J. Pharm. Pharmacol. 1986, 38: 578–582
Received November 25, 1985

Plasma concentrations and pharmacokinetics of midazolam during anaesthesia

P. CREVAT-PISANO, S. DRAGNA, C. GRANTHIL*, P. COASSOLO, J. P. CANO† AND G. FRANCOIS

*INSERM U 278, Faculté de Pharmacie, 27 Boulevard Jean Moulin, 13385 Marseille, and *Département d'Anesthésie-Réanimation, Chu Timone, Marseille, France*

Midazolam and 1-hydroxymidazolam plasma concentrations have been monitored and pharmacokinetic parameters of midazolam estimated during anaesthesia induced and Maintained by its repeated injection according to two protocols ($3 \times 0{\cdot}3$ mg kg^{-1} at 45 min intervals or an induction dose of 0·3 mg kg^{-1} with maintenance doses of 0·15 mg kg^{-1} at 30 min intervals). Minimum plasma concentrations of midazolam measured just before each injection were $258{\cdot}8 \pm 108{\cdot}4$ ng ml^{-1} for the first protocol and $353{\cdot}1 \pm 55{\cdot}2$ ng ml^{-1} for the second protocol; maximum midazolam concentrations, measured 5 min after the last administration, were $1103{\cdot}1 \pm 237{\cdot}9$ ng ml^{-1} and $743{\cdot}0 \pm 103{\cdot}2$ ng ml^{-1}, respectively, suggesting that a continuous infusion of midazolam after a loading dose should be better than repeated injections at keeping the concentration close to the sedative level of 400 ng ml^{-1}. The estimated pharmacokinetic parameters were similar to those already published, except for the β elimination half-life of midazolam ($3{\cdot}24 \pm 0{\cdot}90$ h for protocol 1 and $3{\cdot}34 \pm 1{\cdot}47$ h for protocol 2) which was slightly longer than that reported for single dose studies. The comparison of plasma determinations, obtained either by gas-liquid chromatography or by a radioreceptor assay technique, clearly showed that 1-hydroxymidazolam, even after repeated midazolam administration, was not present at a concentration sufficient to affect the overall pharmacological activity of the parent drug.

Midazolam (Ro 21-3981) is a new imidazobenzodiazepine used as an induction agent for anaesthesia (Baber et al 1982; Forster et al 1980; Fragen et al 1978; Melvin et al 1982; Reves et al 1978, 1979). It has the characteristic properties of benzodiazepines, i.e. sleep inducing, sedative, anxiolytic, anticonvulsant and muscle relaxant effects coupled with a short elimination half-life of about 2 h (Kanto & Klotz 1982; Smith et al 1981) and the water-solubility of its salts (maleinate).

The drug is metabolized in the liver to 1-hydroxy-, 4-hydroxy- and 1,4-dihydroxy-midazolam, which are then mainly glucuronidated before renal elimination (Heizmann & Ziegler 1981). The main metabolite is 1-hydroxymidazolam which is pharmacologically active but markedly less so than the parent drug (Heizmann et al 1983), and has an elimination half-life of about 0·8 h measured during single dose studies (unpublished results).

We set out to monitor midazolam and 1-hydroxymidazolam plasma concentrations, and to estimate pharmacokinetic parameters of the drug during anaesthesia induced and maintained by repeated injections. Plasma concentration of the drug and of its main metabolite were determined selectively using gas-liquid chromatography (GLC). The 'benzodiazepine activity' was also estimated using a radioreceptor assay technique (RRA) and the results obtained with the two analytical methods were correlated.

† Correspondence.

MATERIALS AND METHODS

Patients

Eight patients, 5 males, 3 females, aged 17 to 62 years, 61–73 kg, were anaesthetized for maxillo-facial surgery over at least 1·5 h. Exclusion criteria were emergency procedures, benzodiazepine intolerance, pregnancy, myasthenia and chronic or occasional benzodiazepine treatment.

Experimental procedure

The recommended induction dose of midazolam is 0·2 mg kg^{-1} (Reves et al 1981) corresponding to an induction time of 80 s.

In the present work, midazolam was administered according to two protocols. In protocol 1, four patients received 3 injections of 0·3 mg kg^{-1} at 45 min intervals. In protocol 2, four other patients received a loading dose of 0·3 mg kg^{-1}, and then three maintenance doses of 0·15 mg kg^{-1} at 30 min intervals. The loading dose of 0·3 mg kg^{-1} was established in a previous study. Blood samples were

collected just before each injection and 5, 15, 30 min, 1, 2, 4, 6, 8, 12, 24 h after the last injection. In addition, all patients received rectal pentobarbitone for premedication and a fentanyl-nitrous oxide/oxygen mixture for induction and maintenance of anaesthesia. Three patients also received enflurane because the surgical procedure had been extended.

Analytical procedure

Midazolam and 1-hydroxymidazolam plasma concentrations were measured using an RRA technique (Dorow et al 1982; Hunt et al 1979; Lund et al 1981). Synaptic membranes were prepared as described by Hunt et al and homogenized in ice cold Tris-HCl pH 7·4. Active material (midazolam and 1-hydroxymidazolam) was extracted from plasma using diethyl ether at pH 13. An aliquot of the organic layer was evaporated to dryness and the residue incubated, 25 min at 4 °C, with 400 µl of the membrane suspension (approx. 0·25 mg protein) and 100 µl of [^{3}H]flunitrazepam (approx. 0·5 pmol). The incubation was stopped by addition of 5 ml ice cold buffer (Tris-HCl pH 7·4). Bound radioactivity was isolated by filtration under vacuum through Whatman GF/B filters which were washed with ice cold buffer and placed into 10 ml Ready-Solv EP (Beckman). Bound radioactivity was determined using a liquid scintillation counter. The results, after correction for non-specifically bound radioactivity (as determined by incubation in the presence of 10^{-6} µM unlabelled diazepam), were expressed as 'midazolam equivalents' using a daily 'logit-log' linearized standard curve. The concentration range for this standard curve was selected to cover a 20–80% inhibition of specific [^{3}H]flunitrazepam binding. Quality controls containing only midazolam were analysed together with the unknowns to assess the validity of the RRA technique.

Plasma concentrations of midazolam and its 1-hydroxy derivative were also measured by capillary GLC with electron capture detection (Coassolo et al 1982). After extraction of 0·5 ml plasma at pH 9 with 0·4 ml butyl acetate, the organic layer was evaporated to dryness in 1·0 ml minivial under a stream of nitrogen. The residue was then dissolved in 100 µl acetonitrile—10 µl BSTFA (*N,O*-bis(trimethylsilyl)-trifluoroacetamide) for 1-hydroxymidazolam silylation. After evaporation to dryness, the residue was dissolved in toluene before GLC analysis on a glass WSCOT (25 m × 0·5 i.d.) capillary column coated with CP-Sil 5 as stationary phase. In these conditions, the retention times of midazolam and 1-hydroxymidazolam at an oven temperature of 280 °C were 3·0 and 4·2 min, respectively.

Quality control samples based on midazolam and 1-hydroxymidazolam were analysed together daily with the unknowns to assess the assay validity.

Data analysis

Estimation of the concentrations (nM) of midazolam and 1-hydroxymidazolam, which reduced the specific [^{3}H]flunitrazepam binding by 50% (EC50), were considered to correlate RRA and GLC results obtained as follows:

Benzodiazepine activity in midazolam units as determined by RRA =

$$\text{GLC midazolam concn} + \frac{\text{EC50 midazolam}}{\text{EC50 1-OHmidazolam}} \times (\text{GLC 1-OHmidazolam concn})$$

Pharmacokinetic model. Plasma kinetics were analysed using a linear open two compartmental model. Pharmacokinetic parameters were derived by fitting multiple dose data to the appropriate equations and weighting each data point by the reciprocal of the squared concentrations.

Statistical analysis. The results obtained with RRA and GLC, and the pharmacokinetic parameters calculated during the two protocols, were analysed statistically using Student's *t*-test.

Clinical monitoring. During anaesthesia, all patients were constantly monitored. Arousal from anaesthesia was evaluated using Aldrete's score. Retrograde amnesia was estimated using a memory test performed simultaneously with each sample collection.

RESULTS

Method evaluation

RRA. The extraction recoveries of midazolam and of 1-hydroxymidazolam from plasma were greater than 80%. These percentages were calculated from the (EC50 value after extraction from spiked plasmas)/(EC50 value without extraction) ratios for each compound. The reproducibility of the method was ±6% for midazolam plasma concentrations of 50 ng ml^{-1}. The sensitivity limit was 0·5 ng ml^{-1} in the final incubation volume. The linearity of the technique was good for midazolam concentrations ranging from 0·71 to 7·13 ng ml^{-1} in the final incubation volume.

GLC. The linearity of plasma determination proved satisfactory for midazolam concentrations ranging from 4 to 400 ng ml^{-1} and for 1-hydroxymidazolam concentrations of 3 to 70 ng ml^{-1}. The reproducibility was better than ±10% for midazolam and 1-hydroxymidazolam concentrations of about 4 ng ml^{-1}. The limit of detection was estimated at 1–2 ng ml^{-1} for each compound. The extraction recovery for midazolam and its metabolite was better than 70%.

EC50 values. RRA–GLC correlation

The EC50 values of midazolam and 1-hydroxymidazolam were, respectively, 1·74 and 5·71 nM. The correlation between the RRA and GLC techniques, based on 24 samples, is r = 0·981. The equation of the linear regression curve was Y(RRA) = 0·925 X (GLC) + 13·4, where X = (midazolam concentration) + 0·305 × (1-hydroxymidazolam concentration).

Plasma values of RRA active material and of corrected GLC concentrations were virtually superimposable in all patients; as indicated by the Student's *t*-test ($P < 0·05$), there was no significant difference between the two groups of values. Moreover, correlation between RRA active material and unchanged midazolam determined by GLC was also satisfactory (r = 0·991). Thus 1-hydroxymidazolam did not affect the overall activity of the parent compound.

Plasma levels

The mean of minimum (C_{min} measured just before the second injection) and maximum (C_{max} measured 5 min after the last injection) concentrations of midazolam determined by GLC and RRA are presented in Table 1. For 1-hydroxymidazolam, maximum plasma values during protocol 1 (81 ± 24·5 ng ml^{-1} i.e. 10% of the midazolam maximum values) were obtained 15 min after the last injection. The fourth patient, a chronic alcoholic, was excluded from this estimation because of high levels of 1-hydroxymidazolam compared with the other subjects. During protocol 2, there was a large intersubject variability in 1-hydroxymidazolam concentration, maximum plasma levels ranging from 58·6 to 108·9 ng ml^{-1} and being reached during surgical procedure in one case and 15 min after the last injection in the other cases. In all the patients, concentrations of the metabolite decreased at least as rapidly as those of midazolam post injection. Fig. 1a, b show the plasma concentrations of RRA active material, midazolam and 1-hydroxymidazolam during the two protocols.

Table 1. Mean of minimal (C_{min}) and maximal (C_{max}) plasma concentrations of RRA active material and midazolam determined by GLC during the two protocols.

Concentrations (ng ml^{-1})		P1 (n = 4)	P2 (n = 4)
C_{min}	Midazolam (GLC)	258·8 ± 108·4	353·1 ± 55·2
	Active material (RRA)	238·4 ± 98·8	379·9 ± 72·9
C_{max}	Midazolam (GLC)	1103·1 ± 237·9	743 ± 103·2
	Active material (RRA)	1157·6 ± 322·8	801·8 ± 86·7

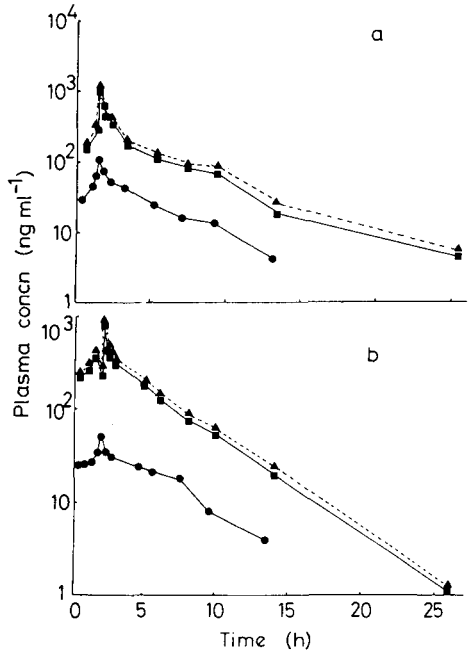

FIG. 1a. Mean plasma levels from the 4 patients on protocol 1 of RRA active material, midazolam and 1-hydroxymidazolam determined by GLC. b: Plasma levels of RRA active material, midazolam and 1-hydroxymidazolam determined using GLC, in one of the patients on protocol 2. Key: (▲), RRA; (■), GLC midazolam; (●), GLC 1-hydroxymidazolam.

Pharmacokinetic parameters

The main pharmacokinetic parameters of RRA active material and of midazolam determined by GLC are presented in Table 2. Student's *t*-test ($P < 0·05$) indicated that there was no significant difference between the two groups of values (protocols 1 and 2). The 1-hydroxylated metabolite was not considered for this data analysis because of its low plasma levels, its EC50 value being higher than that of midazolam and its short elimination half-life.

Table 2. Mean values ± s.e. of the main pharmacokinetic parameters of active material (RRA) and midazolam (GLC) from patients on protocols 1 and 2.

Analysis	Total distribution volume (litre)	Clearance (litre h^{-1})	Elimination half-life (h)
Protocol 1 RRA	78·7 ± 6·8 (70·8–82·9)	24·5 ± 4·2 (19·8–28)	2·95 ± 0·99 (2·04–4·02)
Protocol 1 GLC	74·1 ± 18·6 (70–98)	23·7 ± 4·4 (17·4–31·1)	3·24 ± 0·9 (2·14–4·20)
Protocol 2 RRA	70·7 ± 4 (65–71)	25·5 ± 5·6 (20–33·2)	3·30 ± 0·92 (2·3–4·5)
Protocol 2 GLC	73·1 ± 5·4 (68–80)	27·9 ± 6·4 (21·5–36·6)	3·34 ± 1·47 (2·3–5·5)

Clinical results

The quality of induction was excellent but the intersubject variability was large during maintenance. The local and general tolerance to midazolam was good. Retrograde amnesia, sometimes reported with midazolam (Reves et al 1983) was not observed. Arousal from anaesthesia occurred 2–4 h after the last injection and mean plasma concentration of midazolam at that time was 131·2 ± 4·0 ng ml^{-1} (n = 5).

DISCUSSION

Correlation RRA–GLC

The correlation between the two techniques when respective EC50 values of midazolam and its metabolite were considered was acceptable (r = 0·981). The ratio EC50 midazolam/EC50 1-hydroxymidazolam was slightly lower than that previously reported (Jochemsen et al 1983) but is in good agreement with the data reported by Ziegler et al (1983), thus indicating that the 1-hydroxy metabolite had a less potent effect and a duration of action about half that of the parent compound. The equally good correlation between RRA and unchanged midazolam (GLC) levels proved that 1-hydroxymidazolam, which was extracted as well as midazolam during RRA or GLC, is present in amounts unlikely to affect the pharmacological activity of the parent drug.

Plasma levels

According to a previous study (Crevoisier et al 1984) the midazolam plasma concentration needed to obtain a sedative effect is about 400 ng ml^{-1}. Therefore, it was decided to maintain this level during the surgical procedure. Fig. 2a showed a simulated curve of midazolam plasma levels (GLC) in one of the patients on protocol 1; there were fluctuations around 400 ng ml^{-1} (C_{min} = 224·4 ng ml^{-1} and C_{max} = 1330 ng ml^{-1}). To minimize these fluctuations, protocol 2 was adopted. The simulated curve and experimental data (Fig. 2b) are in good agreement; fluctuations were reduced but not suppressed (C_{min} = 264·4 ng ml^{-1} and C_{max} 618·7 ng ml^{-1}). This suggests that a continuous infusion of midazolam after a loading i.v. dose should be better than repeated injections to maintain a sedative plasma level. Arousal times and corresponding midazolam concentrations were comparable to those reported by Crevoisier et al (1984).

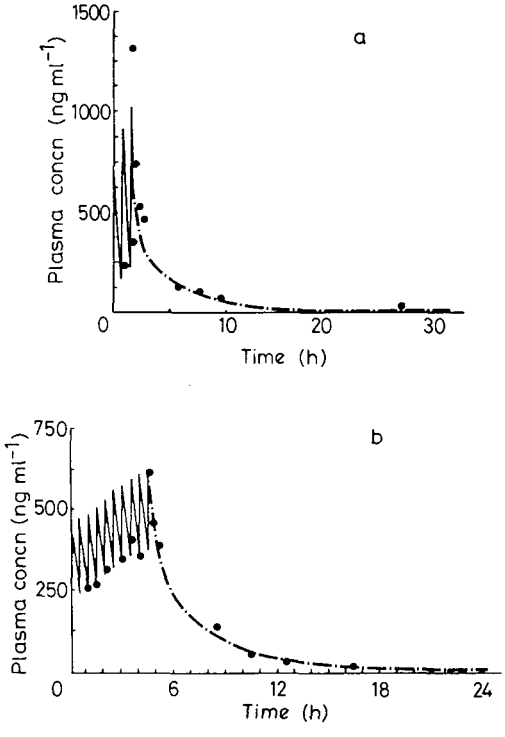

FIG. 2. Simulated curve and experimental values of midazolam plasma levels in one of the patients on protocol 1(a) and protocol 2(b).

Pharmacokinetic parameters

Main pharmacokinetic parameters were similar to whichever protocol and analytical technique were used. Total distribution volume and clearance values of midazolam agreed with previous data (Smith et al 1981). As anticipated, apparent elimination half-life values of midazolam were slightly longer than those reported during single dose studies (Kanto & Klotz 1982; Smith et al 1981). There was no difference in the pharmacokinetic parameters calculated using the serum levels determined by RRA or GLC (Aaltonen et al 1985).

The findings confirmed firstly that RRA was as valid as GLC in the assessment of the pharmacokinetic properties of midazolam, and secondly that 1-hydroxymidazolam, even after repeated doses of midazolam, had only a weak effect on the pharmacological activity of midazolam. Similar observations were made after single dose studies (Jochemsen et al 1983; Aaltonen et al 1985).

CONCLUSION

RRA would seem to be valuable as a tool in investigating binding affinities and pharmacokinetic properties of midazolam and its active metabolite, 1-hydroxymidazolam. But, RRA is not always as acceptable as GLC for kinetic studies of some benzodiazepines, especially for compounds with high levels of active metabolites with long elimination half-lives (e.g., diazepam, clobazam). Nevertheless, plasma concentrations of RRA active material should reflect a better relationship with some clinical or pharmacological effects of benzodiazepines than do levels of the parent compound alone.

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