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Short communication

Nanospheres of cyclosporin A: poor oral absorption in dogs

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Abstract

The cyclic undecapeptide cyclosporin A (CYA) used as first-line therapy in the prevention of xenograft rejection following organ transplantation, is extremely hydrophobic. Marketed formulations employ solubilising agents to facilitate absorption in the gastrointestinal tract. In this study, cyclosporin A nanospheres were prepared by precipitation in an aqueous surfactant solution. The particle matrix consists of the drug itself. Drug was dissolved in acetone and mixed rapidly with an aqueous solution of polysorbate 80 and sodium dodecyl sulphate (SDS). The acetone was evaporated to give a colloidal precipitate of spherical particles. Particle size could be controlled by varying the quantity of starting materials to give nanospheres of Z-average diameters in the range 250–900 nm with low polydispersity. The oral absorption of CYA from these nanospheres was compared to absorption from a microemulsion formulation in the dog. The relative bioavailability of cyclosporin A from nanospheres was only 3%, based on comparison of the area under the blood concentration–time curve (AUC) values for the two formulations.

Keywords: Cyclosporin A; Nanospheres; Oral absorption; Dogs

The formulation of the immunosuppressant drug cyclosporin A (CYA) for oral use presents a considerable challenge as it is practically insoluble in water (Ismailos et al., 1991). It is generally accepted that it cannot be absorbed following oral administration unless it is first solubilised in gastro-intestinal (GI) fluids by appropriate excipients. In this study the absorption of CYA from nanospheres comprising only the drug itself was investigated. Such particles might be absorbed intact across the gut wall and avoid pre-systemic dissolution limitations.

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Extremely hydrophobic drugs such as CYA can be made to form such particles using a precipitation technique as reported by Gaßmann et al. (1994). Drug is dissolved in an organic solvent and mixed rapidly with an aqueous solution of surfactant stabilisers to give a colloidal precipitate of spherical particles. CYA nanospheres were produced using a similar technique. The absorption characteristics of CYA from the colloidal precipitate were assessed in dogs and compared to those of the Neoral® microemulsion formulation.

Particle size has been shown to be an important factor in the extent of absorption of submicron polymeric spheres across the gut wall (Florence and Jani, 1993). Attempts were therefore made to control the diameter of the CYA nanospheres by varying production conditions.

CYA (purity 99.6%) was obtained from Galena (Czech Republic). All organic solvents used were high performance liquid chromatography (HPLC) grade and purchased from Fisher (UK). Polysorbate 80 was obtained from Fluka (UK) and sodium dodecyl sulphate (SDS) from BDH (UK). Double de-ionised water was used throughout. Neoral® was obtained from Sandoz (UK).

CYA nanospheres were prepared by quickly adding a solution of CYA in acetone through 4 ml of an aqueous surfactant solution in a glass vial via a micropipette. A non-ionic (polysorbate 80) and an ionic surfactant (SDS) were used to stabilise nanospheres by steric and electrostatic mechanisms, respectively. The surfactant solution was stirred rapidly using a magnetic bar during addition of the CYA solution. Acetone was evaporated under vacuum at 60°C in a rotary evaporator at constant speed for 5 min then under a steady stream of nitrogen for 2 min. Vacuum pressure, rotary speed, nitrogen flow-rate and SDS concentration (0.01% w/v) were kept constant for each batch of nanospheres produced.

The quantities of either CYA, polysorbate 80 or acetone were varied in turn to investigate the influence of these components upon particle diameter (formulations A–G; Table 1). Where the quantity of acetone was increased to 1 ml, the time of evaporation was increased to 8 min to ensure complete removal of the solvent.

The morphology of CYA nanospheres were assessed by scanning electron microscopy (SEM). A small quantity of the nanosphere suspension was dried and put on to an SEM stub using carbon adhesive discs and transferred to an Emitech K550 sputter-coater. The sample was gold-coated for 3 min at 30 mA and then viewed and photographed under a Philips XL20 scanning electron microscope.

The Z-average diameter and polydispersity of each batch of CYA nanospheres was determined by photon correlation spectroscopy (PCS) using a Zetasizer 3000 (Malvern Instruments, Malvern, UK) equipped with a fixed angle 10 mW helium–neon laser operated at 633 nm. Particle size was measured immediately after production and also after storage at room temperature for 24 h.

The oral absorption of CYA from nanospheres produced as described above was compared to that of Neoral® in two groups of five to ten male beagle dogs (11–16 kg). Each formulation was diluted to a constant volume of 5 ml with water immediately before administration and given at a dosage of 100 mg CYA/dog as a single oral dose. The nanospheres were made using 50 mg polysorbate 80 and 200 μg SDS/dose and had a Z-average diameter of 394 ± 35 nm. Serial blood samples (2 ml) were then taken from the antecubital vein up to 24 h post-dose and mixed with ethylene-diamine tetra-acetic acid as anticoagulant. A polyclonal antibody radioimmunoassay kit was used to determine CYA concentrations in

Table 1
Quantities of starting materials used in nanosphere formulations[a]

Formulation	CYA (mg)	Acetone (ml)	Polysorbate 80 (% w/v)
A	15	0.5	1.5
B	25	0.5	1.5
C	75	0.5	1.5
D	25	0.25	1.5
E	25	1	1.5
F	25	0.5	0.5
G	25	0.5	2.5

[a] Sodium dodecyl sulphate (SDS) was included in all formulations at 0.01% w/v. The volume of aqueous phase was also kept constant at 4 ml.

whole blood (Immunotech, Czech Republic). The bioavailability of each formulation was compared by calculating the area under the blood concentration–time curve (AUC) using the log-trapezoidal method. The maximum blood concentration (C_{max}) reached and the time take to achieve C_{max} (T_{max}) were also determined for CYA nanospheres and the Neoral® microemulsion.

SEM revealed that CYA precipitated as submicron spherical particles using the above method. When the quantities of starting were varied in turn independently, PCS indicated that the quantity of CYA and the volume ratio of the organic phase to the aqueous phase were the chief determinants of particle size (Fig. 1). Fig. 2 shows SEM micrographs of CYA nanospheres produced using different component proportions to give nanospheres of diameter 352 and 824 nm as determined by PCS. The latter were produced by simultaneously increasing the volume of acetone and quantity of CYA used. Within the range of quantities of each component used it was found that the polysorbate 80 concentration could be varied between 0.5 and 2.5% w/v with little effect upon size. Polydispersity ranged from 0.1 to 0.5 and tended to increase slightly upon storage. However, changing the component quantities did not result in any trend in this parameter (Fig. 1). In all batches of nanospheres size increased rapidly in the 24 h period following production (11–32% increase).

Oral absorption studies in dogs showed that CYA could be absorbed from nanospheres but the extent of absorption was markedly less than Neoral®. CYA bioavailability from the nanosphere formulation was only 3%, based on comparison of AUC values for nanospheres and Neoral®. The maximum concentrations achieved also showed considerable variability compared to Neoral® (Table 2). CYA blood concentrations from the nanosphere suspension declined slowly until approximately 11 h post-dose, when a secondary rise in blood concentration was seen. This may have been due to delayed translocation of CYA into the bloodstream from nanosphere 'depots' in the intestinal mucosa or the mesenteric lymphatic system. The low bioavailability seen might also be due to the anionic charge conferred

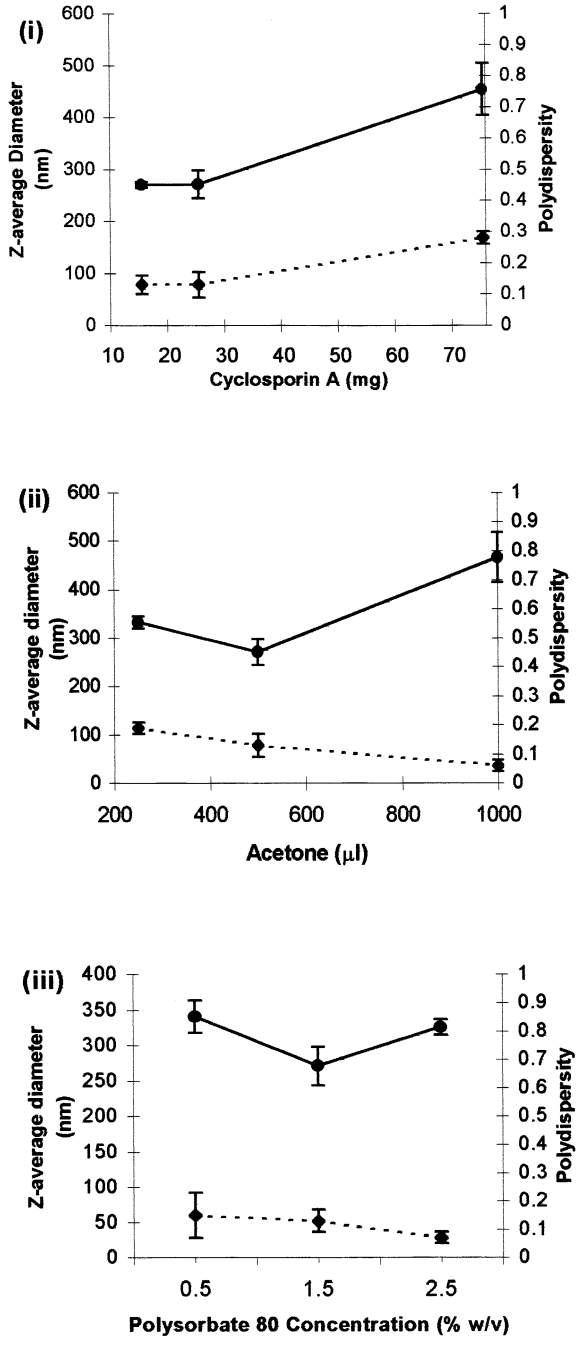

Fig. 1. Effect of varying (i) cyclosporin A (CYA) quantity, (ii) acetone volume, and (iii) polysorbate 80 concentration upon particle size (●) and polydispersity (◆) of CYA nanospheres. Each data point represents the mean of three batches with standard deviations shown.

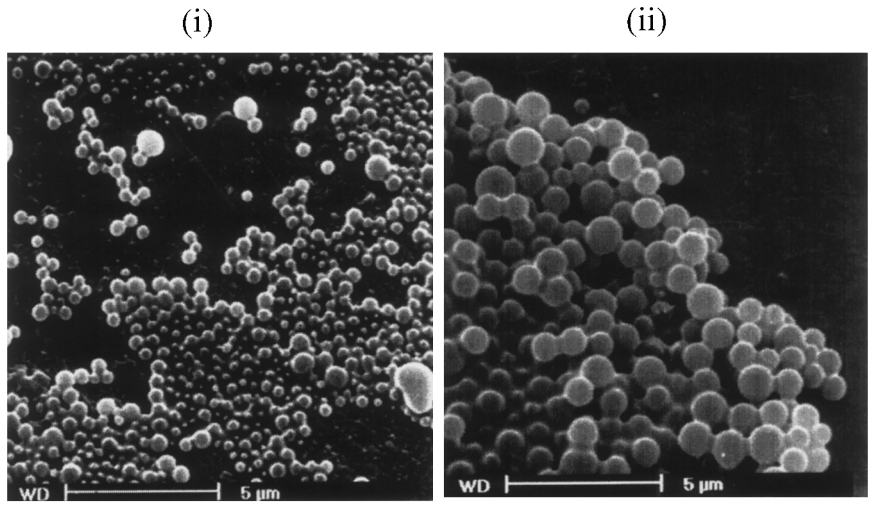

Fig. 2. Scanning electron micrographs of cyclosporin A (CYA) nanospheres with mean Z-average diameters of (i) 352 nm and (ii) 824 nm.

Table 2
Pharmacokinetic parameters for cyclosporin A (CYA) nanospheres and Neoral® given to dogs as a single oral dose of 100 mg CYA[a]

Formulation	AUC (ng h/ml)	Initial C_{max} (ng/ml)	T_{max} (h)
CYA nanospheres	351 (20%)[b]	25 (58%)[b]	2.2 (36%)[b]
Neoral® microemulsion	12 790 (16%)[c]	1689 (26%)[c]	1.9 (37%)[c]

[a] Relative standard deviations are shown in brackets.
[b] $n = 5$.
[c] $n = 10$.

to the particles by the SDS used in the formulation, since association of the sphere to the negatively-charged surface of the intestinal mucosa might be hindered.

In conclusion, therefore, CYA can easily be formulated as nanospheres even in the absence of particle-forming polymers. Particle size can be controlled simply by changing the quantity of the drug itself and other components used in the production of such nanospheres. However, the ability of this system to facilitate oral absorption of CYA in dogs was poor compared to an efficiently-absorbed microemulsion formulation.

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