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# Acute and chronic tianeptine treatments attenuate ethanol withdrawal syndrome in rats

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**Abstract**

Effects of acute and chronic tianeptine treatments on ethanol withdrawal syndrome were investigated in rats. Ethanol (7.2% v/v) was given to adult male Wistar rats by a liquid diet for 30 days. Acute or chronic (twice daily) tianeptine (5, 10 and 20 mg/kg) and saline were administered to rats intraperitoneally. Acute and last chronic tianeptine injections and saline were done 30 min before ethanol withdrawal testing. After 2nd, 4th and 6th hours of ethanol withdrawal, rats were observed for 5 min, and withdrawal signs which included locomotor hyperactivity, agitation, tremor, wet dog shakes, stereotyped behavior and audiogenic seizures were recorded or rated. Locomotor activity in naive (no ethanol-dependent rats) was also tested after acute tianeptine treatments. Acute but not chronic tianeptine treatment attenuated locomotor hyperactivity and agitation in ethanol-dependent rats. Both acute and chronic tianeptine treatment produced some significant inhibitory effects on tremor, wet dog shakes, stereotyped behaviors and audiogenic seizures during the ethanol withdrawal. Our results suggest that acute or chronic tianeptine treatment attenuates ethanol withdrawal syndrome in ethanol-dependent rats and this drug may be useful for treatment of ethanol-type dependence.


*Keywords:* Dependence; Ethanol; Ethanol withdrawal syndrome; Rat(s); Tianeptine

## 1. Introduction

Alcoholism and depression have some common neurochemical substrates (Markou et al., 1998). Alcoholic patients have symptoms of depression (Weissman and Myers, 1980; Grant and Harford, 1995) and some antidepressant drugs are of general use in patients with ethanol dependence. They are mainly indicated in the ethanol withdrawal and the treatment of combined psychiatric disorders (Miller, 1995; Myrick et al., 2001). Recent studies from our laboratory also indicated that some antidepressants had inhibitory effects on some signs of ethanol withdrawal syndrome in rats (Uzbay et al., 2004; Saglam et al., 2004). These findings implied that antidepressant agents might be useful in treatment of ethanol withdrawal syndrome.

Tianeptine is a tricyclic drug that exhibits antidepressant activity in experimental models (Curzon and Datla, 1993) and clinical trials (Guelfi, 1992; Loo et al., 1993; Saiz-Ruiz et al., 1998). The neurochemical properties of tianeptine differ from those of other tricyclic and non-tricyclic antidepressants. Although tianeptine's antidepressant actions have traditionally been assumed to act through serotonergic modulation (Wagstaff et al., 2001), recent studies indicate that antidepressant effects of this drug may be attributable to non-serotonergic mechanisms, including its capacity to buffer excitatory amino acid receptors against stress (Kole et al., 2002). Tianeptine reverses the adverse effects of stress on brain morphology and synaptic plasticity by reducing excessive accumulation of intracellular calcium as a result of stress-induced excitatory amino acid activation (McEwen and Magarinos, 2001). On the other hand, many studies have shown a clear role of excitatory amino acid stimulation in the development of ethanol dependence (Rossetti and Carboni, 1995; Tsai et al., 1995; Hardy et al., 1999). In addition, blockade of NMDA receptors markedly reduces ethanol withdrawal signs in rodents (Morriset et al., 1990; Liljequist, 1991; Thomas et al., 1997). These findings imply that tianeptine may be useful for treatment of ethanol withdrawal signs in alcoholics.

*Abbreviations:* NMDA, *N*-methyl-D-asparatate; SSRI, selective serotonin reuptake inhibitor.

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Limited clinical studies on alcoholic patients and ethanol-dependent animals suggested that tianeptine had some beneficial effects on depression and somatic anxiety appearing after ethanol withdrawal in alcoholic patients (Loo et al., 1988; Malka et al., 1992). File et al. (1993) also suggested that tianeptine was able to reverse the anxiogenic effects of ethanol withdrawal in the social interaction test in rats. However, ethanol withdrawal consists of more than anxiety. Other symptoms such as locomotor hyperactivity, agitation, increased stereotyped behavior and wet dog shakes, tremors and audiogenic seizures also appear during ethanol withdrawal in rodents. These signs are easily quantifiable elements of intense ethanol withdrawal syndrome that indicates development of physical dependence to ethanol (Majchrowicz, 1975; Morriset et al., 1990; Uzbay and Kayaalp, 1995). In addition, no study investigating the effects of chronic tianeptine treatment on ethanol withdrawal syndrome has been published yet.

The main objective of the present study was to investigate the effects of acute and chronic administration of tianeptine on the signs of ethanol withdrawal syndrome in rats.

## 2. Methods

### 2.1. Animals and laboratory

All procedures in this study are in accordance with the Guide for the Care and Use of Laboratory Animals as adopted by the National Institutes of Health (USA). Adult male Wistar rats (214–339 g weight at the beginning of the experiments) were subjects. They were housed in a quiet and temperature- and humidity-controlled room (22±3 °C and 65±5%, respectively) in which a 12-h light/dark cycle was maintained (0700–1900 h light). Exposure to ethanol and all behavioral experiments involved in ethanol withdrawal syndrome were carried out in the separate and isolated laboratories, which have the same environmental conditions with the colony room.

### 2.2. Drugs used in the study

Tianeptine was purchased from Servier Laboratory (France) and dissolved in saline. Tianeptine or saline was injected to rats intraperitoneally at a volume of 1 ml/200 g body weight. Drug solutions were prepared freshly in the morning of each experiment.

### 2.3. Chronic ethanol administration to rats

For chronic ethanol exposure, the rats were housed individually and ethanol was given in the modified liquid diet as previously described (Uzbay and Kayaalp, 1995). At the beginning of the study, rats were given the modified liquid diet without ethanol for 7 days. Then liquid diet with 2.4% ethanol was administered for 3 days. The ethanol concentration was increased to 4.8% for the following 4 days and finally to 7.2% for 30 days. Liquid diet was freshly prepared daily and presented at the same time of the day (1000 h). The weight of the rats was recorded every day, and daily ethanol intake was measured and expressed as g per kg per day. Control rats ($n=8$) were pair fed an isocaloric liquid diet containing sucrose as a caloric substitute to ethanol.

### 2.4. Evaluation of ethanol withdrawal syndrome

At the end of the exposure to 7.2% ethanol-containing liquid diet, ethanol was withdrawn from the diet by replacing the diet with one that did not contain ethanol at 1000 h. Ethanol-dependent rats were then assigned into several groups ($n=8$ for each group). Tianeptine (5, 10 and 20 mg/kg) and saline were injected to the first four groups 30 min before ethanol withdrawal testing. The same doses of tianeptine and saline injections were applied to the next independent four groups of the rats for 30 days of ethanol (7.2%) or isocaloric liquid diet intake. The injections were done twice daily in these groups. Last tianeptine or saline injections were done 30 min before locomotor activity measurements and/or ethanol withdrawal testing.

The rats were then observed for 5 min at 2, 4 and 6 h following withdrawal of ethanol diet. At each observation time, rats were assessed simultaneously for the following behavioral conditions: agitation, tremor, stereotyped behavior and wet dog shakes. Locomotor activities of the rats were also recorded (Opto Varimex Minor, Columbus, OH, USA) as a total of horizontal, vertical and ambulatory activities of the rats and expressed as mean±S.E.M. Wet dog shakes, tremors and audiogenic seizures were assessed as incidence. Wet dog shakes behavior was considered positive if they occurred at least three times during the observation period. Tremor was determined after lifting rats vertically by the tail; positive was assigned to rats showing clearly distinct forelimb tremor when they were rotated 180° around axis of the tail. Grooming, sniffing, head weaving, gnawing and chewing were observed as major stereotyped behaviors during the ethanol withdrawal in the study. Stereotypic behaviors and agitation were scored using a rating scale as previously described (Uzbay et al., 1997; Uzbay et al., 2004) (Table 1).

Each group received a second injection of its original drug given at 6 h after the first injection. After 6 h of withdrawal testing, rats were exposed to an audiogenic stimulus (100 dB) for

Table 1
Rating scale for agitation and stereotyped behavior signs induced by ethanol withdrawal in rats[a]

| Signs | Scoring |
|---|---|
| Agitation | 0: no irritability or aggressive behavior |
| | 1: rats showing mild or moderate irritability |
| | 2: very irritable |
| | 3: handling vocalization and moderately aggressive |
| | 4: handling vocalization and very aggressive |
| | 5: spontaneous vocalization and very aggressive |
| Stereotyped behavior | 0: no stereotyped behavior |
| | 1: rats showing only one stereotyped behavior |
| | 2: two stereotyped behavior |
| | 3: three stereotyped behavior |
| | 4: four stereotyped behavior |
| | 5: all of stereotyped behavior |

[a] Uzbay et al. (1997, 2004).