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Original Contribution

# Protective effects of minocycline, doxycycline and tetracycline on seizure and lethality in a mice cocaine toxicity model ☆

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ABSTRACT

*Introduction:* Acute cocaine intoxication is one of the important causes of admission to emergency department, especially in western countries. We aimed to compare the efficacies of tetracycline, minocycline, doxycycline in the prevention of seizures and deaths in mice due to cocaine intoxication.
*Methods:* In the study, a total of 120 balb-c male mice weighing 25–30 g were randomized into 4 groups as tetracycline 255 mg/kg, minocycline 170 mg/kg, doxycycline 157 mg/kg, 0.5 ml saline (placebo). The doses of tetracycline, minocycline and doxycycline are the calculated ED50 values. The mice in the groups received 93 mg/kg cocaine intraperitoneally 10 min after drug administration. The dose of cocaine is 50% of the lethal dose. After cocaine injection, all mice were observed for 30 min in terms of cocaine toxicity findings. Mortality rates, death times, seizure activities, and seizure onset times of the mice were clinically evaluated in an observational way.
*Results:* There were significant differences among all the groups in terms of seizure and lethality ($p < 0.001$). The ratio of animals with seizures was significantly lower in the *minocycline* (73.3%), and *doxycycline* (73.3%) groups (all $p = 0.040$). The ratio of animals with lethality was significantly lower in the *minocycline* (23.3%) group compared with vehicle ($p < 0.001$).
*Conclusion:* In our acute cocaine intoxication model, minocycline was effective in terms of lethality and preventing seizures, doxycycline was effective in preventing seizures.


## 1. Introduction

Acute cocaine intoxication is one of the important causes of admission to emergency department, especially in western countries. 27% of patients admitted to the emergency department as a drug addict are cocaine addicts [1]. Unlike Turkey, cocaine is the most common fatal illegal substance encountered in patients presenting to the emergency department in the United States [2]. As is known, although the use of cocaine in western countries has been decreasing due to the increasing awareness towards the risks of cocaine use and numerous anti-drug campaigns [3]; in our country, its use is getting increased especially among the people with high income living in big cities [4]. In Turkey, there are very few comprehensive studies that have been conducted on the prevalence of substance use. In a study conducted with university students in Ankara, the rate of hallucinogen and cocaine use was found to be 5% [4].

The effect of cocaine depends on the amount taken, the duration of administration and the route of administration. Stimulation of the cerebral cortex causes symptoms such as restlessness, excitement and increased motor activity, and leads to general sympathetic stimulation. Motor centers in the medulla spinalis are stimulated at toxic doses which results in the emergence of tonic-clonic seizures. Cocaine reduces seizure threshold and facilitates convulsions. Cocaine-related seizures have been found to be associated with morbidity and mortality in cocaine toxicity [5]. Cocaine increases the activity of monoamine neurotransmitters by blocking the dopamine, norepinephrine and serotonin reuptake

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pumps in the central and peripheral nervous systems. Cardiovascular complications, seizures and death are encountered through these mechanisms. There is no active treatment model in cocaine toxicity to reduce toxic effects other than symptomatic treatment. With the aim of reducing toxic effects, many studies have been carried out while many drugs have been tried with different methods. The current treatment approach for acute cocaine toxicity is the use of benzodiazepines. However, because of the fact that benzodiazepines do not have any direct effect on the neurotransmitters and toxication, their use is not sufficient on its own to prevent cocaine toxicity, which leads to a need for combined drug use. Neuroinflammatory response, which is responsible for the pathogenesis of seizures, produced in CNS with the activation of microglia and astrocytes causes the release of proinflammatory cytokines such as interleukin (IL) and tumor necrosis factor (TNF).

Through these cytokines, neuroinflammation occurs leading to the increase in neuronal excitability and so seizures are triggered [6-9]. Besides, in the case of inflammation, metalloproteinases increase and cause tissue damage and apoptosis. Tetracycline group antibiotics inhibit metalloproteinase-9 inhibition that plays a role in restoring tissue damage [10,11], inhibition of proinflammatory cytokines [6] and polypolymerase increasing neuronal excitability [7,8]. This group of antibiotics has been reported to show anti-inflammatory and neuroprotective properties by preventing apoptosis through inhibiting metalloproteinases, and by inhibiting TNF-alpha and pro-inflammatory cytokines. Thus, it has been reported that by reducing excitability, it decreases incidence of seizures, their duration and number [9]. Furthermore, it has been shown that tetracycline group antibiotics reduce infarct areas in myocytes by decreasing proinflammatory cytokines specifically high mobility group box-1, LDH and CK levels [12,13]. Because of their cardioprotective effects, these antibiotics may be thought to reduce cardiotoxic effects of cocaine. Studies have shown that tetracycline group antibiotics protect cardiomyocytes, decrease the inflammatory response and decrease infarct areas [12]. In another study, it was shown that poly polymerase-1 was inhibited in cardiac myocytes and thus myocyte deaths decreased [13]. In this way, cocaine-induced cardiac deaths can be reduced.

In our study, we aimed to compare the efficacies of tetracycline, minocycline, doxycycline in the prevention of seizures and deaths in mice due to cocaine intoxication. As the hypothesis of the study, we suggest that tetracycline group antibiotics will decrease seizure activity and increase survival in cocaine intoxication because of their neuroprotective and cardioprotective effects. To the best of knowledge, our study is the first one on this subject.

## 2. Methods

### 2.1. The design of the study

The study is a prospective, randomized, double-blind, placebo-controlled experimental study. The aim of this study is to investigate and compare the efficacies of tetracycline, minocycline and doxycycline in the prevention of seizures and deaths due to cocaine intoxication in mice. In the study, a total of 120 balb-c male mice weighing 25–30 g were randomized into 4 groups. The randomization process was performed by assigning numbers randomly using a simple allocation strategy. Researchers continued the research study without knowing the treatment groups throughout the study.

### 2.2. Selection of experimental animals

Prior to the study, the approval of Animal Experimentation Ethics Committee of Pamukkale University was obtained. A total of 120 male balb-c mice weighing between 25 and 30 g were used in the study. The reason why we selected male mice was to eliminate the difference that could be caused by the sensitivity of female mice to toxicity. The mice were obtained from the Experimental Animal Laboratory of Pamukkale University. A photo period of 12-h day/12-h night cycle was applied and the mice were fed ad libitum with standard food and water. All experiments were performed in the same laboratory in March 2015.

### 2.3. Study groups and treatments

The distribution of drug doses administered intraperitoneally to each group in our study is as follows:

Group 1: Tetracycline 255 mg/kg.
Group 2: Minocycline170 mg/kg.
Group 3: Doxycycline 157 mg/kg.
Group 4: 0.5 ml saline (placebo).

The doses of tetracycline, minocycline and doxycycline are the calculated ED50 values (the drug dose which constitutes 50% of the maximum effect) in terms of anticonvulsant activity in the previous studies conducted [14].The drugs in all study groups were administered as a pre-treatment before cocaine. In this study, it was planned to give the selected antibiotic groups before cocaine administration by considering similar studies [15]. This method was preferred to evaluate drug efficacy in order to prevent seizures and deaths due to the fact that mice have a rapid seizure following cocaine administration. The study of mice cannot be conceptualized for people in this form, but it is important for them to guide other studies in the future. The mice in the groups received 93 mg/kg cocaine intraperitoneally 10 min after drug administration [15]. The dose of cocaine is 50% of the lethal dose.

### 2.4. Method and procedures

All injections were administered intraperitoneally using a 25 G injector. The drugs were diluted with sterile distilled water before use and put into 1 ml injectors. 2.79 mg/dl cocaine solution was obtained by preparing cocaine with sterile distilled water. Intraperitoneal administration was selected as it is the standard method for rodent models of cocaine toxicity [15]. Because of the necessity that the researchers shouldn't know the drugs administered, in order to imitate the combination group, 0.1 ml of saline was injected in the tetracycline, minocycline, doxycycline and saline groups after the first injection. After 10 min, cocaine was administered at a dose of 93 mg/kg.

### 2.5. Conducting the study and data collection

After cocaine injection, all mice were observed for 30 min in terms of cocaine toxicity findings. The observation time was determined as 30 min because all of the deaths in previous studies [15] occurred in the first 8.5 min on average. The mice were placed in separate observation cages from the beginning of the study. During the observation, mortality rates, death times, seizure activities, seizure onset times of the mice were evaluated. The seizure and lethality were evaluated in an observational way. In clinical evaluation, seizures were evaluated based on rapid and repetitive jumping movements of mice (such as popcorn jumping), tonic-clonic activity and righting reflex parameters. The changes observed in the mice were recorded in the observation scales that had been prepared separately for each group.

### 2.6. Primary data analysis

Chi-squared tests and Fisher's exact tests were used for comparisons where appropriate. Survival analysis was performed, and

Kaplan–Meier survival curves with log rank were generated. Results were considered statistically significant when $p < 0.05$. The data were analysed with Statistical Package for Social Sciences (SPSS) for Windows, Version 22.0. The study was designed to have 80% power to detect a 30% difference in lethality assuming a control group survival of 30%.

## 3. Results

There were significant differences among all the groups in terms of seizure and lethality ($p < 0.001$). The ratio of animals with seizures was significantly lower in the Minocycline (73.3%), and Doxycycline (73.3%) groups compared with vehicle (all $p = 0.040$). The ratio of animals with lethality was significantly lower in the Minocycline (23.3%) group compared with vehicle ($p < 0.001$). The results of the pretreatment with each medication, and placebo on seizure incidence and lethality are shown for each group in Table 1.

Fig. 1 shows that all treatments prolonged the time to lethality, which was only significantly longer in the Minocycline group compared to placebo-treated mice ($p < 0.001$). In addition, time to seizure was also significantly longer in the Minocycline and Doxycycline groups compared to placebo-treated mice (Fig. 2, $p < 0.001$).

## 4. Discussion

The present study demonstrates that pretreatment with Minocycline and Doxycycline reduces seizure activity (all $p = 0.040$); Minocycline reduces lethality ($p < 0.001$) statistically during acute cocaine toxicity. However, Tetracycline did not reduce the prevalence of these outcomes. When the studies in the literature were examined, it was seen that there were many conducted to evaluate the neuroprotective and cardioprotective effects of tetracycline group antibiotics [9-13]. As the hypothesis in our study; we suggested that due to neuroprotective and cardioprotective effects of tetracycline-class antibiotics, they would reduce seizure activity and increase survival in cocaine intoxication. According to the literature review, our study was the first study to evaluate the efficacy of tetracycline group antibiotics in the cocaine intoxication model.

In a study by Wang et al. [14], mice that were given different doses of tetracycline, doxycycline and minocycline were subjected to corneal flow at different frequencies and the seizure activity for 4 h was evaluated. Anticonvulsant activity was found to be significant in all three drugs compared to the control group. Minocycline was revealed to be the most effective drug. In the study, the toxicity of the drugs and dose-response studies were also conducted, and they revealed ED50 values of 254.6 mg/kg for tetracycline, 157.3 mg/kg for doxycycline and 170.3 mg/kg for minocycline. In our study, we used tetracycline group antibiotics in similar doses and found tetracycline ineffective in terms of seizure activity after cocaine use in toxic doses. In our study, tetracycline was found to be effective only in 6 hertz model and ineffective in other electrical studies. Similar to the results of this study, we found doxycycline and minocycline effective in reducing seizure activity.

**Table 1**
Distribution of convulsions and lethality produced by cocaine according to the study groups.

| Variables | Study groups | | | | |
|---|---|---|---|---|---|
| | Tetracycline | Doxycycline | Minocycline | Placebo | *p* |
| Seizure, n (%) | 27 (90) | 22 (73.3) | 22 (73.3) | 28 (93.3) | <0.001 |
| Lethality, n (%) | 15 (50) | 17 (56.7) | 7 (23.3) | 22 (73.3) | <0.001 |

*p*: values comes from chi-squared test.

Previous studies have shown that tetracycline group antibiotics inhibit the inhibition of metalloproteinase-9 which plays a part in repairing tissue damage [10,11], the inhibition of proinflammatory cytokines [6] and poly polymerase derivatives, which are among the nuclear enzymes that increase neuronal excitability [7,8] by playing a role in cell destruction. This group of antibiotics has been reported to show anti-inflammatory and neuroprotective properties by preventing apoptosis through inhibiting metalloproteinases, and by inhibiting TNF-alpha and pro-inflammatory cytokines, and thereby decrease seizure, its duration and its number by reducing seizure excitability [9]. Cocaine was thought to cause tissue damage and apoptosis by increasing metelloproteinases [16]. In a study conducted by Smith et al. [17] on rats, the use of cocaine was observed to increase metalloproteinase activity in brain cells. In addition, it was thought that in toxic doses it lowered the seizure threshold by stimulating the motor centers in the medulla spinalis, and this caused the incidence of tonic-clonic seizures. Brundula et al. [18] evaluated minocycline activity in multiple sclerosis in a study, and minocycline was revealed to decrease microglial activation and apoptosis by reducing metalloproteinases. In the light of these studies, it has been shown that cocaine increases metalloproteinases, thus facilitating inflammation and seizures. The tetracycline group antibiotics, which reduce inflammation and tissue damage, are tested among the drugs that can be used in the treatment of seizures. In our study, using this information, we hypothesized that this group of antibiotics would reduce seizure and lethality in acute cocaine intoxication. Similar to the findings of these studies, in our study we found that these antibiotics reduced seizure and lethality.

In a study conducted on rats, Nogueriaa et al. [19] examined the effects of 10–25–50–100 mg/kg doses of intraperitoneal doxycycline applied to four different groups after the administration of pilocarpine on seizure and lethality. Pilocarpine is an agent that causes seizure with cocaine-like mechanisms in dopamine, serotonin, glutamate, muscarinic and dopaminergic ways [20]. It affects by blocking the dopamine, norepinephrine and serotonin reuptake pumps. Compared to the positive control group, while the most successful group in reducing seizure was the 25 mg/kg doxycycline group, the most successful group in preventing lethality was the 50 mg/kg doxycycline group. In this study, 7 h after the experiment, temporal cortex was examined, and amino acid levels were compared. Compared to the positive control group, doxycycline decreased glutamate and aspartate levels, and increased the amount of GABA. Glutamate has an effect on the development of seizure by facilitating neuronal excitability [21]. It is known that cocaine plays a role in the development of seizure by reversibly binding to postsynaptic GABAA receptors and acting as an inhibitor [22]. Pharmacologically, in cocaine uptake, increased endogenous GABA levels were shown. In the studies, drugs that inhibit GABA uptake (diazepam, phenobarbital) were found to be effective in preventing cocaine-induced seizure [23]. Combining the results of these studies and the results of our own study, it can be suggested that one of the reasons why tetracycline group antibiotics reduce seizure and lethality after pilocarpine, which is similar to cocaine effects is the fact that these drugs reduce glutamate and increase GABA levels. In the study, after the application of doxycycline, the dose of 25 mg/kg was found to be more effective in seizures, and 50 mg/kg dose was revealed to be more effective in lethality, which shows that more dose studies are needed for these drugs. In our study, all the groups were administered the same dose. Similar to our study, new results can be obtained for seizure and lethality by applying different doses.

The most important cause of cocaine-induced death is known to be caused by cardiac toxic effects. In electrophysiological studies, it has been revealed that cocaine acts as class 1 antiarrhythmics by affecting sodium channels and leads to dysrhythmias, acute

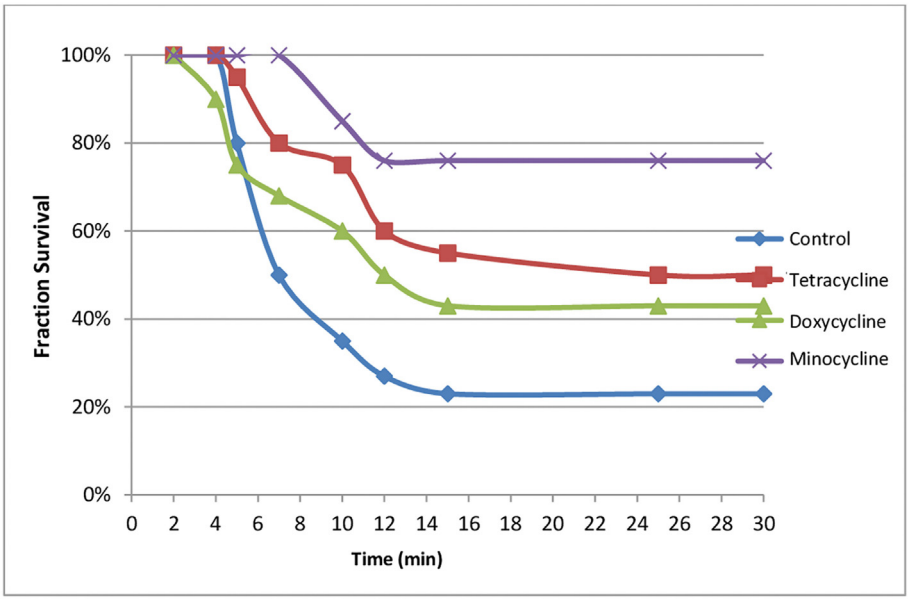


**Fig. 1.** Kaplan-Meier plot for apparent lethality for each treatment.

vasospasm or MI through the exacerbation of chronic atherosclerotic disease [24]. In cardiac research on minocycline, it reduces cell damage by reducing poly polymerase-1 in myocytes, which is similar to its effect in neurons [13]. It has also been shown that minocycline reduces infarct areas in myocytes by decreasing proinflammatory cytokines specifically High mobility group box-1, LDH and CK levels [12]. Accordingly, it can be considered that minocycline shows cardioprotective effects by protecting cardiomyocytes and decreasing inflammatory response and infarct areas, and thus reduces cardiotoxic effects of cocaine. Thus, cocaine-induced cardiac deaths can be reduced. In our study, we can associate the low lethality rate in the minocycline group to the fact that minocycline suppresses cardiovascular events, which are considered among the most common causes of cocaine-induced lethality.

In studies conducted on CNS, minocycline has been shown to inhibit poly polymerase-1, which is one of the nuclear enzymes that are found in the brain tissue and play a role in cell destruction and increase neuronal excitability [7,8]. In a study on rats, Drabek et al. [25] examined brain tissue damage and TNF-α levels of minocycline after prolonged cardiac arrest. In the study, the experimental group was given 90 mg/kg minocycline after hypothermic arrest. An analysis of the cortex, striatum, hippocampus and cerebellum indicated that levels of TNF-α decreased in the minocycline group. Thus, lethality and inflammation in neurons decreased; hence, it reduced the seizure. In a study by Ahmadirad et al. [26], the effects of minocycline on mice with induced seizure by using 37.5 mg/kg pentylenetetrazole were investigated. Minocycline was administered at an intraperitoneal dose of 25 mg/kg, one hour before and after pentylenetetrazol. Then, the hippocampus and priform cortex of the mice were dissected and levels of TNF-α and GABA receptor expression levels were compared with the control group. In the analysis, it was observed that TNF-α gene expression decreased and GABA receptor subunits increased. This explains that minocycline can inhibit the seizure by reducing TNF-α involved in inflammation and by increasing GABA, the major inhibitory neurotransmitter. These studies indicate that minocycline can prevent seizure by reducing neuroinflammation that has a role in the seizure pathogenesis and by reducing cell death. With these

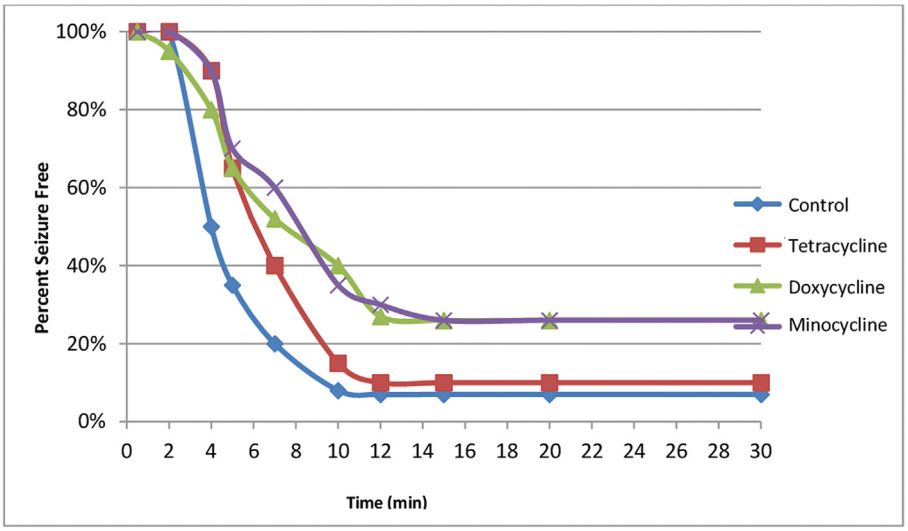


**Fig. 2.** Kaplan-Meier plot for time to seizures for each treatment group.

results, we can explain that minocycline is the most successful drug in our study because it reduces cell lethality and inflammation in both central and cardiac systems.

It is thought that the glutamatergic system has an important role in the cocaine-induced seizure development mechanism. NMDA antagonists reduce cocaine-induced seizure and lethality [21]. In a study on rats by Gonzales et al. [27], the hippocampus was dissected and examined under the stereo microscope, and minocycline was found to reduce the concentration of cytosolic calcium by 40%. Decreased calcium levels have been shown to reduce the amplitude and frequency of glutamate-induced postsynaptic stimulus, making the neuron excitability difficult. Reduction of NMDA receptor gene expression with this mechanism may explain the anticonvulsant activity of minocycline. As is known, cocaine reduces seizure threshold and facilitates the convulsions. In our study, the decrease in seizure activity observed in tetracycline-group drugs can be explained in this way.

Although tetracycline and doxycycline decreased the number of deaths in our study, it was not as effective as minocycline. The reason for this is that we think that minocycline is the most lipophilic and is the best agent for passing the blood brain barrier. Lipophilicity may contribute to the effectiveness of minocycline and doxycycline.

Although an accepted cocaine intoxication model was used in our study, findings obtained from animal studies can only be used to support human studies. Our study also had some limitations. First of all, the mice could not be monitored in terms of blood pressure, heart rate, pulse oximetry and respiratory rate and epileptic activity by EEG. Therefore, especially cocaine-induced cardiorespiratory complications due to cocaine were not revealed. Another limitation of the study is the fact that tetracycline, doxycycline and minocycline were administered only at the doses administered in clinical studies. Lower and higher doses may lead to different results. The doses applicable to humans were not clearly established. Another limitation is that the mechanism of lethality could not be explained and subclinical seizures could not be detected because we could not measure the degree of excitability at the receptor level and we were not able to perform hemodynamic, electrocardiogram, and electroencephalogram monitoring.

## 5. Conclusions

In our acute cocaine intoxication model, minocycline and doxycycline were effective in preventing seizures. While minocycline was effective in terms of lethality, tetracycline and doxycycline were not found to be so. It's being the best lipophilic agent that crosses the blood brain barrier and its cardioprotective and neuroprotective effects characterize minocycline.

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