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Design and evaluation of an oral controlled release delivery system for melatonin in human subjects

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Abstract

Six human subjects were given an oral formulation designed to provide an immediate and controlled release of melatonin (MT). The controlled release formulation consisted of MT-loaded sugar beads coated with 20% Aquacoat®. A computer simulation program (MAXSIM®) was used to estimate the MT dose and ratio of immediate and controlled release MT based on average population pharmacokinetics of MT. When 0.5 mg of MT (immediate release portion of MT, 0.1 mg) was administered to four subjects, average peak plasma MT concentration was reached at about 600 pg/ml and maintained at about 100 pg/ml over 8 h. Observed peak plasma MT concentrations were 3-times greater than predicted by simulation. These results suggest that the MT dose, ratio of immediate release MT to controlled release MT, and the controlled release dosage form must all be considered in order to closely mimic the endogenous plasma MT concentration-time curve. Deconvolution and pharmacokinetic analysis suggested that less than 20% of the orally administered controlled release MT dose reached the systemic circulation from time 0 to 8 h. A good correlation was observed between plasma MT concentration and urinary excretion rate of 6-sulphatoxymelatonin (6-STMT), a major metabolite of MT. As plasma MT concentration increased, the urinary excretion rate of 6-STMT increased concomitantly. This suggests that the urinary excretion rate of 6-STMT may be used as an index of human plasma MT concentration.

Keywords: Melatonin; Coated bead; Immediate release; Controlled release; Computer simulation; Deconvolution; 6-Sulfatoxymelatonin

1. Introduction

Melatonin (MT) is an indole amide neurohormone secreted by the pineal gland in a circadian fashion (Lerner et al., 1959; Waldhauser and Dietzel, 1985). MT plasma concentration is low during the daytime (< 10 pg/ml). It then starts to rise in the late evening and is maintained at 25–120 pg/ml during the night (over 8 h) until it returns to the daytime baseline (Waldhauser and Dietzel, 1985; Lewy and Newsome, 1983). Although the potential significance of introducing exogenous MT at physiological and pharmacological concentrations is still unknown (Petterborg et

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al., 1991; Sack et al., 1991), exogenous MT may have clinical potential in human subjects to treat circadian rhythm disorders, including sleep disorders (Cramer et al., 1974; Arendt et al., 1988; Dahlitz et al., 1991), jet lag (Petrie et al., 1989), shift work syndrome, and seasonal affective diseases (Rosenthal et al., 1984; Waldhauser et al., 1986).

Oral sustained release dosage forms may be of interest in the evaluation of the clinical potential of MT, since sustained input may offset rapid turnover of MT (Strassman et al., 1987; Le Bars et al., 1991). The usual nocturnal secretion pattern of MT over 8 h, coupled with its short half-life, prompted development of a controlled release delivery system for MT. Polymer coating technology was coupled with pharmacokinetic simulation to design an oral controlled release dosage form (Hossain and Ayres, 1992).

The purpose of this study was to develop and evaluate an oral controlled release drug delivery system for MT that could produce a plasma MT concentration-time profile that would mimic the endogenous plasma MT profile found in human subjects. Computer simulation was used to determine the dosing regime of drug and predict plasma concentration-time profiles. Deconvolution and pharmacokinetic analysis were used to analyze plasma MT concentrations. Correlation between the plasma concentration of MT and the urinary excretion rate of 6-sulphatoxymelatonin (6-STMT), a major metabolite of MT, was also investigated.

2. Materials and methods

2.1. Materials

Melatonin was purchased from Regis Chemical Co. (Morton Grove, IL, USA). Core sugar spheres (USP/NF) were purchased from Paulaur Co. (Robbinsville, NJ, USA). Aquacoat® (polymeric ethylcellulose suspension; type ECD-30) was provided courtesy of FMC Corp. (Philadelphia, PA, USA). All other chemicals used were reagent grade and were used without further purification.

2.2. Computer simulation

Plasma MT concentration-time curves were estimated using a computer simulation program (MAXSIM®, version 3.01, Uppsala, Sweden) assuming a one-compartment open model. Pharmacokinetic parameters of MT for MAXSIM® computer simulation were selected from the literature (Table 1). The input (dose) was specified as 0.1 mg MT as immediate release followed by 0.04 mg/h over 8 h. The 0.04 mg/h rate was based on dissolution studies using the coated beads.

2.3. Preparation of coated beads

The apparatus for MT loading onto sugar beads and applying Aquacoat® was a laboratory scale spray coater with a Wurster column (2 inch $\times$ 7 inch, STREA-1, Aeromatic Inc., Columbia, MD, USA). A 0.8 mm nozzle was used. A mixture of MT (1.2 g) with polyvinylpyrrolidone (0.24 g) and hydroxypropylcellulose (0.12 g) as binders in 200 ml of ethanol was applied to 300 g of 8–10 mesh sugar beads to produce MT-loaded beads. The solution was delivered at 4 ml/min using a peristaltic pump. Coating solution was prepared by mixing 100 g of Aquacoat® and dibutyl sebacate (4.5 g) and triethyl citrate (4.5 g) as plasticizers. Coating solution (109 g) was applied to 70 g of MT-loaded beads to produce (theoretically) 20% coatings based on weight gain. Actual weight gain was less due to loss of some polymer material during coating. The inlet temperature and the pressure of atomization air were 50° C and 12–15 lb/inch^2, respectively.

Dissolution studies were performed in triplicate using the USP dissolution apparatus I (basket method) at 37 $\pm$ 0.5° C. The stirring rate was 50

Table 1
Selected pharmacokinetic parameters of MT for computer simulation in human subjects

K_{el} (h^{-1})	K_a (h^{-1})	V_c (l)	V_d (l)	F	References
0.90	–	13	35.2	–	Iguchi et al. (1982)
–	1.74	–	–	–	Waldhauser et al. (1984)
–	–	–	–	0.1	Lane and Moss (1985)

rpm. 3 g of coated beads (containing 7.47 mg MT) were placed in the basket. Dissolution medium for the first 2 h was 900 ml of enzyme-free simulated gastric fluid (pH 1.4) followed by enzyme-free simulated intestinal fluid (pH 7.4). Dissolution samples were collected for 24 h with equal volume replacement of temperature-equilibrated media. $T_{50\%}$ (time to 50% drug release) was 4 h with 85% drug release after 12 h.

2.4. In vivo study protocol

Six human volunteers (five males and one female) participated in the study after signing an informed consent form approved by Oregon Health Sciences University (Table 2). All subjects were admitted to the Clinical Research Center at 10 a.m. on the day of the study. Subjects fasted at least 2 h before the study. Four subjects were given one gelatin capsule that contained 0.1 mg of immediate release MT and one gelatin capsule that contained 0.4 mg of controlled release MT on 20% Aquacoat® coated beads. This was designated as the 'low dose' (0.5 mg total dose). Two subjects were given two capsules of both the immediate and controlled release MT. This was designated as the 'high dose' (1.0 mg total dose of MT).

Blood samples were collected through an indwelling intravenous catheter at 0 (10:30 a.m.), 0.5, 1, 1.5, 2, 3, 4, 6, and 8 h after MT administration. Urine was collected over 2-h intervals. Total urine volume was measured and an aliquot was saved for subsequent measurement of 6-STMT concentration. Plasma MT concentrations were determined by high-sensitivity GC/MS (Lewy and Markey, 1978). Urinary 6-STMT concentrations were determined by radioimmunoassay (Aldhous and Arendt, 1988).

2.5. Deconvolution and pharmacokinetic evaluation

Input rates (μg/h) and cumulative amounts of MT absorbed were estimated using an interactive deconvolution program (PCDCON, version 1.0, courtesy of Dr Gillespie, College of Pharmacy, University of Texas). Plasma MT concentrations following an intravenous bolus of MT (Iguchi et al., 1982) were used for impulse response specification of MT. Plasma MT concentrations obtained after oral administration of our MT controlled release delivery system were used for the input response specification of MT.

Table 2
Vital statistics of six human subjects

Subject no.	Sex	Age (years)	Weight (kg)	Height (cm)
1	M	49	82	182.9
2	M	32	102	185.4
3	M	30	73	177.8
4	F	30	49	165.0
5	M	30	59	169.5
6	M	45	65	180.3
Mean ± S.D.		36 ± 9	71.7 ± 18.7	176.7 ± 8.1

Noncompartmental pharmacokinetic parameters for MT including AUC (area under the drug concentration-time curve), AUMC (area under the moment curve), and MRT (mean residence time, AUC/AUMC) were calculated using RSTRIP II (Micromath Scientific Software, Salt Lake City, UT, USA). This program employs a least-squares minimization procedure based on a modification of Powell's algorithm. The estimated bioavailability, F_{EST}, of MT following the oral administration of our controlled release delivery system was obtained by dividing the AUC in our study (AUC_{DDS}) by the AUC following the intravenous injection of MT (AUC_{IV}) reported by Iguchi et al. (1982) (see Eq. 1). It is noted that such an estimate across studies is risky. F_{EST} may be useful to establish trends, but it is not useful for obtaining absolute bioavailability. The relative oral bioavailability, F_{REL}, of MT from the oral controlled release delivery system was estimated by dividing the AUC in our study (AUC_{DDS}) by the AUC from a different study (AUC_{PO}) reported by Waldhauser et al. (1984), in which MT was given orally in an immediate release formulation (see Eq. 2). The cautions associated with F_{EST} also apply to F_{REL}. The equations used are as follows:

$$F_{EST} = \left(\frac{AUC_{DDS}}{D_{DDS}}\right) \div \left(\frac{AUC_{IV}}{D_{IV}}\right) \quad (1)$$

$$F_{REL} = \left(\frac{AUC_{DDS}}{D_{DDS}}\right) \div \left(\frac{AUC_{PO}}{D_{PO}}\right) \quad (2)$$

where D_{DDS} is the MT dose given orally in our controlled release formulation, D_{IV} denotes the MT dose given by intravenous injection in the study by Iguchi et al. (1982) and D_{PO} is the MT dose given orally as an immediate release formulation in the study by Waldhauser et al. (1984).

2.6. Statistical analysis

Regression analysis was used to calculate the slope, correlation coefficient (r), and coefficient of determination (r^2). Aptness of the linear models was evaluated by comparing F-test for lack of fit (Neter et al., 1985). Estimators of the linear regression parameters were obtained according to the methods of least-squares minimization.

3. Results and discussion

3.1. Analysis of plasma MT concentration-time curve

Before administering the oral controlled release delivery system to human subjects, plasma MT concentrations were predicted using computer simulation (MAXSIM®). Computer-simulated plasma MT concentration vs time profiles for the new dosage form are shown in Fig. 1. A conventional (immediate release) MT capsule (curve 1) does not produce the desired sustained MT concentration because MT has a short half-life (Iguchi et al., 1982; Waldhauser et al., 1984). Curve 2 shows that a mixture of 0.1 mg MT for immediate release and beads coated with 20% Aquacoat® containing 0.4 mg MT for controlled release are predicted to produce a plasma MT concentration vs time profile that mimics the profile known to be produced by the endogenous release of MT by the pineal gland during the night.

The observed mean plasma MT concentration-time profile after administration of MT to human subjects is shown in Fig. 2. The study was conducted during the day because the normal daytime concentration of MT is below 10–30 pg/ml. Thus, the contribution of endogenous MT to the concentration-time profile shown in Fig. 2 is negligible. When the low dose of MT (0.5 mg) was administered to four subjects, average peak plasma MT concentration was reached at about 600 pg/ml and was maintained above 100 pg/ml over 8 h. When the high dose of MT (1.0 mg) was administered to two subjects, aver-

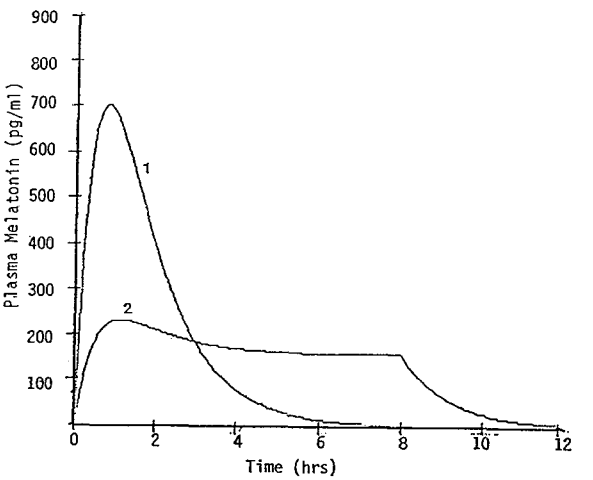

Fig. 1. Computer-simulated plasma MT concentration-time curve. (1) 0.5 mg immediate release MT only. (2) 0.1 mg immediate release MT and 0.4 mg controlled release MT.

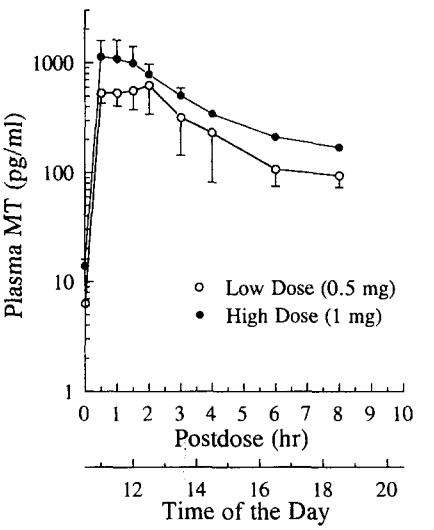

Fig. 2. Mean plasma MT concentration-time profiles after administration of oral controlled release delivery system to six human subjects at two different doses. Values are expressed as mean ± S.D. ($n = 4$ for low dose, $n = 2$ for high dose).

Table 3
Noncompartmental pharmacokinetic parameters for MT following administration of the oral controlled release delivery system to human subjects

Values	Low dose (0.5 mg)				Mean ± S.D. [a]	C.V. [b] (%)	High dose (1 mg)		Mean ± S.D.	C.V. [b] (%)
	1	2	3	4			5	6		
Slope (h^{-1}) [c]	0.505	0.089	0.278	0.162	0.259 ± 0.18	70.3	0.345	0.179	0.262 ± 0.117	44.8
AUC [d]	3729	2127	2063	1538	2364 ± 947	40.1	4742	3820	4281 ± 652	15.2
AUMC [e]	13205	5202	4985	4503	6974 ± 4164	59.7	10966	19812	15389 ± 6255	40.6
MRT [f]	3.54	2.44	2.42	2.93	2.83 ± 0.53	18.7	2.32	5.18	3.75 ± 2.02	53.9
C_{max} [g]	774.8	744.4	628.2	456.7	651.0 ± 144.1	22.1	1666.2	689.8	1178 ± 690.4	58.6
T_{max} [h]	1.77	0.72	1.21	0.58	1.07 ± 0.54	50.4	0.72	0.45	0.59 ± 0.19	32.6
F_{EST} [i]	0.30	0.17	0.16	0.13	0.19 ± 0.07	39.6	0.19	0.15	0.17 ± 0.03	16.6
F_{REL} [j]	1.28	0.73	0.71	0.53	0.82 ± 0.32	39.9	0.82	0.66	0.74 ± 0.11	15.3

[a] Standard deviation.
[b] Coefficient of variation, C.V. (%) = S.D./mean · 100.
[c] Slope of terminal points by least-square regression.
[d] Area under the curve (pg h ml^{-1}).
[e] Area under the moment curve (pg h^2 ml^{-1}).
[f] Mean residence time (h), MRT = AUC/AUMC. MRT for i.v. data was 0.71 h when data of Iguchi et al. were analyzed by RSTRIP II.
[g] Observed maximum plasma MT concentration (pg/ml).
[h] Time to reach maximum plasma MT concentration (h).
[i] Estimated bioavailability based on the i.v. data of MT in different subjects, AUC_{IV} = 247.6 pg h ml^{-1}, dose = 10 μg (Iguchi et al., 1982).
[j] Relative bioavailability based on the oral data of MT in different subjects, AUC_{PO} = 464480 pg h ml^{-1}, dose = 80 mg (Waldhauser et al., 1984).

age peak plasma MT concentrations were doubled as expected, and the terminal slope was parallel with the terminal slope from the low dose. The general shape of the plasma MT concentration-time profile was similar for the two different doses.

The rate and extent of in vivo MT release (Fig. 3) were obtained by deconvolution of the plasma concentration-time profile. The rapid initial input rate results from the immediate release MT. As expected, the high dose of MT provided a larger input rate than the low dose. Estimated cumulative amount of MT input after oral administration of the controlled delivery system is shown in Fig. 4. The slope for cumulative amount input was steeper during the first 2 h, followed by a slower cumulative input for the next 6 h. The cumulative amounts input at the low and high dose were approx. 95 and 160 μg, respectively. This suggests that only 16–19% of the administered dose of MT reached the systemic circulation over the 8 h study period.

The noncompartmental pharmacokinetic parameters obtained using RSTRIP II are summarized in Table 3. Mean residence time (MRT) has been defined as the mean time for intact drug to transit through the body. The apparent terminal slopes were prolonged, as would be expected for

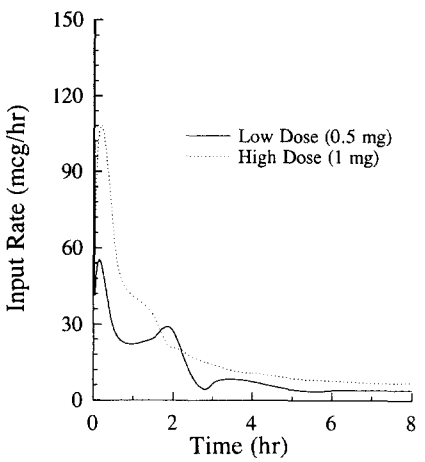

Fig. 3. Estimated MT input rate (μg/h) by deconvolution analysis resulting from an oral controlled release delivery system at two different doses of MT.

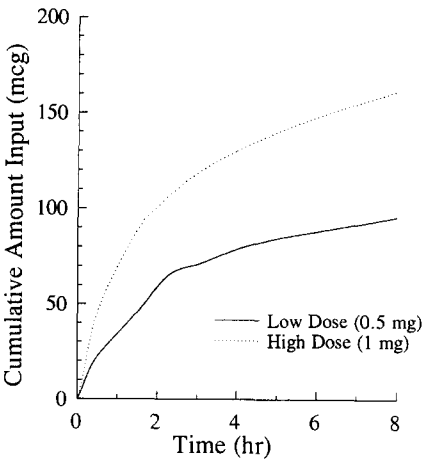

Fig. 4. Estimated cumulative amount input (μg) of MT by deconvolution analysis resulting from an oral controlled release delivery system at two different doses of MT.

a controlled release preparation. MRT calculated from intravenous data from a different study (Iguchi et al., 1982) was 0.71 h. Our oral dosage form produced an MRT 5-times the value reported for intravenous MT. The observed MRT ranged from 2.4 to 5 h. Estimated bioavailability (F_{EST}) was about 19 and 18% for low and high MT dose, respectively (see Eq. 1). These results were consistent with the cumulative amount of MT input as estimated by deconvolution (Fig. 4). The relative bioavailability (F_{REL}) in this study was about 82 and 74% for low and high dose MT, respectively (see Eq. 2).

The observed plasma MT concentration-time profile was compared with computer simulated curve projections obtained previously (Fig. 5). The observed maximal plasma MT concentrations were 3-fold greater than the MT concentrations predicted by computer simulation. The reason for the difference in simulated vs observed plasma MT concentrations is not clear. The difference may reflect lack of correlation between in vitro and in vivo release from the dosage form or limitations in using pooled literature pharmacokinetic data for computer simulations.

The urinary excretion rate of 6-STMT was plotted following administration of sustained release MT (Fig. 6). Urinary excretion rates of 6-STMT during the daytime in subjects not re-

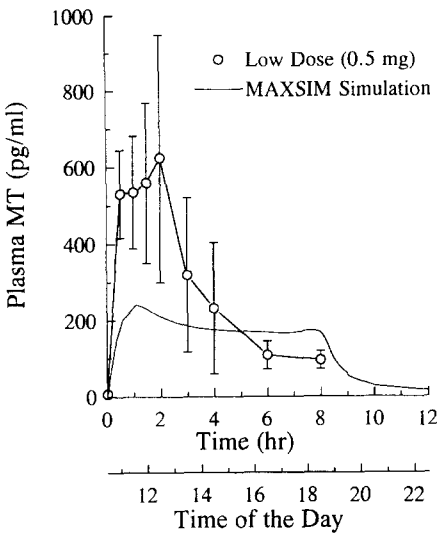

Fig. 5. Comparison of the profiles of observed plasma MT concentrations (mean ± S.D., $n = 4$) and computer-simulated plasma MT concentration at low dose of MT.

ceiving MT were low (< 200 ng/h). When the oral controlled release delivery system of MT was administered, the urinary excretion rate of 6-

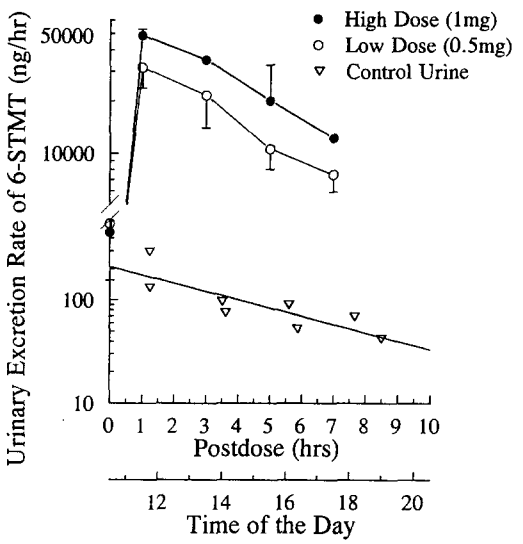

Fig. 6. Urinary excretion rate (ng/h) of 6-STMT determined at the midpoints of each urine collection interval after administration of the oral controlled release delivery system to six human subjects. Values are expressed as mean ± S.D. ($n = 4$ for low dose, $n = 2$ for high dose).

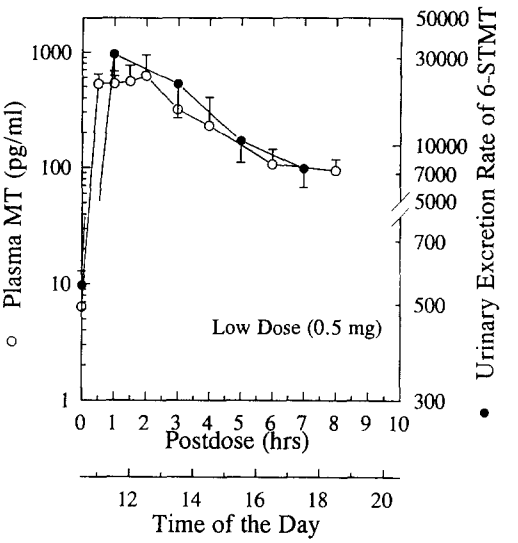

Fig. 7. Comparison of the profiles of plasma MT concentration and urinary excretion rate of 6-STMT determined at the midpoints of each urine collection interval at the low dose of MT (0.5 mg) in human subjects. Values are expressed as mean ± S.D. ($n = 4$).

STMT increased compared to the control urine. The terminal slopes of urinary excretion rate profiles of 6-STMT were parallel for the two different doses. Correlation between plasma MT and urinary 6-STMT excretion rate has been reported elsewhere (Nowak et al., 1987; Brown et al., 1991; Lee et al., 1994). Urinary 6-STMT excretion rate may be a valuable non-invasive method to assess human MT plasma concentrations. Plasma MT concentration and urinary 6-STMT excretion rate profiles as a function of time at low dose and high dose MT are shown in Fig. 7 and 8, respectively. The profiles of plasma MT and urinary excretion rate of 6-STMT are parallel at each dose. As expected, urinary 6-STMT reflects the behavior of plasma MT concentration. Cumulative amounts of urinary 6-STMT excreted over 6 h were compared at two different doses of MT (Fig. 9). As MT dose was doubled, urinary 6-STMT also doubled. Fig. 10 shows the relationship between urinary excretion rate of 6-STMT and plasma MT concentration in six human subjects. The linear relationship between urinary excretion rate of 6-STMT and

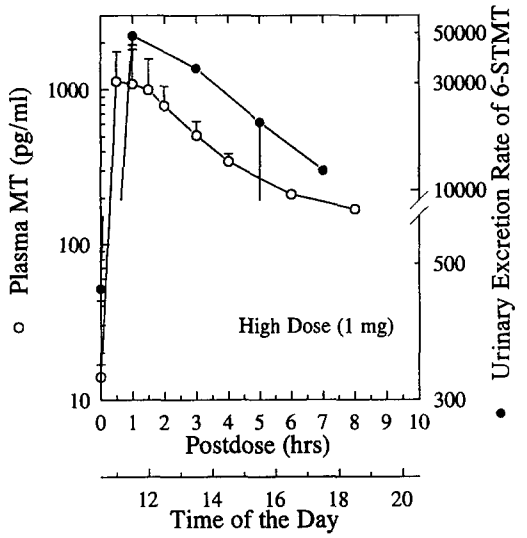

Fig. 8. Comparison of the profiles of plasma MT concentration and urinary excretion rate of 6-STMT determined at the midpoints of each urine collection interval at the high dose of MT (1.0 mg) in human subjects. Values are expressed as mean ± S.D. ($n = 2$).

plasma MT concentration was significant ($F_{1,18} = 2.28$, $r^2 = 0.56$, $p < 0.001$). Furthermore, the linear relationship was much greater when only post

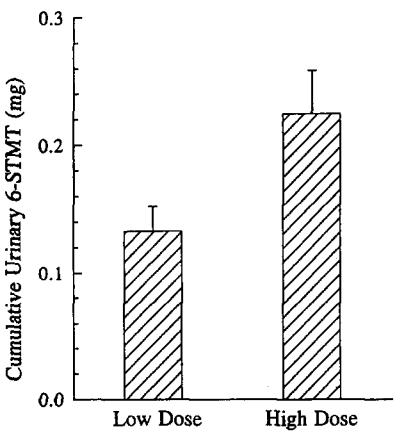

Fig. 9. Cumulative amount of urinary 6-STMT (mg) over 6 h at two different doses of MT. Values are expressed as mean ± S.D. ($n = 4$ for low dose, $n = 2$ for high dose).

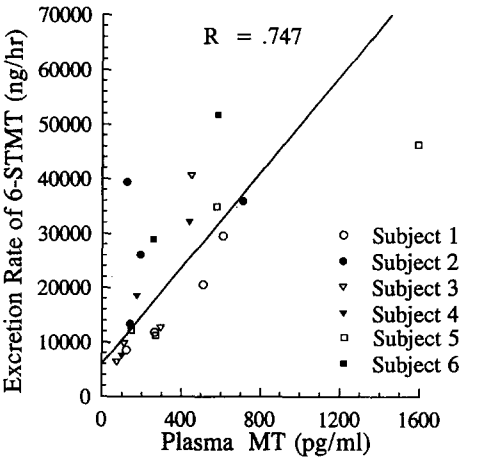

Fig. 10. Relationship between plasma MT concentrations and urinary excretion rates of 6-STMT determined at the midpoints of each urine collection interval in six human subjects.

peak plasma MT concentrations were correlated ($r = 0.838$).

4. Conclusion

An oral preparation designed to release 0.1 mg MT immediately and 0.4 mg MT from coated beads in a controlled release fashion over 8 h produced about 600 pg/ml average peak plasma MT concentrations and then maintained MT concentrations above 100 pg/ml for 8 h. The input rate and cumulative amount of MT absorbed were estimated using plasma MT concentrations and a deconvolution program. Deconvolution and pharmacokinetic analysis across studies revealed that less than 20% of the MT dose administered reached the systemic circulation. The low bioavailability may result from extensive first pass metabolism (Lane and Moss, 1985) and/or incomplete drug release from the coated beads. Peak observed plasma MT concentrations were about 3-times greater than predicted by computer simulation. Urinary excretion rates of 6-STMT were statistically correlated with plasma MT concentrations. Urinary 6-STMT excretion rates may be used as a non-invasive method to assess pineal

gland activity or MT dosage form effects in humans.

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