Pharmacokinetics and pharmacodynamics following single and repeated nightly administrations of loprazolam, a new benzodiazepine hypnotic

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1  The pharmacokinetics of oral loprazolam and pharmacodynamic responses on the morning following nightly drug administration were examined after single and after seven consecutive 1 mg doses in six non-fasting healthy subjects.
2  The serum concentration-time profiles for unchanged loprazolam measured by a specific high pressure liquid chromatography/gas chromatography (h.p.l.c./g.c.) method and for benzodiazepine material measured by radioimmunoassay (RIA) were qualitatively similar although RIA levels were consistently higher.
3  Approximate elimination half-life of unchanged loprazolam after single doses was 8.0 h. For RIA measured material, approximate half-life was 11.7 h following acute administration and 12.8 h after seven consecutive doses.
4  Compared to results after single doses, maximum serum concentration and AUC were greater following 1 week's treatment. Using RIA results, the increases were 27.2% (95% CL 6.9 to 47.4%) and 35.1% (95% CL 15.8 to 54.3%) respectively and using h.p.l.c./g.c. data, 11% (95% CL − 22.6% to 44.5%) and 41% (95% CL − 50.9 to 133.0%).
5  After repeated doses of loprazolam, there were no significant changes with respect to results after single doses in psychomotor function assessed objectively by critical flicker fusion threshold or choice reaction time, or in sleep quality or behaviour on awakening assessed subjectively by 10 cm analogue scales.
6  Mean time to maximum serum concentration was 4.95 h with considerable interindividual variability (range 1–12 h) and there was a lag time of 1–1.5 h before drug was detectable in blood.
7  Thus, although the elimination half-life of loprazolam suggests a short to intermediate duration of action, after night time administration following food, the present formulation exhibits slow and irregular absorption, and appreciable serum concentrations persist the next day. On repeated doses there is evidence of slight accumulation. It is postulated that this benzodiazepine has the pharmacokinetic characteristics of a hypnotic with anxiolytic properties the following day.

Keywords benzodiazepine hypnotic loprazolam pharmacodynamics pharmacokinetics

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Introduction

Loprazolam (Dormonoct, Roussel Laboratories) is a new water soluble 1,4 benzodiazepine with hypnotic properties in man (Boyd & Ankier, 1983; Hindmarch & Clyde, 1980; Salkind & Silverstone, 1983). Therapy with a hypnotic calls for an approach which differs fundamentally from that where a constant drug effect is required. An intermittent action restricted to the night and avoiding residual effects during the day is desirable. Since these properties are at least to some extent related to circulating active drug, appreciable levels of drug or metabolite should be present during the period of intended sleep but little should persist on awakening, necessitating an agent which displays rapid absorption and elimination. Furthermore, a hypnotic should show minimal accumulation on repeated night time use.

This study was carried out to examine the pharmacokinetics of oral loprazolam at the usual clinical dose (1 mg) in healthy subjects after single doses and following repeated administrations for 1 week. In addition, we investigated the degree of accumulation of loprazolam and attempted to determine whether this was of pharmacodynamic significance. To provide information analogous to clinical circumstances, on each occasion the drug was administered at night after an evening meal.

Methods

Subjects

Six healthy males, age 22–37 years, weight 60.3–91.4 kg, none of whom smoked tobacco, participated in the study. The subjects gave written consent for the procedure after full explanation of its purposes and risks. The protocol was approved by the hospital ethics committee.

Study design

The study was carried out in two phases consisting of (a) single doses and (b) repeated doses of loprazolam administered nightly for 7 consecutive nights. The subjects were allocated randomly to the phases, each subject taking each treatment regimen in a balanced, crossover fashion. The treatments were separated by a 2 week drug free interval.

Drugs

Loprazolam was administered as 1 mg tablets. Other drugs, including alcohol, were not permitted from 24 h before each treatment until after completion of each study phase.

Procedure

Loprazolam 1 mg was taken with water (150 ml) at 21.30 h, 2 h after an evening meal and thereafter the subjects refrained from driving or operating machinery until the next morning. The subjects were admitted to hospital overnight on the day of administration of the single dose and on day 7 of repeated dose administration. On these occasions the evening meal was consumed at a constant time (19.30 h); subjects were allowed free choice of meal in the first phase and this was reproduced exactly in the second. The volunteers remained supine until 10 h after treatment, at which time a light breakfast was consumed. Thereafter normal activities were resumed.

On study days, venous blood samples were taken immediately before treatment (0) and at 0.25, 0.5, 0.75, 1.0, 1.5, 2.0, 3.0, 4.0, 6.0, 8.0, 12.0, 24.0, 36.0 and 48.0 h after treatment. Serum was separated as soon as possible and stored at -20°C until analysis. Psychomotor testing, and subjective assessments of sleep and early morning behaviour were made 12 h before drug administration (12 h before the first dose of the repeated dosing regimen), and 12 and 36 h after drug administration (12 and 36 h after the seventh consecutive dose of the repeated dosing regimen). Volunteered and inquired adverse reactions were recorded 12 h and 18 h after each dose of loprazolam.

Analysis

Drug concentrations Serum loprazolam concentrations were assayed using a specific technique involving the separation of loprazolam from other benzodiazepine material using high pressure liquid chromatography (h.p.l.c.) followed by gas chromatography (g.c.) as described by Stevens et al. (1983). Duplicate samples were also analysed by a radioimmunoassay (RIA) method. The RIA was developed by raising antibodies against conjugates of the drug to the carrier protein bovine serum albumin. The coefficient of variation of this method is 8–13% over the assay range with between batch variation of 5–14%. Although relatively specific for loprazolam, the RIA may show cross-reactivity with a number of putative metabolites including the piperazine N-oxide of loprazolam (156%), desmethyl loprazolam (75%) and 4-hydroxy loprazolam (1160%). Both methods are
sensitive to levels of approximately 1 ng ml⁻¹, and determinations less than this were taken to be below the limit of detection of the method used.

**Pharmacokinetic parameters** Maximum serum concentration (Cₘₓ) and the time taken to achieve maximum serum concentration (tₘₓ) were measured directly. Where possible, the serum levels of loprazolam were fitted to a sum of exponentials as shown in the general equation 1. This was achieved using an iterative least squares curve-fitting programme written for a Hewlett Packard 1000 series minicomputer (Ings et al., 1980). The initial estimates were obtained automatically using a ‘feathering’ technique and reciprocal weighting factors were used where appropriate.

\[
C_i = \sum_{i=1}^{n} C_i e^{-\lambda_i t_i}
\]

where \(C_i\) = serum concentration

\(t_i\) = time

\(C_i\) = coefficient of the ith term

\(\lambda_i\) = exponential constant of the ith term

For the input phases of the profiles (absorption) the exponential term became negative.

The half-life (tₜ) for each declining phase was calculated from equation 2.

\[
t_{1/2} = \frac{0.693}{\lambda_i}
\]

Where curve-fitting was impossible an estimate of tₜ was obtained by linear regression of the post-peak serum levels with time. In those cases where the serum concentration fell below the limit of detection of the assay and increased subsequently, a value for tₜ could not be obtained. The area under the serum concentration-time curve for one dosing interval (AUC₂₄) was calculated using the trapezoidal rule (Yeh & Kwan, 1978). Where 24 h Cₘᵢ was below the minimum level of detection of the analytical method and curve-fitting was possible, 24 h Cₘᵢ was estimated using equation 1. The AUC between the last measurable time point and 24 h was then determined by the trapezoidal rule. Where curve-fitting was not possible, AUC to the last measurable time point (AUC₁) was calculated again using the trapezoidal rule.

The ratios of both AUC₂₄ (or AUC₁) and Cₘₓ after repeated doses to the corresponding values after single doses have been taken as a measure of the accumulation of loprazolam during repetitive dosing. The ratios have been expressed as percentage increases (i.e. ratio \(-1 \times 100\)).

**Psychomotor testing** After pre-experiment training to preclude learning, psychomotor function was assessed by critical flicker fusion threshold (CFFT) and choice reaction time (CRT) using the Leeds Psychomotor box (Parkway Electronics, Leeds). These methods which are objective measures of arousal and central nervous system integration of discrete units of sensory data have been described by Hindmarsh (1979). CFFT was detected using ascending and descending scales in a set of four light emitting diodes in foveal fixation at a distance of 1 m. The threshold measured was the mean of six values on each occasion. CRT was estimated using an automated test in which one of five lights was illuminated at random and the subject responded by pressing the appropriate button to extinguish the stimulus light. The response measured was the mean time to extinguish 30 stimulus presentations.

**Subjective assessment** Subjective assessments of sleep quality and early morning behaviour (residual or hangover effects) were made using a series of 10 cm line analogue scales. The aspects of sleep and early morning behaviour assessed were: ease of getting to sleep (sleep latency); perceived sleep quality (restfulness, wakefulness, depth of sleep); integrity of behaviour on awakening (alertness, balance, clearheadedness, co-ordination). In all cases, these scales were designed such that high scores represented beneficial effects, i.e. improved quality of sleep and lack of residual effects.

**Statistics** Comparisons of the pharmacokinetic parameters after single and multiple doses of loprazolam were made using analysis of variance allowing for subject, phase and treatment effects (Cochran & Cox, 1957). In addition 95% confidence limits (CL) were calculated to determine the precision of the comparisons. The RIA and h.p.l.c./g.c. derived pharmacokinetic data were compared by Student’s t-tests for paired observations. The results of the objective psychomotor tests and the subjective ratings of sleep quality and residual effects 12 h before and 12 and 36 h after single doses were compared with those at equivalent times before and after repeated doses again using Student’s t-tests for paired observations, with Bonferroni correction for multiple comparisons (Miller, 1966). Since, on study nights subjects were admitted to hospital and underwent pharmacokinetic investigation, comparison of pharmacodynamic parameters after loprazolam with those before loprazolam was not valid.
Results

Pharmacokinetics

Mean serum loprazolam concentrations derived from h.p.l.c./g.c. and by RIA after single and repeated doses are presented in Table 1 and the concentration vs time relationships are illustrated in Figure 1. Derived pharmacokinetic parameters are given in Table 2.

H.p.l.c./g.c. analysis For maximum serum concentration and mean time to reach maximum serum concentration, the results were similar after single and repeated doses. There was marked intersubject variability particularly in the latter parameter (3–12 h on acute administration and 1–8 h on chronic dosing). Mean elimination half-life for loprazolam after single doses was 8 h and the estimate following seven consecutive administrations was 3.5 h. Half-lives could be calculated in only five subjects after single doses and in three subjects after repeated doses, however, making comparisons between the different conditions of the study unreliable. Since curve-fitting was not generally possible, AUC$_{24}$ could not be estimated in those cases where serum levels had declined below the level of detection within 24 h of dosing. Where curve-fitting was possible, AUC$_{\infty}$ was only marginally greater than AUC$_{t}$ indicating that the additional area was unlikely to represent a major portion of total AUC. The mean AUC$_{t}$ after repeated dosing (50.0 ng ml$^{-1}$ h) was greater than AUC$_{t}$ after single doses (35.5 ng ml$^{-1}$ h) but the difference was not statistically significant. Estimated accumulation for maximum serum concentration was 11% (95% CL – 22.6 to 44.5%) and for AUC$_{t}$ was 41% (95% CL – 50.9 to 133.0%).

RIA analysis Curves were fitted satisfactorily to a sum of exponentials using a first order absorption and first order elimination term. Mean peak serum concentration was greater after repeated doses than after single doses and the difference reached the level of statistical significance ($P < 0.05$). There was no significant difference between the treatment regimens with respect to the time to reach maximum serum concentration and once again there was considerable interindividual variability (2–8 h on acute dosing and 2–12 h following repetitive administrations). Mean elimination half-lives were 11.7 h (single doses) and 12.8 h (repeated doses). There was a statistically significant increase in AUC$_{24}$ between single and repeated administrations ($P < 0.01$). Estimated accumulation assessed by the increase in maximum serum concentration was 27.2% (95% CL 6.9 to 47.4%) and using the increment in AUC$_{24}$ was 35.1% (95% CL 15.8 to 54.3%).

Comparison of h.p.l.c./g.c. and RIA analyses No important differences were observed with respect to maximum serum concentrations and the time taken to reach these levels. RIA estimated AUC and half-lives were considerably greater and the increments were statistically significant ($P < 0.05$) after repeated doses. These differences are of uncertain con-

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>H.p.l.c./g.c.</th>
<th>RIA</th>
<th></th>
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<tr>
<td></td>
<td>Single doses</td>
<td>Repeated doses</td>
<td>Single doses</td>
</tr>
<tr>
<td>0</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>0.25</td>
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<td>ND</td>
<td>ND</td>
</tr>
<tr>
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<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>0.75</td>
<td>ND</td>
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</tr>
<tr>
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<tr>
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</tr>
<tr>
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<td>3.1 ± 1.41</td>
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</tr>
<tr>
<td>8.0</td>
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<td>2.6 ± 1.93</td>
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<tr>
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<tr>
<td>48.0</td>
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</table>

ND, below detection limit of the essay. In calculating mean values, individual results < 1 ng ml$^{-1}$ were taken as zero.
Figure 1  Mean serum loprazolam concentration-time profiles after single (---) and seven repeated (-----) doses in six healthy subjects using results from h.p.l.c./g.c. and RIA analyses. Loprazolam 1 mg administered at time zero. Standard deviations omitted for clarity (see Table 1).

Table 2  Mean ± s.d. results for pharmacokinetic parameters in six healthy subjects after single and repeated doses of loprazolam 1 mg. Drug concentrations measured by h.p.l.c./g.c. and RIA

<table>
<thead>
<tr>
<th>Parameter</th>
<th>H.p.l.c./g.c.</th>
<th>RIA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Single doses</td>
<td>Repeated doses</td>
</tr>
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<td>$C_{\text{max}}$ (ng ml$^{-1}$)</td>
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<td>4.6 ± 2.07</td>
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<td>$t_{\text{max}}$ (h)</td>
<td>5.0 ± 3.63</td>
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<td>$t_{\text{c}}$ (h)</td>
<td>8.0 ± 3.35†</td>
<td>3.3 ± 3.6‡</td>
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<tr>
<td>AUC (ng ml$^{-1}$ h)#</td>
<td>35.5 ± 22.26</td>
<td>50.0 ± 26.89</td>
</tr>
</tbody>
</table>

*P < 0.05, **P < 0.01 vs single doses in analysis of variance †n = 5, ‡n = 3

#AUC to last measurable time point for h.p.l.c./g.c. results and AUC during one dosage interval for RIA results

sequence, however, due to the difficulties with curve-fitting encountered using information derived from the h.p.l.c./g.c. assay. The extent of accumulation estimated by data from each analytical method was not significantly different as can be seen by the overlap in the 95% CL of the increases in both maximum serum concentration and AUC.

Side effects and laboratory screens

Adverse reactions unrelated to the hypnotic properties of the drug were not encountered. There were no clinically significant abnormalities in laboratory screens at the end of either treatment regimen.

Psychomotor tests and subjective assessments of sleep quality and integrity of behaviour on awakening

These results are shown in Table 3. At no time point was there a significant difference between single and repeated doses for any of the parameters examined ($P > 0.05$ in all cases). Compared to single doses, following repeated doses there was a consistent trend for values to be higher 12 h after drug administration. These findings suggest that repeated nightly administrations of loprazolam are not associated with deterioration in sleep quality, or increased adverse residual effects whether assessed by objective tests or subjectively.

Discussion

The serum loprazolam profiles derived from h.p.l.c./g.c. and RIA data were qualitatively similar after both single and repeated doses. Blood levels developed slowly, there was no indication of a distinct distribution phase and the post-peak serum concentration-time curves were complex suggesting that each calculated terminal half-life was a hybrid of at least two elimination phases. Half-lives were estimated from serum concentrations at relatively few time points over a period equivalent to between one and two half-lives and thus must be considered approximations. The lower limit of sensitivity of both assays (1 ng ml$^{-1}$) was quite high in relation to the mean peak serum concentrations (4.0–5.1
These factors undoubtedly contributed to the difficulties experienced in defining pharmacokinetic parameters.

Serum drug levels measured by RIA were consistently higher than those of unchanged loprazolam as assessed by chromatography and the differences were particularly marked at late time points where the RIA method may be subject to interference. Since this assay is specific for loprazolam-like benzodiazepines, it must be concluded that the distortion of the serum profiles compared to those of the parent drug represents benzodiazepine structure(s) which are presumably metabolite(s) of loprazolam. The major biotransformation product in man is the piperazine N-oxide (Illing & McLean, unpublished data) which occurs in appreciable concentrations (approximately 50% of those of unchanged loprazolam) and has an elimination half-life of the same order as the parent drug but the time course of its appearance would tend to accentuate levels measured by RIA during the terminal elimination phase of loprazolam. When the RIA method is used to assess loprazolam concentrations, the appearance of this or other metabolites could lead to spurious prolongation of half-life and exaggerate apparent accumulation. However, since the piperazine N-oxide of loprazolam may have pharmacological activity approaching that of the parent drug (Miller, unpublished data), its presence in biological fluid must be considered in the evaluation of this benzodiazepine. Compared to loprazolam levels after single doses, the maximum serum concentrations and AUC were increased after seven consecutive nightly doses. For RIA data, the increments were statistically significant and estimated accumulation was approximately 30%. The increased serum levels after repeated doses of loprazolam were not reflected in changes in the objective assessment of psychomotor function, or in subjective assessment of sleep quality or residual effects on performance. However, these evaluations were not carried out until 12 h after drug administration. Thus the results of this study do not preclude the possibility that pharmacokinetic accumulation of loprazolam or its metabolites may be associated with increased unwanted 'hangover' effects on the morning following night time use.

Our findings provide evidence that the absorption of loprazolam can be slow. Mean time to peak serum concentration was considerably greater than that usually quoted for benzodiazepines used as hypnotics (Breimer, 1979;
concentration-effect relationships marked lag time, and Curry & Whelpton, 1979; Greenblatt et al., 1981; Kangas & Breimer, 1981) and there was a marked lag time, on average 1–1.5 h after drug administration, before loprazolam was detectable in serum. Although onset of activity depends on concentration-effect relationships which are unknown for loprazolam, rate of absorption is usually the rate limiting factor in the transfer of drug molecules to their sites of action in the central nervous system, after oral dosing (Curry & Whelpton, 1979). Thus loprazolam is unlikely to have a rapid pharmacological effect. In this study, however, the hypnotic was administered at night after food, which is the usual clinical practice, rather than in the morning after an overnight fast which is the rule in pharmacokinetic studies. Diurnal variability in the absorption of benzodiazepines has been reported (Chamberlain et al., 1981) and this may lead to delayed peak levels when the drug is taken at night. Furthermore, secondary peaks of unchanged loprazolam and RIA measured material occurred frequently. Similar observations have been made with loprazolam (Stevens et al., 1983) and other benzodiazepines (Breimer et al., 1977; Kangas & Breimer, 1981; Korttila & Kangas, 1977) and may relate to ingestion of food. Where secondary peaks represent the maximum serum concentrations of the drug, they may have an important influence on the assessment of absorption, but whether they denote pharmacologically active drug remains to be established. Until comparisons with other benzodiazepine hypnotics after night time dosing and food are available, it is premature to ascribe undue importance to the rate of absorption and pharmacokinetic profiles of loprazolam in the circumstances of this study.

Loprazolam 1 mg satisfies certain pharmacokinetic requirements for a useful hypnotic agent. It is eliminated relatively rapidly and there is trivial accumulation after repeated nightly doses. Our half-life estimates place loprazolam in the category of benzodiazepines expected to have short-intermediate duration of action (Greenblatt et al., 1981). However, the slow development of peak serum levels and the absence of a marked distribution phase leading to appreciable serum drug concentrations 12 h after ingestion support the conclusion that loprazolam 1 mg may have a persistent effect on performance on the day following night time administration (Nicholson & Stone, 1983). Amrein et al. (1983) have attempted to determine the potential use of benzodiazepines on the basis of their pharmacokinetic profile using the residual fraction—the quotient of drug concentration at 12 h after oral intake to maximum drug concentration. In the study reported here, residual fraction for unchanged loprazolam after single doses was 0.44 and after repeated doses 0.47. Inspection of the results of another study of the pharmacokinetics of unchanged loprazolam (Stevens et al., 1983) allows the calculation of similar residual quotients. Furthermore, in clinical use, loprazolam taken at night is associated with a mean decrease in daytime anxiety (Morgan & Oswald, 1982). These observations suggest a potential role for loprazolam as a hypnotic with anxiolytic properties the next day.

There were marked interindividual differences in absorption rate of loprazolam as assessed by time to peak concentration and maximum serum concentration achieved. Biopharmaceutical factors governing the rate and extent of absorption are important in this respect (Breimer, 1979). It is not yet determined whether the irregularities and variability in loprazolam absorption are due to the physico-chemical characteristics of loprazolam or to its pharmaceutical formulation but such diversity in pharmacokinetic factors may have important consequences in terms of intersubject variability in onset and extent of drug action.

In conclusion, loprazolam is absorbed slowly after ingestion at night following food and although its elimination half-life suggests a short to intermediate duration of action appreciable drug concentrations persist the next day. On repeated doses, there is a minor degree of pharmacokinetic accumulation. It would appear that the principal role of loprazolam may be in the management of disturbed sleep secondary to daytime anxiety.

References


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