P-078 **Surface modified pseudo-nanocapsules for improved absorption of cyclosporine A**

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INTRODUCTION AND OBJECTIVES

A novel formulation based on LBL technology has been developed in our laboratory to avoid the intrinsic drawback of early developed formulations for oral delivery of peptides (CsA) with improved therapeutic efficacy, specificity, tolerability and therapeutic index. In order to enhance bioavailability and to reduce inter-subject variability of CsA by improving solubility, permeability and reducing the efflux transportation of CsA from intestinal membrane, an attempt has been made to improved bioavailability of CsA by surface modification. The preparations of CsA available in the market contain cremophor and other solvents which are reported to be potentially toxic and therefore it has been envisaged that having a formulation of CsA devoid of cremophor and solvents would be more desirable.

MATERIALS AND METHODS

Sodium alginate (SA), Glycol chitosan (GC) and Pluronic F-68 (PF-68) were purchased from Sigma. Lipid (PE-PEG 2000) was purchased from Lipoid AG Germany. $CaCl_2.2H_2O$ and Na_2CO_3 (Hi media, Mumbai) were used without further purification.

Fabrication of CsA loaded capsules by alternate assembling Uniform, porous and spherical $CaCO_3$ CP with a narrow polydispersity index (PDI) was synthesized and CsA loading ($CaCO_3$ CsA) was done as reported by Gupta et al. 2008. CsA loading was determined by the RP-HPLC method. Sequential deposition of PE's over the preformed $CaCO_3$-CsA (Size: 4.5 micron, Zeta potential: 3.2mV) was carried out using previously described method (Wang et al., 2006; Gupta et al.,2008) with slight modification followed by surface modification with 0.2%w/v of PEPEG2000 (Lipoid AG, Germany).

In-vitro characterization The CsA loading in $CaCO_3$CP was characterized by IR (Perkin Elmer, Germany) and TGA (TG/DTA, Perkin Elmer, Germany).

In-vitro drug release by dialysis method LBL-CsA and other formulations containing CsA were redispersed in 1 ml of phosphate buffer containing 0.05% w/v of Tween 80 and placed in the treated dialysis bag (cut off mol. wt. 12000 Dalton, Sigma) which was tied securely at both the ends. The bags were suspended initially for 2h in 199 ml of simulated gastric fluid (SGF, pH=1.4) and release was further extended for 48h in same volume of simulated intestinal fluid (SIF, pH 7.4) and placed in shaker bath at speed of 100 rpm at 37 °C. At specified time intervals the sample was collected and analyzed for CsA using RP-HPLC. The medium was replaced with one ml fresh buffer after each determination.

Biochemical evaluation of intestinal damage One milliliter of formulations was placed in pretreated fresh ileal loop of small intestine of Wistar rat for 2h and intestinal damage was assessed The positive and negative controls used were 1% triton x-100 and plain saline respectively. The concentration of lactate dehydrogenase (LDH) in the fluid was determined using protocol as provided in LDH-UV kit (Sigma).

In-vivo performance A single dose of different formulations equivalent to 5mg/ kg (prepared as 1mg/ml concentration and about ~1.0 ml) was administered by gavage to overnight fasted Wister rats (weighing 200±25mg). Serum was collected immediately from the blood (colleted at different time) by centrifugation for 5 min at 3,000rpm. Serum CsA level was monitoring using developed and validated bio-analytical method for estimation of CsA using RP-HPLC method.

Statistical analysis The data between different formulations were compared for statistical significance by one-way ANOVA followed by Turkey-Kramer multiple comparisons test using Graph Pad Instat software (GraphPad Software, CA, USA). A p value< 0.05 was considered significant.

RESULTS AND DISCUSSION

A novel microencapsulation technology based on LBL assembly of oppositely charged PE's has been established to form supramolecular multilayer assemblages of PE's. The technique utilizes the electrostatic attraction and complex formation between oppositely charged PE's over $CaCO_3$-CsA. IR spectrum (Fig.1) of the $CaCO_3$-CsA reflects the characteristic absorption bands of CsA and $CaCO_3$ without obvious new bands, which indicates that CsA encapsulation/adsorption in nanopores of $CaCO_3$ is of physical in nature (Wang et al., 2006). The thermal decomposition temperature of CsA was increase by approx two fold (400˚C±100˚C to 700˚C±50˚C) upon entrapment in $CaCO_3$ CP indicates that some interaction would have occurred between CsA and $CaCO_3$, which may be attributed to the formation of ceramics, since mass transfer of heat is uniform throughout the CP shown in Fig 2.

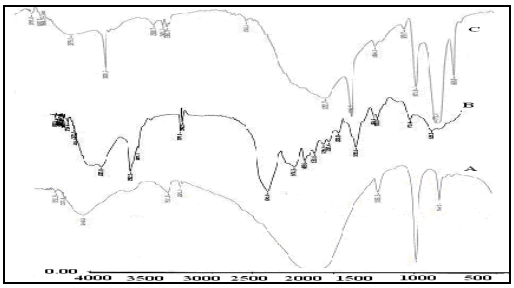

Fig. 1. IR data of A: $CaCO_3$ CP; B: CsA; C: $CaCO_3$-CsA respectively.

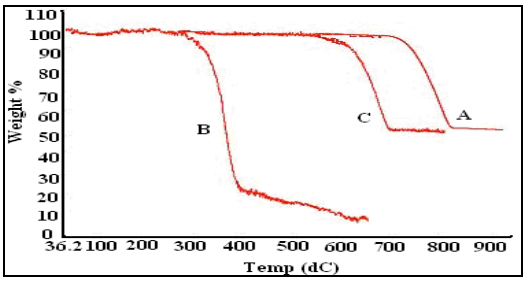

Fig. 2. TGA data of A: $CaCO_3$ CP; B: CsA; C: $CaCO_3$-CsA respectively.

Electrophoretic mobilities of $CaCO_3$ CP and fabricated microcapsules at every layering evidenced layer alteration followed by surface modification (data not shown). The maximum drug loading was found to be 74.8±2.43%. The step of surface modification of the SA/GC multilayered shells with PEPEG2000 involves adsorption or possible hydrophobic interaction between them (by zeta potential change from +22.4 to +6.3 mV) which, provide the additional stability to the system and make the system near biocompatible as well and help in intestinal absorption. These LBL-CsA exhibited controlled in vitro release of CsA for nearly constant rate and CsA was found to keep inside the capsules in water and SGF (pH 1.4) during 2h, while 75–80% of CsA can be revealed in supernatant after 48h of incubation in SIF (Fig. 3).

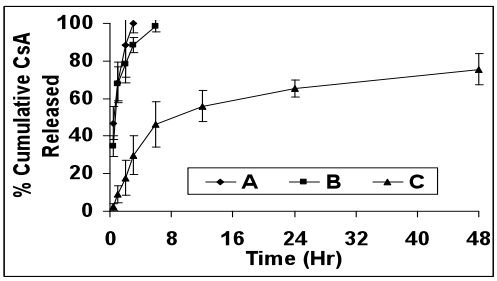

Fig. 3. In-vitro release study.

While all other formulation released completely within 3hr. Interestingly, it has been revealed that the LDH release (an indicator of membrane damage) was not significant in any of the developed formulation as compared to positive control (1% Triton-X) as well as plain CsA and is expected to be a good vehicle for oral administration on CsA (Fig. 4).

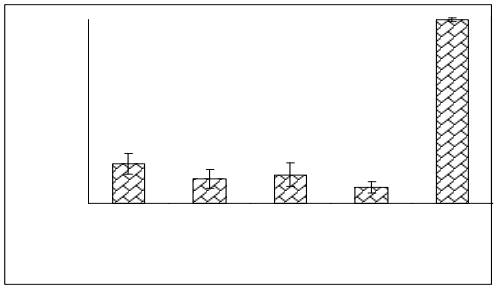

Fig. 4. Effect of different formulations on LDH in ileal loop of rat small intestine.

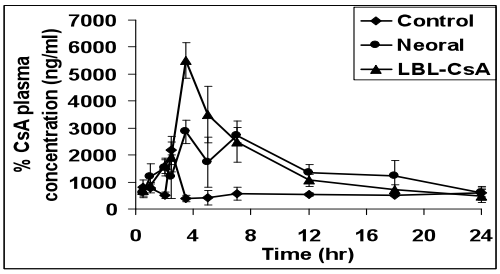

Fig. 5. Comparative in vivo plasma concentration Vs. time profiles of CsA administered orally as Sandimmune Neoral® , LBL-CsA and CsA as control (all values reported are mean±S.D., n=3).

Table 1: Pharmacokinetic parameters of CsA on oral administration of different formulations

Formulation code	C_{max}(µg/ml)	T_{max} (hr)	$AUC_{0\text{-}Inf}$ (µg.h/ml)
CsA	2.170±.510	2.5 ±0.58	14.41 ±3.38
LBL-CsA	4.812±.847	3.5± 0.39	49.89±8.27
Neoral	2.870± .430	3.5 ± 0.63	41.90 ±10.27

CONCLUSION

The relative bioavailability of novel LBL-CsA was found to be ~120.63% as compared to Sandimmune Neoral® without any intestinal damage. It is concluded that LBL-CsA is a viable carrier for improved oral delivery of CsA with safety consideration. Together these results indicate that LBL-CsA could be a potential alternative to the existing cyclosporine treatment.

REFERENCES

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