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Abstract

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Antihyperalgesic Effect of the GABA_A Ligand Clobazam in a Neuropathic Pain Model in Mice: A Pharmacokinetic–Pharmacodynamic Study

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Abstract: Facilitation of spinal GABAergic inhibition with benzodiazepines (BZDs) reverses pain sensitization in animals; however, the use of BZDs in man is limited by their sedative effect. The antihyperalgesic effects of GABA_A agonists are mediated by GABA_A receptors containing α2 subunits, whereas sedation is linked to α1 subunit-containing receptors. α2 and α3 selective GABA_A receptor modulators have been tested in animals but are not yet available for use in human beings. Clobazam is a 1,5-BZD, which exhibits less cognitive side effects than other benzodiazepines. Here, we studied its antihyperalgesic effects in a mouse model of neuropathic pain. Clobazam showed a dose-dependent antihyperalgesic effect in the chronic constriction injury (CCI) model of neuropathic pain, peaking at 1 hr after administration and lasting for 4 hr with no relevant sedation at a dose of 3 mg/kg. At higher doses, the antihyperalgesic effect was stronger, but sedation became significant. The blood and brain kinetics of clobazam were linear over the range of doses tested with a short half-life of the parent compound and a ready penetration of the blood–brain barrier. Clobazam blood concentrations decreased rapidly, falling below the limit of detection at 120 min. after drug application. Its main metabolite, N-desmethyl-clobazam, showed more delayed and prolonged pharmacokinetics, partly explaining why antihyperalgesia persisted when clobazam was no longer detectable in the blood. Considering its therapeutic margin and its pharmacokinetic properties, clobazam would be a valuable compound to assess the role of the GABAergic pathway in pain transmission in human beings.

Diminished synaptic inhibition in the spinal cord critically contributes to central sensitization, a key phenomenon in chronic inflammatory and neuropathic pain. The role of glycineric and γ-aminobutyric acid (GABAergic neurons in this process has been widely described [1,2]. Therefore, facilitation of the spinal GABAergic input is a rational approach to compensate for diminished inhibitory pain control. In fact, the antihyperalgesic effect of several GABA_A agonists such as muscimol or of positive allosteric modulators such as diazepam has been demonstrated in animals [3].

In human beings, research on the analgesic effect of BZDs is scarce and controversial. In clinical research, diazepam has been used as an active placebo in a study seeking to demonstrate the analgesic effect of fentanyl in patients with chronic non-cancer neuropathic pain [4]. On the other hand, clonazepam is widely used in practice to treat neuropathic pain and has demonstrated efficacy in myofascial pain, temporomandibular joint dysfunction, cancer-related neuropathic pain and in stomatodynia when used topically [5–8]. However, the use of BZDs in chronic pain is rather limited by their side effects, such as sedation, memory impairment and dependence.

Advances in the understanding of the molecular diversity of GABA_A receptors have suggested that the therapeutic index might become improved through the development of subtype-selective or partial BDZ-site agonists [9–12].

Benzodiazepine-sensitive GABA_A receptors contain at least one of the following α subunits α1, α2, α3 or α5, together with two β subunits and a γ2 subunit in a 2:2:1 stoichiometry [13,14]. Work in GABA_A receptor point-mutated mice has shown that the sedative action of BDZs is mainly mediated by GABA_A receptors containing α1 subunits [15], whereas α2- and α3-containing GABA_A receptors were found to be responsible for the anxiolytic properties [16] and largely responsible for the spinal antihyperalgesic actions of classical BDZs [3,17]. In animals, α1-sparing (non-sedative) BDZ agonists showed an antihyperalgesic activity in inflammatory and neuropathic pain models without losing efficacy after repeated treatment [17,18]. Such compounds are under clinical development but are not yet available for use in human beings [19].

Clobazam is a 1–5 BDZ prescribed in all forms of anxiety and in epilepsy. It seems to exert less cognitive and psychomotor side effects compared with clonazepam and lorazepam in a wide range of pharmacodynamic tests in man [20,21]. Therefore, clobazam may be a suitable compound to test the antihyperalgesic effect of GABA_A agonists in exploratory pain studies in human beings. Although an antihyperalgesic action of clobazam in mice is likely, it has not been proven so far.
In a set of experiments, we therefore investigated the antihyperalgesic and sedative effects of clobazam in a neuropathic pain model in mice and correlated this to its pharmacokinetic properties.

Materials and Methods

Drugs. Clobazam (Urbanyl®; Sanofi Adventis, Meyrin, Switzerland) or vehicle was suspended in 0.5% methyl cellulose and 0.9% NaCl and administered orally in a total volume of 10 ml/kg. Doses of 3, 10 and 30 mg/kg were tested.

Neuropathic pain. The chronic constriction injury (CCI) model was used in 7- to 8-week-old mice. Unilateral constriction injury of the left sciatic nerve just proximal to the trifurcation was performed as described previously [22]. Anaesthesia was induced and maintained by 2% isoflurane (Provet AG, Lyssach, Switzerland), combined with oxygen (30%). The sciatic nerve was exposed at the mid-thigh level proximal to the sciatic trifurcation by blunt dissection through the biceps femoris. 5 to 7 mm of nerve was freed of adhering tissue, and three chronic gut ligatures (4/0) (Ethicon, New Brunswick, NJ, USA) were tied loosely around the nerve with about 1 mm spacing. The ligatures were tied until they elicited a brief twitch in the hindlimb. The incision was closed in layers.

Mechanical sensitization. Mechanical sensitivity was assessed with electronic von-Frey filaments (IITC, Woodland Hills, CA, USA). Four or five measurements of paw withdrawal thresholds (PWTs) were made for each time-point and averaged. The system is able to measure, store and display the test readings in grams based upon the amount of pressure applied. Measurements of PWTs of the injured paw and of the contralateral paw were taken alternately. Mechanical sensitization was measured before and 7 days after surgery.

The first experiment was set to assess the effect of three doses of clobazam on mechanical sensitization and on sedation and to measure clobazam brain concentrations. Clobazam (3, 10, 30 mg/kg) or vehicle was administered orally, and mechanical sensitization was assessed for 4 hr after drug administration (n = 6 mice / dose). Immediately after behavioural test, mice were killed for clobazam determination in the brain. Brains were dissected and frozen at −20°C until further processing.

The second experiment was set to determine clobazam pharmacokinetics and to correlate it with its antihyperalgesic effect. To limit the number of mice required and as the analytical method was sensitive enough, only two doses were chosen. Clobazam (3 and 10 mg/kg) was administered orally, and mechanical sensitization was assessed for 4 hr after clobazam administration (n = 6 mice / dose), and the pharmacokinetics of clobazam and N-desmethyl clobazam in whole blood was determined using the dried blood spots (DBS).

Four microlitres of whole blood was collected, through a small incision of the mouse tail and spotted on DBS filter cards (Whatman, Dassel, Germany), using a volumetric micropipette (Eppendorf, Hamburg, Germany). Sampling was performed at 0, 0.25, 0.5, 0.75, 1, 2 and 3 hr after clobazam administration. The blood spots were allowed to dry at room temperature for 2 hr and then packed in a sealable plastic bag-containing desiccant until analysis.

Four hours after drug administration, mice were killed to determine clobazam and its metabolite concentration in the brain.

Locomotor activity. In a third experiment, locomotor activity was measured for 1 hr starting at 1 hr after oral administration of vehicle or clobazam (3 and 10 mg/kg) during the early light phase of the day-night cycle. Mice (n = 10) were placed in individual circular enclosures (diameter 20 cm) equipped with four photocells 15 min. before testing. Motor activity is expressed as the total number of photocell crossings during a 1-hr period.

Pharmacokinetics. Clobazam concentrations were measured in whole blood at 0.25, 0.5, 1, 1.5, 2 and 4 hr after oral intake.

Clobazam and N-desmethyl-clobazam determination in whole blood performed with DBS (second experiment). Full pharmacokinetic profiles of clobazam and its metabolite N-desmethylclobazam were obtained from individual mice with the use of dried blood spot (DBS) technique. This procedure offers the potential to use low blood sample volumes (4 μl in this study), making it particularly attractive for use in mouse studies where blood volume is very limited. Moreover, this approach reduces the number of animals required and the problem of animal-to-animal variations when one animal is killed per time-point [23].

Extraction procedure. Disc with the whole blood spot (exactly 4 μL) was punched from the DBS card and transferred into an HPLC glass vial tube with an insert (total volume 200 μL). A 100 μL of the working internal standard solution in MeOH was added, and the tube was vortex-mixed for about 2 min. The disc was removed from the vial, and 5 μL of the supernatant was injected into the LC–MS/MS system.

Analytical method. All experiments were performed with an API 4000 triple quadrupole mass spectrometer (AB Sciex, Concord, ON, Canada) controlled by Analyst 1.5.1 software. The mass spectrometer was operated in the multiple reaction monitoring (MRM) mode with positive electrospray ionization. The MRM transitions were 301.1 → 259.1, 287.1 → 244.8 and 292.1 → 249.8 with a dwell time of 150 ms for clobazam, desmethylclobazam and IS (desmethylclobazam-d5), respectively. The instrument was directly coupled with an Agilent series 1100 (Waldbronn, Germany) LC system. Chromatography was performed on a Phenomenex Kinetex C18 analytical column (50 mm × 2.1 mm, 2.6 μm; Torrance, CA, USA) preceded by a KrudKatcher ultra in-line filter, 0.5 μm. Flow rate was 0.5 mL/min using gradient elution conditions. The method was fully validated before application to this pharmacokinetic study.

Clobazam and N-desmethyl-clobazam brain determination.

Analytical method. Brains were weighed and homogenized in 2 ml of deionized water. The final volume was adjusted to 5 ml with water. Hundred microlitres of internal standard was added to brain homogenate samples, and extraction was performed with Oasis® HLB SPE columns (Wafers, Wexford, Ireland). The samples were loaded and cartridges were washed with 1 ml of formic acid 0.1%-acetonitrile (85-15 v/v). The cartridges were dried under vacuum. Compounds of interest were eluted with 1 ml of methanol. After evaporation, residues were reconstituted in 100 μl of formic acid 0.1%-acetonitrile (80-20 v/v), and 20 μl was injected onto the HPLC system.

Separation and quantification were performed as described for blood concentrations determination.

Pharmacokinetic parameters were estimated by a non-compartmental method using WinNonlin® version 5.2 (Pharsight, Mountainview, CA, USA).

Statistical analysis. Drug effects at time-point t were expressed as percentage maximum possible effect (%MPE) calculated from comparisons of PWTs of the injured side obtained before surgery, and before and after drug treatment.

To calculate areas under the curve (AUC), baseline (prior to drug treatment) PWTs thresholds of the lesioned paw on day 7 after nerve injury were averaged for each group, and the AUC for each group was calculated using the trapezoidal rule.

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ligation were subtracted from all later withdrawal thresholds. Statistical comparisons were made with one-way analysis of variance (ANOVA) followed by Scheffe’s post hoc test. *p values < 0.05 were considered significant.

Results

**Antihyperalgesic effect of clobazam in neuropathic mice.**

Antihyperalgesic effect of clobazam was evaluated in mice, which had undergone CCI surgery of the left sciatic nerve. After surgery, the operated mice developed progressive behavioural signs of mechanical sensitization (quantified as a decrease in the PWT in response to stimulation with von-Frey filaments). Mechanical PWTs decreased from $3.48 \pm 0.09$ g pre-surgery to $1.44 \pm 0.11$ g, respectively, (mean ± S.E.M., n = 6); *p < 0.001 ($p = 0.00008$).

On day 7 after surgery, when sensitization of the ipsilateral paw had reached a plateau, clobazam was administered orally and mechanical sensitivities of the ipsi- and contralateral paws were assessed for 4 hr. Clobazam significantly increased ipsilateral PWTs in a dose-dependent manner with a maximum effect 1 hr after administration that lasted for 4 hr. PWTs of the contralateral paw were not affected (fig. 1A).

The overall effect as observed with the calculated AUCs for the injured paw in clobazam-treated mice (3 mg/kg: 1.89 ± 0.54 g/h; 10 mg/kg: 2.22 ± 0.29 g/h; 30 mg/kg: 3.2 ± 0.25 g/h) was clearly improved and different from that of vehicle-treated mice (0.09 ± 0.14 g/h) [3 and 10 mg/kg: *p < 0.05; 30 mg/kg: *p < 0.001; ANOVA followed by Scheffe’s post hoc test, $F (3, 11) = 17.42$] (fig. 1B).

**Pharmacokinetic profile.**

The mean blood concentration curves of clobazam and N-desmethyl-clobazam after 3 mg and 10 mg/kg and the related %MPE are presented in fig. 2. After oral intake, clobazam quickly appeared in the systemic circulation [mean (±S.D.) $T_{\text{max}}$ in min. = 25 (7)], demonstrating rapid distribution. The mean (±S.D.) $T_{\text{max}}$ of N-desmethyl-clobazam was 102 (73) min. (table 1).

Clobazam blood (determined in the second experiment) and brain (determined in the first experiment) pharmacokinetics was linear over the dosage range tested (tables 1 and 2). Clobazam half-life in blood was much shorter than N-desmethyl-clobazam half-life [mean half-life (±S.D.) 39 (±6) versus 264 (±51) min.].
After 4 hr, the brain concentration of clobazam at the dose of 3 mg/kg was under the limit of detection while the mean (±S.D.) brain concentration of N-Desmethyl-clobazam was 0.86 (0.18) μg/g. The respective brain concentrations after 10 mg/kg administration were 0.011 (0.005) and 2.97 (0.61) μg/g.

Pharmacokinetic–pharmacodynamic relationship. Figure 3 shows the effect versus concentration curves for clobazam and N-desmethyl-clobazam at 3 and 10 mg/kg. The blood concentration–effect relationship of clobazam showed an anticlockwise hysteresis loop, while the blood concentration–effect relationship of N-desmethyl-clobazam showed a clockwise hysteresis loop. This configuration suggests an early effect of the parent compound relayed by the metabolite.

Sedation. The sedative effect of clobazam was evaluated in individual automated circular enclosures. At the dose of 3 mg/kg, clobazam did not impair motor activity. Significant reduction was observed only at 10 mg/kg [p < 0.01; ANOVA followed by Scheffe’s post hoc test, F (2, 29) = 6.68] (fig. 4).

Discussion

Our results confirm that like other non-selective GABA A ligands such as diazepam, clobazam has an antihyperalgesic effect in the CCI model in mice. Clobazam exhibited a dose-dependent effect, which was well correlated to its blood and brain concentrations. After oral intake, clobazam rapidly appeared in the systemic circulation and in the brain (peak value at $T_{max}$ 25 and 30 min. in blood and brain, respectively), demonstrating a fast absorption and distribution with ready penetration of the blood–brain barrier. Its half-life in mice was markedly shorter than the half-life in human beings (39 min. versus 16–50 hr) and similar to previously reported values [24]. In the systemic circulation, the $T_{max}$ of the main metabolite N-desmethyl-clobazam was 102 (±73) min., and its half-life 264 (±51) min. after a dose of 3 mg/kg and in similar ranges after 10 mg/kg.

To quantify the antihyperalgesic effect of clobazam and to compare it to its pharmacokinetics, we calculated the %MPE and showed that it was reached at 1 hr, which is consistent with the pharmacokinetic profile of the parent compound ($T_{max}$ = 30 min. in the brain). However, the persistence of the effect for 4 hr, although plasma and brain concentrations of clobazam were undetectable after 2 hr despite a sensitive analytical method, suggests a possible role of N-desmethyl-clobazam. In fact, the time course of the antihyperalgesic effect was best matched when the plasma levels of clobazam and of N-desmethyl-clobazam were taken into account. Besides, N-desmethyl-clobazam was tested in vitro on cultured cerebral neurons of rats and exhibited a dose-dependent enhancement of GABA-activated currents identical to that of clobazam [25].

As expected, in this mouse model of neuropathic pain, clobazam had a strong and significant antihyperalgesic effect.
The magnitude of this effect was comparable to the effect of diazepam [17] and of the α2/α3 – subtype-selective BZD-site ligand HZ166 [18]. Similar to what is described in the literature for diazepam [3] and α2–α3 selective compounds [9,17,18], clobazam did not modify the response of the non-injured paw, suggesting that a facilitation of GABA_A receptor-mediated inhibition at the spinal cord level is involved in the observed antihyperalgesia.

Clobazam is a non-selective BZD, which appears to show relatively little cognitive and psychomotor side effects at therapeutic doses in human beings. Unlike other non-selective BZDs such as diazepam or clonazepam, which possess a 1–4 chemical structure, clobazam has a 1–5 chemical structure which may contribute to its better side effect profile [26]. However, the selectivity of clobazam has not been determined. The reduced effect on psychomotor performance of clobazam (10 and 20 mg) compared with clonazepam (0.5 and 1 mg) was demonstrated in healthy volunteers [26] as well as, compared with lorazepam (1 mg), in anxious patients [20,21]. Our experiment corroborates these findings because the activity of the mice, recorded for 1 hr from the peak effect, was not significantly decreased at the dose of 3 mg/kg, which already displayed a notable antihyperalgesic effect. When the doses were increased up to 10 mg/kg or higher, the sedation became significant.

The use of selective compounds targeting the α2 and α3 subunits of the GABA_A receptor would give an opportunity to separate the desired antihyperalgesic effects from the undesired sedative effects, which are mainly mediated by α1-containing GABA_A receptors. Such compounds were successfully tested in rats (L-838,417, for example [17]). They were, however, not further developed in human beings for pharmacokinetic reasons such as a poor bioavailability and short half-lives. More recently, these positive results were replicated with HZ166, a novel 8-substituted triazolo- and imidazobenzodiazepine, which also showed a better pharmacokinetic profile [18].

Compounds that have entered clinical trials include TPA023 and more recently MRK-409 [27,28]. However, the development of MRK-409 was terminated because, unlike in pre-clinical tests, it failed to exhibit anxiolytic activity at non-sedative doses [28].

In summary, clobazam exhibits pronounced antihyperalgesic activity at a dose that does not cause significant sedation. As long as subtype-selective compounds suitable for use in human beings are lacking, clobazam is a good candidate for proof-of-principle studies addressing antihyperalgesic properties of BZDs in human experimental pain models.

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References


27 Atack JR. GABA(A) receptor subtype-selective efficacy: TPA023, an alpha2/alpha3 selective non-sedating anxiolytic and alpha5IA, an alpha5 selective cognition enhancer. CNS Neurosci Ther 2008;14:25–35.