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Intranasal Dexamethasone: a New Clinical Trial For The Control of Inflammation and Neuroinflammation in Covid-19 Patients

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Research Article

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INTRANASAL DEXAMETHASONE: A NEW CLINICAL TRIAL FOR THE CONTROL OF INFLAMMATION AND NEUROINFLAMMATION IN COVID-19 PATIENTS

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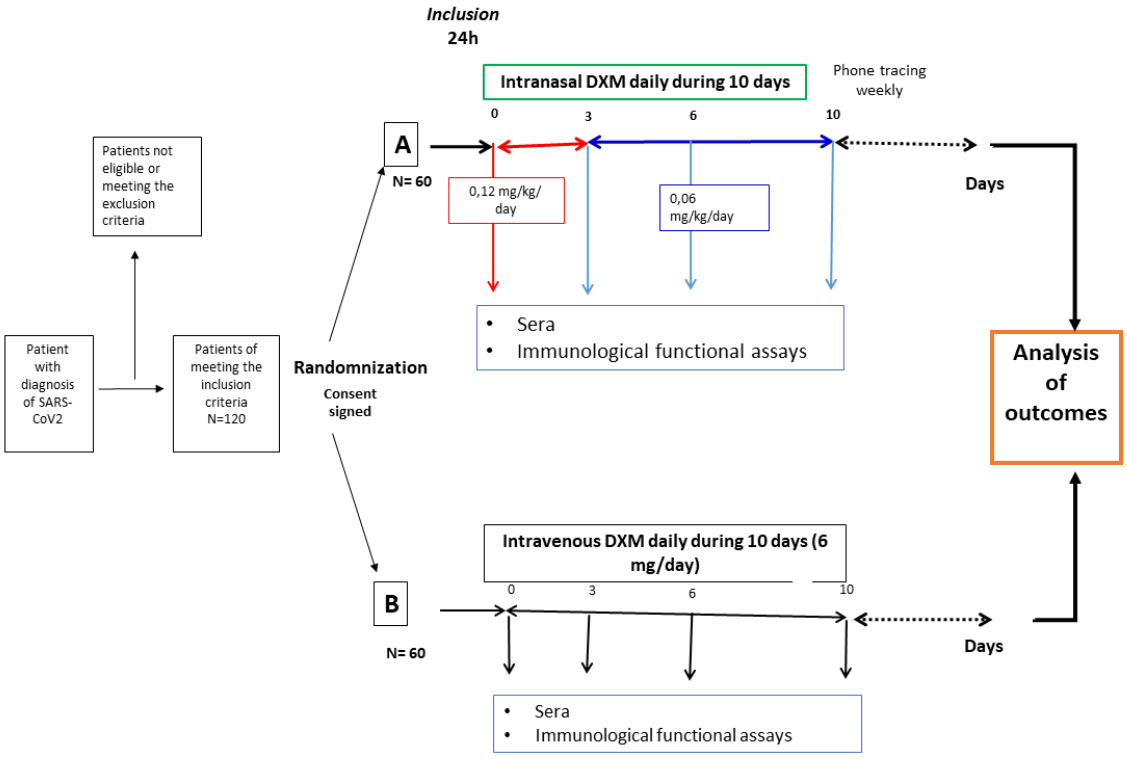
Graphical Abstract



Highlights

- REVIVAL is a controlled, open-label multicentric study to compare the standard low doses of intravenous dexamethasone with low doses weight-adjusted of intranasal dexamethasone
- Intranasal dexamethasone can reach more effectively than intravenous the respiratory tract
- Intranasal dexamethasone can reach the central nervous system in therapeutic concentrations even at low doses
- REVIVAL aims to add to the control of systemic inflammation, the control of neuroinflammation to reduce central failures and sequelae

Abstract

COVID-19 has produced more than 176 million infected individuals and almost 3.2 million deaths worldwide. The infection results in a dysregulated systemic inflammation, multi-organ dysfunction, and critical illness. Cells of the central nervous system (CNS) are also affected triggering a dysregulated neuroinflammatory response.

Low doses of glucocorticoids (GCs) orally or intravenously administered has been proved to reduce mortality of moderate and severe COVID-19 patients. However, low doses administered by those routes do not reach therapeutic levels in the CNS. In contrast, if dexamethasone is administered by the intranasal route can result in therapeutic doses in the CNS even at low doses of the GC.

Methods: This is an approved multicentric randomized controlled protocol to compare the effectiveness of low doses of intranasal dexamethasone versus intravenous administered in adult moderate and severe COVID-19 patients. The protocol is conducted in five health institutions in Mexico City. A total of 120 patients will be randomized in two groups (intravenous vs intranasal) at 1:1 ratio, both groups will be treated with these dexamethasone schemes for 10 days. The primary outcome of the study will be clinical improvement, defined as a statistically significant higher reduction in the NEWS-2 score in intranasally versus intravenously dexamethasone treated patients. The second outcome will be the reduction in mortality during hospitalization.

Conclusions: This protocol is currently undertaken to improve the efficacy of the standard therapeutic dexamethasone regimen for-moderate and severe COVID-19 patients.

Trial registration: ClinicalTrials.gov identifier: NCT04513184 Registered November 12, 2020 and was approved by COFEPRIS with identifier DI/20/407/04/36. People are currently being recruited.

Keywords: Dexamethasone, intranasal administration, inflammation, neuroinflammation, COVID-19

Background

So far, the outbreak of COVID-19 has caused more than 176 million infected individuals and almost 3.2 million deaths worldwide (https://coronavirus.jhu.edu/map.html) with a current global case-fatality ratio of 2.1%, the most affected geographic region are the Americas with a case-fatality ratio of 2.6%.

Several factors predict a poor outcome for COVID-19 patients, such as comorbidities (diabetes, hypertension, obesity) and aging with an underlying dysregulated inflammatory response[1]. Other relevant factors include SARS-CoV-2 neurotropism/neuroinvasiness [2-9]. In fact, the viral RNA was observed in the brain of patients that deceased by severe acute respiratory syndrome due to COVID-19 infection [10-12]. Likewise, it was reported evidence of astrocytic activation and neuronal damage in severe COVID-19 patients, which present elevated plasmatic levels of GFAP and NfL [13]. Other authors have evaluated astrocytes [14] and neurons in 2D o 3D cultures showing an extensive infection [15,16]. The infection of cells of the Central Nervous System results in the expression of PAMPs and DAMPs that trigger a neuroinflammatory response. The exacerbated systemic inflammation with the consequent breakdown of the blood-brain barrier and the migration of cells and peripheral inflammatory mediators also contribute to increase to the in situ generated neuroinflammatory response. Together, this dysregulated and sustained neuroinflammation can add to peripheral damage, central (CNS) damage, which may contribute to the multi-organ dysfunction and death [10,12].

Natural history of SARS-CoV-2 infection

A clinical staging system has been proposed in SARS-CoV-2 infection as follow, early infection (Stage I, mild), pulmonary involvement (Stage IIa, moderate) without hypoxia, or with hypoxia (Stage IIb), and finally Stage III (systemic hyperinflammation) [17] (Figure 1).

After exposure to SARS-CoV-2, virus gains host access through the nasal cavity and respiratory airway. During early infection (Stage I), mild and non – specific symptoms may be observed (fever, malaise, and asthenia), upon this prodromic phase virus binds its target ACE2, TMPRSS2 [18, 19] and more recently NRP-1 [20,21]. These receptors are highly present on several tissues including the olfactory neuroepithelium (less in the sensitive olfactory neurons) and lung [19-22], consequently, the infection can be established in the lungs (Stage II) and lead to viral pneumonia, cough, and fever with or without hypoxia. Here the SARS-CoV-2 PAMPs will be recognized by TLR3, TLR7 and TLR8 in the endosome but also in the RIG-1 like receptor in the cytosol [23]. The virus can also reach the CNS through the olfactory and trigeminal nerves terminal. Once in the CNS it can infect and damage endothelial, pericytes and neural cells that expressed ACE2, NRP-1 receptors [20, 21] promoting neuroinflammation (Figure 1). CNS viral involvement is related to headache, dizziness, and ataxia, but infection also may progress to the whole brain including the brainstem [5, 6]. Finally, in a minority of infected-patients disease progresses to Stage III where a hyperinflammatory syndrome (the sustained production of proinflammatory cytokines including IL-1β and TNFα) is observed, with mitochondrial and lysosomal damage, expressing elevated proinflammatory cytokines, reactive oxygen species (ROS), and the hyperactivation of P2X7 receptors. These processes induce inflammasome activation (which increased IL-6 levels) and lead to pyroptosis which

139 determines a persistent inflammatory cycle by disseminating viral antigens and RNA in the
140 circulation. Thereafter, it is possible the generation of immune complex and its deposition in
141 target organs [23-25]. During this phase, sustained neuroinflammation may exacerbate the
142 neuronal injury, therefore spreading damage and contributing towards central respiratory
143 failure besides other signs of systemic organ involvement resulting in multi-organ
144 dysfunction [17].

145 A crucial strategy to treat COVID-19 patients seems to be the control of neuroinflammation
146 and systemic inflammation. For this purpose, it is important to consider how the virus invades
147 the human organism. The most frequent form is the intranasal route which allows a direct
148 access to both, the respiratory and the central nervous systems through neural pathways [5; 15-
149 18]. Coronaviruses including SARS-CoV-2 can infect brainstem neurons associated with
150 cardio-respiratory control, which induces central alterations of pulmonary function [5; 26-29]. In
151 fact, COVID-19 neurological clinical symptoms particularly nausea, vomiting, and dysgeusia
152 appear to involve the dorsal vagal complex (DVC) and the nucleus tractus solitary (NTS)
153 linked to the control of several autonomic functions [26]. The NTS is a well-known target of
154 neuro-immune activation [33], and its ascending projections reach the hypothalamus
155 (hypothalamic paraventricular nucleus) involved in the HPA axis activation while other NTS
156 projections come to the rostral ventrolateral medulla (RVM), which controls respiratory and
157 cardiovascular functions [34].

158 The viral infection in respiratory and central nervous system cells promotes the expression
159 of pathogen-associated molecular patterns (PAMPs) and damage-associated molecular
160 patterns (DAMPs) signals that in turn trigger inflammasome and oxidative stress [23,35]. Later

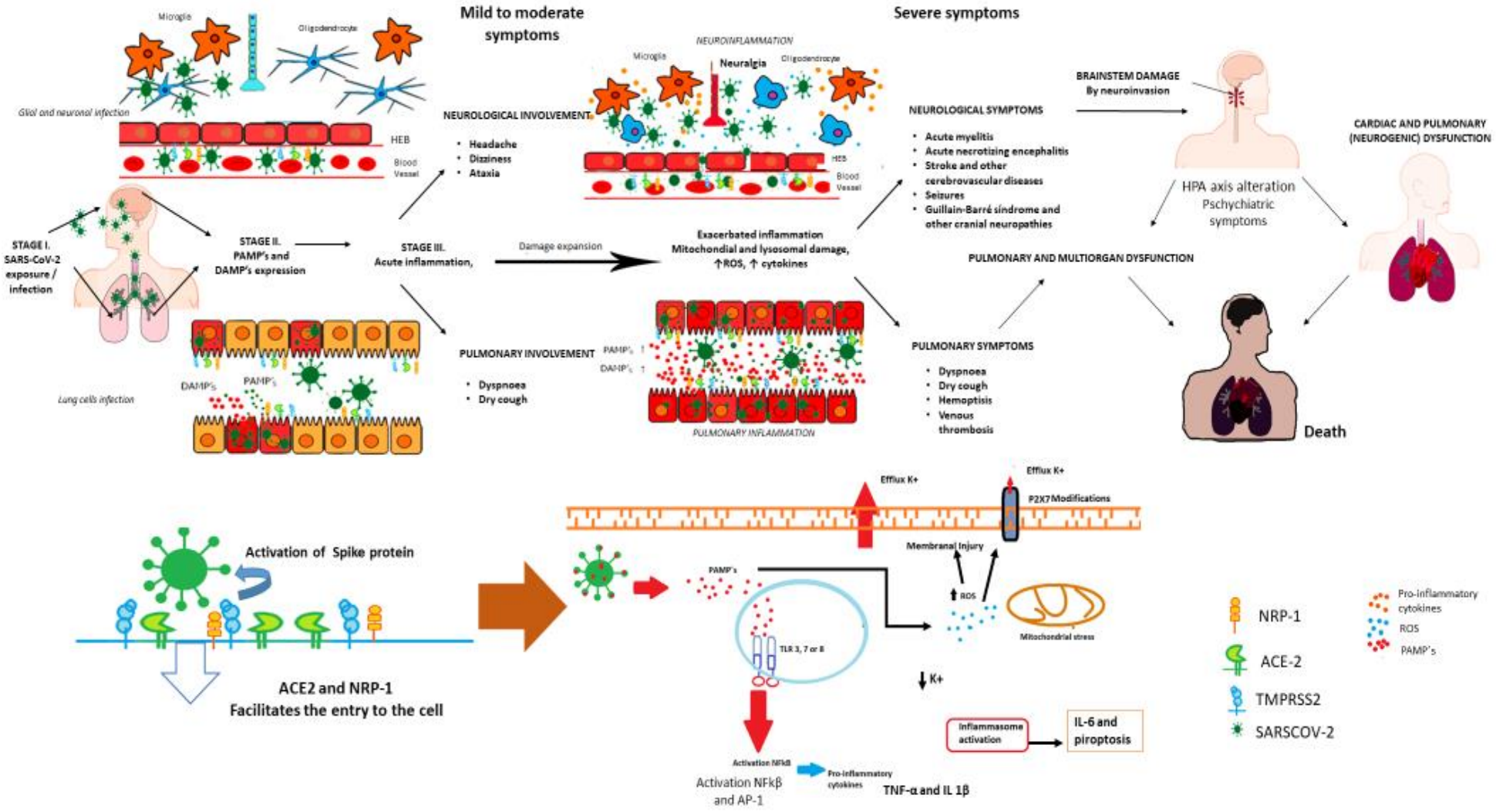
during infection, inflammatory response may become dysregulated extending the initial damage caused by the infection.

Adrenal affection in SARS-CoV-2 infection

Critically ill-patients of different pathologies frequently show adrenal insufficiency which may increase morbidity and mortality [36, 37]. COVID-19 might affect the hypothalamic-pituitary-adrenal (HPA) axis as well. Hypothalamic and hypophysis tissues do express ACE2 and can therefore be viral targets [38]. The virus may directly damage the hypothalamus as well as the pituitary leading to hypothalamo-pituitary dysfunctions. In fact, since SARS outbreak in 2003, it was observed that coronavirus affects the HPA axis, and vasculitis was demonstrated by autopsy studies in several organs including adrenal glands, particularly adrenal cortical cells undergo degeneration and necrosis [39]. Although the full spectrum of COVID-19 endocrinological manifestations within long-term is still unclear, several endocrine alterations have been reported in SARS survivors, as well as hypocortisolism, and hypothyroidism, and low levels of dehydroepiandrosterone, which suggested a transient hypothalamic-pituitary dysfunction [40]. Recently, an Arabian study in 28-patients reported the adrenal response to an acute COVID-19 infection, the median morning cortisol level was 196 (31-587) nmol/L, the ACTH median level of 18.5 (4-38ng/L). Interestingly, severe forms patients had lower cortisol and ACTH [41]. In addition, in other autopsy studies, edema, neuronal degeneration and evidence of viral genome were found in the hypothalamus [42] Thus, in the presence of subacute thyroiditis or adrenal insufficiency, corticosteroid therapy should help in reduced high amounts of thyroid hormones, and replace adrenal function, improving the evolution of these patients, regardless the route of administration.

Rationale

Dexamethasone sodium phosphate (ALIN, injectable solution. Chinoin Laboratory) is a highly soluble glucocorticoid with a neutral pH 7-8.5, which did not injury the nasal mucosa. This synthetic steroid is an anti-inflammatory and immunomodulator drug that inhibits prostaglandins and leukotrienes synthesis, platelet activation, and coagulation through regulation of transcriptional factors such as NFK-β y AP-1 [43,44]. In addition, it can sensitize the cells to extracellular ATP during NLRP3 induction, which enhances the release of proinflammatory molecules [45]. In addition, it has been reported that DXM exerts important neuroprotective effects as rescue the neurovascular integrity during neuroinflammation [46].


Mild to moderate symptoms
Severe symptoms
Microglia
Oligodendrocyte
Glial and neuronal infection
HEB
Blood Vessel
NEUROLOGICAL INVOLVEMENT
Headache
Dizziness
Ataxia
NEUROINFLAMMATION
Neuralgia
NEUROLOGICAL SYMPTOMS
Acute myelitis
Acute necrotizing encephalitis
Stroke and other cerebrovascular diseases
Seizures
Guillain-Barré síndrome and other cranial neuropathies
BRAINSTEM DAMAGE
By neuroinvasion
HPA axis alteration
Pschychiatric symptoms
CARDIAC AND PULMONARY (NEUROGENIC) DYSFUNCTION
STAGE I.
SARS-CoV-2 exposure / infection
STAGE II.
PAMP's and DAMP's expression
STAGE III.
Acute inflammation,
Damage expansion
Exacerbated inflammation
Mitochondial and lysosomal damage,
↑ROS, ↑ cytokines
PULMONARY AND MULTIORGAN DYSFUNCTION
DAMP's
PAMP's
Lung cells infection
PULMONARY INVOLVEMENT
Dyspnoea
Dry cough
PULMONARY INFLAMMATION
PULMONARY SYMPTOMS
Dyspnoea
Dry cough
Hemoptisis
Venous thrombosis
Death
Activation of Spike protein
ACE2 and NRP-1
Facilitates the entry to the cell
Efflux K+
P2X7 Modifications
Membranal Injury
ROS
Mitochondrial stress
TLR 3, 7 or 8
↓K+
Inflammasome activation
IL-6 and piroptosis
Activation NFkβ and AP-1
Pro-inflammatory cytokines
TNF-α and IL 1β
NRP-1
ACE-2
TMPRSS2
SARSCOV-2
Pro-inflammatory cytokines
ROS
PAMP's

193 **Figure 1.**

Dexamethasone a potent anti-inflammatory drug

Considering that complications of COVID-19 result from exacerbated and uncontrolled peripheral inflammation and neuroinflammation, derived from the so-called cytokine storms, at least three important and key points have been considered in the use of DXM for the treatment of victims of the Coronavirus: the timing, the dose, and the route of administration of the steroid. First, the drug would not be applied from the beginning of the infection, the time at which the inflammation favors the host. It should be given to promote the installation of an adaptive immune response and thus control the infection. A low dose of DXM (6 mg per patient for 10 days) applied to quickly and effectively control pulmonary inflammation with minimal negative side effects [47]. In addition, the intranasal route would allow direct access of the DXM to the CNS, thereby controlling the sustained neuroinflammation provoked by damage to infected astrocytes, neurons and microglia during the progression of COVID that cause the fatal central respiratory and cardiac failure in these patients.

It is well known that drugs administered intranasal usually permit higher bioavailability in CNS without the need of BBB pass or hepatic degradation, in comparison with similar intravenous doses administered in experimental models [55, 56-58]. In addition, the administration of DXM by this route induces an inflammatory control by arriving directly to the respiratory system, more effectively and quickly than by using intravenous route [56-59]. DXM prevents the binding of ACE2 to spike protein of SARS-CoV-2 and can bind LYS353, an active residue of RBD [60], and reduces ACE2 expression in several types of cells by suppressing type I interferon expression [61], can also downregulate neutrophils extracellular traps, possibly through Toll-like receptor regulation [62]. It is known that hyper inflammation

is related to high levels of NETs which is related to ARDS in which neutrophilia predicts thrombosis and poorer outcomes [63, 64].

METHODS

Trial design

The “REVIVAL trial” an interventional study, phase 2, multicentric randomized controlled in adult patients with confirmed COVID-19 diagnosis was designed to evaluate the efficacy of low doses of intranasal DXM compared to intravenous administration in patients of five COVID-19 referral centers in Mexico City. This protocol is supported in part by the Institutional grant "Programa de Investigación para el Desarrollo y la Optimización de Vacunas, Inmunomoduladores y Métodos Diagnósticos del Instituto de Investigaciones Biomédicas", UNAM (DGAPA-UNAM, PAPIIT IN201020), as well as by another specific grant provided by the Mexican Ministry of Foreing Affairs (Secretaria de Relaciones Exteriores) and Mexican Agency for International Development Cooperation (AMEXCID) with identifier: 318.01 fund MEX-CHI. This trial is being coordinated at the Department of Immunology of the Biomedical Research Institute, UNAM.

Settings

This clinical trial is being conducted at the following Institutions “Hospital General de México Dr. Eduardo Liceaga”, “Instituto Nacional de Neurología y Neurocirugía Manuel Velasco Suárez”, “Instituto Nacional de Cardiología Ignacio Chavez”, “COVID-19 unit at Citibanamex” and “Hospital Central Militar” all of them in Mexico City.

Eligibility criteria

Inclusion criteria includes patients of both sexes, (non-pregnant female) 18 years of age and under 90 years, with presumptive SARS-CoV-2 infection with more than 5 days of clinical evolution and with moderate to severe symptoms requiring oxygen support or high flux mechanical ventilation (NEWS-2 $\geq$ 5), abnormal CT- chest scan CO-RADS >3. Patients diagnosed with atypical pneumonia, confirmed by chest images and oxygen saturation (SpO_2) less than 93% in ambient air or when a ratio of the partial pressure of oxygen (PaO_2) and the fraction of inspired oxygen (FiO_2) (PaO_2: FiO_2) was 300 mm Hg or less, and a confirmatory RT- PCR SARS-CoV-2 positive test. These patients will be allocated into the experimental group or the control group in a ratio 1:1 (two arms) (Fig. 2) according to the randomization.

Exclusion criteria includes patients with RT-PCR SARS-CoV-2 negative test, those receiving previously GCs at high doses, by oral or intravenous administration, or severely immunosuppressed as in AIDS, pregnancy; autoimmune disease patients as well as those who have received outpatient treatment with steroids for more than 72 hours prior to hospital admission, older than 90 years, or with DXM allergy, risk for glaucoma or recurrent respiratory diseases.

Elimination criteria will be considered in case of voluntary withdrawing or lacking informed consent, or imminent risk of death within 48 hrs.

The pharmacovigilance staff of each hospital will perform a continuous monitoring each 72 hours during the period of study (including all adverse events).

Interventions

Groups and comparators

The study will be carried out in two groups, group A (experimental) that will receive intranasal DXM, and group B (Control) that will receive intravenous DXM (Fig.2), based on the previously reported data, where the intranasal administration can reach the brain and bloodstream more quickly and efficiently [56-59]. Group A will receive daily intranasally DXM at a dose of-0.12 mg / kg for the first three days, that will be followed by seven days at a dose of 0.06mg / kg. Group B will receive daily 6mg intravenous DXM. In both groups, a close follow-up will be done by the pharmacovigilance staff every 72 hours, they will assess whether it is appropriate for the patients to continue within the protocol.

Procedures

A double follow-up form (written and online) will be filled for each patient, and completed at the end of treatment or fatal outcome after randomization, whatever occurs first. Besides a daily clinical evaluation, blood and saliva samples will be collected every third day during the whole treatment period, to perform ancillary tests as SARS-CoV-2 viral load, functional immunological assessment (lymphocyte cytometry, cytokines / chemokines profile), as well as cortisol levels, among other analysis. All human samples will be stored at -70ºC until use. All patient's personal data and medical information will be treated in a strictly confidential way. Only the lead investigator and the hospital coordinator investigators will have access.

Participants

The sample includes 120 adult patients between 18 and 90 year-olds, both sexes with moderate and severe forms of COVID-19.

Sample size and Randomization

The sample size was calculated with EPIDAT version 3.1.2 software, with the option "Sample size and surveillance curves" with an estimation of 50% increase in the proportion of patients free of mechanical ventilation [intranasal DXM 70% vs intravenous DXM 45%]. This value was estimated based on the data of the COVID-19 patients registered in Mexican hospitals with a confidence of 95%, power of 80% and proportion of losses of 10%, with these characteristics is obtained 60 per group. The randomization will be making with Sealed Envelope software. This software is a freeware [Online] available from: https://www.sealedenvelope.com/simple-randomiser/v1/lists [Accessed 5 May 2020]. This study is a multicenter randomized controlled trial. (Fig. 2)

Confidentially

Each patient who agrees to participate in the protocol will be assigned an identification number, which will be used to check out throughout the procedure. This code identifies the hospital of origin and the patient identification number. All the information collected during the procedure will be confidential and used for the research purpose only and follow up for any adverse effect.

Outcomes

The expected primary outcome is clinical improvement, defined as a two-point improvement of ordinal scale regarding the initial NEWS-2 score. The secondary expected outcome includes a reduction in mortality that will be follow-up during treatment (after randomization), as well as a reduction of the time required for mechanical ventilation and the length time of patient's stay in hospital. Viral load, several other physiological parameters and the immune-inflammatory profile will also be evaluated before and after treatment (see above).

Data collection and management

The patient receives an informed consent letter, where the characteristics of the procedure are detailed, if he accepts, the letter will be signed and the patient will be randomized; saliva and nasopharyngeal sample will be taken to know the viral load and treatment will begin as indicated in figure 2; a clinical history will be made based on the initial results and physical inspection of the patient.

The samples taken will be sent for specialized analysis following standardized operating procedures (SOP's) for the analysis.

Plans to promote participant retention and complete follow-up

All participants in the research protocol will receive specialized medical care, by monitoring continuously clinical, neurological, and neuropsychological studies. These evaluations will be carried out to monitor the evolution of the disease at 1, 3, 6, and 12 months after COVID-19. Those participants presenting some functional decline post COVID will be received

medical treatment and neurorehabilitation.

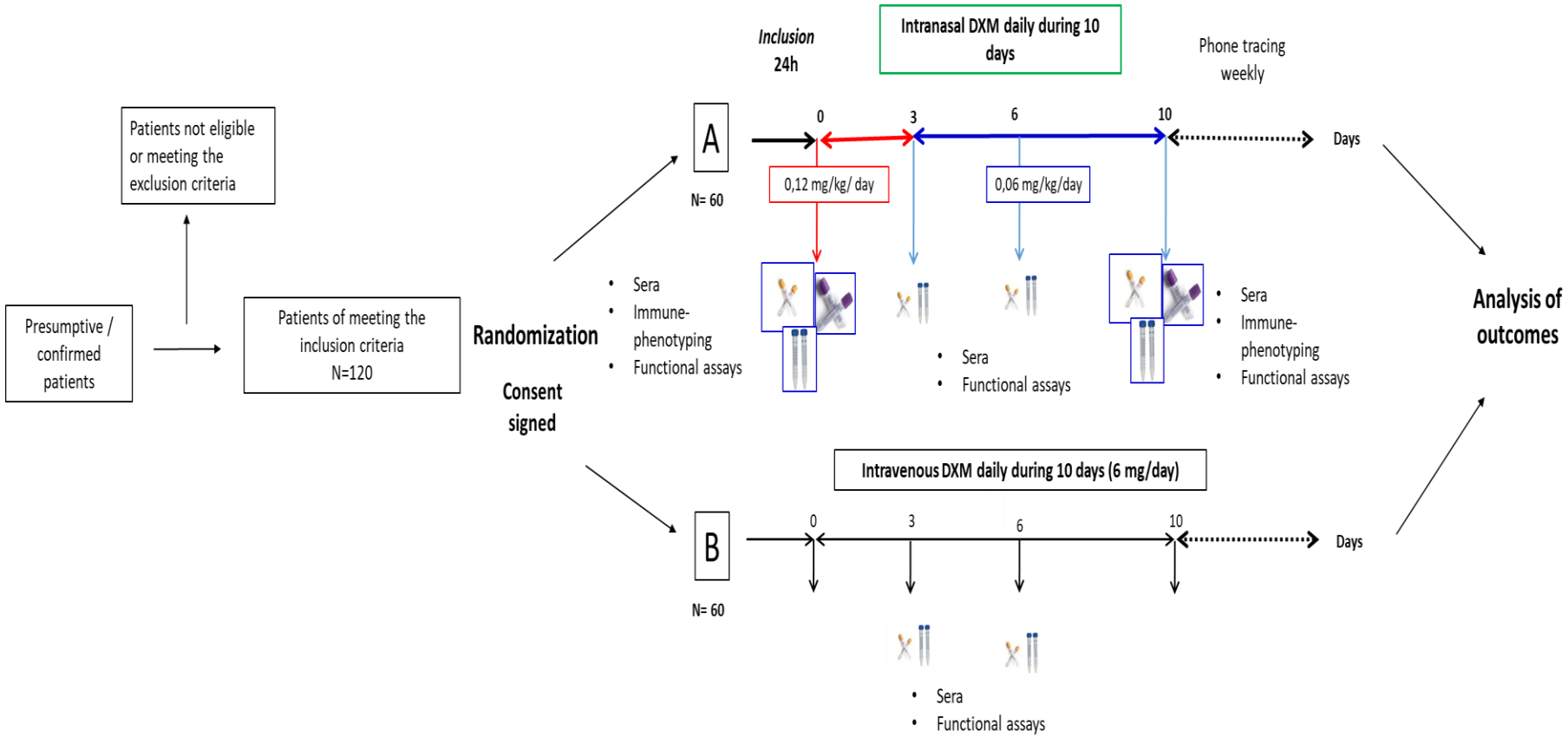
Likewise, for patients who present an adverse effect or health problem during its participation in the dexamethasone treatment study or derivate to it upon hospitalization, the General Hospital of Mexico Dr. Eduardo Liceaga will take care of the necessary treatment and/or care until their resolution. In addition, patients will be monitored every 3 months for 1 year after the study

Management

The information collected during the procedure will be documented physically and digitally in an exact and precise way. Each complete patient report will be used by researchers in conjunction with the molecular and immunological tests to analyze the outcomes. The information collected will be treated as confidential, and only the global results will be published without showing the names of the patients, in case the data is required, the information can **be** request to the researchers with valid reasons.

Analysis of outcomes

A database will be built, and a descriptive statistic will be performed. The data distribution will be analyzed and compared with DXM route of administration with a multivariable analysis: nested ANOVA with repeated measures and Markov test. The analysis will be done with a R software (4.0.0, Arbor Day). A statistical difference with $P < 0.05$ will be considered significant.



341

342 **Figure 2.**

343

5. Conclusions

Intranasal DXM at low doses could be a more effective therapeutic option to control inflammation and neuroinflammation during ARDS in severe and critical forms of SARS-CoV-2 infection. In addition, it could aid the HPA axis upon this severe stress condition. DXM in low doses applied by systemic route although beneficial for COVID-19 patients, cannot reach effective therapeutic concentration in the CNS to control neuroinflammation. In contrast, intranasal administration of DXM is highly effective to control neuroinflammation as demonstrated in experimental models of several inflammatory conditions [44-47]. Therefore, in the REVIVAL trial clinical protocol, we propose boosting the effect of DXM treatment at low doses in COVID-19 through an intranasal route of administration to reach CNS at therapeutic doses that may effectively reduce the morbidity and mortality in severe or critical COVID-19 patients, even more than that reported data in the RECOVERY trial.

A randomized study in hospitalized COVID-19 patients (moderate and severe forms), the intranasal DXM at low doses (clinicaltrials.gov id: NCT04513184) is being tested. The clinical evolution and respiratory parameters of the patients receiving intranasal DXM (experimental treatment) is compared with recommended treatment of 6 mg of intravenously DXM (https://www.covid19treatmentguidelines.nih.gov/). Considering the prevalence of metabolic syndrome and obesity in Mexico, a therapeutic scheme weight-adjusted at low dose is being applied i.e., three-day schedule of 0.12 mg/kg and 7 days at 0.06 mg/kg. If the current approach results less prone to adverse effects but enough to reach CNS and control neuroinflammation as we hypothesized, there will be direct interest to extent this protocol to

several COVID hospitals of the National Healthy System in Mexico. In addition, it will be mandatory to increase the initial sample size (preliminary results) to publish it and share it with the International scientific community.

6. Declarations

6.1 Study Status

The study was registered under the platform Clinical Trials from NCBI in August 2020, and was approved by COFEPRIS in Mexico with identifier DI/20/407/04/36. People are currently being recruited.

6.2 Ethics declarations

This study was reviewed and approved by the Committees of Ethics, Research and Biosecurity of the five Hospitals committees. Hospital General de México "Dr. Eduardo Liceaga" (DI/20/407/04/36), Instituto Nacional de Neurología y Neurocirugía (INNN 31/20) and Instituto Nacional de Cardiología (INCICh: 20-1167), temporary unit for COVID Citibanamex, and Hospital Central Militar (FM/DI/107/SR/2020), and is approved for COFEPRIS with identifier DI/20/407/04/36.

All participants will provide a written informed consent before enrollment and all the work will be conducted according to the Helsinki statements.

6.3 Availability of data and materials

Data and materials are not available at this moment, because the work being considered is the first approach to a clinical trial currently started. When the study will be completed, the

dataset obtained and analyzed will be available from the corresponding author only by reasonable request.

6.4 Funding

This work were supported by Dirección General de Personal Académico, UNAM (DGAPA-UNAM, PAPIIT IV201020), by the Institutional program "Programa de Investigación para el Desarrollo y la Optimización de Vacunas, Inmunomoduladores y Métodos Diagnósticos del Instituto de Investigaciones Biomédicas" U.N.A.M as well by the Mexican Ministry of Foreing Affairs (Secretaria de Relaciones Exteriores) and Mexican Agency for International Development Cooperation (AMEXCID) with identifier: 318.01 fund MEX-CHI.

6.5 Conflict of interest

The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

7. Author's contributions

Study concept and design: GC, ES, HB, MR, JH, MCC

Data adquisition and interpretation: GC, ES, HB, MR, JH, MCC, AJR, DAM, MFMM, LVTA, RLBC, RMW, LERG, KIC, EGV, MRC, YL, MLHM, MLH, KMQ, ASM, SHD, IGRZM, AMC, INMS, EBS, AFP, MJFM, PSHH, JC, LH, NAF, MH, MPT, GM, HJ, EEA, GR, ROA, SOF, SRM, JAHA, JCT, AFR, HB, MCR, RJB, GS, JLA, GF, JPL.RIAR, DMR, LRRA, RAAB

Manuscript drafting: GC, ES, HB, MR, JH, JAHA, MCR, RJB, GS, JLA, GF, JPL

Critical revision of the manuscript for important intellectual content: GC, ES, HB, MR, JH, JAHA, MCR, RJB, GS, JLA, GF, JPL

8. Consent for publication

Not applicable

9. Acknowledgements

Not applicable

10. List of abbreviations

DVC: Dorsal Vagal Complex

NTS: Nucleus Tractus Solitary

HPA: Hypothalamic Pituitary Adrenal axis

RVM: Rostral Ventrolateral Medulla

DAMP: Damage-Associated Molecular Patterns

PAMP: Pathogen-Associated Molecular Patterns

DXM: Dexamethasone

GCs: Glucocorticoids

ACE2: Angiotensin-Converting Enzyme 2

TMPRSS2: Transmembrane Protease Serine 2

CNS: Central Nervous System

ROS: Reactive Oxygen Species

426 **NFK-B**: Nuclear Factor K beta

427 **AP-1**: Activator Protein 1

428 **ARDS**: Severe Acute Respiratory Distress Syndrome

429 **BBB**: Blood Brain Barrier

430 **NET**: Neutrophil Extracellular Traps

431

Figure Legends

Figure 1. Inflammatory phenomenon associated with SARS-CoV-2 infection and its neurological and respiratory manifestations. The SARS-CoV-2 virus enters mainly by air and reaches the lungs through direct ventilation and the CNS through the olfactory and trigeminal nerve, the entry of the virus is facilitated by NRP-1, ACE2 receptors and the protein S activation by TMPRSS2. In the CNS, the virus infects neurons, glial cells, and endothelial cells, increasing the permeability of the BBB, and may cause cerebral edema and intracranial hypertension, as well as neuroinflammation. If the viral infection continues, the damage spreads throughout the body causing heart and systemic failure. This damage is associated with an increase in neuroinflammation, directed by microglia and oligodendrocytes, causing damage to the brain stem, and causing a dysfunctional state of the heart and lung. Likewise, in the lung, due to exacerbated inflammation and intravascular coagulation, respiratory arrest is induced that can lead to the patient death. The inflammation is conducted by the cellular activation trough TLR3, 7 and 8 for components from the virus (PAMPS) and subsequent production of pro-inflammatory cytokines (TNFα and IL 1β) and generation of ROS; those ROS can be able to modify the P2X7 receptor in the brain and activate the inflammasome by the decrease of K^{+}. The activation of inflammasome increases the production of IL-6 and pyroptosis.

Figure 2. Outline of the REVIVAL trial clinical protocol. Initially, patients will be informed about the clinical trial, if they accept and sign the consent, they will be randomized using the Sealed envelope® software. Group A receive DXM intranasally obtaining serum and a swab on days 0, 3, 6 and 10 post treatment. On the other hand, group B receive intravenous DXM, obtaining the same samples on the same days 0,3,6 and 10. Throughout the study, the patients

455 are monitored. Once the results are obtained, these are analyzed to define if exist a
456 statistically difference between groups.

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Supplementary Files

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