Review

# Estimated doses of melatonin for treating deadly virus infections: focus on COVID-19

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**Running title**: Melatonin dose and viral infection

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## ABSTRACT

Increased evidence implies that melatonin may be a promising molecule for combating COVID-19 due to its potent antioxidative, anti-inflammatory and immunoregulatory capacities. A frequently asked question concerns the suitable dosage of melatonin for deadly virus infections including COVID-19 patients. The golden standards for a suitable dosage of medicine are safety and effectiveness. By reviewing the pharmacokinetics as well as animal studies and clinical trials of melatonin in the deadly viral infections and sepsis, we estimate that a dose of 8 mg/kg/day of melatonin is suitable for COVID-19 patients, especially for the severe cases. To maintain an elevated melatonin serum level lasting longer and smoother, this daily dose can be divided into 5 sub-doses with the initial dose of doubling over the other sub-doses. The recommended dose is in the ranges used to treat septic patients clinically and is devoid of any adverse effect; thus, it is safe. This dose is calculated from an effective dose which significantly reduces the mortality of virus-infected mice and is, therefore, assumed to be effective for COVID-19 severe patients. In our opinion, a dose or a medicine which can only improve the symptoms of mild or moderately severe patients of COVID-19 lack biological significance since virus infection is a self-limited disease and most of the patients with mild or moderate symptoms will recover by themselves whether treated or not. A meaningful treatment is to target the severe patients and significantly reduce the resulting mortality. The suggested melatonin dose is, thus, mainly recommended for the severe COVID-19 patients. The possibility of using suppositories for the delivery of highly dosed melatonin is also addressed, since long-term experience with this treatment is available for another disease.

**Key words**: melatonin, virus, COVID-19, infectious diseases, pharmacokinetics, bioavailability, half-life ($T_{1/2}$).

## 1. INTRODUCTION

Since the outbreak of COVID-19 in Wuhan, China at the end of 2019, it has stormed all European and American countries. In Africa and in the heavily populated India, the infections

are still more clustered, but seem to approach the logarithmic phase. Millions of people have been afflicted and hundred thousand of cases of mortality have been recorded. WHO has classified COVID-19 as a world pandemic. Even though scientists work extremely hard trying to develop effective therapies for COVID-19, only limited success has been achieved to date. There is no doubt that by far the best remedy to shut down this pandemic is vaccine; however, the large-scale availability of a suitable vaccine is many months or years away. During this window period, the majority of individuals may become infected by SARS-CoV-2, a serious perspective for the especially vulnerable individuals over 60 years (1). To identify effective medicines for afflicted individuals and to lower the mortality are still a daunting task for scientists and physicians. Currently, the research focuses on anti-viral drugs, especially on repurposing some established antiviral drugs including anti-influenza virus drugs, lopinavir, ritonavir, the anti-Ebola virus drug, remdesivir, and anti-malaria drugs which exhibit anti-corona virus activity *in vitro* (2, 3). A clinical trial has reported no significant effects having been found in COVID-19 patients treated with anti-influenza drugs, lopinavir and ritonavir (4). Anecdotal information has indicated that chloroquine/hydroxychloroquine may be effective to prevent and treat patients with mild to moderately severe symptoms. However, the published data received only limited support towards these claims (5). For the anti-Ebola virus agent remdesivir, the data are still too limited to judge. For example, among the first 12 COVID-19 patients in USA, 3 of them have been treated with this agent and no conclusion has been drawn and its potential hepatotoxicity is a matter of concern (6). In a case report, it seems that remdesivir has exhibited beneficial effects in a moderately severe patient (7). Currently, several clinical trials have been going on in China and in other countries. The results of these trials will provide reliable information on the treatment effects of remdesivir on COVID-19 in the near future. The low efficacy of anti-viral drugs on COVID-19 is not unexpected. Anti-influenza medicines may be taken as an example. Scientists all over the world have put great efforts for developing anti-influenza drugs. However, due to increased drug resistance and continuously occurring mutations of the virus, we are still in lack of ideal medicines to target this disease and new vaccines have to be repeatedly adapted to the actually appearing viral subtypes, while additional research is going on to develop differently targeting antibodies and other drugs (8, 9). The drugs in the market can only mitigate the mild to moderate symptoms if used in the early stage of the flu, and have reduced effects in the patients with severe symptoms or those who have predisposed complications (10, 11). Thus, the clinical significance is limited, also because viral infectious diseases are self-limiting and the mild to moderately severe patients develop self-recovery without treatment. For the viral infectious diseases, the key is to reduce the severe symptoms, i.e., the massive tissue and organ injury and, finally, to control the mortality. It has been speculated that the severe symptoms are beyond viral cytotoxicity *per se* but result from the overreaction of the innate immune response that causes destructive inflammation, as also observed in severe disease progression of corona virus infections (12). This may be one of the reasons for why antiviral drugs have failed to be effective in severe patients. To compensate the shortcomings of the anti-viral drugs, a more generalized and less virus-specific therapy which instead targets severe symptoms of the viral infection should be considered. Melatonin is a suitable candidate. Melatonin possesses an excellent antioxidative and anti-inflammatory capacity and it balances the overshooting innate immune response while promoting the adaptive immunity (13-16). Currently, an increasing number of publications has suggested or strongly recommended the use of melatonin to combat COVID-19, especially in the severe cases (17–20). Thus, many questions are asked concerning its suitable

dosages for successfully treating this deadly virus infection. Here, we will review the literature and also provide our opinions to scientists and physicians on this question.

## 2. SAFETY CHARACTERISTICS OF MELATONIN

Melatonin should not be considered as a man-made substance (although most of its commercial products contain synthetic melatonin), but rather as a naturally occurring molecule, since it is present in almost all organisms from the bacteria to the human (21). The majority of cells in a human body can synthesize melatonin, as recently shown in the mitochondria (22). In addition, the daily food we consume contains significant amounts of melatonin and these include rice, wheat, bread, meat, fish, eggs, vegetables, fruits, wine and coffee. From this point of view, melatonin is safe to our body in general. Many animal studies have documented its safety. Its oral $LD_{50}$ (Lethal Dose, 50% or median lethal dose) in mice was determined to be around 1,250 mg/kg (23) or simply could not be identified when applying extremely high quantities (24). The study of reproductive toxicology showed that female rats who received an extremely large dose of melatonin (200 mg/kg/day) during entire gestation did not show any side effects to the mothers nor to their pups and the results even indicated that the neonates from the melatonin-treated mothers had a lower incidence of deformations than the controls (25). The results from human trials are consistent with the animal studies discussed above. Here, we only mention several human studies which used extremely high melatonin dosages for a quite long period. Nordlund & Lerner (26) treated patients with 1,000 mg melatonin/daily for 3 months and they did not find obvious side effects in these subjects. Voordouw *et al.* (27), testing melatonin as a contraceptive medicine, treated 12 women with 300 mg melatonin/daily for 4 months and no significant side effects were reported. Weishaupt *et al*. (28) gave to severely ill ALS patients 300 mg melatonin daily for 2 years, without any adverse effects. For the acute melatonin treatment, the dose can be as high as 50 mg/kg for surgical patients, who tolerated this extremely high melatonin dose well and without serious side effects (29). All data indicate that large doses of melatonin, whether given chronically or for acute treatment will not cause intolerable or uncontrollable side effects and that the safety margin of melatonin for humans can be up to 3,750 mg/day for a 75kg individual (29).

## 3. POTENTIAL SIDE EFFECTS OF MELATONIN

The commonly reported side effects of melatonin are headaches, dizziness, nausea and drowsiness in a small portion of consumers (30). Except for drowsiness, which is related to sleep-inducing/sedating properties of melatonin and which especially occurs upon incorrect circadian timing of intake, the other symptoms are typically observed in countless clinical studies on other drugs, even in the placebo groups. In fact, clinical trials found these side effects to be equally distributed in both melatonin and placebo groups (31, 32). Some anecdotal reports claim that melatonin may cause vivid dreams or nightmares. These side effects of melatonin may be dose-related (33), since different melatonin doses cause non-identical activities. For example, low doses of melatonin are more efficient in promoting sleep onset than higher ones, but higher doses induce more drowsiness reflecting an elevated sedative potential (34).

A case report indicated that a diarrhea might have been caused by melatonin (35] and another case report suggested that melatonin might attribute to a manic episode in an adult male (36). The latter observation may have been related to a specific chronotype, but this cannot be

generalized, since bipolar disorder has a circadian dimension and phase shifts in individuals with shortened or lengthened spontaneous period lengths respond differently (37). In fact, these claims have not been confirmed in state-of-the-art clinical trials. Recently, the sudden death of a melatonin-receiving infant has been reported, which was ruled as undetermined. Surprisingly, the melatonin level in peripheral blood had reached a concentration of 1,400ng/mL (38). Whether the death was associated with melatonin has remained unclear since his/her surviving twin also received melatonin supplementation and the authors stated that "the conclusion of the cause and manner of death in this case could not be more definitive".

The potential side effects of melatonin on autoimmune diseases are an intriguing issue since melatonin is considered as an immunoregulator. The results from extensive animal studies are in favor of protective effects by melatonin on autoimmune diseases, but this is not central to this review. Here we focus on the clinical reports related to this issue. An early case report showed that melatonin supplementation worsened symptoms of a patient with Crohn's disease (39). Crohn's disease is an autoimmune disease in the intestine. The authors speculated that melatonin being an immune enhancing agent promoted the autoimmune reaction in this case. In a following study, the same group observed that the worsened clinical manifestation of a patient with chronic colitis was again associated with melatonin intake (40). In contrast, current clinical trials have reported the beneficial effects of melatonin on the chronic irritable bowel diseases (CIBD) including colitis ulcerosa (41, 42). The protective mechanisms are attributed to the strong anti-inflammatory effects of melatonin. Rheumatoid arthritis is another common autoimmune disease often encountered in the older population. Sulli *et al*. (43) and El-Awady *et al*. (44) have observed that the serum melatonin levels in the patients are higher than those of matched control subjects. In addition, melatonin levels have been found positively associated with the severity of the symptoms of the disease. The warning information is that the patients with rheumatoid arthritis should be cautious when taking melatonin. It is not clear whether the increased melatonin is the response of patients to stress, since stress can increase melatonin production (45), although this is not generally so and the opposite has been also observed (46). Currently, Forrest *et al*. (47) went a further step to treat rheumatoid arthritis patients with melatonin. The results showed that this treatment did not worsen the clinical manifestations of the patients but, slowly built up the antioxidant defense of the patients. The results indicate that increased melatonin in rheumatoid arthritis patients is probably not the cause but the consequence of the disease. Real caution is due in autoimmune hepatitis. Two case reports indicated that melatonin may be a potential cause and worsening factor in this disease (48, 49). A similar phenomenon has also been found upon treatment with ramelteon (50), a melatonin receptor agonist.

The different responses of melatonin to autoimmune diseases may be explained by its immune regulatory effects. As mentioned above, melatonin suppresses the overreaction of innate immunity and associated inflammation but promotes the adaptive immune response to facilitate the antibody production. Generally, melatonin can exert context-dependently both anti-inflammatory and proinflammatory actions, with a particular preference for the anti-inflammatory side in high-grade inflammation and in degenerative neuroinflammation (15, 51). If the highly upregulated innate immunity and the resulting inflammation are the main causes of autoimmune diseases such as in the CIBD (41, 42), melatonin exhibits beneficial effects. In the neurodegenerative disease amyotrophic lateral sclerosis (ALS) (28), melatonin reduced the oxidative damage and was moderately beneficial in a murine model. Other examples for prevailing anti-inflammatory actions can be found elsewhere (52, 53). However, if the activated T and B cells (adaptive immune system) and the increased autoantibodies are the main cause of

an autoimmune disease such as autoimmune hepatitis, in which the self-attack is triggered by T-helper cell-mediated liver autoantigen recognition and B-cell production of autoantibodies (54), melatonin may worsen the situation. We should address that the side effects listed here are mainly from the limited numbers of case reports and anecdotal claims and only few from clinical trials. To interpret these data requires great precaution.

Another undesired side effect of melatonin concerns type 2 diabetes. Contrary to antidiabetic actions of melatonin in nocturnal rodents, melatonin reduces glucose tolerance in the diurnally active human (55–57). This effect is strongly aggravated in homozygous carriers of the G allele of the melatonin receptor $MT_2$ (58). The reason of this previously unexpected relationship has been identified and is related to (i) the higher affinity of melatonin to the $MT_2$ variant relative to wild-type and (ii) to the progressive overexpression of the variant $MT_2$ in pancreatic β-cells, which becomes critical in individuals over 45 years (59). Especially under these extreme conditions, melatonin strongly suppresses adenylyl cyclase and, as cAMP is required for insulin secretion, the organism does not respond to elevated glucose. However, this problem can be easily controlled in hospitalized patients, regardless of whether the genotype of $MT_2$ is known or not. The requirement is simply to determine blood glucose levels and administer, if necessary, insulin. Therefore, nothing speaks against the use of high melatonin in diabetics with COVID-19.

## 4. INTERACTIONS OF MELATONIN WITH OTHER SUBSTANCES

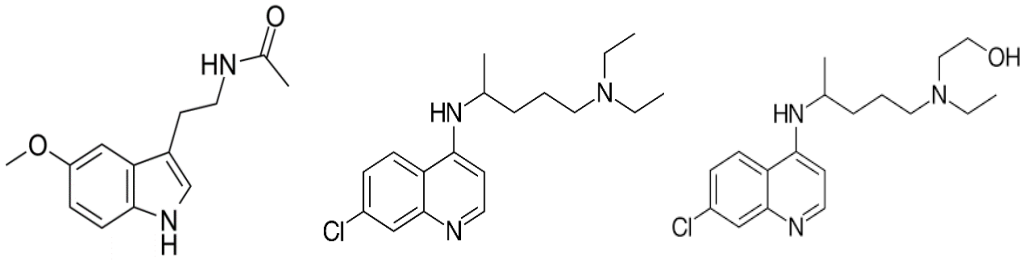
As always, drug interactions have to be considered, because they may limit the use of a medicament in practice. The earliest pertinent report showed that the antihypertensive β-adrenergic antagonist, propranolol, inhibited the nocturnal melatonin production by blocking the β-adrenergic receptor in pinealocytes (60). Additional side effects of β1-adrenoceptor antagonists in the brain, such as disrupted sleep, nightmares and hallucinations have been related to depressed nightly melatonin secretion, however only in terms of correlation and not by direct evidence (61, 62). Benzodiazepines, the most commonly used hypnotic and anxiolytic medicines, also suppress melatonin production in humans (63, 64). The mechanism has remained partially unclear and may be based on GABAergic mechanisms in the pineal gland (65), with additional possibilities concerning the suprachiasmatic nucleus (66), which controls the mammalian pineal and also contains benzodiazepine binding sites. Long term use of benzodiazepines disrupts the melatonin circadian rhythm; therefore, this has been related to increased body weight and visceral adiposity (67). On the reverse, melatonin has also been shown to interfere with GABA receptors and, thus, benzodiazepine binding sites in several brain regions (68). The influences of coffee consumption on melatonin levels in human are inconsistent. A trial showed that the use of coffee significantly increased the nocturnal serum melatonin level, which was interpreted in terms of competition between caffeine and melatonin for the same catabolic enzyme, CYP1A2 (69). Generally, all CYP1A2 inhibitors such as fluvoxamine, cimetidine, ciprofloxacin and enoxacin as well as, to a lesser extent, competing substrates such as flutamide, mirtazapine, olanzapine and tacrin can, in principle, delay the catabolic decay of melatonin, which would be not at all a problem under conditions of severe COVID-19 treatment. However, CYP1A2 inducers such as barbiturates, primidone and rifampin may lead to an undesired enhancement of melatonin catabolism. In contrast, another study on interference by coffee indicated that it might reduce human melatonin production and urinary 6-hydroxymelatonin excretion (70). An additional trial reported that there was no significant difference in the salivary melatonin level after oral intake of 200 mg of caffeine twice a day for 7 days, compared to control (71). There

are several anecdotal claims related to cautions of melatonin interactions with anticoagulants, anti-platelet drugs, antihypertensive medicines and anticonvulsants, etc., but there are no clinical studies to support these claims.

At the moment, accumulated evidence has indicated that the increased blood coagulation tendency has a negative relationship to the symptoms of COVID-19 (72) and anticoagulants are recommended to reduce the severity of the COVID-19 patients (73). Melatonin also exhibits the anticoagulating activity and has been suggested to treat the Ebola virus infection (74). From this perspective, it should not be a problem to use melatonin with other anticoagulants in COVID-19 treatment. In addition, melatonin has a short $T_{1/2}$ (see below) and if physicians identify the bleeding tendency in patients and if this bleeding tendency is related melatonin, melatonin withdraw will achieve rapid results due to its short $T_{1/2}$. Therefore, the concomitant use of anticoagulants and melatonin is safe, at least, it will not cause prolonged bleeding problem after its withdraw.

Here, we shall briefly address potential interactions of melatonin with chloroquine/hydroxychloroquine (CQ/HCQ), since these drugs have been suggested and tested for preventing and treating COVID-19. We have realized that melatonin and chloroquine/hydroxychloroquine have a similar planarity of their chemical structures (Figure 1). In fact, a common binding site for both melatonin and CQ exists, the human quinone reductase 2 (hQR2, or NQO2) (75), which had formerly been assumed to be a third melatonin receptor ("$MT_3$"). The full spectrum of functions of hQR2 has not been clarified. Both quinone reductases 1 and 2 act on several xenobiotics as well as endogenous quinones and are thought to be detoxifying enzymes, but QR2 differs from QR1 by reducing catecholamine quinones (76). Another difference concerns the consequences of their inhibition. While QR2 gene disruption causes increased resistance to para-quinone toxicity, QR1 gene disruption leads to increased para-quinone toxicity (76). In terms of redox biology, quinones and their reduction products, semiquinone radicals and hydroquinones, are easily interchangeable and can undergo organic redox cycling, as discussed for another naphthalenic compound, the melatonergic agonist agomelatine (77). According to their deviating substrate specificities, inhibition of QR2 and QR1 have different outcomes. Therefore, it seems likely that QR2 is associated with reactive oxygen species (ROS) generation and inflammation, whereas its inhibition may contribute to protection in inflammatory diseases (78). Several different inhibitors of QR2 have been identified, among them, besides melatonin, its precursor *N*-acetylserotonin (79) and resveratrol, which was more potent than the indoleamines (80). The diversity of QR2 inhibitors is not surprising, because an enzyme with broad substrate specificity will likely bind many other molecules that may be inhibitory if they are unsuitable as substrates. Structurally, melatonin and chloroquine occupy the same catalytic site of the hQR2 (81, 82) with a comparable affinity of $K_m$s in the range of µM (83–85). Functionally, both molecules have the capacity to reduce cofactor of the enzyme, FAD to $FADH_2$, modify the conformation of hQR2 and inhibit this enzyme (81, 86). There are some differences regarding the inhibiting manner of these two molecules. Kinetic data indicate that melatonin is a competitive inhibitor against the enzyme-specific substrate *N*-methyldihydronicotinamide (NHeM) and a noncompetitive inhibitor versus the classic substrate menadione (84), whereas for chloroquine these properties are reversed (85). Interactions of melatonin and chloroquine have been observed in biological systems in cultured cells cotreated with chloroquine and melatonin, the former partially blocked the melatonin-induced autophagy flux in a wound-healing model (87). In an animal study, melatonin and hydroxychloroquine produced synergistic effects by inhibiting tumor growth via autophagy facilitation which is

otherwise mediated by $MT_2$ (88). There is no evidence for binding of the chloroquines to the $MT_1$ and $MT_2$ melatonin receptors and the interference may be confined to QR2 binding. Inhibition of hQR2 does relate to reduced ROS formation and inflammation (89, 90), but the relevance of melatonin and the chloroquines to COVID-19 appears to be fundamentally different. While initial data on melatonin treatment are highly encouraging, even in severe cases (see this special issue), the efficacy of hydroxychloroquine has been substantially disputed in a recent multicenter trial (91). Authors report somewhat higher mortality in hydroxychloroquine-treated than in untreated patients. Anyway, the chloroquines are known for toxic effects, especially cardiotoxicity (92) and retinotoxicity (93). In the mentioned trial (91), cardiac function had been monitored and there was no sign of overdose, as far as authors had followed the suggestions in a paper they are referring to (94). These doses are in the range of those usually applied in malaria suppression and rheumatoid arthritis (95), whereas higher doses are dangerous. Chloroquine has been even misused for committing suicide (96), whereas a suicide with highest doses of melatonin is virtually impossible.




**MT** **CQ** **CQOH**

**Fig. 1. Structural comparisons of melatonin and chloroquine/hydroxychloroquine.**
*MT: melatonin, CQ: chloroquine, CQOH: hydroxychloroquine.*

## 5. SOME PARAMETERS OF PHARMACOKINETICS OF MELATONIN IN HUMANS

Pharmacokinetics of melatonin have been extensively studied in animal models and also in human clinical trials. Here, we only discuss the human data. Harpsøe *et al*. (97) published an overround review to deal with pharmacokinetics of melatonin in 2015. It is not necessary to repeat their overview. Thus, the data in this review are extracted partially from their publication and partially obtained from other results published since 2015. To simplify our presentation, only the three most important parameters of pharmacokinetics of melatonin are selected including $T_{max}$ (the time from melatonin delivery to its serum maximum concentration), $T_{1/2}$ (the time from its serum maximum concentration till the decay to its half level) and bioavailability. These parameters provide references as to estimate the clinical dosages and the delivery frequency of melatonin to the patients with deadly virus infections such as in COVID-19. The $T_{max}$ of melatonin for orally administration is 46.8 ± 21.5 min (n = 22). This number is calculated from healthy subjects, excluding the potentially interfering factors of smoking, slow-release preparations, coffee consumption and melatonin combinations with other additive agents. The typical $T_{1/2}$ of melatonin for oral administration is 52.8 ± 18.1 min (n = 21). Data from the trials with extremely long $T_{1/2}$ (over 100 min) are excluded. The values of $T_{max}$ and $T_{1/2}$ are calculated using data extracted from Harpsøe *et al*. (97) combined with Galley *et al*. (98) and Anderson *et al*. (99, 100). The bioavailability of melatonin for humans is considerably lower than that in experimental animals. This should be taken into consideration when comparing with dosages used in animals. The bioavailability of melatonin for humans is 18.9% and ranges from 8.7-33%.

These numbers are obtained from the data extracted from the review of Tan *et al*. (45). Currently, Anderson *et al*. (99) have reported an extremely low bioavailability (3%) of melatonin in humans and this value is not included in our calculation. The relatively low bioavailability of melatonin in humans reflects the high first-pass effect (rapid metabolism by liver when melatonin first passes it) or the potential entering of melatonin to the enterohepatic circulation, since extremely high melatonin levels have been detected in the bile of humans (101). For the convenience of readers, these parameters of melatonin pharmacokinetics are graphically presented in Figure 2. There are few trials on the elderly population, but a couple of clinical trials have investigated the pharmacokinetics of melatonin in neonates. The $T_{1/2}$ of melatonin in neonates is remarkably prolonged compared to adults and ranges from 7.98-15.82 hr. (102, 103). This significantly prolonged $T_{1/2}$ should be taken a consideration when melatonin is used in the neonates. One should be also aware that pharmacokinetic data depend on the formulation (cf. controlled/extended release), mode of administration (cf. infusion, suppositories) and can become dosage-dependent, especially when very high doses are applied.

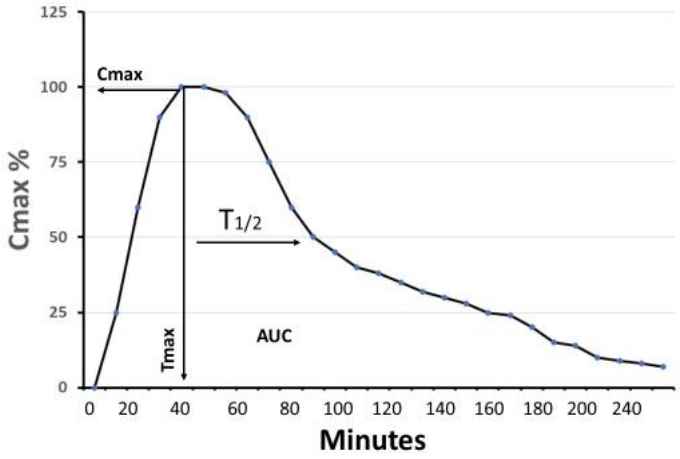



**Fig. 2. Illustration of pharmacokinetics of orally administered immediate-release melatonin.**

*$C_{max}$: the serum maximum melatonin concentration after delivery; here this parameter is expressed as 100% for the easy understanding, $T_{max}$: the time from melatonin delivery to its serum maximum concentration, $T_{1/2}$ (the time from its serum maximum concentration decays to its half level), AUC: area under the curve. $T_{max}$ = 46.8 ± 21.5 min and $T_{1/2}$ = 52.8 ± 18.1 min as indicated in the graph.*

## 6. APPARENT EFFECTIVE DOSES OF MELATONIN IN ANIMALS WITH DEADLY VIRAL INFECTION AND IN HUMAN SEPSIS

There are no clinical trials that have investigated the potential effects of melatonin on virus infections yet. Here, we have listed the details of some animal studies related to protective effects of melatonin on virus infections (Table 1), which can be used as references to justify the use of melatonin in human trials, particularly in COVID-19.

In addition, we have listed the clinical trial information related to melatonin treatment on sepsis in Table 2. The clinical manifestations of sepsis are similar to those in virus infections. Our focus is to address dosages used in these trials and try to justify the suitable dose which will be recommended for the use in deadly virus infections including the COVID-19.

**Table 1. Information of melatonin used in the animals with deadly viral infections.**

| Virus | type | Disease | Animal | Melatonin doses | Results | Ref. |
|---|---|---|---|---|---|---|
| RSV | -ssRNA | Lung infection | Mice | 5mg/kg, trice/day for 3 days after infection. | ↑ SOD, GSH; ↓ TNF-α, NO, MDA, ·OH. | (104) |
| VEEV | +ssRNA | Encephalo-myelitis | Mice | 0.5 mg/kg/day, 3 days before and 5 days after infection. | ↓ mortality from 100% to 45%, ↓ virus load. | (105) |
| VEEV | +ssRNA | Encephalo-myelitis | Mice | 1 mg/kg/day. | ↓ mortality from 100% to 16%, ↓ virus load, ↑ IgM. | (106) |
| VEEV | +ssRNA | Encephalo-myelitis | Mice | 0.5 mg/kg /day. | ↓ mortality from 100% to 75%, ↓ neuron oxidative damage and apoptosis. | (107) |
| RHDV | +ssRNA | Rabbit hemorrhagic disease | Rabbits | 10, or 20 mg/kg i.p. at 0, 12, and 24 hr. after infection. | ↓PARP1, Caspase3/8/9, Bax, Cyto C, GSSG/GSH, TBARS. ↑ Bcl-2, Bcl-xl (high dose is more effective). | (108) |
| RHDV | +ssRNA | Rabbit hemorrhagic disease | Rabbits | 10, or 20 mg/kg i.p. at 0, 12, and 24 hr. after infection. | ↓ TLR4, TNF-α, IL-6, ↑ NF-κB. | (109) |
| SFV | +ssRNA | Encephalitis | Mice | 0.5 mg/kg (sc.) 3 days before and 10 days after infection. | ↓ mortality from 100% to 44%. | (110) |
| WNV | +ssRNA | West Nile fever | Mice | 0.17mg/kg (i.m.) 2 days before and 8 days after infection. | ↓ mortality from 75% to 31%. | (110) |
| Influenza A virus | -ssRNA | Influenza | Mice | 20 or 200 mg/kg/48 hr (10 or 100 mg/kg/24 hr.). | 20 mg slightly, 200mg significantly ↓ mortality (Fig. 3). | (111) |

*RSV: respiratory syncytial virus, VEEV: Venezuelan equine encephalomyelitis virus, RHDV: rabbit hemorrhagic disease virus. SFV: Semliki Forest virus, WNV: West Nile virus.*

**Table 2. Information of melatonin used in the human sepsis.**

| Treatment | N (T/C) | Doses | mg/kg/ day | Results | Ref. |
|---|---|---|---|---|---|
| RDS of infants | 60/60 | 10 doses (each of 10 mg/kg)/72 hr. (the first four doses separated by 2-hr intervals, the fifth and the sixth separated by 4-hr intervals, the seventh and eighth by 8-hr intervals and the ninth and 10th separated by 12-hr intervals (iv). | 33 | Two died in controls, no death in melatonin treated. No adverse effects. ↓ TNF-α, IL-6, IL-8, NO. | (112) |
| Sepsis of newborns | 10/10 | A total of 20 mg melatonin (2 doses of 10 mg separated by a 1-h interval) orally. Average weight of 3.5 kg. | 5.7 | 3 of 10 died in controls, no death in melatonin treated. ↓ MDA+4-HAD. | (113) |
| Surgical neonates | 10/10 | 10 doses (each of 10 mg/kg) /72 hr. (the first 4 doses separated by 2-hour intervals, the fifth and the sixth separated by 4-hour intervals, the seventh and eighth by 8-hour intervals and the ninth and tenth separated by 12-hour intervals, each infusion taking 2 hours). (iv). | 33 | ↓ TNF-a, IL-6, IL-8, NO. WBC, ANC, CRP. Improvement of clinical outcome. | (114) |
| Daytime endotoxemia (adults) | 12/12 | 100 mg (an 8-hour infusion) (assumed 70 kg/adult). | 1.4 | ↓ IL-1β, YKL-40. | (115) |
| phase I dose escalation study for sepsis | 20 | 20, 30, 50, and 100 mg for individuals in each group. Average weight of 76 kg (select highest dose). | 1.3 | No subject reported nausea, headache, vomiting, diarrhea, or abdominal pain. Some subjects reported drowsiness. | (98) |
| Nighttime endotoxemia (adults) | 12/12 | 100 mg (an 8-hour infusion) (assumed 70 kg/adult). | 1.4 | No changes of proinflammatory cytokines. | (116) |
| Neonatal sepsis | 25/25 | 20 mg given through the oro/naso-gastric tube. Average weight of 2.44 kg. | 8.2 | improvement of sepsis score, clinical and laboratory outcome. | (117) |
| Neonatal sepsis | 20/20 | 20mg melatonin as single dose by nasogastric tube. Average weight of 2.47 kg. | 8.1 | One died in control, no death in melatonin treated. ↓ hs-CRP, ↑ platelets. Improved clinical outcome. | (118) |
| Average | | | 11.5 (05-33) | | |

*RDS: respiratory distress syndrome, MDA: malondialdehyde, 4-HDA: 4-hydroxylalkenals, CRP: C-reactive protein, ANC: absolute neutrophil count, hs-CRP: high sensitive C-reactive protein. N: sample size, T/C: treated/control. When calculation of the average amounts of melatonin (mg/kg), we did not weigh the different administration routes (iv or orally). It is difficult to make such an adjustment since, in some studies, the infusion time is 2 hours and others 8 hours. In addition, the bioavailability of orally administered melatonin for individuals shows substantial differences. Thus, we assume in this context that the amounts of melatonin given in these different routes are equally efficient. This assumption is not perfect, but it is the simplest way to provide a reference value to readers.*

## 7. ESTIMATED MELATONIN DOSAGE FOR THE DEADLY VIRUS DISEASES, PARTICULARLY IN COVID-19

Currently, the frequently asked question is what doses of melatonin are suitable for COVID-19 treatment. This is a tough question to answer since COVID-19 is a completely new disease and there are no clinical data or even animal studies of melatonin on this disease. This review is not to definitely answer this question but to provide information in the simplest way as possible to researchers and physicians, in order to quickly figure out the range of adequate doses of melatonin used in deadly virus infections, especially COVID-19. In other words, readers can select a suitable dosage based on safety margin, pharmacokinetics and doses already used in septic patients or animal studies listed in the tables 1 and 2. In addition, we also provide our opinions.

For melatonin, the usual dosing guideline refers to applications that are entirely different from anti-viral therapies. The general dose for sleep or jetlag is 3 mg to be taken half an hour before bedtime. No one knows how this 3 mg melatonin has been selected for food supplement purposes in the US. In the European Union, the European Medical Agency (at that time, EMEA) restricted controlled-release melatonin (Circadin®) to 2 mg, largely for reasons of caution concerning long-term treatment necessary in insomnia. However, to date there is no solid academic data to support these regulations. The anecdotal claim is that this dose reflects the night peak of physiological melatonin in the circulation. However, the circulating melatonin level, although being of relevance to receptor saturation, does not consider the real melatonin levels in other sites of the body. For example, the melatonin levels in the third ventricle or in the bile are several orders of magnitude higher than those in the serum (119). We have hypothesized that the circulatory melatonin is, in a sense, kind of a residue of the melatonin generated in the pineal gland, which is primarily targeting the suprachiasmatic nucleus via the CSF. In other words, there is no convincing standard melatonin dose and the origin of the 3 mg melatonin standard is a mystery. In addition, the destructive inflammation and massive pathological alterations occurring in the severe COVID-19 patients require adequate measures that are not satisfied by the so-called physiological levels of melatonin. The suitable doses should be based on the animal studies and clinical trials of sepsis listed in Table 1 and 2. The effective doses of melatonin listed in Table 1 are considerably variable. This may be due to the different types of viruses and different animal models and, thus, variable responses to melatonin have to be expected. However, we strongly suggest to consider the dose selected by Huang *et al*. (111) used to treat the H1N1 virus-associated deadly influenza. In this study, the authors selected two melatonin doses and found that melatonin exhibited dose-related effects as to reduce the mortality caused by the H1N1 influenza virus. Melatonin, at the dose of 10 mg/kg/day (20 mg/kg /48 hr.), had a demonstrable

but only slight effect, whereas the dose of 100 mg/kg/day (200 mg/kg/48 hr.) substantially reduced the mortality. This effect was even more profound when melatonin was given early or cotreated with an antiviral medicine (ribavirin) (Figure 3). If we convert this murine to the human dose according to standard dose translation, based on the surface area by dividing a factor 12.3 (120), the calculated equivalent human dose is 8.1 mg/kg/day (100/12.3 = 8.1). This dose is very similar to the dose used in two neonatal septic trials (8.1 and 8.2 mg/kg/day listed in Table 1, last 2 trials), although, in this case, the neonatal/adult dose translation would have to be considered. The average effective dose for human sepsis obtained in Table 2 is 11.5 mg/kg/day with a range from 0.5 – 33 mg/kg/day (Table 2). The converted dose from mouse to human of 8.1 mg/kg/day is within the range of average effective dose for human sepsis. Importantly, this dose should not cause obvious adverse effects, according to these clinical trials. Thus, the estimated dose to treat the deadly virus infectious diseases including COVID-19 is around 8 mg/kg/day. For a 75 kg individual, the daily dose is 600 mg (75 x 8 = 600). Based on the relative short $T_{1/2}$ of melatonin, it seems preferable to divide the 600 mg to 5 partial doses for maintaining continuously elevated melatonin levels. Circadian issues do not appear to be of major importance, since the concentrations needed for suppressing the overshooting inflammation have anyway to exceed the receptor-saturating levels. The initial dose is 200 mg to quickly increase the serum melatonin level, whereas the maintenance doses given 4 hours later ($T_{max}$ + around 3 $T_{1/2}$), i.e., second dose of 100 mg, and thereafter every 5 hours 100 mg for the rest of the treatment day. Apart from efficacy, the most important property of a drug has to be its safety and to be devoid of additional damage to the patients, especially those in severe condition. Melatonin in this dosage is remarkably safe even to the neonates (Table 2). This dose of 8 mg/kg/day can be used in the severe COVID-19 patients. Even if its efficacy would turn out to be insufficient, it will not cause any harm over several days of administration. For the mild to moderately severe patients, this dose may be reduced.

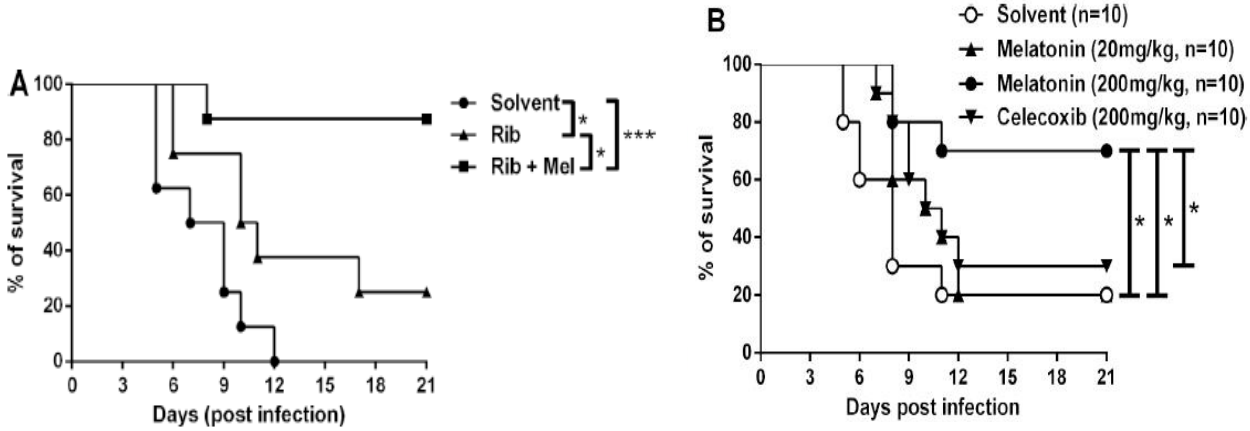



**Fig. 3. Effects of melatonin on mortality of mice inoculated with H1N1 influenza virus.**

*A. Infected mice were treated with solvent (n = 4), 20 mg/kg ribavirin (Rib) (n = 18) or 20 mg/kg ribavirin plus 100 mg/kg melatonin (Rib + Mel) (n = 18) daily for 5 days. B. Melatonin or celecoxib were given at 6 h before intranasal infection with the virus and given twice again at day 2 and 4 post-infection. The doses mentioned in B are for 48 hr. The numbers should be 10 mg/kg/day or 100 mg/kg/day as mentioned in the text. *p < 0.05, ***p < 0.01. The figure is modified from Ref. 111.*

## 8. SUPPOSITORIES, AN ALTERNATE MODE OF ADMINISTRATION

While melatonin is mostly given orally to humans, another possibility is the use of suppositories, which contained 300 mg in the original study in which this application was chosen (28). This procedure was developed for and used in a long-term study over 2 years in ALS patients, in order to facilitate the administration to individuals with the ALS-typical difficulties of swallowing (28). When following the levels of blood melatonin, a rapid and very strong increase was observed after application in the evening, but the daytime levels decreased relatively slowly and remained considerably elevated at the diurnal timepoints of measurement. It seems that this method of administration is associated with a certain slow-release effect, due to a slower dissolution of the wax carrier. Moreover, rectal administration is known to reduce, in other tested drugs, the first-pass metabolism by up to two thirds (121). Therefore, melatonin given by this procedure may attain a higher bioavailability. The procedure of melatonin-containing suppository production is described and had been granted a meanwhile expired patent (28, 122). The safety of this application is shown by the long duration of the study, carried out in severely ill patients. The advantage of enteral administration could be that the number of treatments per day might be reduced, perhaps, to 2 daily applications or only 1. This could be more convenient to both the caregivers and the patients especially with artificial ventilation. One may also consider variants, such as two different suppositories, a higher and a lower dosed one, for the use in the evening and after lunch, respectively. The optimal dosing has to be empirically found out. However, there should not be a risk for overdosing.

## 9. CONCLUSION

An approved dosage for treating COVID-19 patients with melatonin does not yet exist. However, the experience with other deadly viral diseases in animals and with sepsis in humans can be taken for an approximate suggestion for clinical practice. The relative uncertainty about the exact doses in mild, moderate and severe cases of this disease should not be seen as a major problem and not as a reason of concern. The most inopportune situation could result from an insufficient dose, but the reverse, overdose, is not a serious risk because of the extremely good tolerability of melatonin. Interactions with other drugs are mostly restricted to changes in melatonin levels, which are, however, not dangerous. Drug interactions that substantially increase the actions of other medicaments have, to the best of our knowledge, not been reported. Clinicians should not hesitate to try melatonin in the combat against COVID-19. In case of success, the suitability of melatonin should be remembered after the time of the actual “corona crisis”, as soon as other deadly viral diseases with overshooting inflammatory responses emerge.

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## AUTHORSHIP

DXT and RH both wrote and edited this article.

## CONFLICT OF INTEREST

Authors declare no conflict of interest.

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