Neoral Oral Solution by oral administration.

(Table II), while in rat whole blood and human liver homogenate a more rapid breakdown was observed, with a $t_{1/2}$ of about 9.9 minutes (Table I) and 2.2 minutes (Fig. 2), respectively. Descriptive pharmacokinetic parameters of two compartment models with lag time were obtained by using WinNonlin Program. The results as shown in Table III and Fig. 3 demonstrated that greater bioavailability of KI-306 is achieved as compared with Sandimmune Neoral Solution, as indicated by the higher AUC and C_{max} than the conventional Neoral. The AUC and C_{max} were increased nearly 1.5 fold. The kinetic value was also seen with T_{max} of about 1.43 and 2.55 hours for KI-306 and Neoral, respectively (Fig. 3).

Therefore, this type of the prodrug may, even if administered in the lesser amount, achieve the equivalent or superior to the conventional drugs and may greatly reduce side effects such as nephrotoxicity, hypertension, hyperkalemia and the like. In view of the results of enzyme kinetic study, prodrugs, cyclosporine conjugates, has the same pharmacological usage as cyclosporine itself.

Table III. Pharmacokinetic parameters of KI-306 and Neoral upon oral administration to rats at 7 mg/kg of CsA equivalent

	KI-306		NEORAL	
	Mean (μg/mL h)	CV (%)	Mean (μg/mL h)	CV (%)
AUC	32.79	19.85	21.40	10.03
C_{max}	1.77	4.40	1.08	3.26
T_{max}	1.43	11.35	2.55	16.08

AUC, area under the plasma concentration-time curve; C_{max}, maximum plasma concentration; T_{max}, time to reach; CV, coefficient of variation.

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