Central effects during the continuous osmotic infusion of a benzodiazepine (triazolam)

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1 The effects of relatively constant plasma levels of a rapidly eliminated benzodiazepine (triazolam) were studied in young healthy males to determine whether tolerance to certain effects may develop over a relatively short period of time. The drug was given over a period of 30 h (2 days and 1 night) at zero-order rate using a rectal osmotic pump.

2 Performance was measured at 2 hourly intervals during the day and was continuously impaired during the infusion, though there was a rapid recovery when the infusion ceased. All tasks were affected, in particular mental arithmetic and letter cancellation, but there was some improvement in performance during the second day. The normal circadian improvement in performance may have contributed to this effect, but some degree of tolerance to the effect of the drug cannot be excluded.

3 Overnight there was a marked reduction in wakefulness, suppression of slow wave sleep, and delay to the onset and reduction in the duration of rapid eye movement sleep. During the night after infusion there was less slow wave sleep and increased wakefulness.

4 The experimental design may prove useful in the study of tolerance to drugs.

Keywords benzodiazepines sleep performance tolerance pharmacokinetics

Introduction

Some degree of tolerance or adaptation to the central effects of benzodiazepines may develop either with single doses of drugs which are slowly eliminated (Greenblatt *et al.*, 1979) or with repetitive ingestion (Mattila *et al.*, 1978; Church & Johnson, 1979; Seppala *et al.*, 1980). However, there is limited information on the processes involved, and it is in this context that we have assessed psychomotor function during relatively constant plasma concentrations of a rapidly inactivated benzodiazepine, triazolam. A rectal osmotic pump was used to attain a constant plasma concentration. This system delivered a drug solution at zero-order rate (infusion) in an aqueous environment. Steady state levels and sensitivity to the drug were assessed for each volunteer, and then performance and sleep were studied before, during and after infusion in a placebo controlled study.

Methods

The study was carried out in healthy male volunteers aged between 22 and 30 years. The factors involved in attaining steady state levels of a rapidly eliminated benzodiazepine by means of an osmotic pump were determined initially, and then the effects of steady state plasma levels on performance and on sleep were studied (Figure 1). The protocol was approved by the Committee for Medical Ethics of the University Hospital of Leiden, and the subjects gave written informed consent.

Pharmacokinetic study on steady state plasma levels

An ALZA pump (length 45 mm, external diameter 13 mm, volume 2 ml) based on the osmotic principle (Theeuwes & Yum, 1976) was used to achieve constant drug plasma concentrations

Figure 1 Design of the experiment in which central effects were studied. The subjects slept from 23.00 to 07.00 h before, during and after infusion. Performance tests (▨) were carried out from 08.00–21.30 h each day. The pump (indicated by arrow) was inserted at 09.00 h on day 1 and taken at 18.00 h on day 2.

(De Leede *et al.*, 1981; Breimer *et al.*, 1984). Drug solution was infused from the pump into an aqueous environment at zero-order rate (65 $\mu\text{L/h}$) for at least 24 h. Triazolam (0.75–5.0 mg/ml) was dissolved in the drug vehicle which was a mixture of propylene glycol:water:alcohol (50:40:10 weight %). In the experiments the pump was incubated in isotonic saline for 5 h before rectal insertion, and the rates were 25 $\mu\text{g/h}$ in four and 65 $\mu\text{g/h}$ in five of the nine subjects.

The rectal pump was inserted at 09.00 h on the first day of the experiment and replaced at 22.30 h the same day by one with an identical rate. This was withdrawn at 18.00 h the next day—31 h after the beginning of the infusion. Blood samples were taken through a flexible cannula in a forearm vein during the first day at 0.75, 1.5, 3, 5, 7, 9, 11 and 13 h, and by venepuncture during the second day at 24, 27, 31, 33, 35 and 37 h after insertion. Heparin solution (5000 iu/ml) was added to the samples, and after separation the plasma was stored at -20°C . Concentrations of triazolam were measured by capillary gas chromatography (Jochemsen & Breimer, 1981), and some samples were analysed using a radio-receptor technique (Jochemsen *et al.*, 1982).

Elimination half-lives were calculated by least squares regression analysis of the terminal log linear part of the plasma concentration *vs* time curves. Clearance values were calculated using the pumping rate of the delivery system determined *in vitro*, and the measured steady state concentration. The rate for the subsequent experiments was chosen using these values on the basis that the desired steady state plasma concentration was proportional to the product of the steady state concentration and the clearance value.

Performance studies on central effects

Effects of steady state plasma levels on sleep and on performance were studied in seven of the

subjects. Experiments with the drug vehicle (placebo) and with the drug were separated by between 2 and 4 weeks. In three subjects triazolam was used and in four subjects the placebo was used in the first experiment. The pump was inserted at 09.00 h on day 1 and finally withdrawn at 16.00 h on day 2. Blood samples were taken at 13.00, 17.30 and 21.30 h on the first day, and at 08.30, 12.00, 16.00 h on the second day. Performance was measured over half-hour sessions from 08.00, 10.00, 11.00, 13.00, 15.00, 17.00, 19.00 and 21.00 h each day (Figure 1), and each session contained all tests. The experiment was single-blind. A light breakfast was eaten around 09.15 h on each day.

Digit symbol substitution (DSS) One of a series of 50 sheets each with 200 randomised digits (0–9) arranged in 10 rows on each side was presented. In the space below each digit subjects were required to write the corresponding symbol indicated by a code at the top of each page. They were given 2 min per side to complete as many substitutions as possible. The number correct on each sheet was scored.

Symbol copying (SC) One of a series of 50 sheets each with 200 randomised symbols (used with DSS) arranged in 10 rows on each side was presented. Subjects were required to copy each symbol in the space provided and were allowed 1 min per side to copy as many symbols as possible. The number correct on each sheet was scored.

2-letter and 6-letter cancellation One of a series of 50 (2-letter test) or 40 (6-letter test) sheets with 1200 randomised letters arranged in 40 columns was presented. Each sheet had either 2 or 6 target letters printed at the top. Subjects working down each column in turn were given either 2 min (2-letter test) or 5 min (6-letter test) to cancel as many target letters as possible. The number of columns completed, and the number

of correct cancellations/possible cancellations was scored.

Logic One of a series of 50 different sheets of 100 problems was presented. Each problem took the form that, if: $B > A$: $A = F$: $F < E$: $E > D$. Then $B < D$. Subjects were required to state whether the solution was True (T), False (F) or Indeterminate (?). They were given 3 min to complete as many problems as possible, and the number correct/number attempted was scored.

Mental arithmetic One of a series of 50 different sheets with 192 sums was presented, and each sum required the addition or subtraction of three 3-digit numbers. Written calculations were not permitted. Subjects completed as many sums as possible within 10 min, and the number correct and number attempted were recorded.

Assessment of mood

Subjects assessed their mood on visual analogue scales related to a 100 mm line. The assessments and extremes of the scale were: I am very wide awake – Extremely sleepy; I am very tense – Very relaxed; I am very calm – Extremely anxious; I am very energetic – Very lethargic; I am very dull – Very alert; I have no ability to concentrate – Complete ability to concentrate; I am highly efficient – Completely useless. For all assessments the subjects were instructed to mark the line at a point which corresponded with their present state.

Sleep electroencephalography

Nocturnal sleep was recorded before the experiment, during infusion and after the pump was withdrawn (Figure 1). The subjects slept in single bedrooms. In an adjoining room three channels of electroencephalographic (EEG) activity (C4-A1, P1-T5 and OzPz-03), were recorded on

a Van Gough EEG machine together with the electro-oculogram, the electromyogram and respiration. 'Lights out' was between 23.00 h and midnight, and subjects were awoken if necessary at 07.00 h. The recordings were scored into 30 s epochs according to the criteria of Rechtschaffen & Kales (1968) by two analysts blind to the experimental condition.

Statistical analysis

The results of the performance tests were analysed initially by a mixed model analysis of variance (ANOVA) in which the fixed effects were drug at two levels (placebo and triazolam), days at two levels and times within day at 8 levels, and subjects was the random effect. A correction was made for drug order, which was not balanced over the subjects, by including it as a covariate in the analysis. To ensure that the assumptions of ANOVA (normality and homoscedacity) were not violated, transformations of the raw measures (Box & Cox, 1964) were considered, and the residuals were examined (Anscombe, 1961). A square root transformation was selected for the mental arithmetic test, but no transformation was necessary for the other variables. If the F -ratio for the drug by time interaction was significant ($P < 0.05$), differences between placebo and triazolam at the eight times within a single day were tested (Dunn, 1961). The standard error for comparisons was obtained as a composite of the appropriate error terms in the ANOVA table.

A separate analysis was carried out on performance decrements due to triazolam at times of blood sampling, using log blood concentration as a covariate. When the error terms for comparisons between means at the six times, corrected for blood concentration, were homogeneous, Tukey's T method (Kendall & Stuart, 1976) was used to test differences between the means. However, linear trends on

Figure 2 Plasma concentrations (ng/ml) of triazolam (Subject B) in the pharmacokinetic study. Closed and open circles indicate measurement by gas liquid chromatography and by radioreceptor techniques respectively. Arrows indicate insertion and removal of pump.

Figure 3 Plasma concentrations (ng/ml) of triazolam (Subject G) in the initial pharmacokinetic (●) and subsequent performance (▲) study. Closed and open triangles indicate measurement by gas liquid chromatography and by radio-receptor techniques respectively. Arrows indicate insertion and removal of pump.

day 1 and day 2 were tested as planned comparisons. All other tests between the means were suggested by inspection of the data and were made using Scheffe's S method (1959).

The sleep measures were analysed by a three factor ANOVA in which the fixed effects were drug at two levels and night at three levels, corresponding to before, during and after infusion. Transformations were selected by the above procedure, and where necessary means were back-transformed for presentation. If the *F*-ratio for the drug by time interaction was significant ($P < 0.05$) differences in sleep between triazolam and placebo on each of the 3 nights were tested by Dunn's method.

Results

Pharmacokinetics

With the osmotic pumps an almost model zero-order infusion curve with constant steady-state levels was obtained in three subjects, and an example of such a profile is shown in Figure 2. In the other subjects concentrations were more variable, but still relatively close to those desired

(Figure 3).

In the performance study it was intended to achieve concentration levels which would produce drowsiness without sleep. Since there were large interindividual differences in both clearance and apparent sensitivity, the dose administered was calculated for each individual, and with the aid of the clearance value the dose (pumping rate) was calculated. The desired and achieved plasma concentrations for the performance study are given in Table 1. In three subjects the concentrations were quite constant, whereas in two subjects there were rather large fluctuations. In a further two subjects concentrations were quite constant on both days, but they were higher by a factor of two on the second day. The reason for this difference is not clear, but it may be related to fluctuations in clearance or in rate of drug delivery.

When the infusion ceased concentrations followed linear elimination in all subjects, and the mean elimination half-life was calculated as 2.2 h with a range of 1.4–2.9 h. Similar results were obtained with gas chromatographic and radio-receptor methods, indicating that active metabolites were unlikely to contribute to the effects of the infusion of triazolam.

Table 1 Plasma concentrations (ng/ml) of triazolam during the performance study

Time after insertion of pump (h)	Subject						
	D	B	K	J	V	G	M
4.0	3.6	2.4	8.2	6.4	2.2	2.3	1.4
8.5	4.4	2.8	3.1	8.1	3.2	2.8	1.3
13.5	5.3	2.7	2.4	13.8	3.6	3.4	1.7
23.5	6.6	5.7	3.8	9.7	3.1	4.2	3.5
27.0	3.7	4.7	5.0	10.6	3.1	4.2	3.2
31.0	2.5	5.4	4.5	9.9	3.2	3.1	3.2
Desired value	3.5	3.6	6.0	10.0	3.5	4.5	4.5

Performance

Effects of triazolam on performance are given in Tables 2a and b and illustrated in Figures 4 and 5. Mental arithmetic and letter cancellations were impaired from 1 h (10.00 h), number of symbols copied was reduced from 2 h (11.00 h), and substitutions on digit symbol were reduced from 4 h (13.00 h) after the beginning of infusion. Performance on the logic test was not impaired until 10 h (19.00 h) after the infusion commenced. Impaired performance on these tests were at a similar level throughout the first day.

During the second day there were improvements in performance, but at the same time there were indications of changes in triazolam concentration: the log plasma concentrations obtained from the values in Table 1 when examined by analysis of variance showed some

time differences ($P = 0.082$), with peak concentration occurring after 23.5 h at the time of greatest impairment of performance. To allow for these differences, performance on the six occasions drug concentration data were available was corrected for triazolam by analysis of covariance (Table 3). With digit symbol substitution ($P < 0.05$), mental arithmetic and 2- and 6-letter cancellation ($P < 0.01$) performance improved from 08.00 h (23 h) to 15.00 (30 h). Six-letter cancellation at 15.00 h on day 2 was better than that of the mean of the assessments from 13.00 to 21.00 h on day 1 ($P < 0.05$). With removal of the pump performance at all tasks recovered quickly.

Performance at mental arithmetic and 2-letter cancellation correlated with blood levels ($P < 0.05$), as did digit symbol substitution, symbol copying and 6-letter cancellation ($P < 0.1$). Subjective assessments were reduced to three

Table 2a Effect of triazolam on various measures of performance during first day of infusion (means for subjects)

Measures	Time (h) of assessment							
	08.00	10.00	11.00	13.00	15.00	17.00	19.00	21.00
Digit symbol substitution	0.5	-19.4	-22.7	-42.1 ***	-37.5 **	-30.5 *	-34.5 **	-35.7 **
Symbol copying	5.2	-11.3	-30.4 *	-31.4 *	-29.0 *	-23.0	-34.0 **	-33.4 **
Mental arithmetic	-0.8	-11.3 *	-11.2 *	-16.7 **	-14.0 **	-12.8 *	-13.8 **	-15.3 **
Logic	4.0	-3.0	-5.3	-8.3	-9.5	-10.2	-12.1 *	-11.1 *
2-letter cancellation	-22.0	-96.4 *	-113.6 ***	-181.9 ***	-124.3 ***	-113.2 ***	-105.4 **	-137.6 ***
6-letter cancellation	-16.8	-230.5 ***	-329.4 ***	-357.5 ***	-315.8 ***	-269.7 ***	-285.5 ***	-291.0 ***

Table 2b Effect of triazolam on various measures of performance during second day of infusion (means for subjects)

Digit symbol substitution	-52.1 ***	-44.6 ***	-50.1 ***	-33.4 ***	-33.1 **	-14.0	-8.3	-7.3
Symbol copying	-44.5 ***	-41.1 ***	-42.3 ***	-34.0 **	-32.1 *	-19.3	-11.0	-5.4
Mental arithmetic	-20.7 ***	-22.0 ***	-20.5 ***	-17.0 **	-11.2 *	-13.0 *	-9.8	-3.2
Logic	-13.7 *	-13.4 *	-13.4 *	-13.8 *	-10.8	-8.0	-6.8	-5.2
2-letter cancellation	-207.0 ***	-177.2 ***	-170.6 ***	-96.2 *	-105.7 **	-21.1	-40.2	22.1
6-letter cancellation	-361.5 ***	-296.8 ***	-282.2 ***	-270.2 ***	-174.8 **	-103.0 *	-25.1	19.5

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

Figure 4 Number of substitutions in digit symbol test over the 2 day experiment with placebo (●) and with triazolam (■) (means for seven subjects) (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$). Arrows indicate insertion (09.00 h day 1) and removal (18.00 h day 2) of pump.

Figure 5 Number of cancellations on 6-letter cancellation test over the 2 day experiment with placebo (●) and with triazolam (■) (mean for seven subjects) (* $P < 0.05$; ** $P < 0.01$; $P < 0.001$). Arrows indicate insertion (09.00 h day 1) and removal (18.00 h day 2) of pump.

principal components (factors). The first factor related increased sleepiness and lethargy and alertness efficiency and ability to concentrate, the second to calmness and the third to less tenseness and irritability and increased sociability and happiness. Together they accounted for 75% of the variance.

Subjective sleepiness increased over the first day, and the subjects considered themselves sleepy on day 2, although there was a linear reduction in the assessment from 08.00 to 15.00 h.

Sleep

Sleep measures and sleep stages are given in Tables 4 and 5, and illustrated in Figure 6. There were no differences between baseline nights obtained before infusion of placebo and triazolam.

There was no increase in total sleep time during infusion of triazolam, but sleep onset was markedly shorter ($P < 0.001$) and the sleep efficiency index was improved ($P < 0.001$). The number of awakenings was also reduced ($P <$

Table 3 Effect of infusion of triazolam on performance measures (differences from placebo) (means corrected for blood level, $n = 617$)

Time after insertion of pump (h)	Digit symbol substitution	Symbol copying	Mental arithmetic	2-letter cancellation	6-letter cancellation
Day 1					
4.0	45.0	25.4	15.5	196.9	378.1
8.0	32.2	22.4	13.9	127.0	288.6
12.0	36.3	33.2	15.3	140.7	295.2
Day 2					
23.0	48.9	47.4	20.7	190.4	338.7
28.0	31.2	35.0	16.3	84.8	254.7
30.0	32.5	35.1	11.1	101.8	169.4

Significance levels; * $P < 0.05$; ** $P < 0.01$ **Table 4** Effect of infusion of solution and triazolam on various sleep measures (means for six subjects)

Measure	Transformation	During infusion		After infusion	
		Solution	Triazolam	Solution	Triazolam
Total sleep time (min)	$\sqrt{(480 - x)}$	371.3	414.3	378.9	317.2 *
Sleep onset latency (min)	$\log(x + 2)$	28.1	4.0 ***	25.2	42.5
Sleep efficiency index	$\log(1 - x)$	0.90	0.97 ***	0.89	0.74 *
REN/NREM ratio		0.28	0.12 ***	0.28	0.26
Latency (min) to stage 3		15.0	22.3	12.0	18.3
Latency (min) to stage REM	$\log x$	100.8	205.5 *	95.6	65.7
Number of awakenings	$\sqrt{x}$	8.5	2.0 ***	8.0	10.2

Significance levels for comparisons between triazolam and placebo * $P < 0.05$, *** $P < 0.001$
Solution used for infusion of triazolam was propylene glycol:water:ethanol (50:40:10 weight %).**Table 5** Effect of infusion of solution and triazolam on duration (min) of sleep stages over the whole night (means for six subjects)

Sleep stage	Transformation	During infusion		After infusion	
		Solution	Triazolam	Solution	Triazolam
Awake	$\log(x + 0.5)$	10.9	6.1 *	16.0	51.1 *
1	$\sqrt{x}$	26.8	13.7 *	26.3	43.7 *
2		196.8	307.7 ***	198.6	179.1
3 + 4		68.8	44.7 *	70.3	36.1 **
REM		79.2	45.2 **	83.1	70.2

Significance levels for comparisons between triazolam and placebo * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$
Solution used for infusion of triazolam was propylene glycol:water:ethanol (50:40:10 weight %).

Figure 6 Hypnograms of a subject from 'lights out'. (a) with placebo from 23.00 h the night before the experiment. (b) during infusion of triazolam from 23.00 h day 1 to 07.00 h day 2 and (c) after withdrawal of pump from 23.00 h day 2.

0.001), and the duration of awake activity and drowsy (stage 1) sleep were shortened ($P < 0.01$ and 0.05 respectively). There was less slow wave sleep ($P < 0.05$), and rapid eye movement (REM) sleep was delayed ($P < 0.05$) and reduced ($P < 0.01$). There was a compensatory increase in the duration of stage 2 sleep ($P < 0.001$).

Sleep during the recovery night after withdrawal of the pump was different from that after infusion of the placebo. Total sleep time and the sleep efficiency index were reduced ($P < 0.05$), and durations of awake activity and drowsy (stage 1) sleep were increased ($P < 0.05$). Slow wave sleep was reduced ($P < 0.01$), but there were no obvious changes in REM sleep.

Discussion

In the present study improvements in digit symbol substitution, mental arithmetic and letter cancellation were observed during the second day, and, despite a relatively constant plasma concentration, 6-letter cancellation was better during the second day at 15.00 h compared with the assessments between 13.00 and 21.00 h on day 1. These improvements could have been

dependent on relatively low levels of performance early during the morning of the second day. There were relatively high plasma levels of triazolam after overnight rest, while even minimal plasma levels may lead to a significant impairment of performance a short time after sleep or when superimposed upon the relatively low level of performance inherent in the circadian variation of performance. In previous studies with a rapidly eliminated diazepam (brotizolam) we observed impaired performance 9.5 h (09.00 h) after overnight ingestion, whereas, with the same dose, there was no change in performance 8 h (16.30 h) after early morning ingestion (Nicholson *et al.*, 1980).

It is well established that the oral ingestion of triazolam may lead to delay in the appearance of rapid eye movement (REM) sleep, though in doses used clinically this delay may not lead to a reduction in the duration of REM sleep over the whole night. However, the present studies show that when blood levels are maintained over the whole night delay in the appearance of REM sleep may be prolonged, and that suppression of REM sleep will continue. Further, that though clinical doses of triazolam may only reduce the number of delta waves (Johnson & Spinweber, 1981) continuous infusion will reduce slow wave

sleep to around two thirds of control values. These observations would suggest that the suppression of rapid eye movement and slow wave sleep by a benzodiazepine is likely to be related to plasma concentrations.

During the night after infusion there were marked changes in sleep. Sleep onset latency was not altered, but slow wave sleep was reduced, and this change was likely to be related to the early part of the night when residual levels of triazolam persisted. However, total sleep time and the sleep efficiency index were reduced and awake and drowsy sleep increased. Such increased levels of wakefulness would be expected after a prolonged period of sedation with a relatively high plasma level of a benzodiazepine, though it is of interest that there were no obvious changes in REM sleep during the withdrawal night.

In summary, the effect of a continuous osmotic infusion of triazolam was sustained over the first day and over the whole of the first night, but there was an improvement in performance during the next day. Performance during the infusion may have been influenced by circadian variation in performance, but even so the possibility that sustained levels may be accompanied by some tolerance cannot be excluded.

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