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The influence of the time of day on midazolam pharmacokinetics and pharmacodynamics in rabbits

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ABSTRACT

Background: This study evaluates the time-of-day effect on midazolam and 1-OH midazolam pharmacokinetics, and on the sedative pharmacodynamic response in rabbits. Also, circadian fluctuations in rabbits' vital signs, such as the blood pressure, heart rate and body temperature were examined. The water intake was measured in order to confirm the presence of the animals' diurnal activity. The secondary aim involved the comparison of two methods of data analysis: a noncompartmental and a population modeling approach.
Methods: Twelve rabbits were sedated with intravenous midazolam 0.35 mg/kg at four local times: 09.00, 14.00, 18.00 and 22.00 h. Each rabbit served as its own control by being given a single infusion at the four different times of the day on four separate occasions. The values of the monitored physiological parameters were recorded during the experiment and arterial blood samples were collected for midazolam assay. The pedal withdrawal reflex was used as the measurement of the sedation response. Two and one compartmental models were successfully used to describe midazolam and 1-OH midazolam pharmacokinetics. The categorical pharmacodynamic data were described with a logistic model.
Results and conclusions: We did not find any time-of-day effects for the pharmacokinetic and pharmacodynamics parameters of midazolam. For 1-OH midazolam, statistically significant time-of-day differences in the apparent volume of distribution and clearance were noticed. They corresponded well with the rabbits' water intake. The noncompartmental and model-based parameters were essentially similar. However, more information can be obtained from the population model and this method should be preferred in chronopharmacokinetic and chronopharmacodynamic studies.

Introduction

Midazolam is a commonly used sedative agent in the intensive care unit (ICU) settings worldwide. It is an intermediate-to-high-extraction drug; therefore, clearance is expected to be dependent on the hepatic blood flow. On the other hand, midazolam undergoes extensive metabolism by isoforms of the cytochrome P450 3A subfamily (e.g., CYP3A4 and CYP3A5) to a major hydroxylated metabolite (1-OH-midazolam), so the CYP activity can also be considered as a factor influencing its pharmacokinetics (PK) [14,38]. Both, over- and undersedation, are common problems in the ICU setting. Complications related with the inappropriate use of analgesic and sedative agents in the ICU patients are common [22,34]. High interindividual variability in the PK of midazolam still poses a problem in clinical settings. Therefore, the knowledge of the PK of midazolam and its metabolites may serve as a valuable tool for developing optimal infusion regimens for intensive care patients [14]. One of the open questions is the influence of the circadian clock on midazolam sedation. The results

Abbreviations: AUC, area under the curve; AUMC, area under the first moment curve; BT, body temperature; DBP, diastolic blood pressure; FOCEI, first-order conditional estimation with interaction; HR, heart rate; ICU, intensive care unit; IIV, interindividual variability; IOV, interoccasion variability; LD, light–dark cycle; MBP, mean blood pressure; MOF, NONMEM objective function; NCA, noncompartmental analysis; PK, pharmacokinetics; PD, pharmacodynamics; SBP, systolic blood pressure; VPC, visual predictive check.

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of recent studies have suggested that the time-of-day may be an important factor influencing both the PK and pharmacodynamics (PD) of sedatives [7,9,15,26,33,40]. There was some circadian variability of oral midazolam given to six healthy subjects noted [21]. However, for the intravenous route of administration the data are equivocal [20,39]. Midazolam is a highly protein-binding (albumin) drug (96–98%), which undergoes significant first-pass oxidative metabolism in the liver and intestine [17,29]. Therefore, the potential circadian profile of this drug may differ, depending on the route of administration.

We examined the effects of the administration time on the PK and PD of midazolam and its active metabolite with reference to circadian rhythms during sedation in New Zealand white rabbits. The other aim of our study was to assess the circadian rhythmicity in the rabbits' vital signs, as well as to characterize the daily activity of the laboratory rabbits. The chronopharmacokinetics and chronopharmacodynamic data were analyzed by means of two approaches: the noncompartmental analysis and nonlinear mixed effect (population) modeling. The former method is based on the statistical moment analysis and leads to the determination of basic PK parameters (like clearance and volume of distribution at steady state). The latter method is based on a mathematical PK/PD model and leads to the estimates of all important PK/PD parameters along with their interindividual variability in the population of animals under study.

Rabbits are not very often used in chronopharmacokinetic or chronopharmacological studies, especially due to their unimodal or bimodal pattern of activity [18]. However, recent data concerning the molecular clock, which regulates the circadian rhythmicity in mammals, provide better insight into the nature of the circadian rhythms in rabbits. Also, it is suggested that feeding conditions synchronize the circadian clock of laboratory rabbits [5,6,13,16,40]. To our knowledge, our study is the first to examine the chronobiology of the drug on rabbits supplemented with a circadian analysis of the animals' physiological and behavioral parameters.

Materials and methods

Animals

The experimental protocol was approved by the Local Ethical Committee for Animal Research and it also adhered to the recommended ethical criteria for biological rhythm research on animals proposed by Portaluppi [32]. Twelve healthy New Zealand white rabbits with the average weight of 3.6 ± 0.3 kg (mean ± SD) were used. They were housed individually in stainless steel cages under controlled environmental conditions. The room temperature and relative humidity were controlled, at 20–22 °C and 50–60%, respectively. The animals were maintained under a 12-h light:12-h dark cycle (LD) for 1 month before treatments to habituate them to experimental conditions (light-on: 07.00–19.00 h). They were provided with 125 g of commercial pelleted diet/day, once daily between 08.00 and 12.00 h, and tap water ad libitum. The water intake was controlled five times a day (07.00, 11.00, 15.00, 19.00 and 23.00 h) in order to measure the activity of rabbits (the greater intake – the higher activity). Midazolam (Polfa S.A., Poland) was administered intravenously via short bolus (0.35 mg/kg) at four local times: 09.00, 14.00, 18.00 and 22.00 h. Each rabbit served as its own control by being given a single drug dose at each of the four different times of treatment on different days. The washout period between each timed injection was two weeks. The experiments were conducted during the months of March and April.

The animals were fasted on the day of sedation. Just before infusion, the rabbits were weighed and placed into restraining cages. The hair over the auricular artery and on the tail was removed, and the skin was cleaned with alcohol. A 22G catheter was inserted percutaneously into the central auricular artery and fixed with tape. The catheter was flushed with heparin saline and fixed to the skin. Midazolam (Midanium, Polfa S.A., Poland) was administered as a bolus injection at a dose of 0.35 mg/kg to the marginal vein of the opposite ear. Warm fluids (38 °C) were infused after each blood sampling. The arterial catheter was attached to a Philips IntelliVue MP5 monitoring system with a Philips M1567A catheter. Throughout each session, the body temperature was monitored by means of a rectal probe and maintained at ~38.5–39 °C by means of a heating lamp and FIR Therapeutic Pad (MHP-E 1220, 38 °C).

The first arterial blood sample was taken and initial vitals were measured before commencing injection of the drug. The animals were oxygenated with 100% oxygen at 3 L/min via a facial mask; the oxygen flow was continued until the animals recovered completely. The sedation monitoring included the rectal temperature (digital thermometer of Philips IntelliVue MP5 monitor) as well as the cardiac and respiratory status. The heart rate was monitored and recorded from the curve of the arterial blood pressure. The blood oxygen saturation was monitored from the shaved tail by pulse-oximetry.

In order to monitor the level of sedation, two basic reflexes, i.e. the pedal withdrawal reflex and corneal reflex, were tested. The reflexes were tested in the following periods: initially, 20, 40, and 60 s, and every successive minute afterwards until full recovery. The loss of the corneal reflex indicates dangerously deep anesthesia, when cardiac arrest may develop, whereas the pedal withdrawal reflex is useful to verify the level of sedation [37,43,44]. In this study, the recorded endpoint was the loss and return of the pedal withdrawal reflex, whereas the corneal reflex was always retained during the experiments. The presence and absence of the pedal withdrawal reflex were identified as follows: (+) if the pedal withdrawal reflex was present, and (−) if the pedal withdrawal reflex was absent.

Arterial blood samples (1.5 ml) were collected at the following time points: before beginning of the infusion, after 1, 5, 10, 15, 20, 30, 45, 60, 90, 120 and 150 min after midazolam administration. The lost blood volume was restored by adequate saline infusion (0.9% NaCl, Polfa, Poland). Blood samples were transferred into heparinized tubes and immediately centrifuged, with the plasma samples stored at 4 °C until analysis.

Midazolam assay

Plasma samples were analyzed for midazolam and 1-OH-midazolam by means of validated high-pressure liquid chromatography (Agilent 1200 series, Waldbronn, Germany) coupled with a triple quadrupole mass spectrometer, equipped with an electrospray ionization source (Agilent 6410B, Wildnington, Delaware, USA). The mass spectrometer was working in the MRM mode and three reactions were recorded for each compound. The applied column was Zorbax Eclipse XDB C18 Rapid Resolution HT 4.6 mm × 50 mm, 1.8 μm (Agilent, USA). The mobile phase was: formate buffer pH 3.2 [A] and 0.1% formic acid in acetonitrile [B] (Merck, Darmstad, Germany). The flow rate was 0.5 ml/min. The gradient was programmed as follows: 90% [A] and 10% [B] for 1 min, followed by a linear change to 20% [A] and 80% [B] in 6 min, then 20% [A] and 80%[B] was held for 1.5 min. Midazolam, 1-OH-midazolam and diazepam D5 (internal standard) were purchased from Crilliant (Round Rock, TX, USA). Abselut Nexus (Agilent, USA). Solid phase extraction columns (60 mg/3 ml) were used to midazolam and metabolite extraction according to the manufacturer's procedure. The extraction recovery (% + SD) was 91.1 ± 3.5 and 86.8 ± 2.8 for midazolam and 1-OH-midazolam, respectively. The intraday precision (RSD, %) at 20 ng/ml standard was 5.3 and 7.2 for