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Evaluation of Ampicillin and Ceftriaxone on Ethanol Consumption as Compared to Naltrexone in Alcohol Dependant Rats

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Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

Original Research Article

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ABSTRACT

Context: Extracellular glutamate level in reward centre of brain increases during ethanol drinking sessions. Hence, it can be hypothesized that drugs which decrease extracellular glutamate might have deaddictive properties. It has been shown that β-lactam antibiotics are potent stimulators of glutamate transporter 1(GLT1) expression. Previous studies have shown that ceftriaxone decreases ethanol consumption but this has not been compared to standard line of treatment (naltrexone). Also, no study was conducted for testing ampicillin even if in an in-vitro experiment ampicillin has shown to increase GLT1 levels more than ceftriaxone. Hence, our study's objectives were to compare efficacy of ceftriaxone and ampicillin with naltrexone on ethanol consumption in rats.
Methods: Permission of ethics committee was taken. Study was divided into two parts. Part I included standardization of model & Part II included 8 groups of six rats each. Group 1: vehicle control, Group 2: 1mg/kg/d naltrexone, Group 3: 100mg/kg/d ceftriaxone, Group 4: 200mg/kg/d ceftriaxone, Group 5: 100mg/kg/d ampicillin, Group 6: 200mg/kg/d ampicillin were given i.p injections for 15 days and Group 7: 200mg/kg ceftriaxone & Group 8: 200mg/kg ampicillin i.p. single dose. Parameters measured were ethanol & water intake per day for 15 days.
Results: Groups 2 to 8 showed statistically significant decrease in ethanol intake as compared to vehicle control. Also, group 3 & 4 showed an increase in water consumption

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as compared to Group 1.
Conclusions: Our study recommends that drugs acting on glutamate pathways like ceftriaxone and ampicillin can be explored for treatment of alcohol dependence.

Keywords: Glutamate transporter 1; ceftriaxone, ampicillin; beta lactam antibiotics; ethanol.

KEY MESSAGES

New treatment avenues are being explored for the treatment of alcohol dependence. One new avenue is to decrease synaptic glutamate by increased transcription of glutamate transporter 1 (glt1). Ceftriaxone has been proven to increase glt1 transporter and decrease alcohol consumption in rats. However, ampicillin in an invitro study has been shown to increase glt1 transporter more than ceftriaxone. Also both these drugs have not been compared to the standard line of therapy like naltrexone. Our study for the first time shows that ampicillin decreases ethanol consumption in rats and both ceftriaxone and ampicillin decrease ethanol consumption which is comparable to naltrexone.

1. INTRODUCTION

National Council on Alcoholism and Drug Dependence and the American Society of Addiction Medicine defines alcoholism as a primary, chronic disease with genetic, psychosocial and environmental factors influencing its development and manifestations characterized by impaired control over drinking, preoccupation with drug alcohol, use of alcohol despite adverse consequences and distortions in thinking, most notably denial [1].

Chronic alcoholism results in increased risks of cardiovascular & cerebrovascular accidents. Ethanol results in hepatic & pancreatic dysfunction, as also anemia and impotence in men [2].

The current therapy of alcohol addiction consists mainly of the USFDA approved drugs namely disulfiram, acamprosate & naltrexone [2]. These drugs although efficacious and relatively safe have multitude problems. For instance, if ethanol is ingested while taking disulfiram, the inhibition of aldehyde dehydrogenase causes increased levels of acetaldehyde, resulting in disulfiram-ethanol reaction which, in severe cases, may lead to congestive heart failure (CHF) or even death. Acamprosate is associated with gastrointestinal upset, dizziness, confusion and headache. Adverse effects of naltrexone are headache, depression, fatigue, nervousness, insomnia etc. All the three drugs lead to hepatic, renal and cutaneous impairement [3]. Thus, there is a dire need for efficacious and safe drugs for alcohol deaddiction.

Insights into pathophysiology of alcohol dependence have revealed that several aspects of alcohol addiction involve changes in the glutaminergic transmission. These studies have demonstrated that increase in the extracellular glutamate level in the reward centre of the brain occurs during ethanol drinking sessions [4]. The glutamatergic neurons project from amygdala and prefrontal cortex on the mesolimbic reward pathway from which reciprocal dopaminergic projections arise [5]. Release of dopamine in nucleus accumbens results in the subjective feeling of reward. Thus, dopamine results in pleasure and causes the drug abusers to repeat this behavior of drug consumption to re-experience this pleasure, resulting in addiction [6]. Hence, it can be hypothesized that drugs that decrease extracellular

glutamate might have de-addictive properties by decreasing the release of dopamine in the reward centre.

Glutamate, the principal excitatory neurotransmitter, once released in the synapse, is inactivated by its uptake by the astroglial glutamate transporter 1 (GLT1). Rothstein et al have shown that β-lactam antibiotics are potent stimulators of GLT1 expression, the action being mediated through increased transcription of the GLT1 gene. The increase in GLT1 expression is three times with ceftriaxone and four times with ampicillin [7]. Furthermore, other investigators substantiated on this lead by conducting an experimental study on rats and showed that when ceftriaxone was given in doses of 25, 50, 100 and 200 mg/kg there is a decrease in ethanol consumption.[8] But, only the doses of 100 mg/kg and 200 mg/kg of ceftriaxone showed an increase in the expression of GLT1 transporter [8]. Hence these two doses of ceftriaxone were chosen for our experiment. Although the magnitude of increase of GLT1 expression was higher with ampicillin as compared to ceftriaxone, still no experimental study has been conducted to evaluate the effects of ampicillin in decreasing alcohol consumption. Doses of ampicillin were taken to be same as ceftriaxone to see whether the in-vitro effect of higher GLT1 activation by ampicillin is translated in an in-vivo experiment. Moreover, researchers have shown in in-vitro studies even a single dose of ceftriaxone and ampicillin lead to induction of gene for synthesis of GLT1. This induction had resulted in rise in levels of GLT1 even 5 days after the dose.[7] Hence, it was decided to test single high doses of ceftriaxone and ampicillin also. Further, there have been no studies in which this newly found use of beta-lactam has been compared to existing drug treatment for alcohol addiction (e.g. naltrexone or acamprosate). In comparison between these two standard drugs, naltrexone is a better deaddicting agent because acamprosate's beneficial effects were limited upto a particular dose. It caused weight loss and its efficacy was not associated with sustained effect beyond its discontinuation [9]. Hence, the present study was conducted to compare the efficacy of ceftriaxone and ampicillin with naltrexone in potentially alcoholic rats.

2. MATERIALS AND METHODS

2.1 Ethical Consideration

The study was conducted after obtaining the approval of the Institutional Animal Ethics Committee (AEC/06/2011). Committee for the purpose of control and supervision on experiments on animals (CPCSEA) guidelines were followed during the entire experiment.

2.2 Animals

63 male Wistar rats bred in the Central animal house of Seth G.S. Medical College and KEM Hospital were utilized for the study. Of these, 15 animals were used for standardization of the animal model and the remaining 48 animals were used for conducting the actual study. They were maintained under standard laboratory conditions on 12-hour day/night cycle. The animals were acclimatized to the laboratory conditions prior to experimentation. All the experiments were carried out between 10:00 hours to 16:00 hours at ambient temperature. Food was provided ad libitum and water was provided as per the protocol.

2.3 Description of Animal Model

Albino rats of the Wistar strain (135–150 g) were screened for potential alcoholism after subjecting them to forced swim test for 15 minutes. A typical response in the forced swim test consists of rats swimming actively for some time, pause for some time, and then continue swimming. Rats were allowed to swim in 20cm deep water in a large plastic tub. The period of inactivity (passive swimming) with the head seldom protruding over the surface of the water, was recorded for each rat. This period of inactivity is directly proportional to the depressive like state. The very active rats were characterized by an inactive period, shorter than 130 seconds and the less active ones by a period of 300 seconds or longer. The less active rats are the potential alcoholics. These potentially alcoholic rats were used for the study. They were kept singly in polypropylene cages with free access to food and 20% w/v ethanol in water to drink for a period of 15 days (Period 1). For the next 15 days they had access to two bottles: one containing water and the other 20% w/v ethanol in water (Period 2). This induces alcohol dependence in rats as explained by Sukul et al [10]. During the period of standardization, after the first two periods, the alcohol and water consumption was monitored for a period of 15 days. The average consumption of ethanol and water of those 15 days was calculated and is mentioned in Table 1.

The study was conducted in two parts:

Part I of the study consisted of standardization of the aforementioned animal model using 15 rats. We standardized the model since this model was never used in our experimental setup before.
Part II consisted of inducing alcohol dependence using the same model.

This was followed by dividing the 48 rats into the following eight groups:

1) Group 1 (Control): vehicle control (Normal Saline) i.p. (intraperitoneally) for 15 days
2) Group 2 (Positive control): 1mg/kg/d naltrexone i.p. for 15 days
3) Group 3: Given 100mg/kg/d ceftriaxone i.p. for 15 days
4) Group 4: Given 200mg/kg/d ceftriaxone i.p. for 15 days
5) Group 5: Given 100mg/kg/d ampicillin i.p. for 15 days
6) Group 6: Given 200mg/kg/d ampicillin i.p. for 15 days
7) Group 7: Given 200mg/kg/d ceftriaxone i.p. single dose
8) Group 8: Given 200mg/kg/d ampicillin i.p. single dose

The daily consumption of ethanol and water by each rat and their weights were recorded for 15 days (Period 3). The study was assessed by a third person who was blind to our experiment, so it was an assessor blind study.

3. RESULTS

3.1 Part I

A total of 15 animals were used for standardisation.

Table 1. Results of standardisation of the model

Parameters analyzed	Mean ± SD
Alcohol (g/kg/d)	3.92±2.53
Water (g/kg/d)	101.6 ±15.49

3.2 Part II

Fig. 1 depicts the daily intake of ethanol (g/kg of body weight) by the eight groups expressed as mean ± SD were 3.84±1.65 for the vehicle control group, 0.84±0.18 for the naltrexone positive control group, 1.55±0.12 and 0.92±0.17 for the ceftriaxone 100mg/kg and 200mg/kg dose respectively, 0.83±0.27 & 0.78±0.13 for the ampicillin 100mg/kg & 200mg/kg dose respectively and1.21±0.40 and 0.98±0.28 for single doses of ceftriaxone & ampicillin respectively.

Ceftriaxone 100mg/kg group and 200mg/kg group rats drank 60% and 76% less ethanol as compared to vehicle control rats and this was statistically significant. Also, ampicillin 100mg/kg group and 200mg/kg group rats drank 78% and 80% less alcohol as compared to vehicle control rats and this too was statistically significant.

Even though not statistically significant, the higher dose of ampicillin showed a decrease in the ethanol consumption as compared to naltrexone.

As seen in Fig. 1, both ceftriaxone and ampicillin show a dose dependent decrease in ethanol consumption.

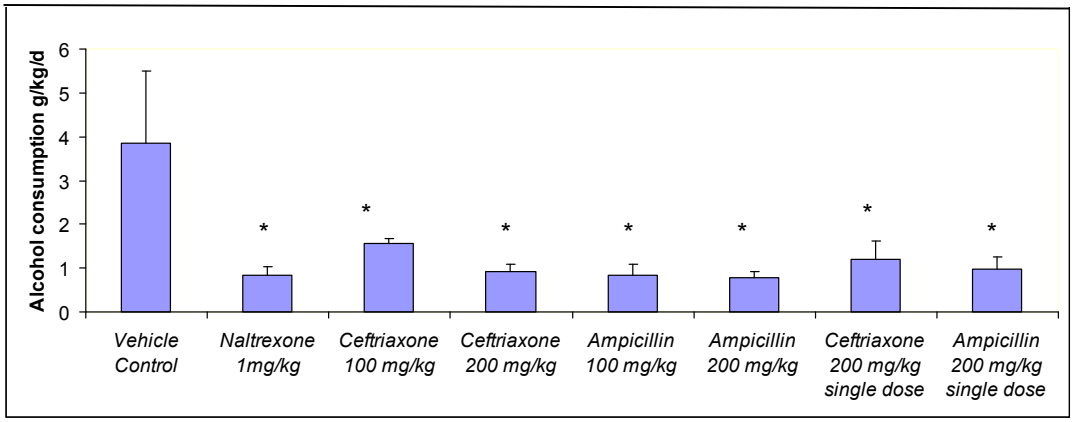

Fig. 1. Alcohol consumption

**p< 0.001 as compared to Vehicle control using one way ANOVA and post hoc Tukey's test*

As shown in Fig. 2, the daily intake of water (g/kg of body weight) expressed as mean ± SD is 76.83±25.84 for vehicle control group, 122.16±12.45 for the naltrexone positive control group, 129.83±11.21 and 102.16±19.38 for the ceftriaxone 100 mg/kg and 200 mg/kg doses respectively, 90.83±12.51 and 99.33±4.76 for the ampicillin 100 mg/kg and 200 mg/kg doses respectively and 79.66±14.23 and 90.5±12.86 for single doses of ceftriaxone and ampicillin respectively. study drugs reduced alcohol consumption without reducing water consumption. The study drugs reduced alcohol consumption without reducing water consumption.

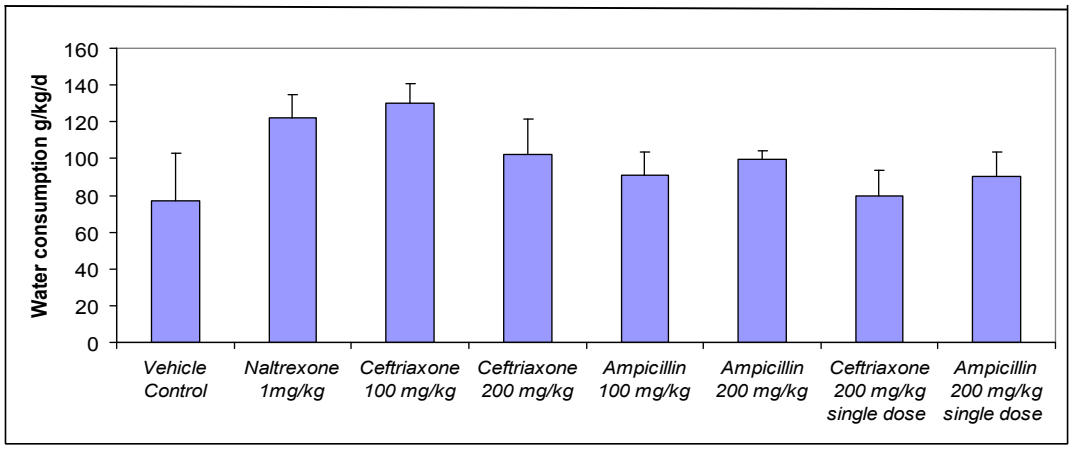

Fig. 2. Water consumption

4. DISCUSSION

Alcohol addiction is on a rise in India [11]. The treatment part is divided into three different components: de-addiction drugs, anti-craving drugs and psychotherapy. Due to adverse effects of the present set of drugs, newer domains of receptor action and drugs is the need of the hour.

The standardization of the animal model revealed ethanol consumption after alcohol dependence to be 3.92±2.53 g/kg/d as shown in Table 1. This is comparable to the ethanol consumption shown by Wistar rats as shown by Palm et al. The ethanol consumption in these Wistar rats after inducing dependence ranged from 1.84 g/kg/d to 3.85 g/kg/d [12].

Our study revealed that ceftriaxone, in doses of 100 mg/kg & 200 mg/kg, leads to a decrease in the ethanol consumption of alcohol dependent rats, as compared to the vehicle control. We can hypothesize that these doses may have increased GLT1 expression which might have decreased craving as shown previously [8]. However, our study, shows a dose dependent decrease in ethanol consumption by ceftriaxone which is statistically comparable to naltrexone. This sort of finding can be taken forward in future for managing alcohol addiction in patients not responding to other drugs.

This mechanism of action of ceftriaxone i.e. increased GLT1 expression has been used by a number of other researchers in different paradigms of addiction of drugs other than alcohol. Ceftriaxone has been shown to block methamphetamine-triggered reinstatement of conditioned place preference and significantly increased GLT1 levels in the medial prefrontal cortex and nucleus accumbens thereby modulating the expression of the glutamate transporter in a critical region of the cortico-striatal addiction circuitry and attenuating drug seeking behavior in rats [13]. Also, ceftriaxone attenuates amphetamine-induced hyperactivity and behavioral sensitization suggesting its efficacy against the adverse effects of amphetamine [14]. For cocaine addiction paradigms, it has been found that there is a decrease in expression and function of GLT1 transporter in the rat nucleus accumbens core following cocaine self-administration. Ceftriaxone restores glutamate reuptake and attenuates the increase in synaptically released glutamate that accompanies cocaine-primed reinstatement [15]. Similarly; relapse to cocaine seeking behavior depends on increased

glutamate transmission in the mesocorticolimbic motive circuit, including prefrontal cortex (PFC) and nucleus accumbens (NAcc). Ceftriaxone-induced blockade of cocaine relapse was associated with an increase in GLT1 expression in both PFC and NAcc [16]. Repeated morphine administration produces mechanical allodynia and thermal hyperalgesia which are signs of opioid induced hyperalgesia (OIH) and reduces spinal GLT-1 expression in mice. Ceftriaxone inhibited OIH by causing upregulation of GLT1 expression [17]. Also, repeated ceftriaxone administration blocks development of tolerance to morphine antinociception through GLT-1 activation [18].

Similarly, this increased expression of GLT1 transporter has been explored in other neurological conditions not related to addiction like Huntington's disease [19], Parkinson's' disease [20], spinal muscular atrophy [21] and tuberous sclerosis [22].

Our study, for the first time, exhibited the efficacy of ampicillin at both 100mg/kg & 200 mg/kg doses, causing a dose dependent decrease in ethanol consumption. Ampicillin was shown to increase GLT1 expression in an in-vitro study [7].

This property of Ampicillin of increased GLT1 expression has been shown to be useful in improvement in cellular hypoxic tolerance [23] and protecting neurons from ischaemic damage [24].

Crossing of the blood brain barrier is a prerequisite for this action of ceftriaxone & ampicillin. A previous study reported that ampicillin & ceftriaxone can cross the blood-brain barrier which resulted in clinical efficacy against a variety of brain infections, including bacterial meningitis and brain abscesses [25].

Our study for the first time shows that ampicillin does translate its in-vitro efficacy of increased GLT1 expression as compared to ceftriaxone in an in-vivo experiment and both the doses of ampicillin showed a trend of higher decrease in ethanol consumption as compared to the highest dose of ceftriaxone used, even though the results are statistically not different. The challenge with both ampicillin and ceftriaxone is the same of drug resistance. These results add to literature of evidence implicating the GLT1 activation as a viable target for the control of alcohol abuse. Specifically, we demonstrated for the first time effects on alcohol addiction of ampicillin and in comparison to the standard of care. These data highlights the importance of testing ceftriaxone and ampicillin in individuals with different ethanol drinking profiles, including humans. We haven't done the GLT1 levels analysis by using any in-vitro method which has been previously done in other studies. Also, one of the best models of addiction is the operant model. However, in our set-up we could use the aforementioned model only.

Our study recommends that drugs acting on glutamate pathways like ceftriaxone and ampicillin can be explored more and tried to include in the armatarium of the drugs used in the crusade of alcohol addiction.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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