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Chronopharmacological Study of Antihistamines in Man with Special References to Terfenadine

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Summary. A double blind, placebo-controlled, randomized, single dose, cross-over chronopharmacological study of two antihistamines (Terfenadine 20 and 60 mg and Clemastine 1 and 3 mg) was undertaken in 10 healthy volunteers (8 males and 2 females), 21–28 years of age. Drug or placebo was administered at 07.00 h or 19.00 h at one week intervals to subjects with diurnal activity from 07.00–23.00 h and nocturnal rest. The responses measured before and at fixed intervals after each dose of drug or placebo were surface area measurement of skin reaction (wheal and erythema) to intradermal histamine, self-rating for sleepiness using a visual analogue technique, random number addition and eye-hand skill tests. Circadian variation in the response to I. D. histamine 2 μg and vigilance and psychomotor skills were validated. Chronopharmacological changes in the inhibitory effects of the antihistamines Terfenadine and Clemastine on the skin reaction to intradermal histamine were documented. The time from drug administration to maximal effect and the duration of effect was longer with both drugs when administered at 07.00 than at 19.00 h, and the degree of maximal inhibition was greater when the drugs were administered at 19.00 h. Dose-related inhibition of the histamine skin reaction was obtained with both drugs; Terfenadine 60 mg had approximately equivalent inhibitory activity to that of Clemastine 3 mg. Only Clemastine 3 mg had a significant central depressant effect, as shown by self-rating of sleepiness and random number addition. Terfenadine 60 mg administered at 19.00 h tended to produce a lower sleepiness score than did the placebo. A chronotherapeutic optimization approach to a Terfenadine dosage schedule is proposed.

Key words: Terfenadine, clemastine, chronopharmacological effects, circadian rhythms, antihistamines, dermal reaction, psychomotor score, central depression, skin reactions.

Circadian change in the skin reaction (erythema and wheal) to intradermal histamine has been demonstrated in man [1, 2] and in the guinea pig [3]. In the latter study circadian rhythms were shown to persist, even though the animals were in constant environmental conditions, i. e. without any known synchronizer.

The duration of the inhibitory effect of the antihistamine Cyproheptadine on the skin reaction to histamine was found [4] to vary with the time of administration of a single 4 mg dose. For example, when administered at 07.00 h the duration of the inhibitory effect on the wheal reaction was 17.50 ± 0.93 h (SEM), as compared to 8.6 ± 1.32 h when administered at 19.00 h. These chronopharmacological differences show that physiological circadian rhythms must be taken into consideration when the time course of pharmacological effects is studied.

The aim of the present investigation was to study the chronopharmacology of two antihistamines; Terfenadine, a new compound that is reported to be free of central nervous system depression [13, 14, 16], and Clemastine, a standard antihistamine that was included for reference purposes. The protocol was designed to evaluate any drug effect on skin reaction to intradermal (I. D.) histamine and on psychomotor function.

The structural formulae of the compounds are:

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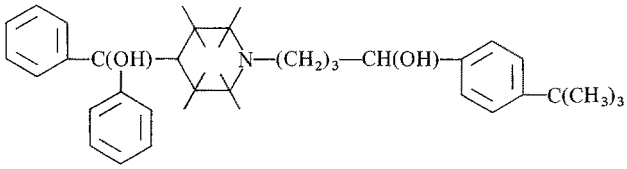
– Terfenadine



– Clemastine

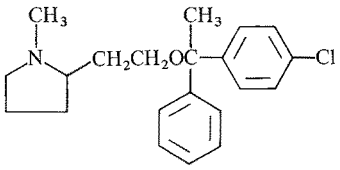


Table 1. Subject's characteristics (Student)

	Sex	Age (years)	Weight (kg)	Height (cm)	Student activity
1	M	26	57	170	architecture
2	M	21	68	170	medecine
3	M	27	77	180	medecine
4	M	23	70	179	architecture
5	F	26	54	162	economics
6	M	22	50	160	medecine
7	F	22	50	155	literature
8	M	23	74	180	literature
9	M	24	64	176	architecture
10	M	28	73	182	medecine

Material and Methods

Subjects

Ten subjects, 8 males and 2 females, aged 21 to 28 years volunteered for the study. Their individual characteristics are presented in Table 1. All were healthy as judged by normal routine clinical and biological examinations prior to entry into the study, including complete blood counts, erythrocyte sedimentation rate, blood glucose and urea, serum transaminases, and serum Na, K, Cl and Ca. None were on other medications, including oral contraceptives (O. C. S.). Particular care was taken to avoid test periods on days of menstruation, since both O. C. S. and menstruation have been shown to modify the skin reaction to histamine [6].

Study Protocol (modified from a previous study, [4]) was first a double blind, placebo-controlled comparison of single daily doses of Terfenadine 20 and 60 mg and Clemastine 1 and 3 mg ('double dummy' packaging), administered to the subjects at 07.00 h at intervals of at least one week. The subjects received the drugs or placebo in random order (5 × 5 Latin Square). The second part of the study was open and consisted of administration of Terfenadine 20 and 60 mg and Clemastine 3 mg at 19.00 h at intervals of 1 week. Only 4 of the subjects were available for the tests with Terfenadine 20 mg and Clemastine 3 mg in the second part of the study.

The experiment was carried out on Saturdays and Sundays from the end of November 1976 to the end of January 1977 in order to avoid any possible interference by circannual rhythms [7].

On test days, the subjects were active from 07.00 to 23.00 h and slept from 23.00 to 07.00 h, except they were awakened for a histamine wheal test at 03.00 h.

The diet was controlled on test days only as follows: breakfast (08.00 h) 500 calories, lunch (12.30 h) and dinner (20.00 h) 750 calories each (proteins 17%, lipids 40%, carbohydrates 43%). No alcoholic beverages were allowed on test days.

Drug Dosage and Administration

The doses of Terfenadine were based upon the work of Hüther [5], who demonstrated antihistamine activity at 20 mg and maximum activity at 60 mg. Similar published data was not available for Clemastine, but the recommended dose is 1 mg 2–3 times a day.

Single doses of a drug or placebo were ingested at 07.00 or 19.00 h at intervals of one week.

Intradermal histamine tests were performed at 07, 11, 15, 19, 23 and 03 h. After each injection (except at 03.00 h) the following tests were performed: self-rating of sleepiness, eye-hand skill and random number addition.

Response Variables

Skin Reactions to Intradermal Histamine. A commercial preparation of histamine (Allergopharma Joachim Ganzer KG, Reinbeck, Germany) was diluted on each test day with 0.9% saline to provide 2 μg histamine for each 0.1 ml intradermal (ID) injection, the dose found optimal by Hüther et al. [5]. Injections were made on the flexor surface of the forearm. The injections were made by the same physician in each subject using precision syringes and needles (Terumo Disposal Syringe with 26 G needles, Jintan Terumo Co. Ltd., Tokyo, Japan) and the injection sites were at least 6 cm apart. Precise limits both of erythema and wheals were delineated on the skin with a ball point pen 10 min after the I. D. injec-

tion. The outlines were removed from the skin with transparent celophane adhesive tape and applied to the case report form, from which they were reproduced on transfer paper, cut out, weighed and re-expressed as actual surface area in sq. cm.

Self Rating of Sleepiness. The subject was instructed to draw a vertical line on a horizontal rectangle (22 × 5 mm), imagining the rectangle as a meter on which the further to the right the mark the more sleepy the subject felt, and the more to the left the more alert he or she felt. Scores (0–22) were afterwards obtained by measuring the distance in mm from the left to where the mark was made.

Eye-Hand Skill Test. Using an instrument designed by Halberg, consisting of a flat metal box with a steel tube protruding vertically from it, the subject placed 25 calibrated cylindrical metal bearings in his left hand, started the chronometer (placed face down), and with his right hand fed the bearings one by one into the tube. The time necessary to complete the task was recorded.

Random Number Addition Test. Five squares of 25 random numbers were displayed on a sheet of paper and the subject was instructed to start a chronometer (placed face down) and correctly to add the numbers two by two and to write down the sums. The score was the time required to perform the task.

Statistical Analysis

Student's t-test and analysis of variance.

Results

Validation of the Circadian Rhythm of the Response Variables

Skin Reaction to Intradermal Histamine. The mean 24 h wheal and erythema responses to I. D. histamine during the placebo period were first calculated for each subject from the 7 time periods, as well as the percentage variation in the reactions at each time during the 24 h.

The mean (± SEM) percent variation in wheal (upper curve) and erythema (lower curve) reactions for the group (n = 10) for each time period are shown in Figure 1. It is evident that there was a circadian rhythm both in the wheal and erythema responses to I. D. histamine. The peak response occured at about 23.00 h with a trough 12 h earlier or later.

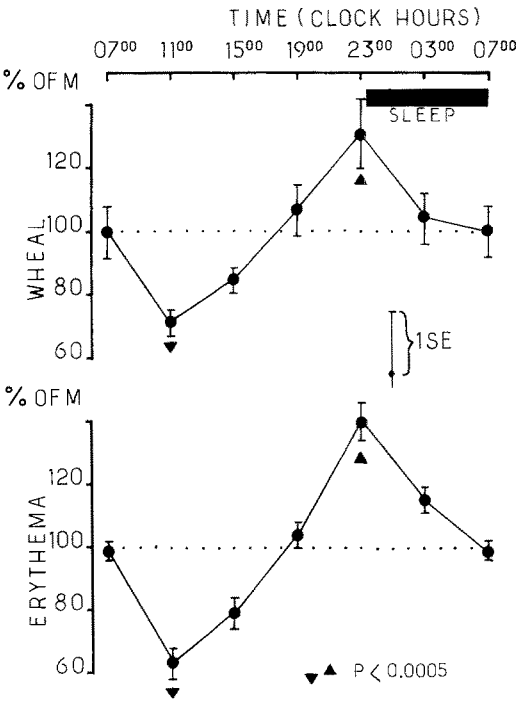


Fig. 1. Circadian rhythm of skin reactions to intradermal injection of histamine

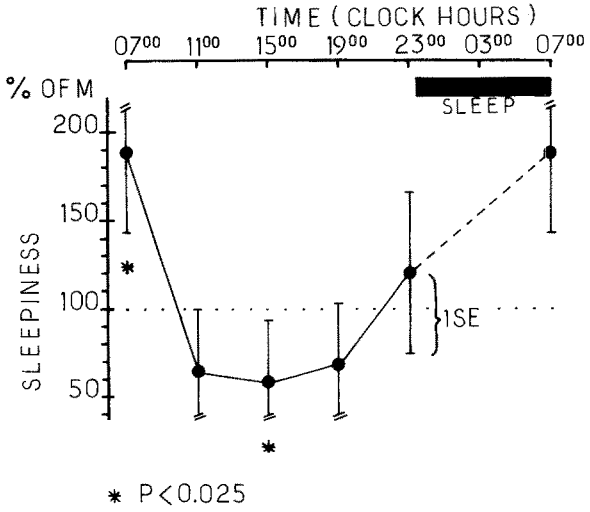


Fig. 2. Circadian rhythm of sleepiness (self-rating)

The mean 24 h areas for wheal and erythema were 1.55 ± 0.06 and 10.36 ± 3.25 sq. cm., respectively. The peak to trough differences were statistically significant ($p < 0.0005$).

Sleepiness. Individual 24 h mean values and percent variation from the 24 h mean for each time period (except during sleep periods) were calculated and the

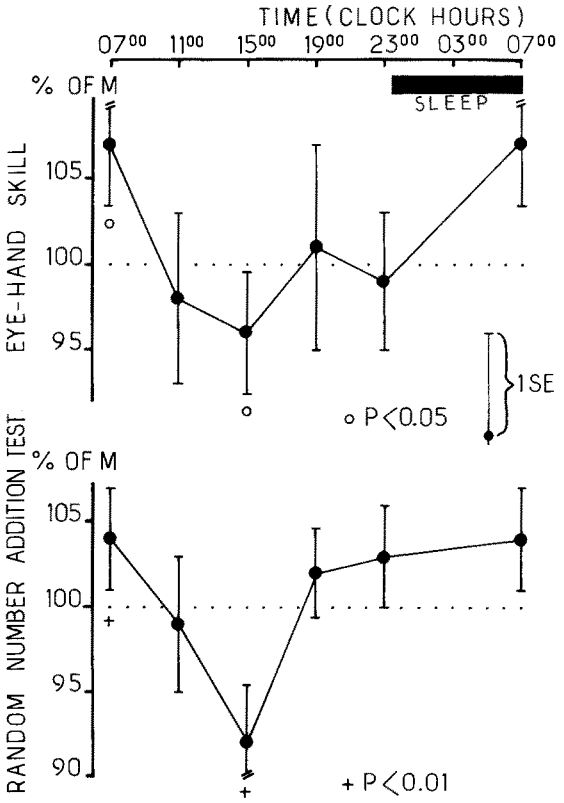


Fig. 3. Time (clock hours)

mean (± SEM) percent age variations for the group are presented in Figure 2.

The circadian peak occured at 07.00 h and the trough (maximum vigilance) at between 11–19.00 h. The peak to trough differences were statistically significant ($p < 0.025$).

Eye-Hand Skill and Random Number Addition Scores. Group mean percentage variations from the 24 h mean eye-hand skill scores (upper curve) and random number addition scores (lower curve) are shown in Figure 3. The data is expressed as mean percentage deviation (± SEM) for each period from the 24 h mean ($n = 10$). The best performance (shortest duration) occured at 15.00 h for both tests, which corresponds to the period of least sleepiness (Fig. 2). Peak to trough differences for both tests were statistically significant ($p < 0.05$ and 0.01, respectively).

Effect of the Antihistamines on the Response Variables

Validation of Drug Dosages. The mean percent reduction (± SEM) from placebo values of the erythema and wheal reactions to I. D. histamine for each time point is presented in Figure 4 for Terfenadine 20 and 60 mg, and in Figure 5 for Clemastine 1 and 3 mg. From the curves it appears that Terfenadine 60 mg and Clemastine 3 mg had approximately equivalent antihistamine effects.

Effects on the Skin Reactions to I. D. Histamine. Mean (± SEM) values were computed from individual time series for (a) delay (hours) from dosing to peak inhibition, (b) maximum percent inhibition compared to placebo, and (c) total duration (hours) of inhibitory effect after single doses of Terfenadine 20 or 60 mg or Clemastine 3 mg administered at 07.00 and 19.00 h (Table 2).

The peak effect after Terfenadine 60 mg or Clemastine 3 mg occured by the 9th–13th h after dosing at 07.00 h, whereas it occured by the 4–5th h after dosing at 19.00 h; the differences were statistically significant. The maximum percentage inhibition produced by both drugs was significantly greater when they were administered at 19.00 h than at 07.00 h.

The duration of the antihistaminic effect on the skin was significantly longer when the drugs were administered at 07.00 h than when they were administered at 19.00 h.

Effect on Vigilance. The data in Table 3 is based upon comparison of mean 24 h individual sleepiness scores from the placebo control and drug treatment days. Only Clemastine 3 mg showed a statistically significant difference (increase) in sleepiness scores as compared to placebo. In fact, when administered at 07.00 h, Clemastine 3 mg increased the mean sleepiness score by over 25% in 7 of 10 subjects. Half the patients who received Terfenadine 60 mg at 19.00 h had a sleepiness score 25% or more lower (more vigilance) than the placebo group. There were no complaints of nocturnal sleep disturbance.

Effect on random number addition and eye-hand skill tests. These effects were calculated by comparison of scores 4 and 8 h after the 07.00 h dose of each drug (and 4 h after the 19.00 h dose of Terfenadine 60 mg): the data is presented in Tables 4 and 5. The only statistically significant change was an increase in the time required to perform the random number addition 8 h after the 3 mg dose of Clemastine, when 4 out of 10 subjects had >15% increase in score.

Discussion

During the placebo days, the control curves for skin reaction to I. D. histamine, random number addition

Table 2. Chronopharmacological changes in parameters characterizing the inhibitory effect of Terfenadine and Clemastine on the skin reaction to intradermal injection of histamine. E = Erythema; W = Wheal

a. Span of time (h) to reach maximum depth of inhibition ($\bar{X} \pm SEM$)

Agent (dose)			Time of dosing		P
			07.00 h	19.00 h	
Terfenadine	(20 mg)	E	10.1±4.6	5.0±3.2	>0.05
		W	8.0±4.8	5.0±3.3	>0.05
	(60 mg)	E	11.3±3.1	4.3±1.0	<0.01
		W	9.7±3.3	4.1±0.9	<0.025
Clemastine	(3 mg)	E	10.8±3.8	4.0±0.8	<0.025
		W	13.5±3.8	5.5±3.0	<0.025

b. Maximum inhibition as a percentage of control ($\bar{X} \pm SEM$)

Agent (dose)			Time of dosing		P
			07.00 h	19.00 h	
Terfenadine	(20 mg)	E	−40.0±5.9	−57.0±7.9	<0.025
		W	−22.0±4.3	−52.0±3.7	<0.0005
	(60 mg)	E	−68.0±5.8	−80.3±3.5	<0.025
		W	−38.5±5.2	−66.2±3.3	<0.0005
Clemastine	(3 mg)	E	−54.9±3.7	−71.2±3.8	<0.0125
		W	−31.3±4.3	−53.7±4.3	<0.0005

c. Total duration of inhibitory effect in hours ($\bar{X} \pm SEM$)

Agent (dose)			Time of dosing		P
			07.00 h	19.00 h	
Terfenadine	(20 mg)	E	22.6±1.7	14.5±3.0	<0.005
		W	18.6±2.6	13.0±2.2	<0.025
	(60 mg)	E	26.8±2.4	19.3±1.2	<0.0025
		W	23.0±2.5	14.6±2.4	<0.005
Clemastine	(3 mg)	E	25.0±3.6	15.0±3.2	<0.01
		W	21.0±4.2	12.5±3.8	<0.05

Table 3. Change in self-rating of sleepiness

Agent (dose)	Time of dosing[a]	Increase in sleepiness as % of control (placebo)	N° of subjects/total with individual mean of sleepiness rating[b]		
		$\bar{X} \pm SEM$	increased $X > 25\%$	equal $X \pm 25\%$	decreased $X < -25\%$
Terfenadine (20 mg)	07.00 h	7% ± 34	3/8	3/8	2/8
Terfenadine (60 mg)	07.00 h	1% ± 14	2/10	8/10	2/10
	19.00 h	4% ± 39	2/10	3/10	5/10
Clemastine (1 mg)	07.00 h	4% ± 33	3/8	3/8	2/8
Clemastine (3 mg)	07.00 h	43% ± 23	7/10	3/10	0/10

[a] When the agent was ingested at 07.00 h, self-rating of sleepiness was recorded at 11.00, 15.00, 19.00, 23.00 and 07.00 h; when ingested at 19.00 h, self-rating were recorded at 23.00, 07.00, 11.00, 15.00 h (not at 04.00 h)

[b] The figure ± 25% as confidence limits results from statistical analysis of data recorded both during the placebo days and immediately before treatment with any compound

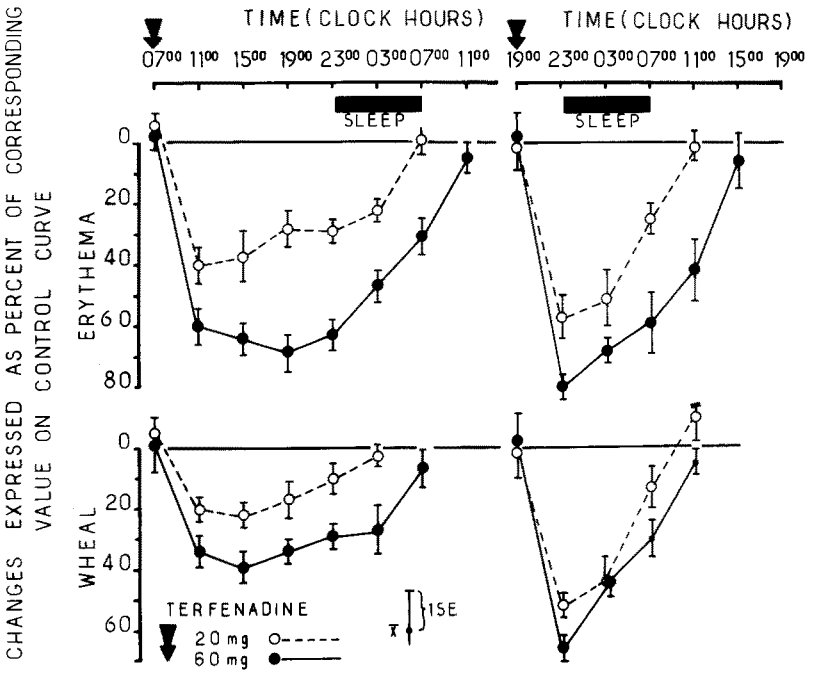


Fig. 4. Daily changes in terfenadine-induced inhibition of the local skin reactions to histamine: I. D. 2 μg/0.1 ml

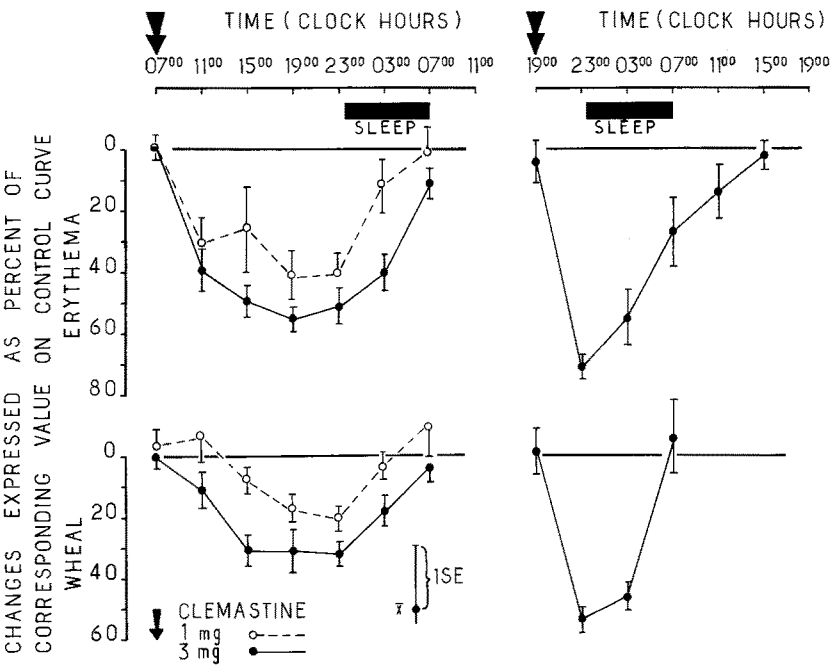


Fig. 5. Clemastine induced inhibition of the local skin reactions to histamine: I. D. 2 μg/0.1 ml

and eye-hand skill tests were in agreement with those published previously [1, 2, 6, 8, 9, 10]. The circadian rhythm of self-rating of sleepiness has not previously been used in this context, but self-rating has proven acurate and reliable in the quantification of circadian rhythms of subjective variables, such as mood, physical vigour, fatigue and dyspnoea [8, 10, 11]. The control curves further demonstrate that the subjects were healthy from a chronobiological point of view and that circadian changes in response variables must be taken into account when evaluating pharmacological effects of drugs [12].

Chronopharmacological changes in the inhibition of skin reactions to I. D. histamine following Terfenadine and Clemastine are similar to those demonstrated previously with Cyproheptadine [4]. The agents produce principally (perhaps exclusively with Terfenadine) H_1-receptor blockade. Despite differences in their chemical structure, their antihistamine effects varied identically with the timing of ad-

Table 4. Change in the duration of the random number addition test

Agent (dose)	Time of dosing	Time of measurement	Change in duration as % of control $\bar{X} \pm SEM$	N° of subjects/total having individual mean X[a] better $X < -15\%$	equal $X \pm 15\%$	poorer $X > +15\%$
Terfenadine (20 mg)	07.00 h	11.00 h	+4%±5	–	7/8	1/8
		15.00 h	+4%±3	–	7/8	1/8
Terfenadine (60 mg)	07.00 h	11.00 h	−2%±5	2/10	8/10	–
		15.00 h	−2%±9	2/10	8/10	–
Terfenadine (60 mg)	19.00 h	23.00 h	+8%±6	2/10	5/10	3/10
Clemastine (1 mg)	07.00 h	11.00 h	+7%±5	1/8	6/8	1/8
		15.00 h	+7+±5	–	6/8	2/8
Clemastine (3 mg)	07.00 h	11.00 h	+3%±5	1/9	6/9	2/9
		15.00 h	+16%±6	–	6/10	4/10

[a] The figure ± 15% as confidence limits results from statistical analysis of data recorded during the placebo days and immediately before ingestion of any agent

Table 5. Change in the duration of the eye-hand-skill test

Agent (dose)	Time of dosing	Time of measurement	Changes in duration as % of control $\bar{X} \pm SEM$	N° of subjects/total having individual mean X[a] better $X < -15\%$	equal $X \pm 15\%$	poorer $X > +15\%$
Terfenadine (20 mg)	07.00 h	11.00 h	+3%±6	1/8	4/8	3/8
		15.00 h	−5%±4	2/8	6/8	–
Terfenadine (60 mg)	07.00 h	11.00 h	−2%±4	3/10	5/10	2/10
		15.00 h	−5%±4	3/10	6/10	1/10
Terfenadine (60 mg)	19.00 h	23.00 h	+8%±6	2/10	5/10	3/10
Clemastine (1 mg)	07.00 h	11.00 h	+6%±6	1/8	5/8	2/8
		15.00 h	−2%±6	–	8/8	–
Clemastine (3 mg)	07.00 h	11.00 h	+3%±6	2/9	5/9	2/9
		15.00 h	+2%±6	1/10	7/10	2/10

[a] The figure ± 15% as confidence limits results from statistical analysis of data recorded both under placebo and immediately before ingestion of any agent

ministration (07.00 vs 19.00 h). Therefore, their chronopharmacological changes may be related to circadian rhythms of target organ sensitivity rather than to circadian rhythms of absorption, distribution, metabolism and excretion or other metabolic functions.

Other studies have shown that Terfenadine has little or no undesirable central depressant effect, as evidenced by lack of sleepiness, drowsiness, or altered psychomotor function [14, 16]. The present results are in agreement with those reports, even though different investigational methods were employed.

Based upon the results of this clinical chronopharmacological study of Terfenadine, a chronotherapeutic optimization approach is suggested. Theoretically, 24 h antihistamine protection could be obtained with Terfenadine 60 mg administered at 07.00 h and 20 mg (or 30 mg) at 19.00 h, thereby reducing the potential risk of a decrease in night time sleepiness.

References

1. Reinberg, A., Sidi, E., Ghata, J.: Circadian reactivity rhythms of human skin to histamine or allergen and the adrenal cycle. J. Allergy **36**, 273–283 (1965)
2. McGovern, P. J., Smolensky, M. H., Reinberg, A.: Circadian and circamensual rhythmicity in cutaneous reactivity to histamine and allergenic extract. In: Chronobiology in allergy and immunology, J. P. McGovern, M. Smolensky and A. Reinberg, (eds.), pp. 79–116. Springfield, Illinois: C. C. Thomas 1977

3. Scheving, L. E., Sohal, G. S., Enna, D., Pauly, J. E.: The persistance of a circadian rhythm in histamine response in guinea pigs maintained under continuous illumination. Anat. Rec. **175**, 1–6 (1973)
4. Reinberg, A., Sidi, E.: Circadian changes in the inhibitory effects of an antihistaminic drug in man. J. invest. Dermatol. **46**, 415–419 (1966)
5. Hüther, K. J., Renftle, G., Barraud, N., Burke, J. T., Koch-Weser, J.: Inhibitory activity of Terfenadine on histamine-induced skin wheals in man. Europ. J. clin. Pharmacol. **12**, 195–199 (1977)
6. Smolensky, M. H., Reinberg, A., Lee, R. E., McGovern, J. P.: Secondary rhythms related to hormonal changes in the menstrual cycle; special reference to allergology. In: Biorhythms and human reproduction. M. Ferin, F. Halberg, R. Richart, R. Vande Wiele (eds.), pp. 287–306. New York: John Wiley 1974
7. Reinberg, A.: Aspects of circannual rhythms in man. In: Circannual Clocks, Annual Biological Rhythms. E. T. Pengelley (ed.), pp. 423–505. New York: Academic Press 1974
8. Halberg, F.: Chronobiology. Ann. Rev. Physiol. **31**, 675–725 (1969)
9. Reinberg, A., Apfelbaum, M., Assan, R.: Chronophysiologic effects of a restricted diet (220 cal/24 h as casein) in young healthy but obese women. Internat. J. Chronobiol. **1**, 391–404 (1973)
10. Reinberg, A., Vieux, N., Laporte, A., Ghata, J., Migraine, C., Abulker, C., Dupont, J., Nicolai, A.: Ajustement de rythmes circadiens physiologiques d'opérateurs d'une raffinerie, lors de changement d'horaire travail-repos tous les 3–4 jours. Arch. Malad. Prof. Med. Trav. **37**, 479–494 (1976)
11. Reinberg, A., Guillet, P., Gervais, P., Ghata, J., Vignaud, D., Abulker, Ch.: One month chronocorticotherapy (Dutimelan 8–15 mite) control of the asthmatic condition without adrenal suppression and circadian rhythm alteration. Chronobiologia **4**, 295–312 (1977)
12. Reinberg, A., Halberg, F.: Circadian chronopharmacology. Ann. Rev. Pharmacol. **11**, 455–492 (1971)
13. Kinsolving, C. R., Munro, N. L., Carr, A. A.: Separation of the CNS and H_1 receptor effects of antihistamine agents. Pharmacologist **15**, 221–226 (1973)
14. Clarke, C. H., Nicholson, A. N.: Performance studies with antihistamines. Br. J. clin. Pharmac. (1978) in press.
15. Reinberg, A.: Advances in human chronopharmacology. Chronobiologia **3**, 151–166 (1976)
16. Moser, L., Hüther, K., Koch-Weser, J., Lundi, P. V.: Comparison of the effects of Terfenadine, Diphenhydramine and placebo alone and in combination with diazepam or alcohol on psychomotor skills and subjective sensations. (to be published)

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