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Evaluation of metered dose inhaler (MDI) formulations of ciclosporin

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Abstract

Our purpose was to evaluate metered dose inhaler (MDI) formulations of ciclosporin (cyclosporine) for aerodynamic properties, chemical stability and bioactivity. Ciclosporin formulations (0.1, 0.5 and 1.0% w/w) were prepared in hydrofluoroalkane (HFA) propellants (134a and 227) containing 3 and 6% ethanol. Aerodynamic properties of the MDI formulations were analysed using an eight-stage Andersen cascade impactor and respirable mass and non-respirable mass, mass median aerodynamic diameter (MMAD) and geometric standard deviation (GSD) were determined from the impaction profiles. The chemical stability of 0.1% ciclosporin in HFA 227 containing 3% ethanol formulation stored at room temperature and 40°C was evaluated by HPLC at 0, 14, 30 and 90 days. The bioactivity of ciclosporin MDI formulations was evaluated by determining the ciclosporin-mediated inhibition of interleukin-2 (IL-2) release from human Jurkat cells stimulated with phorbol 12-myristate 13-acetate (PMA). As ethanol concentration increased from 3 to 6%, respirable mass decreased from 2.3 mg per five actuations to 0.04 mg per five actuations for HFA 227 formulations, and from 1.5 mg to 0.09 mg per five actuations for HFA 134a formulations. The MMAD for both HFA 134a and 227 formulations increased with an increase in ciclosporin concentration. HPLC analysis showed ciclosporin to be extremely stable in HFA 227 at room temperature and 40°C. Stimulation of Jurkat cells with PMA released significant amounts of IL-2, which was inhibited by ciclosporin in a dose-dependent manner. This study shows the feasibility of developing chemically stable and bioactive HFA-based MDI formulations of ciclosporin.

Introduction

The metered dose inhaler (MDI) is the most widely used inhalation delivery device for the treatment of asthma and obstructive pulmonary diseases due to its portability, durability, reliability, long shelf life and cost effectiveness (Keller 1999). Due to the phasing out of chlorofluorocarbons (CFCs), alternative MDI systems containing hydrofluoroalkanes (HFAs) have been developed. The new MDI systems are being investigated for delivery of small molecule drugs as well as peptides and proteins.

Pulmonary transplantation is the most effective technique that is used to treat end-stage pulmonary disease. Despite improved survival with a number of immunosuppressants, such as ciclosporin (cyclosporine), azathioprine and tacrolimus, the majority of lung transplant patients experience acute rejection. Acute rejections are normally managed with pulse-dose corticosteroids or cytolytic therapy. However, a large number of these patients develop persistent rejections, which most of the time end up with chronic rejection or death due to increased systemic immunosuppression and development of bronchiolitis obliterans (Griffith et al 1993; Paradis et al 1993; Trulock 1993). To improve the outcome of pulmonary transplantation, scientists are exploring the application of currently used medication via the inhalation route.

Ciclosporin is a cyclic undecapeptide that is used for treatment of allograft rejection, especially in the lungs, kidneys and liver. The most important immunosuppressive mode of action of ciclosporin is the blocking of early events of T-cell activation, in particular the inhibition of interleukin-2 (IL-2) production (Freed et al 1991). Currently, for treatment of lung rejection, ciclosporin is mainly administered orally and intravenously. Due to potential instability throughout the gastrointestinal tract and adverse side effects associated with ciclosporin, such as nephrotoxicity and hepatotoxicity, delivery of this agent via the inhalation

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route has been investigated (Blot et al 1995; Mitruka et al 2000; Rohatagi et al 2000; Burckart et al 2003). Burckart et al (2003) studied the lung deposition of ciclosporin after intravenous and aerosol administration in lung transplant patients and the results demonstrated that concentrations of ciclosporin after nebulization were higher than those after intravenous infusion. Additionally, aerosolized ciclosporin proved to be a valuable therapy for treating allograft rejection in lung transplant patients by reducing the side effects associated with conventional therapy (Burckart et al 2003).

Myrdal et al (2004) reported chemical stability of ciclosporin in HFA 134a for a period two of years and it was found to be stable in different container closure systems (aluminium, epoxy-coated and stainless steel) and storage configurations (upright and inverted). The biological activity of ciclosporin has been studied through inhibition of IL-2 release from mitogen-stimulated human Jurkat cells. Upon stimulation, Jurkat cells release a significant amount of IL-2 and treatment with ciclosporin has been shown to significantly reduce IL-2 release (Heidecke et al 1988; Mukerjee et al 2001). Although studies of aerosolized ciclosporin have been reported and chemical stability of ciclosporin in HFA 134a has been investigated, there has been no report so far on the use of HFA 227 for ciclosporin aerosolization and its associated stability. Further, the bioactivity of ciclosporin has not been assessed for aerosolized drug, or its activity in HFA formulations. Both HFA 134a and HFA 227 propellants have been approved as alternatives to CFC; extensive research has been conducted with HFA 134a as compared with HFA 227. Moreover, ciclosporin stability in HFA 134a has been established. Therefore, as an alternative to HFA 134a, further investigation of HFA 227 is required to study any changes or similarities in ciclosporin MDI performance and stability.

The objective of this study was to illustrate the potential of developing MDI formulation of peptides using ciclosporin as a model drug. MDI formulations of ciclosporin in HFA-based propellant systems (HFA 134a and 227) were evaluated and characterized for aerodynamic properties, such as respirable mass and respirable fraction, mass median aerodynamic diameter (MMAD) and geometric standard deviation (GSD). In addition, we evaluated the chemical stability and biological activity of ciclosporin in HFA 227 propellant. Since this is the first study to evaluate bioactivity of aerosolized ciclosporin by inhibition of IL-2 release from stimulated Jurkat cells, the method was first validated by evaluating the bioactivity of aqueous ciclosporin.

Materials and Methods

Materials

Ciclosporin was kindly provided as a gift by Panacea Biotech (India). Phorbol 12-myristate 13-acetate (PMA) and all tissue culture chemicals were purchased from Sigma Chemical Company (St Louis, MO). Human IL-2 ELISA Kit was purchased from BD Pharmingen (San Diego, CA). The six-stage viable impactor and eight-stage Andersen cascade impactor, Mark II were obtained from Grasbey Andersen (Smyrna, GA). MDI glass vials, continuous valves and non-continuous valves were kindly provided by 3M Pharmaceuticals (St Paul, MN). Human Jurkat A3 (a T-cell leukaemia cell line) was obtained from the laboratory of Dr Chang-Yan Cheng at Boston University (Boston, MA). Water, acetonitrile, and *tert*-butyl methyl ether were purchased from Fisher Scientific (Atlanta, GA). All other chemicals were of reagent grade.

Formulation of ciclosporin MDI

Ciclosporin formulations were prepared in HFA 134a and 227 propellants with varying ciclosporin (0.1, 0.5, and 1.0% w/w) and ethanol (3 and 6% w/w) concentrations. The required amounts of ciclosporin and ethanol were weighed directly in clean 15-mL glass vials. The vials were then crimped with continuous valves and HFA 134a or 227 was then added from a pressure burette attached to a filling machine to bring the final weight in each vial to 12 g. The formulations were then placed on a platform shaker (Innova 200, New Brunswick Scientific, Edison, NJ) at 150 rev min^{-1} and allowed to shake overnight.

Characterization of ciclosporin MDI formulations

The aerodynamic particle size distribution of ciclosporin formulations was analysed using an eight-stage Andersen cascade impactor, Mark II (Grasbey Andersen, Smyrna, GA). The formulations were primed five times before analysis. Five shots were then fired (at 10-s intervals) into the impactor, which was set at a flow rate of 28.3 L min^{-1}. The deposited drug was rinsed from the impactor plates with 5 mL acetonitrile–water (50:50). The samples were then analysed by HPLC method as described below. The results obtained from the HPLC analysis were used to determine the MMAD and GSD, which were calculated using an established software in our laboratory (Haynes et al 2003). The respirable mass was calculated as the cumulative mass of drug collected on Andersen stages having a cut off diameter less than 4.7 μm. Respirable fraction was calculated as the ratio of the total drug deposited on various stages of the impactor, including drug collected from the actuator and throat, jet stage, impactor stages 0–7 and the filter. These results were used to select a propellant system for our stability and bioactivity studies.

High-performance liquid chromatography (HPLC) analysis of ciclosporin

The HPLC system consisted of a Waters autosampler (717 plus), two pumps (model 515) and a UV detector (model 996 PDA). Sample analysis was performed on a Vydac C-18 column, which was maintained at 80°C. Acetonitrile–water (70:30) was used as the mobile phase at a flow rate of 1.0 mL min^{-1} with an injection volume of 100 μL and UV detection was performed at 210 nm. Quantification was done by peak area using a standard curve that was prepared daily. Assay validation was performed by a seven-point standard curve with linearity coefficient (r^2) of 0.999. System suitability test was performed during each analysis to determine the reproducibility of the assay.

Stability of ciclosporin MDI formulations

Ciclosporin formulations (0.1% ciclosporin and 3% ethanol w/w) were prepared in HFA 227 propellant. The formulations were stored at room temperature (25°C) and 40°C, and samples were collected at 0, 14, 30 and 90 days. At each time point, the vials were opened and the propellant was evaporated. The drug was reconstituted with acetonitrile–water (50:50) and samples were analysed by HPLC using acetonitrile–water–*tert*-butyl methyl ether (450:500:50 v/v), pH 6, as the mobile phase. The retention time of for the ciclosporin peak was at 33 min. Quantification was done by peak area using a standard curve that was prepared daily. System validation was performed by a seven-point standard curve with a coefficient of variation (r^2) value of 0.994. To verify system precision and reproducibility, a suitability test was performed before each analysis.

Treatment of Jurkat cells with ciclosporin

Jurkat cells were grown in RPMI 1640 medium supplemented with 10% fetal bovine serum and 1% penicillin–streptomycin. For production of IL-2, jurkat cells were washed three times in serum-free RPMI medium (SFM) and subsequently used for stimulation by PMA. For treatment with ciclosporin, 2 mL of cell suspension containing 6×10^6 cells, as prepared above, was added and stimulated with PMA (5 μg mL^{-1}). Ciclosporin stock solution (30 μg mL^{-1}) was prepared in dimethyl sulfoxide (DMSO) and RPMI 1640 medium and subsequently added to the cells at different dilutions to final concentrations of 0.6, 1.2 and 2.5 μg mL^{-1}. The cells were then incubated for 24 h in a six-well culture plate. After incubation at 37°C in 5% CO_2, the cell suspension was centrifuged for 10 min and the supernatant was collected for IL-2 analysis. Release of IL-2 from Jurkat cells was measured by human IL-2 ELISA kit (BD Pharmingen, San Diego, CA). The formulations and results were used as standards during analysis of biological activity of MDI ciclosporin.

Treatment of Jurkat cells with ciclosporin MDI

Jurkat cells (6×10^6) were placed in 10 mL of SFM in sterile glass petri plates suitable for the six-stage viable impactor. The plates were placed on the fourth stage of the impactor and the cells were exposed to one shot of ciclosporin formulations (0.025, 0.05, 0.1% w/w ciclosporin, 3% ethanol w/w in HFA 227). After exposure, the cells were incubated for 10 min and then 2 mL of the cell suspension was placed in a six-well culture plate and incubated for 24 h. After incubation at 37°C in 5% CO_2, the cell suspension was centrifuged for 10 min and the supernatant was collected for IL-2 analysis. Release of IL-2 from Jurkat cells was measured by human IL-2 ELISA kit (BD Pharmingen, San Diego, CA). In addition, the bioactivity of ciclosporin MDI formulations kept at various storage conditions (25°C and 40°C for a period of up to 90 days) was investigated by the same procedure used to analyse fresh ciclosporin MDI samples.

Statistical analysis

One-way analysis of variance followed by Tukey's Multiple Comparison Test were used to determine the significance of difference in the bioactivity of aqueous and aerosolized ciclosporin. The statistical analysis was performed using GraphPad PRISM version 3.0 software (San Diego, CA).

Results

Characterization of ciclosporin MDI formulations

The aerodynamic particle size data for the various ciclosporin formulations are summarized in Table 1. There was an increasing trend in the MMAD values as ciclosporin concentration was increased from 0.1% w/w to 1% w/w in both HFA 134a and HFA 227 propellants (Table 1). Figures 1A and 1B represent the respirable mass for ciclosporin formulations for both HFA 227 and HFA 134a. Respirable mass is the mass of drug with particle size < 4.7 μm. For both propellants, the respirable mass decreased with an increase in ethanol concentration. An increase in ethanol concentration (3 to 6% w/w) resulted in a significant decrease in respirable mass from 2.3 mg/five actuations to 0.04 mg/five actuations and from 1.5 mg/five actuations to 0.09 mg/five actuations in HFA 227 and HFA 134a, respectively. The respirable fraction of ciclosporin formulations is represented in Figures 2A and 2B. The respirable fraction is the total amount of drug deposited on the throat and impaction plates (particles size > 4.7 μm). For both propellants, the respirable fraction decreased with an increase in ethanol concentration.

Chemical stability of ciclosporin MDI formulations

The chemical stability of ciclosporin formulations in HFA 227 at room temperature (25°C) and 40°C at different time intervals (0, 14, 30 and 90 days) was evaluated. HPLC analysis showed that ciclosporin was stable in HFA 227 at both storage conditions. No degradation products were observed in the HPLC chromatograms (data not shown).

Table 1 Mass median aerodynamic diameter (MMAD) and geometric standard deviation (GSD) for ciclosporin MDI formulations

Formulation	MMAD (μm)	GSD
0.1% Cs, 3% EtOH/134a	0.7 ± 0.02	1.8
0.1% Cs, 6% EtOH/134a	0.7 ± 0.08	1.9
0.1% Cs, 3% EtOH/227	0.8 ± 0.15	2.4
0.1% Cs, 6% EtOH/227	0.8 ± 0.11	2.1
0.5% Cs, 3% EtOH/134a	1.3 ± 0.21	2.0
0.5% Cs, 6% EtOH/134a	1.4 ± 0.22	2.1
0.5% Cs, 3% EtOH/227	1.4 ± 0.02	1.9
0.5% Cs, 6% EtOH/227	1.6 ± 0.08	1.9
1% Cs, 3% EtOH/134a	1.5 ± 0.09	2.1
1% Cs, 6% EtOH/134a	1.7 ± 0.08	2.0
1% Cs, 3% EtOH/227	2.1 ± 0.02	2.2
1% Cs, 6% EtOH/227	2.1 ± 0.13	2.1

MMAD and GSD data obtained from the impaction profiles of ciclosporin MDI formulations containing 0.1, 0.5 and 1% w/w ciclosporin (Cs) in 3 and 6% w/w ethanol (EtOH) using HFA 134a or HFA 227.

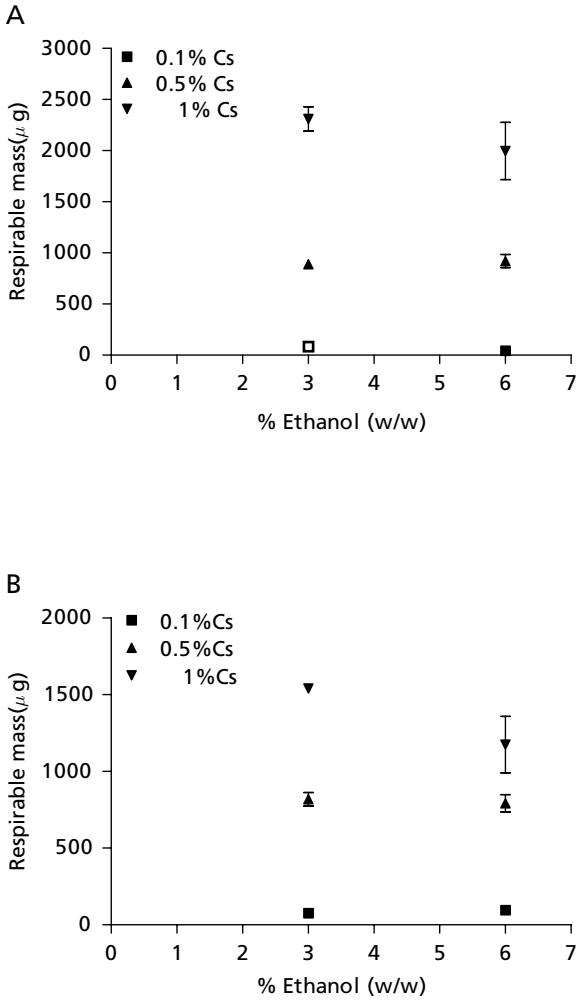

Figure 1 Respirable mass per five actuations for HFA 227 (A) and HFA 134a (B) formulations prepared with 0.1, 0.5 and 1% w/w ciclosporin (Cs) in 3 and 6% w/w ethanol (n = 3).

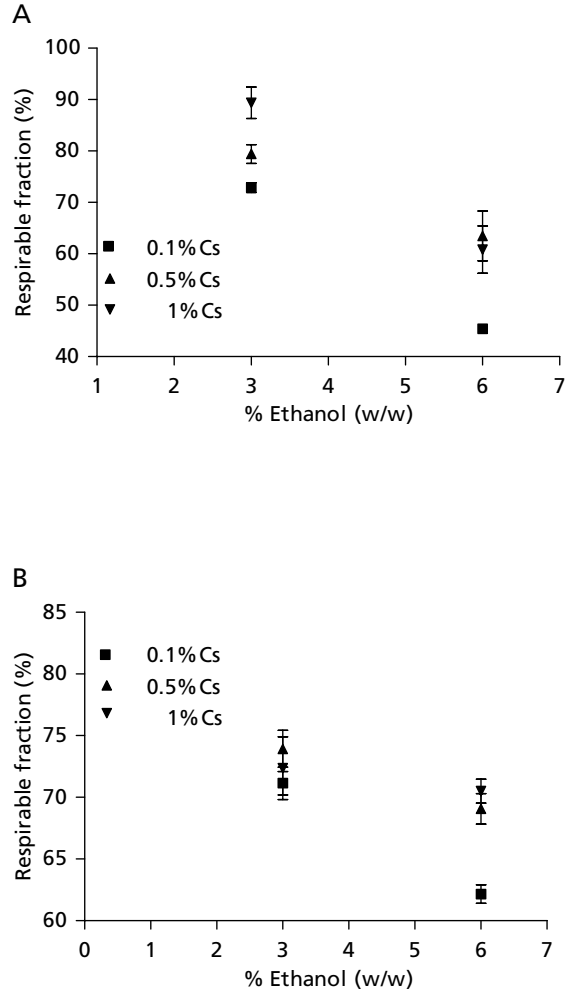

Figure 2 Respirable fraction per five actuations for HFA 227 (A) and HFA 134a (B) formulations containing 0.1, 0.5 and 1% w/w ciclosporin (Cs) in 3 and 6% w/w ethanol (n = 3).

Inhibition of IL-2 release by ciclosporin

A T-cell stimulating agent, PMA, was used to stimulate Jurkat cells for production of IL-2. Incubation with PMA (5 μg mL^{-1}) was performed in SFM and upon stimulation; Jurkat cells released a significant amount (approximately 20 pg mL^{-1}) of IL-2, which was quantified by ELISA. Inhibition of IL-2 release by ciclosporin is represented in Figure 3. In the presence of ciclosporin (0.6, 1.2 and 2.5 μg mL^{-1}), IL-2 release was inhibited by 43%, 60% and 73%, respectively. The percent inhibition of IL-2 release by ciclosporin was reported as the average value of three experiments. These results indicate that ciclosporin inhibits IL-2 release from Jurkat cells upon PMA stimulation and the inhibition was dependent upon ciclosporin concentration.

Inhibition of IL-2 release by ciclosporin MDI formulations

Upon exposure to one shot of ciclosporin MDI formulations (0.025, 0.05 and 0.1% ciclosporin w/w), IL-2 release was inhibited by 79, 81 and 93%, as compared with the PMA-

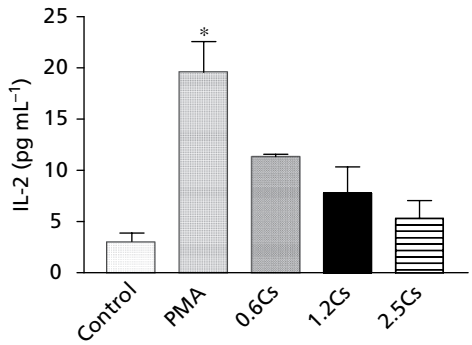

Figure 3 Inhibition of interleukin-2 (IL-2) release by aqueous ciclosporin (Cs) in PMA (5 μg mL^{-1})-stimulated Jurkat cells. Jurkat cells were treated with 0.6, 1.2 and 2.5 μg mL^{-1} Cs and incubated for 24 h. IL-2 release was measured by ELISA. Data expressed as mean ± s.d., n = 3. *P< 0.05, PMA-treated cells vs control.

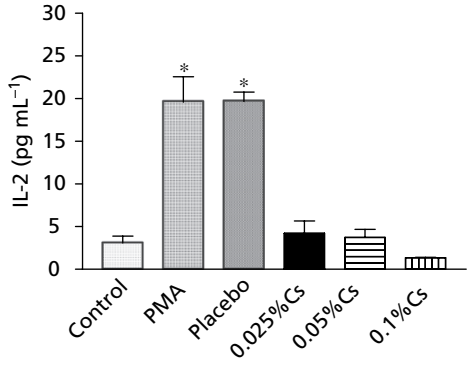

Figure 4 Effect of aerosolized ciclosporin (Cs) on IL-2 release from PMA ($5\,\mu g\,mL^{-1}$)-stimulated Jurkat cells. MDI formulations of ciclosporin were prepared with 0.025, 0.05 and 0.1% w/w Cs, 3% w/w ethanol in HFA 227 propellant and PMA-stimulated Jurkat cells were exposed to the aerosolized ciclosporin using six-stage viable impactor. Following exposure to aerosolized ciclosporin, the cell suspension (2 mL) was incubated in six-well plates for 24 h. Subsequently, the release of IL-2 in the supernatant was determined using an IL-2 ELISA kit. Data expressed as mean ± s.d., n = 3. $^*P < 0.05$, PMA-treated cells vs control.

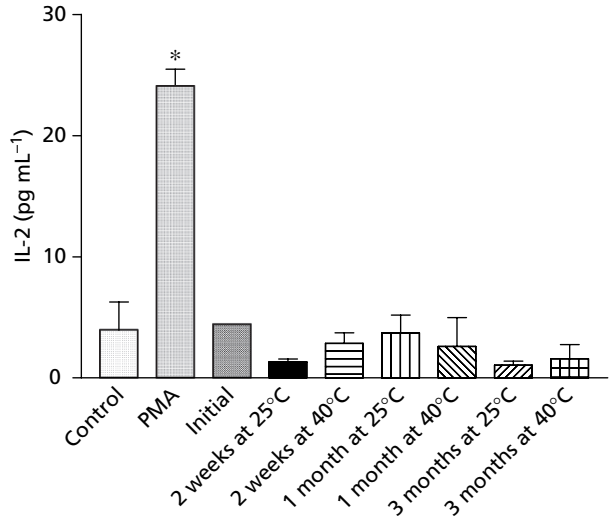

Figure 5 Effect of aerosolized ciclosporin from MDI formulations stored at room temperature (25°C) and 40°C for 90 days on IL-2 release. MDI formulations of ciclosporin were prepared with 0.1% w/w Cs and 3% w/w ethanol in HFA 227 propellant. At each time point, IL-2 release was estimated by following the same procedure as described in Figure 4. Data expressed as mean ± s.d., n = 3. $^*P < 0.05$, PMA-treated cells vs control.

stimulated cells. Experiments were also performed with placebo MDI containing HFA 227 and 3% w/w ethanol formulation in the same way as described above and we observed that there was about $19.7\,pg\,mL^{-1}$ of IL-2 released from Jurkat cells, which was similar to that of PMA-stimulated cells (Figure 4). Thus our results with placebo formulation indicate that MDI formulation ingredients (HFA 227 propellant and ethanol) did not contribute to IL-2 release inhibition by ciclosporin.

Inhibition of IL-2 release by ciclosporin MDI stability formulations

Jurkat cells were stimulated with PMA ($5\,\mu g\,mL^{-1}$) and exposed to 0.1% ciclosporin w/w formulations that were stored at different conditions (25°C and 40°C). We observed a similar trend as with aqueous ciclosporin (unaerosolized form), that upon stimulation of Jurkat cells with PMA there was a significant increase ($P < 0.05$) in IL-2 release (84%), and after treatment with aerosolized ciclosporin IL-2 release was inhibited by 89% (Figure 5). Furthermore, our results showed that at different time points (0, 14, 30 and 90 days) and different storage conditions (25°C and 40°C), ciclosporin maintained its bioactivity as indicated by the similar levels of IL-2 inhibition as compared with the fresh ciclosporin MDI samples. The observed IL-2 inhibition at different storage conditions was found to be not statistically significantly different from the initial inhibition ($P > 0.05$).

Discussion

The main purpose of this study was to investigate the feasibility of developing an MDI formulation using ciclosporin as a model peptide drug. Inhalation delivery of ciclosporin by nebulization has been extensively studied, and this made this drug an ideal model for our studies. This study demonstrates that ciclosporin can be formulated as an MDI formulation in HFA-based systems with suitable aerodynamic characteristics. We evaluated the aerodynamic properties of ciclosporin formulations with varying ciclosporin and ethanol concentrations in HFA 134a and 227. Our findings confirmed previous results (Myrdal et al 2004) that for both propellants, there was a decrease in respirable mass as ethanol concentrations were increased. The MMAD for HFA 227 formulations was found to be higher (about 0.2 μm) than that of HFA 134a formulations. Lung deposition of aerosol particles in the lungs is mainly governed by the aerosol's particle size. Clinical data by Corcoran et al (2004) indicated that peripheral deposition of aerosolized ciclosporin is preferred for improved pulmonary function. This formulation has been reported to have a MMAD of 1.2 μm (Burckart et al 2003). The actual deposition pattern of nebulized ciclosporin in the previous studies was not reported, although Leach et al (2002) reported that an HFA 134a-based steroid formulation with an MMAD of 1.1 μm deposited uniformly throughout the lungs, whereas CFC-based formulations with MMAD of about 3 μm deposited primarily in the large central and intermediate airways. From our studies, HFA 227 formulations were able to produce particles with MMAD of 0.8–2.1 μm. The particle size difference of 0.2 μm between HFA 134a and HFA 227 formulations may not be of clinical significance; however it is beyond the scope of this paper to discuss the exact implication on clinical settings. We observed that there was more drug deposition from HFA 227 formulations than from those of HFA 134a.

In our study, we also evaluated the chemical stability of MDI ciclosporin in HFA 227 at 25°C and 40°C for a period of 90 days. Our results showed that ciclosporin was stable in

HFA 227. Although physical stability studies were not conducted, the formulations were periodically checked for clarity, colour change and aggregation within the three months of chemical stability testing. At the end of three months, the formulations maintained their initial appearance and chemical stability analysis by HPLC showed no detectable degradation. In addition to chemical stability, our study also showed that ciclosporin was biologically active in fresh MDI formulations and it also maintained activity after storage. Several studies have been reported where researchers have used the inhibitory effect of ciclosporin on IL-2 production as a measure of ciclosporin bioactivity (Stein et al 1999; Mukerjee et al 2001). Assays such as ELISA and IL-2 reporter gene assay (IL-2 RGA) have been developed to investigate the bioactivity of ciclosporin through IL-2 inhibition. ELISA has been the most commonly used technique due to its simplicity and data reproducibility.

In this study, we used human Jurkat cells, which upon PMA stimulation released a significant amount of IL-2. PMA was selected over phytohaemagglutinin (PHA) as a mitogen stimulant based on our preliminary studies, which showed that Jurkat cells released more ($>20\,pg\,mL^{-1}$) IL-2 upon PMA stimulation compared with PHA (approx. $4.5\,pg\,mL^{-1}$). Ciclosporin inhibited IL-2 release from Jurkat cells in a dose-dependent manner. Stimulation of Jurkat cells with PMA resulted in a significant (about 6-fold) increase in IL-2 release, and upon treatment with ciclosporin IL-2 release was inhibited. These results confirmed the findings of previous researchers (Stein et al 1999; Mukerjee et al 2001) and provided us with an analytical tool with which to monitor the bioactivity of aerosolized ciclosporin. We analysed the bioactivity of freshly prepared ciclosporin MDI formulations in HFA 227 system. PMA-stimulated Jurkat cells were exposed to one shot of each formulation (0.025%, 0.05% and 0.1% w/w ciclosporin in HFA 227) and incubated for 24 h. As shown in Figure 4, an increase in the ciclosporin concentration in HFA 227 from 0.025% to 0.1% resulted in an increase in IL-2 inhibition from 79% to 93%, as compared with Jurkat cells treated with PMA alone. These results showed that aerosolization of ciclosporin did not affect its bioactivity. In addition, we evaluated the bioactivity of MDI ciclosporin stability formulations that were stored in glass vials for 90 days at 25°C and 40°C. We found that ciclosporin maintained its activity by inhibiting IL-2 release from PMA-stimulated Jurkat cells to the same extent as that of the freshly prepared MDI formulation (Figure 5). Previously we have used the six-stage viable impactor for studying the cytotoxicity of aerosolized methotrexate (Shaik et al 2002) and aerosolized cyclooxygenase-2 inhibitors (Haynes et al 2003). In this study, we used the same method for exposing the Jurkat cells to the aerosolized ciclosporin from an MDI formulation. However, we observed a greater inhibition of IL-2 release from aerosolized ciclosporin as compared with the aqueous ciclosporin, which may be attributed to loss of activity due to colloidal precipitation of ciclosporin in DMSO with SFM dilutions and probably due to improved cellular uptake of aerosolized ciclosporin from HFA 227 formulations. However, literature data suggest that aerosolized ciclosporin exhibits slower elimination than intravenous infusion in lung transplant patients (Burckart et al 2003). The slower elimination has been attributed to increased uptake by alveolar macrophages and increased binding to epithelial cell membranes (McAllister et al 1996). Based upon these previous findings, we believe that the cellular kinetics of ciclosporin are different between aerosol and solution dosage forms. We ruled out the possibility of any inhibitory contributory effect of formulation ingredients (HFA 227 and ethyl alcohol), as we found that placebo formulation demonstrated similar release of IL-2 to that of PMA treated cells under the same experimental conditions.

Our results indicate that HFA 227-based MDIs may be developed for suitable peptide drugs like ciclosporin. Aerosolized ciclosporin may find application in improving the survival and extending the period of rejection-free survival in lung-transplant patients (Iacono et al 2006). Additional applications of aerosolized ciclosporin include overcoming drug resistance and enhancement of drug cytotoxicity as studied by Koshkina et al (2004) and in the treatment of airway inflammation (Xie et al 2002). Further, the formulation development approach used for ciclosporin may be applied for other suitable peptide drugs, and the in-vitro bioactivity measurement technique as proposed in this study, as well as in our previous studies, will be useful in screening the various formulations in the development stage and monitoring the quality control of formulations upon storage. However, further pre-clinical studies are needed to evaluate the potential of HFA-based delivery of peptide drugs in suitable animal models.

Conclusion

In conclusion, we demonstrated the feasibility of developing chemically stable and bioactive HFA-based MDI formulations of ciclosporin. For the first time we report a simple technique that provides an assessment of biological activity of aerosolized ciclosporin.

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