Arch. Pharm. Res. Vol.20, No.6, pp. 555-559, 1997

Batch Variation and Pharmacokinetics of Oral Sustained Release Melatonin-loaded Sugar Spheres in Human Subjects

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(Received July 28, 1997)

The three different batches of an oral sustained release melatonin (MT) delivery system were prepared by aqueous-based fluid-bed coating of the sugar spheres for the evaluation of *in vitro* release characteristics and plasma concentration profiles in human subjects. The MT contents in 20% coated sugar spheres of three batches (B1, B2 and B3) were 3.3±0.08, 2.4±0.1 and 2.5±0.13 mg per gram of coated sugar spheres, respectively. The release profiles of three different batches had a very similar fashion. However, the release half-lives ($T_{50\%}$) of MT from B1, B2 and B3 was 3.70±0.2, 5.2±0.2 and 4.9±0.07h, respectively. Plasma concentration profiles of sustained release 0.2mg melatonin-loaded sugar spheres containing 10% immediate release melatonin in gelatin capsules (B1 and B2) were then evaluated in human subjects. The *in vivo* plasma concentration profiles of the two batches (B1 and B2) were very similar each other and located between the physiological endogenous ranges. The time to reach the peak concentration (T_{max}) was more advanced in case of B1 when compared to B2. However, there was no statistically significant difference in the maximum concentration (C_{max}) and the area under the curve (AUC) between B1 and B2. The AUC of melatonin-loaded sugar spheres containing 10% and 20% immediate release MT in human subjects had a good linearity between dose and AUC, regardless of the fraction of immediate release MT, indicating the first order elimination process of MT within these doses. The current oral sustained release MT delivery system may be utilized to treat circadian rhythm disorders if it is proven to be more clinically useful when compared to immediate release MT.

Key words : Batch variation, Melatonin; Aqueous-based fluid-bed coating; Immediate release; Sustained release; Pharmacokinetics

INTRODUCTION

Melatonin (MT) is a neurohormone secreted by the pineal gland in circadian rhythm (Waldhauser and Diezel, 1985). The clinical usefulness of exogenous MT has been widely investigated in the treatment of disorder sleep syndrome, jet-lag, seasonal affective disease, shift work syndrome, and other circadian disorders (Miles *et al.*, 1988; Yu and Reiter, 1993). Various dosage forms to deliver MT were widely investigated (Benes *et al.*, 1993; Lee *et al.*, 1994; Lee *et al.*, 1995; Konsil *et al.*, 1995; Lee and Min., 1996). However, the dose, time of administration and dosage formulation are very important for the clinical application of MT (Cassone *et al.*, 1986; Lewy *et al.*, 1992; Lee *et al.*, 1994; Lee *et al.*, 1995; Garfinkel *et al.*, 1995; Wurtman and Zhadanova, 1995). It was reported that the sustained release MT treatment was more clinically useful to initiate and maintain the sleep in the elderly insomniacs compared to immediate release or conventional therapy (Haimov *et al ., 1995; Garfinkel et al.*, 1995; Wurtman and Zhadanova, 1995). The sustained release dosage forms must be designed to deliver MT immediately and in a controlled fashion over 8-10 h because of its short half-life so that the physiologically produced endogenous MT plasma concentrations are mimicked (Lee *et al.*, 1995; Lee and Min., 1996). The dosage forms of MT may be helpful in the future because no commercial dosage forms of MT that mimic endogenous MT circadian rhythm are currently available although conventional tablet, solution and tea have been marketed as a supplementary

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nutrient in the United States.

Recently, we prepared sustained release MT-loaded sugar spheres using fluid-bed coating techniques (Lee *et al.*, 1996). The sustained release dosage forms containing total 0.5 or 1.0mg MT (20% immediately and 80% in a controlled fashion) produced delayed/retarded plasma concentration profiles. We also observed good prediction of plasma MT concentration using a deconvolution/convolution modeling technique after oral administration of sustained release MT formulations (Lee *et al.*, 1995). However, the plasma concentration curves were maintained higher than physiological endogenous plasma concentration. Therefore, it would be interesting to vary a total dose and a portion of immediate/sustained release MT dosage regimen thereby producing endogenous plasma concentration. Furthermore, the batch variations of sustained release MT-loaded sugar spheres in terms of *in vitro* release and *in vivo* plasma concentration are needed. The *in vitro* and *in vivo* batch variation of dosage forms have to be validated so that manufacturing process can be confirmed with reliability and reproducibility.

The purpose of present work was to prepare three different batches (B1, B2, B3) of sustained release MT-loaded sugar spheres using aqueous-based fluid-bed coating techniques. The *in vitro* release characteristics were evaluated between batches. The plasma concentration profiles by varying total dose and fraction of immediate/sustained release MT between batches were also evaluated in human subjects.

MATERIALS AND METHODS

Materials

Melatonin (MT) was purchased from Regis Chemical Company (Morton Grove, IL, USA). Core sugar spheres as a substrate for MT loading was provided from Paulaur Co. (Robbinsville, NJ, USA). Polyvinylpyrrolidone (MW 40,000) and hydroxypropylcellulose (MW 300, 000) from Aldrich (Milwaukee, WI, USA) were used as a binder for loading of MT on sugar spheres. Aquacoat® (aqueous ethylcellulose suspension) containing 30% solid was kindly provided courtesy of FMC (Philadelphia, PA, USA). The triethyl citrate and dibutyl sebacate as plasticizers were purchased from Sigma Chem. Co. (St. Louise, MO, USA) and Aldrich (Milwaukee, WI, USA), respectively. Methanol was purchased from EM Industries, Inc. (New Jersey, USA). All other chemicals were of reagent grade and used without further purification.

Preparation of sustained release melatonin-loaded sugar spheres

The three different batches (B1, B2, B3) of sustained release MT-loaded sugar spheres were prepared according to the same procedure as reported previously (Lee *et al.*, 1996). Briefly, MT (1.2 g) in a mixture of polyvinylpyrrolidone (0.24 g) and hydroxypropylcellulose (0.12 g) as binder in 200 ml of ethanol was loaded onto 8~10 mesh sugar spheres (300 g) at 40°C with a continuous fluidizing air supply. Dibutyl sebacate (0.45 g) and triethyl citrate (0.45 g) as plasticizers were added to 100 g of Aquacoat® for the preparation of coating solution. The resulting solution was then diluted with distilled water (1:1 w/w). The MT-loaded sugar spheres (90 g) were 20% coated with coating solution (124.4 g) using fluid-bed coater (Niro Aeromatic-Fielder AG, Bubendorf, Switzerland). The theoretical coatings resulted in 20% level, based on the solid content of Aquacoat®.

The final coated beads were dried in the coating chamber for 1h and then further air-dried in hood.

In vitro release characteristics

The release characteristics of MT from 20% coated sugar spheres were studied in triplicate using a dissolution apparatus type I Basket method (Fine Scientific DST600A, Seoul, Korea) in the simulated gastric (pH 1.4±0.1, NaCl-HCl buffer solution) for 2h followed by simulated intestinal fluids (pH 7.4±0.1, phosphate buffer solution) thereafter. The simulated gastric and intestinal fluids were prepared according to Lee and Lee (1995). The dissolution samples (1 ml) were collected at a given interval with replacement of equal volume of dissolution media, and were filtered through a millipore membrane filter. The concentration of MT released from three different batches of sustained release sugar spheres as a function of time was determined using a reverse phase HPLC as mentioned previously (Lee and Min, 1996). The release half-life (time to release 50% of drug) was designated as $T_{50\%}$.

Preparation and pharmacokinetics of melatonin-containing gelatin capsules

The gelatin capsules containing total 0.2 mg MT (10% or 20% immediately release fraction) were prepared. The coated sugar spheres of B1 and B2 containing MT were used as a sustained release portion of MT, respectively. The one gelatin capsule was administered to two different groups of human subjects after giving informed consent. Four people were given one gelatin capsule in case of B1 and six people in case of B2 around 10-11 AM. Subjects were fasted overnight and at least three hours before the study. The subjects were healthy with no medication. The blood samples were collected through an indwelling intravenous catheter. The plasma sample were stored at -40°C until analysis. The plasma concentration of MT

was determined by radioimmunoassay using RIA kit (Elias Usa, Inc., Osceola, WI, USA). The AUC of MT was calculated using RSTRIP® program (Micromath Scientific Software, Salt Lake City, UT, USA). The T_{max} and the C_{max} were directly read from the plasma concentration profiles.

Statistical analysis

The data were compared for the statistical significance ($p<0.05$) by the analysis of variance test followed by the multiple comparison of the Least Significant Difference (LSD) between groups.

RESULTS AND DISCUSSION

The MT-loaded sugar spheres were previously prepared and then evaluated in the human subjects for the purpose of mimicking the endogenous plasma concentration profiles (Lee *et al.*, 1995; Lee *et al.*, 1996). However, *in vitro* and *in vivo* characteristics of dosage forms between batches have to be validated so that manufacturing process can be confirmed with reliability and reproducibility. The three different batches of sustained release MT-loaded sugar spheres were prepared and then *in vitro/in vivo* characteristics were evaluated in human subjects.

The MT contents in 20% coated sugar spheres of three batches (B1, B2 and B3) were 3.3±0.08, 2.4±00.1 and 2.5±00.13 mg per gram of coated sugar spheres, respectively. The MT contents of B1 was higher when compared to B2 and B3. The release characteristics of sustained release MT-loaded sugar spheres between three different batches in simulated gastric fluid for 2h followed by simulated intestinal fluid are given in Fig. 1. The amount of MT release was normalized as percentage because MT contents per gram of coated sugar spheres were different. The percentage of MT released was independent on pH. In addition, the release profiles of three different batches had a very similar pattern. The $T_{50\%}$ of MT from B1, B2 and B3 was 3.7±00.2, 5.2±00.2 and 4.9±00.07 h, respectively. There was a statistically significant difference in $T_{50\%}$ between B1 and B2, and also between B1 and B 3. However, it is necessary to validate whether about 10% difference of *in vitro* MT released between B1 and B2 (B3) was correlated with *in vivo* plasma concentration profiles.

Plasma concentration profiles of sustained release 0.2 mg melatonin-loaded sugar spheres containing 10% immediate release melatonin between B1 and B2 in human subjects are given in Fig. 2. The plasma concentration profiles of the two batches (B1 and B2) were very similar each other. The concentrations obtained from two different batches of coated sugar spheres were also located between the physiological endogenous ranges. The T_{max} and C_{max} of B1 and B2 were found to be 0.5 h and 1.0h; 117±21 pg/ml and 108±33 pg/ml, respectively. The T_{max} was more advanced in case of B1 when compared to B2, indicating *in vitro* release behavior (see Fig. 1). There was no significant difference of C_{max} between B1 and

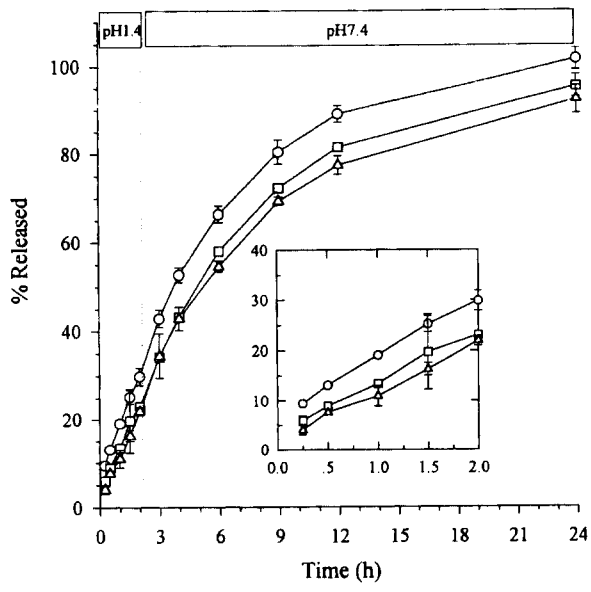

Fig. 1. Release characteristics of sustained release melatonin-loaded sugar spheres from three different batches in simulated gastric fluid for 2h followed by simulated intestinal fluid. Batch 1 (○); Batch 2 (□); Batch 3 (△).

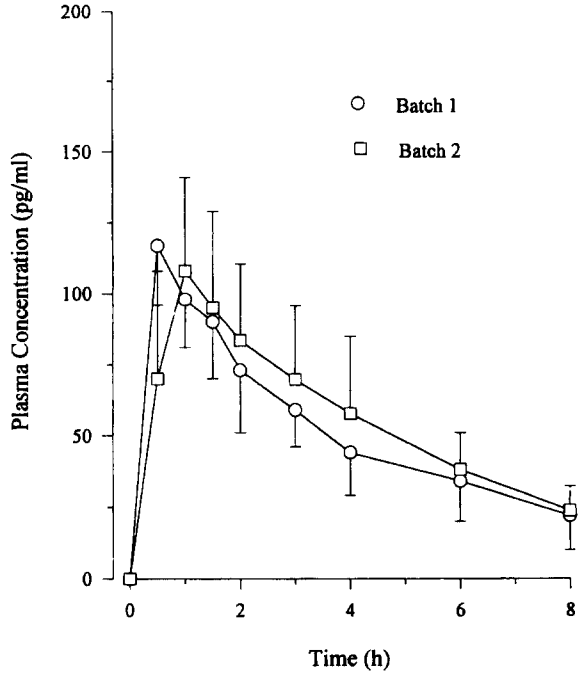

Fig. 2. Plasma concentration profiles of sustained release 0.2 mg melatonin-loaded sugar spheres containing 10% immediate release melatonin in human subjects. Batch 1 (○); Batch 2 (□).

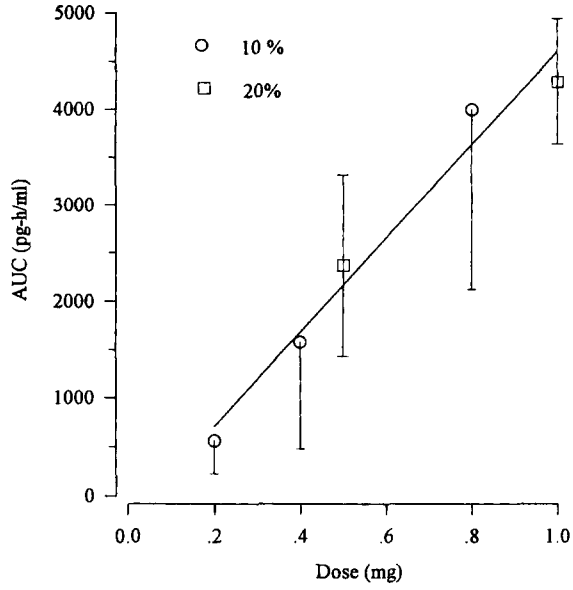

Fig. 3. Area under the curve (AUC) of sustained release melatonin-loaded sugar spheres as a function of loading dose containing 10% (○) and 20% (□) immediate release melatonin in human subjects.

B2. The calculated AUC of B1 and B2 were 515.5± 206.2 and 555.6±334.9 pg-h/ml, respectively. No statistically significant difference of AUC between two batches was observed.

On the other hand, effect of the dose and fraction of immediate release MT between two batches on AUC were also compared. AUC of sustained release MT-loaded sugar spheres as a function of MT loading dose containing 10% and 20% immediate release melatonin in human subjects is shown in Fig. 3. There was a good linearity between dose and AUC (r^2=970), regardless of fraction of immediate release MT, indicating the first order elimination process of MT within these doses. *In vitro* and *in vivo* summary statistics of three different batches of melatonin-loaded sugar spheres are given in Table 1.

There might be an *in vitro/in vivo* correlation of coated sugar spheres between B1 and B2. We also previously investigated *in vitro/in vivo* correlation of sustained release coated sugar spheres containing various amount of MT (Lee *et al.*, 1995). However, it is hard to expect in vivo difference of plasma profiles because the statistical significance of in vitro release property ($T_{50\%}$) was observed due to small standard deviation between batches (see Table 1). Instead of that, the dissolution requirement for sustained release MT-loaded sugar spheres must be tolerant. For example, USP pharmacopoeia states that not more than 35%, between 35% and 70% and not less than 85% of the extended release phenytoin sodium capsules dissolves in 30, 60 and 120 min. respectively.

Base on these results, total dose and fraction of immediate/sustained release MT are important to mimic the endogenous plasma concentration profiles. However, the clinical significance of MT in sustained release only or immediate/sustained release preparation needs to be validated in terms of sleep disorders, jet lag and shift worker syndrome. Regardless of that findings, the current oral sustained release MT delivery system may be used to treat circadian rhythm disorders in the future if it is proven to be more clinically useful when compared to immediate release MT as reported previously (Haimov *et al.*, 1995; Garfinkel *et al.*, 1995; Wurtman and Zhadanova, 1995).

Table I. *In vitro* and *in vivo* summary statistics of three different batches of melatonin-loaded sugar spheres

Batch	Contents (mg/g spheres)	$T_{50\%}$[1] (h)	AUC (pg-h/ml)	C_{max} (pg/ml)	T_{max} (h)
B1	3.3±0.08[2]*	3.7±0.2*	515.5±206.2	117±21	0.5±0.05
B2	2.4±0.10	5.2±0.2	555.6±334.9	108±33	1.0
B3	2.5±0.13	4.9±0.07	--	--	--

[1]Time to release 50% of drug.
[2]Mean±standard deviation.
*Statistically significant from other batches ($p<0.05$).

ACKNOWLEDGEMENT

This work was supported in part by the research grants from Korea Ministry of Health and Welfare (#HMP-96-D-7-0049). The part of this work was presented at the 1996 annual meeting of the Korean Society of Pharmaceutics.

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