Melatonin "high dose" to prevent inflammatory storm in the COVID-19 pandemic.

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Fast Track Report

"Melatonin, analgesic hormone that relieves inflammation, among many other effects"(1, 2)

The author wishes to state that this is not his field of expertise and is not a consultant on the subject; his work is has been conducted in an attempt to help, like many other colleagues in the context of the pandemic and is based on a thorough review of experimental and clinical literature and on consultation with top level experts, for which reason he considers that the protocol is reliable.
Queries must be submitted to the relevant authorities. The author merely recommends that, since there is no clinical experience, it is necessary to conduct a prospective study measuring the recommended variables that were identified in different disease scenarios and in this way confirm if humankind has found what is being sought.

Introduction

Melatonin and its effects on lung lesions, inflammatory storm and COVID-19 related mortality.

Lung involvement is considered to be the first cause of death in the current coronavirus pandemic. The severity of the inflammatory storm triggered by the viral infection has been identified as the main factor in fulminant lung conditions. Intervening in an early phase might help to prevent or attenuate the consequences of this entity and would help reduce the number of deaths. Further, it would prevent the most severe scenario by attenuating the high impact created by the demand for resources that are difficult to plan when it is impossible to stop the admission of patients to critical areas where health-care professionals are the most exposed. This demand has reached such magnitude and scarcity of resources is so dramatic that in the first economy in the world the FDA has authorized simultaneous mechanical ventilation of up to 4 patients in one ventilator (1-4).

Elderly patients show significantly higher mortality and cardiovascular diseases create high risk of refractory response and secondary infections. The severity of lung lesion has been confirmed in retrospective mortality studies conducted in Wuhan. Out of a total of 68 fatal cases, 36 patients (53%) died of respiratory insufficiency, 5 patients (7%) of myocardial damage and circulatory failure, 22 (33%) of both causes and 5 of unknown cause.

Another study of 155 consecutive confirmed patients of COVID-19 in Zhongnan Hospital, Wuhan non-remission of clinical and radiological findings was observed within the first 10 days of hospitalization in 50% of the COVID-19 +patients,

The value of investigating the use of old drugs

At present, apart from the valuable measures that have been implemented (isolation, early detection, protection equipment for health-care staffand support treatment based on protocols to avoid freewill implemented to maintain patients alive), no specific treatments have been developed with proven efficacy to stop or attenuate the trigger of the inflammatory process.

Yadi Zhou et al (4) promote resorting to old drugs given the time, cost and failures implied in the development of a new drug. Of a list of 16 molecules that can be used, they focus on 3 potential combinations, i.e. sirolimus plus dactinomycin, mercaptopurine plus melatonin and toremifene plus emodin. Melatonin is phylogenetically known as an old molecule whose origin has been traced back 2.5 to 3.1 billion years (5). This molecule, characterized by notable versatility, is synthetized by the pineal gland and other organs, including the GI tract, retina, thymus, bone marrow, and leucocytes. Melatonin and its metabolites have proven

significant immunomodulating, anti-inflammatory, anti-bacterial effects and protection of mitochondrial homeostasis, causing these effects with direct action on receptors and indirectly through antioxidant and anti-nitricoxide actions among others including free radical scavenger, upregulation of anti-oxidant pathways, reduction of pro-inflammatory cytokine level, increased level of anti-inflammatory cytokines and intervening on the activity of the angiotensin converting enzyme.

These effects warrant many indications, but it should be noted that high doses transform this molecule into a potent anti-inflammatory drug that might attenuate or stop the inflammatory response that produces lung damage and may eventually contribute to repair tissue as has been proven by world experts (Cardinali and others) in similar scenarios as the one currently affecting the world at large.

Monitoring of the inflammatory response evolution in the COVID-19 pandemic and immunity level in the exposed population

Considering that the immune and inflammatory status and response contribute to define the severity of lesions in COVID-19 patients, management of these patients and treatment decision-making should be based on the monitoring of accurate plasma indicators that provide actual information.

Analyzing the measurement of predictive indicators, the role of CD4, as well as the CD4/CD8 ratio has been studied in other pathologies in which the immune response is a critical factor in the final therapeutic outcome. $CD4^+$ was significantly greater in patients with good immune response than in non-responders (INRs) or in those with insufficient response (IIRs) ($P < 0.001$).

The SARS- CoV-2 infection may primarily affect T lymphocytes, specially CD4+T y CD8+ T cells, resulting in the reduction in number as well as in the production of IFN-γ. The study by Qin Ning et al. showed that the importance of immunological markers is determined by their correlation with the severity of the COVID-19 disease. (3-9)

The group of patients that becomes refractory to treatment and experiences clinical deterioration and oxygen desaturation showed a significant difference as compared to the general infected population. In the refractory population, LDH and PCR levels reached significantly higher values, thus have been identified as potential predictors to monitor evolution.

In the follow-up of clinical and laboratory parameters in studies by Qiurong (150 cases), Wang (138 cases) and Xiao-Min (91 cases), it was possible to identify refractory cases with a highly statistical difference. This valuable information resulting from such studies is helpful to organize health care of the vulnerable global population that has not yet suffered the surge of the COVID-19 impact. Laboratory parameters with predictive value identified in the studied populations were: neutrophils (P=0.017), AST or TGO (P=0.004), LDH (P=0.017), C-reactive protein (CRP, P=0.001), reduction of the platelet level (P=0.049) and albumin (P=0.001).

The refractory group, as mentioned above, had a greater incidence of bilateral pneumonia (P=0.031) and pleural effusion (P=0.006) which, as mentioned at the beginning, are the main causes of death that medicine should attempt to anticipate, which in turn would mean solving

2 problems at the same time, namely loss of lives and an unprecedented economic and logistic demand that current infrastructure cannot meet.

Rx, CT and ultrasound and clinical data have proven helpful and this is indicative of the need for careful surveillance and clinical selection of the pulmonary semiology in light of the huge deficit of resources and availability of Rx equipment worldwide. It should be noted that of 91 patients that developed pneumonia, 61 (67.03%) were bilateral, and 25 (27.47%) were unilateral.

It is useful to review the study by Qian-Yi Peng that describes 5 ultrasound findings typical of the lung and pleural lesion by COVID19 and this would help reduce the need to mobilize complex patients that could be assessed and monitored at bedside.

Proven efficacy

Melatonin "high dose" by oral or intravenous route has shown potent pharmacological actions. There are numerous references on clear and well-documented benefits in sepsis, severe viral and bacterial infections, Ebola fever and others. Melatonin in high concentrations has shown bactericidal activity against Gram-positive and Gram-negative organism, including Methicillin-resistant Staphylococcus Aureus and Carbapem-resistant germs.

Melatonin increases the blood concentration of IL-10 (anti-inflammatory cytokines). Likewise, it reduces high levels of myeloperoxidase (MPO) activity in polymorphonuclear pulmonary infiltrates. Melatonin has been used to treat secondary lesions related to this type of infectious diseases and severe inflammatory processes that affect the lung causing SDRA, pneumonitis and bilateral pneumonia due to its preventive and therapeutic effects. It has also proven efficacious in the management of premature babies with severe pulmonary distress. Melatonin has been widely studied in cases of ionizing-radiation related pneumonitis. Finally, this molecule has shown systemic and intrathecal effects used to treat migraine, peri-operatory and cancer pain. In the present context, this effect that has also been investigated by Cardinali would warrant its use due to the presence of severe myalgias, one of the most frequent symptoms of COVID-19 infection.

A systematic review and meta-analysis of 5057 articles revealed that melatonin acts in anti-tumor therapies, including effects such as tumor remission and global survival improvement. Melatonin restitutes the function of alveolar epithelium impaired by bleomycin and besides enhancing tolerance to the toxic effects of cytostatic agents, has proven the synergic effects of cytostatic drugs in cancer (pancreas, stomach, lung, ovary and breast), significantly reducing mortality.

Although there is no time for historical or anecdotal data, it is worth noting that in 1926 Berman reported improved resistance to infectious diseases in kitten fed for 2 years with pineal glands of young bulls. Care should be taken when considering melatonin analogues or agonists on MT(1)/MT(2) receptors such as ramelteon without proven activity on free radicals.

It is interesting to note the differences in affinity to receptors that might explain the high doses required to cause effects in diseases such as multiple sclerosis or lateral amyotrophic sclerosis, among others, in which doses greater than 50 mg have been used with no reported toxicity.

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Proven safety

Studies in volunteers administering high doses of more than 50 mg and 100 mg PO, IV have proven high levels of safety beyond any discussion. Reference is made to a recent publication by Prof. Dr. Daniel Cardinali, who has 238 citations in Pubmed and is endorsing this proposal as well as other experts who have no doubt that higher doses should be used in scenarios like this. In said publication Cardinali reports using doses greater than 50 mg in pathologies like MS, ALS, and other diseases in humans. Melatonin does not produce respiratory depression nor cardiac arrythmias and no serious effects have been reported to date, so much so that it is sold over the counter for sleep disorders.
Dose-scaling experiments have confirmed remarkable non-toxicity of melatonin in humans at doses of up to 100 mg. Melatonin has also been used in high doses in healthy individuals, athletes and people with pathologies without adverse effects. In healthy women, doses of up to 300 mg daily have been used for 4 months; in healthy individuals 80 mg per hour during 4 hours and worth mentioning is the experience in multiple sclerosis at doses of 50 to 300 mg daily for 4 years. (8-10)

Rationale

In Italy, an excellent study was conducted in 2013 showing its role in serious infectious viral diseases. Melatonin has validated its efficacy and safety in bacterial and viral sepsis in severe conditions with serious lung lesions caused by the sites and mechanism of action of the virus. It is considered that the preservation of the pulmonary function mainly depends on the indemnity of type II alveolar epithelial cells, and this has been correlated with mitochondrial integrity. The de-coupling and inappropriate mitochondrial function leads to the production of free-radical induced cell death. In this respect, melatonin reduces the production of oxygen free radicals and prevents apoptosis and senescence of these epithelial-alveolar cells.

Melatonin has significantly reduced ionizing radiation-induced toxicity with the participation of these mechanisms. COVID-19 virus binds with high affinity (ten times higher than SARS-CoV) to the ACE2 (angiotensin II converting enzyme) to activate the inflammasome NLRP3, causing a violent immune response with a potentially lethal 'cytokine storm'. SARS-CoV-2 produces a huge inflammation, also attributed to the release of large amounts of interleukin 1β (IL-1β). Melatonin produces effects on calmodulin which in turn affects the expression of ECA2, thus regulating the expression of this receptor and confirms another target highly involved in the relation of the virus with alveolar involvement. Additionally, this mechanism could explain the greater sensitivity of the elderly to this infection as the human body produces high concentrations of melatonin during the first three decades of life.

Also, melatonin acts by other mechanisms as a free radical scavenger and on nitric oxide as mentioned earlier, and it is understood that elderly individuals are more vulnerable to such severe inflammation mechanisms.

Melatonin has significantly reduced mortality and helped recover the function of the alveolar epithelium caused by bleomycin in experimental models. Also, well-documented experiences in cancer in humans has proven preventive action given its numerous neuromodulation actions on immunity and on other systems with benefits in pancreatic, lung, breast and gastric cancer where it is no longer a mere promise. (11-32)

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Based on existing references on the efficacy of melatonin in different types of lung lesions, including those caused by viral or bacterial infections, toxic, radiation-induced and pharmacological and its proven high safety profile, the author proposes the preventive use of melatonin in high doses in populations exposed to COVID 19 to try and attenuate or block the inflammatory storm and consequent lung lesion.

Summary of mechanisms

Melatonin reduces the oxidative stress lung damage caused by radiation. Part of the effect is mediated by receptors, whereas it has other receptor-independent mechanisms, like cytoprotection, free radical scanvenger and nitric oxide synthase inhibitor, among others.

Specifically in COVID-19 infection, melatonin may prevent and reduce the lung lesion induced by viruses or bacteria.

The role of ECA2 is the transformation of angiotensin I in angiotensin 1-9 and angiotensin II in angiotensin 1-7. These final products have vasodilation, antifibrotic and anti-inflammatory effects and promote natriuresis. Therefore, they reduce arterial pressure by counterregulating the action of angiotensin II. It has been observed that the serious cases of COVID-19 present very high levels of angiotensin II. Further, the level of angiotensin II has been correlated with the viral load of SARS-CoV-2 and pulmonary damage. This disbalance of the renin-angiotensin-aldosterone system might be related to the ACE2 inhibition by the virus. It should be noted that this effect has already been observed in the SARS outbreak in 2003.
The inflammatory storm is expressed by a massive cytokine release, as shown in several studies and has been recognized as a very important mechanism of action of melatonin. The immunomodulatory actions of melatonin and the inhibiting action against the production and activation of proinflammatory mediators is another mechanism that has been described in several studies on its anti-inflammatory effects.

All of these mechanisms and action sites of melatonin in the same action sites as these viral, bacterial infections, ventilator-induced pneumonia, neonatal sepsis with pulmonary failure, radiation-induced toxicity, cytostatic toxicity, cancer lesions and others, support the need to urgently give an opportunity to high-dose melatonin in the prevention and ultimately in the treatment of COVID 19 infection. There is irrefutable evidence of the safety of this drug after administration of 50 or 100 mg in human volunteers as well as in patients with severe pathologies such as MS, ELAM, gastric, pancreatic cancer and others. (33-60)

Material and methods

Type of design. It is proposed to do a "prospective cohort" evaluation and it is possible to compare against historical controls and affected populations with similar characteristics when there is data available.
In this case there is a possibility to conduct several multicenter studies, there being at present many interested groups waiting for a study models, however with the limitation of not having availability of the drug at the required concentrations. However, there is availability of this molecule worldwide as it is being used for diverse indications as stated earlier.

Ethics committee and informed consent

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In order not to infringe local legal and ethical standards effective in each health care institution when treating patients and/or health care professionals (medical doctors, nurses, physical therapists) that have the indication of this preventive regime, it is recommended that each patient/and or health care member about to implement this proposal should request intervention of the bioethics committee. For the same reason, it is recommended to get an informed consent from the medical group or the health care center involved, adding all relevant information and stating that the patient or the responsible person in good health may have the document available declaring to have read it and having been informed and understood the scope and risk of the therapeutic proposal seeking his/her welfare.

Clinical record

Data registry. General data and then monitoring data to follow evolution, including age, gender, clinical history with co-morbidities, exposure to COVID-19 (history of recent travel and degree of isolation, address, isolation requirement on the grounds of numerous family), symptoms at admission and severity, laboratory values and Rx at admission according to the Standardized Nursing Registry Table and record of indications and specific treatments for COVID-19.

Monitoring of evolution parameters

This monitoring will be done based on the evaluation of data recorded in a Standardized Table by the health care personnel (See Standardized Table). This table will be used to record 3 daily controls, one per nursing shift, that will include minor and major clinical and laboratory predictors and melatonin doses (See Annex 1). These data will have prognostic value and will help to identify if the evolution is positive or negative and in decision-making as reflected in the classification of populations to apply the **initial dose indications** in each case (See Annex 2) to determine in an objective manner what risk group each patient belongs to in each phase of evolution.

ANNEX 1.Predictor to monitor melatonin treatment

Recording in Standardized registry and nursing monitoring table (See Standardized Table)

Minor clinical predictors:

1) Stable symptoms like cough, breathing difficulty or shortness of breath, myalgia
2) Fever
3) Improvement of radiological alterations
4) Oxygen saturation maintained above 93%

Major clinical predictors:

a) Moderate to severe increase of minor clinical predictors
b) Respiratory frequency greater than 30 bpm
c) Pulse oximeter (Sat O2) ≤93% at rest
d) Partial pressure of oxygen (PaO2) / fraction of inspired oxygen (FiO2) ≤300mmHg (1mmHg=0.133kPa).

Biochemical predictors:

a) Neutrophils and lymphocytes
b) GPT
c) LDH

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d) Ferritin
e) PCR
f) Platelets
g) CD4 /CD8
h) D-dimer

Decision-making criteria as per data profile in the Standardized Monitoring Table (See registry and monitoring Table 1) :

a) Nursing table parameters **with stable or descending values for 3-6 consecutive days**is indicative of favorable evolution. This is also considered a check-list for home treatment follow-up.

b) Parameters in nursing table with ascending values is indicative of **unfavorable evolution**and indication of refractory response: it is recommended to assess at shorter intervals to assign a risk group and change dosing patterns.

ANNEX 2. Classification of AT-RISK POPULATION and initial dosing proposal

AT-RISK POPULATION: Individuals older than 60 with concomitant pathologies, specially cardiopathies, respiratory diseases, immunosuppression and individuals with nutritional deficiency without age limit, and health professionals taking care of patients

For the purpose of selecting the melatonin dose, 2 populations will be defined and assigned their respective RISK GROUPS, as follows:

"Infected population" identified as RED Code with 2 risk groups, and
"Health care staff" identified as BLUE Code with 2 risk groups

a) **"infected population"** identified as RED Code with 2 risk groups and one special group of refractory patients treated in critical areas (intensive care units and operating rooms);

b) **"health care personnel"** identified as BLUE Code with 2 risk groups and one special critical areas group (intensive care units and operating rooms); and a non-exposed high risk group

Melatonin dosing options will be seen next to each Risk Group for each one of the 2 populations.

The administration of melatonin will always be scaled rapidly within the high dose range, seeking to be "**appropriate for the response**". This means that the appropriateness of the response will depend on the systematic evaluation of measurements entered in the nursing table to make quick-scale adjustments according to the severity of the entity being treated.

Based on the **Standardized Nursing Registry Table**, that contains clinical and laboratory predictors, decisions will be made to define to which degree of the proposed risk group the

infected patient or the health care professional will be assigned to after each evaluation as per the status of prognostic predictors.

The first goal is to determine in the shortest possible time if there is a favorable response to melatonin at the proposed dose, then scaling the dose to determine what is the dose that really produces an effect or if an effect can be obtained at a lower dose. This will make it possible to provide information to all participants in real time and accelerating the positive outcome of a study by sharing results in a context of pandemic. (54-60)

Initial dosing by risk group

"Dosing must be based on monitoring so that it is appropriate to the response"

Medication should always be administered **at bedtime**, most particularly in the case of health care staff involved in the care of patients.

RED Code POPULATION : COVID19 confirmed or suspected + : Predictors (+ o -) 2 GROUPS

1) **Absence of predictors and COVID19 + test**
   DOSE: 10 mg Day 1; 25 mg Day 2, 50 mg Day 3 and follow-up according to clinical predictor monitoring. IF PREDICTORS ARE IDENTIFIED, THE PATIENT IS MOVED TO GROUP 2
   (see Standardized Nursing Registry and Monitoring Table)

2) **Minor clinical predictors + with or without previous test (urgent testing)**
   DOSE: 25 mg Day 1; 50 mg Day 2, 100 mg Day 3 and follow-up according to clinical predictor monitoring
   (see Standardized Nursing Registry and Monitoring Table)

SPECIAL GRUP: COVID19 + PATIENTS IN CRITICAL CARE AREA UNDER MECHANICAL VENTILATION (Intensive Care Units and Operating Rooms):
50 mg IV every 12 hours and follow-up monitoring major and biochemical predictors.
If no favorable response is observed (clinical and biochemical predictors), increase the dose by 50% every 24 hours.

BLUE CODE POPULATION: COVID19 – : and Absence of clinical predictors
(Prior mandatory detection test)
Health care staff with high exposure during the management of COVID19 patients.

2 Groups of exposed professionals (young and older caretakers) and a third group of health-care professionals NOT exposed (potential reserve and general at-risk population).

1) **Individuals younger than 40 years of age, absence of predictors and COVID19 (-) test**
   DOSE: 10 mg Day 1, 25 mg daily at bedtime as from Day 2. Daily monitoring for early detection of clinical predictive criteria. If a predictor is determined, moves to the corresponding RED Code group
   (see Standardized Nursing Registry and Monitoring Table)

2) **Individuals above 40 years of age, absence of predictors and COVID 19 (-) test**
   DOSE: 10 mg Day 1, 25 mg Day 2, 50 mg Day 3 and 75 to 100 mg daily at bedtime as from Day 4.
   Daily monitoring for early detection of clinical predictive criteria.
   (see Standardized Nursing Registry and Monitoring Table)

NON-EXPOSED HIGH- RISK GROUP. Individuals above 60, poor immunity and individuals with deficient nutritional

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status. Dosing as per Group 2.

SPECIAL GRUP: HEALT CARE PERSONNEL WORKING IN CRITICAL CARE AREA (Intensive Care Units and Operating Rooms):
(Prior mandatory detection test) 25 mg PO Day 1 and scaling to 50 mg Day 2 and 75 to 100 mg at initiation of 12 hour-rest. If assessment determines immunological deficit or age above 60 move to Group 2 regime and it is recommended to be replaced in duties by lower risk individuals.

	08:00to 16:00 hs	16:00 to 24:00 hs	0 to8:00 hs
Minor clinical predictors			
Cough	Número de episodios x turno		
Respiratory difficulty and shortness of breath	0 to 10 Verbal numerical scale		
Resp. Frequency – 30 bpm	N of breaths per minute		
Myalgia (muscular pain) Measuring muscular pain	0 to 10 Verbal numerical scale		
Oxygen therapyYES/NO	Liters x minute continuous/intermittent YES / NO		
Sat. > 93%	%		
Rx / ULTRASOUND / Clinic	YES / No Unilateral / Bilateral		
Major Clinical Predictors			
PCm			
Resp. Frequency >30 bpm	N of breaths per minute		
Sat.>93%	%		
PaFi> 300	PO2 insp/FiO_2		
Severe deficit of ventilatory mechanics	PCO2 >60 and clinical criteria		
Mechanical respiratory assistance	YES / NO		
Biochemical prognostic predictors			
Neutrophils	Measured values / normal values		
Limphocytes T (severe reduction in refractory cases)	Measured values / normal values		
Urea	Measured values / normal values		
AST (TGP)	Measured values / normal values		
LDH	Measured values / normal values		
PCR (C-Reactive Protein)	Measured values / normal values		
Ferritin	Measured values / normal values		
Platelets	Measured values / normal values		
Albumin	Measured values / normal values		
interleukin-6 (IL-6)	Measured values / normal values		
Cardiac troponin	Measured values / normal values		
Cd4+T (reducción severa en casos refractarios)	Measured values / normal values		
Cd8+T (reducción severa en casos refractarios)	Measured values / normal values		

D Dimer	Measured values / normal values		
f IL-2R, IL-6, IL-10, and TNF-α	Measured values / normal values		
Daily melatonin high dose	Dosing appropriate to the response		

Standardized nursing registry and monitoring table

Measured values / normal values: Indicate measurement method or reference value.
Highlighted in bold letter:laboratory variables accessible in most laboratories that have proven to be have early predictive prognostic value of good or poor evolution

In COVID19 + patients in good general condition and descending predictors, home monitoring can be made with telephone or video-conference follow-up.

Industrial production options.
Melatonin IV and PO high dose, master formulas. If available in the higher clinical risk group or in those with the worse immune response and eventually respiratory distress it is recommended to administer IV or by nasogastric tube. Other option could be authorizing master formula preparations at the high capsule dose of 10, 25, 50 and 75 mg.

Registry of adverse effects, toxicity
It is recommended to record every secondary or adverse event occurred during the use of the drug. Communicate every response attributed to the use of the drug and associated medication. Record idiosyncratic responses and allergic events attributed to the drug and record associated medication.

The author's intention is to request special authorization given the exceptional present situation with the relevant consideration of applicable agencies (ANMAT, FDA and others) and Public Health Ministries to authorize the production, facilitate access and promote Preventive High Dose Melatonin in suggested at-risk populations.

Disclosure: The author has no conflicts of interest

I dedicate this proposal to preventive medicine and to all investigators like Prof Dr. Daniel Cardinali (238 PUBMED publications on melatonin), CONICET Emeritus Investigator who has supported this proposal and contributed with his valuable studies an extraordinary powerful tool that deserves to be evaluated in the current scenario.

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