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The effect of olanzapine pretreatment on acute cocaine toxicity in mice

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Abstract

Background—Acute cocaine poisoning causes neuroexcitation and can be fatal. The toxic effects of cocaine can be attenuated by antagonists of serotonin, muscarinic cholinergic and dopamine receptors. Olanzapine, an atypical antipsychotic medication, is an antagonist of these receptors. The objective of this study is to evaluate the efficacy of olanzapine pretreatment for attenuation of acute cocaine toxicity using a mouse model.

Methods—Eighty male CF-1 mice were randomly assigned to olanzapine (1 mg/kg) or placebo pretreatment. Fifteen minutes later, all animals received 103 mg/kg intraperitoneal cocaine.

Results—Overall mortality was 11% for olanzapine treated animals and 45% for placebo. Olanzapine also appeared to alter the characteristics of seizures due to cocaine.

Conclusions—In this model of acute cocaine toxicity, olanzapine pretreatment attenuated acute cocaine toxicity. Olanzapine should be evaluated further as a potential treatment for acute cocaine poisoning.

Introduction

Cocaine neuroexcitation and toxicity can be attenuated by antagonists of the dopaminergic, serotonergic and muscarinic cholinergic receptors.^{1–3} Benzodiazepines are the current treatment of choice for acute cocaine poisoning,⁴ but they do not directly interact with any of these neurotransmitter systems. Olanzapine is a widely-used parenteral antipsychotic medication that is an antagonist of the D1 and D2 dopamine, muscarinic cholinergic, and 5HT_{2A} serotonin receptor.⁵ As olanzapine is a direct antagonist of several receptors that contribute to the toxic effects of cocaine, it may offer an alternative treatment for acute cocaine poisoning. The objective of this preliminary study is to determine if pretreatment with olanzapine attenuates cocaine toxicity. Our hypotheses are that olanzapine will decrease the proportion of animals reaching our endpoint of irreversible toxicity and prolong survival of cocaine poisoned mice. If pre-treatment attenuates cocaine neurotoxicity, further studies can be performed to determine if olanzapine is effective once toxicity is established.

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Methods

This was a laboratory study using male CF-1 mice weighing between 25 and 40 g. We used male animals to decrease variability in susceptibility to toxicity reported in female mice.⁶ All experiments were reviewed by our local animal use committee and complied with U.S. animal care guidelines. The animals were housed in groups of 5 with free access to food and water on 12 hour light/dark cycles. They were allowed to acclimate for a minimum of 7 days prior to all experiments.

In a preliminary study we used the up and down method to identify an appropriate dose of olanzapine.⁷ This protocol is designed to minimize the number of animals required to identify an effective dose. Animals are pre-treated with increasing doses of olanzapine and then administered an LD 90 (115 mg/kg based on previous work from our group) of cocaine. The dose of olanzapine is systematically increased or decreased depending on the response of the animal. The results are then analyzed by a computer program to determine the dose (with 95% confidence intervals) for prevention of toxicity in 50% of animals (ED50).

Once we identified a dose that appeared to be effective, we performed a randomized trial comparing olanzapine (1mg/kg) to placebo (saline). All injections were administered by intraperitoneal injection using a 25 g needle. Intraperitoneal administration was selected as it is the standard method for rodent models of cocaine toxicity.^{4;2;8} Olanzapine and saline were administered as 0.2 ml 15 minutes prior to cocaine. The cocaine was given as a precise weight based dose of 103 mg/kg dissolved in normal saline in a concentration that required 0.2 ml for a 30 g mouse. Olanzapine as Zyprexa IntraMuscular (Eli Lilly and Company, Indianapolis IN) was purchased from our hospital pharmacy and cocaine HCL was purchased from Sigma-Aldrich (St. Louis MO).

Following the cocaine injection, an investigator continuously observed the animals for signs of cocaine toxicity for 60 minutes. To minimize animal suffering, we used irreversible toxicity instead of lethality as our primary endpoint. Irreversible toxicity was defined as a seizure lasting longer than 8 minutes, a respiratory rate less than 40% of pretreatment, or inability to ambulate 30 minutes after cocaine administration. In our experience, animals that have these effects will not recover and therefore are euthanized with sodium pentothal (150 mg/kg intraperitoneal) to minimize suffering. Animals that did not reach irreversible toxicity by 30 minutes go on to full recovery by 60 minutes and were counted as survivors.

A secondary outcome was seizures. Based on our previous work we defined seizures as a tonic/clonic episode followed by a loss of righting reflex.⁸ The observer noted the time of onset of all seizures at the start of tonus. The observer also made general observations about the activity of the animals following cocaine treatment.

Our main outcome was reported as a relative risk, defined as (number of olanzapine animals experiencing the outcome/total olanzapine animals)/ (number of control animals experience the outcome/total number of control animals). Statistical significance was defined as a 95% confidence interval for the relative risks that excluded 1. Our secondary outcome was the time to irreversible lethality in control compared to olanzapine treated animals. These were compared using a log-rank test. A sample size of 40 animals per treatment provides 80% power to detect a 30% change in survival assuming a 50% survival in the placebo group.

Results

The initial study population included 40 mice per group. Two animals in the olanzapine group and one animal in the placebo did not receive a complete injection and were excluded. The

total number of animals completing the protocol was 38 for the olanzapine group and 39 for the control group.

The overall proportion of animals that developed irreversible toxicity was 46.2% (95% CI 30 to 63%) in the control group and 10.5% (2.9 to 24%) in the olanzapine group. The relative risk of irreversible toxicity for olanzapine-treated animals relative to control animals was 0.23 (0.09 to 0.69). The median time to irreversible toxicity was undefined in both groups as fewer than 50% of the animals in either group reached irreversible toxicity. Survival analysis showed longer survival (Chi square for Log Rank=12.9, $p<0.0001$) in olanzapine treated animals (Figure 1).

Olanzapine had an unexpected effect on cocaine-induced activity and seizures. Untreated animals would have uncontrolled running, and generally have a tonic-clonic seizure with loss of righting reflex (our initial definition of seizures for this study). Animals treated with olanzapine had some uncontrolled running followed by a loss of righting reflex that appeared to be a seizure, but no tonic-clonic activity and therefore did not meet our a-priori definition of seizure. Using our standard definition of seizures, the rate of seizures in the first olanzapine treated animals was 16% vs 55% in control animals ($n=20$ /group). However, after recognizing that olanzapine-treated animals were likely having seizures without tonic-clonic activity, we refined our definition of seizures and applied this definition to the second group of 20 animals. When seizures were defined as uncontrolled running followed by loss of righting reflex, the rate of seizures was 100% for olanzapine treated animals and 70% in control animals ($n=20$ /group). As this was not a planned analysis, we did not perform a statistical comparison between the treatments.

Discussion

Pretreatment with olanzapine dramatically attenuates cocaine toxicity. Previous studies using a similar model and cocaine dose found that the relative risks of toxicity for ziprasidone or diazepam treated animals relative to controls are only 0.5 and 0.4 respectively.⁸ This suggests that olanzapine may attenuate toxicity more effectively than the standard treatment. However, direct comparisons are needed to make a definitive statement.

Studies evaluating other antipsychotic medications have also reported protective effects. While studies of haloperidol, a typical antipsychotic, have reported no effect, these studies were limited in sample size and when the results of these studies are combined, there is a modest protective effect.^{9;10} Ziprasidone, an atypical antipsychotic, is equivalent to diazepam for prevention of cocaine toxicity.⁸ Ziprasidone and olanzapine have similar binding at dopamine, alpha adrenergic, and 5HT-2 and 6 serotonin receptors, and different binding at the muscarinic cholinergic receptor and monoamine transporters.⁵ This is concordant with prior studies suggesting that the dopamine, sigma and serotonergic systems mediate cocaine toxicity.^{1;3}

Olanzapine appears to change the manifestation of seizures from cocaine. This was a surprising finding, as previous studies of antipsychotic medications have reported a decrease in seizures. It is possible that the decrease reported in these studies may in fact be a change in the manifestations rather than prevention of seizures. It is also possible that differential 5-HT receptor binding between olanzapine and the agents used in previous trials could be responsible.¹¹ Further studies using EEG monitoring will be required to verify these observations.

While our study focused on cocaine neurotoxicity, cocaine overdose can also produce cardiotoxicity by direct antagonism of fast sodium channels¹² and vascular effects through vasoconstriction.¹³ Our study was not designed to determine if the irreversible toxicity was attributable to neurological or cardiovascular toxicity. However, the olanzapine does not have significant effects on cardiac sodium channels¹¹, suggesting that the effects we observed are

not due to direct cardiac effects. Olanzapine has relatively high affinity for alpha1 adrenergic receptors¹⁴, so we cannot exclude antagonism of peripheral alpha receptors as the mechanism for the observed protective effect.

Our study has several limitations. The first is the use of an animal model that may not translate directly to humans. However, mice are a standard model of cocaine toxicity and this dose of olanzapine is similar to the human dose when scaled using body surface area (this would be approximately 6 mg in a 70 kg human).¹⁵ Therefore we believe these results are promising enough to warrant further study. Second, we used a pre-treatment model and intraperitoneal cocaine and olanzapine administration. We selected pre-treatment as it offers the best chance of drug delivery and assurance of adequate serum concentrations. We used intraperitoneal administration as it is a standard method for studies of cocaine toxicity and allows comparison of our results to those of other studies. Next, we were only able to directly compare olanzapine to placebo rather than to diazepam. Our Animal Use and Care Committee felt that having a second control group would result in excessive animal and was not appropriate for a preliminary study already limited by pre-treatment. They recommended that direct comparisons be performed in a more clinically relevant model.

Conclusion

Olanzapine attenuates cocaine neurotoxicity in a mouse model. Olanzapine appears to alter the seizures by cocaine, further study is needed to determine if there is EEG evidence of ongoing seizures. If olanzapine does not decrease seizures, it may not be useful for treatment of cocaine toxicity. However, as olanzapine has a direct effect on the neurotransmitter systems responsible for cocaine toxicity,¹⁻³ we suggest that further studies are warranted to evaluate the potential role of olanzapine for acute cocaine poisoning in humans.

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Figure 1.

Apparent survival curves for olanzapine and placebo treated mice. Olanzapine treated mice have a significantly prolonged apparent survival (X^2 for Log-Rank test =12.9, 1 degree of freedom; $p<0.001$). Apparent survival is defined as a seizure less than 8 minutes duration, respiratory rate less than 40% of pretreatment after the seizure, or inability to ambulate 30 minutes after cocaine administration