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Cerebral and plasma kinetics of a high dose of midazolam and correlations with its respiratory effects in rats☆

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Abstract

Benzodiazepine poisoning causes coma and respiratory depression. Our objective was to determine whether, and to what extent, arterial blood gas disturbances correlated with blood or cerebral kinetics of midazolam. A 160 mg kg^{-1} single dose of midazolam was infused intravenously over 20 min in catheterized male Sprague–Dawley rats. Midazolam kinetics was simultaneously determined in plasma and brain using striatal microdialysis. Midazolam concentrations were measured using a high-performance liquid chromatographic assay with ultraviolet detection. Midazolam (160 mg kg^{-1}) reproducibly induced deep coma with respiratory acidosis. Plasma midazolam kinetics was well described by a bi-exponential model, with an elimination half-life of 6.4 ± 1.8 h. The striatal dialysate concentration peaked at 50.0 ± 8.9 min after the end of infusion, with a significant delay to peak concentration compared to plasma. Respiratory depression, assessed by the elevation in $PaCO_2$, was more closely correlated with midazolam striatal dialysate rather than plasma kinetics. These results suggest a central mechanism for midazolam respiratory effects at toxic doses in rats. In conclusion, our study showed a delayed onset in peak $PaCO_2$ and pH effects after the slow infusion of a toxic dose of midazolam in rats. The effects on arterial blood gases were better correlated with midazolam

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striatal concentrations than with plasma concentrations. This study may contribute to better understanding of benzodiazepine-induced respiratory depression in poisonings.

Keywords: Midazolam; Acute poisoning; Respiratory depression; Pharmacokinetics; Pharmacokinetic–pharmacodynamic relationships; Arterial blood gases; High-pressure liquid chromatography; Cerebral microdialysis

1. Introduction

In normal subjects, hypnotic doses of benzodiazepines (BZD) have little effect on respiration (Charney et al., 2001). By opposite, in predisposed individuals (i.e., with upper airway obstruction syndrome, drug addiction or poisonings), BZD may induce marked respiratory depression (Charney et al., 2001; Reves et al., 1985; Alexander and Gross, 1988; Clergue et al., 1981).

Acute BZD poisoning represents a frequent cause of admission to the intensive care unit (ICU) (Hojer et al., 1989). Morbidity and even mortality occur as a result of coma and respiratory depression (Mokhlesi et al., 2003). Patients severely intoxicated with BZD usually require mechanical ventilation, especially in the case of concomitant central nervous system depressant or antidepressant drug co-ingestion (Charney et al., 2001; Gaudreault et al., 1991). Deaths caused by BZD alone are uncommon (Buckley and McManus, 2004; Drummer et al., 1993). However, respiratory depression has been reported in pure flunitrazepam poisonings (Mignée et al., 1980). Furthermore, in forensic reports, various BZD had been considered either the causative factor or a significant contributory factor in the deaths (Buckley and McManus, 2004).

Midazolam-induced respiratory effects have been studied in healthy volunteers. A sedative intravenous (i.v.) dose of midazolam does not change the ventilatory response to isocapnic-sustained hypoxia (Dahan and Ward, 1991), while it may decrease the ventilatory and mouth occlusion-pressure responses to CO_2 (Forster et al., 1980). Midazolam administration results in upper airway obstruction (Montravers et al., 1994), tidal volume and minute ventilation decrease (Alexander et al., 1992), and diaphragmatic contractility reduction (Molliex et al., 1993). Moreover, central apnea may occur during the first few minutes after i.v. administration of midazolam ($0.1\,mg\,kg^{-1}$) followed by obstructive apnea (Montravers et al., 1992).

While drug poisonings involving BZD are a well-recognized cause of both morbidity and mortality, the mechanism of BZD-induced respiratory depression remains poorly investigated in this setting. We recently demonstrated that the mechanism of respiratory depression in drug-induced coma involving BZD in humans is related to an increase in the upper airway resistance, with snoring, flow limitation and obstructive apnea, resulting in an increase in the resistive work of breathing (Gueye et al., 2002).

The results of studies in humans, which have reported on the relationships between plasma BZD concentrations and their corresponding neurological effects, are conflicting. This may be in part related to the complex metabolism of numerous BZD to active metabolites. After oral, intramuscular, and intravenous midazolam administration, a close relationship has been found between the plasma or serum concentration and psychometric findings (Kanto, 1985). Relationships of sigmoidal shape has been reported by Mandema et al. in healthy subjects between the effects of midazolam assessed using saccadic eye movement test or EEG activity, and the corresponding plasma concentrations of both midazolam and its rapidly cleared α-hydroxy-metabolite (Mandema et al., 1992). Following three serial i.v. injections of $0.05\,mg\,kg^{-1}$ of midazolam at 20 min intervals in healthy volunteers, Sunzel et al. reported sigmoidal relationships between the observed increase of $PaCO_2$ and the corresponding midazolam plasma concentrations (Sunzel et al., 1988). In contrast, no correlations were found between tidal volume or respiratory rate with plasma concentrations.

To our knowledge, the pharmacokinetics–pharmacodynamic relationships of midazolam respiratory effects and the corresponding plasma concentrations have not been addressed at doses inducing coma. In a previous study, we showed in rats that the median intravenous lethal dose (LD_{50}) of midazolam was $357\,mg\,kg^{-1}$ and the minimal dose inducing coma

130 mg kg^{-1} (Gueye et al., 2000). We also reported that at very high doses, death occurred rapidly following injection in relation to apnea and deep coma. We, thus, used an intraperitoneal 160 mg kg^{-1} dose of midazolam, which caused deep coma with a delayed onset of respiratory failure, as assessed by a significant increase in $PaCO_2$ and a significant decrease in pH (Gueye et al., 2000). The aim of the present study was to determine whether respiratory acidosis correlated more closely with blood or brain concentrations.

2. Materials and methods

All experiments complied with the ethical guidelines established by the National Institutes of Health and the French Minister of Agriculture. Sprague–Dawley male rats (Iffa-Credo, France), weighing between 250 and 300 g at the time of experimentation, were housed in a light- and temperature-controlled animal care unit with free access to food and water until 12 h prior to experimentation. Following experimentation, euthanasia was performed using carbon dioxide.

2.1. Arterial blood gas study

Rats ($N = 10$) were anesthetized with ketamine (Ketalar®) 70 mg kg^{-1} and xylazine (Rompum®) 10 mg kg^{-1} intraperitoneally. Femoral venous and arterial catheters were placed 24 h prior to drug administration. On the following day, rats were placed in Plexiglas cylinders designed with several openings to prevent CO_2 rebreathing. Midazolam (generously provided by Hoffman-LaRoche, France) was diluted at a concentration of 20 mg ml^{-1} in physiologic saline, adding 0.1 M hydrochloric acid to obtain a pH of 3.3. Rats received 160 mg kg^{-1} i.v. midazolam by an infusion pump (Harvard Instruments-PHD 2000, USA) over 20 min. Arterial blood samples (200 μl) were obtained before and at 5, 30, 60, 120, 180, and 240 min after drug administration, and immediately measured using a blood gas analyzer (ABL 300, Radiometer, France). An 800 μl volume of saline was infused at 120 min to compensate the sampled blood.

2.2. Plasma kinetics study

Catheterized rats ($N = 7$) received 160 mg kg^{-1} i.v. midazolam by an infusion pump over 20 min. Based on previously reported plasma elimination half-lives of midazolam of approximately 20–30 min (Yamano et al., 2000; Tuk et al., 1999; Watanabe et al., 1998; Sunzel, 1989), blood samples (300 μl) were collected through the arterial catheter before injection, during the infusion (at 5, 10, and 20 min), and after the infusion (1, 2, 5, 10, 20, 30, 60, 120, 180, and 240 min), then centrifuged (10 min, 3500 × *g*), and stored at −20 °C until quantification. As performed in the arterial blood gas study, an 800 μl volume of saline was infused at 120 min to compensate the sampled blood.

2.3. Cerebral kinetics study

Following anesthesia, a microdialysis probe (CMA/12 Microdialysis AB, Stockholm) was placed in the striatal area using stereotaxic surgery (Mégarbane et al., in press; Desrayaud et al., 1996). Rats ($N = 4$) were allowed to recover for 6 days, underwent venous catheterization as described above, and on day 7, the cerebral kinetics study was performed. Animal and drug preparations and administration were identical to the studies above. Microdialysis was performed using physiologic saline at an infusion rate of 1.5 μl min^{-1}. Polyether–ether–ketone microdialysis catheters (Phymep, France) of 0.12 mm internal diameter and 48 cm length were employed. Samples were obtained every 20 min, beginning 4 min after i.v. drug injection (to compensate for catheter dead space) during 4 h. Saline flow was maintained all the time through the probe. Samples were stored at −20 °C until quantification. The efficiency of the CMA/12 probes, using the in vitro relative loss, was 7.0 ± 0.8%. The anatomical location of the dialysis probe was confirmed by microhistopathology of formaldehyde-fixed brain specimens in all animals following sacrifice.

2.4. Midazolam quantification assays

For blood samples, a methanol solution of flunitrazepam (generously provided by Hoffman-LaRoche, France) was used as internal standard (final concentration: 0.1 μg ml^{-1}) and added to plasma prior to extraction. Midazolam was extracted from 100 μl of plasma by adding 4.5 ml of dichloromethane/*n*-hexane (90/10, v/v) mixture and 1 ml of phosphate buffer 1 M (pH 10.8). The mixture was vortexed during 1 min before centrifugation (15 min, 2800 × *g*). The organic phase

(4 ml) was transferred into a clean tube and evaporated to dryness under an air stream at 35 °C over 10 min. After complete evaporation of the solvent, the residue was dissolved in 100 μl of the mobile phase, shaken for 30 s and 20 μl volume of the mixture was injected in the chromatographic system. The plasma absolute recovery of midazolam was determined at 86.5 ± 1.4% in the concentration range of 0.5–4 mg l^{-1}. To guarantee comparative recovery rate in further assays, a plasma control with 2 mg l^{-1} midazolam was systematically prepared with plasma samples.

Analyses were performed using a high-pressure liquid chromatographic (HPLC) assay with reverse phase, coupled with an UV-detector (ThermoQuest, France) and fitted with an autosampler (Spectra-Physics SP8875, ThermoQuest, France). Chromatographic separation was carried out on a capillary column (250 mm × 4.6 mm, filled with Spherisorb 80 Å, ODS2, 5 μm, Prolabo, France). The mobile phase was a mixture of phosphate (0.05 M KH_2PO_4 diluted with distilled water, pH 6.0), acetonitrile, and methanol (35/30/35, v/v/v), using a 1.2 ml min^{-1} flow rate. Measurement of the UV absorption was performed at 245 nm. Appropriate detection potentials were selected to maximize the signal intensity: between +0.2 and +0.5 V for plasma samples and between +0.1 and +0.5 V for striatal samples.

The method was validated using the generally retained criteria of optimization for HPLC, including specificity, reproducibility of migration time, linearity, and sensitivity as well as within-run and between-run precision. In the chromatogram obtained after preparation of blank plasma, no additional peaks interfered with the measured midazolam and internal standard. The compounds were well separated with a migration time of 4.2 min for flunitrazepam and 8.9 min for midazolam. For plasma samples, the linearity of the method (peak height ratio of the drug/internal standard versus drug concentration) was evaluated over a concentration range of 20–120 mg l^{-1}. Regression analysis performed by the least-squares method gave the following formula: $y = 0.5227x - 0.0025$, with an excellent correlation coefficient ($r^2 > 0.999$). This equation was determined by five calibrations obtained on different days. To determine between-run and within-run precisions, injections were realized 10 times for two plasma controls (30 and 100 mg l^{-1}). Coefficients of variation for within-run precision were excellent, 2.7% and 0.6%, respectively. For between-run, coefficients of variation were below 10%.

For striatal samples, 75 μl of the flunitrazepam solution, prepared as mentioned above, was added to 25 μl of the dialysate. The chromatographic procedure was similar, as previously described for plasma. However, due to low concentrations found in striatum, an additional standard curve was prepared, showing linearity in the concentration range 0.025–0.5 mg l^{-1} ($y = 0.0039x + 0.0082$, $r^2 > 0.999$, $N = 5$). For precision study ($N = 10$), two controls (0.03 and 0.4 mg l^{-1}) were used and all coefficients of variation ranged from 5% to 10%.

The limit of detection was 0.008 mg l^{-1} (coefficient of variation: 12.0%) and the limit of quantification 0.025 mg l^{-1} (coefficient of variation: 8.6%).

2.5. Pharmacokinetic and statistical analysis

Results are expressed as mean ± S.E.M. Pharmacokinetic studies were performed using Kinetica® software (InnaPhase, Philadelphia). We measured the peak concentration (C_{max}). Using a compartmental analysis, we determined the distribution and elimination half-lives ($T_{1/2\alpha}$ and $T_{1/2\beta}$). Using a non-compartmental analysis, we determined the observed area under the curve (AUC), the steady-state volume of distribution (VD_{SS}), the mean residence time (MRT), and the total body clearance (Cl).

Comparisons of midazolam effects on arterial blood gases to baseline values were performed using repeated measures one-way analysis of variance (ANOVA) and Dunnett's multiple comparison tests. All tests were performed using Prism version 2.0 (GraphPad Software, San Diego, CA, USA). Statistical significance was considered for $P < 0.05$.

3. Results

All rats receiving 160 mg kg^{-1} of midazolam over 20 min developed within 5–10 min after the beginning of the infusion, a prolonged hypotonic coma lasting more than 8 h. Significant respiratory depression, as assessed by an increase in arterial $PaCO_2$ (6.34 ± 0.17 versus 5.27 ± 0.18 kPa, $P = 0.01$) and a decrease in arterial pH (7.34 ± 0.01 versus 7.42 ± 0.01, $P = 0.01$), was observed at 5 min after the end of

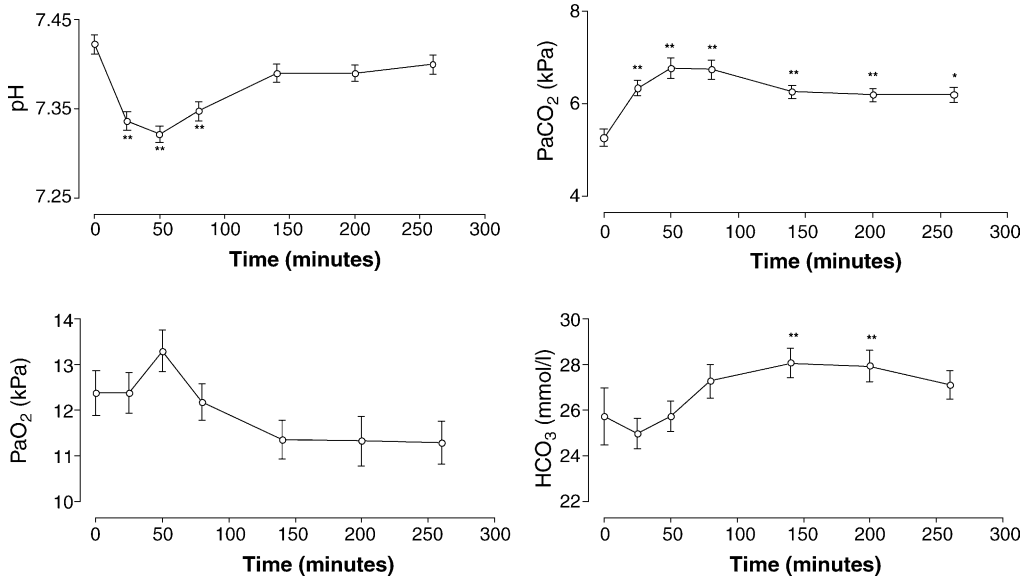

Fig. 1. Effects of 160 mg kg^{-1} midazolam on the arterial pH, $PaCO_2$, PaO_2, and blood bicarbonate concentrations in rats ($N = 10$). Values represent mean ± S.E.M. at each post-injection time interval. Time zero denotes baseline values (before injection). At each time, values were compared to the baseline value using repeated measures one-way analysis of variance (ANOVA) and Dunnett's multiple comparison tests. $^{*}P < 0.05$, $^{**}P < 0.01$.

infusion, in comparison to the baseline (Fig. 1). The maximal respiratory depression assessed by $PaCO_2$ was observed 30 min post-infusion and reached a plateau during the following 30 min (6.77 ± 0.21 and 6.75 ± 0.21 kPa, respectively). Thereafter, while there was a progressive decrease in $PaCO_2$, the values remained significantly different from the baseline values until the end of the experiment, at 240 min post-injection. The lowest arterial pH was observed at 30 min after the end of infusion, in comparison with the baseline value (7.32 ± 0.01 versus 7.42 ± 0.01, $P = 0.01$). While arterial pH increased at 60 min post-injection (7.35 ± 0.01), it remained significantly different from the baseline value ($P = 0.01$). Thereafter, arterial pH was no longer significantly different from baseline. Concomitant significant increases in plasma bicarbonate were seen as well (Fig. 1). There was no significant effect of midazolam on PaO_2 at any time.

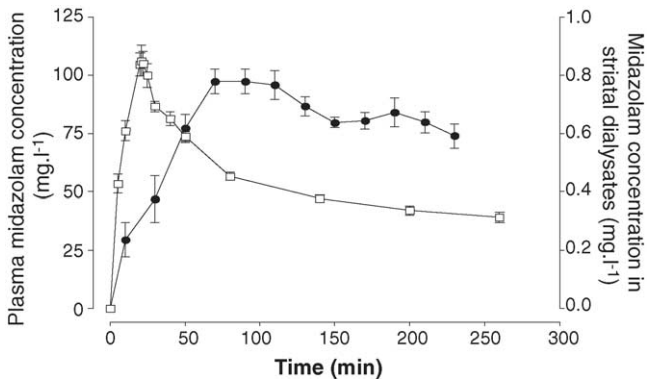

Fig. 2. Kinetics of midazolam in plasma (open square) and in striatal dialysates (filled circle) in rats treated with 160 mg kg^{-1} midazolam infused over 20 min. Seven rats were used for plasma and four for striatal kinetics. Concentrations are presented as mean ± S.E.M.

Table 1
Pharmacokinetics of plasma midazolam concentrations in rats ($N = 7$) after 160 mg kg^{-1} midazolam i.v. infusion over 20 min

Parameters	Mean ± S.E.M.
Peak plasma concentration C_{max} (mg l^{-1})	120.9 ± 6.0
Distribution half-life $T_{1/2\alpha}$ (min)	6.4 ± 1.8
Elimination half-life $T_{1/2\beta}$ (min)	288.6 ± 44.3
Area under the curve (AUC) (mg min l^{-1})	13940 ± 289
Mean residence time (MRT) (min)	399.6 ± 61.5
Steady-state distribution volume (Vd_{ss}) (l kg^{-1})	2.06 ± 0.10
Total body clearance (Cl) (ml min^{-1} kg^{-1})	5.7 ± 0.6

Values represent mean ± S.E.M.

Kinetics of midazolam in plasma was well described by a bi-compartmental open model with a rapid distribution phase ($t_{1/2\alpha}$: 6.4 ± 1.8 min) and a slow elimination phase ($t_{1/2\beta}$: 288.6 ± 44.3 min) (Fig. 2 and Table 1). Striatal dialysate kinetics showed a slow distribution of midazolam into the brain extracellular liquid, with a peak of 0.80 ± 0.03 mg l^{-1} reached at 90.0 ± 11.5 min after the start of infusion. Thus, there was a delay of 70.0 ± 8.9 min between striatal dialysate and plasma midazolam peak concentrations. A plateau phase of striatal dialysate concentrations was then observed over a period of 40 min. The mean observed AUC value in striatum was 147.7 ± 4.5 mg min l^{-1}, corresponding to a ratio of striatal dialysate to blood midazolam concentration of 1.1%.

The maximal respiratory effect of midazolam assessed by alteration in $PaCO_2$ level was not reached immediately after the end of infusion and was not concomitant with the peak plasma midazolam concentration. There was a 30 min delay between the maximum increase in $PaCO_2$ and the end of midazolam infusion. Thereafter, while there was a significant decrease in plasma midazolam concentrations, $PaCO_2$ remained at a plateau maximal value between 30 and 60 min after the end of infusion.

There was better correlation between the $PaCO_2$ level and the cerebral kinetics than with plasma kinetics. Fig. 3 shows a counter-clockwise relationship between $PaCO_2$ and plasma midazolam concentrations, while there is a clockwise relationship with striatal dialysate concentrations.

4. Discussion

In the literature, measurement of midazolam concentrations has been achieved using different assays, specifically, gas (Fisher et al., 1995) or liquid phase chromatography (Chan and Jones, 1993). However, these methods, developed for routine clinical practice, necessitate large sample volumes. We tested a highly sensitive technique for the measurement of midazolam concentrations in small samples, better adapted for the study of plasma and cerebral microdialysis toxicokinetics in rats. One limitation of our study results from the fact that we measured midazolam only, and not its metabolites, some of which (including α-OH-midazolam) exhibit as potent an activity as midazolam with regard to sedative activity (Tuk et al., 1999). However, the respiratory depressant activity of midazolam metabolites in rats has not been addressed to our knowledge.

Plasma kinetics of midazolam administered at pharmacological doses have been determined in humans (Mandema et al., 1992; Greenblatt et al., 1989; Sunzel

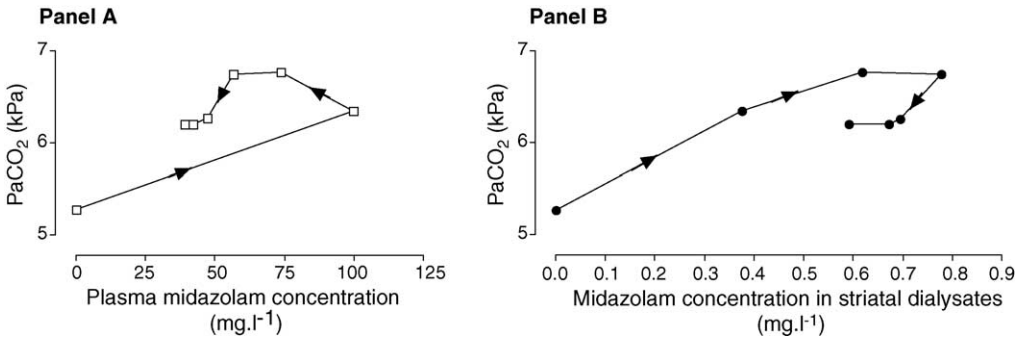

Fig. 3. Pharmacodynamic–pharmacokinetic correlation between $PaCO_2$ and plasma midazolam concentration (Panel A) and striatal dialysate midazolam concentration in (Panel B) rats administered 160 mg kg^{-1} midazolam i.v. over 20 min.

et al., 1988; Reves et al., 1985; Kanto, 1985; Vree et al., 1981; Allonen et al., 1981) and animals (Yamano et al., 2000; Tuk et al., 1999; Watanabe et al., 1998; Sunzel et al., 1988; Vree et al., 1981). However, to date, no study has been conducted at elevated doses. We previously determined i.v. midazolam LD_{50} in Sprague–Dawley male rats to be $411 \pm 125\,mg\,kg^{-1}$, when administered via the tail vein (Gueye et al., 2000). Thereafter, we showed that a $160\,mg\,kg^{-1}$ dose of midazolam administered intraperitoneally consistently induced a deep coma with respiratory acidosis. In this study, we administered the $160\,mg\,kg^{-1}$ dose by an i.v. infusion over 20 min. The aim of this study was to compare kinetics in plasma and brain microdialysis, particularly the respective C_{max} and peak time (T_{max}). To increase the reproducibility of our results, we selected i.v. infusion. Midazolam's kinetic profile at high doses appears to be different from that at therapeutic doses in rats. After i.v. administration of 2.5–10 mg kg^{-1} doses, large and rapid distributions were reported, with a distribution half-life ($T_{1/2\alpha}$) of 2.3 ± 0.5 min (Sunzel, 1989) and a steady-state apparent distribution volume (VD_{SS}) of 1.6–2.4 l kg^{-1} (Tuk et al., 1999; Björkman et al., 1996). In this study, we found values within the same range (6.4 ± 1.8 min and $2.06 \pm 0.10\,l\,kg^{-1}$), confirming the rapid and large distribution of midazolam. However, regarding the elimination phase, we found an elimination half-life of 288.6 ± 44.3 min, about 10-fold the 20–30 min half-life reported for therapeutic doses (5–10 mg kg^{-1}) (Yamano et al., 2000; Tuk et al., 1999; Watanabe et al., 1998; Sunzel, 1989). Similarly, we found total body clearance of $5.66 \pm 0.62\,ml\,min^{-1}\,kg^{-1}$, about 2.5–18 times lower than those obtained at therapeutic doses (12.7 ± 1.1, 71.6 ± 5.5, 74.3 ± 2.8, and $101.0 \pm 7.7\,ml\,min^{-1}\,kg^{-1}$) (Yamano et al., 2000; Higashikawa et al., 1999; Tuk et al., 1999; Watanabe et al., 1998). It should be noted that the duration of our study was planned considering an anticipated elimination half-life of about 30 min. One limitation of our study is its inability to accurately describe the late phase of the time course of plasma midazolam concentration results, given the observed prolonged 288 min half-life, in relation to our study duration of only 240 min. It is also noteworthy that other studies were based on different sampling protocol duration of 90 (Watanabe et al., 1998) and 150 min (Tuk et al., 1999). As midazolam elimination mainly occurs via metabolism, our data suggest that midazolam metabolism was saturated at such high concentrations, as previously reported in humans (Allonen et al., 1981) and dogs (Vree et al., 1981), but not yet verified in rats.

The capacity of BZDs to cross the blood–brain barrier is influenced by binding to plasma proteins, liposolubility, ionization constants, and cerebral blood flow (Colburn and Jack, 1987; Arendt et al., 1987). Midazolam crosses the blood–brain barrier and is distributed to the brain extracellular liquid and cellular components. Midazolam is more extensively distributed to the brain, in comparison with other BZDs due to its non-ionized presentation at physiological pH (pK_a: 6.15), and its high lipophilicity (octanol/water coefficient: 475) (Gerecke, 1983). Surprisingly, in our study after i.v. infusion of a $160\,mg\,kg^{-1}$ dose, there was a slow distribution into the striatal extracellular liquid, with a C_{max} obtained only after 90 min from the start of infusion and 70 min after reaching the plasma C_{max}. These results are startling for an anesthetic agent used to induce quick sedation. However, as microdialysis measures only midazolam free fraction in the extracellular liquid, the delay to the T_{max} corresponded in fact to the time needed by the striatal compartment to achieve a steady state between entrance (blood–brain barrier crossing, which may be rapid) and elimination (slow equilibrium between the free fraction in striatal extracellular liquid and the bound fraction to brain tissues). The fraction of free midazolam that passes from the peripheral circulation to the striatal dialysate, and which is assessed by the observed ratio of free striatal/total plasma AUC value, was about 1/100 in our study. Consistently, after an intraperitoneal administration of $50\,mg\,kg^{-1}$ midazolam, Arendt et al. found a ratio of total brain/free plasma midazolam concentrations of 33.9 (Arendt et al., 1987). This elevated ratio is due to midazolam low plasma protein-unbound fraction (around 3.5%) and high affinity to brain tissues (Mandema et al., 1991; Björkman et al., 1996). Considering its high liposolubility (Gerecke, 1983) and large steady-state volume of distribution ($2.06 \pm 0.1\,l\,kg^{-1}$), our data are in agreement with Arendt's results and confirm that at high doses, midazolam concentrates within the brain. We, thus, may think that rat decapitation technique, which allows measurement of midazolam concentrations in the whole brain tissues, would demonstrate parallel midazolam brain and blood kinetics.

At this high dose, infused over 20 min, although there was a delay in striatal distribution of free midazolam, our study showed a similar delay of 30–60 min following the initiation of infusion to the maximum of respiratory depression, assessed by an increase in $PaCO_2$. Thus, there was a better correlation between the $PaCO_2$ values and the free striatal midazolam concentrations than between the $PaCO_2$ values and the total plasma midazolam concentrations. However, another limitation of our study results from the site where microdialysis was performed. Indeed, microdialysis was performed in the striatum while neuronal control of breathing is in the brainstem (Feldman and Smith, 1995). However, even with this limitation the $PaCO_2$ values correlated better with striatal than with plasma concentrations. Cerebral kinetics seems particularly adapted to study the correlation between BZD dose and respiratory effects. A pharmacological mechanism, which may explain the disappearance of pharmacological effects of BZD, is redistribution from the central to the peripheral compartment (Greenblatt et al., 1989). Reves et al. found that in humans, diazepam-induced electroencephalogram alterations disappeared 7 h following administration while the elimination half-life of diazepam was 33 h (Reves et al., 1985). Our study showed a paralleled slow decrease in $PaCO_2$ values and midazolam striatal dialysate concentrations. In a previous study in two healthy volunteers, a PET scan revealed that the region of the brain where diazepam was redistributed most rapidly was the brainstem. However, at the 160 mg kg^{-1} dose of midazolam our study did not show any discrepancy between $PaCO_2$ and striatal concentrations. This finding calls for clarification of the existence of a redistribution phenomenon at toxic doses in the diminution of effect. Finally, the paralleled slow decrease in $PaCO_2$ values and striatal midazolam concentrations do not suggest any acute tolerance to the respiratory effects induced by midazolam.

The mechanism of BZD-related respiratory depression in human is still misunderstood. BZD may cause central and obstructive apnea as well as decreases in tidal volume and minute ventilation. Upper airway obstruction seems to play a major role during sedation with midazolam (Montravers et al., 1994) or acute BZD poisonings (Gueye et al., 2002). An increase in inspiratory intercostal and expiratory abdominal muscle activities and a decrease in diaphragmatic contractility appear to compensate midazolam-related alteration of breathing pattern (Molliex et al., 1993; Montravers et al., 1992). These effects are generally reversed with flumazenil, a specific BZD antagonist on the γ-aminobutyric acid (GABA)-A receptors (Gueye et al., 2002; Molliex et al., 1993; Gross et al., 1991). They are thought to be CNS-mediated, with little effect on respiratory mechanics (Reves et al., 1985). Central apnea, reported with midazolam within the first minutes after i.v. infusion (Montravers et al., 1992; Reves et al., 1985) reinforces this hypothesis. Our results describing a good correlation between $PaCO_2$ increase and striatal midazolam concentrations suggest a central mechanism for midazolam respiratory effects at toxic doses in rats. However, our study did not allow us to clarify whether the delayed time-course of respiratory effects characterized with an increase in $PaCO_2$ resulted from a specific toxicological effect of midazolam or from a non-specific midazolam-induced sedation. Midazolam induces an immediate sedation considered as its pharmacological effect. The delay between the respiratory effects and the peak of plasma kinetics may suggest that respiratory depression is an indirect effect of sedation.

In conclusion, our data show that 160 mg kg^{-1} of midazolam administered intravenously over 20 min to rats presents a very different kinetic profile than that seen at pharmacological doses, consisting of a long elimination half-life associated with a diminished total body clearance, probably related to the saturation of the liver metabolism pathways. Midazolam kinetics demonstrates a long-lasting distribution. Our data support a delayed onset in peak $PaCO_2$ and pH effects after the slow infusion of a toxic dose of midazolam. The effects on arterial blood gases are better correlated with midazolam striatal concentrations than with plasma concentrations. This study may thus contribute to better understanding benzodiazepines-induced respiratory depression in poisonings.

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