

ORIGINAL ARTICLE

Analgesic effect of clobazam in chronic low-back pain but not in experimentally induced pain

J. Schliessbach¹, P.H. Vuilleumier¹, A. Siegenthaler², L. Bütikofer³, A. Limacher³, P. Juni⁴, H.U. Zeilhofer^{5,6}, L. Arendt-Nielsen⁷, M. Curatolo⁸

1 University Department of Anesthesiology and Pain Medicine, Inselspital, Bern University Hospital, Bern, Switzerland

2 Chronic Pain Management, Lindenhof Group Bern, Lindenhof Hospital, Bern, Switzerland

3 Clinical Trials Unit Bern, Department of Clinical Research and Institute of Social and Preventive Medicine, University of Bern, Bern, Switzerland

4 Department of Medicine, Applied Health Research Centre, University of Toronto, Toronto, ON, Canada

5 Institute of Pharmacology and Toxicology, University of Zurich, Zurich, Switzerland

6 Institute of Pharmaceutical Sciences, ETH Zurich, Zurich, Switzerland

7 Centre of Sensory Motor Interaction SMI, School of Medicine, University of Aalborg, Aalborg, Denmark

8 Department of Anesthesiology and Pain Medicine, University of Washington, Seattle, WA, USA

Correspondence

Juerg Schliessbach

E-mail: juerg.schliessbach@insel.ch

Funding sources

This study was funded by the Swiss National Science Foundation SNF in the context of the Special Program for University Medicine SPUM 33CM30_140339.

Conflict of interest

None declared.

Accepted for publication

11 February 2017

doi:10.1002/ejp.1032

Abstract

Background: Chronic pain is frequently associated with hypersensitivity of the nervous system, and drugs that increase central inhibition are therefore a potentially effective treatment. Benzodiazepines are potent modulators of GABAergic neurotransmission and are known to exert antihyperalgesic effects in rodents, but translation into patients are lacking. This study investigates the effect of the benzodiazepine clobazam in chronic low-back pain in humans. The aim of this study is to explore the effect of GABA modulation on chronic low-back pain and on quantitative sensory tests.

Methods: In this double-blind cross-over study, 49 patients with chronic low-back pain received a single oral dose of clobazam 20 mg or active placebo tolterodine 1 mg. Pain intensity on the 0–10 numeric rating scale and quantitative sensory tests were assessed during 2 h after drug intake.

Results: Pain intensity in the supine position was significantly reduced by clobazam compared to active placebo (60 min: 2.9 vs. 3.5, $p = 0.008$; 90 min: 2.7 vs. 3.3, $p = 0.024$; 120 min: 2.4 vs. 3.1, $p = 0.005$). Pain intensity in the sitting position was not significantly different between groups. No effects on quantitative sensory tests were observed.

Conclusions: This study suggests that clobazam has an analgesic effect in patients with chronic low-back pain. Muscle relaxation or sedation may have contributed to the effect. Development of substances devoid of these side effects would offer the potential to further investigate the antihyperalgesic action of GABAergic compounds.

Significance: Modulation of GABAergic pain-inhibitory pathways may be a potential future therapeutic target.

1. Introduction

Agonists at the benzodiazepine binding site of GABA_A receptors (short: benzodiazepines) have been used to treat low-back pain since their introduction on the market in the early 1960s (Hingorani, 1966;

Moll, 1973), but this historical interest was mainly related to their muscle-relaxant properties. It was not until 20 years later that they were ascribed a genuine analgesic action at a spinal level in rats and humans (Goodchild and Noble, 1987; Goodchild and

Serrao, 1987). Edwards et al. 1990 subsequently demonstrated that this effect was mediated by the benzodiazepine-GABA receptor complex in the spinal cord. However, despite promising initial results, benzodiazepines have never gained as wide an acceptance in pain therapy as other drugs, likely due to their liability to addiction and tolerance development and their sedative side effects.

In the past few years, advances in pain research have again attracted attention on spinal inhibitory pathways. A loss of inhibitory GABAergic and glycinergic transmission plays a key role in inflammatory and neuropathic pain states (Zeilhofer, 2008). These are frequently accompanied by exaggerated pain sensation like hypersensitivity or allodynia, and spontaneous pain (Curatolo et al., 2006). As benzodiazepines facilitate GABAergic inhibition through positive allosteric modulation of GABA_A receptors, they should be able to compensate for this loss of inhibition. Initial studies have indeed shown that benzodiazepines reverse hyperalgesia in rodent models of inflammatory and neuropathic pain but do not alter pain perception in uninjured tissues (Knabl et al., 2008, 2009). These effects have subsequently been attributed to α 2-subunit containing GABA_A receptors (Knabl et al., 2008), which are devoid of sedative effects, while sedation is mainly mediated by the α 1-subunit containing receptors (Rudolph et al., 1999).

This growing body of evidence provides a new rationale for the use of drugs that enhance GABAergic transmission, especially when a state of central hypersensitivity is present that might favourably respond to such inhibitory modulation. Since central hypersensitivity is quite common in chronic pain patients with a point prevalence of 18%–35% (Schliessbach et al., 2013), at least a part of patients can be expected to benefit from antihyperalgesic treatment by GABA modulation.

Recent work in genetically modified mice indicates that spinal antihyperalgesic actions of benzodiazepines only occur at significantly higher doses than sedative effects (Ralvenius et al., 2015). It is likely that subtype-selective (i.e. α 1-sparing) compounds will overcome this limitation, but such compounds are not yet available for use in humans. Clobazam is a 1-5-benzodiazepine that – albeit not α 2-specific – is less sedative than the more common 1-4-benzodiazepines (Wildin et al., 1990) and produces antihyperalgesia without strong sedation in mice (Besson et al., 2013). The aim of the present study was therefore to investigate for the first time the analgesic effects and side effects of clobazam in

chronic low-back pain patients and to examine its effects on the pain system using quantitative sensory tests. Positive results would encourage further research on the use of GABAergic compounds that are devoid of sedation and tolerance for the treatment of chronic pain.

2. Methods

2.1 Patients and setting

This study was part of a larger project investigating the efficacy of different drugs in chronic low-back pain, the study protocol of which has been published (Siegenthaler et al., 2015). This randomized, placebo-controlled, double-blinded cross-over study was carried out at the University Department of Anesthesiology and Pain Medicine of the Inselspital Bern, Switzerland, according to good clinical practice guidelines and the Helsinki Declaration. The study was approved by the local ethics committee (KEK 213/09) and registered with clinicaltrials.gov (NCT01179828). Patients were recruited by advertisement in local newspapers and gave written informed consent prior to the tests. Consecutive patients aged between 18 and 80 years with chronic low-back pain of at least 3 months duration were included. Exclusion criteria were pain intensity at rest $<3/10$ on the numerical rating scale (NRS) at the time of testing (whereby 0 = no pain and 10 = worst pain imaginable), suspected radicular pain (as defined by leg pain associated with an MRI finding of a herniated disc or foraminal stenosis), signs or suspicion of neurological dysfunction at the tested sites, pregnancy (as assessed by pregnancy test), breast feeding, ongoing treatment with an antidepressant, opioid or anticonvulsant, intake of centrally active substances (including drug or alcohol abuse), known allergy or pharmacological contraindications to clobazam or tolterodine (active placebo), multisite or widespread pain, systemic inflammatory or rheumatological disease, and major depression (Beck Depression Inventory short form score >9). Current analgesic medication had to be stopped 1 week before the first experiment. Only acetaminophen and ibuprofen were allowed as rescue medication until 24 h before the experiment. Patients unable to stop their analgesic regimen were not recruited.

2.2 Study medication

A single oral dose of clobazam 20 mg was compared to 1 mg of the active placebo tolterodine. Tolterodine

is an anticholinergic drug used to treat hyperactive bladder disorders. It is a specific antagonist at muscarinic M2- and M3-receptors and should be devoid of any analgesic effects, but mimics some of the sedative side effects such as blurred vision, drowsiness and sleepiness. This allowed for better blinding of patients and investigators than an ineffective placebo. Drugs were concealed by the hospital pharmacy using red-coloured hard gelatin capsules (LGA, La Seyne sur Mer, France) and packed in semiopaque plastic flasks labelled with the subject number, session number, lot number and expiry date. Neither the subject nor the investigators were aware of which flask contained which substance. Randomization was as well performed by the hospital pharmacy using a computer-generated random list. All pharmaceutical processes strictly followed the good manufacturing practice (GMP) guidelines.

2.3 Experimental procedure

2.3.1 General aspects

The experiment took place in two sessions separated by a minimum wash-out period of 1 week. All tests were performed on the more painful body side. In case of bilateral pain or pain in the midline, the test side was randomly selected. Patients were placed comfortably in the supine position in a quiet room.

2.3.2 Pain intensity

The intensity of low-back pain was rated on a 0–10 NRS (0 = no pain, 10 = worst pain imaginable) in both sitting and supine position at baseline and in intervals of 30 min after drug intake for up to 2 h. A patients global impression of change scale (PGIC) was assessed at the same time by a seven-point Likert scale ranging from '1 = very much improved' over '4 = no change' to '7 = very much worse'. Nausea, dizziness and fatigue were assessed on a 0–10 NRS as well.

2.3.3 Quantitative sensory tests (QST)

Measurements were performed in the following order: pressure pain detection threshold (PPDT), pressure pain tolerance threshold (PPTT), electrical single-stimulus pain threshold (ESPT), electrical repeated-stimulus pain threshold (ERPT – assessing temporal summation), pain intensity on electrical train-of-twenty stimulation (0–10 NRS), heat pain detection threshold (HPDT), heat pain tolerance threshold (HPTT) and cold pain detection threshold

(CPDT). Conditioned pain modulation (CPM) was assessed using an electrical train-of-five burst at an intensity 1.2 times the ERPT as the test stimulus and the cold pressor test at the contralateral hand as the conditioning stimulus. With the exception of electrical train-of-twenty stimulation, triplicate measurements were made. The measurements were performed at baseline as well as 60 and 120 min after drug intake.

2.3.3.1 Pressure pain detection and tolerance thresholds (PPDT and PPTT). PPDT and PPTT were recorded using an electronic pressure algometer (Somedic AB, Horby, Sweden) with a probe tip of 1 cm². Pressure was increased at a rate of 30 kPa/s up to a maximum of 1000 kPa. The subject stopped the measurement by pressing a button when the sensation started to be painful (PPDT) and when the painful sensation became intolerable (PPTT), respectively. Both PPDT and PPTT were recorded in intervals of 1 minute between measurements.

2.3.3.2 Electrical single and repeated pain thresholds (ESPT and ERPT). ESPT and ERPT were performed using a computer-controlled constant current stimulator (Digitimer Ltd, Welwyn Garden City, UK). Bursts of five 1 ms square wave impulses within 25 ms (perceived as one single stimulus) were delivered via 2 AgCl electrodes placed distal to the lateral malleolus in the innervation area of the sural nerve. The current intensity was increased from 1 mA in steps of 0.5 mA until the sensation was rated as painful (ESPT). For ERPT, the stimuli were repeated five times at a frequency of 2 Hz. Current intensity of all 5 stimuli was increased in steps of 0.5 mA until the last 2–3 stimuli were perceived as painful, indicating temporal summation threshold.

2.3.3.3 Electrical train-of-twenty. The arithmetical mean of three ERPT assessments at baseline was used to deliver 20 identical stimuli over 10 s with a frequency of 2 Hz. This stimulus intensity remained constant over the two subsequent measurements at 60 and 120 min. Subjects rated the maximal and final pain intensity during this stimulation on a 0–10 NRS. A decrease in pain intensity in the subsequent measurements would be indicative of an analgesic effect and a difference between maximal and final pain intensity during the 20 stimulations was considered a feature of endogenous pain modulation. Patients whose pain ratings decreased during the train-of-twenty stimulation were defined

as T20 decreaseers in contrast to those whose pain ratings remained constant over all 20 stimuli.

2.3.3.4 Temperature pain thresholds (HPDT, HPTT, CPDT). Temperature pain thresholds were assessed using a thermode (TSA II, Medoc, Ramat Yishai, Israel) with a probe surface of 3×3 cm. All measurements started at 30.0°C , and the rate of temperature change was $1^{\circ}\text{C}/\text{s}$. Subjects stopped the measurements by pressing a button when the warm sensation turned to pain (HPDT) or when the pain became intolerable (HPTT) or when the cold sensation started to become painful (CPDT). In any case, the measurements were stopped at a temperature of 50.5°C for HPTT or 0°C for CPDT respectively. Measurement sites were the lateral aspect of the lower leg (dermatome L5) and the radial surface of the proximal forearm (dermatome C6). For modelling, CPDT was dichotomized into those that did not feel pain until 0°C ('at limit') and those that interrupted the measurement due to pain above 0°C ('above limit').

2.3.3.5 Conditioned pain modulation (CPM). CPM was assessed using the cold pressor test at the hand contralateral to the tested side. Subjects immersed their hand in ice saturated water ($1.5 \pm 1^{\circ}\text{C}$), until the cold pain reached an intensity of 7/10 on the NRS. Five electrical stimulations at an intensity 1.2–1.5 times the ERPT were delivered three times in intervals of 10 s and rated by the subject on a 0–10 NRS. This was performed before and during the cold pressor test. A decrease in pain ratings during the cold pressor test was considered an indication of CPM.

2.3.4 Descriptive variables

The following descriptive variables were assessed on a questionnaire before the first study session: age, sex, body mass index (BMI), pain duration in years, history of surgery due to the painful condition, average pain intensity during the last 24 h on a 0–10 NRS, pain-related life interference from the multidimensional pain inventory (MPI), catastrophizing scale and Beck Depression Inventory (BDI).

2.3.5 Statistical analysis

Primary outcome measure was the intensity of low-back pain on a 0–10 NRS 2 h after drug intake. The proportion of drug responders in both

arms was used as a secondary outcome measure. Drug responders were defined as having $>30\%$ pain reduction, which is usually considered a clinically meaningful effect. Changes in pain thresholds as assessed by the sensory measurements and drug-induced side effects were additional outcome measures.

Continuous and ordinal variables that were roughly normally distributed (NRS and CPM) or normally distributed after log-transformation (PPDT, PPTT, ESPT and ERPT) were analysed by linear mixed models with treatment group, time point and their interaction as covariates. The models were adjusted for baseline values and treatment phase (clobazam first vs. placebo first) in order to account for a possible learning effect. A carry-over effect was excluded by design (wash-out period between the phases) and was not tested for. A random intercept was added for each subject (to account for intrasubject correlation) and a random intercept and slope for each subject in each treatment phase (to account for repeated measures). Correlations between subsequent measurements were modelled with a first order autoregressive correlation structure. The treatment effect was calculated over all time points (joint p -value) and at each time point based on marginal models and is presented as mean difference or geometric mean ratio (if data were log-transformed). Margins are statistics calculated from predictions of a previously fit model at fixed values of some covariates and averaging over the remaining covariates. Margins were calculated at each time point and contrasted for clobazam and placebo. Correction for multiple testing was not attempted due to the hypothesis-generating nature of the study.

Dichotomous variables (drug responders, T20-decreaseers, dichotomized CPDT) were modelled by separate logistic GEEs (generalized estimating equations) at each time point with treatment group and phase as covariates and subject ID as panel variable. The treatment effect at each time point is presented as odds ratio. HPDT and HPTT which were truncated at 50.5°C were analysed by separate mixed tobit regression models at each time point with treatment group and phase as covariates and subject ID as panel variable. The treatment effect at each time point is presented as mean difference. Sample size considerations were made for the primary outcome parameters of the main project and are published in the study protocol (Siegenthaler et al., 2015). Statistical analyses were performed using STATA (STATA Corp., College Station, TX, USA).

3. Results

3.1 Effect on low-back pain

A total of 49 patients completed the experiment; their characteristics are shown in table 1. A detailed flow chart displaying recruitment and randomization can be found in the study protocol (Siegenthaler et al., 2015). A significant effect on pain of clobazam compared to placebo was observed in the supine position (joint $p = 0.036$ over all time points, treatment effect at 120 min: -0.68 on a 0–10 NRS, 95%-CI: -1.15 to -0.21 , $p = 0.005$). Substantial pain reduction was as well observed in the sitting position over time, but with no difference between clobazam and placebo (joint $p = 0.450$ over all time points, treatment effect at 120 min: -0.09 on a 0–10 NRS, 95%-CI: -0.56 to 0.37).

Twenty-nine patients (62%) responded to clobazam after 120 min, and twenty (43%) to placebo in the supine position (OR: 2.17, 95% CI 1.00 to 4.74, $p = 0.051$). In the sitting position, there was no significant difference between clobazam and placebo. The time course of pain reduction and proportion of drug responders are illustrated in Figure 1A–D, and the pain ratings and treatment effects across all time points are displayed in Table 2 for both supine and sitting position.

After 120 min, the PGIC decreased to 3.4 in both arms (95% CI: 3.2–3.7 for clobazam and 3.2–3.6 for placebo), thus showing a slight trend to improvement for both clobazam and placebo with no differences between drugs (joint $p = 0.389$).

3.2 Side effects

Despite the use of an active placebo, fatigue and dizziness occurred more frequently with clobazam

(Table 3). A total of 33 and 20 patients reported some degree of fatigue and dizziness, respectively, in the clobazam session compared to only 22 and 8 patients in the tolterodine session ($p = 0.052$ and $p < 0.001$, respectively). However, the intensity estimates on the 0–10 NRS were low, the maximal value being 1.8 for fatigue in the clobazam experiment. In order to examine the influence of side effects on drug efficacy, a linear mixed model with additional adjustment for nausea, fatigue and dizziness was fitted (Table 4). A trend to better effect of clobazam compared to placebo could still be observed 1 h after drug intake ($p = 0.058$) and became significant after 2 h (treatment effect -0.59 on the 0–10 NRS, 95% CI: -1.09 to -0.09 , $p = 0.02$). Again, this effect could only be demonstrated for pain in the supine position. However, the joint p -value was not significant anymore after adjustment for side effects ($p = 0.156$).

3.3 Quantitative sensory tests

No significant effects of clobazam could be observed in any of the sensory tests. The detailed results of the measurements are shown in Table S1 and the time courses of electrical and pressure tests are displayed in Figure 2.

4. Discussion

4.1 The results in the context of existing literature

This study demonstrates that a single oral dose of clobazam 20 mg was more effective than placebo in reducing chronic low-back pain. This effect was only observed in the supine and not in the sitting position. Furthermore, none of the mechanistic experimental pain measures (QST) were significantly influenced by clobazam compared to placebo.

Several animal studies have demonstrated that GABA agonists reverse pain and hyperalgesia in inflamed tissues or after nerve injury, but leave pain thresholds of healthy tissues unchanged (Knabl et al., 2008; Zeilhofer et al., 2009; Di Lio et al., 2011; Besson et al., 2013). This has led to the assumption that benzodiazepines by themselves do not act as analgesics. Instead, they increase synaptic inhibition in pathologically altered (i.e. sensitized) neuronal circuits and hence exert an antihyperalgesic effect.

Two earlier studies have investigated this hypothesis in humans (Vuilleumier et al., 2013; Besson

Table 1 Patient characteristics. Continuous/ordinal data are presented as means and standard deviations, binary data as numbers and percentages.

Number of patients	49 ^a
Age (years)	54.3 ± 15.8
Sex (female)	29 (59%)
BMI (kg/m ²)	27.0 ± 4.6
Pain duration (years)	12.1 ± 11.7
Previous surgery due to pain (yes)	8 (17%)
Average NRS (0–10)	5.3 ± 1.9
Impairment of daily life (0–10)	2.8 ± 1.4
Catastrophizing score (0–5)	1.9 ± 1.3
Beck Depression Inventory (0–21)	2.6 ± 2.3

NRS, numeric rating scale.

^aOne patient only completed clobazam phase.

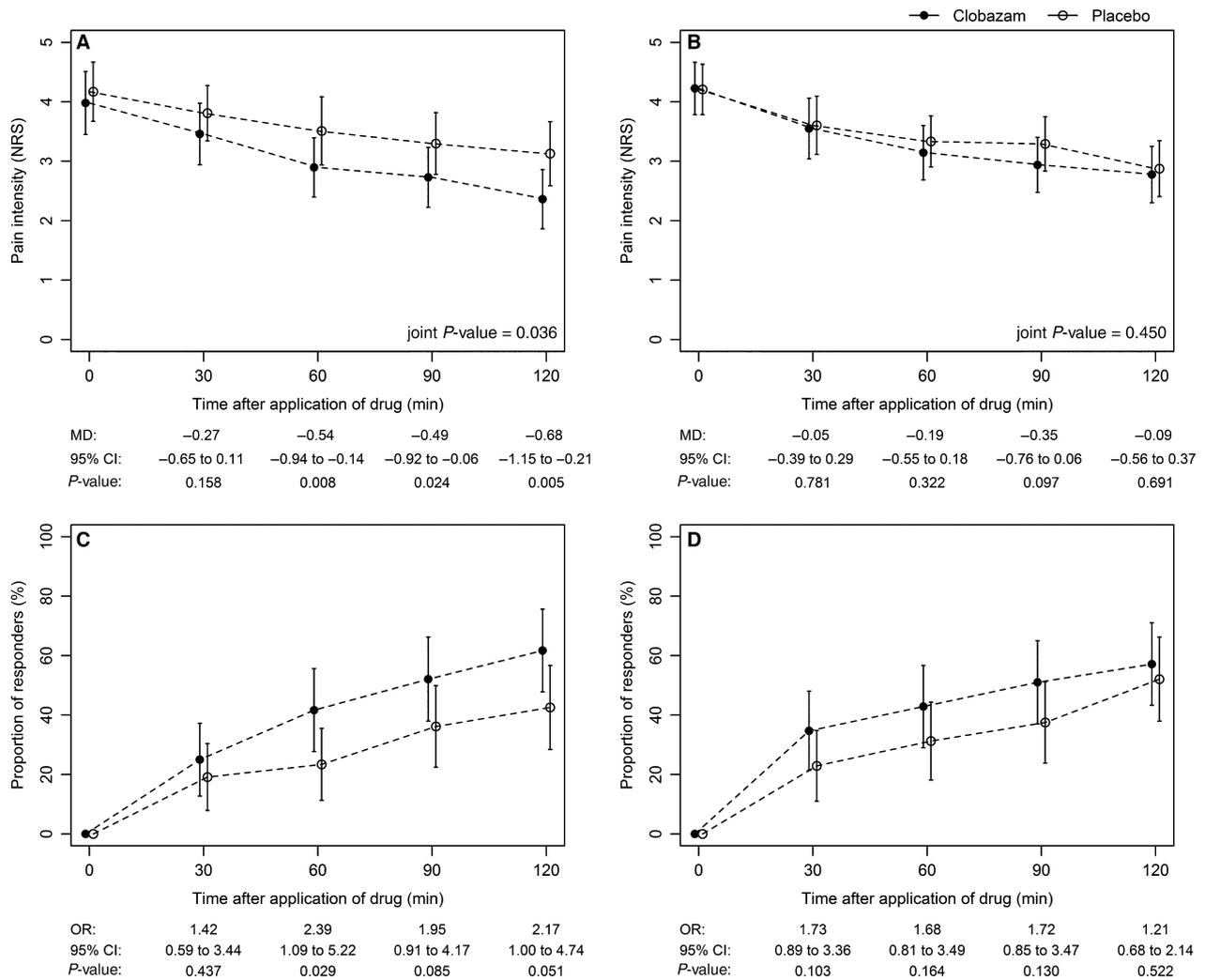


Figure 1 Effect of clobazam versus placebo on (A and B) pain intensity (0–10 numeric rating scale, NRS) and on (C and D) the proportion of responders (reduction in NRS >30%) in (A and C) supine or (B and D) sitting position. MD, mean difference; OR, odds ratio.

et al., 2015). Both of them compared clobazam and clonazepam to tolterodine in experimental models of inflammation and hyperalgesia. Vuilleumier et al. performed intradermal capsaicin injection and Besson et al. used a model of experimental sunburn injury at the forearm. The former study found that the area of capsaicin-induced hyperalgesia increased over time in the tolterodine session, but both clobazam and clonazepam seemed to abolish this increase in hyperalgesia. In the latter study, the area of sunburn hyperalgesia decreased significantly faster with both clobazam and clonazepam compared to tolterodine. These findings support the notion that benzodiazepines exert an antihyperalgesic effect. In both studies, remotely measured QST, such as nociceptive withdrawal reflex of the leg, pressure pain thresholds at the toe or

cutaneous electrical thresholds at the ankle remained unchanged. This confirms to some extent the hypothesis that benzodiazepines do not affect pain thresholds in uninjured tissues. Only heat pain threshold at the site of sunburn injury was influenced by clonazepam in Besson’s study. In the present study, all QST were performed at the leg or forearm, where tissue injury or inflammation was unlikely to be present. For other benzodiazepines, e.g. tetrazepam, there is some evidence from earlier randomized controlled trials (Arbus et al., 1990; Salzmann et al., 1992), showing that tetrazepam was superior to placebo in terms of pain relief and functional improvement. This effect was ascribed to the muscle relaxant properties of tetrazepam. Whether an antihyperalgesic effect of the drug was responsible as well, was not discussed at that time.

Table 2 Pain ratings and treatment effects for clobazam versus active placebo over time. Responders were defined as having >30% pain reduction on a 0–10 numeric rating scale (NRS, 0 = no pain, 10 = worst pain imaginable).

	Time (min)	Clobazam Mean (95% CI)	Placebo Mean (95% CI)	Treatment effect ^b		
				Difference (95% CI)	p-value	Joint p-value
NRS (supine)	30	3.5 (2.9, 4.0)	3.8 (3.3, 4.3)	−0.27 (−0.65, 0.11)	0.158	0.036
	60	2.9 (2.4, 3.4)	3.5 (2.9, 4.1)	−0.54 (−0.94, −0.14)	0.008	
	90	2.7 (2.2, 3.2)	3.3 (2.8, 3.8)	−0.49 (−0.92, −0.06)	0.024	
	120	2.4 (1.9, 2.9)	3.1 (2.6, 3.7)	−0.68 (−1.15, −0.21)	0.005	
NRS (sitting)	30	3.6 (3.0, 4.1)	3.6 (3.1, 4.1)	−0.05 (−0.39, 0.29)	0.781	0.450
	60	3.1 (2.7, 3.6)	3.3 (2.9, 3.8)	−0.19 (−0.55, 0.18)	0.322	
	90	2.9 (2.5, 3.4)	3.3 (2.8, 3.7)	−0.35 (−0.76, 0.06)	0.097	
	120	2.8 (2.3, 3.2)	2.9 (2.4, 3.3)	−0.09 (−0.56, 0.37)	0.691	

	Time (min)	Number of patients (%)		Treatment effect ^c	
				Odds ratio (95% CI)	p-value
Responders (supine) ^a	30	12 (25.0%)	9 (19.1%)	1.42 (0.59, 3.44)	0.437
	60	20 (41.7%)	11 (23.4%)	2.39 (1.09, 5.22)	0.029
	90	25 (52.1%)	17 (36.2%)	1.95 (0.91, 4.17)	0.085
	120	29 (61.7%)	20 (42.6%)	2.17 (1.00, 4.74)	0.051
Responders (sitting)	30	17 (34.7%)	11 (22.9%)	1.73 (0.89, 3.36)	0.103
	60	21 (42.9%)	15 (31.3%)	1.68 (0.81, 3.49)	0.164
	90	25 (51.0%)	18 (37.5%)	1.72 (0.85, 3.47)	0.130
	120	28 (57.1%)	25 (52.1%)	1.21 (0.68, 2.14)	0.522

^aMissing data for 1 patient at all time points in both treatment phases and for 1 patients at 120 min in clobazam phase.

^bEstimated by linear mixed models, adjusted for baseline value and treatment phase.

^cEstimated by logistic gee, adjusted for treatment phase.

Table 3 Intensity and frequency of side effects over all time points at patient level. Intensity was rated on a 0–10 numeric rating scale (NRS with 0 = no side effect, 10 = worst imaginable side effect) and averaged per patient, frequency was obtained by dichotomization (NRS No side effect = 0 versus side effect NRS \geq 1 at any time point). Data are presented as means and SD or numbers and percentage. Differences between treatment groups were analysed by Wilcoxon matched-pairs signed-ranks and McNemar tests for intensity and frequency respectively.

	Clobazam N = 49	Placebo N = 48	p-value
Nausea intensity	0.1 \pm 0.5	0.1 \pm 0.4	0.655
Fatigue intensity	1.8 \pm 1.9	0.9 \pm 1.2	<0.001
Dizziness intensity	0.7 \pm 1.2	0.2 \pm 0.7	<0.001
Nausea frequency (yes)	8 (16%)	7 (15%)	1.000
Fatigue frequency (yes)	33 (67%)	22 (46%)	0.052
Dizziness frequency (yes)	20 (41%)	8 (17%)	<0.001

4.2 Sedation, muscle relaxation or antihyperalgesia?

Clobazam had an effect on low-back pain in the supine position. Apart from the hypothesized antihyperalgesic action, two other explanations may be possible based on the pharmacologic action of benzodiazepines: sedation and muscle relaxation. As the

side-effect-adjusted analysis suggests, sedation clearly contributed to the observed effect, but is unlikely to be the sole explanation since the treatment effect remained observable – although less significant. Furthermore, if sedation had been a major contributor, it should have influenced the QST at least to some extent. For muscle relaxation, the degree of contribution cannot easily be quantified, partly because the exact mechanism remains unknown. Animal experiments found α 1- and α 5-subunit-containing GABA receptors to mediate muscle relaxation (Milic et al., 2012), but α 2-subunits have been implicated as well (Crestani et al., 2001). Furthermore, there are peripheral benzodiazepine receptors that cause increased muscle contraction instead of muscle relaxation (Chiou and Chang, 1994). In human volunteers, midazolam has simultaneously produced weakened grip strength and increased bite force (Huang et al., 2012), suggesting that both muscle relaxation and increased contractility might be present at the same time, with different effects in different muscle groups. A recent systematic review investigated the effect of muscle relaxants in low-back pain (Abdel Shaheed et al., 2016). The authors concluded that there was insufficient evidence for benzodiazepines. Other centrally active muscle

Table 4 Effect of clobazam versus active placebo on pain adjusted for nausea, fatigue and dizziness and their interaction with treatment group in a linear mixed model.

	Time (min)	Mean (95% CI)		Treatment effect Mean difference (95% CI)	p-value	Joint p-value
		Clobazam	Placebo			
NRS (supine)	30	3.5 (2.9, 4.0)	3.8 (3.3, 4.3)	-0.18 (-0.60, 0.25)	0.411	0.165
	60	2.9 (2.4, 3.4)	3.5 (2.9, 4.1)	-0.43 (-0.87, 0.01)	0.058	
	90	2.7 (2.2, 3.2)	3.3 (2.8, 3.8)	-0.40 (-0.87, 0.08)	0.100	
	120	2.4 (1.9, 2.9)	3.1 (2.6, 3.7)	-0.59 (-1.09, -0.09)	0.020	
NRS (sitting)	30	3.6 (3.0, 4.1)	3.6 (3.1, 4.1)	-0.10 (-0.49, 0.29)	0.606	0.561
	60	3.1 (2.7, 3.6)	3.3 (2.9, 3.8)	-0.19 (-0.61, 0.22)	0.361	
	90	2.9 (2.5, 3.4)	3.3 (2.8, 3.7)	-0.35 (-0.80, 0.10)	0.131	
	120	2.8 (2.3, 3.2)	2.9 (2.4, 3.3)	-0.11 (-0.60, 0.37)	0.655	

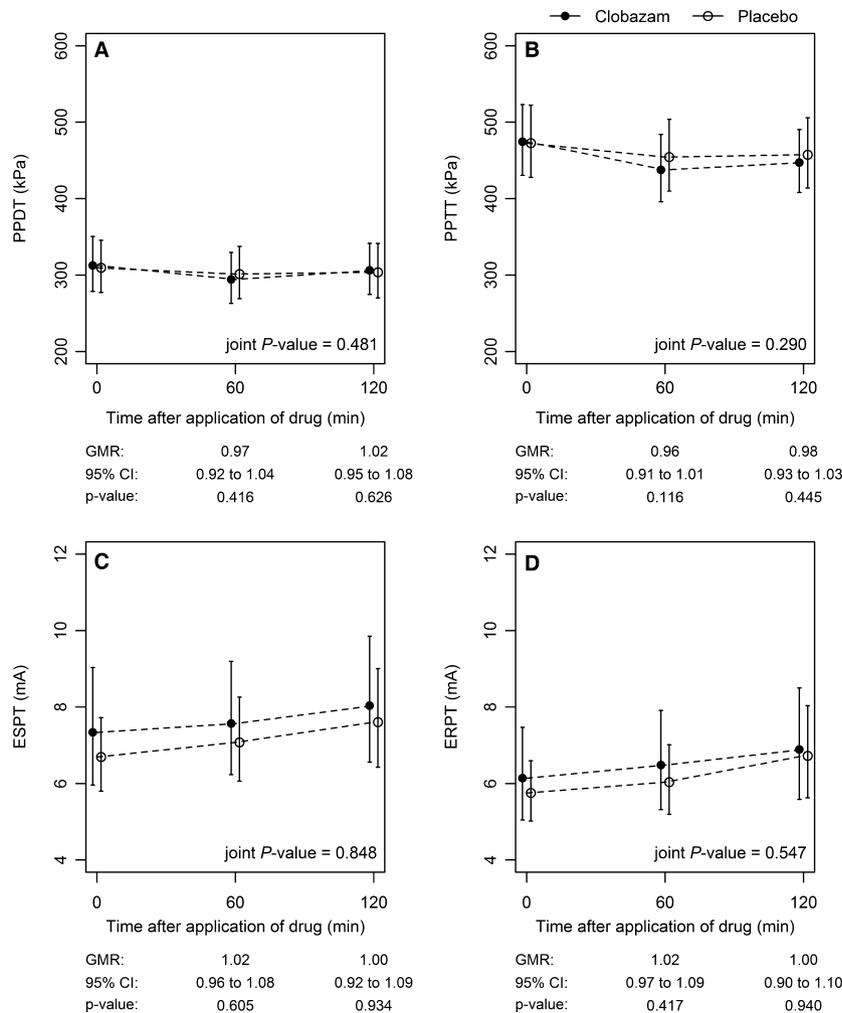


Figure 2 Effect of clobazam versus placebo on (A) pressure pain detection thresholds, (B) pressure pain tolerance thresholds (PPTT), (C) electrical single pain threshold (ESPT) and (D) electrical repeated pain threshold (ERPT). GMR, geometric mean ratio.

relaxants, such as eperisone, pridinol, tizanidine or carisoprodol, were ineffective in chronic low-back pain.

Unfortunately, muscle relaxation was not assessed in the present study. A relevant muscle-relaxing effect of clobazam can therefore not be ruled out.

The question arises, however, why clobazam was only effective in the supine and not in the sitting position. Undoubtedly, some degree of muscle relaxation is present in the supine position and might partly explain the observed effect. The above-cited studies (Crestani et al., 2001; Huang et al., 2012; Milic et al., 2012), which all demonstrated reduced grip strength in animals and humans, suggest that muscle relaxation by benzodiazepines is measurable also during voluntary muscle contraction. When applying this line of thinking to the present results, a muscle-relaxing effect of clobazam should as well be present in sitting position, since sitting is a form of voluntary muscle contraction. However, the present results show no effect of clobazam in the sitting position. Finally, an action of medications on pain via muscle relaxation relies on the assumption that muscle tension is relevant in chronic low-back pain, but the presence and relevance of muscle tension in chronic low-back pain remains uncertain.

The hypothesized antihyperalgesic effect of benzodiazepines may offer a third possible explanation for the observed results. When patients are in supine position, mechanical strain on the low-back is diminished and direct nociceptive input, e.g. arising from facet joints or intervertebral discs may be reduced. The persistence of pain in this situation may be partly due to hyperexcitability of neuronal structures in the sense of central hypersensitivity, and therefore be more amenable to antihyperalgesic effects of clobazam. Conversely, when patients sit up, the effect of gravity increases mechanical strain on the lower back and more direct nociceptive input is present that can no longer be inhibited by clobazam.

4.3 Strengths and limitations

The fact that side effects occurred more frequently in the clobazam arm raises the concern that tolterodine may not have been an adequate active placebo. As an alternative, first-generation antihistamines are sometimes used [e.g. as a comparator for pregabalin in (Markman et al., 2015)]. However, antihistamines are not without problems, as they possess many unspecific central effects such as serotonin- and noradrenalin-reuptake inhibition, sodium channel blockade (Cole et al., 2011) and even potentiation of opioid effects (Carr et al., 1985). An influence of antihistamines on pain or QST can therefore not be ruled out. In the absence of an ideal active placebo, we chose tolterodine as it has no documented analgesic effect, even if it only partly mimicked the side effects of clobazam. In our opinion, this is still an

advantage over an inactive placebo with no side effects at all. Another concern may be the observation time of only 2 h. But the data from Besson et al. (2015) support the notion that the maximal effect occurs within that time period.

4.4 Conclusion

In summary, a single oral dose of clobazam effectively reduced chronic low-back pain in supine position without altering pain sensitivity at remote sites. This is in line with existing studies in animals and human volunteers that suggest an antihyperalgesic effect of benzodiazepines in experimental pain. However, the unspecific side effects of benzodiazepines such as sedation and muscle relaxation limit this conclusion to some extent. A substance that enhances GABAergic inhibition without causing these side effects would offer the opportunity to study these mechanisms in more detail. Perhaps this can be achieved by the development of new, $\alpha 2$ -subtype-selective compounds. In any case, this study must not be understood as a legitimation to routinely prescribe benzodiazepines for chronic low-back pain. But they support the hypothesis that GABAergic modulation of nociceptive pathways may be effective for some chronic pain conditions.

Acknowledgements

We thank Marco Eschenmoser and his team from the hospital pharmacy for their time and effort in the preparation of the study medication.

Author contributions

A.S., M.C., P.J. H.U.Z. and L.A.N. conceptualized and designed the study. J.S. and P.H.V. acquired the data. Statistical analysis was made by L.B. and A.L. All authors interpreted the data. J.S. and M.C. drafted the manuscript. All authors critically revised the manuscript and agreed to the final version.

References

- Abdel Shaheed, C., Maher, C.G., Williams, K.A., McLachlan, A.J. (2016). Efficacy and tolerability of muscle relaxants for low back pain: Systematic review and meta-analysis. *Eur J Pain* 21, 228–237.
- Arbus, L., Fajadet, B., Aubert, D., Morre, M., Goldberger, E. (1990). Activity of tetrazepam (Myolastan®) in low back pain. A double-blind trial v. placebo. *Clin Trials J* 27, 258–267.
- Besson, M., Daali, Y., Di Lio, A., Dayer, P., Zeilhofer, H.U., Desmeules, J. (2013). Antihyperalgesic effect of the GABA(A) ligand clobazam in a neuropathic pain model in mice: A pharmacokinetic-pharmacodynamic study. *Basic Clin Pharmacol Toxicol* 112, 192–197.

- Besson, M., Matthey, A., Daali, Y., Poncet, A., Vuilleumier, P.H., Curatolo, M., Zeilhofer, H.U., Desmeules, J. (2015). GABAergic modulation in central sensitization in human: Randomized placebo controlled pharmacokinetic-dynamic study comparing clobazam to clonazepam in healthy volunteers. *Pain* 156, 397–404.
- Carr, K.D., Hiller, J.M., Simon, E.J. (1985). Diphenhydramine potentiates narcotic but not endogenous opioid analgesia. *Neuropeptides* 5, 411–414.
- Chiou, L.C., Chang, C.C. (1994). Pharmacological relevance of peripheral type benzodiazepine receptors on motor nerve and skeletal muscle. *Br J Pharmacol* 112, 257–261.
- Cole, J.B., Stellpflug, S.J., Gross, E.A., Smith, S.W. (2011). Wide complex tachycardia in a pediatric diphenhydramine overdose treated with sodium bicarbonate. *Pediatr Emerg Care* 27, 1175–1177.
- Crestani, F., Low, K., Keist, R., Mandelli, M., Mohler, H., Rudolph, U. (2001). Molecular targets for the myorelaxant action of diazepam. *Mol Pharmacol* 59, 442–445.
- Curatolo, M., Arendt-Nielsen, L., Petersen-Felix, S. (2006). Central hypersensitivity in chronic pain: Mechanisms and clinical implications. *Phys Med Rehabil Clin N Am* 17, 287–302.
- Di Lio, A., Benke, D., Besson, M., Desmeules, J., Daali, Y. et al. (2011). HZ166, a novel GABAA receptor subtype-selective benzodiazepine site ligand, is antihyperalgesic in mouse models of inflammatory and neuropathic pain. *Neuropharmacology* 60, 626–632.
- Edwards, M., Serrao, J.M., Gent, J.P., Goodchild, C.S. (1990). On the mechanism by which midazolam causes spinally mediated analgesia. *Anesthesiology* 73, 273–277.
- Goodchild, C.S., Noble, J. (1987). The effects of intrathecal midazolam on sympathetic nervous system reflexes in man—a pilot study. *Br J Clin Pharmacol* 23, 279–285.
- Goodchild, C.S., Serrao, J.M. (1987). Intrathecal midazolam in the rat: Evidence for spinally-mediated analgesia. *Br J Anaesth* 59, 1563–1570.
- Hingorani, K. (1966). Diazepam in backache. A double-blind controlled trial. *Ann Phys Med* 8, 303–306.
- Huang, M.Y., Matsuura, N., Kaneko, Y., Ichinohe, T. (2012). Midazolam increases bite force during intravenous sedation. *J Oral Maxillofac Surg* 70, e458–463.
- Knabl, J., Witschi, R., Hosl, K., Reinold, H., Zeilhofer, U.B. et al. (2008). Reversal of pathological pain through specific spinal GABAA receptor subtypes. *Nature* 451, 330–334.
- Knabl, J., Zeilhofer, U.B., Crestani, F., Rudolph, U., Zeilhofer, H.U. (2009). Genuine antihyperalgesia by systemic diazepam revealed by experiments in GABAA receptor point-mutated mice. *Pain* 141, 233–238.
- Markman, J.D., Frazer, M.E., Rast, S.A., McDermott, M.P., Gewandter, J.S. et al. (2015). Double-blind, randomized, controlled, crossover trial of pregabalin for neurogenic claudication. *Neurology* 84, 265–272.
- Milic, M., Divljakovic, J., Rallapalli, S., van Linn, M.L., Timic, T., Cook, J.M., Savic, M.M. (2012). The role of alpha1 and alpha5 subunit-containing GABAA receptors in motor impairment induced by benzodiazepines in rats. *Behav Pharmacol* 23, 191–197.
- Moll, W. (1973). Therapy of acute lumbospondyl syndromes through optimal muscle relaxation using diazepam. Results of a double-blind study on 68 cases. *Med Welt* 24, 1747–1751.
- Ralvenius, W.T., Benke, D., Acuna, M.A., Rudolph, U., Zeilhofer, H.U. (2015). Analgesia and unwanted benzodiazepine effects in point-mutated mice expressing only one benzodiazepine-sensitive GABAA receptor subtype. *Nat Commun* 6, 6803.
- Rudolph, U., Crestani, F., Benke, D., Brunig, I., Benson, J.A. et al. (1999). Benzodiazepine actions mediated by specific gamma-aminobutyric acid(A) receptor subtypes. *Nature* 401, 796–800.
- Salzmann, E., Pffringer, W., Paal, G., Gierend, M. (1992). Treatment of chronic low-back syndrome with tetrazepam in a placebo controlled double-blind trial. *J Drug Develop* 4, 219–228.
- Schliessbach, J., Siegenthaler, A., Streitberger, K., Eichenberger, U., Nuesch, E., Juni, P., Arendt-Nielsen, L., Curatolo, M. (2013). The prevalence of widespread central hypersensitivity in chronic pain patients. *Eur J Pain* 1–20.
- Siegenthaler, A., Schliessbach, J., Vuilleumier, P.H., Juni, P., Zeilhofer, H.U., Arendt-Nielsen, L., Curatolo, M. (2015). Linking altered central pain processing and genetic polymorphism to drug efficacy in chronic low back pain. *BMC Pharmacol Toxicol* 16, 23.
- Vuilleumier, P.H., Besson, M., Desmeules, J., Arendt-Nielsen, L., Curatolo, M. (2013). Evaluation of anti-hyperalgesic and analgesic effects of two benzodiazepines in human experimental pain: A randomized placebo-controlled study. *PLoS ONE* 8, e43896.
- Wildin, J.D., Pleuvry, B.J., Mawer, G.E., Onon, T., Millington, L. (1990). Respiratory and sedative effects of clobazam and clonazepam in volunteers. *Br J Clin Pharmacol* 29, 169–177.
- Zeilhofer, H.U. (2008). Loss of glycinergic and GABAergic inhibition in chronic pain – Contributions of inflammation and microglia. *Int Immunopharmacol* 8, 182–187.
- Zeilhofer, H.U., Mohler, H., Di Lio, A. (2009). GABAergic analgesia: New insights from mutant mice and subtype-selective agonists. *Trends Pharmacol Sci* 30, 397–402.

Supporting Information

Additional Supporting Information may be found online in the supporting information tab for this article:

Table S1 Effect of clobazam versus active placebo on quantitative sensory tests.