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"Cannabinoid pulmonary drug delivery systems for the treatment of COVID-19"

Joseph Patrick McGrail Gámiz

Trabajo Fin de Grado

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ABSTRACT

The Coronavirus Disease 2019 (COVID-19), caused by the Severe Acute Respiratory Distress Syndrome (SARS-Cov-2), was identified for the first time in late 2019 in China, resulting in a global pandemic of enormous impact. Despite a fast development and implementation of vaccination strategies, and the scouting of several pharmacological treatments, alternative effective treatments are still needed. In this regard, cannabinoids represent a promising approach, since they have been proven to exhibit several immunomodulatory and anti-inflammatory properties, some of them in COVID-19 *in vitro* models. However, they present unfavourable physicochemical properties and side effects due to unspecific biodistribution, including psychotropic effects, which hamper their pharmacological use. These limitations can be overcome by the use of a convenient drug delivery system, and/or through a local modality of administration, specifically the pulmonary route. To this purpose, several key factors determining the efficacy of the technological design must be controlled. This review work intended to assess the feasibility and convenience of a cannabinoid-based pulmonary treatment for COVID-19, ultimately identifying the most convenient drug delivery system for the purpose. To that aim, pharmacological properties of cannabinoids with potential application in COVID-19, as well as the state-of-the-art on pulmonary drug delivery systems, were reviewed. Finally, in the light of the reviewed information, a choice of drug delivery system was rationally proposed.

Key words: COVID-19, cannabinoids, pulmonary delivery, drug delivery systems, drug inhalation device,

INDEX

ABBREVIATIONS

- Δ9-THC: Δ^9-tetrahydrocannabinol.

-ACE2: Angiotensin-converting enzyme 2.

- ARDS: Acute respiratory distress syndrome.

- CBD: Cannabidiol.

- CBDA: Cannabidiolic acid.

-CBG: Cannabigerol.

-CBGA: Cannabigerolic acid.

-CBN: Cannabinol.

-COVID-19: Coronavirus disease 2019.

-DPI: Dry powder inhaler.

-DSPE: Distearoylphosphatidylethanolamine.

-EAE: Experimental autoimmune encephalomyelitis.

-HCV: Hepatitis C Virus.

-MLR: Mixed Lymphocyte Reaction.

-MPs: Microparticles.

-MS : Multiple Sclerosis

-MSDC: Myeloid-derived suppressor cells.

-NPs: Nanoparticles.

-PLGA: Poly Lactic-co-Glycolic acid.

-pMDI: Pressurized metered dose inhaler.

-Poly(I:C): Polyinosinic:polycytidylic acid.

-PPAR: Peroxisome proliferator-activated receptors.

-PS:Polymersomes.

-SARS-CoV-2: Severe Acute Respiratory Distress Syndrome Coronavirus 2.

-SEB: Staphylococcal Enterotoxin B.

-SFCAs: Short Fatty Chain Acids.

-SLN: Solid Lipid Nanoparticles.

-S: Spike protein.

-THCA: Tetrahydrocannabinolic acid.

-THCV: Tetrahydrocannabivarin.

-TMPRSS2: Transmembrane serine protease.

-Tregs: Regulatory T-Cells.

FIGURES

1. INTRODUCTION

Coronavirus disease 2019 (COVID-19), which is caused by the Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), appeared in China in late 2019 from a zoonotic origin. Most cases were asymptomatic or developed only a mild disease. However, a substantial number of patients developed respiratory illness needing hospital care, some of them even presenting severe symptomatology leading to the use of ventilator support (Horby et al., 2021; Huang et al., 2020). This situation led to a global pandemic of enormous scale, resulting in a vast and tragic loss of lives. Many treatments have been applied, including interferon- α and anti-viral drugs like remdesivir (Adnan et al., 2020). Vaccines were designed and produced to fight off the virus, including novel ones such as mRNA-based vaccines that have been proven highly efficient and safe (R.Baden et al., 2021). Notwithstanding, due to the scale of the pandemic, alternative and effective treatments need to be considered. One of them is the use of cannabinoids.

Cannabinoids were firstly identified in the plant *Cannabis sativa*. Cannabinoids found in the very plant are named phytocannabinoids. This term distinguishes them from synthetic cannabinoids and endocannabinoids, the latter being chemically endogenous cannabinoid receptor ligands in mammals. The two most employed phytocannabinoids in the pharmaceutical field by far are Δ9-tetrahydrocannabinol (Δ9-THC) and cannabidiol (CBD) (Vuolo et al., 2019; Berman et al., 2018).

These chemical compounds have been, and are still being explored, as potential pharmaceutical treatments against a wide range of pathologies such as pain, inflammation, multiple sclerosis, cardiovascular disorders, anorexia, to mention a few. Their beneficial effects are mainly due to the activation of two plasma membrane G-protein coupled receptors, CB1 and CB2. (Berman et al., 2018). CBD, for instance, showed anti-inflammatory, analgesic, and immunomodulatory effects through the activation of these receptors. Specific CB2 activation led to a higher controlled release of proinflammatory cytokines, which was found to be useful in conditions that cause airway inflammation and fibrosis, such as asthma (Galiegue et al., 1995; Vuolo et al., 2019). These findings suggest a potential application of CBD in other respiratory syndromes, including COVID-19. Actually, cannabinoids, and particularly Δ9-THC and CBD, have been recently shown to be effective in helping alleviate the symptoms caused by severe cases of COVID-19 through several mechanisms, including reducing the inflammation caused by the cytokine storm, activating PPAR-γ receptors with immunomodulatory effects, and hampering SARS-Cov-2 viral replication and cell invasion (M.Anil et al., 2021; Raj et al., 2021; Esposito et al., 2021).

Nonetheless, cannabinoids present several limitations for the development of pharmaceutical formulations, such as unfavourable physicochemical properties and side effects due to unspecific biodistribution, including psychotropic effects (Brownjohn et al. 2012). Yet, these drawbacks can be overcome with an adequate drug delivery system to control their biodistribution. In addition, a local modality of administration can also contribute to that purpose. Being COVID-19 a respiratory illness, pulmonary administration stands out as highly convenient for a potential cannabinoid treatment, providing fast drug absorption, a localized pharmacological effect and avoidance of first pass metabolism (Chandel et al., 2019), all of them desirable features for a cannabinoid administration. However, several key factors must be considered when designing a pharmaceutical formulation for pulmonary administration, which highly determine the ultimate efficacy of the treatment (Babu et al., 2013).

2. OBJECTIVES

The aim of this work was to assess the potential feasibility and convenience of a cannabinoid-based pulmonary treatment for COVID-19, ultimately identifying the most convenient drug delivery system for the purpose. To that aim, pharmacological properties of cannabinoids with potential application in COVID-19, as well as the state of the art on pulmonary drug delivery systems, were reviewed.

With that overall purpose, the following partial objectives were stablished:

1. Searching and analysing studies on pharmacological properties of cannabinoids related to COVID-19

2. Reviewing current pulmonary drug delivery systems, with focus on their advantages and disadvantages

3. Identifying and comparing the up-to-date available inhalation devices for pulmonary delivery, with emphasis on their pros and cons

4. Concluding about the ideal choice of drug delivery system and inhalation device for a cannabinoid-based treatment against COVID-19

3. METHODOLOGY

This work was carried out with the aid of several scientific databases including Scopus and PubMed, which were accessed through the electronic resources from the web page of the University of Seville.

To perform the search on pharmacological properties of cannabinoids with potential application in COVID-19, the keywords ""Canna*" AND "COVID"" were used, limiting the search to their appearance in Title, Abstract and Keywords sections. Also, only research articles were selected in the search, while no restrictions of year of publication or language were applied.

To investigate the state-of-the-art drug delivery systems for pulmonary administration, the keywords "Pulmonary administration AND drug delivery systems" were used, limiting the search to their appearance in Title, Abstract and Keywords sections. Only works published in the last five years were included in the search, while of no restriction of language was applied. Additionally, the works from the resulting search output were individually selected on account of the impact factor (IF) from the publishing source. IF were consulted in the Journal of Citation Reports (JCR) from the Web of Science (WoS) database, accessed as well through the electronic resources from the web page of the University of Seville.

The searches were conducted from January to May 2021.

4. RESULTS AND DISCUSSION

4.1 Potential Applications of Cannabinoids in COVID-19

A total of 132 research articles were retrieved from the search on cannabinoids and COVID-19, all of them published in 2020 and 2021, clearly stating the relevance of the scope. Most of them were framed within the fields of medicine, pharmacology and alike (Figure 1), while the rest were mostly related to the field of behavioural sciences. The vast majority of the research activity was located in the USA (Figure 1).

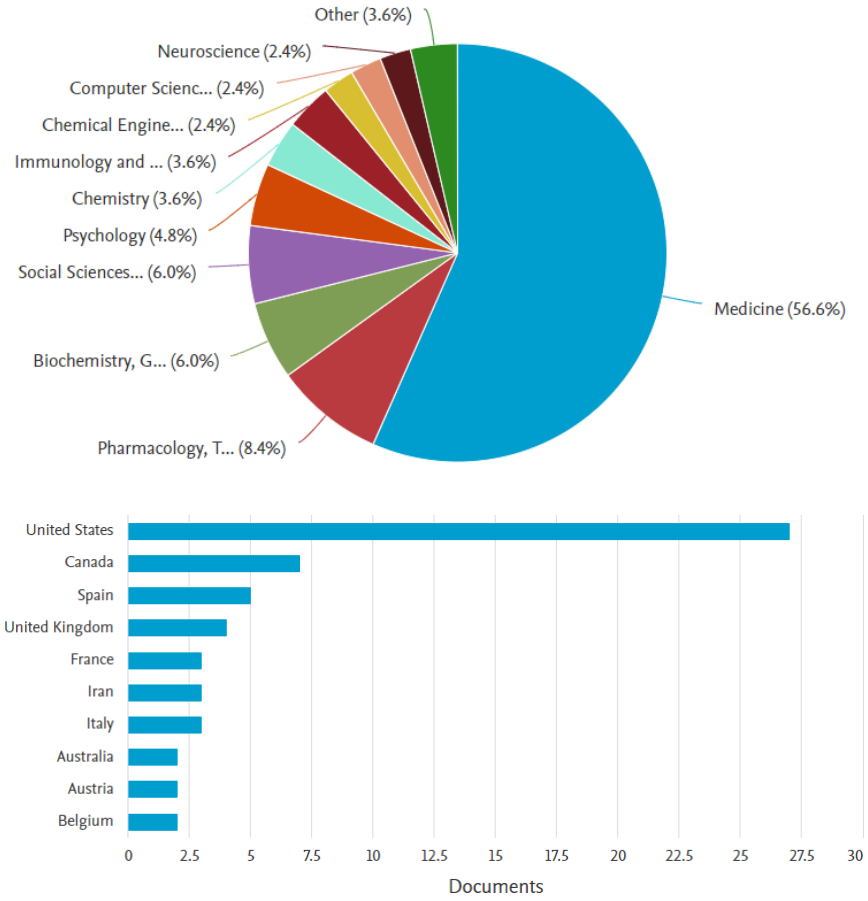

Figure 1. Number of articles published by subject area (top side of the figure) and by country/territory (bottom side of the figure). The graphics were retrieved from Scopus, May 2021

4.1.1 Downregulation of ACE2 and TMPRSS2 (Prophylaxis)

The infection mechanism of SARS-coronavirus 2 (SARS-CoV-2) involves the priming of spike (S) proteins by host cell proteases. S protein priming entails cleavage at the S1/S2 site and the S2´site, which leads to the fusion of the viral and cellular membrane. Specifically, the process takes place upon interaction of the S protein with angiotensin-converting enzyme 2 (ACE2) receptors and

transmembrane serine proteases (TMPRSS2). This mechanism was identified by analysing the similarities between SARS-CoV and SARS-CoV-2 (Figure 2) (Hoffman et al., 2020).

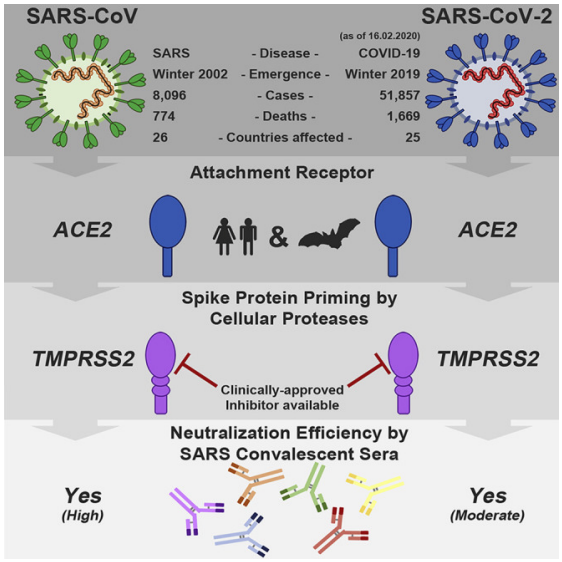

Figure 2. SARS-CoV-2 Cell Entry Depends on ACE2 and TMPRSS2 and Is Blocked by a Clinically Proven Protease Inhibitor (Hoffman, 2020).

Cannabidiol (CBD) is being considered for several pharmacological applications regarding SARS-CoV-2. An interesting potential application would consist of its use for prophylaxis; CBD extracts have been reported to cause down regulation of ACE2 and TMPRSS2 (Wang et al., 2020). This hypothesis was further confirmed with *in vitro* experiments; ACE2 expression was reduced in an alveolar epithelial (A549) cell line by CBD (M. Anil et al., 2021), indicating that the cannabinoid would potentially hamper SARS-CoV-2 infection. Mouthwashes with high concentrations of CBD were proposed for leveraging from this finding (Esposito et al., 2020).

4.1.2 PPAR-γ activation

Peroxisome proliferator-activated receptors (PPAR) are a large family of nuclear receptors/transcription factors, which modify the transcription of target genes upon activation. They are activated by steroids and lipid metabolites, such as CBD (O´Sullivan et al., 2010).

A specific type of PPAR, PPAR- γ, may be of interest since its activation leads to reduced pulmonary inflammation, which has been related to a faster recovery in viral respiratory infections such as influenza (Huang et al., 2019). Despite of being a different virus, SARS-CoV-2 can also lead to increased lung inflammation, which has been associated to most severe cases of

COVID-19 (Huang et al., 2020). Hence, PPAR- γ activation could also be a potential pharmacological target in this case.

Moreover, studies have shown that a significant number of patients who survived COVID-19 presented unresolved decreased lung capacity and pulmonary fibrosis (Esposito et al., 2020). Pulmonary fibrosis is categorised as an alteration in fibroblast phenotypes causing a disproportionate accumulation of extracellular matrix. Interestingly, *in vitro* experiments showed that the activation of PPAR- γ in lung fibroblasts led to an inhibition of a proliferative response (Milam et al., 2007).

CBD has been attributed with PPAR-γ agonist activity, which could be of use for limiting excessive lung inflammation, decreasing in turn the mortality rate of COVID-19 disease. Furthermore, it has been described as a weak agonist (Esposito et al., 2020), which would allow it to prevent the secondary effects associated to full PPAR-γ agonists. Full PPAR-γ agonists, as it is the case of thiazolidinediones, typically pose a higher risk of cardiovascular complications, such as stroke and heart failure (Graham et al., 2010). Thus, exploiting the properties of CBD as a weak agonist, minimizing the risk of undesirable side effects, may represent an interesting approach against COVID-19.

It should also be considered that the anti-inflammatory properties of CBD in the context of pulmonary fibrosis were also evaluated in mice subjected to induced allergic asthma. The mice treated with CBD presented reduced lung inflammation and a lower rate of fibrosis with respect to the control group (Vuolo et al., 2019).

Overall, these findings highlight the great potential of PPAR- γ agonists as a treatment strategy for COVID-19-related pulmonary fibrosis, a common long-term effect derived from the disease, and more specifically that of CBD. The weak agonist properties of this cannabinoid would offer the added value of minimized risk of side effects, typically attributed to full agonists.

4.1.3 Prevention of the Cytokine Storm and ARDS

The cytokine storm, or cytokine release syndrome, is a reaction that takes place when the human body is exposed to an infection or drugs that cause a disproportionate release of inflammatory cytokines (Dzobo et al., 2021). This cytokine storm is key factor in the development of the acute respiratory distress syndrome (ARDS) (Raj et al., 2021). ARDS arises from the excessive activation

of the immune system and, as mentioned before, the cytokine storm. It may result in respiratory and multi-organ failure, leading to death. Importantly, COVID-19 disease stems from the development of ARDS (Nagarkatti et al., 2020). Several strategies involving the use of cannabinoids as means of preventing and treating the cytokine storm, and therefore ARDS, have been identified.

Downregulation of the expression of inflammation-related genes

The capacity of cannabinoids to downregulate the expression of inflammation-related genes has been explored. Kovalchuk et al. (Kovalchuk et al., 2021) studied the effects of several cannabis extracts on UV-exposed artificial human skin. The resulting expression of genes encoding interleukins and pro-inflammatory cytokines with a significant role in the development of ARDS and associated mortality of COVID-19 was analysed. A weak correlation of Δ9-THC concentration in the extract with the downregulated expression of several genes was found, while the potential influence of CBD, cannabigerolic acid (CBGA) and cannabinol (CBN) concentration could not be either confirmed or discarded.

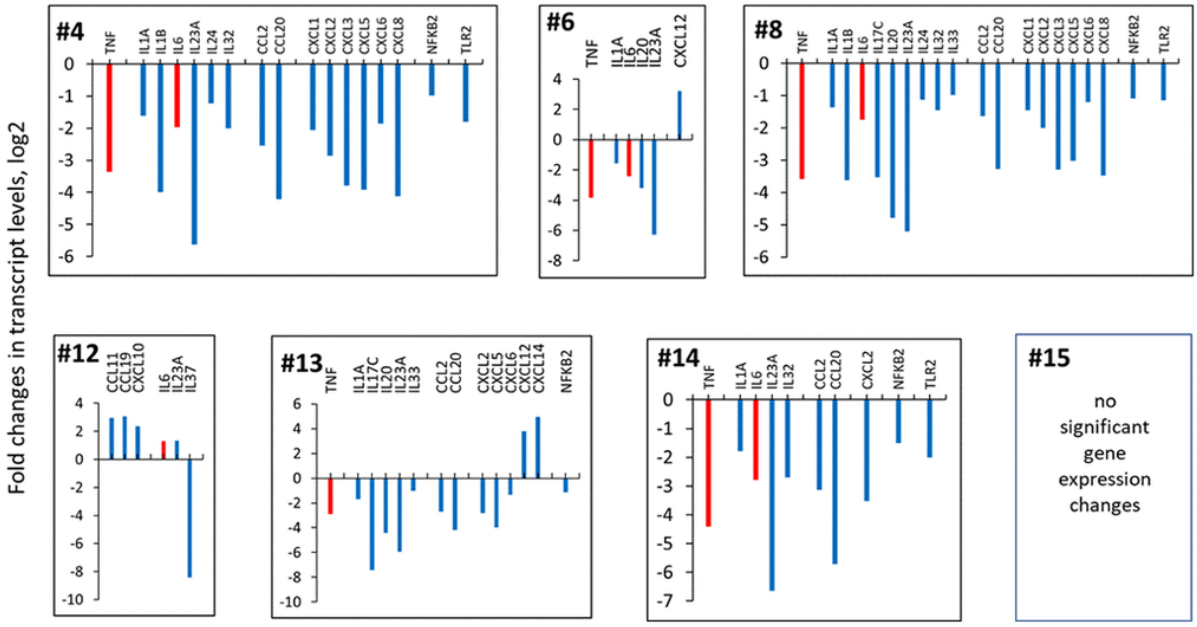

Figure 3. Graphic displaying analysis of global gene expression profiling of Artificial Human 3D skin EpiDermFT™ exposed to Ultraviolet (UV) and treated with different Cannabis Sativa extracts. Extracts 4, 6, 8, 13 and 14 highly downregulated genes such as pro inflammatory interleukins and cytokine, C-C motif chemokines and C-X-C subfamily cytokines that play a role in ARDS. Extract 12 upregulated the genes and 15 did not modify gene expression (Kovalchuk et al., 2021)

Even though the study did not use a tissue model reflecting the usual region plagued by inflammation, as in lung and alveolar tissue, the mechanisms of inflammation and fibrosis count with similarities (Kovalchuk et al., 2021), which point out the potential usefulness of cannabinoids in this scenario. It should still be considered that a SARS-CoV-2 inflammation tissue model should be used in further analysis.

Increase of immunoregulatory cells Tregs and MSDC

The potential effects of cannabinoids against the cytokine storm are also related to regulatory T-Cells (Tregs) and myeloid-derived suppressor cells (MSDC), (Nagarkatti et al., 2020). Tregs are a type of cells that regulate T-Cells anti-inflammatory properties (Robinson et al., 2015). MSDC are a type of cells derived from myeloid lineage that were originally discovered in cancer patients, and were more recently found at locations where inflammation was prevalent. These preferred locations suggested they play a role in suppressing and controlling the inflammatory response (Elliot et al., 2018). Furthermore, the activity of both of these types of cells activity have been shown to be linked to autoimmune disorders, and recent studies have shown a potential related pharmacological effect of cannabinoids (Nagarkatti et al., 2020).

Specifically, Elliot et al. showed CBD could play a role in autoimmune diseases such as multiple sclerosis. They induced experimental autoimmune encephalomyelitis (EAE) was in mice, and those that were treated with CBD presented far milder symptoms than the control subjects. This pharmacological effect was attributed to an observed profound increase of MSDC in CBD-treated mice, and, subsequently, a vast reduction in T-Cell proliferation. This hypothesis was further confirmed when MSDC depletion reversed the positive effects of CBD treatment (Elliot et al., 2018). This effect of CBD could be of use against COVID-19, where the induction of a MSDC would be a beneficial factor in the taming of the cytokine storm and the prevention of the associated excessive.

On the other hand, several findings pointed out the capacity of cannabinoids to induce the formation of Tregs (Nagarkatti et al., 2020). Specifically, Robinson et al. tested their effect on an organ graph rejection cell model, extracted from mice spleens and further subjected to a one-way Mixed Lymphocyte Reaction (MLR). Cell cultures treated with a CB2 selective agonist, O-1996, presented a far higher amount of Tregs than their control counterparts. Furthermore, IL-10 was also found to be boosted in cells treated with the CB2 selective agonist. Analysis showed that IL-10 plays an important role in the reduced proliferation of T-Cells and increase of Tregs. In

the light of these results, cannabinoids were stated as stimulators for the production of IL-10 and Tregs (Robinson et al., 2015)

Overall, based on the involvement of Tregs and MSDC in the reduction of inflammation the reported effects of cannabinoids could be exploited for the treatment of COVID-19. In order to assess their potential, further research should be conducted in COVID-19 patients, including the analysis of Tregs and MSDC levels in response to cannabinoids.

Decrease of infiltrating MNCs

Mono Nuclear Cells (MNCs) are immune cells that play an important role in inflammation processes, including the cytokine storm associated to COVID-19. For instance, a lower number of MNCs and T-Cells in the lungs are expected to provide COVID-19 patients with a lower chance of suffering ARDS (Nagarkatti et al., 2020).

Interestingly, Δ9-THC was found to stimulate apoptosis in MNCs that infiltrate the lungs in mice immune system stimulated by Staphylococcal Enterotoxin B (SEB). A variety of genes related to apoptosis were increasingly expressed, including those encoding caspases, mitochondrial apoptogenic protein 1 and mitochondrial c oxidases (Mohammed et al., 2020). Not only MNCs are induced to apoptosis by Δ9-THC but activated T-Cells as well (Nagarkatti et al., 2020). Once again, this observed effect could be exploited as a treatment strategy for COVID-19.

Increased expression of apelin

Apelin is an endogenous multi-functional ligand that interacts with a type of G-protein coupled receptor known as APJ. Subsequently, the apelinergic system is activated, which results in a suppression of several molecules involved in the immune system, including cytokines that favour the induction of inflammation, such as CCl2, CCL3, CCL4, CCL7 and TNF-α. Given the above-commented association between COVID-19 and the risk of developing the ARDS, targeting a molecule significantly involved in the inflammation process may represent a useful strategy, and CBD was considered as a candidate drug for this purpose (Lopes Salles et al., 2020).

In the work by Lopes Salles et al., ARDS was induced to mice by administration of Polyinosinic:polycytidylic acid (Poly(I:C)). and subsequently several markers of inflammation, including apelin, were evaluated. The study comprised three animal groups; i) a non-ARDS-induced control group; ii) a poly(I:C) ARDS-induced group; iii) a poly(I:C) ARDS-induced group

treated with CBD. At the end of the study, the blood and lung tissue from the animals were evaluated, yielding the results presented in Figure 5 (Lopes Salles et al., 2020).

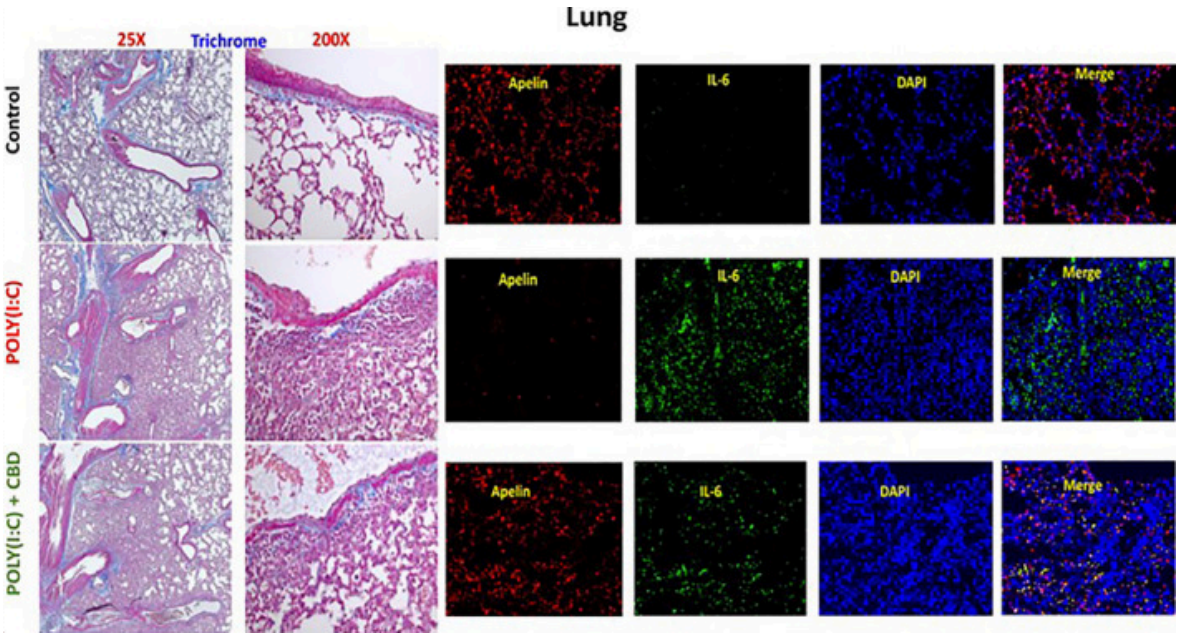

Figure 4. Microscopy images from Masson´s trichome analysis of lung tissues displayed that post intranasal administration of elevated dosage of Poly (I:C) led to elevated devastation of the lung tissue, pulmonary oedema, hypertrophy and fibrosis, while CBD treatment helped maintain the original) state, represented in control image (Images on the left). Immunofluorescence analysis revealed that CBD contributed to maintain normal Apelin levels compared to Poly (I:C) treated tissue, and reduced the amount of IL-6, a pro-inflammatory cytokine (Images on the right) (Red: apelin; green: IL-6; DAPI: cell nuclei) (Lopes Salles et al., 2020).

Figure 4 shows the results of the histological study, where differences between the assayed groups can be observed. Tissue microscopy images (left side of the figure) showed significant inflammation, fibrosis and pulmonary edema in the poly(I:C)-induced group when compared to the control group. In addition, the CBD-treated poly(I:C)-induced group presented a similar appearance to the control group. Immunofluorescence analysis was carried out to confirm the observations. The results (right side of the figure) indicated that the tissue from the poly(I:C)-induced CBD-treated group had a higher amount of apelin along with a reduced amount of IL-6, a pro-inflammatory cytokine, than the poly(I:C)-induced, non-treated group. It is also worth considering that the amount apelin in the CBD-treated group was similar to the control group (Lopes Salles et al., 2020).

Altogether, the results point out the potential usefulness of CBD in the onset of ARDS of COVID-19 patients on account of its capacity to improve lung structure and reduce lung fibrosis and edema, while apelin could constitute a highly useful biomarker of ARDS (Lopes Salles et al., 2020).

Regulation of miRNA expression and histone modifications

Regarding epigenetics, cannabinoids also play a role in regulating the expression of miRNA, and they are responsible for histone modifications in immune cells as well (Nagarkatti et al.,2020). These alterations constitute another pathway for reducing inflammation, which could be of use in reducing the swelling caused by the cytokine storm in COVID-19 patients with severe symptoms.

miRNAs are known as non-coding RNA. They are known to have an important role in the regulation of the immune response. Their regulation of gene expression involves their direct binding to specific sequences located at the 3'UTR region specific mRNAs, leading to their disintegration (Mohammed et al., 2020). Specifically, miR-19, -210 and -223 are linked to pro-inflammatory activity, while fewer miRNAs are known to be anti-inflammatory. For instance, miR-181c is considered to belong to the latter. Research revealed that CBD treatment in mice with EAE downregulated an elevated number of miRNAs linked to inflammatory effects. On the other hand, it upregulated a certain number of miRNAs associated to anti-inflammatory properties (Yang et al., 2019). These results are depicted in Figure 5.

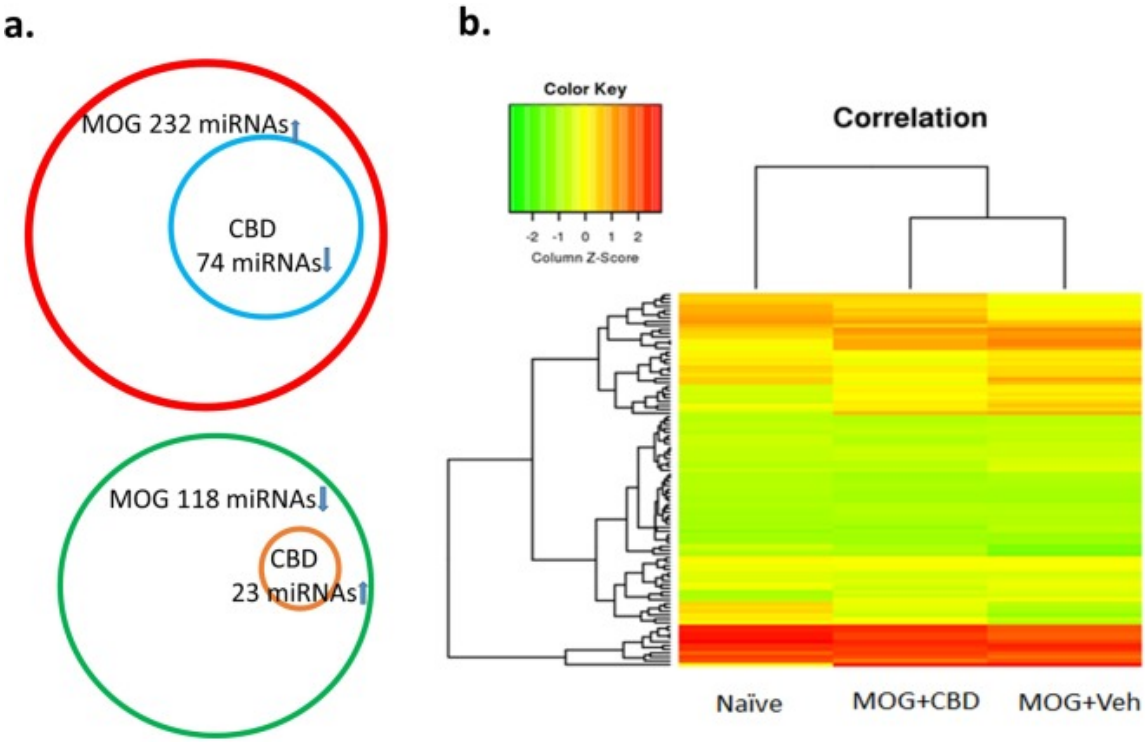

Figure 5. Representation of the expression of microRNA in CD4+ T cells. Myelin Oligodendrocyte Glycoprotein (MOG) with vehicle (Veh) was added to CD4+ T cells to simulate autoimmune disease. A portion of these cells were then treated with CBD and both were compared to a naïve group of cells. The expression of miRNA was obtained by microarray.

a. *Of the 232 MOG induced miRNAs, CBD supressed the expression of 74 of them (top image). MOG caused the suppression of 118 miRNAs and CBD induced 23 of these supressed miRNAs (bottom image).* ***b.*** *Image represents a heat map displaying the expression levels of 1910 miRNAs comparing the three different groups (Yang et al., 2019).*

Through the research of Yang et al, mice with EAE presented 232 miRNAs induced in their activated T-Cells and 118 miRNAs inhibited. CBD suppressed 74 of these induced 232 miRNAs, and reversed the inhibition of 23 out of 118 miRNAs. The decrease of pro-inflammatory miRNAs and increase of anti-inflammatory ones helped reduce the severity of EAE symptoms (Yang et al., 2019). Now, we shall look at the effect of CBD on histone modifications in immune cells.

Additionally, investigations on the influence of CBD on histone modifications shed light on the influence of this mechanism on reducing inflammation. The previously mentioned study performed by Yang et al. analysed the methylation of two histone marks in CD4+ T cells from the EAE-bearing mice; H3K4me3, which induces transcription activation, and H3K27me3, which leads to transcription suppression. Overall, the extent of methylation was not significantly altered, but

the genes that underwent methylation of their histones did. Specifically, CBD led to reduced coverage of H3K27me3 in genes related to anti-inflammatory interleukins IL-4, IL-5 and IL-13, and also resulted in increased coverage of H3K4me3 in the same genes, overall leading to an increased expression of these anti-inflammatory cytokines (Yang et al., 2019).

To sum up, the works by Yang et al. and Mohammed et al. helped elucidate the epigenetic mechanism by which CBD is involved in gene expression leading to controlling inflammation, specifically regarding miRNA expression and histone modification, which could be applied to the treatment of the COVID-19 cytokine storm.

Microbiota protection

COVID-19 patients present significant alterations in gut microbiota. This dysbiosis leads to fewer bacterial symbionts along with an excessive amount of potentially pathogenic bacteria. For instance, *Faecalibacterium prausnitzii*, an anti-inflammatory bacterium, was present in smaller amounts. Moreover, bacteria that downregulate the expression of ACE2, used as the gateway for SARS-CoV-2 cell internalization, were also present in a lower extent (Zuo et al., 2020). Overall, this dysbiosis has been associated with a higher predisposition to SARS-Cov-2 infection and worse disease prognosis.

An interesting aspect of cannabinoids is their effect on microbiota. Δ9-THC and CBD were found to elevate the amount of Short Fatty Chain Acids (SFCAs) in the organism, which are metabolites manufactured by the gut microbiome, including *Faecalibacterium prausnitzii,* with several significant roles in the immune system (Parada Venegas et al. 2019). This was revealed in a study by Al-Ghezi et al., who used a murine model of multiple sclerosis (MS) as inflammatory disease, where SFCAs decreased the formation of pro-inflammatory Th17 and elevate the number of Treg cells, which, as above mentioned, regulate cells such as Th17 cells (Al-Ghezi et al., 2019).

Based on these observations, Δ9-THC and CBD could be useful in controlling excessive inflammation in inflammatory diseases such as MS, which could be potentially translated into treatment strategies against COVID-19.

Effects on COVID-19 models: blocking of SARS-Cov-2 replication and decrease of pro-inflammatory cytokines

While previous sections highlighted the anti-inflammatory properties of cannabinoids assayed for several inflammatory diseases, that could be potentially applied in the management of the cytokine storm and subsequent ARDS developed in COVID-19, this section presents an overview of the results from assessing their pharmacological potential directly in COVID-19 cell culture models.

Recent research by Raj et al. analysed the mechanism by which cannabinoids interfere in the replication of SARS-CoV-2 and lead to its inhibition, with focus on their action over SARS-CoV-2 M^{pro}. SARS-CoV-2 M^{pro} is a protease with a critical role in the lifecycle of the virus; it cleaves translated RNA viral polyproteins, which results in 12 non-structural proteins such as Nsp4 and Nsp16, which play an important role in the replication process. As a consequence, SARS-CoV-2 M^{pro} is considered the most convenient molecular target to block coronavirus replication. To investigate the potential role of cannabinoids, a virtual screening of 32 different types of cannabinoids was carried out to analyse which ones were able to interact with SARS-CoV-2 M^{pro}. The results indicated an interaction of five cannabinoids; tetrahydrocannabinolic acid (Δ9-THCA), Δ9-THC, CBN, CBD, and cannabidiolic acid (CBDA). Consecutively, these molecules were evaluated *in vitro* to measure their antiviral effect against SARS-CoV-2 (Raj et al., 2021).

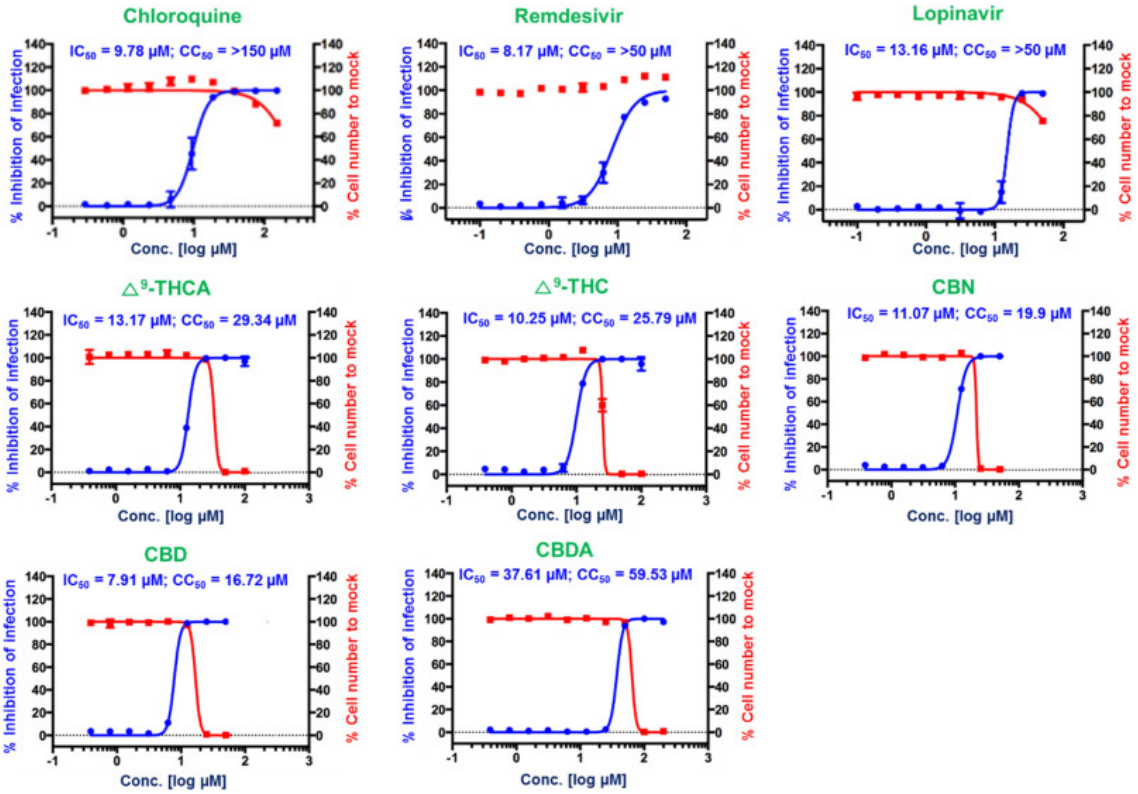

Figure 6. Graphics presenting the dose-response curve analysis of three control drugs (Chloroquine, Remdesivir and Lopinavir) and five different cannabinoids: Δ^9-tetrahydrocannabinolic acid (Δ9-THCA), Δ^9-tetrahydrocannabinol (Δ9-THC), cannabinol (CBN), cannabidiol acid (CBDA), and cannabidiol (CBD). Blue circles represent the inhibitory concentrations against SARS-CoV-2 infection. Red squares represent cell viability, allowing to measure potential toxicity. Half maximal inhibitory concentration (IC50) was used to compare the effectiveness of the SARS-CoV-2 inhibition. Δ9-THC and CBD stood out on account of their low IC_{50}. (Raj et al., 2021)

The results from the evaluation (Figure 6) indicated a highly effective antiviral activity of CBD and Δ9-THC, with IC50 values of 7.91 and 10.25 respectively. In addition, in terms of IC50, CBD outperformed chloroquine, remdesivir and lopinavir, drugs currently used in the treatment of COVID-19 (Raj et al., 2021).

Following this research, M.Anil et al. evaluated the anti-inflammatory effects of cannabinoids over immune response markers associated to COVID-19. To do so, two *C.sativa* extracts were evaluated, namely $F_{CBD,}$ composed of cannabinegerol (CBG), tetrahydrocannabivarin (Δ9-THCV) and mostly CBD.; and $F_{\Delta 9\text{-THC}}$ (composition?). While the later exhibited low anti-inflammatory effects in the epithelial lung cancer cell line A549, F_{CBD} was assayed in the same cell model with

interesting outcomes. The results indicated a decreased secretion of pro-inflammatory cytokines IL-6 and IL-8 in response to the extract, which is depicted in Figure 8.

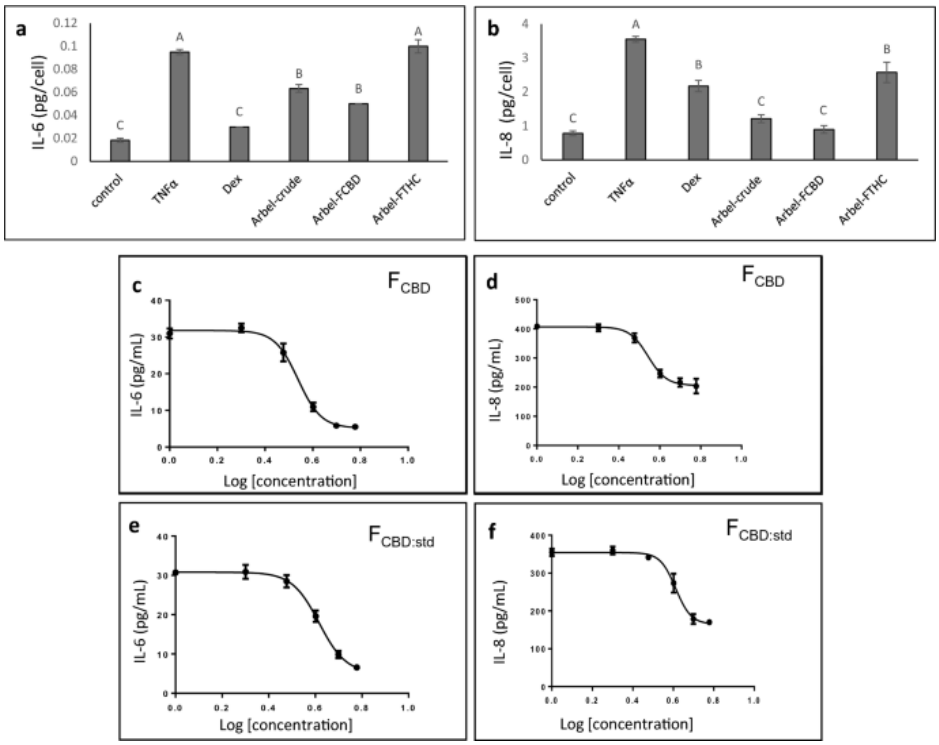

Figure 7. ***a*** *and* ***b.*** *Graphic presenting the levels of IL-6 (a) and IL-8 (b) in the epithelial lung cancer cell line A549. Cells were treated with TNFα to increase interleukin concentration, and subsequently treated with different* C. sativa *extracts to analyse their effect on IL levels. Dexamethasone was used as positive control to compare the anti-inflammatory effects.*
c *and* ***d****. Dose-effect curves representing the decrease of IL-6 and IL-8 levels when A549 cells were exposed to cannabidiol fraction (F_{CBD}).* ***e*** *and* ***f****. Dose-effect curves representing decreased concentration of IL-6 and IL-8 when A549 cells were exposed to standardized cannabidiol fraction ($F_{CBD:std}$), $F_{CBD:std}$ being a combination of phytocannabinoids in the same ratios found in F_{CBD} without other chemical compounds (M.Anil et al., 2021).*

Figure 7 shows a significant decrease in the levels of IL-6 and IL-8 in response to $F_{CBD.}$ Being these two molecules the very major cytokines involved in the COVID-19 cytokine storm, their decrease suppports the potential of cannabinoids as treatment strategy. It should also be considered that other pro inflammatory cytokines, such as CCL2 and CCL7, were decreased as well (M.Anil et al., 2021).

As above mentioned in section "Downregulation of the expression of inflammation-related genes", the studies on artificial human 3D skin where CBD led to a decrease in pro-inflammatory cytokines IL-6, IL-8, CCL2 and CCL7 suggested a potential application of CBD as anti-inflammatory candidate (Kovalchuk et al., 2021), even though the experiments were not carried out in a lung tissue model. At this point, it is worth mentioning that these studies by M.Anil et al., where the same cytokines were decreased in a lung cancer cell model, further support the potential translation of the anti-inflammatory properties of cannabinoids assayed in several inflammatory models to treatment strategies for COVID-19. Certainly, further research on COVID-19 models should be carried out.

4.2 Potential Pulmonary Drug Delivery Systems for Cannabinoids in Covid-19

Even though cannabinoids offer a wide range of pharmacological properties, it must be mentioned that they also present several limitations for their pharmaceutical use, such as unfavourable physicochemical properties (low stability, oily resin nature) and side effects stemming from unspecific biodistribution (Brownjohn and Ashto, 2012).
On one hand, Δ9-THC causes psychoactive effects due to its agonist action over CB1 receptors, highly expressed in the central nervous system (Nagarkatti et al., 2020). Despite these adverse effects, its agonist action over CB1 and CB2 receptors located in immune cells provide it with potent anti-inflammatory effects compared to other cannabinoids. Moreover, Δ9-THC is currently approved by the FDA, and it is used in several cases such as avoiding nausea and vomiting in cancer patients (Mohammed et al., 2020).

On the other hand, CBD is a non-psychoactive cannabinoid that is hence better suited for the development of treatment strategies, since it does not present significant affinity to the CB1 receptors highly expressed in the central nervous system. The downside to CBD is its less potent anti-inflammatory effect, due to its partial agonist effect over CB2 receptors. Indeed, its pharmacological effects are mostly considered to be due to its agonist activity on PPAR- γ receptors, among others (Nagarkatti et al., 2020).

To overcome these limitations from cannabinoids, drug delivery tools can be used for a targeted delivery at the region of interest within the organism, thereby achieving a greater efficacy along with minimized side effects related to undesired biodistribution. For instance, cannabinoid

loaded nanoparticles (NPs) have been produced and evaluated in this regard with promising results (Duran-Lobato et al., 2014; Martin-Banderas et al. 2015). Additionally, local administration modalities can also contribute to maximize the amount of drug reaching the target area while minimizing its presence at inconvenient locations. Particularly, pulmonary administration received special attention for treatments against diseases affecting mainly the lungs, as it is the case of COVID-19.

Pulmonary delivery offers several advantages over traditional systemic delivery on account of its localized, non-invasive nature; direct access to the lungs, decreased risk of development of bacteria resistance against antibiotics, minimized drug side effects, faster absorption, and avoidance of first-pass metabolism (Laube et al., 201). Nonetheless, some disadvantages must be also taken into account, such as the need to use an inhaler device (Chandel et al., 2019).

Altogether, pulmonary delivery represents an attractive modality of administration for cannabinoids as potential COVID-19 treatment, aiming at maximizing their effects while preventing potential psychoactive effects arising from their biodistribution to the central nervous system (Laube et al., 2011, J.Watson et al., 1999). In that regard, a pulmonary drug delivery system would be mandatory. For a successful drug delivery system design, several key factors determining the efficacy of aerosols in reaching their intended destination must be considered, including formulation and particle size of the aerosol, and the delivery device (Chandel et al., 2019).

Next sections will discuss these key factors, with focus on ultimately proposing the most promising drug delivery system prototype for the pulmonary administration of cannabinoids as COVID-19 treatment.

4.2.1 Particulate Drug Delivery Systems

Pulmonary administration requires the use of particulate formulations to ensure an adequate transport within the lungs (Groneberg et al., 2003). Thus, most relevant particulate drug delivery systems are reviewed in the following subsections.

4.2.1.1 Microparticles

Microparticles (MPs) are particles with sizes in the micrometre scale (1-1000 μm). Their physiochemical properties can be tuned to better suit the drug to encapsulate and its intended fate. These properties including their shape, molecular weight, size, surface charge, and hydrophobicity (Osman et al., 2018).

Compared to other systems of lower particle size, such as NPs, MPs offer higher drug loading capacity due to their higher volume, present more extended-release time and, importantly, they have the ideal size for aerosol deposition in the lower respiratory tract (Hu et al., 2018; Wang et al., 2018). Nonetheless, they are also limited by some disadvantages compared to NPs; MPs size hampers them from reaching the alveolar region, since the size required to reach this area is around 0.5 μm. Furthermore, they are susceptible to phagocytosis by alveolar macrophages, limiting the treatments efficacy. Aiming at combining the advantages of both MPs and NPs, MPs loaded with NPs, these in turn loaded with the drug of interest, are receiving increasing attention as a delivery strategy (Osman et al., 2018).

4.2.1.2 Nanocarriers

Nanoparticles

The term nanoparticles (NPs) generally refer to particles or entities within the manometer scale (1-1000 nm) (Osman et al., 2018). Their use as drug delivery systems, also known as nanocarriers, deeply influenced the pharmaceutical technology field on account of the advantages they offer (Torchilin et al. 2012).

Nanocarriers offer a high drug loading capacity and sustained release, which protects the loaded molecule from enzymes, increasing its stability, and delivers it at the target area, including locations in the lung. This performance results in reduced dosing and a better patient compliance. In addition, they can be easily prepared from a wide range of natural, polymeric biodegradable and biocompatible materials, such as poly (lactic-co-glycolic acid) (PLGA), polyethylenimine (PEI) and alginate (Osman et al., 2018; Babu et al., 2013), as well as lipids (Paliwal et al. 2020). Finally, they are usually amenable to surface chemical modifications to allow for an optimized interaction with the desired biological targets, and hence improved biodistribution (Osman et al. 2018).

However, nanocarriers also have some limitations to overcome in their design and production. Firstly, the production process or the degradation products from the polymers above mentioned

may alter or denature a loaded macromolecule. Secondly, several physicochemical parameters determining their successful targeted delivery must be accurately controlled, including an adequate size, inner structure, surface chemistry and ensured biocompatibility. Failing in this endeavour may result in nanocarrier aggregation, adsorption of proteins onto their surface, and inadequate intracellular tracking, ultimately reducing their efficacy (Babu et al., 2013).

Providing the above-mentioned parameters are conveniently selected and controlled, nanocarriers constitute a valuable drug delivery system for cannabinoid-based treatments against COVID-19. Prior works on cannabinoid-loaded NPs tailored for several administration routes and specific biological interactions, conducted by our research team (Martín-Banderas et al., 2012; Durán-Lobato et al., 2014), support further research on this approach.

4.2.2 Lipid Vesicular Delivery Systems

Liposomes

Liposomes are vesicles constituted by phospholipid bilayers, containing an aqueous core with a size ranging from the micrometre to the nanometer scale. Their bilayers are usually composed of natural or synthetic lipids that usually presenting neutral or anionic charge, whereas cationic charge lipids are included in the composition for gene delivery purposes. Single bilayer or multilayers liposomes can be produced for a higher controlled release and improved loading capacity. Liposomes have been widely used for pulmonary administration (Osman et al., 2018; Vyas et al., 2004). The hydrophobicity nature of their bilayers would make them a convenient cannabinoid delivery system considering the lipidic nature of these compounds. Their disadvantages include a questionable mechanical stability and robustness when compared to other lipid vesicular and polymeric delivery systems (Moretton et al., 2015).

Polymersomes

Polymersomes (PS) are vesicles on the manometer scale, formed by self-assembly of amphiphilic block copolymers. Their structure comprises a hollow sphere covered by a bilayer membrane. In this membrane, the hydrophilic polymer domains are in contact with the internal aqueous core and the external aqueous environment, while the hydrophobic polymer domains remain inside the bilayer. They were recently considered a multipurpose nano- delivery system due to their capacity to a accommodate a wide spectrum of drugs due to their composition. Advantages of PS include their improved colloidal and mechanical stability in comparison to liposomes, and the

easiness to chemically modify their polymeric surface (Moretton et al., 2015). Considering PS as a cannabinoid carrier candidate, their hydrophobic regions would contribute to drug loading while the hydrophilic polymeric surface would open possibilities for chemical tuning and specific targeting.

Polymeric micelles

Polymeric micelles, such as those composed of PEG-phospholipids, are polymeric carriers with amphiphilic characteristics. PEG-phospholidpids count with a hydrophilic PEG block and a hydrophobic distearoylphosphatidylethanolamine (DSPE) block. When in contact with aqueous media, a micellar structure is formed. PEG remains at the outer side of the micelle, protecting it from phagocytic uptake, while the DSPE block constitute the nucleus of the micelle. These lipid vesicular delivery systems exhibited the ability to slowly release loaded molecules over an extended period of time, owing to its composition. Polymeric micelles present the advantages of being apt for pulmonary ~~r~~administration and the capacity to control drug release (Gill et al., 2011).

Niosomes

Niosomes are vesicular systems constituted by non-ionic surfactants, such as Span 60 and Span 85. They are osmotically active, moderately stable, and capable to stabilise entrapped drugs. Niosomes are preferentially distributed to the reticuloendothelial system, where the phagocytic cells are present. Therefore, locations presenting higher of these nanocarriers include the liver, spleen and, most importantly, lungs. Therefore, niosomes are convenient drug carriers for diseases that require the targeting of these organs (El-Ridy et al., 2013). Similarly to other nanocarriers, they offer the advantages of being a pulmonary delivery system, though they also share the same disadvantages as some previously mentioned nanocarriers, such as limited stability (Chandel et al., 2019).

4.2.3 Drug Delivery Devices for Inhalation

Effective pulmonary administration requires a complementary interaction between the drug formulation, the patient, and the inhaler device. Incorrect use of the device is the main reason leading to reduced treatment efficacy, which is usually due to incomplete knowledge in how to use the device or a not coordinated activation of the device with inhalation (Laube et al., 2011).

Inhalation devices are generally categorized as nebulizers, pressurized metered dose inhalers (pMDI) and dry powder inhalers (DPI). Each of them present specific advantages and disadvantages, which will be discussed below, aiming at selecting the most convenient option for a cannabinoid-based treatment for COVID-19.

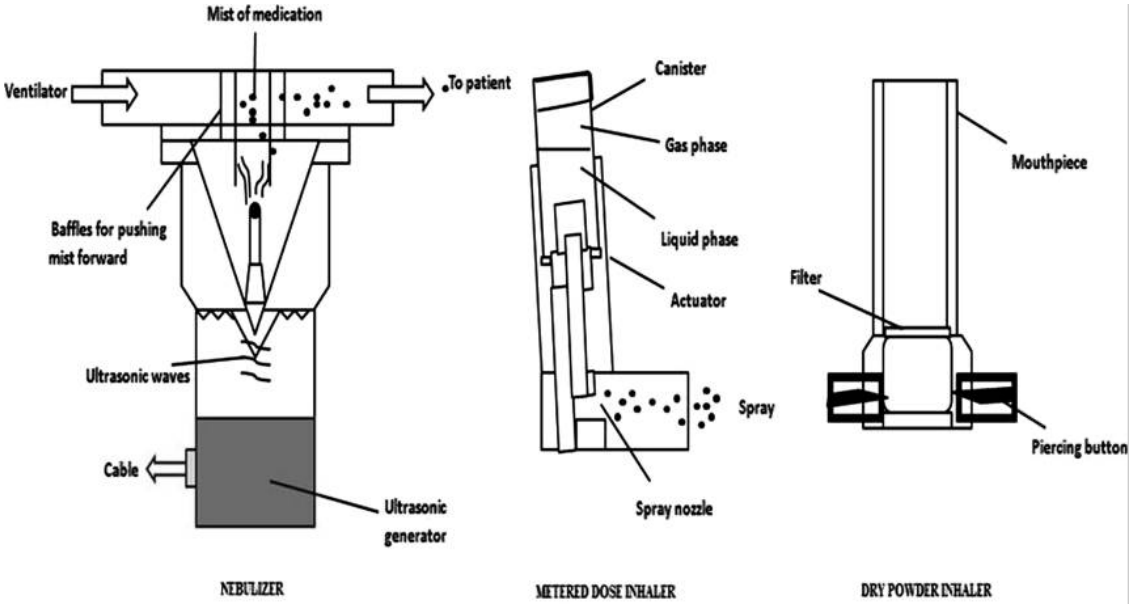

Figure 8. Diagram displaying the structure of three different inhalation devices: Nebulizer, Pressurized Metered Dose Inhaler (pMDI) and Dry Powder Inhaler (DPI) (Jin et al., 2020).

Nebulizers

Nebulizers are a type of inhalation device that produce a polydisperse aerosol, where most of the drug amount is contained in particles ranging from 1 to 5 μm in diameter (O´Callaghan et al., 1997). There are two types of nebulizers depending on how the atomization generating the aerosol takes place (Hess et al. 2008). A first type, the jet nebulizer, employs compressed air for atomization. In this case, the air passes rapidly from the compressor through a small hole (Venturi) to a bigger chamber where its rapid expansion causes a negative pressure. As a result, the liquid is sucked up in the form of ligaments that collapse into droplets. Larger particles impact against a structure known as baffle and return to get re-nebulised. The second type, the ultrasonic nebulizer, generate aerosols with a rapidly vibrating piezoelectric crystal that create waves inside the solution containing the drug. In turn, aerosols are released from the waves (Ibrahim et al., 2015; Laube et al., 2011).

General advantages to nebulizers include their capacity to administer higher doses in comparison to other aerosol devices, and the fact that they do not require coordination between inhalation and activation of the device. Also, the liquid formulations are cheaper and easier to develop than the ones used in MDI and DPI (Ibrahim et al., 2015).

Notwithstanding, both types of nebulizers are limited by several drawbacks, including the need of a bulky compressor, their nosiness, and the temperature drop experienced by the liquid due to liquid evaporation in the droplets. In addition, a significant amount of drug can be lost in the tubing if the formulation is not properly optimized, and the device needs to be assembled and loaded with the drug before each use. Furthermore, in the case of the ultrasonic nebulizers, their cost is higher, they generate a temperature increase in the equipment, potentially affecting the formulation, and their handling is less user-friendly (Laube et al. 2011; Ibrahim et al. 2015).

Pressurized Metered Dose Inhalers (pMDI)

pMDI are the most used inhalers in dealing with respiratory diseases such as asthma. They are usually composed by a canister, metering valve, actuator, and a mouthpiece. The canister contains the drug, which is dissolved in a propellant system, in turn containing liquefied gas. The metering ensures the right magnitude of aerosol is released when the device is activated (Laube et al., 2011).

The main disadvantage of pMDI is the required coordination between activation and inhalation. Also, the dose that can be administered is lower than in the case of a nebulizer. To solve the coordination problem, breath activated pMDI were developed, for instance Easibreath®. In addition, inhalation aids can be added to increase the efficacy of aerosol administration, such as inhalation chambers (Laube et al., 2011). Later, multi-dose liquid inhalers were developed to further overcome these limitations, combining the benefits of pMDIs and nebulizers. For instance, they allowed the administration of similar drug doses as nebulizers. Respimat® is an example of multi-dose liquid inhalers: it transforms solutions into aerosols as a soft mist, while being easier to use than its predecessors (Ibrahim et al., 2015).

Dry Powder Inhalers (DPI)

DPI employ powder formulations for drug delivery, which present higher stability than the liquid formulations used in other devices. Also, DPI do not require coordinating the inhalation and

activation of the device, since they are activated with a sufficiently strong breath (Bell et al. 1971). On the downside, the formulation and manufacturing process of the dry powder must avoid particle agglomeration typically resulting in reduced device effectiveness, which is a challenging task (Ibrahim et al. 2015). Another disadvantage is the fact that some patients do not have the necessary inspiratory force to enable an efficient transport of aerosols from the device to the lungs (Laube et al., 2011).

To overcome the latter disadvantage, new active DPI assist with the inhalation by using mechanisms such as vibration or gas discharge. An example would be The Spiros™ device, which uses a battery powered motor to activate a rotating impeller that generates the aerosol from the dry powder (Laube et al., 2011). Pulmonary administration of NPs using standard DPI as a treatment approach has been proposed by Rawal et al., who employed Rifampicin following this strategy to treat tuberculosis (Rawal et al., 2017). A similar approach could prove highly useful for a cannabinoid-based COVID-19 treatment.

4.2.4 Proposal of a Most Suitable Cannabinoid Pulmonary Delivery Strategy

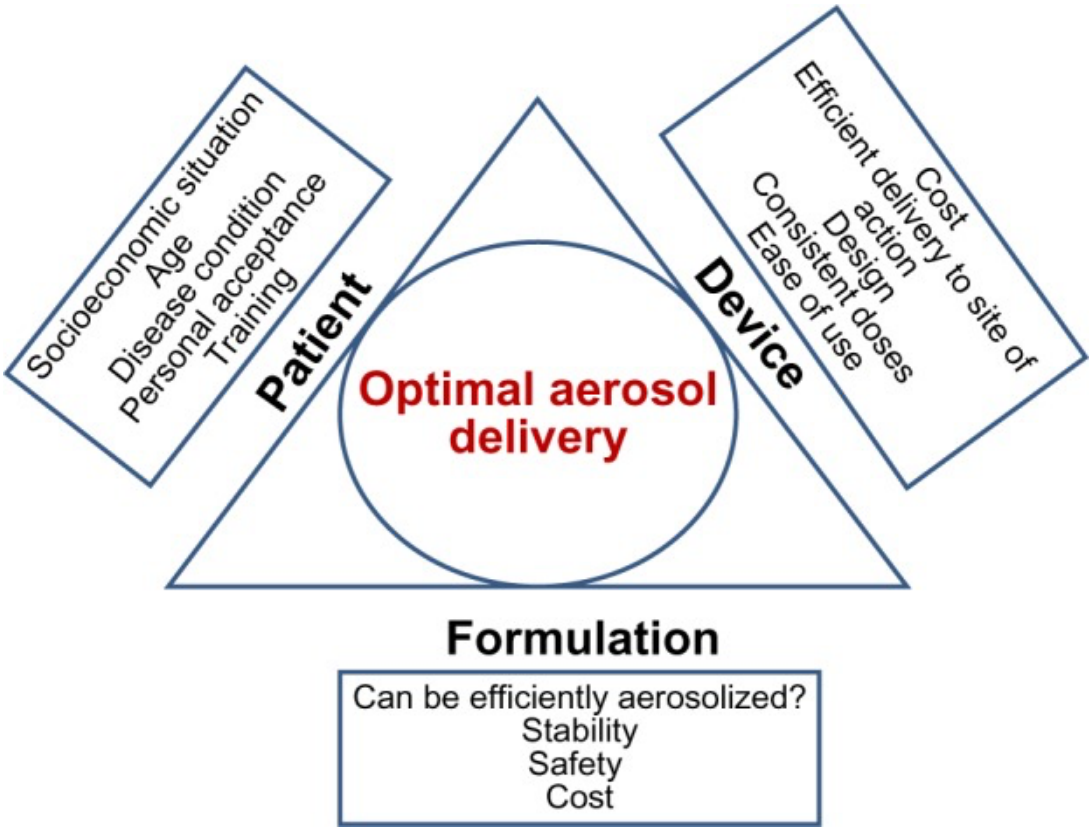

Figure 9. Factors that influence aerosol delivery (Ibrahim et al., 2015).

Based on the information above presented, we would conclude initially using NPs to deliver the cannabinoids through the pulmonary route, specifically PLGA polymer NPs or lipid NPs, on account of their higher stability and capacity for surface chemical modification for specific targeting, as compared to vesicles or MPs. These nanoparticles could be in turn encapsulated inside a microparticle, better suited for the aerosolization process, and as previously described. Regarding the inhalation device, in the case of a cannabinoid-based treatment meant as prophylaxis, a pMDI would be elected on account of its patient-friendly use. On the other hand, if the cannabinoid-based treatment was designed as rescue approach for hospitalized patients with respiratory insufficiency, then a device not requiring action from the patient would be in place. In this case, a nebulizer would be the device of choice.

5. CONCLUSIONS

This bibliographical revision aimed at studying the feasibility and convenience of developing a cannabinoid-based pulmonary treatment for COVID-19 with the aid of a drug delivery system, ultimately proposing the most suitable technological proposal.

The following conclusions were drawn:

- Cannabinoids were found to present several potential applications for COVID-19 treatment, including COVID-19 prophylaxis, based on their immunomodulatory and anti-inflammatory properties that minimize the impact of ARDS and the cytokine storm, and their capacity to reduce viral replication. These effects are mainly triggered by their activation of CB1, CB2 and PPAR- γ receptors.

-In order to enable a pharmacological treatment strategy based on cannabinoids, their unfavourable physicochemical properties and side effects due to unspecific biodistribution, including psychotropic effects, must be overcome. To that purpose, both an adequate drug delivery system and/or a local modality of administration would be helpful. Specifically, pulmonary administration stood out owing to several advantages, such as selective drug deposition on the lungs, fast drug absorption, evading of systemic drug exposure and associated side effects, and avoidance of the first-pass metabolism.

-Regarding technological aspects for pulmonary delivery, cannabinoid-loaded polymeric or lipid NPs, in turn loaded into MPs, were proposed as most the most promising delivery strategy. Specifically, PLGA and lipid NPs would provide a high drug loading, increased stability, controlled release and specific targeting, while MPs would ensure adequate particle deposition in the lungs.

-With regard to devices, pMDI would be selected for prophylaxis approaches in healthy subjects, due to their user-friendly character. However, nebulizers would be selected in the case of treatments for severe cases of COVID-19, were patients are usually unable to inhale properly.

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