RESEARCH PAPER

An intravenous injection of melatonin: formulation, stability, pharmacokinetics and pharmacodynamics

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Abstract

Two different intravenous (IV) formulations for melatonin at strength of 5 mg/mL; one using 2-hydroxypropyl-B-cyclodextrin and propylene glycol to increase solubility and stability, and a second having additional antioxidant and chelating agent to reduce oxidation and hydrolysis were investigated. Both formulations were tested for clarity, pH stability, sterility, endotoxins, particulate matter according to US Pharmacopoeia Revision 31 (USP31), and photostability according to ICH guideline (ICH (Q1B), Option2), accelerated stability at 25°C/75% RH for 6 months, and real time stability for one year at 30°C/75%RH, according to IHC guidelines. The pharmacokinetics of the IV dose were investigated in Wistar rats, and the antioxidant effect determined ORAC analysis of rat plasma. Both formulations passed USP tests for purity and stability, although the formulation without antioxidant exhibited more color and pH change over the testing period. Formulation 2 showed expected pharmacodynamics on injection in rat (20 µg/200 µL/rat), and plasma showed a significant increase ($p<0.05$) of ORAC antioxidant capacity from 10 min after injection. This study provides evidence of suitable intravenous formulations of melatonin for bioavailability or studies.

Introduction

Melatonin, N-acetyl-5-methoxytrypt-amine, is a naturally occurring compound that has been found in all life forms so far examined, from the simplest microorganism, to higher plants and the most complex life forms, including man. It has probably played a physiological role in

organisms for a very long time. It was first isolated from bovine pineal tissue by the dermatologist Aaron Lerner and colleagues of Yale University in 1958 (Lerner et al, 1958).

In humans, melatonin is an endogenous neurohormone and secreted primarily from the pineal gland. It is also a natural antioxidant and potent free radical scavenger (Reiter et al, 2003, Korkmaz et al, 2009, Bonnefont-Rousselot et al, 2010). Melatonin controls circadian rhythms of the body; therefore it is involved in the sleep-wake cycle, functions of the immune and cardiovascular systems, and cell regulation (Reiter et al, 2003, Vijayalaxmi et al, 2002). Age-related reduction of melatonin has been correlated with disturbance of sleep, deterioration of health and chronic diseases related to oxidative damage, including cancer (Megdal et al, 2005).

Currently there is no commercially available intravenous (IV) dosage form of melatonin. As melatonin is considered a drug in most countries, an IV dosage form is required for bioavailability studies of solid or other dosage forms, a requirement for drug registration in these countries. Furthermore, there is also growing interest in melatonin therapies with clinical trials being conducted for sepsis (Gitto et al, 2001), burns (Sahib et al, 2010), ischemic reperfusion (Dominguea-Rodriguez et al, 2007), pre-surgical (Caumo et al, 2009, Borazan et al, 2010), post-surgical (Gitto et al, 2004), cancer (Wang et al, 2012, Seely et al, 2011), preeclampsia (Aversa et al, 2012), cataract and glaucoma (Ismail et al, 2009) radiation protection (Berk et al, 2007), and new areas of investigation such as radiocontrast medium induced nephropathy (Gazi et al, 2006) and paraquat poisoning (Ramirez-Zambrano et al, 2007, Melchiorri et al, 1995) where oral forms may not be appropriate or usable. For example patients undergoing surgery may not be able to ingest melatonin via oral route, and severe cases of burns and radiation exposure may need immediate controlled dosing. Oral melatonin has high first pass metabolism (>90%) in the liver (Lane and Moss, 1985), low and variable absolute human bioavailability (average 8.6% female, 16.8% male, range 1-37%) (Fourtillan et al, 2000) and high inter-subject dose variability (AUC curve of individual subjects varies by up to 25 times among subjects) (Waldhauser et al, 1985), so thus IV administration will be often be preferred for accurate dose control.

There is good evidence to indicate that melatonin solution gradually loses potency at all pH values and is not stable when exposed to light or oxygen. Daya et al (2001) studied the stability of melatonin solutions over a wide pH range (1.2-12) at room temperature and at 37°C over a period of 21 days and found that from days 3 to 21 there was a gradual decrease in potency of melatonin throughout this range of pHs, with the decrease not exceeding 30%. The results of the study indicated that solutions of melatonin are relatively stable at room temperature (20°C) and at 37°C for at least 2 days. Cavallo et al (1995) prepared sterile aqueous solutions of melatonin at various concentrations (1.0-113.0 μg/ml) in pyrogen-free glass vacuum vials stored at room temperature, 4°C, and at -70°C for up to 6 months (Cavallo et al, 1995). It was found that the shelf life of melatonin was approximately 5 months at room temperature. Andrisano et al (2000) identified the photodegradation products of melatonin as 6-hydroxymelatonin (6-OHM) and N1-acetyl N2-formyl-5-methoxykynurenamine (AFMK) and characterized them by NMR, FTIR and mass spectra. Both of these compounds also occur endogenously in the body as products of normal hepatic metabolism and radical

scavenging, and are not considered toxic.

There are thus significant technical challenges to formulating an IV melatonin dose. Melatonin is only slightly soluble in water (1.2-2.4 mg/mL) (Shida et al, 1994, Kandimalla et al, 1999), weakly basic (pKa = 12.7) (He et al, 2005), is light sensitive (Andrisano et al, 2000), and unstable in solution – it hydrolyses to 6OH-melatonin and oxidizes to AFMK (Daya et al, 2001).

There have been many studies that have attempted to improve the melatonin solubility including the stability (Dayal et la, 2003, Lee et al, 1997, Lee et al, 1998). The solubility of melatonin in propylene glycol (PG) solution increases slowly until 40% PG and then steeply increases (Lee et al, 1997). Solubility of melatonin increased linearly with concentration of 2-hydroxypropyl-B-cyclodextrin (2-HPBCD) without increase in PG. Melatonin solubility in mixtures of PG and 2-HPBCD also increased linearity but was less than the sum of its solubility in 2-HPBCD and PG individually. It was also found that the highest mixture of PG at 40% v/v and 2-HPBCD at 30% w/v had comparable solubility to the other vehicles at much higher concentration, and had efficiency of melatonin solubilization (Lee et al, 1997). Furthermore, their study indicated that melatonin solution was degraded following first kinetics and was unstable in strong acid solution (HCl-NaCl buffer, pH1.4) but relatively stable at other pH values from 4-10 at 70°C due to an amide linkage that appears to be sensitive to acid catalyzed hydrolysis in low pH solutions (Lee et al, 1997, Connors et al, 1979). Melatonin in 10% PG was degraded 85 times more quickly than in aqueous solution without PG at 70°C. On the other hand, the degradation rate constant of melatonin in 2-HPBCD was not changed significantly when compared to water. The amide moiety of melatonin may be less sensitive to protonation or acid catalyzed degradation in 2-HPBCD solutions when compared to PG solution (Lee et al, 1997).

This study investigated two different IV formulations for melatonin at a strength of 5 mg/mL; one 2-HPBCD and PG to increase solubility and stability, and a second having additional sodium bisulfite as an antioxidant, and ethylenediamine-tetraacetate sodium (NaEDTA) as chelating agent to reduce oxidation and hydrolysis. Both formulations were tested for clarity, pH stability, sterility, endotoxins, particulate matter according to US Pharmacopoeia Revision 31 (USP31), and photostability according to ICH guideline (ICH (Q1B), Option2), accelerated stability at 25°C/75% RH for six months, and real time stability for one year at 30°C/75%RH, according to IHC guidelines. Finally, the pharmacokinetics of the IV dose were investigated in Wistar rats, and the antioxidant effect determined by ORAC analysis of rat plasma.

Materials and Methods

Melatonin was obtained from Huanggang Innovation Biochemicals Co. Ltd., China (Batch number HY091010). PG and 2-HPBCD (Sigma, Barcelona, Spain) were used in the melatonin injection develop-ment in this study. Preliminary study found that the most suitable ratio between PG and 2-HPBCD was 10% and 20%, respectively. The other vehicle was Sörensen phosphate buffer pH 7.0 since this buffer has isotonicity similarly to human plasma. The Sörensen phosphate buffer solution pH 7.0 consists of 0.07 N monobasic sodium phosphate anhydrous solution 40%v/v, 0.07 N dibasic sodium phosphate solution 60% v/v and sodium chloride 0.46% w/v (Osol, 1980). Therefore the formulation of melatonin injection in this study was developed by mainly using 2-HPBCD as the complexing agent, together

with PG as co-solvent, and Sörensen phosphate buffer to adjust pH and volume.

- *Production and Evaluation of the Product*: The melatonin injection was produced by a GMP certified manufacturer stringently controlled under clean room class 10,000 for mixing and clean room class 100 for filling. The sterile equipment which contacted with the solution was made from stainless steel type 316L. The primary packaging materials, amber glass vials type 1, were sterilized by using dry heat at 200 °C for 2.5 h. The active substance and excipients were dissolved in co-solvents and sterile water for injection until dissolved completely and adjusted to its final volume which was pH checked. In addition, the product was sterilized by final filtration through a membrane (0.2 micron pore size) before aseptic filling and flushed with 100% nitrogen gas throughout the filling in the vials. The evaluation of melatonin injection was performed according to US Pharmacopoeia Revision 31 (USP31), including clarity and pH, sterility test, endotoxin test and particulate matter test. The clarity test was conducted by visual inspection of all filled vials, inspecting for foreign matter against a black and white screen. The pH measurement was performed by pH meter (Model MP 220, Mettler Toledo, Switzerland) with an in-house specification range of 5.0-7.0 for physiologically tolerable pH for injection[36]. The sterility test of the sterile product is defined by the absence of viable and multiplying microorganisms, as specified in USP31 and harmonized with the European and Japanese Pharmacopoeia. The membrane filtration method was used in this study due to accuracy and precision. Twenty vials which sampled randomly dip in 70% ethanol for 15 min, and sterile solution was filtered through Sterility Test Equipment (Membrane Filtration System, Satorius, model SM16316) employing 0.45 μm sterile membrane filter in aseptic condition. The filter was then washed with 100 ml of Sterile Peptone Water, segmented aseptically into two equal parts and individually transferred to Fluid Thioglycollate Medium (USP) and Soybean-Casein Digest Medium (USP). Then they were incubated for not less than 14 d at 32.5± 2.5°C and 22.5±2.5°C, respectively. Fluid thioglycollate medium was selected based upon its ability to support the growth of a wide range of anaerobic and aerobic bacteria and fungi (i.e. yeasts and molds). The bacterial endotoxin test (BET) to detect unsafe levels of microbial cell wall debris, from live or dead gram-negative bacteria used amoebocyte lysate from the horseshoe crab (Lymulus polyphe-mus) to react with endotoxin in the product. Three techniques used were the gel-clot technique (based on gel formation), the turbidimetric technique i.e. development of turbidity after cleavage of an endogenous substrate (Pyrogent-5000 Turbidimetric LAL Lonza, Kit part#N383), and the chromogenic technique (development of color after cleavage of a synthetic peptide-chromogen complex). The particulate matter test was conducted by followed the official method of the USP31. For determination of particulate matter, two procedures, method 1 (light obscuration particle count test) is preferably applied, and method 2 (microscopic particle count test) are specified. In this study, Method 1 was employed to test the particulate matter of melatonin injection. The preparation complies with the test if the average number of particles present in the units tested does not exceed 6000 per container equal to or greater than 10 um and does not exceed 600 per container equal or greater than 25 um.

- *Analytical method validation*: A stability-indicating high performance liquid chromatographic (HPLC) method was developed for melatonin drug substance and melatonin products, assay, content uniformity and dissolution test for the melatonin capsule and also injection as assay. The HPLC method was validated according to the current ICH Guidelines Topic Q2 (R1). This validation included specificity, precision, linearity, range, accuracy and robustness. The HPLC system comprised a ODS4-2 (C18) Phenomenox column (150 mm×4.6 mm× 5 μM) at 30°C, mobile phase acetonitrile:distilled water (40:60) at 1 mL/min, injection volume 20 μl, UV detector at 304 nm, yielding a retention time of melatonin of 3.4 – 3.6 min and a total run time of 5 min.

  Stress testing is important as part of method validation to determine the effect of product degradation of the analytical method being used. It also gives insights into stability issues that are useful for the formulation. The following stress tests were performed. Thermal hydrolysis stress was conducted with 1 mg/ml of melatonin in mobile phase stored at 60 °C and sampled at initial time and after 3, and 7 d. Acid and base hydrolysis stress was analyzed with 1 mg/ml melatonin solution in 1N HCl and 1N NaOH, respectively, stored at 25 °C and sampled at initial time and after 3, and 7 d. Redox stress was conducted using 1 mg/ml solution of melatonin in 3% hydrogen peroxide and stored at 25°C for 3 h. All of the testing samples were randomly sampled, filtered with a 0.2 μm PTFE membrane filter and the degradation peak determined together with the melatonin peak analyzed for purity using HPLC apparatus.

- *Photostability of melatonin injection:* The two formulations of melatonin injection at a strength of 5mg/ml were put in a calibrated photo-chamber (Model KBF ICH720 E2, Binder Co., Ltd., Germany) equipped with both a UVA and cool white fluorescent for the photostability study. The procedure followed the ICH guideline (ICH (Q1B), Option 2). The photo-chamber temperature was controlled in the range of 8-15 °C and 41-45% relative humidity throughout this study. Samples were randomly taken for HPLC analysis.

- *Pharmacokinetics* - Male Wistar rats (*Rattus norvegicus*) rats were chosen at adult age of 20 – 24 weeks with body weight approxi-mately 500 – 600 g. The study was approved by Institutional Animal Ethics Committee. Caging conditions for the experimental rats were as follows: regular light cycles of 12/12 hours light/dark (lights off at 6:00 PM and on at 6:00 AM daily), a room temperature range for rat housing at 22 – 24°C, a relative humidity at the level of rat cages of 50–70%, room ventilation rates of about 15 – 20 air changes per hour, light intensity (during day) for rats below 150 – 300 lux, the sound of 60 dB less that background noise, pressure for noninfectious animal lab at 30 Pascal. The animals (n = 8) were housed (2 or 3 per cage) with free access to food and water in the Northeast Laboratory Animal Center, KKU.

  Melatonin IV formula 1 (20 μg/200 μL/rat) was administered using an IV catheter (no 24) attached to a 1 mL syringe (Nipro, USA). The injection started at the middle or slight distal part of tail. A needle injection catheter was used for rapid blood sample collection with the IV catheter inserted in tail vein and gently pushing of the needle and catheter head.

  Blood samples (300 μL) were collected before, and 10, 20, 30, 60, 120 and 240 minutes after melatonin injection then centrifuged (10 min at

14,000 rpm; room temperature) to obtained plasma (150 µL). The upper organic layer was removed with pipette to a new tube and plasma (100 µL) was used for melatonin analysis, with 50 µL retained for oxygen radical antioxidant capacity assay (ORAC) analysis. The samples were stored at – 80°C until analysis. Melatonin was analyzed by HPLC using a previously validated and published method (Sangkasat et al, 2011).

- *Effect on plasma antioxidant status by ORAC antioxidant capacity assay* - To investigate the effect of the melatonin dose in-vivo, a pharmacodynamics study of the effect on rat plasma antioxidant status was employed, using ORAC assay. Plasma was assayed 0, 10, 20, 30, 60, 120 and 240 minutes after the administration of a 20 µg of melatonin IV injection (formula 2).

The ORAC assay based on the scavenging of peroxyl radicals generated by 2,2-azobis (2-amidino-propane) dihydrochloride (AAPH), which prevents the degradation of the fluorescein probe and, consequently, prevent the loss of fluorescence of the probe was modified from previously reports (Huang et al, 2005, Prior et al, 2005). The reaction was carried out in 75 mM phosphate buffer (pH 7.4) in a 96-well plate. Human serum (20 µL) was mixed and incubated with fluorescein solution (a final concentration of 70 nM) for 15 min at 37°C, followed by APPH solution (a final concentration of 12 mM) and the fluorescence was recorded by fluorescence microplate reader (Spectra max Gemini, Molecular Devices, USA) for 80 min at λ_{ex} 485 and λ_{em} 538 nm. A blank sample and trolox solution (final concentrations of 1-10 µM) were simultaneously tested and all being analyzed in triplicate. The area under curve (AUC) was calculated for each sample by integrating the fluorescence curve. Net AUC was calculated by subtracting the AUC of the blank. The final results were converted to mmol Trolox equivalents/L using Trolox as the reference antioxidant. The curve (AUC) technique that combines both inhibition percentage and the length of inhibition time, making it superior to measurement of inhibition percentage at fixed time[4] (Cao and Prior, 1998, Sofic et al, 2005).

Results and Discussion

The HPLC analysis method validation passed all ICH Guidelines for specificity, precision, linearity, range, accuracy and robustness. Stress testing gave the following results (assay data shown in Table 1).

No degradation product was observed in melatonin thermally stressed at 60°C for 7 d. The peak purity of melatonin in the chromatogram was the relevant evidence. The potency of melatonin gradually decreased over time with acid-base hydrolysis stress tests. The chromatograms of acid hydrolysis stress test did not show any degraded peaks whereas those of base hydrolysis showed another peak at retention time 4.1 min (later than the melatonin peak) that gradually increases over time. The melatonin potency was also dramatically decreased. The potency of melatonin in 3% hydrogen peroxide was still stable for 3 h as shown in Table 1. However a pale yellow solution was observed. This result corresponds to a previous study (Carampin et al, 2003). The interaction of melatonin with hydrogen peroxide leads to the opening of the indole ring with the formation of AFMK, an orange oily color (Harte et al, 2003), a metabolite of melatonin and also has potent antioxidant activity (Meike et al, 2009). Since only a little AFMK formed, the solution observed was a pale yellow.

Both formulations of melatonin injection at strength of 5 mg/ml

passed tests for assay, clarity, pH stability, sterility, bacterial endotoxins, and particulate matter. Formula 1 had a pH of 7.10 and formula 2 pH of 6.64.

From Table 2, a negligible change in pH of formula 1 from pH of 7.10 to 6.98 over 31 days was observed. However the pH of the second formulation decreased dramatically from pH 6.64 to 5.5 by day 31. A sterile solution of formulation two without melatonin and filled in amber glass and stored in the photo-chamber showed no significant change in the color of solution and pH over the same time period.

Table 1 The percentage of melatonin remaining in the IV solution after storage with stress conditions.

Stress	Storage time	% melatonin mean	SD
Heat	0 d	100.8	0.1
	1 d	100.5	0.2
	3 d	100.7	0.2
	7 d	100.0	0.1
Acid	0 d	100.2	0.1
	1 d	93.0	0.1
	3 d	92.3	0.2
	7 d	86.9	0.1
Base	0 d	100.4	0.2
	1 d	91.9	0.2
	3 d	91.1	0.1
	7 d	87.4	0.2
Redox	0 h	100.3	0.1
	3 h	100.1	0.1

The rate of change in color, from colorless to pale yellow and decrease in pH of formula 2 was far slower than that of formula 1. This is undoubtedly due to the antioxidant substance sodium bisulfite added formula 2, reducing formation of yellow/orange AFMK, (the other hydrolysis product 6-hydroxymelatonin is a white powder or colorless solution) and reducing the hydrolysis reaction to 6-hydroxymelatonin. This later reaction requires addition of OH to melatonin and explains the decrease in pH. Although the potency of melatonin solution in both formulae illustrated a gradual decline throughout the study, their potency still remained within the specification requirement (90.0-110.0%) by day 31.

Table 2 Comparison of 2 formulas of melatonin IV solutions stored in photo-chamber for 31 d.

	Days	Assay (%)	pH	Appearance
Formula 1	0	95.5	7.10	Clear
	1	97.3	7.05	Clear
	2	94.5	7.04	Clear
	7	93.1	7.00	Pale yellow clear
	17	91.7	6.98	Yellow clear
	31	91.1	6.98	Yellow clear
Formula 2	0	97.6	6.64	Clear
	1	97.6	6.52	Clear
	2	96.3	6.53	Clear
	7	95.7	6.38	Clear
	17	94.4	6.16	Pale yellow clear
	31	92.6	5.55	Pale yellow clear

Note: Blank solutions remained at pH6.67 and clear appearance for 31 d.

Table 3 shows the result of the accelerated stability study of the melatonin injection at the strength of 5 mg/ml and storage in 25°C/75%RH for six months. It was observed that the color of the two formulations changed from clear solution to yellow solution in formula 1 and changed to pale-yellow in the other one. Even through the potency still met the specification, the appearance was changed. Therefore the proposed shelf-life should be based on the real time data. The yellow color is undoubtedly due to formation of AFMK from residual dissolved oxygen reacting with melatonin. AFMK is a naturally occurring metabolite from the endogenous metabolism of melatonin in animals, and a product of radical scavenging, particularly in the brain. The latter is particularly important as a natural protectant for the brain against free radical damage. As such, AFMK is non-toxic, and an important antioxidant in its own right.

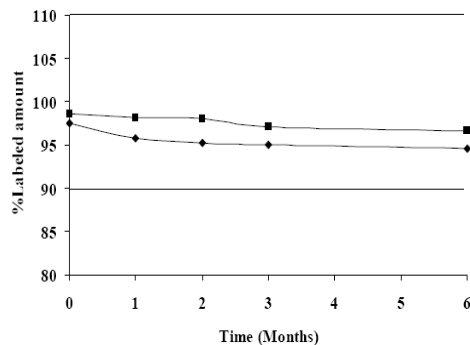
Some colorless 6-hydroxymelatonin is undoubtedly also formed by a hydrolysis reaction (resulting in production of protons that explains the decreasing pH), but again is a natural, non-toxic metabolic product of melatonin, and also a potent antioxidant in its own right. It would seem that the antioxidant in formula 2 limits the amount of AFMK formed by oxidation of melatonin, but cannot prevent hydrolysis.

Table 3 Summary of the accelerated stability study of the two formulations of melatonin injection for six months.

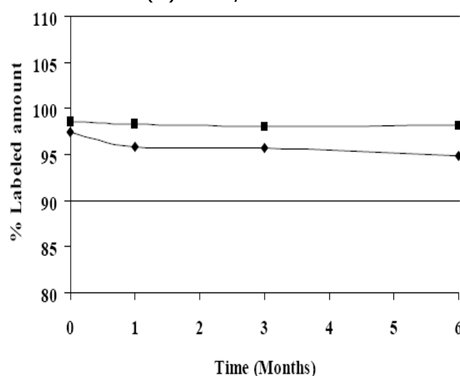
	Month	Appearance	%Labeled amount	pH
Formula 1	0	Clear	97.5	7.10
	1	Yellow	95.8	6.93
	2	Yellow	95.2	6.93
	3	Yellow	95.0	6.88
	6	Yellow	94.6	6.81
Formula 2	0	Clear	98.6	6.64
	1	Clear	98.2	6.00
	2	Clear	98.0	5.83
	3	Pale-yellow	97.1	5.59
	6	Pale-yellow	96.6	5.50

Table 4 Summary of the real time stability study of formulas of melatonin injection for 12 months.

	Month	Appearance	%Labeled amount	pH
Formula 1	0	Clear	97.5	7.10
	1	Pale-yellow	95.9	6.99
	3	Pale-yellow	95.7	6.89
	6	Yellow	94.9	6.85
	9	Yellow	94.1	6.93
	12	Yellow	93.5	6.89
Formula 2	0	Clear	98.6	6.64
	1	Clear	98.3	6.37
	3	Clear	98.1	6.21
	6	Clear	98.2	5.96
	9	Clear	96.0	5.82
	12	Clear	96.3	5.73



(a) 30°C/75%RH.



(b) 40°C/75%RH

Figure 1 Percent labeled amount of the melatonin injections stored at (a) 30°C and (b) 40°C controlled 75% RH. (♦Formula 1; ■ Formula 2)

The real time stability study of melatonin injection for six months is presented in Table 4. The data indicate that the color of solution in formula 1 obviously changed whereas formula 2 remained a clear solution.

The levels of melatonin detected in rat plasma versus time are shown in Figure 2 for a 20 µg/200 µL/rat dose, fitted to a non-compartment model fitted using Kinetica Version 5 (Thermo Scientific) (Table 5).

A previous study using male Sprague Dawley rats showed that the volume of distribution of melatonin was 1050 mL/kg with its clearance of 2011 mL/h/kg and a half life of 0.33 h for a 10 mg/kg dose (Yaleswalem et al, 1997). These values are somewhat

higher than the present study, perhaps due to the much larger dose, and the young age of the rats used.

Injection of a 20 µg/200 µL dose in rat gave a significant increase in plasma ORAC value ($p < 0.05$) from 10 min after IV injection of melatonin until the last point assayed at 240 minutes (Figure 3). The effect of melatonin on antioxidant status, lipid peroxidation (TBARS) and lipid profile daily has previously been demons-trated in rat (Subramanian et al, 2007) with administration of melatonin for 45 days at two doses (0.5 and 1.0 mg/kg body weight).

Table 5. Fitted pharmacokinetic parameters for a 20 µg/200 µL/rat dose (N=8)

Parameter	Mean	SD	Remark
AUC (ng/mg.min)	1965.1	958	area under the concentration-time curve
Lz	0.0146		slope of the terminal phase using log scale
$t_{½}$ (min)	102.1	70	half-life of elimination
MRT (min)	130.3	78	mean residence time = $AUMC_{tot}/AUC_{tot}$
Clearance	12.4 1491	6 730.0	mL/min mL/hr/kg
Vz (mL)	1444.5	883	apparent volume of distribution during the terminal phase
Vss (mL)	1288.2	559	apparent volume of the plasma compartment at steady state
C_{max} (ng/mL)	37.1	13	maximum concentration
R	-0.9552		log linear regression coefficient
Slope (min^{-1})	0.00702		slope of the linear equation on log-transformed data

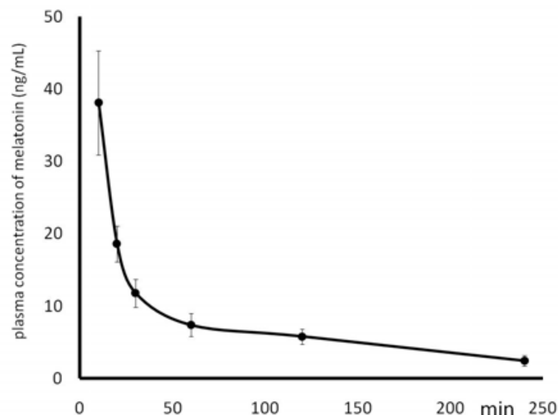


Figure 2 Pharmacokinetic profiles of melatonin intravenous injection at a 20 µg/200 µL/rat dose (n = 8).

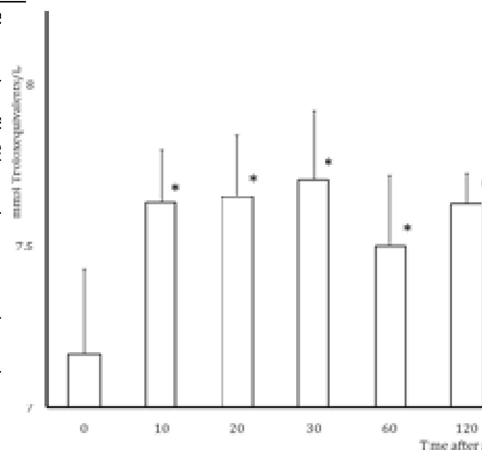


Figure 3 Mean and SD from ORAC assay for antioxidant capacity of rat plasma after the administration of 20 µg of melatonin IV injection (n = 8). * $p < 0.05$ compared to base-line data at 0 min.

Concomitantly, melatonin superoxide dismutase, catalase and glutathione peroxidase as well as increased glutathione levels. This current study shows that the effects occur at much lower dose and very rapidly, detectable from only 10 minutes after administration.

Conclusions

This study provides evidence of suitable intravenous formulations of melatonin at a strength of 5 mg/mL using 2-hydroxypropyl-B-cyclodextrin and propylene glycol to increase solubility and stability, one also containing additional antioxidant and chelating agent to reduce oxidation and hydrolysis. Both formulations passed USP tests for purity and stability, although the formulation without antioxidant exhibited more color and pH change over the testing period. The formulations showed expected pharmacokinetics for a 20 µg/200 µL IV dose in Wistar rats. The antioxidant effect determined by ORAC analysis of rat plasma showed a significant increase ($p < 0.05$) of ORAC antioxidant capacity from 10 min after injection, however, other tests such as FRAP should be used to confirm this result. Also, safety data is required prior to conducting bioavailability or clinical studies. Therefore, 2-hydroxypropyl-B-cyclodextrin could form inclusion complex and enhance melatonin solubility in water and used in the formulation of its injection. The pharmacokinetic profile as well as plasma antioxidant activity suggested a potential for use in clinical study.

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