Acta Neuropsychiatrica 2014

DOI: 10.1017/neu.2013.23

ACTA NEUROPSYCHIATRICA

Chronic oral carbamazepine treatment elicits mood-stabilising effects in mice

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Kara NZ, Karpel O, Toker L, Agam G, Belmaker RH, Einat H. Chronic oral carbamazepine treatment elicits mood-stabilising effects in mice.

Objective: The underlying biology of bipolar disorder and the mechanisms by which effective medications induce their therapeutic effects are not clear. Appropriate use of animal models are essential to further understand biological mechanisms of disease and treatment, and further understanding the therapeutic mechanism of mood stabilisers requires that clinically relevant administration will be effective in animal models. The clinical regimens for mood-stabilising drugs include chronic oral administration; however, much of the work with animal models includes acute administration via injection. An effective chronic and oral administration of the prototypic mood stabiliser lithium was already established and the present study was designed to do the same for the mood stabiliser carbamazepine.
Methods: Mice were treated for 3 weeks with carbamazepine in food. ICR mice were treated with 0.25%, 0.5% and 0.75%, and C57bl/6 mice with 0.5% and 0.75%, carbamazepine in food (w/w, namely, 2.5, 5.0 or 7.5 g/kg food). Mice were then tested for spontaneous activity, forced swim test (FST), tail suspension test (TST) and amphetamine-induced hyperactivity.
Results: Oral carbamazepine administration resulted in dose-dependent blood levels reaching 3.65 μg/ml at the highest dose. In ICR mice, carbamazepine at the 0.5% dose had no effect on spontaneous activity, but significantly reduced immobility in the TST by 27% and amphetamine-induced hyperactivity by 28%. In C57bl/6 mice, carbamazepine at the 0.75% dose reduced immobility time in the FST by 26%.
Conclusions: These results demonstrate a behaviourally effective oral and chronic regimen for carbamazepine with mood stabilising-like activity in a standard model for mania-like behaviour and two standard models for depression-like behaviour.

Keywords: animal models, anticonvulsants, bipolar disorder, depression, mania, mice, mood-stabilisers

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Accepted for publication March 26, 2013

First published online 29 May, 2013

Significant outcomes

- We established a chronic oral regimen of carbamazepine (CBZ) that has behavioural mood stabilising-like effects in mice.
- We demonstrate that chronic oral administration of CBZ results in dose-relevant blood levels, anti-manic-like effects in the amphetamine-induced hyperactivity test and antidepressant-like effects in the forced swim test (FST) and the tail suspension test (TST).

Limitations

- The results are based on very few behavioural models, one for mania-like and two for depression-like behaviour. Additional models and tests are recommended.
- Detected blood levels are lower than clinically relevant levels in humans; however, this might be related to differences in kinetics of the drug between mice and humans.

Introduction

Despite the high prevalence of bipolar disorder (BPD) reaching lifetime prevalence of 1–3% in the adult general population, remarkably little is known regarding its pathophysiology or the means by which effective medications exert their therapeutic effects (1). Moreover, the classical mood-stabilising drugs including lithium salts and the anticonvulsants, CBZ and valproic acid, were discovered by serendipity and their therapeutic mechanisms of action are not yet understood (1).

One important approach to gain better understanding into the underlying pathophysiology of BPD and the mechanism of its treatment is to explore the biological targets of known medications utilising animal models (2). A number of animal models and tests are used to study BPD and the effects of known and novel treatments (3); however, one limitation in this respect is the lack of an animal model that includes both the depression and the manic poles of the disorder and the oscillation between them (4). Therefore, the common practice is to use separate models for depression and for mania including the FST, the TST and the amphetamine-induced hyperactivity test that have been intensely utilised and were demonstrated to be useful in identifying possible mood-stabilising effects (5–7).

An attempt to understand the therapeutic mechanism of action of a drug requires that not only will the drug have an effect in a relevant animal model, but also that it will be administered in a way that is similar to its use in the clinic (2,8). Hence, for mood stabilisers, it is important to establish a drug regimen in animals that will have behavioural effects following chronic oral administration. Such a regimen was established for the prototypic mood stabiliser lithium in mice (9). With this protocol, lithium is given in food starting with a 2% concentration for 5 days and followed by a 4% concentration for 10 days. This protocol, with some small variations in different laboratories, was demonstrated to result in clinically relevant lithium blood levels as well as therapeutic-like effects in behavioural models (9–11). Yet, such protocols were not clearly established for other mood stabilisers.

The effect of the anticonvulsant mood stabiliser CBZ in animal models related to BPD was rarely examined, and past results are not highly consistent. For example, in the amphetamine-induced hyperactivity test, acute administration of CBZ reduced hyperactivity in mice in one study (12), but similar doses had no effect in mice in another one (13), and chronic CBZ regimen in rats had no effect as well (14). In the FST, chronic administration of CBZ to rats resulted in a decrease of immobility time in one study (15), but not in another (16), although both studies used similar doses, regimens and rats. We did not identify studies that tested acute or chronic CBZ administration in the TST.

In light of the scarcity of studies and contradicting results regarding the effect of chronic CBZ in behavioural tests relevant to BPD, the aim of the present study was to establish a chronic oral administration regimen of CBZ in mice that will elicit mood stabilising-like activity in models of depression and mania.

Methods

Animals

Eight- to ten-week-old male ICR mice (Experiments 1 and 2) or C57bl/6 (Experiment 3) were group housed in standard mice cages in an animal colony room with a 12/12 h light/dark cycle, with constant temperature at 23 ± 1°C and *ad libitum* food and water. All experimental procedures followed the Israeli guidelines for the treatment and care of experimental animals and were approved by the Ben-Gurion University of the Negev IACUC (protocol #IL-56-08-2012).

Drugs

CBZ (Sigma, St Louis, MO, USA) was added to powdered food and thoroughly mixed using a Kitchen-Aid® mixer for at least an hour. For Experiment 1, CBZ was administered for 3 weeks at three dose regimens: (1) first week at 0.25% (w/w) and last 2 weeks at 0.5% (w/w); (2) 3 weeks at 0.5% w/w; and (3) first week at 0.5% (w/w) and last 2 weeks at 0.75% (w/w). The control group received regular powdered food. These doses were selected on the basis of previous work in different contexts and on a preliminary work in our lab (17). The 3-week schedule was selected based on clinical time span expected for the drug to elicit therapeutic response (1), animal results with similar mood stabilisers (18) and preliminary results from our lab. Mice were weighed every other day during the treatment period.

Experiment 1 presented us with two problems. (1) Mice did not float in the FST (see procedure below), and when not active they sank under water and were immediately rescued by the experimenter. (2) Mice treated with the highest CBZ dose [0.75% (w/w), namely, 7.5 g/kg food] showed an average weight loss of 6.5% after 3 days of treatment. To overcome these problems, in Experiment 2 we replaced the FST with the TST and used CBZ doses not higher than 0.5% (w/w). Furthermore, we added Experiment 3 to evaluate the effects of CBZ in the FST in a different strain of mice (C57bl/6).

https://doi.org/10.1017/neu.2013.23 Published online by Cambridge University Press

In these mice, there was no weight reduction in the 0.75% CBZ dose and mice did not sink in the FST. We therefore tested the C57bl/6 mice in the FST with the original dose range.

Behavioural tests

Spontaneous activity test. Mice were placed individually in the centre of a transparent Plexiglas box ($37.5 \times 37.5 \times 45$ cm) for 30 min and their behaviour was digitally recorded. Distance moved was analysed using an automated acquisition and analysis software (Ethovision, Noldus Inc., Wageningen, the Netherlands).

FST. Mice were placed for a 6-min session in a glass cylinder (22 cm diameter and 30 cm high) filled with water at a temperature of 22 ± 1°C such that the mouse cannot touch the bottom or climb out of the cylinder. Sessions were digitally recorded, and the duration of immobility during the last 4 min of the session was scored by an experimenter blind to the treatment groups. Immobility was defined as the time spent by a mouse floating or making only those movements necessary to keep its head above the water.

TST. Mice were suspended by the tail from a hook placed 30 cm above a padded table, using an adhesive tape for a 6-min session. Sessions were digitally recorded, and the duration of immobility during the last 4 min of the session was manually scored by a blind observer. Immobility was defined as the time spent by a mouse hanging passively and completely motionless.

Amphetamine-induced hyperactivity. Mice were injected with amphetamine (1.5 mg/kg dissolved in saline to a 10 ml/kg injection volume) or saline (control) and placed in the centre of a transparent Plexiglas box ($37.5 \times 37.5 \times 45$ cm) for a 30-min session. Their behaviour was recorded and analysed as described above for the spontaneous activity test.

Blood levels

At the end of Experiment 1, five mice from each group were euthanised using 20% isoflorane, and blood was withdrawn using a heart puncture. CBZ blood levels were measured by a fluorescence polarization immunoassay kit (Roche Diagnostics, Mannheim, Germany), according to the manufacturer's instructions, run on a Cobas 400 apparatus (Roche Diagnostics, Rotkreuz, Switzerland).

Statistics

When more than one dose of CBZ was tested, the data were analysed using a one-way analysis of variance (ANOVA). When ANOVA showed a significant effect, it was followed by *post-hoc* LSD tests. When only one dose of CBZ was tested and compared with control, data were analysed using a Student's *t*-test. The amphetamine-induced hyperactivity test was analysed using a two-way ANOVA with amphetamine and CBZ as main factors. The ANOVA analysis was followed by *post-hoc* LSD tests.

Results

General observations of mice under the experimental treatment regimen did not indicate any gross sickness behaviour, changes in home cage behaviour or in fur conditions.

Experiment 1

CBZ at the 0.25% or 0.5% doses had no effects on spontaneous activity, not for the total activity in 30 min and not for analysis across the session in 10-min time bins [distance in cm in a 30-min session – regular food: 9134 ± 582; 0.25% CBZ: 7615 ± 766; 0.5% CBZ: 9807 ± 913; repeated measures ANOVA across three 10-min time bins: CBZ effect: $F(3, 16) = 1.22$, $p = 0.33$, NS; time effect: $F(3, 32) = 59.2$, $p < 0.001$; CBZ × time interaction: $F(6, 32) = 0.61$, $p = 0.72$, NS]. Data from additional tests were not available for Experiment 1 because of the issues that arose from the FST (see the Methods section for details).

Experiment 2

Chronic oral administration of 0.5% CBZ resulted in a 27%, statistically significant reduction in immobility time in the TST [Fig. 1a; $t(33) = 2.31$, $p = 0.027$] and a 28%, statistically significant reduction in amphetamine-induced hyperactivity [Fig. 1b; two-way ANOVA with amphetamine and CBZ as main factors: amphetamine effect – $F(1, 36) = 16.8$, $p < 0.001$; CBZ effect – $F(1, 36) = 1.83$, $p = 0.18$; amphetamine × CBZ interaction – $F(1, 36) = 4.9$, $p = 0.033$; *post hoc*: control food/amphetamine group different from all other groups]. Further analysis of the amphetamine groups across time (in 10-min time bins) shows that the effects of CBZ does not change across the session [repeated measures ANOVA, CBZ × time interaction: $F(2, 36) = 0.47$, $p = 0.63$, NS].

Experiment 3

Treatment of C57bl/6 mice with 0.75% but not lower CBZ doses resulted in a 26%, statistically significant

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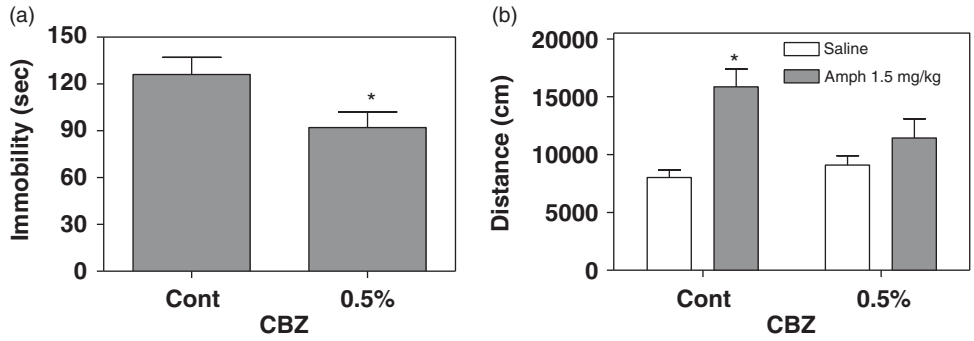

Fig. 1. Chronic oral administration of 0.5% carbamazepine (CBZ) in food resulted in a significant reduction in tail suspension test immobility time (a) and in amphetamine-induced hyperactivity (b) in ICR mice. Results are presented as means ± SEM, Cont = control group; amph = amphetamine. *$p < 0.05$ compared with Cont (a) and compared with all other groups (b).

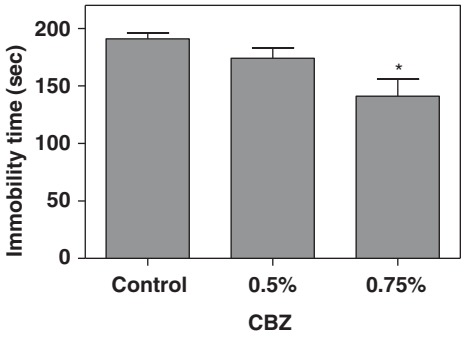

Fig. 2. Chronic oral administration of 0.75% carbamazepine (CBZ) in food resulted in a significant reduction in forced swim test immobility time in C57bl/6 mice. Results are presented as means ± SEM. *$p < 0.05$ compared with other groups.

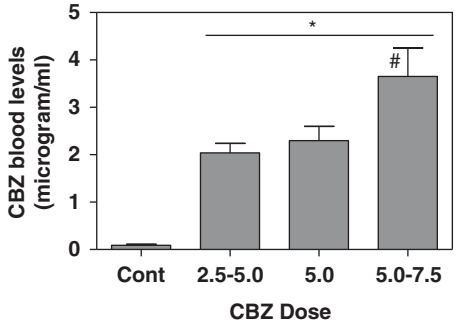

Fig. 3. Chronic oral administration of carbamazepine (CBZ) resulted in dose-relevant blood levels. *Significant difference from control; #significant difference from all other groups.

reduction in FST immobility [Fig. 2; ANOVA $F(2, 13) = 5.46$, $p = 0.02$; *post-hoc* LSD: CBZ 0.75% different from other groups, $p < 0.05$].

Blood levels

Oral administration of all doses of CBZ resulted in detectable blood levels of the drug that were significantly higher compared with control [Fig. 3; ANOVA: $F(3, 16) = 18.03$, $p < 0.0001$, *post-hoc* LSD, all CBZ groups different from control] and with a significant dose/concentration correlation ($r = 0.86$, $p < 0.0001$).

Discussion

The objective of this work was to establish a chronic oral administration regimen of CBZ that elicits mood stabilising-like effect in mice models of depression and mania. The objective was achieved as chronic oral administration of 0.5% (w/w) CBZ in food to ICR mice resulted in a mood stabilising-like effect, including an anti-manic-like effect in the amphetamine-induced hyperactivity test and in an antidepressant-like effect in the TST.

Our initial plan was to study mice in the FST; however, unfortunately, and for an unknown reason, ICR mice treated chronically with CBZ did not float well and showed a tendency to sink when not active. We therefore switched from the FST to the TST. The limited floating ability of ICR mice treated with CBZ cannot be attributed to generalised motor impairment, as these mice were not different from treated control mice in the spontaneous activity, and showed reduced immobility (increased activity) compared with controls in the TST, which is also a motor-dependent test. The floating impairment of these mice in the FST is therefore an open question at this time. CBZ administration to rats was not reported to induce similar problems (16), and acute CBZ was not reported to affect FST behaviour in Swiss mice (19). We did not find any previous reports on the effects of CBZ on swimming in ICR (CD-1) mice. We therefore added an FST experiment with C57bl/6 mice (Experiment 3) where mice did not sink, and the results also show that chronic oral administration of CBZ results

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in an antidepressant-like effect in the FST (Fig. 2). There is a difference in the effective concentration of CBZ between ICR and C57bl/6 mice; however, such differences in dosing between diverse strains are not uncommon.

The regimen established in the present study has three advantages: (1) it is closely related to the human administration paradigm being oral and chronic; (2) it is technically simple as the drug is administered in food; and (3) it is significantly less stressful to animals, compared with chronic administration *via* injections or *via* oral gavage. It is important to note that our experiments attempted to mimic the prophylactic effects of CBZ, as chronic treatment preceded the induction of manic-like and depression-like behaviours. This is important because the effect of CBZ in patients is well established for acute mania but not so much for acute bipolar depression. Yet, the drug's prophylactic effect is established for both poles of the disorder (20,21).

Interestingly, blood level of CBZ after treatment with the dose that was effective in the behavioural experiments (0.5%) was lower than the human therapeutic range. Whereas the therapeutic range for patients is 4–12 μg/ml, the mean blood level in our 0.5% group was only 2.3 μg/ml and only the 0.75% group was close to the human therapeutic levels, with a 3.7 μg/ml concentration. The relatively low blood levels in the 0.5% group may cast some doubt on the results, but these results are not unique. For example, in a study related to the anticonvulsant effects of CBZ in rats, chronic administration of 30 mg/kg/day of the drug resulted in blood levels between 2 and 3 μg/ml, but in a significant behavioural effect reducing seizure frequency in over 50% (22). Therefore, it is possible that the steady state concentrations of CBZ that are sufficient to induce behavioural changes in rodent models are lower than the doses used in the clinic.

A large body of work demonstrates the effects of lithium in a variety of doses and regimens in the FST and the amphetamine-induced hyperactivity test. Most importantly, some of these studies are based on chronic oral administration, which is the most relevant to the clinical realm (9,10). We now present a comparable method of clinically relevant administration of CBZ to mice and suggest that it will now be possible to use this method to gain better understanding of the therapeutically relevant biological effects of CBZ.

Acknowledgements

The study was partially supported by a US–Israel Binational Science Foundation (BSF) grant to HE (grant #2011313).

Authors contributions

Ms. Kara coordinated the experiments, took part in the execution of the experiments and led the analysis and the writing of the initial draft of the manuscript. Ms. Toker and Ms. Karpel took part of the initial planning and the execution of the experiments, and were involved in the analysis of the data and the writing of the initial draft. Profs Agam and Belmaker were responsible for the initial planning of the experiments and their execution and were involved in data analysis and in writing the manuscript. Prof. Einat was involved in the execution of the experiments and was in charge of data analysis and of the final writing, editing and submission of the manuscript.

Statement of Interest

None.

Ethical Standards

The authors assert that all procedures contributing to this work comply with the ethical standards of the relevant national and institutional guides on the care and use of laboratory animals. All experimental procedures followed the Israeli guidelines for treatment and care of experimental animals and were approved by the Ben-Gurion University of the Negev IACUC (protocol # IL-56-08-2012).

References

1. Belmaker RH. Bipolar disorder. N Engl J Med 2004; **351**:476–486.
2. Gould TD, Quiroz JA, Singh J, Zarate CA, Manji HK. Emerging experimental therapeutics for bipolar disorder: insights from the molecular and cellular actions of current mood stabilizers. Mol Psychiatry 2004;**9**:734–755.
3. Einat H. Modelling facets of mania – new directions related to the notion of endophenotypes. J Psychopharmacol 2006; **20**:714–722.
4. Einat H. Different behaviors and different strains: potential new ways to model bipolar disorder. Neurosci Biobehav Rev 2007;**31**:850–857.
5. Cox C, Harrison-Read PE, Steinberg H, Tomkiewicz M. Lithium attenuates drug-induced hyperactivity in rats. Nature 1971;**232**:336–338.
6. Cryns K, Shamir A, Shapiro J et al. Lack of lithium-like behavioral and molecular effects in IMPA2 knockout mice. Neuropsychopharmacology 2007;**32**:881–891.
7. Einat H, Yuan P, Szabo ST, Dogra S, Manji HK. Protein kinase C inhibition by tamoxifen antagonizes manic-like behavior in rats: implications for the development of novel therapeutics for bipolar disorder. Neuropsychobiology 2007;**55**:123–131.
8. Gould TD, Einat H. Animal models of bipolar disorder and mood stabilizer efficacy: a critical need for improvement. Neurosci Biobehav Rev 2007;**31**:825–831.

https://doi.org/10.1017/neu.2013.23 Published online by Cambridge University Press

9. O'Brien WT, Harper AD, Jove F et al. Glycogen synthase kinase-3beta haploinsufficiency mimics the behavioral and molecular effects of lithium. J Neurosci 2004;**24**:6791–6798.
10. Bersudsky Y, Shaldubina A, Belmaker RH. Lithium's effect in forced-swim test is blood level dependent but not dependent on weight loss. Behav Pharmacol 2007;**18**:77–80.
11. Kovacsics CE, Gould TD. Shock-induced aggression in mice is modified by lithium. Pharmacol Biochem Behav 2009;**94**:380–386.
12. Arban R, Maraia G, Brackenborough K et al. Evaluation of the effects of lamotrigine, valproate and carbamazepine in a rodent model of mania. Behav Brain Res 2005;**158**: 123–132.
13. Kalinichev M, Dawson LA. Evidence for antimanic efficacy of glycogen synthase kinase-3 (GSK-3) inhibitors in a strain specific model of acute mania. Int J Neuropsychopharmacol 2011;**6**:1–17.
14. Elphick M. Effects of carbamazepine on dopamine function in rodents. Psychopharmacology (Berlin) 1989;**99**:532–536.
15. Barros HM, Leite JR. The effects of carbamazepine on two animal models of depression. Psychopharmacology (Berlin) 1987;**92**:340–342.
16. Kitamura Y, Akiyama K, Kitagawa K et al. Chronic coadministration of carbamazepine together with imipramine produces antidepressant-like effects in an ACTH-induced animal model of treatment-resistant depression: involvement of 5-HT(2A) receptors? Pharmacol Biochem Behav 2008; **89**:235–240.
17. Shaldubina A, Einat H, Szechtman H, Shimon H, Belmaker RH. Preliminary evaluation of oral anticonvulsant treatment in the quinpirole model of bipolar disorder. J Neural Transm 2002;**109**:433–440.
18. Chen J, Cai F, Cao J, Zhang X, Li S. Long-term antiepileptic drug administration during early life inhibits hippocampal neurogenesis in the developing brain. J Neurosci Res 2009;**87**:2898–2907.
19. Szymczyk G, Zebrowska-Lupina I. Influence of antiepileptics on efficacy of antidepressant drugs in forced swimming test. Pol J Pharmacol 2000;**52**:337–344.
20. Post RM, Ketter TA, Uhde T, Ballenger JC. Thirty years of clinical experience with carbamazepine in the treatment of bipolar illness: principles and practice. CNS Drugs 2007;**21**:47–71.
21. Fountoulakis KN, Grunze H, Panagiotidis P, Kaprinis G. Treatment of bipolar depression: an update. J Affect Disord 2008;**109**:21–34.
22. Ali A, Dua Y, Constance JE, Franklin MR, Dudek FE. A once-per-day, drug-in-food protocol for prolonged administration of antiepileptic drugs in animal models. Epilepsia 2012;**53**:199–206.

https://doi.org/10.1017/neu.2013.23 Published online by Cambridge University Press