Research Paper

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GABAergic modulation in central sensitization in humans: a randomized placebo-controlled pharmacokinetic–pharmacodynamic study comparing clobazam with clonazepam in healthy volunteers

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Abstract

Positive allosteric modulators of $GABA_A$ receptors (GAMs) acting at specific subtypes of $GABA_A$ receptors effectively restore compromised spinal pain control in rodents. Studies addressing a similar antihyperalgesic effect in humans are sparse and are hampered by sedative effects of nonselective GAMs available for use in humans. We present results from a randomized controlled double-blind crossover study in 25 healthy volunteers, which addressed potential antihyperalgesic actions of clobazam (CBZ) and clonazepam (CLN) at mildly sedating equianticonvulsive doses. Clobazam was chosen because of its relatively low sedative properties and CLN because of its use in neuropathic pain. Tolterodine (TLT) was used as an active placebo. The primary outcome parameter was a change in the area of cutaneous UVB irradiation–induced secondary hyperalgesia (ASH), which was monitored for 8 hours after drug application. Sedative effects were assessed in parallel to antihyperalgesia. Compared with TLT, recovery from hyperalgesia was significantly faster in the CBZ and CLN groups ($P = 0.009$). At the time point of maximum effect, the rate of recovery from hyperalgesia was accelerated by CBZ and CLN, relative to placebo by 15.7% (95% confidence interval [CI] 0.8-30.5), $P = 0.040$, and 28.6% (95% CI 4.5-52.6), $P = 0.022$, respectively. Active compounds induced stronger sedation than placebo, but these differences disappeared 8 hours after drug application. We demonstrate here that GAMs effectively reduce central sensitization in healthy volunteers. These results provide proof-of-principle evidence supporting efficacy of GAMs as antihyperalgesic agents in humans and should stimulate further research on compounds with improved subtype specificity.

Keywords: $GABA_A$ receptors agonists, Benzodiazepines, Clobazam, Central sensitization, Healthy volunteers, Pharmacokinetic–pharmacodynamic

1. Introduction

Pharmacological management of chronic pain is still an unmet medical need. In light of the recent progress in our understanding of the neurobiology of chronic pain states, mechanism-based approaches have been advocated.[34] In the last decade, diminished synaptic inhibition in the spinal cord has been recognized as an important contributor to central sensitization,[46–48] a key phenomenon in chronic inflammatory and neuropathic pain. According to this concept, facilitation of GABAergic inhibition in the spinal dorsal horn should be a rational approach to compensate for diminished inhibitory pain control. In fact, antihyperalgesic effects of several $GABA_A$ receptor agonists, such as muscimol, or of positive allosteric modulators of $GABA_A$ receptors, such as benzodiazepine site agonist (BDZs), have been repeatedly demonstrated in rodents.[20] New insights into the contribution of defined subtypes of $GABA_A$ receptors to different clinical effects of BDZs have suggested that their unwanted effects might be reduced through the development of agents acting only at subtypes of $GABA_A$ receptors.[24,28,49,50]

Benzodiazepine site agonist-sensitive $GABA_A$ receptors contain 1 or 2 of the following α subunits: $\alpha1$, $\alpha2$, $\alpha3$, or $\alpha5$, together with 2 β subunits and 1 $\gamma2$ subunit in a 2:2:1 stoichiometry.[2,3] Work in $GABA_A$ receptor point-mutated mice has shown that the sedative action of BDZs is mainly mediated by $GABA_A$ receptors containing an $\alpha1$ subunit ("$\alpha1GABA_ARs$"),[35] whereas $\alpha2$ and $\alpha3$-containing $GABA_A$ receptors were found to mediate the anxiolytic properties[10,22] and to be largely responsible for the spinal antihyperalgesic actions of classical BDZs.[19,30] In rodents, $\alpha1$-sparing nonsedative BDZ agonists showed antihyperalgesic activity in inflammatory and neuropathic pain models.[11] Such compounds are not yet available for use in human.

Sponsorships or competing interests that may be relevant to content are disclosed at the end of this article.

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In humans, clinical research on analgesic effects of BDZ is scarce and controversial. Clonazepam (CLN) is the most widely used BDZ in patients with neuropathic pain.[9] However, its specific effect on central sensitization has not been studied under well-defined experimental conditions in humans and its use in clinical practice is limited by adverse effects, such as sedation, memory impairment, and dependence.

Clobazam (CBZ) is a 1,5 BDZ recommended for all forms of anxiety and epilepsy. It exerted less cognitive and psychomotor side effects compared with CLN and lorazepam in a wide range of pharmacodynamic tests in healthy volunteers and patients.[14,29,38] According to recent work, CBZ displays significantly higher affinity for $\alpha 2GABA_AR$ than for $\alpha 1GABA_AR$.[18] We previously demonstrated its antihyperalgesic effect in mice,[6] and a recent exploratory study in healthy volunteers has suggested an antihyperalgesic effect in capsaicin-induced hyperalgesia.[41] Hence, CBZ seems to be a suitable compound to test the antihyperalgesic effect of $GABA_A$ receptor modulators in humans.

We present here the results from a randomized double-blind placebo-controlled and crossover pharmacokinetic–pharmacodynamic study addressing the effect of CBZ and CLN on central sensitization induced by exposure to ultraviolet B (UVB) light.

2. Methods

2.1. Study design

This randomized, double-blind, placebo-controlled, crossover design trial included a screening visit followed by 3 test sessions separated by wash-out periods of at least 2 weeks. At screening, the minimal erythema dose (MED) and cytochrome P450 2C19 (CYP2C19) genotype and phenotype were determined for all volunteers. Each of the 3 test sessions started on day 1 with the evaluation of preirradiation mechanical and thermal pain thresholds followed by a standardized UVB-exposure procedure. The dose was set to 3 times the predetermined MED. On day 2, baseline values were measured 20 (±1) hours after UVB exposure. Study drug or comparators were administered 1 hour later (**Fig. 1**). Pharmacodynamic assessments were done in a standardized sequence at baseline and 1, 2, 4, 8, 12, and 24 hours after drug administration. Pharmacokinetic samplings were done at 0.5, 1, 2, 4, 8, 12, and 24 hours after drug administration.

The trial was conducted at the Clinical Investigation Unit of the Geneva University Hospitals. Computer-generated randomization was done in blocks of 6 in a consecutive number series by an independent pharmacist. Each volunteer was allocated to 1 of the 6 possible treatment sequences comparing the study drugs CBZ, CLN, and an active placebo. UVB exposure and all pharmacodynamic outcome measurements were made by the same physician. Subjects and experimenters were blinded. The local ethics committee and the Swiss regulatory authorities approved the protocol. Healthy volunteers were recruited through local advertisement. All volunteers provided written informed consent.

2.2. Study drugs

Study drugs were repacked from commercial preparations into identical capsules and relabeled by the Pharmacy Department of the Hospital to ensure blinding. Drugs were administered orally in fasted condition. Clobazam is a 1,5 BDZ, recommended in all forms of anxiety and epilepsy. It has a 90% bioavailability. Maximal plasma concentrations are reached 2 hours after oral administration. Clobazam biotransformation into the active metabolite *N*-desmethylclobazam (NDMC) is catalyzed by CYP2C19 and CYP3A4. *N*-desmethylclobazam is inactivated by CYP2C19. Clobazam and NDMC half-lives are 20 and 50 hours, respectively.[32] Dosing was based on antiepileptic therapeutic equivalency with CLN.[23] Each volunteer received 20 mg of CBZ.

Clonazepam is a 1,4 BDZ, which is used for adjuvant therapy in patients with chronic neuropathic pain. Dosing was based on common clinical practice in pain management. Each volunteer received 1 mg of CLN.

Tolterodine (TLT) is an anticholinergic compound that lacks antinociceptive properties in humans. Tolterodine is mildly sedative and was used as an active placebo. Tolerability and manufacturing constraints determined the administered dose. Each volunteer received a standard dose of TLT (1.37 mg).

2.3. UVB-induced erythema pain model

The UVB-induced erythema pain model elicits a peripheral and a central area of stable hyperalgesia.[12] At screening, an individual MED was determined for each volunteer through a standardized and automated procedure with the Quantel handheld 308-nm excimer laser. At each session, volunteers were exposed to 3 times the predetermined MED with the same device on a 2.5 × 2.5-cm area on the volar side of the left forearm. A different area was exposed in each session. Adjacent to the local inflammatory response, an area of secondary hyperalgesia to pinprick (ASH) occurred within 20 hours after exposure.[12]

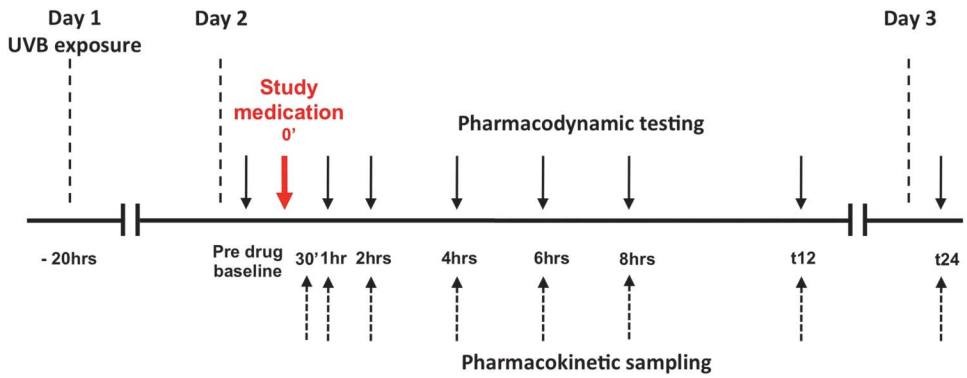

Figure 1. Study flowchart: volunteers were exposed to UVB irradiation on their forearms 20 hours before study drug administration (days 1). On day 2, predrug baseline measures were obtained and the study drug was applied. Pharmacodynamic testing and venous blood sampling were done at the indicated time points.

2.4. Volunteers

Healthy white, type III skin phototype, nonsmoker or light smoker (<10 cigarettes per day), 18- to 50-year-old, male volunteers were included. To be randomized, volunteers needed to show local secondary hyperalgesia to pinprick after UVB skin exposure at screening (see Primary endpoint for details). Exclusion criteria included any concomitant illness, current or history of drug or alcohol abuse, regular intake of alcohol exceeding 3 units a day or 21 units a week, recent psychotropic drug intake (over the preceding month), sun allergy or hypersensitivity, and current and regular intake of any drugs that might affect nociception.

2.5. Primary endpoint

The primary endpoint was the ASH, which was mapped on the skin surrounding the erythema with a hand-held probe (235-mN von Frey filament).[5,21] The standardized pressure elicited with this filament is not reported as painful under nonsensitized conditions. Volunteers were asked to keep their eyes closed while the probe was applied. Pressure was applied with the probe at regular distances (5 mm) along 8 lines starting 5 cm outside the sensitized skin and radiating back toward the center of the square-shaped erythema until the volunteer reported a definite change in sensation from normal to "different," "burning," or "unpleasant." The exact locations were marked and the mapping of the 8 points (octagon) was transferred onto parafilm, which was subsequently cut and photocopied. The total surface (octagon) was computed based on the weight of its paper format. The area of primary hyperalgesia (6.25 cm^2 of exposed skin) was subtracted from the octagon to obtain the ASH.[16,17] A null value was imputed in the case of negative values.

2.6. Secondary endpoints

2.6.1. Subjective sedation

Subjective feeling of sedation was rated by volunteers on a 0- to 100-mm visual analog scale (VAS).

2.6.2. Saccadic eye movements

Saccadic eye movements to target, assessed according to Wilson method, were used as an objective measure of sedation.[1] Surface electrodes were placed at the lateral canthus of the subjects' eyes to record eye movement-related changes in the corneoretinal potential. Volunteers were in a sitting position and instructed to track a red target appearing alternatively at random intervals and variable durations either on the left or right side of a 24'' monitor positioned 58 cm from the volunteers. The average peak velocity, latency, and relative inaccuracy were computed from a 15 saccadic eye movement sequence recorded at each time point.

2.6.3. Digital symbol substitution test

Psychomotor performance was assessed using a subscale of the Wechsler Adult Intelligence Scale.[13] The digital symbol substitution test (DSST) evaluates the ability to concentrate and identifies altered information processing performance. A series of simple calligraphic symbols are arbitrarily linked to 9 digits (1-9) according to a predefined code. Subject was instructed to associate the correct symbol to its corresponding digit over the 2-minute time. The total number of correct symbols drawn was recorded. Different versions of the test (ie, different symbol-digit codes) were used at each assessment to limit learning bias.

2.6.4. Mechanical pain thresholds

Increasing pressure was delivered manually at a constant rate with a von Frey electronic device (Bioseb, Id-Tech Bioseb; Chaville, France). Mechanical pain thresholds (MPTs) in the areas of primary and secondary hyperalgesia were defined as the least amount of pressure eliciting a painful sensation. The value was the arithmetic mean of 3 measurements.

2.6.5. Thermal perception and pain thresholds

A microcomputer-driven 9-cm^2 Peltier contact thermode, driven by a thermal sensory analyzer (Medoc Advanced Medical Systems, Ramat-Yishai, Israel), was used to identify thermal thresholds.[45] The thermode-stimulating surface was placed over the area of primary hyperalgesia and secured by a Velcro band. For each standardized sequence and for both hot and cold, the linear rate of change was set at 1°C/s, with a baseline temperature set at 32°C (temperature ranged from a minimum of 0°C to a maximum of 50°C). The cold threshold was evaluated first. Perception and pain thresholds were assessed according to the method of limits (arithmetic mean from 4 measurements).

2.6.6. Nociceptive flexion reflex

Single rectangular electrical impulses (0.5 milliseconds) were delivered through a pair of surface electrodes placed over the volunteer's sural nerve with 6- to 10-second interstimulus intervals using a constant current stimulator at variable intensities (1-100 mA) (Nicolet Viking IV; Nicolet, Madison, WI). Electromyographic responses were recorded using a second pair of surface electrodes placed over the tendon of the ipsilateral biceps femoris muscle. Response signals were considered positive if their corrected computed surface reached at least 0.15 mVms. The objective pain threshold was defined as the intensity of the current eliciting 50% positive responses in a series of 20 to 30 stimulations and was obtained by fitting Hill equation to the percentage of positive responses. Subjects were told that the sensation intensity could randomly increase, decrease, or stay the same, with stimulus repetition occurring independent of their response.[37,42]

2.6.7. Subjective pain thresholds

After electrical stimulation of the sural nerve, volunteers were asked to rate their painful sensation and affective pain experience on 2 numerical scales, ranging from nothing to very strong pricking or burning sensation (7) for the painful sensation, and from nothing (0) to unbearable pain (7), for the affective component. Thresholds were calculated using the same algorithm as described above for the nociceptive flexion reflex (NFR).

2.7. Genotyping

2.7.1. CYP2C19 genotyping

Three single-nucleotide polymorphisms of the CYP2C19 gene were genotyped, CYP2C19*2 (c.681G>A), CYP2C19*3 (c.636G>A), and CYP2C19*17 (g.-806C>T). Genomic DNA was extracted from whole blood, and genotyping was performed by real-time polymerase chain reaction using commercially available sequence-specific fluorescent probes.

2.7.2. CYP2C19 and CYP3A4 phenotyping

Microdoses of midazolam (0.1 mg) and omeprazole (2 mg), prepared by the Pharmacy Department of the Hospital, were given orally to volunteers, and plasma samples were collected 2

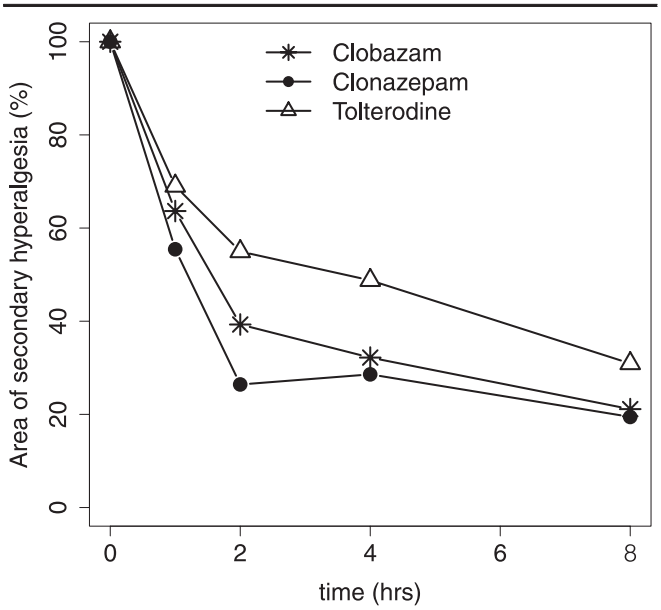

Figure 2. Relative reduction of the area of secondary hyperalgesia to pinprick (ASH) over the first 8 hours of testing. The ASH decreased by 69% in the tolterodine (TLT) arm and by 79% and 81% in the Clobazam (CBZ) and clonazepam (CLN) arms, respectively. The rate of recovery was greater with CLN when compared with TLT ($P = 0.009$).

hours after intake. CYP2C19 and CYP3A4 phenotypes were identified based on the metabolic ratios of midazolam/1-OH-midazolam and omeprazole/OH-omeprazole, respectively.[8]

2.8. Pharmacokinetic analysis

Clobazam and NDMC plasma concentrations were measured using a validated LC–MS/MS assay.[41] Blood samples (6 mL) were collected in EDTA tubes at 0.5, 1, 2, 4, 6, 8, 12, and 24 hours after drug administration. Pharmacokinetic parameters were estimated with WinNonlin version 4.1 (Pharsight Corporation, Sunnyvale, CA).

2.9. Statistical analyses

Based on the results of Ing Lorenzini et al.[16,17] and Gustorff et al.,[12] we assumed that our study drug would reduce the size of the hyperalgesic skin areas by 30% (corresponding to an effect size of 0.8). Based on this assumption, we calculated that 18 volunteers would be required to achieve a statistical significance level of 5% and a statistical power of 90%. To allow a 30% drop-out, we included and randomized 25 volunteers.

Data were expressed as means and SD for continuous outcomes. Missing data points were omitted. Linear mixed-effect models were used to assess the independent effects of time and drugs (fixed effects) adjusted on the session order factor and with a random effect on subjects. When assessing the independent effects on the primary endpoint, time was used as a continuous variable with a linear and quadratic effect (while it was used as a categorical parameter for the secondary endpoints). Differences in the ASH and secondary endpoints between 2 by 2 drugs at 2 and 8 hours after administration were assessed using the paired t test or the Wilcoxon paired test. Statistical analyses were performed using the R software (version 2.13.1, from the R Foundation for Statistical Computing, Vienna, Austria). All statistical tests were 2 sided with a significance level of 0.05.

3. Results

3.1. Phenotyping and genotyping

Twenty-five volunteers were included, and 23 completed the study. One drop out occurred because of lack of secondary hyperalgesia. The other subject withdrew consent. All but 2 volunteers were genotyped and phenotyped as CYP2C19 extensive metabolizers. One volunteer carried the CYP2C19*17/*2 variant and an omeprazole/OH-omeprazole ratio of 0.6, which may indicate an ultrarapid metabolizer (UM) genotype and phenotype. One volunteer had an omeprazole/OH-omeprazole ratio of 4.9, which is the borderline for a CYP2C19 poor metabolizer phenotype [8]. All the volunteers were CYP3A4 extensive metabolizers.

3.2. Area of secondary hyperalgesia

The UVB irradiation caused visible erythema in the UVB-exposed skin area. Before any drug intake and 20 hours after exposure, the mean (±SD) mechanical threshold in this area before was 47.5 (±30.5) g. This compared to 146.9 (±75.7) g before UVB exposure. Similarly, heat pain thresholds in this area dropped from 43.2 (±1.7)°C to 39.2 (±1.7)°C. The mean (±SD) area of secondary pinprick hyperalgesia (ASH) before drug intake was 21.15 (±19.15) cm^2. No blisters or skin damage exceeding the intended range occurred in any of the volunteers. No adverse effect of the study drugs was reported except mild sedation, which was expected.

We observed shrinking of the ASH over the first 8 hours of testing by 69% after placebo and by 79% and 81% in the CBZ and CLN

Table 1

Mean differences in changes of outcome parameters from baseline to 2 hours after drug application (evolution mean differences) between the active compounds and placebo and between clobazam (CBZ) and clonazepam (CLN).

	CBZ vs placebo	P	CLN vs placebo	P	CLN vs CBZ	P
Size of the area of secondary hyperalgesia (%)	**15.7** (0.8 to 30.5)	0.04	**28.6** (4.5 to 52.6)	0.022	**12.9** (−8.0 to 33.7)	0.214
Peak velocity, deg/s	**34.6** (9.5 to 59.8)	0.01	**54.1** (23.9 to 84.2)	0.001	**31.6** (4.6 to 58.6)	0.024
Feeling sedated VAS, mm	**10.4** (1.1 to 21.4)	0.074	**26.3** (15 to 37.7)	<0.001	**15.9** (2.4 to 29.4)	0.023
DSST, number of words	**3.2** (−3 to 9.3)	0.296	**11** (4.5 to 17.5)	0.002	**7.9** (1.2 to 14.6)	0.023
Primary hyperalgesia, kPa/cm^2	**7** (−3.4 to 17.4)	0.177	**−6.8** (−19.3 to 5.7)	0.0268	**13.8** (−0.2 to 27.2)	0.053
Cold perception thresholds, °C	**0.4** (−0.2 to 1)	0.169	**0.5** (−0.1 to 1.2)	0.094	**0.1** (−0.5 to 0.7)	0.665
Heat perception thresholds, °C	**−0.5** (−1 to −0.1)	0.030	**−0.4** (−1.2 to 0.4)	0.264	**0.1** (−0.6 to 0.7)	0.841
Heat pain thresholds, °C	**−0.4** (−1 to −0.3)	0.268	**−1.2** (−2.5 to 0)	0.048	**0.9** (−0.1 to 1.8)	0.077
NFR threshold, mA	**−1.4** (−6 to 3.3)	0.541	**0** (−4.5 to 4.5)	0.992	**1.4** (−2.3 to 5.1)	0.453

Data are expressed as evolution mean differences (95% confidence interval) between 2 × 2 drugs. Statistical analyses were made using the paired *t* test.
DSST, digital symbol substitution test; NFR, nociceptive flexion reflex; VAS, visual analog scale.

arms, respectively (**Fig. 2**). ASH was not significantly different between the 3 drugs at baseline. The time effect was significant for all 3 drugs with a significant negative slope and significant positive quadratic effect, indicating that there was a significant reduction with time and that this reduction was significantly slowing over time, The reduction in ASH was significantly faster under CLN compared with TLT (difference in slope was −3.5, $P = 0.009$).

Differences between the effect of the active compounds and the placebo were maximal at 2 hours after drug administration. At this time point, the absolute mean differences of the ASH relative reduction from baseline, between active compounds and placebo were 15.7% (95% confidence interval [CI] 0.8-30.5), $P = 0.040$, and 28.6% (95% CI 4.5-52.6), $P = 0.022$, for CBZ and CLN, respectively (**Table 1**). For both compounds, this difference decreased to 10% within 8 hours, which was no longer statistically significant.

3.2. Sedation

Objective sedation measured by saccadic peak velocity and subjective sedation reported on a VAS demonstrated that CBZ and CLN were more sedative than placebo at t = 2 hours (**Fig. 3**). The mean differences in the absolute decrease of peak velocity at t = 2 hours between active compounds and placebo were 34.6 (95% CI 9.5-59.8) deg/s, $P = 0.010$, and 54.1 (95% CI 23.9-84.2) deg/s, $P = 0.001$, for CBZ and CLN, respectively. The mean differences of "feeling sedated" on a VAS between the same time points were 10.4 (95% CI 1.1-21.4) mm, $P = 0.074$, and 26.3 (95% CI 15.0-37.7), $P < 0.001$ (**Table 1**). Significant differences in sedation between treatment groups were no longer apparent 8 hours after drug application with the exception of CLN, which still impaired saccadic eye movements at this time point (mean decrease of the peak velocity 29 [95% CI 7.6-50.3] deg/s, $P = 0.011$). At 2 hours after drug application, DSST performance was significantly impaired only by CLN (mean difference of recalled words between CLN and placebo, 11 [95% CI 4.5-17.5], $P = 0.002$; **Table 1**).

3.3. Peripheral sensitization and nociceptive flexion reflex

Very small but clinically irrelevant changes in thermal and MPTs in the area of primary hyperalgesia were observed in the CLN arm. The NFR was not changed (**Table 1**).

3.4. Pharmacokinetic data

The mean plasma concentration-vs-time profiles of CBZ and NDMC are shown in **Figure 4**. The pharmacokinetic data were best described using a noncompartmental model (**Table 2**). Inclusion of the values of the UM and PM volunteers did not change the mean PK parameters values.

4. Discussion

We demonstrated here that in healthy volunteers, CBZ and CLN, at equipotent doses, chosen on the basis of their anticonvulsant effect, decreased UVB-induced secondary hyperalgesia. This finding is in line with previous results from our group, reporting an antihyperalgesic effect of CBZ in the chronic constriction injury model in mice[6] and with a recent exploratory study suggesting an antihyperalgesic effect of CBZ and CLN on capsaicin-induced central sensitization in healthy volunteers.[41]

As expected, CLN and, to a lesser extent, CBZ had significant initial effects on objective and subjective measures of sedation. The DSST performance results followed the same trend but were less consistent, probably due to learning effects. The smaller sedative effect of CBZ is consistent with a recent report that compared the affinities of CBZ, NDMC, and zolpidem to α1GABA$_A$Rs and α2GABA$_A$Rs.[18] According to this study, CBZ and NDMC have higher affinities to α2GABA$_A$Rs than to α1GABA$_A$Rs, whereas CLN has similar affinities to both subtypes, and zolpidem binds α1GABA$_A$Rs with higher affinity than α2GABA$_A$Rs. Therefore, CBZ seemed to be a suitable tool compound to further study the role of GABA$_A$ receptors in human pain pathways.

Maximum antihyperalgesic effects occurred 2 hours after drug administration. This time point corresponds to the maximal plasma levels of CBZ. In mice, we have previously shown that the antihyperalgesic effect of CBZ correlated best with plasma levels, when both CBZ and its main metabolite NDMC were taken into account.[6] A substantial contribution of NDMC to antihyperalgesia also seems likely because NDMC and CBZ cause a similar dose-dependent enhancement of GABA-activated currents in cultured

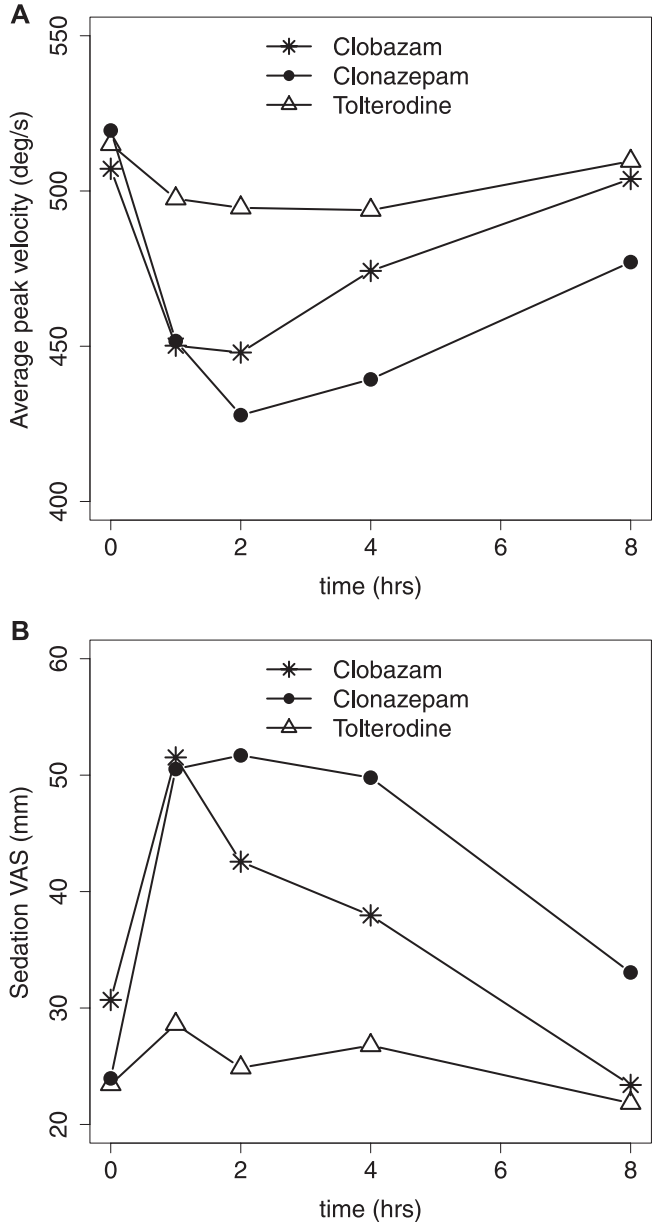

Figure 3. Objective (A) and subjective (B) sedation over the first 8 hours of testing. (A) Mean change in peak velocity (deg/s) over time. (B) Mean change in "feeling sedated" on a visual analog scale (mm) ranging from 0 to 100 mm over time.

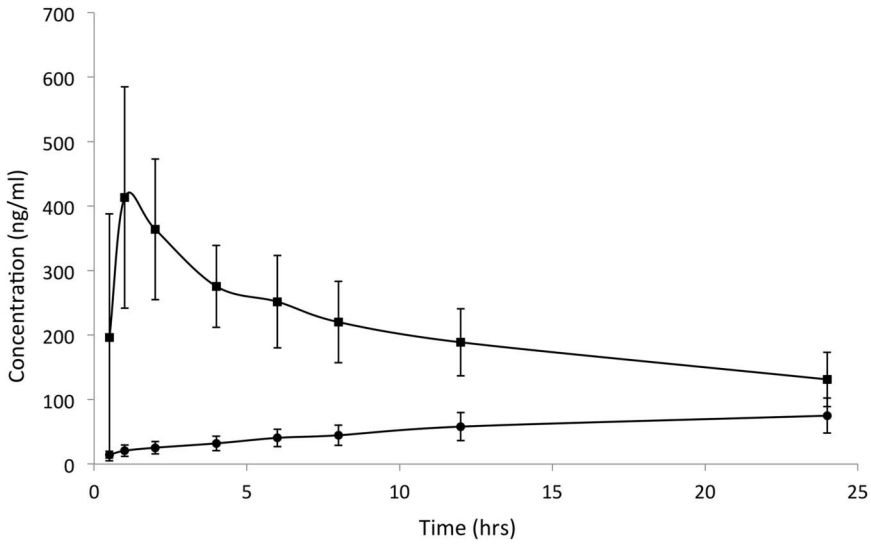

Figure 4. Clobazam (CBZ) and *N*-desmethylclobazam (NDMC) plasma concentration vs time CBZ was given at a dose of 20 mg orally. Mean plasma concentrations (±SD) of CBZ (■) and NDMC (●) were obtained from 23 volunteers at the indicated time points.

rat cerebral neurons.[26] At subsaturating concentrations, NDMC may exert even stronger effects on spinal $GABA_A$Rs than CBZ, as NDMC's affinity to $\alpha 2GABA_A$Rs, which predominate in the spinal cord,[31] is higher than that of CBZ.[18] In this study, the duration of secondary hyperalgesia was too short to verify the contribution of NDMC to antihyperalgesia because NDMC plasma levels rose only slowly and reached maximum values only after 23.5 (±2.4) hours when ASH was no longer detectable. Future studies should be designed to specifically assess the contribution of NDMC to antihyperalgesic and sedative effects in humans.

Clobazam and NDMC are metabolized mainly by CYP3A4 and CYP2C19. To investigate whether variants of the activity of these enzymes contributed to the variability in plasma concentrations, subjects were genotyped and phenotyped for these 2 enzymes. Only 2 volunteers had genotypic and phenotypic results suggesting abnormal CYP2C19 activity. However, their CBZ and NDMC plasma concentrations still fell within the range of extensive metabolizers, and therefore their pharmacodynamic responses, after a single administration, were not affected.

Clobazam and CLN did not significantly affect primary hyperalgesia when assessed by thermal and mechanical stimulations applied to the UVB-exposed skin. This finding is consistent with the lack of a peripheral antihyperalgesic action of BDZs. The lack of effect on the NFR is in line with results obtained in mice that showed that spinally applied BDZ lack analgesic efficacy against acute nociceptive pain[19] and with previous human studies that found no effect on the NFR in healthy volunteers after 4 days of pretreatment with low-dose diazepam compared with placebo.[43]

One limitation of this study is related to the variability and rather short duration of ASH, compared with the results of previous work.[12,16,17] Several explanations may be considered. First, the interindividual variability of ASH was high although the UVB dose was adjusted according to the individual MED,[7,12] and therefore its stability could have been overestimated in the past. Second, the area of primary hyperalgesia found in this study was similar to that described in previous reports with a dramatic decrease in the thermal and mechanical thresholds after radiation and before any drug intake. However, to obtain an ASH stable for 10 hours, Gustorff et al.[12] irradiated a larger spot (5-cm-diameter spot) on the upper part of the leg, leading to a mean ASH, which had twice the size of that found in our study (59.95 [±16.45] vs 21.15 [±19.15] cm^2). Third, ASH was mapped with a stronger force von Frey filament that was used in previous studies (150 g). Smaller discriminating power of repeated 150-g pressure stimuli may have obscured changes in ASH, because in our experience, the repeated use of a 110-g stimulus turned out to be already painful in nonirradiated skin. Fourth, we used TLT as an active placebo to ensure the initial double-blinding regarding sedation. Although anticholinergic drugs are not known to be antihyperalgesic in humans, an effect on ASH cannot fully be excluded. Finally, potentially higher skin temperatures at the thigh as compared with the forearm may have led to stronger and more prolonged UVB-induced secondary hyperalgesia in previous studies that used the thigh instead of the forearm.

Another potentially more important limitation pertains to the concept of pain models. Recent discussions have raised concerns about differences between stimulus-evoked pain and spontaneous pain in the study of chronic pain.[4,39,40] None of the pain models used to date in healthy volunteers mimics all the clinical features of chronic inflammatory or neuropathic pain.[39] Furthermore, a study on spontaneous pain in an experimental human pain model suggested that mechanisms underlying spontaneous pain and sensitization may actually differ and that commonly used sensitization tests may not be good predictors for effects on spontaneous pain.[36] This debate underlines the

Table 2

Pharmacokinetic parameters of clobazam (CBZ) and *N*-desmethylclobazam (NDMC) in whole blood, after 20 mg intake.

Pharmacokinetic parameters	CBZ	NDMC
$T_{1/2}$, h	23.9 (±9.5)	NC
T_{max}, h	1.3 (11.1)	23.5 (±2.4)
C_{max}, ng/mL	486.8 (190.9)	73.7 (±26.7)
AUC, h·ng/mL	9682* (±4230)	1234† (±431)

Data are expressed as mean (±SD).
* AUC_{inf}.
† AUC_{last}.
NC, not calculated.

difficulty of translational approaches, and stresses the need for validating potential drug targets in patients instead of healthy volunteers.[39]

The present proof-of-concept study demonstrates that facilitation of GABAergic synaptic transmission has a statistically significant effect on experimental hyperalgesia in humans. These results are in good agreement with preclinical studies that have demonstrated pronounced antihyperalgesic activity of diazepam in genetically modified mice, which had been rendered resistant to its sedative effects,[19,20,30,44] and in non- or less-sedative BDZ ligands in wild-type mice and rats.[11,15,19,20,25,27,33] Our finding of a weak antihyperalgesic activity in humans at mildly sedating doses is consistent with the idea that dose-limiting sedation underlies the lack of clinically relevant antihyperalgesia by classical BDZs in patients. Our data hence provide an additional impetus for the development of subtype-selective nonsedative BDZ site agonists for potential use in patients with pain. At present, it remains unknown what degree of antihyperalgesia can be achieved in humans with such drugs. Furthermore, it is unknown whether the antihyperalgesic potential described here predicts analgesic efficacy for treating spontaneous ongoing pain (see also Ref. 36).

Taken together, our data suggest that facilitation of GABAergic inhibition by agonists at the BDZ binding site of $GABA_A$ receptors may be a promising yet hitherto unexploited analgesic strategy. It will be interesting to see if future studies with improved nonsedating BDZs support this concept.

Conflict of interest statement

The authors have no conflicts of interest to declare.

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