

ORIGINAL ARTICLE

Dose assessment of melatonin in sepsis (DAMSEL2) study: Pharmacokinetics of two doses of oral melatonin in patients with sepsis

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Abstract

Sepsis is defined as a dysregulated host response to infection, and high-dose melatonin has been proposed as a treatment due to its antioxidant and anti-inflammatory properties. However, there are no data describing the pharmacokinetics of high-dose oral melatonin in critically ill patients. We undertook an open-label trial to determine the tolerance of melatonin administration in these patients and pharmacokinetic analysis, to inform a planned randomised controlled trial. Two cohorts of critically ill patients with sepsis due to community-acquired pneumonia received either 20 or 50 mg oral melatonin liquid as a single dose. Blood samples and clinical measures were analysed over the next 24 h. Melatonin was well tolerated and there were no adverse events. Pharmacokinetic modelling showed that a semiphysiological model, which incorporates saturable first-pass hepatic extraction, was a good fit for our data. Maximum levels of melatonin were extremely high in patients receiving the 50 mg dose and levels of the major metabolite were much lower than expected and not different from those seen after 20 mg, suggesting saturation at the higher dose. We conclude that 20 mg seems a suitable dose of liquid melatonin in patients with sepsis.

KEYWORDS

clinical trial, melatonin, pharmacokinetics, sepsis

1 | INTRODUCTION

Sepsis is defined as a life-threatening organ dysfunction due to a dysregulated host response to infection and is the main cause of death in intensive care units in the United Kingdom.¹ The infection can be bacterial, viral or fungal.² Deaths from SARS-CoV-2 infection (COVID-19) are often the result of sepsis and the immune responses

to COVID are broadly similar to those seen with sepsis due to other respiratory viruses.³

Oxidative stress in patients with sepsis has been consistently described over the last 20 years (reviewed in Macdonald et al.⁴). Recent reviews also postulate oxidative stress involvement in COVID-19 sepsis.^{5–7} Mitochondrial dysfunction initiated by oxidative stress is generally accepted as playing a major role in sepsis.^{8,9}

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and the potential benefit of antioxidants which specifically protect mitochondria during sepsis has been recognised.^{10,11} Melatonin is a potent antioxidant which accumulates in mitochondria after exogenous administration and both its metabolites and reaction products are also antioxidants.¹² In addition, it augments endogenous antioxidant defence systems.¹³

There is compelling evidence from animal studies showing that melatonin is beneficial in sepsis, with decreases in inflammatory mediators, reduced oxidative stress and mitochondrial damage, reductions in biomarkers of organ dysfunction, and improved survival reported in preclinical sepsis models.^{14–16} Melatonin has been proposed as a potential treatment for sepsis^{14,17} and more recently as a therapy for COVID-19.^{18–20} Low-dose melatonin has been given to critically ill patients with the aim of restoring sleep patterns²¹ and there has been one clinical trial of high-dose oral melatonin as part of a study of antioxidant therapy in patients with septic shock.²² Protocols for trials of intravenous melatonin in COVID-19 sepsis have recently been published.^{23–25}

Oral melatonin given to healthy subjects at doses up to 100 mg is well tolerated but with low oral bioavailability and rapid clearance.²⁶ Circulating melatonin levels after oral dosing show marked interindividual variability^{23,26} due to variable first-pass extraction in the liver because of genetic differences in cytochrome P450 enzymes which convert melatonin to the 6-hydroxymelatonin metabolite (6-OHM) and the sulphotransferase (SULT) enzymes which are responsible for sulphation of 6-OHM.²⁷ Pharmacokinetic data, specifically after high-dose oral liquid melatonin in patients with sepsis, are lean, and so we therefore undertook an open-label trial to provide information on how well high doses of oral liquid melatonin are tolerated in these patients, what levels of melatonin and 6-OHMS are achieved at each dose, and a suitable dosing interval, to inform a planned funded randomised placebo-controlled trial. We also undertook pharmacokinetic modelling to compare the oral liquid formulation with our existing data from oral capsules given to healthy subjects.

2 | METHODS

2.1 | Participants and study design

The study was classed as a Clinical Trial of an Investigational Product (CTIMP) and a Clinical Trial Authorization was obtained from the Medicines and Healthcare Regulatory Authority (MHRA), in addition to a favourable ethical opinion from the Scotland A Research Ethics Committee. Participant

recruitment took place in 2018, before the COVID-19 pandemic.

The trial was prospectively registered at <https://www.isrctn.com/ISRCTN70688534> and was an open-label two-dose cohort study.

Written informed consent was obtained either from the patient, or, since most of the patients were not able to consent for themselves, their legal representative—a welfare guardian, a welfare attorney, a near relative or close friend, a clinical person not involved in the study, or another independent person nominated by the healthcare provider, according to the Medicines for Human Use (Clinical Trial) Regulations 2004. Trial Steering and Data and Safety Monitoring Committees with external chairs were established. The study was sponsored jointly by the University of Aberdeen and NHS Grampian and was monitored by NHS Grampian. The work was funded by the Chief Scientist Office of Scotland (Reference number ETM/538).

This was an open-label pharmacokinetic and safety study of high-dose oral liquid melatonin in patients with sepsis due to community-acquired pneumonia, with the first cohort of five patients receiving a single 50 mg dose of oral liquid melatonin, then, after approval from the Data and Safety Monitoring Committee, we planned that a second cohort of five patients would receive 100 mg melatonin. All adult patients admitted to either the intensive care unit (ICU) or medical high dependency unit (MHDU) at Aberdeen Royal Infirmary, Aberdeen, UK, were screened (Figure 1). Patients who fulfilled the criteria for sepsis with clinical suspicion of community-acquired pneumonia and the presence of chest X-ray changes consistent with pneumonia in a 24-h period were eligible. The inclusion and exclusion criteria are listed in Table 1. We did not recruit any patients with evidence of hepatic dysfunction, those who were likely to have immunosuppression, such as those with metastatic cancer, or who had been treated with steroids.

2.2 | Intervention

Participants received an oral liquid containing 1 mg/ml melatonin (Apotek Produktion & Laboratorier) as a single dose, either by nasogastric tube or orally as a drink. For safety reasons we did not have more than one patient in the study at any one time. We also paused recruitment between the two dose cohorts to allow data analysis and safety reporting, on the recommendation of the Data and Safety Monitoring Committee.

The composition of the liquid melatonin is provided in Supporting Information: Table 1. Blood samples were taken from indwelling cannulae before, and 10 and 30 min, then 1, 2, 6, 12 and 24 h after melatonin administration. Usual

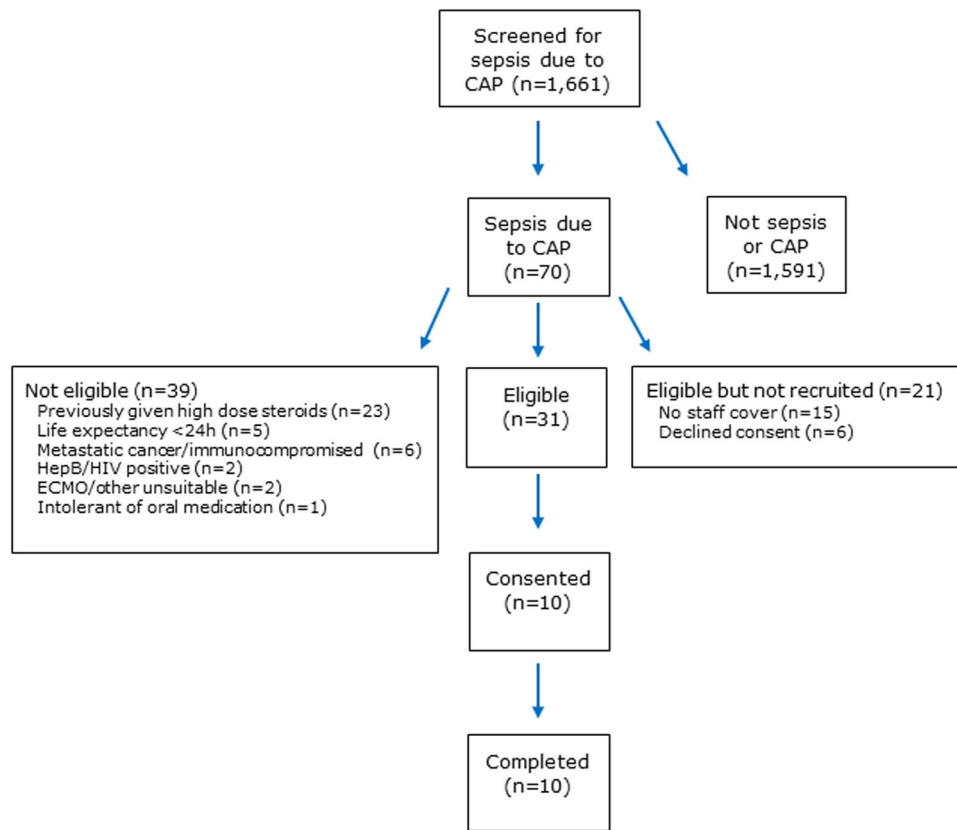


FIGURE 1 Consolidated standards for reporting of trial (CONSORT) diagram showing the flow chart of recruitment to DAMSEL2. DAMSEL2, dose assessment of melatonin in sepsis.

TABLE 1 Trial inclusions and exclusion criteria

Inclusion criteria	Exclusion criteria
Life expectancy of at least 24 h	Life expectancy <24 h
Able to tolerate oral medication	Unable to tolerate oral medication
Age > 16 y	Age < 16 y
Admitted to ICU or MDHU with clinical suspicion of sepsis and X-ray changes consistent with pneumonia <i>plus</i> two of the following in a 24 h period Leucocyte count $<4 \times 10^9/L$ or $>12 \times 10^9/L$ Temperature $<36.0^\circ C$ or $>38^\circ C$ Heart rate >90 beats/min Respiratory rate >20 breaths/min or mechanically ventilated	Treatment with steroids (>20 mg/day prednisolone or equivalent before ICU admission) Women of child-bearing potential without a negative pregnancy test or a history of surgical sterilisation
Bilirubin $<80 \mu\text{mol/L}$	Cancer or immunosuppression Overt hepatic failure Known to be HIV or hepatitis B positive Receiving fluvoxamine or nifedipine Known to be hypersensitive to trial medication and/or excipients

Abbreviations: ICU, intensive care unit; MDHU, medical high dependency unit.

clinical care continued, and admission acute physiological and chronic health evaluation (APACHE) II scores, along with heart and respiratory rates, mean arterial pressure, core temperature, leucocyte count and various biochemical measures were recorded. Participants remained in the study for 24 h. Adverse events were recorded and assessed for severity, expectedness, causality and seriousness. To assess whether melatonin administration caused drowsiness, the Richmond Agitation and Sedation score (RASS)²⁸ was recorded at baseline, and hourly after melatonin administration, for 24 h.

2.2.1 | Melatonin and 6-OHM sulphate analysis

Blood samples were collected into 5 ml clot activator-serum separator vacutainers, allowed to clot and then centrifuged at 1300 g for 10 min at room temperature within 2 h of collection. Serum was then stored at -80°C until assay, within 1 month. Melatonin was measured in duplicate by chromatography-tandem mass spectrometry, which is highly specific and described in detail by us previously.²⁶ 6-OHMS was measured in duplicate using a commercially available competitive enzyme immunoassay (Abbexa Ltd.) according to the manufacturer's instructions. There is no cross-reactivity with native melatonin or other metabolites and the between-assay precision in our hands was 10.8% (coefficient of variation, $n=6$).

2.2.2 | Population pharmacokinetic modelling

We undertook population pharmacokinetic modelling of both our previous data after administration of melatonin given as oral gelatine capsules to healthy young men (DAMSEL1),²⁶ combined with the data from the patients with sepsis presented here (DAMSEL2), to characterise the differences between melatonin given as capsules or as a liquid.

The serum melatonin concentrations from patients with sepsis and the previously published serum and urine concentrations from healthy subjects were fitted using the FOCE-I estimation algorithm in NONMEM® (Version 7.4; GloboMax LLC). The 'tidyverse' package (Version 1.1.1.; Wickham H. 2017) in R® (R Foundation for Statistical Computing) was used to graphically assess the goodness-of-fit (GOF) of the candidate models and for simulations.

Interindividual variability on the typical population parameter estimates was assumed to be log-normally

distributed. Residual unexplained variability was modelled using an additive error model in the logarithm of the observed and predicted concentrations. Inclusion of covariates in the model was driven by graphical evaluation of the relationship between random effects (ETAs) and the covariates. Covariates tested for inclusion in the model were: weight (kg), age (years), serum creatinine (mg/dl), melatonin dose (mg), formulation (capsule or liquid) and subject group (healthy subjects or patients with sepsis).

During model building, modifications to the model were accepted only if they resulted in a significant decrease in the objective function value (i.e., $\Delta\text{OFV} < -3.84$ for one additional parameter). Non-nested models were compared using the Akaike Information Criterion. Internal model validation for the final model was based on GOF plots based on the normalised prediction distribution errors.²⁹

2.3 | Statistical analysis

All model parameters are reported as typical values with associated 95% confidence intervals (CIs) derived from log-likelihood profiling.

Data are presented as median and full range and shown as individual raw data points. Statistical analysis was undertaken using Analyse-It statistical add-in for Microsoft Excel using Wilcoxon–Mann–Whitney testing as appropriate.

3 | RESULTS

3.1 | Participants

The Consolidated Standards of Reporting Trials (CONSORT) diagram (Figure 1) shows the recruitment details of patients with sepsis. In total, 1661 patients were screened (667 in ICU and 994 in MHDU); 70 were identified as having sepsis due to community-acquired pneumonia and were therefore potentially eligible (29 in ICU and 41 in MHDU). Thirty-nine patients met the exclusion criteria and were not recruited, mainly due to previous high-dose steroid administration before ICU/MHDU admission. Of the remaining 31 patients, 21 were eligible but not recruited due to the reasons shown in the CONSORT diagram (Figure 1), and 10 were recruited, 5 to each dose cohort. Baseline clinical and biochemical parameters are shown in Table 2.

In the original protocol, it was planned that the first dose cohort of patients would receive 50 mg melatonin

and then for the second cohort we would escalate to a dose of 100 mg. However, analysis of samples from the first dose cohort revealed extremely high melatonin levels and lower than expected 6-OHMS levels (see below), suggesting saturated metabolism. Although the very high circulating levels of melatonin were not considered to be harmful, after consultation with the trial Data and Safety Monitoring Committee, the dose of melatonin for the second cohort was decreased to 20 mg rather than increasing to 100 mg. Thus, the two final dose cohorts were 20 and 50 mg. The first dose cohort (50 mg) comprised five patients (all male, aged 54–70 years) and the second dose cohort (20 mg) comprised five patients (4 male/1 female, aged 45–83 years). All subjects completed the study protocol and there were no deaths during the 24 h of the study.

TABLE 2 Clinical parameters (worse value in 24-h period)

Measure	Cohort 1 (50 mg)	Cohort 2 (20 mg)
Age (years)	60 [54–70]	50 [45–83]
Temperature (°C)	38.8 [37.2–38.9]	38.5 [38, 39]
Heart rate (beats/min)	111 [85–180]	105 [98–167]
Respiratory rate (breaths/min)	30 [26–35]	28 [24–30]
Leucocyte count ($\times 10^{12}/L$)	12.6 [6.4–14.0]	11.7 [7.4–19.7]
Mean arterial pressure (mmHg)	131 [86–134]	110 [95–158]
Admission APACHE II score	14 [9–29]	18 [6–20]
Arterial blood lactate (mmol/L)	1.3 [1.0–2.3]	1.1 [0.9–1.2]
Bilirubin ($\mu\text{mol}/L$)	11 [5–41]	11 [6–19]
Creatinine ($\mu\text{mol}/L$)	86 [52–107]	73 [59–92]
Sodium (mmol/L)	142 [136–147]	136 [130–137]
Potassium (mmol/L)	3.7 [3.2–4.1]	4.0 [3.7–4.5]

Note: Median [range].

Abbreviation: APACHE II, acute physiological and chronic health evaluation.

TABLE 3 Pharmacokinetic measures

Measure	Cohort 1 (50 mg)		Cohort 2 (20 mg)	
	Melatonin	6-OHMS	Melatonin	6-OHMS
C_{max} (ng/ml)	1465 [986–1928]	6.6 [6.4–8.6]	240 [146–350]	4.7 [3.8–8.0]
T_{max} (min)	30 [10–60]	120 [60–120]	30 [10–60]	60 [30–120]
C_{min} (ng/ml)	0 [0–0]	0.07 [0.04–0.16]	0 [0–1.6]	0.02 [0.02–0.03]

Note: Median [range].

Abbreviation: 6-OHMS, 6-hydroxymelatonin sulphate.

Recruitment of healthy volunteers in DAMSEL1 has been fully reported previously.²⁶

3.2 | Adverse events

All participants in DAMSEL2 tolerated the melatonin very well. Only one participant (50 mg dose cohort) was awake and able to take the liquid orally; in all others, melatonin was administered by nasogastric tube. There were no incidents of vomiting or diarrhoea, no changes in clinical parameters and no other acute effects in the 24 h after dosing. There were four serious adverse events in four separate patients, all of which were expected in this critically ill population and none of which were considered to be related to the study drug. Administration of melatonin had no effect on RASS in any participant.

3.3 | Pharmacokinetic data

3.3.1 | Melatonin and 6-OHM sulphate concentrations

Serum melatonin levels after both doses were highly variable between individuals, increased rapidly, peaked between 10 and 60 min and returned to pre-dose levels by 12–24 h (Table 3). Maximum melatonin levels (C_{max}) in the patients with sepsis who received 50 mg liquid melatonin were extremely high, with a median [range] concentration of 1465 [986–1928] ng/ml, which was considerably higher than we had found previously with the same dose in healthy subjects using melatonin in gelatine capsules.²⁶ At the lower melatonin dose (20 mg), C_{max} levels in patients with sepsis were significantly lower than levels seen after the higher dose (240 [146–350] ng/ml, $p = .008$, Figure 2) but again much higher than in the healthy subjects given melatonin in capsules.²⁶ Serum levels of 6-OHMS also increased rapidly, but the time to maximum concentrations (T_{max}) was longer than for melatonin and levels returned to baseline by 6–12 h (Table 3). The C_{max} of 6-OHMS after

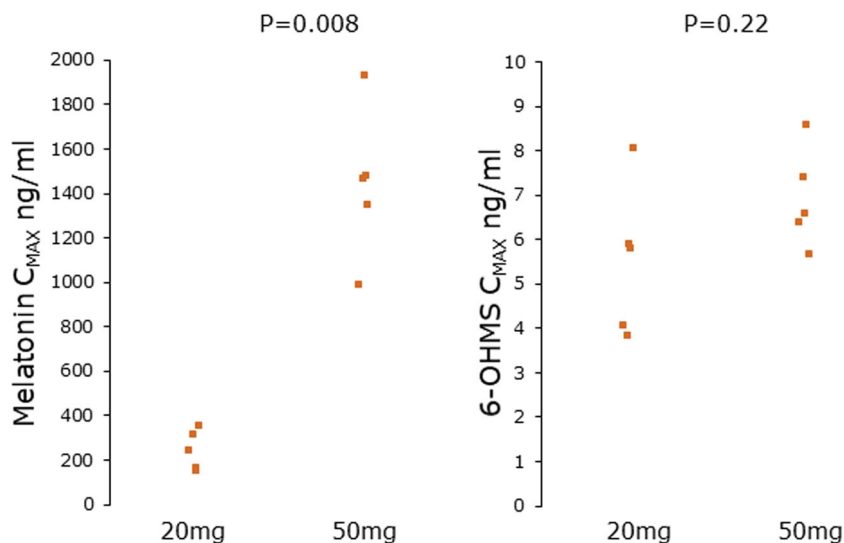


FIGURE 2 Maximal concentrations (C_{max}) of serum melatonin and 6-hydroxymelatonin sulphate (6-OHMS) in patients with sepsis after 20 or 50 mg doses. Individual data points are shown. p Values are from Wilcoxon–Mann–Whitney testing.

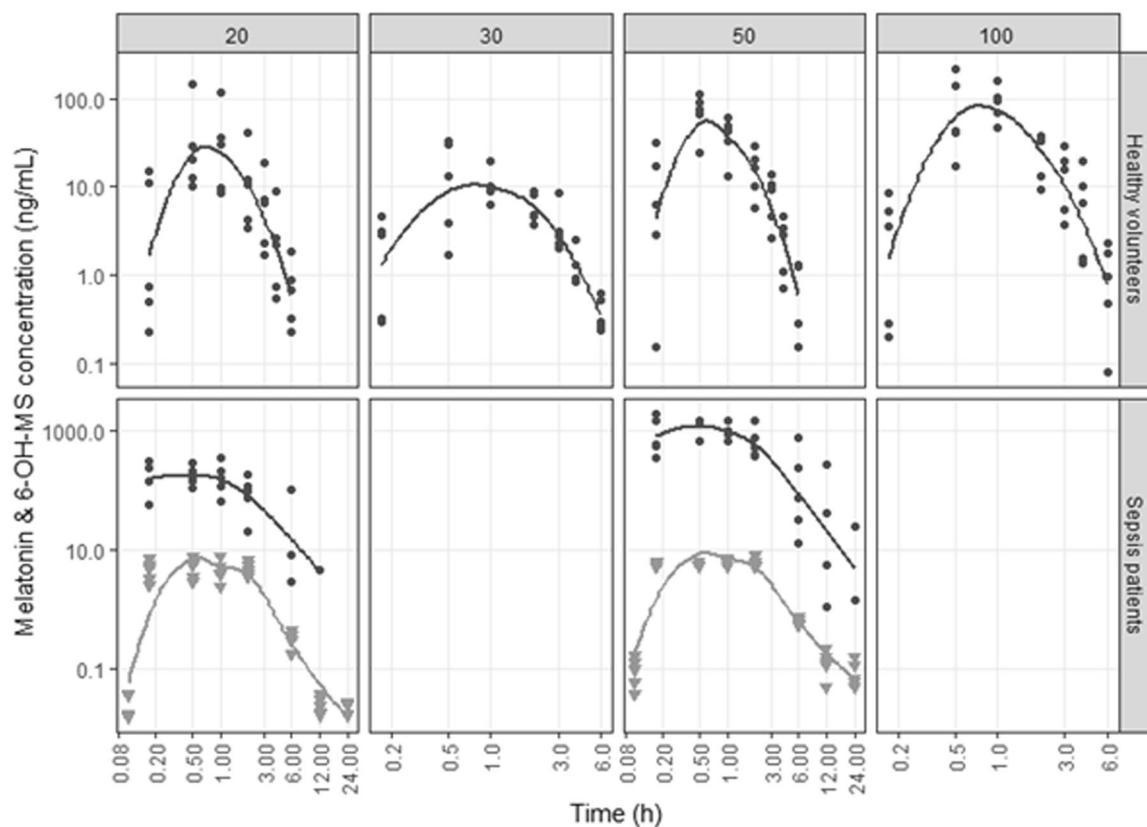


FIGURE 3 Observed melatonin (black circles) and 6-hydroxymelatonin sulphate (6-OHMS) concentrations (grey triangles) in serum after the administration of capsules in healthy subjects (top row) or an oral solution in patients with sepsis (bottom row). The solid black and grey lines are a nonparametric smooth to the data.

the 50 mg dose in patients with sepsis were not significantly different from C_{max} in the 20 mg dose cohort ($p = .22$, Figure 2) and much lower than we had found in healthy subjects given the same melatonin dose as capsules.²⁶

Figure 3 shows the serum concentrations of melatonin from the patients with sepsis (DAMSEL2) after oral liquid melatonin compared to those from healthy volunteers previously reported in DAMSEL1 after oral melatonin capsules.²⁶ In both healthy subjects and patients with

sepsis, serum concentrations increased nonlinearly with dose. In healthy subjects, the mean C_{\max} increased 1.5-fold for a 2.5-fold increase in the dose (49.5 ng/ml compared to 74.6 ng/ml after a dose of 20 or 50 mg, respectively), whereas, in patients with sepsis, the average C_{\max} increased 5.4-fold for a 2.5-fold increase in the dose (268 ng/ml compared to 1440 ng/ml after 20 or 50 mg doses, respectively). Substantial interindividual variability was observed after the liquid dosing in patients with sepsis, as we have previously reported for healthy subjects given capsules.²⁶

Different modelling approaches were attempted to describe the serum melatonin concentrations and are described in more detail in the Supporting Information. As a starting point, we explored three different modelling approaches. The first approach was based on a one-compartment model with linear input and elimination from the central compartment. The second approach was based on a 'first-pass effect model' previously described by Taft et al.³⁰ and the third was a semiphysiological pharmacokinetic model incorporating saturable first-pass hepatic extraction as described by Gordi et al.³¹

We found that the model that best described the data was based on the model structure proposed by Gordi et al.,³¹ depicted in Figure 4 and Supporting Information Figures 1–4. Model equations and assumptions underlying

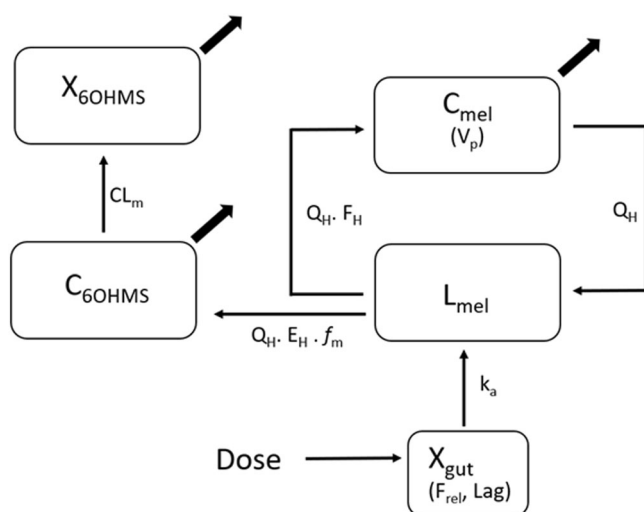


FIGURE 4 Schematic representation of the final model. The solid right-upward pointing arrows denote compartments where samples were taken. C_{6OHMS} , metabolite compartment; C_{mel} , the plasma compartment; CL_{int} , intrinsic hepatic CL; CL_m , 6-OH-MS clearance; E_H , hepatic extraction ratio; f_m , fraction metabolised; f_u , the fraction unbound for melatonin; F_{rel} , relative bioavailability; F_H , fraction escaping the hepatic extraction; k_a , first-order absorption rate constant; L_{mel} , liver compartment; Lag , absorption lag time; Q_H , liver plasma flow; X_{6OHMS} , urine 6-OH-MS compartment; X_{gut} , gut compartment.

this model are described in detail in Supporting Information: Table 2. During modelling, we found that the relative bioavailability (F_{rel}) and the absorption rate constant (k_a) for capsules were lower compared to the liquid melatonin formulation ($F_{rel} = 19\%$ vs. 100% , $k_a = 0.11$ vs. $0.28/\text{min}$) and that there was a statistically significant lag time in melatonin absorption from capsules (8.8 min; 95% CI: 8.4–9.6 min). We also found that the intrinsic hepatic clearance (CL_{int}) in patients with sepsis was 30% (95% CI: 13%–64%) of the CL_{int} in the healthy subjects and that the patients with sepsis had detectable endogenous 6-OHMS levels in pre-dose samples (44 pg/ml; 95%CI: 31–63 pg/ml). No other covariate relationships were identified.

The parameter estimates for the final model are shown in Table 4 alongside the 95% CIs according to log-likelihood profiling. There was high uncertainty in the estimates for k_a , CL_{int} and K_m , as seen from the width of the 95% CI (Table 4). Other parameters were estimated with good precision with relative standard errors of <30%. The GOF graphs shown in Figure 5 and Supporting Information Figures 1–4 demonstrate that the final model describes the observed data well. The estimated hepatic extraction ratio for all individuals is shown in Figure 6, with much lower hepatic extraction in the patients with sepsis who received melatonin as a liquid.

4 | DISCUSSION

The aim of this study was to define the pharmacokinetics of oral liquid melatonin in patients with sepsis due to community-acquired pneumonia, to inform the decisions for the dose and dosing interval for a planned funded randomised controlled trial. We also used data from our previous study in healthy subjects given the same dose of melatonin as oral gelatine capsules to compare the pharmacokinetic properties of the different formulations. We found that oral liquid melatonin was very well tolerated at doses of 20 and 50 mg in two cohorts of critically ill patients with sepsis. Maximum serum levels of melatonin were extremely high in patients receiving the 50 mg dose; several fold higher than seen in our previous study in healthy subjects, where melatonin was given as capsules.²⁶ In contrast, levels of the major metabolite were much lower than expected in patients with sepsis after the 50 mg dose, and not different from those seen after 20 mg.

Oxidative stress and uncontrolled inflammation are hallmarks of sepsis^{2,4,9} and melatonin has been proposed as a potential treatment for sepsis due its remarkable antioxidant and anti-inflammatory activities.^{14,17} We have shown previously that high doses of oral melatonin are without side effects in healthy subjects.²⁶ However, it

TABLE 4 Pharmacokinetic parameters

PK parameter	Estimate (95% CI)
F_{rel} (%)	$\begin{cases} \text{Solution} = 100\% \\ \text{Capsules} = \theta_1 \cdot 100\% \end{cases}$
θ_1	0.19 (0.12; 0.31)
Lag-time (min)	$\begin{cases} \text{Solution} = 0 \\ \text{Capsules} = \theta_2 \end{cases}$
θ_2	8.8 (8.4; 9.6)
k_a (/min)	$\begin{cases} \text{Solution} = \theta_3 \\ \text{Capsules} = \theta_4 \end{cases}$
θ_3	0.11 (0.051; 0.28)
θ_4	0.28 (0.10; 0.68)
V_p (L)	42 (35; 48)
CL_{int} (L/min)	$\begin{cases} \text{Healthy subjects} = \theta_5 \\ \text{Sepsis patients} = \theta_5 \cdot \theta_6 \end{cases}$
θ_5	11 (8.9; 220)
θ_6	0.30 (0.13; 0.64)
K_m ($\mu\text{g/ml}$)	1.3 (0.53; 13)
CL_m (L/min)	29 (23; 36)
Baseline (pg/ml)	$\begin{cases} \text{Healthy subjects} = 0 \\ \text{Sepsis patients} = \theta_7 \end{cases}$
θ_7	44 (31; 63)
Between-subject variability (CV% ^a)	
k_a	130 (88; 210)
CL_{int}	72 (51; 100)
Lag-time	8.7 (4.5; 16)
F_{rel}	68 (37; 120)
Residual unexplained variability (SD) ^b	
Additive error—melatonin	0.35 (0.29; 0.42)
Additive error—6-OHMS	0.80 (0.68; 0.97)
Additive error—urine 6-OHMS	0.87 (0.63; 1.5)

Abbreviations: Baseline, pre-dose 6-OHMS concentrations; CL_{int} , intrinsic clearance from the liver compartment; F_{rel} , relative bioavailability; k_a , absorption rate constant; K_m , liver melatonin concentration where CL_{int} is half-maximal; Lag-time, absorption lag-time; V_p , volume of distribution of the central compartment.

^aCV (%) is calculated according to: $\sqrt{\omega^2} \cdot 100\%$, where ω^2 is the estimated variance in NONMEM.

^bSD is calculated as the square root.

is not easy to administer melatonin as capsules in critically ill sedated patients and so we undertook the current study to investigate the use of a liquid formulation of melatonin for administering to sedated ventilated patients via nasogastric tube. We found that the liquid was both simple to administer by nasogastric tube and

also sufficiently palatable for oral ingestion. There were no side effects even in these critically ill patients, and no effect on sedation levels as shown by the absence of change in sedation scores.

There is no consensus on the dose or dosing interval/frequency of oral melatonin as a treatment for sepsis. We modelled the serum melatonin data from patients with sepsis given liquid melatonin and from our previous study of healthy subjects given melatonin capsules, using three different models and found that the semiphysiological pharmacokinetic model described by Gordi et al.,³¹ which incorporates saturable first-pass hepatic extraction, was a good fit for our data. In our study in healthy subjects, DAMSEL1, we gave doses of up to 100 mg without side effects and the original intention for patients with sepsis in DAMSEL2 was to start at 50 mg in the first dose cohort then escalate to 100 mg. However, we found that the serum melatonin levels were considerably higher than we had seen in healthy subjects given the same dose as capsules.²⁶ Whilst there is no published evidence that high melatonin levels were likely to be harmful, we also had no evidence that they were not harmful in a critically ill patient population, so we opted to give 20 mg to the second patient cohort rather than increasing to 100 mg as planned. We found that levels of the metabolite after 20 mg were similar to those seen after 50 mg,²⁶ despite the difference in serum levels, suggesting saturation of hepatic metabolism. This was confirmed by the population pharmacokinetic modelling that showed that a semiphysiological pharmacokinetic model that incorporates saturable first-pass hepatic extraction, was a good fit for our data. As a consequence of the saturable metabolism, as shown in Figure 6, the hepatic extraction ratio was inversely correlated with the absorption rate of the formulation, such that the faster absorption for the liquid formulation resulted in a lower hepatic extraction ratio and hence more melatonin escaping hepatic first-pass metabolism, resulting in very high serum melatonin levels but low metabolite levels. Our modelling revealed that the intrinsic hepatic clearance in patients with sepsis is only 30% of that in healthy volunteers and that this (in part) explains these observations. We excluded patients with biochemical evidence of hepatic dysfunction.

A systematic review³² of studies reporting melatonin pharmacokinetic data in healthy subjects reported the use of doses ranging from 0.3 to 100 mg, given as i.v. preparations, tablets, capsules, powder or as a solution in corn oil. There were wide ranges in reported pharmacokinetic variables, with substantial variability in study designs/methods, notably formulation, dose and assay method. Another study in healthy volunteers used 10 mg melatonin in gelatine capsules, as we used in DAMSEL1²⁶ and again

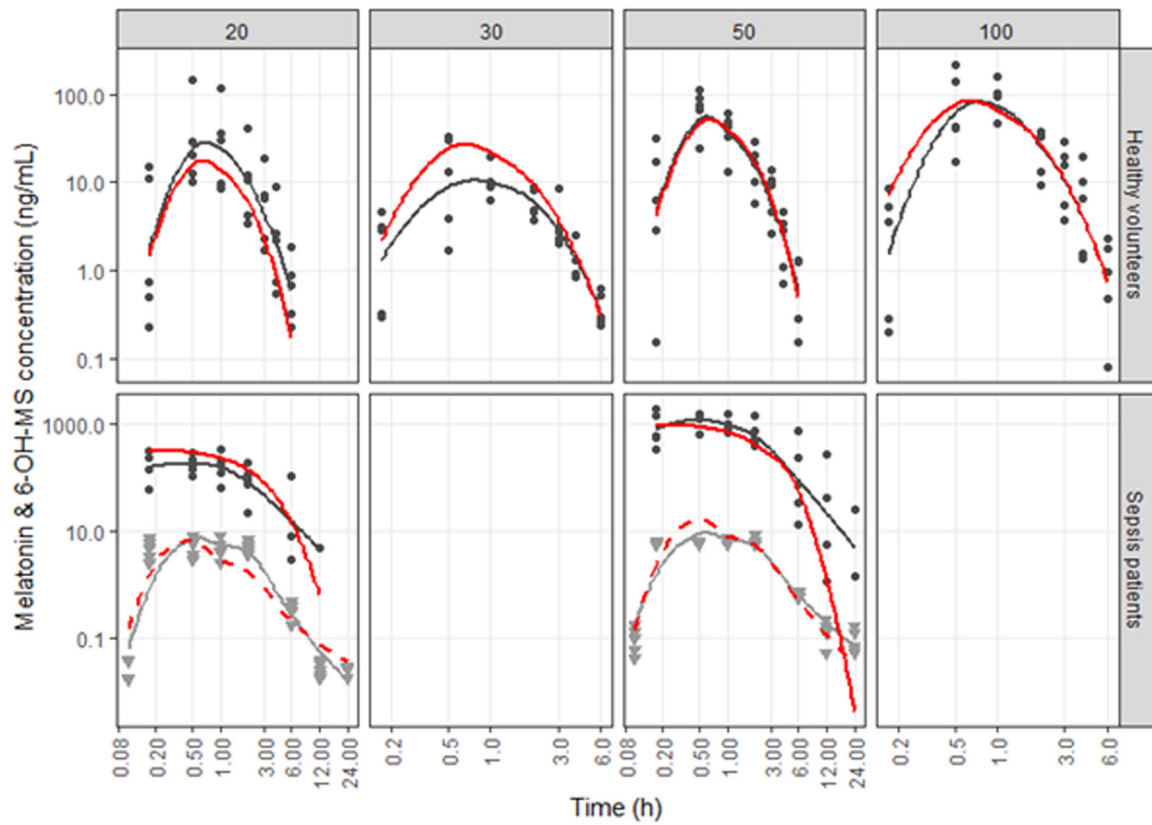


FIGURE 5 Observed melatonin (black circles) and 6-hydroxymelatonin sulphate (6-OHMS) concentrations (grey triangles) in serum after the administration of capsules in healthy subjects (top row) or an oral solution in patients with sepsis (bottom row). The solid black and grey lines are a nonparametric smooth to the data. The solid and dashed red lines are the median predicted melatonin and 6-OHMS concentrations according to the final model.

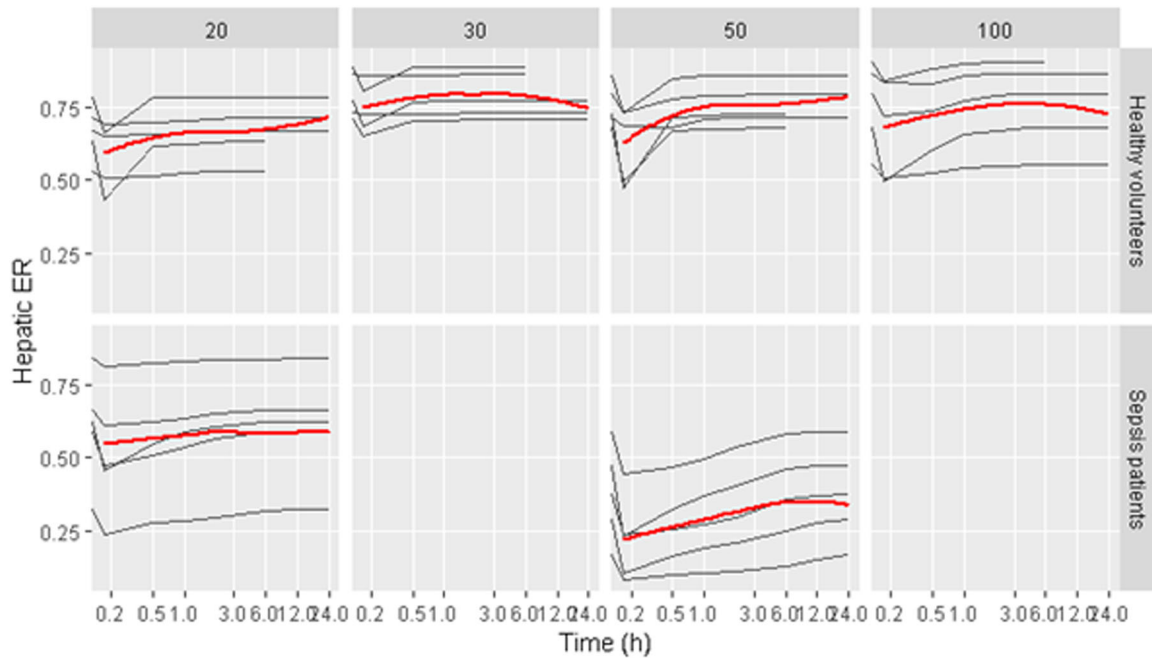


FIGURE 6 The estimated hepatic extraction ratio against time for the healthy subjects (top rows) and patients with sepsis (bottom rows) according to the final model. The solid red lines denote the trajectories of the median of the estimated hepatic extraction ratios.

reported pronounced interindividual variation.³³ Melatonin metabolites were not measured.

Pharmacokinetic data after oral melatonin administration in critically ill patients are sparse. However, a recent study compared melatonin pharmacokinetic parameters after low doses of melatonin given to three groups of ICU patients by three different routes: as lyophilised powder or crushed tablets, both given as a slurry in water by nasogastric tube, or a jellified micro-emulsion of melatonin applied transdermally (all providing 3 mg melatonin). Absorption was much faster and serum melatonin levels higher after the lyophilised powder in water compared to crushed tablets or transdermal application.³⁴ Faster T_{\max} and higher C_{\max} values have been reported in critically ill patients than in healthy subjects^{33–35} and this was assumed to be due to altered hepatic and/or renal dysfunction, although more likely related to the different formulations and dose as we report here. Tablets given by nasogastric tube have to be crushed and made into a slurry and this affects the transit and absorption characteristics of the melatonin, making comparisons unreliable. The supplementary data file provides further details of pharmacokinetic parameters.

There are no data to support the choice of dose and dosing intervals in trials in critically ill patients, either previously used or proposed, and most studies have not considered differences between formulations of administered melatonin. In addition, many do not report circulating levels of serum melatonin and none report metabolite levels. No link between oral doses and serum concentrations of melatonin or its main metabolite and clinical effects has been established. The main metabolite after oral administration of melatonin with first-pass hepatic metabolism is itself bioactive, with equal affinity for melatonin receptors and similar antioxidant and anti-inflammatory effects.³⁶ The majority of oral melatonin is converted to 6-OHM in the liver, then largely sulphated by SULTs and excreted in urine. There is some evidence that 6-OHM can be formed enzymatically at extrahepatic sites or generated through a reaction with peroxynitrite or hydroxyl radical, for example, during oxidative stress.³⁷ Studies where melatonin is being given intravenously may not result in the same metabolite pattern. Our study shows that melatonin given as a liquid is quickly absorbed, resulting in higher melatonin and lower metabolite levels than seen with melatonin given as gelatine capsules. At 50 mg, the very high melatonin and low metabolite levels seen suggest that a high proportion of the liquid melatonin is excreted, with little metabolism to 6-OHMS. In the absence of deranged hepatic function, the most likely explanation is the saturation of the metabolic capability.

There has been one recent study of melatonin as a treatment for non-COVID septic shock, where a single dose of 50 mg melatonin was given daily as 10×5 mg capsules via nasogastric tube, as part of a study comparing several antioxidants.²² Little detail is provided as to the difficulty or otherwise of this method of administration and whether the capsules were delivered whole, presumably with water flushing, or emptied and made into a slurry. The lack of melatonin or metabolite levels means there can be no assessment of the amount or time course of the melatonin absorbed. Differences between the sequential organ failure assessment (SOFA) score between an untreated control group and all groups of patients receiving any of the antioxidants, including melatonin, were reported, but SOFA scores decreased anyway during ICU stay and several patients died; it is unclear if these were accounted for in the analysis.²² During the COVID-19 pandemic, melatonin has been proposed as a therapy for sepsis generally¹⁷ and specifically that caused by the SARS-CoV-2 virus.¹⁸ Trial protocols have also been published which propose to give melatonin as capsules (50 mg daily) to moderately ill patients with COVID pneumonia²³ or intravenously every 6 h for 7 days up to a maximum daily dose of 500 mg in critically ill patients with COVID.²⁴ None of these proposed studies intend to report melatonin or metabolite levels and the justification of the doses, formulations and dosing intervals is unclear.

Our study is a small open-label single-centre study in a homogenous group of patients with sepsis due to non-COVID community-acquired pneumonia. All but one of our patients with sepsis were male, entirely due to predefined eligibility criteria. The healthy subjects were all male, deliberately selected as a precaution for safety reasons as there are no data regarding harmful effects of high-dose melatonin on reproduction or pregnancy outcome. Most pharmacokinetic studies in healthy subjects are undertaken solely in males; it is not known whether there are differences in melatonin metabolism between the sexes. The sepsis patients were considerably older than the healthy subjects and this may have impacted to some extent on the data.

We show that the way in which melatonin is given impacts the levels of melatonin and its main metabolite, which may in turn impact its effects. We have clearly shown that using the liquid formulation, a dose of 50 mg results in saturation of the metabolic hepatic capacity causing very high levels of serum melatonin. Our patients had no evidence of hepatic dysfunction, and it is not known whether sepsis itself impacts on the metabolic capacity of high-dose melatonin. We suggest that a dose of 20 mg, with a dosing interval of 8 h may be suitable in our proposed clinical trial, but future studies

should report pharmacokinetic data in particular patient groups specific to doses and formulations of melatonin.

AUTHOR CONTRIBUTIONS

Helen F. Galley and Nigel R. Webster conceived of and designed the study and Nigel R. Webster and Lee Allen were Chief Investigators. Helen F. Galley had overall responsibility for trial management and regulatory requirements, undertook data analysis, contributed to participant recruitment, sample collection and data acquisition, and drafted the manuscript. Lee Allen and Salley P. Galt were responsible for recruitment and contributed to data acquisition. Pieter J. Colin undertook data analysis and pharmacokinetic modelling. All authors contributed to the data interpretation and writing of the article and approved the final submitted version.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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