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Administration of ziprasidone for 10 days increases cocaine toxicity in mice

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Abstract

Background—Long-term treatment with antipsychotic medications alters the regional density of several of the neurotransmitter receptors that mediate cocaine toxicity. However, the effect of either up- or down- regulation of the neurotransmitter receptors on cocaine toxicity is unknown. Here we determined if subacute administration of the atypical antipsychotic ziprasidone altered the toxic effects of cocaine in mice.

Methods—Ziprasidone (4 mg/kg) or placebo was administered to the first two groups of CF-1 mice for 10 days and, then on day 10, an estimated LD50 dose of cocaine (102 mg/kg) was given to these mice. In a third group, in order to produce a ziprasidone withdrawal state, we administered ziprasidone for 10 days, followed by no treatment for 2 days prior to cocaine administration.

Results—There was no significant difference among the three groups in overall survival: (63% in the treatment group, 60% in the withdrawal group and 80% in the placebo group. Survival time was significantly shorter for the withdrawal group than for the control group.

Conclusions—Our study may have been limited by lower than expected serum ziprasidone concentrations and lower than expected lethality from cocaine. However, our findings suggest that administration of an atypical antipsychotic for 10 days may increase the toxic effects of cocaine.

Keywords

Cocaine; ziprasidone; drug interaction

Introduction

Long-term administration of antipsychotic medications alters the regional density of dopamine, glutamate and serotonin receptors in the brain ^{1–5}. These changes, in particular up-regulation of dopamine receptors, have been suggested as the basis for some of the adverse effects that occur with long-term antipsychotic therapy ⁴. In addition to causing adverse effects, increased receptor density could increase the effects of other drugs, such as cocaine, that act through the

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affected neurotransmitter systems. Increased sensitivity to cocaine could result in a previously “safe” dose of cocaine producing life-threatening effects such as seizures or even death. As many patients taking antipsychotic medications also use cocaine ⁶, even a modest increase in susceptibility could be a major public health concern.

The purpose of this study was to determine if administration of ziprasidone, an atypical antipsychotic for 10 days, increases the lethality of cocaine poisoning in mice. We selected an atypical antipsychotic as these drugs are commonly used to treat schizophrenia. We modeled two clinical scenarios: 1) cocaine use by subjects currently taking their antipsychotic medication (ongoing treatment group); and 2) cocaine use by subjects who had recently stopped taking their antipsychotic medication (withdrawal group). We hypothesized that the toxicity from acute cocaine poisoning would be higher for both the ongoing treatment and withdrawal groups, compared to control animals administered saline.

Materials and Methods

We obtained male CF-1 mice (26–35 g initial weight, 29–41 g final weight) from Charles River Laboratories and allowed a minimum 7-day acclimation period prior to the experiments. The mice were housed in Plexiglas cages (2 to 5 per cage) on a 12-hour light-dark cycle with free access to standard rodent food and water. We used computer generated random numbers to assign the mice to one of three experimental groups: saline control (n=40 mice), ongoing ziprasidone treatment (n=40 mice), and ziprasidone withdrawal (n=45 mice). Our local Institutional Animal Care and Use Committee (IACUC) approved all animal procedures, and we followed all NIH guidelines regarding animal welfare.

We prepared ziprasidone mesylate (Pfizer Pharmaceuticals) in saline and administered it at a dose of 0.13 mg in 0.1 ml (4 mg/kg for a 33 gm mouse) via subcutaneous (SC) injection. This dose approximates the therapeutic human dose based on a body surface area comparison. Control animals received 0.1 ml of normal saline. The control and ongoing treatment groups received once-daily injection for 10 days prior to cocaine administration. The final injection was administered four hours before cocaine administration. The withdrawal group received daily injections for ten days and then no injections for a 2-day washout period prior to cocaine administration. We selected two days for the withdrawal period as this would allow clearance of all drug from the brain without allowing enough time for any up-regulated receptors to return to normal levels ⁷. During this withdrawal period, we handled the mice daily to keep them acclimated to handling.

In preliminary dose-response experiments, we determined that 102 mg/kg by IP injection was the apparent LD50 for cocaine in the CF-1 mice. We obtained cocaine HCl from Sigma Chemicals (St. Louis) and dissolved it in normal saline to produce a solution containing 6.5 mg/ml of cocaine HCl. We weighed each mouse immediately prior to administering 0.41 to 0.57 ml of the cocaine solution.

Following cocaine injection, the mice were returned (in groups of 5) to a transparent glass or Plexiglas cage for 60 minutes of continuous observation. The primary endpoint was “apparent survival” at 60 minutes post cocaine administration. This endpoint was selected because all animals that survived for 60 minutes recovered completely in previous experiments. An observer blinded to treatment group recorded the times of seizure activity (tonic-clonic activity, straub tail, loss of righting reflex, uncontrollable running, popcorn jumping) and “apparent lethality” as defined in the next paragraph.

Per our local IACUC guidelines, we did not use death as an endpoint. Instead, we used “apparent lethality”, defined as the toxic effects that occur in an animal that are usually followed by death unless the animal receives treatment. Based on our previous work, animals that

developed irregular respirations for more than 30 seconds, apnea or had seizures longer than 5 minutes were not likely to survive. Therefore, we euthanized animals exhibiting these effects by sodium pentothal injection (150 mg/kg IP) before death occurred and counted them as non-survivors. This allowed us to measure the effects of ziprasidone therapy while minimizing the distress of the experimental animals. However, occasionally animals would die from cocaine toxicity before they could be euthanized. These animals were also counted as non-survivors.

We attempted to measure serum ziprasidone levels in two mice that had received only the 10-day ziprasidone treatment. The samples were obtained 4 hours after the last dose and analyzed by a commercial laboratory using liquid chromatography and mass spectrometry (National Medical Services, 3701 Welsh Rd. Willow Grove, PA 19090).

Data Analysis

We performed two comparisons: controls versus the ongoing treatment group and control versus the withdrawal group. Our primary outcome was the proportion of animals surviving at 60 minutes and our secondary outcomes were number of animals with seizures, time interval to seizure, and time interval to apparent lethality. We calculated the 95% confidence intervals for the difference in proportions between each group. We compared the times to seizure and to apparent lethality between the two groups using a Chi square analysis of the Logrank Trend. These analyses were performed using Prism software (GraphPad Software, San Diego CA). A 2-sided p-value of 0.05 was considered significant for all analyses. Pre-experiment power calculations determined that a sample size of 40 animals per group would have 80% power to detect a 30% increase in lethality, assuming 50% lethality in the control group.

Results

Although we did not formally measure the behavioral effects of ziprasidone (4 mg/kg) injection, we did note some qualitative difference between the treatment groups. Placebo-treated mice appeared moderately excited immediately post-saline injection, followed by rapid resumption of normal activity. By contrast, ziprasidone-treated mice displayed a brief period of post-handling excitation followed by marked sedation (huddling, sleeping, minimal movement) within 15 minutes of ziprasidone injection. The animals returned to normal activity within 4 hours of treatment. The mice developed some tolerance to ziprasidone over the course of the 10-day study, and were less sedated after the first few days of treatment. The mean (SD) weights of animals just prior to cocaine were 35.1 (2.0), 33.4 (2.4), and 34.0 (2.6) gm for the control, ongoing treatment, and withdrawal groups, respectively.

Injection of the mice with an expected LD50 dose of cocaine caused increased locomotor activity in all mice. There were no qualitative differences in activity among the three groups. The proportions of mice in the control, ongoing ziprasidone treatment and ziprasidone withdrawal groups that experienced cocaine-induced seizures were 72%, 90% and 84%, respectively. These percentages were not significantly different. Nor were statistical differences observed in seizure-free survival time between the control and ongoing treatment groups (χ^2 3.8, $p=0.05$) or between the control and withdrawal groups ($\chi^2=2.4$, $p=0.12$; Figure 1).

The survival times for all groups are shown in Figure 2. Animals in the ongoing ziprasidone treatment group had a survival rate of 63% compared to the control group rate of 80% (95% confidence interval of the difference from control = -2 to 35%). The ziprasidone treatment withdrawal group had a survival rate of 60% (95% confidence interval of the difference from control = 0 to 40%). Thus, there was a trend for both ziprasidone treatment groups to have a higher mortality compared to control. The survival time for the ongoing treatment group following cocaine administration was not significantly different from that of the control group

($\chi^2=2.8$, $p=0.09$), whereas the survival time of the withdrawal group was significantly shorter than that of the control group ($\chi^2=4.22$, $p=0.04$).

Blood was drawn from two mice treated using the ongoing treatment regime at four hours post injection on the final day. However, the levels of ziprasidone were below the lower assay detection limit of 4 ng/ml.

Discussion

We found that withdrawal from 10 days of ziprasidone administration shortened the survival of cocaine poisoned mice. A similar, but smaller, effect was detected in the group that was still receiving ziprasidone. The shortened survival time in the withdrawal group supports the hypothesis that administration of the atypical antipsychotic ziprasidone for 10 days followed by a two-day withdrawal period increases the toxicity of cocaine. Although we found an absolute doubling of mortality in the withdrawal group, the lower limit of the 95% confidence interval for the difference between the placebo and withdrawal group was zero, so we cannot exclude that mortality was equal in the two groups. We found similar, but slightly smaller effects in the ongoing ziprasidone treatment group. We also found similar but non-statistically significant effects on the proportion of animals with seizures and the time to seizure onset.

Our findings are in contrast with studies evaluating acute administration of antipsychotics for treatment of cocaine poisoning. Studies have shown that acute treatment with haloperidol^{8,9} and ziprasidone¹⁰ attenuates cocaine toxicity. One explanation of these contrasting findings would be that acute ziprasidone administration antagonizes the receptors responsible for producing the effects observed during acute toxicity, whereas long-term administration of ziprasidone causes an adaptive response (such as up regulation of receptors that ziprasidone antagonizes), thereby increasing the sensitivity of animals to acute cocaine toxicity. Previous studies have shown that long-term treatment with antipsychotic medications alters the density of brain neurotransmitter receptors relevant to cocaine toxicity, including dopamine, glutamate and serotonin receptors¹⁻⁵, so there is support for this hypothesis. Our study was not designed to investigate these mechanisms, but we are planning additional studies to evaluate these hypothesis. However, our study does suggest that patients treated with antipsychotic medications may have increased susceptibility to cocaine poisoning.

There are several reasons why our model may have limited our ability to detect an effect of subacute ziprasidone treatment and withdrawal on mortality. First, despite using a dose scaled to the usual human daily dose (using body surface area), we did not detect therapeutic serum ziprasidone levels at 4 hours. The therapeutic level is reported to be 40–130 ng/ml¹¹. While we only measured these levels in two animals, we expect that all ziprasidone-treated animals had drug levels well below therapeutic throughout their treatment, and likely also had decreased brain receptor occupancy compared to humans taking therapeutic doses of ziprasidone. This might have reduced any receptor regulation that would have occurred in response to receptor occupancy. A higher dose or a continued release of drug, rather than intermittent injections, would promote consistent receptor occupancy, thus increasing the adaptive processes likely responsible for the effects observed here. A controlled release of medication would also produce lower peak serum levels than intermittent injections, and may better reflect the levels observed with oral dosing.

Our power to detect a difference in mortality was also limited by a lower than expected mortality in the placebo group. We identified this dose of cocaine as an LD50 in a previous experiment in this mouse strain, and have successfully used it several times to produce mortality between 50 and 70%^{10,12}. We believe that the lower mortality in the placebo group observed in this study is because the animals were handled daily during the experiments. Unpredictable

stress increases the behavioral effects of cocaine, and this may translate into increased toxic effects¹³. By repeatedly handling the mice, we were making the stress predictable, which would decrease the augmentation that would be caused during a one-time handling and injection. Nonetheless, the relative risk of mortality for the ongoing treatment group was twice that of the control group. In addition, the lower limit of the confidence intervals of the difference in survival was 0% between the control and the withdrawal group and a 2% increase for the control and ongoing treatment group. The consistent directions of these observed effects suggest that administration of ziprasidone for 10 days increases the susceptibility of mice to cocaine toxicity, and that our relatively small sample prevented us from identifying the difference as statistically significant.

Although the main outcome in this study was negative, we believe that the secondary outcomes and the trends in the main outcome are promising enough to warrant future studies. Chronic administration of antipsychotic medications alters the regional density of several neurotransmitter receptors in the CNS, including those for dopaminergic, serotonergic, cholinergic and glutamatergic neurotransmitter systems that contribute to the acute effects of cocaine^{14;15}. Ziprasidone binds with subnanomolar affinity to serotonin 5-HT₂ receptors and with nanomolar affinity to dopamine D₂ receptors¹⁶. Long-term treatment with other atypical antipsychotics has been shown to up-regulate these and other receptors^{1-5;7}. Ziprasidone also binds to the norepinephrine transporter¹⁷, although there is little evidence that the norepinephrine transporter is involved in cocaine toxicity¹⁸. It is also possible that ziprasidone may alter the metabolism or distribution of cocaine.

Other studies have suggested that long-term treatment with antipsychotic medication alters the behavioral response to cocaine. Mice treated with haloperidol have increased locomotor response following cocaine administration^{19;20}. A similar effect has been reported in monkeys²¹, suggesting that increased sensitivity is not a species-specific effect. We are currently planning studies with a larger sample size and a continuous infusion of antipsychotic medication to maximize our chance of detecting a difference between these groups.

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Reference List

1. Tarazi FI, Zhang K, Baldessarini RJ. Olanzapine, quetiapine, and risperidone: long-term effects on monoamine transporters in rat forebrain. *Neurosci Lett* 2000;287:81-4. [PubMed: 10854717]
2. Tarazi FI, Zhang K, Baldessarini RJ. Long-term effects of olanzapine, risperidone, and quetiapine on serotonin 1A, 2A and 2C receptors in rat forebrain regions. *Psychopharmacology (Berl)* 2002;161:263-70. [PubMed: 12021829]
3. Tarazi FI, Baldessarini RJ, Kula NS, Zhang K. Long-term effects of olanzapine, risperidone, and quetiapine on ionotropic glutamate receptor types: implications for antipsychotic drug treatment. *J Pharmacol Exp Ther* 2003;306:1145-51. [PubMed: 12829726]
4. Tarazi FI, Florijn WJ, Creese I. Differential regulation of dopamine receptors after chronic typical and atypical antipsychotic drug treatment. *Neuroscience* 1997;78:985-96. [PubMed: 9174067]
5. Lee H, Tarazi FI, Chakos M, Wu H, Redmond M, Alvir JM, Kinon BJ, Bilder R, Creese I, Lieberman JA. Effects of chronic treatment with typical and atypical antipsychotic drugs on the rat striatum. *Life Sci* 1999;64:1595-602. [PubMed: 10328519]
6. Regier DA, Farmer ME, Rae DS, Locke BZ, Keith SJ, Judd LL, Goodwin FK. Comorbidity of mental disorders with alcohol and other drug abuse. Results from the Epidemiologic Catchment Area (ECA) Study. *JAMA* 1990;264:2511-8. [PubMed: 2232018]

7. See RE, Toga AW, Ellison G. Autoradiographic analysis of regional alterations in brain receptors following chronic administration and withdrawal of typical and atypical neuroleptics in rats. *J Neural Transm Gen Sect* 1990;82:93–109. [PubMed: 1977417]
8. Derlet RW, Albertson TE, Rice P. Antagonism of cocaine, amphetamine, and methamphetamine toxicity. *Pharmacology, Biochemistry & Behavior* 1990;36:745–9.
9. Derlet RW, Albertson TE, Rice P. The effect of haloperidol in cocaine and amphetamine intoxication. *J Emerg Med* 1989;7:633–7. [PubMed: 2625524]
10. Cleveland NJ, Dewitt CD, Heard K. Ziprasidone pretreatment attenuates the lethal effects of cocaine in a mouse model. *Acad Emerg Med* 2005;12:385–8. [PubMed: 15860690]
11. Sachse J, Hartter S, Hiemke C. Automated determination of ziprasidone by HPLC with column switching and spectrophotometric detection. *Ther Drug Monit* 2005;27:158–62. [PubMed: 15795645]
12. DeWitt C, Cleveland NR, Heard K. The effect of amiodarone pretreatment on acute cocaine toxicity. *J Med Toxicol* 2005;1:1–18. [PubMed: 18072095]
13. Haile CN, GrandPre T, Kosten TA. Chronic unpredictable stress, but not chronic predictable stress, enhances the sensitivity to the behavioral effects of cocaine in rats. *Psychopharmacology (Berl)* 2001;154:213–20.
14. O'Dell LE, Li R, George FR, Ritz MC. Molecular serotonergic mechanisms appear to mediate genetic sensitivity to cocaine-induced convulsions. *Brain Res* 2000;863:213–24. [PubMed: 10773209]
15. O'Dell LE, Kreifeldt MJ, George FR, Ritz MC. Serotonin(2C) receptors appear to mediate genetic sensitivity to cocaine-induced convulsions. *Psychopharmacology (Berl)* 1999;146:313–9. [PubMed: 10541732]
16. Schotte A, Janssen PF, Gommeren W, Luyten WH, Van Gompel P, Lesage AS, De Loore K, Leysen JE. Risperidone compared with new and reference antipsychotic drugs: in vitro and in vivo receptor binding. *Psychopharmacology (Berl)* 1996;124:57–73. [PubMed: 8935801]
17. Schmidt AW, Lebel LA, Howard HR Jr, Zorn SH. Ziprasidone: a novel antipsychotic agent with a unique human receptor binding profile. *Eur J Pharmacol* 2001;425:197–201. [PubMed: 11513838]
18. Ritz MC, George FR. Cocaine toxicity: concurrent influence of dopaminergic, muscarinic and sigma receptors in mediating cocaine-induced lethality. *Psychopharmacology (Berl)* 1997;129:311–21. [PubMed: 9085400]
19. Kosten TA, DeCaprio JL, Nestler EJ. Long-term haloperidol administration enhances and short-term administration attenuates the behavioral effects of cocaine in a place conditioning procedure. *Psychopharmacology (Berl)* 1996;128:304–12. [PubMed: 8972550]
20. LeDuc PA, Mittleman G. Interactions between chronic haloperidol treatment and cocaine in rats: an animal model of intermittent cocaine use in neuroleptic treated populations. *Psychopharmacology (Berl)* 1993;110:427–36. [PubMed: 7870913]
21. Howell LL, Byrd LD. Enhanced sensitivity to the behavioral effects of cocaine after chronic administration of D2-selective dopamine antagonists in the squirrel monkey. *J Pharmacol Exp Ther* 1992;262:907–15. [PubMed: 1388204]

Figure 1.
Time to seizure curves for placebo, ongoing treatment and withdrawal groups. There were no statistical differences between groups.

Figure 2. Survival curves for placebo, ongoing treatment and withdrawal groups. Survival time was significantly longer for placebo animals than for withdrawal animals.