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J. Pharm. Pharmacol. 1994, 46: 611–613
Received August 11, 1993
Accepted December 15, 1993

Pharmacokinetics and brain distribution of zolpidem in the rat after acute and chronic administration

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Abstract—The pharmacokinetics of zolpidem were studied after single dose, administered for either 7 or 28 days to rats. Thirty minutes after the last dose, animals were killed and the brain removed. The highest concentrations in plasma, which were observed at the first sampling time (0·5 h) were 2341 ± 540 (day 0), 1956 ± 325 (day 7) and 2908 ± 1369 ng mL^{-1} (day 28). Corresponding AUC values of 1742 ± 488, 1583 ± 422 and 2683 ± 1249 ng mL^{-1} h were found. MRT increased significantly from 0·46 ± 0·06 h on day 0 to 0·67 ± 0·02 h on day 28. The cerebral levels showed no significant change during the chronic administration (766 ± 285, 685 ± 171 and 887 ± 264 ng g^{-1}, respectively). No modification of the principal kinetic parameters was detected up to the 28th day of treatment.

In recent years, zolpidem (Fig. 1), a novel non-benzodiazepine hypnotic agent belonging to a new chemical series, the imidazopyridines, has been developed and used clinically (Arbilla et al 1985; Depoortere et al 1986; Benavides et al 1988). The activity of benzodiazepines is mediated by their interaction with central ω (BZD) modulatory sites associated with the $GABA_A$-receptor complex (Bosmann et al 1978; Braestrup & Nielsen 1981). The preferential affinity of zolpidem is for the ω_1 site of the global GABA receptor (Langer & Arbilla 1988; Benavides et al 1988), but the most recent results suggested that zolpidem sites are associated to at least three subtypes of receptors (Benavides et al 1993).

In separate clinical studies, zolpidem was generally well tolerated (Palminteri & Narbonne 1988; Licciardello & Licini 1992). Zolpidem did not cause signs of withdrawal symptoms or tolerance (Schlisch et al 1991; Maarek et al 1992) with chronic treatment. A careful use and study of zolpidem in the treatment of insomnia is needed to clarify this trend and in particular to compare zolpidem with benzodiazepines in a widespread double-blind study.

Pharmacokinetic factors may be one of the elements of the tolerance. Time-limited pharmacokinetic properties of zolpidem for no more than two weeks have been published in various species (Thénot et al 1988; Langtry & Benfield 1990), and recently for twenty-one days in haemodialysed uraemic patients (Fillastre et al 1993), although, the treatment is usually for at least one month. Chronic administration of zolpidem does not modify its absorption rate (Durand et al 1992), but a modification of its clearance is possible.

The present study was carried out to ascertain the plasma pharmacokinetic parameters after acute, subchronic (7 days) and chronic (28 days) intraperitoneal administration in the rat. A brain distribution study complemented the pharmacokinetic findings for plasma.

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Materials and methods

Animals. Male Sprague-Dawley rats, 400–450 g (Depré, France), were housed four per cage with free access to food and water under a 12-h light/12-h dark cycle. The animals were acclimatized for one week before the start of the experiments.

Study design. In the acute experiment, rats (five per group) received a single intraperitoneal injection of zolpidem (6·22 mg kg^{-1} as the hemitartrate salt corresponding to 5 mg kg^{-1} of the free base), which was administered dissolved in physiological saline solution (0·25 mL/100 g). Zolpidem was a gift from Synthelabo (Paris, France). In the chronic experiments, rats received early morning injections of 5 mg kg^{-1} zolpidem (at the same hour) for 7 days or for 28 days.

Venous blood samples were taken from the tail into tubes containing lithium heparin, at 0·5, 1, 1·5, 2, 3, 4, 6, 8, 10, and 24 h after administration. Plasma was quickly separated and stored

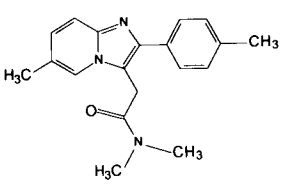

FIG. 1. Chemical structure of zolpidem.

at −20°C until analysis. Brain distribution was measured on day 0, day 7 and day 28. Thirty minutes after the administration animals were killed by decapitation. Brains were removed rapidly on ice, weighed, and homogenized in a glass tube containing distilled water and trichloroacetic acid (0·6 M). Extracts were frozen at −20°C until analysis.

Analytical method. Plasma and tissue levels of zolpidem were determined by HPLC by the method of Guinebault et al (1986). Serum (100 μL) and brain homogenate (500 μL) samples were mixed with internal standard solution of carbamazepine (5 mg L^{-1} in methanol). Zolpidem was extracted against non-stabilized diethyl ether (600 μL) for 1 min. The upper organic phase obtained after centrifugation (5000 *g*, 0·5 min), was transferred to a 5 mL conical glass tube and evaporated to dryness at 37°C under a gentle stream of nitrogen. The residue was then dissolved in 50 μL methanol, and an aliquot (20 μL) was injected directly into the HPLC system.

Chromatography was performed with a Varian Model 5000 liquid chromatograph (Varian, Sunnyvale, USA), equipped with a 20 μL universal loop injector (Valco Instruments, Houston, USA) and a Kratos Spectroflow 783 variable-wavelength UV detector (Ramsey, USA). Separation and quantification were achieved on a reversed-phase system with a C_8 column (Lichrospher 100 RP8, 5 μm, 250 mm × 4 mm i.d., Merck, Darmstadt, Germany) linked to a C_8 precolumn (Vydac Reverse Phase, Varian, Sunnyvale, USA). The lower limit of detection was 8 ng mL^{-1} and reproducibility, over the calibration range, was better than 6%.

Data analysis. The plasma terminal half-life ($t_{\frac{1}{2}}$) was estimated from the terminal phase by linear regression with a computer program (Shumaker 1986) based on the ESTRIP procedure (Brown & Manno 1978). The area under the plasma concentration time curve (AUC) and the first moment of the curve (AUMC) were calculated by the linear trapezoidal rule with the addition of the extrapolated part: $AUC_t + (C_t/\lambda_z)$, where AUC_t is the area from T = 0 to the last concentration C_t estimated and λ_z is the terminal slope. The apparent volume of distribution (Vd) was determined from the ratio: (dose × AUMC)/AUC^2 and the total clearance (CL) was calculated according to: dose/AUC. The mean residence time (MRT) was AUMC/AUC, the terminal half-life ($t_{\frac{1}{2}}$) was obtained from $0{\cdot}693/\lambda_z$. The values of the peak plasma level (C_{max}) and of the time to peak (t_{max}) were raw data.

Statistical analysis. Comparison of pharmacokinetic parameters was by an unpaired *t*-test with $P < 0{\cdot}05$ considered significant.

Results

Fig. 2 shows blood zolpidem curves constructed from raw data. The computed pharmacokinetic parameters are summarized in Table 1. The maximal concentrations were observed in the first sample (30 min) under both acute and chronic treatment. After the single intraperitoneal administration, the concentration of zolpidem in plasma was 2341 ± 540 ng mL^{-1} and at the end of the repeated administration period (day 28) 2908 ± 1269 ng mL^{-1}. The estimated AUC, Vd and CL values showed no significant differences between single, subchronic or chronic administration. On days 0 and 7 and 28, the $t_{\frac{1}{2}}$ values were not significantly different. MRT increased significantly between day 7 and day 28 ($P < 0{\cdot}05$). Plasma concentration as a function of time showed a superposition for the three periods of treatment (Fig. 2). It was observed that the drug was undetectable in plasma 24 h after the last injection.

Brain concentrations after intraperitoneal administration (single, 7 days, 28 days) are shown in Table 2. Independently of the duration of the treatment, zolpidem was present in brain 30 min after injection at a constant concentration.

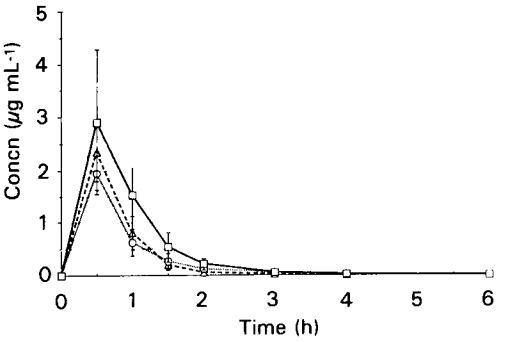

FIG. 2. Mean plasma concentration–time profiles of zolpidem (5 mg kg^{-1}) obtained in five rats after acute and chronic administration (7 days, ○; 28 days, □). Day 0, △.

Table 1. Pharmacokinetic parameters after acute and chronic treatment (5 mg kg^{-1}) (n = 5).

Parameters	Day 0	Day 7	Day 28
C_{max} (ng mL^{-1})	2341 ± 540	1956 ± 325	2908 ± 1369
AUC (ng mL^{-1} h)	1734 ± 489	1564 ± 414	2662 ± 1254
Vd_{ss} (mL)	104·9 ± 24·9	113·4 ± 25·2	87·2 ± 25·2
CL (mL^{-1} h)	141 ± 36	137 ± 54	95 ± 28
MRT (h)	0·46 ± 0·06	0·51 ± 0·12*	0·67 ± 0·22†*
$t_{\frac{1}{2}}$ (h)	0·51 ± 0·06	0·55 ± 0·18	0·68 ± 0·19

*$P < 0{\cdot}05$ compared with day 0. †$P < 0{\cdot}05$ compared with day 7.

Table 2. Mean brain concentrations (ng g^{-1}) of zolpidem in rats, after intraperitoneal doses of 5 mg kg^{-1} zolpidem.

Experiment	n	Concn (ng g^{-1})
Single injection	5	770 ± 285
Daily dosing for 7 days	5	700 ± 170
Daily dosing for 28 days	5	890 ± 260

Discussion

A hypnotic drug should ideally be prescribed for a period as short as possible (Nicholson 1986). In practice, however, general practitioners prescribe hypnotics for a longer period. According to the pharmacokinetic data (Durand et al 1992) it can be said that the administered dose equals a dose of two tablets in man. This strong dose was administered in order to detect the existence of an accumulation phenomenon. Furthermore, none of the metabolites detected in brain or in plasma showed any hypnotic or sedative activities (Depoortere et al 1986), and the hypnotic activity is considered to be due to the unchanged compound.

Between day 7 and day 28, a statistically significant increase in the MRT was observed. This may be due to a decreased hepatic metabolism which was very important (Thénot et al 1988). It has been noted that in patients with liver cirrhosis, the $t_{\frac{1}{2}}$ increased even after a single dose (Bianchetti et al 1988). The other parameters were constant after single, subchronic and chronic administration. In the multiple-dose study, serum concentrations before the first dose on days 7 and 28 were similar. These data indicate that zolpidem did not accumulate in plasma during or after multiple dosing, since the AUC remained constant.

The brain is the target organ of a hypnotic agent such as

zolpidem, and its clinical effect cannot be attained without penetration of the blood-brain barrier. Zolpidem crosses the blood-brain barrier rapidly after a single dose (Garrigou-Gadenne et al 1989). After oral intake zolpidem is very rapidly absorbed, because of its appropriate physicochemical properties for rapid absorption ($pK_a = 6{\cdot}16$, $\log P = 2{\cdot}42$) and is distributed in a relatively small volume, thus permitting the rapid (within 20 to 30 min) achievement of high concentrations at the site of action (Durand et al 1992). It explains our decision to explore brain concentration 30 min after administration. The distribution in the brain appeared homogenous after acute, subchronic or chronic intraperitoneal administration in the rat. The chronic injection did not produce any accumulation.

In conclusion, after chronic administration, zolpidem did not accumulate in plasma and in brain, but we noted a significant increase of MRT. Nevertheless, development of tolerance, if any, may depend not only on pharmacokinetic factors but also on pharmacological factors, particularly on the manner in which drug interacts with its receptors (Sanger & Zivkovic 1992).

As shown in this study, no dramatic modifications of pharmacokinetics could alone account for an eventual change in the pharmacologic effect of zolpidem during a chronic high-dose treatment.

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