Tiagabine - GABA transporter type 1 inhibitor attenuates tactile allodynia in oxaliplatin- and streptozotocin-induced neuropathic pain without impairing cognition

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INTRODUCTION

Gamma-aminobutyric acid (GABA) is the main inhibitory neurotransmitter in the mammalian central nervous system. GABAergic neurotransmission regulates numerous physiological functions, including seizure threshold, mood and muscle tension. GABA is also involved in the modulation of pain and it has been shown that chronic pain may result from a decreased GABA concentration in the central nervous system. Several ways to restore a physiological GABA level are being used nowadays, and the inhibition of GABA uptake is one of mainstreams in this area. Although many GABA transport inhibitors (GAT inhibitors) have been discovered, so far, only tiagabine, a selective inhibitor of GAT1 isoform with affinity of 13 nM, is used in clinic as an antiepileptic drug for the treatment of partial seizures. Of note, this drug is being currently assessed for other (non-epileptic) indications, such as chronic pain, anxiety and depression.

The aim of this research was to investigate antiallodynic and antihyperalgesic properties of tiagabine in two mouse models of neuropathic pain, i.e., a model of streptozotocin (STZ)-induced painful diabetic neuropathy, and chemotherapy-induced peripheral neuropathy caused by oxaliplatin. Preliminary safety pharmacology studies of tiagabine in neuropathic animals were also conducted using locomotor activity, rotarod and passive avoidance tests.

MATERIALS AND METHODS

CHEMICALS

Tiagabine hydrochloride (Sigma Aldrich Poland) at doses (2-8 mg/kg) was dissolved in 0.9% saline and injected intraperitoneally to neuropathic (STZ- or oxaliplatin-treated) mice 60 min before behavioral tests. Control mice received 0.9% saline solution.

ANIMALS

All tests were performed in male Albino-Swiss CD-1 mice weighing between 18 - 22 g. The animals were housed at room temperature of 22±2 °C, under light/dark (12:12) cycle. The animals had free access to food and water. Each experimental group consisted of 8-10 animals. Immediately after the *in vivo* assay the animals were euthanized by cervical dislocation. The procedures of animal maintenance and treatment were approved by the Local Ethics Committee of the Jagiellonian University in Krakow (ZI/862/2013).

DATA ANALYSIS

Results are shown as mean ± SEM. For the statistical analysis one-way ANOVA or repeated measures ANOVA, followed by Dunnett's, Tukey's or Bonferroni *post hoc* tests were used. P<0.05 was considered significant.

Abstract

GABA is involved in pain control. In this study using mouse models of neuropathic pain we showed that tiagabine, the inhibitor of GABA reuptake from the synaptic cleft, attenuates tactile allodynia and heat hyperalgesia in diabetic mice and has antiallodynic properties in chemotherapy-induced neuropathic pain model. Tiagabine does not impair learning or motor functions in neuropathic mice.

BEHAVIORAL TESTING PROTOCOLS

DIABETIC NEUROPATHY

Induction of diabetes: STZ 200 mg/kg (a single i.p. injection)

↓

Selection of diabetic mice by measuring blood glucose levels 1h before STZ injection and repeatedly 7, 14 and 21 days after STZ injection

Diabetic mice: blood glucose level > 300 mg/dl

↓

Pain tests

Von Frey test (tactile allodynia) | Hot plate test (thermal hyperalgesia: 56 °C)

↓

Safety pharmacology studies in diabetic mice

Locomotor activity test | Motor coordination (rotarod test) | Learning and memory (passive avoidance task)

CHEMOTHERAPY (OXALIPLATIN)-INDUCED NEUROPATHY

Induction of toxic peripheral neuropathy: oxaliplatin 10 mg/kg (a single i.p. injection)

↓

Selection of neuropathic mice

Measurement of baseline pain sensitivity using von Frey test

↓

Pain tests (3h and 7 days after oxaliplatin)

Von Frey test (tactile allodynia) | Cold plate test (thermal hyperalgesia: 4 °C)

↓

Safety pharmacology in neuropathic mice

Locomotor activity test | Motor coordination (rotarod test) | Learning and memory (passive avoidance task)

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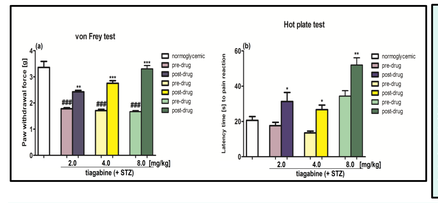
RESULTS

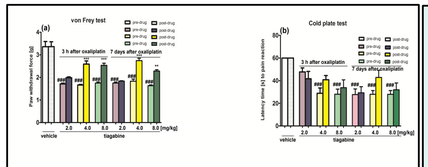
DIABETIC NEUROPATHY



In diabetic mice tiagabine significantly and dose-dependently attenuated tactile allodynia in the von Frey test and was antinociceptive in the hot plate test.

Fig. 1 Antiallodynic (1a) and antihyperalgesic (1b) properties of tiagabine in STZ–induced diabetic neuropathic pain in mice. Results are shown as mean paw withdrawal threshold in response to mechanical stimulation (von Frey test) and latency time to pain reaction (hot plate test). Statistical analysis: one-way ANOVA, followed by Tukey's *post hoc* test. Significance: ### p<0.001 (vs. normoglycemic control mice); * p<0.05, ** p<0.01, *** p<0.001 (vs. pre-drug value in neuropathic mice).

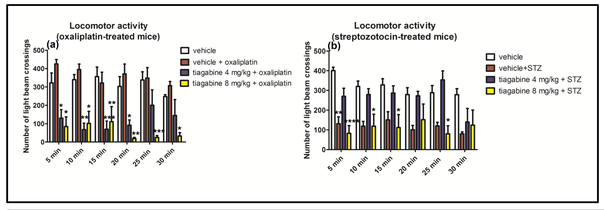
CHEMOTHERAPY (OXALIPLATIN)-INDUCED NEUROPATHY



Tiagabine (4, 8 mg/kg) attenuated both acute-phase and late-phase tactile allodynia induced by oxaliplatin but it did not affect cold hyperalgesia in this model.

Fig. 2 Antiallodynic (2a) and antihyperalgesic (2b) properties of tiagabine in oxaliplatin-induced neuropathic pain in mice. Results are shown as mean paw withdrawal threshold in response to mechanical stimulation (von Frey test) or latency time to pain reaction (cold plate test). Statistical analysis: one-way ANOVA, followed by Tukey's *post hoc* test. Significance: ### p<0.001 (vs. vehicle-treated group); ** p<0.01, *** p<0.001 (vs. pre-drug value in neuropathic mice).

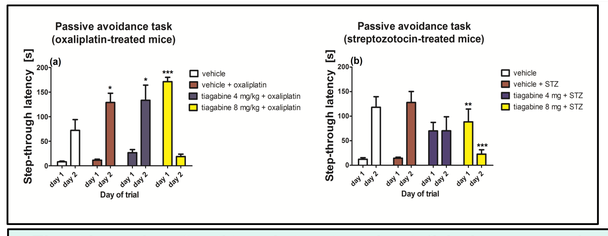
SAFETY PHARMACOLOGY STUDIES



Tiagabine at 4 mg/kg did not impair locomotor functions of neuropathic mice. The dose 8 mg/kg significantly reduced animals' locomotor activity.

In the rotarod test tiagabine did not cause any motor deficits in mice, either.

Fig. 3 Effect of tiagabine on locomotor activity in oxaliplatin-treated mice (3a) and in STZ-treated mice (3b). Results are shown as the number of light-beam crossings at the respective time-point. Statistical analysis: repeated measures ANOVA, followed by Bonferroni *post hoc* comparison. Significance: * p<0.05, ** p<0.01, *** p<0.001, **** p<0.0001 (vs. vehicle-treated mice).



Tiagabine at 4 mg/kg did not induce learning deficits in neuropathic mice as measured in the passive avoidance task. A dose 8 mg/kg impaired cognition in diabetic mice.

Fig. 4 Influence of tiagabine on contextual learning and fear-motivated memory in oxaliplatin-treated mice (4a) and STZ-treated mice (4b) measured using passive avoidance task. Results are shown as mean step-through latencies in the acquisition trial (day 1) and the retention trial (day 2). Statistical analysis: repeated measures ANOVA, followed by Bonferroni *post hoc* comparison. Significance: * p<0.05, ** p<0.01, *** p<0.001 (vs. control at the respective day of testing).

CONCLUSIONS

We showed that GAT1 inhibition with the use of tiagabine attenuates tactile allodynia in diabetic neuropathic pain and tactile allodynia but not cold hyperalgesia in oxaliplatin-induced neuropathic pain

Dose 4 mg/kg is devoid of serious adverse effects, such as sustained sedation, while the dose of 8 mg/kg induces severe and long-lasting sedation and cognitive deficits in neuropathic mice

None of doses tested impairs motor coordination of neuropathic mice