Finally, a report of a spirodiketopiperazine-based CCR5 antagonist, E913, has appeared [7]. Like the previous compounds, E913 **(vi)** is a potent inhibitor of MIP-1α binding to CCR5 expressing cells (IC_{50} = 0.002 μM). It is capable of inhibiting laboratory, clinical and drug-resistant strains with an IC_{50} value of 0.03–0.06 μM. As expected, the anti-HIV activity of the compound was specific for M-tropic, but not T-cell tropic, virus.

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Drug Delivery

A once-a-day dosing formulation of Cyclosporin A

A common problem in organ transplantation is subsequent organ rejection. This problem is alleviated by the administration of immunosuppressive agents. One of the most important immunosuppressive agents in use is Cyclosporin A (CsA), a highly lipophilic cyclic peptide. CsA is an immunosuppressant of choice because of its lack of myelotoxicity when compared with other immunosuppressive agents [1,2]. A drawback of CsA is the need for careful blood-level monitoring; levels above the therapeutic window cause serious adverse effects, such as nephrotoxicity and neurotoxicity, whereas levels below the therapeutic window cause episodes of organ rejection.

Disadvantages of current formulation

CsA is currently marketed as a pre-microemulsion concentrate (NEORAL®; Novartis AG, Basel, Switzerland), which is administered every 12 h. Within the dosing interval, CsA blood levels drop to a point where high episodes of organ rejection occur. It is crucial, therefore, that patients comply with this twice-a-day dosing schedule to achieve success. A once-a-day dosing regimen that maintains sufficient blood levels throughout the 24 h dosing interval would potentially increase patient compliance and, at the same time, decrease episodes of organ rejection. The premicroemulsion concentrate formulation alone cannot be used for a once-a-day formulation because CsA would be released at too high a level during part of the dosing interval, resulting in serious toxicity. To achieve a once-a-day CsA formulation, it will probably be necessary to develop a controlled release formulation that will maintain blood levels of CsA within the therapeutic window for the entire 24 h period.

Kim and coworkers have recently reported on the design of a once-a-day dosing regimen of CsA [1]. Their strategy was to combine a new formulation of CsA premicroemulsion concentrates (preME) and a novel technology for enteric-coated solid-state premicroemulsion concentrates of CsA (sME). The co-administration of preME and sME, containing a total of 200 mg of CsA, provided blood levels of CsA above the minimum therapeutic level for ~24 h with a peak level comparable to that of preME.

New formulations of CsA

Enteric-coated sME were prepared by coating preME with enteric carrier-polymers, such as sodium alginate (AL), EUDRAGIT® L100 (EuD; Rohm GmbH, Darmstadt, Germany), and cellulose acetate phthalate (CAP). PreME consisted of CsA, medium-chain triglyceride, and a mixture of surfactants and cosurfactants. Medium-chain triglycerides were selected as oil because they have been reported to improve the intestinal absorption of various active compounds. Propylene carbonate (ProC) was used as a cosurfactant to solubilize CsA. The surfactants consisted of a mixture of Polyoxol 40 [hydrogenated castor oil 40, CREMOPHOR 40® (Cre); BASF, Ludwigschafen, Germany], mono- and diglyceride (Gly), and poloxamer 124 (Pol) in a 5:1:1 weight ratio. Cre was chosen as the major surfactant because it has been reported to enhance the intestinal permeability of drugs. Pol and Gly were added in equal amounts to improve the stability of the microemulsions. The composite mixture of all these surfactants and cosurfactants (Smix) was considered in ternary phase diagrams with water and oil.

A ternary phase diagram of Smix, water and oil was constructed to help develop an optimal formulation of preME. Various proportions of oil, Smix and water were tested and the phase of each mixture was determined visually. The composition of CsA:oil:Smix (10:18.5:71.5%) formed microemulsions regardless of the amount of water, according to the constructed phase

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diagram. *In vivo*, preME could diffuse into variable volumes of gastrointestinal fluids. It is desirable that preME form microemulsions regardless of the volume it diffuses into, so the composition of preME stated earlier was chosen for further experiments. This composition proved to form microemulsions spontaneously in various aqueous media regardless of pH or ionic strength.

Various enteric-coated preMEs were formulated and tested *in vitro* for their release rates. The results of these studies will not be discussed in detail here, except to state that considerable control over release rate can be achieved by the choice of enteric coating. The enteric coatings tested included EuD, CAP and AL.

New formulations prove advantageous

PreME and sME formulations were tested *in vivo* using beagle dogs. CsA given in preME and various sME formulations showed significantly different pharmacokinetic profiles. Oral absorption profiles were similar between preME and NEORAL. The area under the curve (AUC) values were 3274.7 ± 221.1 ng·h ml^{-1} for preME and 3238.3 ± 466.9 ng·h ml^{-1} for NEORAL. PreME produced blood levels of CsA lower than 100 ng ml^{-1}, the minimum therapeutic level, within 8 h following oral administration. This is also a problem with the current NEORAL regimen; in human studies, blood CsA levels dropped to below 100 ng ml^{-1} within 8 h. It is believed that this contributes to the 45% organ rejection rate among transplant patients. This high incidence of organ rejection indicates the need for dosage forms and dosing regimens that will provide a minimum effective blood level of CsA throughout the day.

Enteric-coated sME produced delayed oral absorption rates compared with preME. The oral absorption of CsA depended on the nature of the enteric-coated polymers. Of the enteric coatings, EuD possessed some of the most interesting properties from a formulation perspective. EuD-coated sME showed T_{max} at 4 h whereas preME showed T_{max} at 2 h following administration. Among the polymers, EuD showed the highest AUC (2506.4 ± 670.9 ng·h ml^{-1}) and C_{max} (475.88 ± 130.39 ng ml^{-1}).

An initial once-a-day dosing regimen

As a once-a-day dosing regimen, preME (100 mg CsA) was orally co-administered with sME (100 mg CsA). Double peaks were observed at 2.5 h (1262.46 ± 314.27 ng ml^{-1}) and 6 h (1044.25 ± 23.79 ng ml^{-1}), which were attributed to preME and sME, respectively. It is important to note that the peak levels after the administration of a total of 200 mg CsA did not significantly differ from the peak levels after the administration of only 100 mg CsA in a preME formulation. The combined dosing regimen of preME and EuD-coated sME provided blood levels over the minimum therapeutic level for a prolonged period. After 24 h, the blood level concentration of CsA was 82.51 ± 28.28 ng ml^{-1}, which is close to the minimum therapeutic level.

A once-a-day dosing regimen, with a combined total dose of 200 mg CsA, administered as a combination of preME and sME, provided blood levels of CsA in the therapeutic range for ~24 h, and retained a C_{max} level comparable with that observed when preME is administered alone. As highlighted by the authors, these preclinical results need to be investigated further in a clinical setting. If proven, this once-a-day dosing regimen holds promise for reducing episodes of organ rejection and, at the same time, increasing the compliance of outpatients treated with cyclosporin A.

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