The actions of melatonin on the sleep-wake cycle were investigated by means of a randomised, double-blind, placebo-controlled trial in 8 subjects with a delayed sleep phase syndrome attending a sleep disorders clinic. In randomised order the subjects received placebo or melatonin 5 mg daily for 4 weeks with a 1 week washout period between the treatments. Drug or placebo was given at 2200 h, 5 h before the mean time of sleep onset determined by pretrial sleep logs. In all 8 subjects sleep onset time (mean advance 82 [range 19-124] min; p <0·01) and wake time (117 [10-187] min; p <0·01) were significantly earlier during melatonin treatment than during placebo. Mean total sleep time was slightly less on melatonin (8 h 12 min) than on placebo (8 h 18 min; p <0·01). Alertness acrophase calculated from the subjects’ ratings of alertness made every 2 h while awake was unaltered. Melatonin may act as a phase-setter for sleep-wake cycles in subjects with a delayed sleep phase syndrome.


Introduction

There is increasing evidence that human circadian organisation is influenced by melatonin. Melatonin secretion occurs during the night and is inhibited by light. Early studies therefore concentrated on possible effects of melatonin on sleep, and suggested that melatonin given by mouth or intranasally in the late afternoon or early evening could induce sleep, although results were conflicting. Later evidence suggests that melatonin may not be a hypnotic, but that it alters the timing of the sleep–wake or rest–activity cycles through its effects on circadian organisation. Melatonin can reduce the psychological and physiological effects of jet-lag and resynchronise sleep when it is disrupted in some totally blind subjects.

Weitzman and colleagues described a syndrome of delayed sleep phase insomnia. 30 of 450 patients presenting with insomnia were unable to fall asleep at the desired clock time, but had little or no difficulty if bedtime was delayed 3–5 h. Sleep duration was normal, but forced early rising was followed by drowsiness and dysphoria. The group as a whole did not have a specific psychiatric disorder, but psychiatric and psychological problems were common and the syndrome was associated with substantial educational, welfare, marital, and social problems. Attempts to treat the disorder by phase advance to an early bedtime failed, but a programme of progressive delay in sleep–onset time resulted in successful rescheduling of the sleep–wake cycle. Thorpy described a further 22 adolescents with a similar syndrome. Here we report the actions of melatonin on the...
sleep-wake cycle in 8 subjects with the delayed sleep phase syndrome.

Subjects and methods

We studied 8 caucasian men attending the Maudsley Hospital sleep disorders clinic with an established diagnosis of the delayed sleep phase syndrome (table I). The diagnostic criteria used were a primary complaint of inability to fall asleep and wake spontaneously at the desired clock time; phase delay of the major sleep episode in relation to the desired time for sleep; symptoms present for at least 12 months; and absence of medical, psychological, or psychiatric factors sufficient to explain the symptoms.

All subjects kept a home sleep log for 4 weeks before the study which confirmed the clinical diagnosis. Polysomnography showed normal sleep variables in all cases except for the delayed time of sleep onset. Pretrial mean sleep-wake times, alertness self-rating scores during wakefulness, motor activity, and melatonin cyclicity were documented in a stable light/dark environment with fixed bedtime (2300 h) and waking time (0800 h) over 5 days (figure).

Plasma melatonin and urinary 6-sulphatoxymelatonin (aMT6s) profiles, pulse duration, and peak concentrations were within the normal range for men aged 20-60 years living under normal light-dark environmental conditions (acrophase data for plasma melatonin in normal subjects, summer: mean 0315 [SD 1.00]). The trial was randomised, double blind, and placebo controlled.

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Results

Pretrial mean delay in sleep onset time (from a conventional reference sleep onset time in late adolescence of 2300 h) was documented in home sleep logs as 209 (range 110-544) min (table II). Before the trial, mean total sleep time was 9 h 9 min (range 3 h 36 min–12 h 33 min).

Comparison of the effects of melatonin and placebo over 4 weeks was based on within-subject differences in changes of sleep log timings and alertness self-rating scores, and the subject’s overall assessment. The 1-week washout period between the treatments was not included in the analysis. Comparisons were made at the end of the trial by Student’s t test (two-tailed). The profiles of alertness rhythms, plasma melatonin, and urinary aMT6s were examined for acrophase (estimated peak time) by cosinor analysis (pretrial assessment: figure).
Melatonin advanced the sleep phase in all subjects. Sleep onset time was advanced by a mean of 82 (range 19–124) min and wake time by 117 (10–187) min compared with placebo; these differences were significant (p = 0.002, p = 0.001, respectively). Mean bed time was slightly but not significantly (p = 0.22) earlier. Mean total sleep time was reduced by 34 min (p = 0.07). Alertness acrophase during wakefulness was not changed significantly (p = 0.49). No order effect of melatonin and placebo was observed.

Polysomnography (on open treatment, with fixed bedtime 2300 h) showed that NREM sleep latency (latency from bedtime to stage 2), but not REM sleep latency (latency from stage 2 to REM), was shorter after melatonin than before treatment (p = 0.005).

Melatonin had no next-day hangover effect as determined by either subjective assessment or alertness rating scales. The sleep phase advance occurred 1–2 days after melatonin was started, and phase delay occurred 1–2 days after the drug was stopped. After the trial 6 subjects correctly identified the melatonin period. The active compound was identified by advance in sleep phase (4 subjects) or by a minor presleep sedative effect (2 subjects).

No definite adverse effect of melatonin was reported except that 1 subject reported headache during melatonin but not placebo. Blood pressure, pulse rate, full blood count, urine analysis, and results of standard automated biochemical tests were comparable on melatonin and placebo except that subject 1 had a high alkaline phosphatase concentration on melatonin treatment (650 IU/1), which fell to 360 IU/1 after 20 weeks of continued melatonin treatment after the trial.

**Discussion**

Melatonin induced an advance in sleep–wake but not alertness acrophase timings in these subjects with the delayed sleep phase syndrome. Most hypnotic, anxiolytic, and sedative drugs have specific effects on sleep structure as well as on the initiation and maintenance of sleep.18 Although reports of the hypnotic properties of melatonin in low dose (2–10 mg) in normal subjects are conflicting, melatonin in high dose (80 mg) rapidly induces sleep, with the characteristic sleep profile of a hypnotic drug.19 With the low melatonin dose (5 mg) we used, sleep onset and wake times were advanced towards conventional times with a slight reduction, rather than increase, in total sleep time. These findings may indicate a phase-setting effect rather than, or in addition to, a hypnotic action of melatonin in the delayed sleep phase syndrome.

Melatonin is rapidly absorbed after oral administration, and peak plasma concentrations occur within 60 min, although sleep onset was delayed for 3–4 h after dosage.19 Animal studies show that there is a narrow window for the phase-setting actions of melatonin on rest–activity cycles, and the timing of administration of melatonin for a phase-setting as opposed to hypnotic effect may be important.31 There is now evidence from human jetlag studies as well as from studies in blind subjects that melatonin has a phase-setting effect for several human circadian rhythms including sleep–wake, rest–activity, cortisol, and temperature cycles.5,6,13,14,20 Many animal studies have shown that photoperiodism and timing of seasonal reproductive activity, as well as gonadal development, are under the control of a sensitive retinal-hypothalamic-pineal-melatonin system.5,6 The duration of the melatonin pulse gives important seasonal information to many animals. Although human beings are less photosensitive than many animals, changes in melatonin profile with the light–dark cycle may be important determinants of many features of human circadian rhythmicity.

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**REFERENCES**

Therapeutic effects of genetically engineered toxin (DAB_{486}IL-2) in patient with chronic lymphocytic leukaemia

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In DAB_{486}IL-2 the receptor-binding domain of native diphtheria toxin is replaced by human IL-2 sequences. This recombinant fusion protein is selectively cytotoxic for cells bearing high-affinity IL-2 receptors—e.g., leukaemic cells. A patient with chronic lymphocytic leukaemia who did not respond to gamma interferon and conventional antileukaemic drugs has responded to DAB_{486}IL-2.


DAB_{486}IL-2 is a recombinant fusion protein in which the receptor-binding domain of native diphtheria toxins has been replaced with human interleukin-2 (IL-2) sequences, and it is selectively cytotoxic for cells that bear the high-affinity receptor for IL-2.

1. The cytotoxic process consists of: (a) the binding of the fusion protein to the high-affinity IL-2 receptor; (b) receptor-mediated endocytosis, during which the fusion protein is apparently processed by a cellular protease at Arg,194 and (c) delivery of fragment A to the cytosol, where it catalyses ADP-ribosylation of elongation factor 2, thus producing inhibition of protein synthesis in the target cell. The introduction of one molecule of fragment A to the cytosol is lethal. The high-affinity IL-2 receptor is composed of at least two glycoprotein subunits: a 55 kDa (p55, Tac antigen) low-affinity subunit, and a 75 kDa (p75, Tac antigen) intermediate-affinity subunit. Once bound to either the high or intermediate form of the receptor, native IL-2 is rapidly endocytosed, whereas DAB_{486}IL-2 selectively binds to only the high-affinity receptor, although the kinetics of internalisation are similar to that of IL-2 itself (Waters C, unpublished). The expression of the high-affinity IL-2 receptor by a variety of haematological malignancies—for example, chronic lymphocytic leukaemia (CLL)—and the restricted expression of this receptor on normal cells suggest that the IL-2 receptor might be an attractive target in the design of therapeutic strategies.

DAB_{486}IL-2 is being evaluated in phase I clinical trials in patients with chemotherapy-resistant malignancies expressing IL-2-receptor (p55). Although this recombinant fusion toxin is specifically cytotoxic for leukaemic cells freshly withdrawn from patients with acute T-cell leukaemia and in a variety of preclinical models, the design of successful therapeutic approaches with this and other novel fusion toxins will require an in-depth understanding of its pharmacokinetics, which will be reported elsewhere.

The patient, a 60-year-old man with Rai stage III CLL diagnosed in 1984, had been treated with gamma-interferon, combination chemotherapy (cytarabine, cisplatin, and dacarbazine), and fludarabine. He had a history of medically controlled mild congestive heart failure, mild renal insufficiency, and numerous episodes of pneumonia.

The protocol was approved by the Institutional Review Board of the MD Anderson Cancer Center under a Food and Drug Administration sponsored investigational new drug licence and the patient gave informed consent for the study. At start of DAB_{486}IL-2 therapy the patient had a leukocyte count of around 24 280/µl, a bone-marrow leukaemic infiltrate of 64%, peripheral and retropertioneal lymphadenopathy, and splenomegaly. 55% of the patient’s leukaemia cells expressed low-affinity IL-2 receptor as determined with an immunofluorescent antibody that binds p55 (anti-CD 25, Becton-Dickinson) and Scatchard analysis revealed the presence of high-affinity receptor. The plan for the phase I trial was for treatment every 4 weeks as follows: a) a first course consisting of three daily intravenous bolus injections of 0.05 mg/kg per day DAB_{486}IL-2, followed about a week later by seven daily injections at the same dose; b) a second cycle starting at day 28, at a dose of 0.1 mg/kg per day; and then c) a maintenance course consisting of 0.1 mg/kg per day for seven days every 28 days. In the patient described here the second course was delayed from day 28 to day 45 because of a community-acquired pneumonia; his other treatments, which varied somewhat from the original plan, were: course 2, days 45-47 and 56-62; course 3, days 80-86; and course 4, days 113-119.

The only side-effect attributable to DAB_{486}IL-2 was a transient increase in aspartate and alanine aminotransferase activities. No changes were noted in normal lymphocyte markers or in in-vitro lymphocyte function tests.

Concentrations remained above 20 ng/ml, the effective cytotoxic concentration for high-affinity IL-2 receptor bearing cells in vitro, for more than an hour at the 0.1 mg/kg dose; the half-life was short, there was no accumulation of DAB_{486}IL-2 with repeated daily doses, and DAB_{486}IL-2 seems to have a distribution equivalent to plasma volume soon after administration. The patient did not show an antibody response to either native diphtheria toxin or the fusion toxin when this was examined after each of the first three treatment cycles, and circulating soluble IL-2 receptor (sIL-2R),
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