The Potential Use of Cyclosporine Ultrafine Solution Pressurised Metered Dose Inhaler in the Treatment of COVID-19 Patients

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Abstract

Introduction: COVID-19 serious respiratory problems start when the virus reaches the alveolar level, where type II cells get infected and die. Therefore, virus inhibition at the alveolar level would help preventing these respiratory complications.

Method: Literature search was conducted to collect physicochemical properties of small molecule compounds that could be used for the COVID-19 treatment. Those compounds were selected that have low melting point, soluble in ethanol, hydrogen-bond donors and acceptors.

Results: There are severe acute respiratory syndrome coronavirus inhibitors with physicochemical properties suitable for the formulation as ultrafine pressurised metered dose inhaler (pMDI). Mycophenolic acid, Debio 025 and cyclosporine A are prime candidates among these compounds. Cyclosporine A (hereafter cyclosporine) is a potent SARS-CoV-2 inhibitor, and it has been used for the treatment of COVID-19 patients, demonstrating improved survival rate. Also, inhalation therapy of nebulised cyclosporine was tolerated, which was used for patients with lung transplants. Finally, cyclosporine has been formulated as a solution ultrafine pMDI. Although vaccine therapy has started in most countries, inhalation therapies with non-immunological activities could minimise the spread of the disease and used in vaccine-hesitant individuals.

Conclusion: Ultrafine pMDI formulation of cyclosporine or Debio 025 should be investigated for the inhalation therapy of COVID-19.

Keywords: COVID-19, Cyclosporine A, Inhalation, Pressurised Metered Dose Inhaler, Regulatory Tests, Approved Drugs.

List of Abbreviations

Analytical evaluation threshold: AET

Fine particle fraction; FPF

Limit of detection: LoD

Nasal high flow: NHF

Pressurised metered dose inhalers: pMDIs

Severe acute respiratory syndrome coronavirus 2: SARS-CoV-2

Valved holding chambers: VHCs

1. Introduction

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection and the resulting coronavirus disease 2019 (Covid-19) has affected the human population across the globe with devastating health consequences. At the time of preparing this paper (September 2021), there have been 219m cases, with 4.55m deaths. So far, the USA has the highest toll of cases, followed by India, Brazil, the UK, Russia, and France. All of these countries have passed a grim milestone of 100,000 deaths. Effective interventions are required to end this pandemic. Wearing a mask is a simple and effective preventative measure to prevent the spread of SARS-CoV-2. However, this has not been effective. Several national lockdowns have been implemented to reduce the spread of the disease, with severe economic consequences that have resulted in 50% of individuals experiencing declines in household earnings. The concerns for the economy (and for "what the way out" of these lockdowns is going to be) have resulted in 42% of populations (UK, Italy, Spain) being at a substantial risk of stress, anxiety, and depression.[1]

Coronaviruses are enveloped, single stranded RNA viruses. These casue repiratory, gasterointestinal and neurological symptoms. The initial steps of coronavirus infection involve the precise binding of the coronavirus spike protein to the host-cell surface receptors. The spike proteins have been identified for several coronaviruses including SARS-CoV-2. The angiotensin- converting enzyme 2 is the receptor that utilised by SARS-CoV-2 for entry. Following this interaction, the spike protein drops and fuses with the host-cell membrane, which is faciliated by the cell- surface serine protease TMPRSS2.[2] This enzyme expression is high in the adult human repiratory tract.[3, 4] It has been shown that inhibition of TMPRSS2 was sufficient to block the entry of SARS-CoV-2 into human respiratory cell line.[5] Although cyclophins play a key role in the replications of viruses, whether cyclophins are important for

SARS-CoV replication is a matter of debate.[6] On the other hand, Cyclosporine A (hereafter cyclosporine) has shown remarkable antiviral activities against SARS-CoV-2.[7] Cyclospoine and alisporivir (Debio 025) are potent inhibitors of cyclophins and reduce SARS-CoV-2 replication.[7, 8] It should be noted that cyclosporine is a well known immunosuppressive drug, while alisporivir (Debio 025) with almost identical physicochemical properties to cyclosporine is a non-immunosuppressive drug.[8] There is a conception that cyclosporine cannot be used in patients with COVID-19 because of its strong immunosuppressive properties.[8] However, the cyclosporine exerts it immunosuppressive effects at much higher doses compared to its antiviral activities.[7]

The vaccination programme has started since December 2021. As of 10th of September 2021, a total of 5,352,927,296vaccine doses have been administered (WHO, https://covid19.who.int). The BNT162b2 (also known as Pfizer/BioNTech) vaccine is one of the approved vaccines, which is a lipid nanoparticle–formulated, nucleoside-modified RNA (modRNA) encoding the SARS-CoV-2 full-length spike, modified by two proline mutations to lock it in the prefusion conformation.[9] The phase II clinical trial indicated that a two-dose regimen (30 µg each dose) of BNT162b2 conferred 95% protection against Covid-19 in persons 16 years of age or older. The ChAdOx1 nCoV-19 (AZD1222) is another vaccine against SARS-CoV-2, which has been approved for emergency use by the UK regulatory authority, Medicines and Healthcare products Regulatory Agency.[10] The ChAdOx1 nCoV-19 (AZD1222) is a chimpanzee adenoviral vectored vaccine with a full-length SARS-CoV-2 spike insert.[10]

There are challenges for roll-out of the Covid-19 vaccination worldwide. For example, immediate[11] or delayed[12] injection-site reactions have been reported for the developed COVID-19 vaccines. In addition, raw materials for mRNA vaccines (e.g., the Pfizer–BioNTech and Moderna) are in short supply, and also require an extremely difficult

manufacturing process.[13] Vaccine hesitancy is another obstacle.[14] A study found that majority of the majority of US population are undecided on whether or not to take COVID-19 vaccinations, mainly due to the perception of its effectiveness.[14] Furthermore, subjects with common variable immunodeficiency may present impaired vaccine responses.[15] Moreover, immunocompromised patients may not recover fast enough from COVID-19, and consequently, prolonged replication of the virus in these patients will lead to the evolution of SARS-CoV-2.[16]

The evolution of SARS-CoV-2 has occurred since its initial emergence. It has been shown that SARS-CoV-2 spike protein variants with mutations in the receptor-binding domain escape monoclonal antibodies or convalescent plasma.[17] SARS-Cov-2 variants with a D614G mutation (spike aspartic acid–614 substitution to glycine) have become dominant in the global pandemic, and D614G mutations increase infectivity of the virus and transmit significantly faster.[18, 19] Fortunately, these variants were antigenically similar.[19] On the other hand, SARS-CoV-2 B.1.1.7 has 8 spike mutations in addition to D614G.[20] This variant emerged from South East of England in September 2020.[21] The strain has now spread to over 50 countries and carries a 35% increased hazard of death.[22]Furthermore, SARS-CoV-2 B.1.351 (501Y.V2) emerged in late 2020 in Eastern Cape, South Africa with enhanced transmissibility.[23] This strand has 9 spike protein mutations.[20] Now the SARS-CoV-2 delta (B.1.617.2) variant has higher transmissibility compared to the alpha variant; and this puts greater burden on health-care services than the alpha variant.[24] As vaccines focus on the spike protein, the mutations in spike proteins may render the vaccines less efficacious.[25] The above observations warrant investigations on therapeutic approaches that are based on non-immunological activities.

The aim of this paper is to identify small molecules that can be investigated for the formulation of solution ultrafine pressurised metered dose inhalers (pMDIs). In particular,

inhalation therapy of nebulised interferon β-1a (SNG001) showed rapid recovery of patients admitted to hospital with COVID-19 symptoms.[26] Also, inhalation therapy using unfractionated heparin has been suggested for patients with COVID-19 admitted to hospital.[27] However, there is a need for an inhalation therapy that can be used widespread for subjects who have just tested positive for COVID-19.

2. Potential Candidates for Ultrafine pMDI for Treatment of COVID-19

SARS-CoV-2 induced the death of epithelial cells, in particular alveolar type II cells.[28] The destruction of these cells leads to scars in the lungs and respiratory symptoms.[29, 30] Therefore, the infection/replication of SARS-CoV-2 could be prevented in type II alveolar epithelial cells (pneumocytes) by delivery of aerosols to lung alveoli. Among inhalers, ultrafine solution pMDIs can deliver significant amounts of drug aerosol particles to alveoli.[31-33]

Research has shown that increasing drug melting point reduces solubility in HFA 134a.[34] For example, progesterone with a melting point of 129°C is soluble in HFA 134a with the concentration of approximately 1% w/w and this increases to 10% w/w with the addition of 20% ethanol.[35] Therefore, a melting point of 150°C or less may be considered as a desired property for solubility in HFA based propellants. Other factors are hydrogen bonding (hydrogen-bond donors and acceptors), which had a significant effect on the solubility in HFA 134a, as well as significant effect on cosolvent (ethanol) solubilisation.[35] Finally, previous work indicated that compounds with logP in the range of 1.88 <logP< 9.85 showed solubility (with or without ethanol) in HFA 134, although this was not a determining parameter.[35] Table 1 presents physicochemical properties of small molecules with coronavirus antiviral activity.

Table 1: Physicochemical properties of small molecule compounds with coronavirus antiviral activity.

No	Drug	CAS	Melting point °C	Ethanol solubility	Mw	logP	H-donor	H-acceptor	IC_{50} µM	EC_{50} µM
1	Ribavirin	36791-04-5	345	< 1 mg/ml	244.2	-1.8	4	7	80 µg/ml[36] (SARS-CoV HKU39849)	9.99 ± 2.97 µg/ml[37] (MERS-CoV)
2	Methylprednisolone	83-43-2	232	5mg/ml	374.5	1.9	3	5	Effectiveness shown in clinical trial[38]	
3	Mycophenolic acid	483-60-3	141	Soluble	320.3	3.2	2	6	0.53[39] (MERS-CoV)	0.87[40] (SARS-CoV-2)
4	Hexamethylene amiloride	1428-95-1	224	NA	311.77	1.3	3	5	9.38[41] (FCoV)	1.34[42] (HCoV-229E)
5	Chloroquine	54-05-7	87	insoluble	515.9	4.6	1	3	5.2[39] (MERS-CoV)	4.1[43] (SARS-CoV)
6	Chlorpromazine	50-53-3	177	soluble	318.9	5.2	0	3	9.15[39](MERS-CoV)	8.8[43] (SARS-CoV)
7	Loperamide	53179-11-6	222	53.7 mg/ml	477	5	1	3	5.9[39] (MERS-CoV)	5.9[43] (SARS-CoV)
8	Lopinavir	192725-17-0	124	soluble	628.8	5.9	4	5	17[39] (MERS-CoV)	17.1[43] (SARS-CoV)
9	Nutlin-3	548472-68-0	NA	100 mg/ml	581.5	5.2	1	5	NA	6.9[44] (MERS-CoV)
10	Hydroxyzine	68-88-2	200	NA	374.9	2.36	1	4	NA	14.4[44] (MERS-CoV) [pamoate salt]
11	Amodiaquine	86-42-0	206	2 mg/ml	355.9	2.6	2	4	6.2[39] (MERS-CoV)	2.1[44] (MERS-CoV)
12	Saracatinib	379231-04-6	84	100 mg/ml	542	4.1	1	10	6.2[39] (MERS-CoV)	2.9[44] (MERS-CoV)
13	Sotrastaurin	425637-18-9	NA	2 mg/ml	438.5	2.6	2	6	NA	9.7[44] (MERS-CoV)

14	Acetophenazine	2751-68-0	167	NA	411.6	2.6	1	6	NA	11.2[44] (MERS-CoV)
15	Dosulepin	113-53-1	131	Freely Soluble	295.4	4.49	0	2	NA	3.4[44] (MERS-CoV) [hydrochloride salt]
16	Methotrimeprazine (Levomepromazine)	60-99-1	117	NA	328.5	4.7	0	4	NA	2.5[44] (MERS-CoV) [Maleate salt]
17	Emetine	483-18-1	54	NA	480.6	4.7	1	6	0.01[39] (MERS-CoV)	0.051[45] (SARS-CoV)
18	Mycophenolate mofetil	128794-94-5	93	Sparingly	433.5	3.2	1	8	1.58[46] (HCoV-OC43)	1.54[47] (HCoV-OC43)
19	Cyclosporine A	59865-13-3	298	Soluble 200 mg/ml	1202.6	7.5	5	12	0.24[7] (SARS-CoV2) and 3.05[48]	>50[CC50][7] (SARS-CoV2)
20	Debio 025	254435-95-5	NA	Soluble	1216.6	7.9	5	12	NA	0.46[8]
21	Phenazopyridine	94-78-0	139	NA	213.24	1.9	2	5	NA	1.9[47] (HCoV-OC43)
22	Pyrvinium	7187-62-4	NA	NA	382.5	5.9	0	1	NA	3.21[47] (HCoV-OC43) [Pamoate salt]
23	Monensin	17090-79-8	103	Soluble (25 mg/ml sodium salt)	670.9	4.2	4	11	NA	3.81[47] (HCoV-OC43) [Sodium salt]
24	Mefloquine	49752-90-1	174	42 mg/ml	378.31	3.6	2	9	7.89[41] (FECV1683)	15.55[45] (SARS-Cov)
25	Amodiaquine	86-42-0	206	2 mg/ml	355.9	2.6	2	4	6.21[39] (MERS-CoV)	2.1[44] (MERS-CoV)

										[Amodiaquine dihydrochloride]
26	Aloxistatin (E64D)	88321-09-9	126	68 mg/mL	342.43	2.3	2	5	200µg/ml[49] (MHV-A59)	1.275[50]
27	Gemcitabine hydrochloride	122111-03-9	287	Poorly Soluble	299.66	0.28	4	6	NA	4.95[43] (SARS-CoV)
28	Tamoxifen	10540-29-1	97	20 mg/ml	371.5	7.1	0	2	10.12[39] (MERS-CoV)	92.88[43] (SARS-CoV) [citrate salt]
29	Terconazole	67915-31-5	126	Sparingly	532.5	4.8	0	7	12.20[51] (MERS-CoV)	15.27[50] (SARS-CoV)
30	Fluspirilene	1841-19-6	187	Soluble	475.6	5.6	1	5	7.5[51] (MERS-CoV)	5.96 [45] (SARS-CoV)
31	Thiothixene	5591-45-7	114	NA (soluble in methanol)	443.6	3.8	0	6	9.3[51] (MERS-CoV)	5.32[45] (SARS-CoV)
32	Fluphenazine	69-23-8	200	1 mg/ml	437.5	4.4	1	8	5.87[51] (MERS-CoV) [hydrochloride salt]	21.43[45] (SARS-CoV) [hydrochloride salt]
33	Promethazine	60-87-7	60	soluble	284.4	4.8	0	3	11.80[51] (MERS-CoV) [hydrochloride salt]	7.54[45] (SARS-CoV) [hydrochloride salt]
34	Astemizole	68844-77-9	149	5 mg/ml	458.6	6	1	5	4.88[51] (MERS-CoV)	5.59[45] (SARS-CoV)
35	Chlorphenoxamine	77-38-3	128	NA	303.8	4.1	0	2	12.65[51] (MERS-CoV) [hydrochloride salt]	20.03[45] (SARS-CoV) [hydrochloride salt]

36	Triflupromazine	146-54-3	25	soluble	352.4	5.54	0	6	5.76[51] (MERS-CoV) [hydrochloride salt]	6.39[45] (SARS-CoV) [hydrochloride salt]
37	Clomipramine	303-49-1	192	soluble	314.9	5.19	0	2	9.33[51] (MERS-CoV) [hydrochloride salt]	13.24[45] (SARS-CoV) [hydrochloride salt]
38	Imatinib mesylate	220127-57-1	211	Freely soluble	589.7	3	10	7	17.69[51] (MERS-CoV) [hydrochloride salt]	9.82[45] (SARS-CoV) [hydrochloride salt]
39	Dasatinib	302962-49-8	280	Slightly soluble	488	3.6	3	9	5.47[51] (MERS-CoV) [hydrochloride salt]	2.10[45] (SARS-CoV) [hydrochloride salt]
40	6-Mercaptopurine	50-44-2	313	0.2 mg/ml	152.18	0.01	2	2	26.9[43] (MERS-CoV)	NA
41	6-Thioguanine	154-42-7	>360	Slightly	167.19	-0.07	3	2	24.4[43] (MERS-CoV)	NA
42	N-Ethylmaleimide	128-53-0	45.5	50 mg/ml	125.13	0.9	0	2	45.0[43] (MERS-CoV)	NA
43	Silvestrol	697235-38-4	138	7 mg/ml	654.66	1.6	4	13	NA	0.003[52] (HCoV-229E)
44	Disulfiram	97-77-8	71	15 mg/ml	296.5	3.9	0	4	24.1[53] (SARS-CoV)	NA
45	8-(Trifluoromethyl)-9H-purin-6-amine (F2124-0890)	2993-05-7	349[a]	NA	203.12	1.6	2	7	10.9[54] (SARS-CoV)	NA
46	Ag7088 (Rupintrivir)	223537-30-2	170	NA	598.7	3.1	3	9	>50[55] (SARS-CoV)	NA
47	GC376	1416992-39-6	NA	NA	507.5	-3.07	4	8	4.35[55] (SARS-CoV)	NA

48	GC375	NA	662[a]	NA	488	0.95 [a]	NA	NA	4.66[55] (SARS-CoV)	NA
49	GC373	NA	477 [a]	NA	417	0.92 [a]	NA	NA	3.48[55] (SARS-CoV)	NA
50	SSAA09E3	52869-18-8	498 [a]	NA	327	3.7	1	3	NA	9.7[56] (SARS-CoV)
51	SSAA09E2	NA	412[a]	NA	300	1.18[a]	NA	NA	NA	3.1[56] (SARS-CoV)
52	SSAA09E1	5351-71-3	NA	NA	199.3	1.6	2	3	NA	6.7[56] (SARS-CoV)
53	Ciclesonide	126544-47-6	60	54 mg/ml	540.7	5.3	1	7	NA	EC_{90} (5.1)[57]

The 50% inhibitory concentration (IC_{50})

The clinical relevance of antiviral *in vitro* activity (defined as half-maximal effective concentration [EC_{50}])

[a]Predicted

From the list of APIs in Table 1, only cyclosporine has been formulated as an ultrafine solution pMDI.[58, 59] Furthermore, cyclosporine has been administered as a nebuliser in clinical trials,[60, 61] and dry powder formulations have been developed.[62] Cyclosporine has been used for the treatment of COVID-19 demonstrating an improved survival rate of the patients.[63] It has been suggested by other investigators to be evaluated in patients with COVID-19.[64, 65]

3. Regulatory Requirements to Bring an Inhaler of Approved Drug into Clinical Trials

Only ciclesonide is an approved ultrafine solution pMDI among APIs in Table 1,[66] but there are certain candidates in Table 1 that are approved drugs. The European Medicines Agency then requires the following *in vitro* tests on pMDIs prior to clinical trials.[67]

3.1.Physical characterisation

This is referred to the shape of the drug and excipient crystals, in addition to the propellant solubility, size distribution, charge, density and rugosity of the particles (related to the surface roughness of the particles and implies a deviation of the roughness profile from the main line[68]). However, physical characterisations are valid, when the formulation is a suspension pMDI, but for a solution pMDI propellant solubility would be more important.

3.2.Minimum fill justification

Minimum fill justification is related to the content of the inhaler. A pMDI should deliver at least the labelled amounts, and final doses should have the same fine particle dose as the other doses from the inhaler. It should be noted that USP states that the net weight of the pMDI should contain the labelled amounts of the drug.

3.3.Extractables and leachables

Leachables and extractables are impurities related to the product.[69] These compounds may be taken by the patient because of product contact with the packaging components such as gasket of pMDI valves, or during the manufacturing of the product. These impurities are safety concerns for patients. Controlled Extraction Studies are required to identify potential leachables.[70] Currently, there are manufacturers that supply pMDI valves such as Aptar Pharmaceuticals or aluminium canisters such as Presspart (Blackburn, UK). Usually, pMDI valve manufacturers have conducted these studies and optimised the valve components to ensure low leachable and extractable levels with a wide a range of formulations. For example, ethylene propylene diene monomer has been introduced to reduce levels of leachable and extractables.[71] Despite of these, leachable studies are required to ensure the safety of the pMDI formulation. The purpose of leachable studies is to establish a correlation between leachable and extractables. This would help to list the maximum amounts of leachables over product shelf-life. It should be noted that pMDIs have been identified as a drug product that there is almost 1:1 correlation between extractables and leachables.[72] To conduct the leachable studies, the Analytical Evaluation Threshold (AET) should be established, which usually is based on the Safety Concern Threshold of 0.15 µg/day. The AET is the limit that an analytical chemist should be able to detect a particular extractable/leachable. Then, for a cyclosporine pMDI with 200 actuations and six actuations per day, the estimated AET is 5 µg/can. Sample orientation should be considered in the leachable studies as when the content of pMDI is in contact with the valve (downward valve position) the level of leachable may be different from the opposite storage orientation (upward valve position). Furthermore, the storage conditions recommended by ICH Q1A (R2) are usually used for leachable studies. Minimum leachable study duration is six months at 40°C with 75% relative humidity.[73]

Routine Extractable Testing also should be conducted[74] to ensure that leachable levels and profiles are maintained within accepted limits.[70] This is important, as the valve

manufacturers may alter the manufacturing process and the residue of new chemicals on the valves such as processing aid additives may affect the aerosol performance of the pMDI. Then the leachables (both identity and quantity) should be evaluated by toxicologists to identify potential safety concerns allowing optimisation during product development process, and before stability studies.

3.4.Delivered dose uniformity & fine particle mass through container life

The pMDI inhaler should be tested for delivering the same dose from the first dose (post priming) until the last labelled dose.[67] The life of container can be divided into three stages: beginning, middle and end;[75] with three doses at the beginning (post priming), 4 doses at the middle, and three doses at the end of the container life.[76] The USP <601> indicates that 9 out of 10 doses should be in the range of 85%-125% of the labelled doses, and none outside of the range 65%-135% of the labelled dose.[76] However, it has been criticised that USP-compendia small sampling provides useful information only about the samples, and it may not be useful for large batches. Small sample sizes between 10-30 would be more suitable for less variable batches.[77] For delivered dose uniformity, sample shots may be taken at the beginning and end of container life. For example, 10 shots at the beginning and 10 shots at the end of container life for 10 canisters.[77]

3.5.Fine particle mass with spacer use

SARS-CoV-2 contaminates the nasal passage as well as the lungs. Therefore, spacers with facemasks could be useful for delivery of the drug to the lungs via the nasal cavity too. Spacers and valved holding chambers (VHCs) are devices that can be used with pMDIs. Spacers are large volume devices that provide a distance between the patient's mouth and pMDI actuator, as well as the ability to hold the aerosol cloud following the actuation of the pMDI. Spacers can be as simple as toilet-paper tubes. VHCs are like spacers in terms of size,

but they contain a valve that opens during inhalation and closes during exhalation. This is to avoid pushing the remaining aerosol cloud in the chamber to the environment. Therefore, the aerosol could stay within the chamber for next round of inhalation. VHCs can range in volume from 50-750 ml. VHC plus pMDI was attached to a standard cascade impactor inlet,[78] or an oropharyngeal model[79] and this assembly was connected to a cascade impactor to determine fine particle dose and aerodynamic particle size distribution.

VHCs are mainly designed to eliminate the coordination between inhalation and actuation for pMDIs and to reduce drug deposition in the throat. The aim of using VHCs for COVID-19 treatment is to target the virus in the nasal passage. Therefore, respiratory facemasks should be used with VHCs. The facemask should be comfortable for the patient.[80] An in vitro model has been developed to measure respirable dose from a VHC-facemask for a small child.[81] It should be noted that for the purpose of COVID-19 treatment, an updated *in vitro* method should also be developed to evaluate the use of a VHC-facemask for an adult. Nasal high flow (NHF) oxygen therapy may be used for COVID-19 patients as a replacement for mechanical ventilation. A pMDI with spacer can be linked to NHF therapy, and an *in vitro* method utilising an adult nasal cast has been developed to measure respirable dose.[82]

Clement International has introduced new A2A spacer, which is a pocket sized VHC. The A2A spacer has a volume of 210 ml and is also collapsible, which improves portability. This VHC is made from anti-microbial, low-static, transparent plastic.

3.6.Particle size distribution

Aerosol particle size plays a key role in penetrating into the lungs following oral/nasal inhalation. It is widely accepted that particles with the aerodynamic diameter in the range of 1-5 μm can penetrate the lungs via inhalation. However, for the purpose of COVID-19 treatment that aerosol particles need to reach the alveolar levels, the aerodynamic diameter

may be reduced to less than 1 µm,[83] which is also called extra fine particle fraction (EFPF),[84] a size that can be achieved by ultrafine aerosol drug delivery systems. The USP <601> provides details about the use of impactors to determine the aerodynamic particle size distribution of pMDIs. It has been shown that the use of oropharyngeal models provided fine particle masses from pMDIs closer to clinical evaluations,[79] instead of using the standard induction port. Therefore, these induction ports may also be used to ensure that required viral inhibitory doses reach the lungs, in particular the alveolar levels.

3.7.Single dose fine particle mass

It is required to measure the fine particle mass of a single dose. This can be challenging when the dose per actuation is low, and the analytical limits of detection and quantification are not low enough. As only fine particle mass is required, the abbreviated impactors (with only two stages) may be employed.[84] There is no indication about which single dose in the EMA guidelines, but FDA 2018 guideline states one dose from the beginning and one dose from the last labelled dose. However, it may be recommended to include the first dose, the middle and the final dose.[85] Then for 200-dose pMDIs the doses will be 1st, 100th, and 200th.

3.8.Actuator deposition

The amount of the drug deposited on the actuator must be determined, and the ex-valve dose should match the label dose claim. The drug deposition on the actuator depends on the actuator nozzle design.[86, 87] Furthermore, the type of actuator material (nylon, polyethylene terephthalate, polyethylene–high density, polypropylene copolymer, and tetrafluoroethylene) and nozzle design (exit orifice design: flat or cone) affected the charge of aerosol particles,[88] which consequently may change drug deposition on the actuator.

3.9.Shaking requirements

This test is for suspension pMDI, therefore, it is not further discussed here.

3.10. Initial and repriming requirements

The way that pMDI valves are designed, the pMDIs require priming before taking the first medication. This is because a drug dose is kept within the valve-metering chamber, before the pMDI actuation in readiness for subsequent use. Capillaries usually are used in the valves to fill the metering chamber. If the orifices of capillaries are greater than a threshold (based on the Rayleigh-Taylor theory), then the formulation may drain back from the metering chamber to the canister, if the pMDI is not used for a long-time.[89] Then, the amounts of the drug in the metering chamber may change, and as a result priming is required. Interestingly, the addition of ethanol to a pMDI formulation would reduce loss of prime due to the decrease in the vapour pressure of the formulation. This was clearly evident for the Qvar in comparison to the Ventolin.[89] Furthermore, the inclusion of ethanol in the formulation may reduce loss of prime due to the temperature cycling moving from a warm environment to a cold environment and vice versa.[90] Therefore, for solution pMDIs of cyclosporine (or any other active ingredients) that would contain ethanol, the loss of prime is less expected. It should be noted that patients may forget to prime the pMDI with at least one visible plume.[91] Therefore, as consistent doses would be required for COVID-19 treatments, then primeless valves such as Aptar DF30 valves would be more appropriate for these formulations.

3.11. Cleaning requirements

Powder residue forms on the pMDI actuator when the device is used. The accumulation of powder could affect the delivered dose. Therefore, a suitable cleaning procedure should be identified to maintain a consistent drug delivery from the pMDI under conditions of normal patient usage, in accordance with recommendations for priming, dosing intervals, and typical dosing regimen. Delivered dose uniformity, fine particle mass, and size

distribution data should be determined to support the cleaning instructions. Several types of cleaning methods have been identified. The patient leaflet information for the Qvar instructs to clean the pMDI using dry tissue or cloth to remove the drug deposition from actuator every week. The use of solvents like water is not recommended as the residues of liquid droplets in the actuator nozzle may affect the aerosol performance of the device. On the other hand, washing the actuator under warm running water and then drying are instructed for the Salamol pMDI. The patient leaflet states proper drying of the actuator and ensuring removal of water droplets from the actuator. The cleaning process should not alter the charge profile of the actuator, as this may lead to reduced emitted dose.[88, 92, 93] Spacers also need to be washed, when used with pMDIs. Detergent-washing the spacer with drip-drying is recommended.[94] This is to avoid electrostatic charges being generated on the surface of the plastic spacer, as the electrostatic charge decreased drug delivery.[95]

3.12. Low temperature performance

As aerosol delivery depends on the evaporation of the propellant, then the ambient temperature may affect the emitted dose.[96] It was shown that *in vitro* lung dose depended on the ambient temperature, with decreasing the lung dose by decreasing the ambient temperature.[97] In particular cold weather may change the size of generated aerosols for ultrafine solution pMDIs. The *in vitro* lung dose dropped to the 80% of labelled dose for an ultrafine solution pMDI like Qvar at 0°C compared to 20°C ambient temperature.[97] It was shown that the addition of ethanol to the pMDI formulation reduced variation in the delivered dose by 15°C change in the environmental temperature.[90] On the other hand, the emitted dose from a suspension pMDI did not change considerably by significant change in the environmental temperature.[98] Interestingly, the plume distance reduced by decreasing storage

temperature to -10°C.[99] It should be noted that the cold temperature of a pMDI plume cloud may cause patient discomfort, leading to reduced drug delivery to the lungs.[100] Therefore, in a cold whether the patient may experience more discomfort in using their pMDIs. However, the use of ethanol in the pMDI formulation would give a warmer plume compared to only HFA propellant.[100]

3.13. Performance after temperature cycling

The particles of suspension formulations may irreversibly agglomerate, a phenomenon called Ostwald Ripening. Temperature cycles may accelerate this irreversible particle agglomeration, as the solubility of drug generally tends to increase by increasing temperature and vice versa, and Ostwald Ripening can be accomplished readily by temperature cycling.[101] The temperature cycling can be for 6 weeks between -5°C and 40°C,[102] or 3-4 weeks between -10°C to -20°C and 40°C with a 12-hour cycle change.[103] Furthermore, high temperatures may lead to the leakage of valve silicon oil into the formulation and potentially affecting the aerosol particle size distribution. In addition, temperature variations may affect the loss of prime, and the addition of ethanol in the formulation may inhibit loss of prime.[90] Although it appears that the temperature cycle test would be more concerned of suspension pMDIs, the temperature cycle may affect the solubility of the drug in solution pMDIs, leading to precipitation of the drug.[101] Then, the drug particles may affect the pMDI valve performance.[102]

3.14. Effect of environmental moisture

EMA requires testing the effects of environmental moisture on the performance of pMDIs, although it is more relevant to capsule based DPIs. It was shown that high environmental humidity affected the initial atomization process and the early stages of aerosol generation.[104] However, it was shown that the evaporation of HFA 227ea was not

affected by changing the environment moisture from <10% to 100%. The addition of 15% ethanol did not change the evaporation rate.[105] On the hand, the *in vitro* throat deposition increased for HFA BDP solution formulation (containing glycerol) by increasing the relative humidity (RH) from 35% to 80%.[106] However, increasing the humidity from <10% to >98% was found to increase drug deposition from pMDIs in a holding chamber.[107] Interestingly, it was found that RH affected the morphology of generated particles from a solution pMDI with the formation of spherical particles in dry air, but porous particles in humid air (RH=50%).[108] The MMAD may change by humidity, if extensive porosity is created within the generated particles.[108] It was determined that droplet lifetime is highly correlated to the throat deposition.[109] Therefore, if the RH affects droplet lifetime, the delivered dose to the lungs would change.

3.15. Robustness

As pMDIs are portable, then the aerosol performance of inhalers should be tested under patient use conditions and accidental mechanical stresses, such as dropping the device or shocks. Furthermore, the devices require cleaning, which could potentially cause mechanical stresses during disassembling and re-assembling. Mechanical stresses (loads) have been applied alongside of the actuator (longitude), as the valve and the actuator nozzle are in this direction. Loads up to 60 kg in 10 to 20 kg increments were applied to both the Qvar and Ventolin pMDIs for two different type of packaging material: high-density-polyethylene and polypropylene. Application of 100 kg load collapsed the Ventolin pMDIs. It was found that loading of 60 kg decreased the ED from the Qvar 40 from 40µg to 28 µg. This was due to the substantial occlusion of the actuator nozzle. Although fine particle fractions (FPFs) remained at 69% before and after applying mechanical stress. While the Ventolin HFA showed decrease in ED at 80 and 90 kg loads. As before, FPFs remained relatively unchanged.[110]

3.16. Delivery device development

This is required when prototypes are used in clinical trials, and for mass production, different machinery is going to be employed. The pMDIs usually are assembled using small-scale bottle crimper.[59] Therefore, factory-produced pMDIs need to be evaluated for emitted dose, fine particle fraction and fine particle mass.

3.17. Inclusion of Dose Counter

It is required (FDA) or encouraged (EMA) by regulatory authorities to include dose counter for pMDIs. There are two purposes for the inclusion of dose counters:1) Informing the patient that device is running out of medication, as patients may continue using the inhaler after the active drug has been depleted.[111] 2) To prevent patients from over or under actuation of the labelled dose, as they may forget whether daily doses have been taken or not. It is required for dose counters to be accurate, which means one numerical value must increase when one dose is actuated. In addition, the dose counter should be rugged, which implies that the device should record accurately the number of doses when multiple actuations are taken. Finally, a dose counter should be ergonomic, which indicates that the dose counter should integrate well with the inhaler and should not affect handling or using the device, compromising patient compliance.[112] The dose counter mechanism should not allow the reset of counts when that last dose is taken. Preferably, the dose counter should prevent actuating the inhaler. The dose counter accuracy and ruggedness may be assessed by comparing the dose counter records with manual records. The ergonomics can be evaluated through user satisfaction assessments.[113] The usefulness of integrated dose counter has been shown with 92% of patients expressed satisfaction.[114] Although dose counters may record the actuation of the inhaler, they do not measure the inhalation of the aerosols. Therefore, electronic devices like the e-diary may be used with the inhaler.[112] However, if inhalers are

used for the treatment in a pandemic, then the use of e-diary devices may not be economically possible. Therefore, patients should be encouraged to use their inhalers through social media and broadcasts.

3.18. Stability studies

The stability of a pMDI should be studied for 24 months at 25°C and 60% RH as recommended by ICH Q1A(R2).[115] The sampling points could be month 1, 3, 6, 12, 15, 18, 21 and 24. For this evaluation, batch size should be > 1000 canisters.[115] The stability of cyclosporine was shown in pMDIs with Spraymiser™ valves over 24 months in either upright or inverted positions at room temperature.[59] Although it was found that the concentration of cyclosporine increased, due to propellant leak. The stability of cyclosporine pMDI (0.1% cyclosporine and 3% ethanol in HFA 227) was also shown; and the formulations were stored at room temperature (25°C) and 40°C with sampling points on 0, 14, 30 and 90 days.[58] These studies indicate an acceptable chemical stability of cyclosporine pMDIs. As there is an urgent need for non-immunological therapies for COVID-19 treatment, the accelerated stability studies could be conducted for duration of 6 months with environmental conditions of 40°C ± 2°C/75%RH ± 5% RH.[115]

4. Clinical Trials

As cyclosporine pMDI inhaler has not been tested before in humans, then the clinical trial would begin by recruiting about 30 infected subjects, and similar number as control subjects.[60] The inclusion criteria would be to have tested positive for COVID-19 and without or initial symptoms. When multicentre clinical studies are conducted, the key outcomes should be standardised to allow meta-analysis.[27] The trial can be a single-centre, double-blinded, and placebo-control.[60] After eligibility is confirmed and consent obtained, patients

should be allocated a unique patient identification number and assigned to one of two treatment groups according to a double-blind randomisation (1:1) schedule: active treatment (cyclosporine) or placebo (vehicle only), administered along with local standard-of-care treatment. Eligible participants should be aged 18 years or older, before admission to hospital. To conduct a desired clinical trial, the PREPARE guide helps to successfully develop the clinical trial from research questions to protocol completion.[116] It is important to set good research questions to solve clinical problems. Then, the primary outcome measure of the clinical trial could be "Reduction of the severity of the disease by using cyclosporine ultrafine pMDI in the patients that are at the early stages of the disease, and consequently reduction in hospital admissions over 14 days of drug administration". The secondary outcomes would be the rate of SARS-CoV-2 eradication after a certain period (7 days), proportion of clinical failure leading to hospital admission, reducing spread of the SARS-CoV-2 to family members when a patient has tested positive, and safety and tolerability of the investigational drug product. Safety data should be collected, which includes adverse events that occur during the treatment, severe adverse events, and premature discontinuation of treatment. The trial should be terminated prematurely if in the opinion of investigators, an unacceptable risk to the safety and welfare of patients is posed by the continuation of the study, as the result of reviewing the safety data. An individual trial patient should be discontinued permanently from the study for either pregnancy, patient withdrawal consent, or intolerable adverse events. To ensure patient safety, a Data Safety Monitoring Committee should be available to review and assess safety data/information as when required. Cyclosporine and placebo pMDIs should be supplied GMP grades for the clinical trials, which can be achieved by contract manufacturers.

A protocol has been published to compare the pneumatic exacerbations between ciclesonide administration and symptomatic treatment in COVID-19 patients and determine

the efficacy of ciclesonide.[117] This protocol was designed for a randomised controlled trial. The protocol considers measurements of SARS-CoV-2 genome copies over time between the control group and ciclesonide group. This can be achieved by performing RT-PCR on nasopharynx samples with a limit of detection (LoD) of about 100 copies of viral RNA per millilitre of transport media,[118] on Day 1, Day 2, Day 4, Day 8, Day 15, Day 22, and Day 29. Other assays may have LoDs much higher than 100 copies of viral RNA, which may lead to false negative test results. Adherence to the treatment is crucial for the reliability of the outcomes. This may be achieved by meeting (face-to-face when needed) for training the subjects in using the inhaler (and valved volume holding chambers), in addition to monitoring the progress of the disease. These meetings can take place when the subjects attend for sampling the nasopharynx for the viral load measurements.

5. Concluding Remarks and Future Work

Cyclosporine inhibits SARS-CoV-2 at low concentrations and has shown improved survival in COVID-19 patients, but with high oral doses leading to serious side effects. Therefore, an inhalation therapy of cyclosporine may benefit COVID-19 patients. The inhalation therapy of cyclosporine has been tolerated in lung transplant patients, using nebulisers. As SARS-CoV-2 causes respiratory complications at the lung alveolar levels, then an ultrafine pMDI formulation would deliver the desired therapeutic amounts to the alveoli and protect the lungs. Ultrafine pMDI formulation of cyclosporine has been developed, as well as DPI formulations. Therefore, regulatory studies need to be conducted for cyclosporine ultrafine pMDI to evaluate this formulation in Phase I/Phase II of COVID-19 trials. Although the COVID-19 pandemic may be over by the time of regulatory approval, coronavirus

infections are repeatedly happening. It has been indicated that cyclosporine may not be suitable for patients with COVID-19 because of its strong immunosuppressive properties. Alisporivir may be considered and this compound has physicochemical properties similar to cyclosporine, but without immunosuppressant effects.

The use of live attenuated vaccines may carry a risk of infection and should therefore be avoided during the use of immunosuppressive drugs such as siponimod. Hence, a non-immunological preventative medicine is needed for coronavirus infections.

6. Acknowledgements

I would like to thank Dr Lisa Spencer at Liverpool Aintree Hospital, Gonçalo Farias, and Adam Fisher at Aptar Pharmaceuticals for constructive discussions in developing this paper. Also I would like to thank Miss Tara Alamdari for proof-reading of the article.

7. Conflict of Interest

The author claims no conflict of interest.

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