Citalopram and Tianeptine: Pharmacological Interventions in alcohol withdrawal-syndrome and serotonin insufficiency in Albino Wistar male rats

Iffat Ara1,2* and Samina Bano1

1. Clinical Biochemistry and Psychopharmacology Research Unit, Department of Biochemistry, University of Karachi, Karachi-75270, Pakistan
2. Faculty of Allied Health Sciences, School of Medical Lab Technology, Minhaj University, 365-M, Model Town, Lahore, Pakistan

Info Article

Submitted: 19-02-2019
Revised: 19-03-2019
Accepted: 19-04-2019
*Corresponding author
Iffat Ara
Email: iffatarak@hotmail.com

ABSTRACT

The present study aims to investigate the neurochemical and behavioral effects of alcohol withdrawal (AW) syndrome in rats. Serotonergic antidepressants such as Citalopram and tianeptine at a dose of 20mg/ kg were tested separately for their ability to prevent seizures in AW groups of rats. Brain regional tryptophan (TRP), 5-hydroxytryptamine (5-HT; serotonin), 5-hydroxyindoleacetic acid (5-HIAA) concentrations were determined using high-performance liquid chromatography connected to the fluorimetric detector. Data analyzed showed increases brain TRP concentrations in all the three regions (amygdala, hippocampus and hypothalamus) after citalopram administered alcohol withdrawn rats; however, tianeptine increases brain TRP concentrations only in amygdala and hippocampus. Increases in brain serotonin concentrations were seen in all the regions only in citalopram administered alcohol withdrawn rats. Further, tianeptine was shown to increase 5-HT turnover in all the regions, however, citalopram appeared to increase 5-HT turnover only in the hippocampus. It is concluded that the citalopram and tianeptine behave differently on the intrinsic pathway of serotonin metabolism that appeared to be compensated by the pattern of 5-HT release. Further, it could be inferred that exposure of serotonergic agents might restore serotonin reuptake mechanism that is desensitized following chronic alcohol exposure. Thus the inclusive approach of the serotonergic system plays an undoubtled role in the pharmacological management of AW syndrome.

Keywords: Serotonin, antidepressants, tryptophan, tianeptine, citalopram

INTRODUCTION

Alcohol withdrawal syndrome characterized by a set of symptoms such as tremor, anxiety, restlessness, insomnia that begins as early as 6h after the initial decline from peak intoxication and the seizures may occur in untreated patients in acute alcohol withdrawal. Many alcohol-dependent patients also experience psychologic problems, such as depression, anxiety, and impulsivity related to serotonin dysfunction (Pihl and Le Marquand, 1998; Thompson, 1978). Interestingly In alcohol withdrawn rats behavioral signs such as audiogenic seizures, locomotor hyperactivity, agitation, and wet dog shakes and tremors have been reported. (Hunter et al., 1975; Uzbay, 2008; Oretti, 1996).

Behavioral deficit during alcohol withdrawal syndrome in rats (Majchrowicz, 1975; Uzbay and Kayaalp, 1995) constitutes the involvement of both neuroendocrine and serotonergic system. Behavioral adaptation during alcohol dependence involves the activation of corticotrophin releasing hormone (CRH) system (Sarnyai et al., 2001) in the hypothalamus that projects to the serotonergic system in limbic brain areas such as hippocampus and amygdala (Koob, 2000; Oscar- Berman, 2000).

Recently, neuroimaging technology has made scientists enable to report shrinkage of total
brain size (Banerjee, 2014; Zahr and Pfefferbaum, 2017; Harper et al., 2005). and changes in brain structures (Rosenbloom and Pfefferbaum, 2008). These brain areas are cerebral cortex and subcortical areas such as the limbic system that supports feeling of emotions include amygdala, the thalamus (signals communication within the brain), the hypothalamus (releases hormones in response to stressful stimulus and is important for behavioral physiological functions) and the hippocampus that constitutes learning and memory functions (Oscar-Berman, 2000).

Tianeptine a selective serotonin reuptake enhancer (SSRE) is a modified tricyclic antidepressant that has been shown to be therapeutically active in patients of depression with a history of alcohol dependence (Hindmarch, 2001; Favre et al., 1997). Studies on stereochemistry in drug action revealed that l isomer is therapeutically more active form than d isomers of tianeptine and its neuroprotective effects become more challenging on 5-HT-mediated behavioral deficits that alter stress-induced morphological changes (Oluyomi et al., 1997; Brink et al., 2006; Liu et al., 2011). However, contradictory evidence proves it as a drug of abuse and addiction because of its structural similarity with antidepressant amphetamine. The existing uncertain profile of tianeptine regarding its safety suggests its co-administration with hypnotics could be useful (Vadachkoria et al., 2009; Calabozo et al., 2016).

Citalopram is a selective serotonin reuptake inhibitor (SSRI), comprises 1:1 ratio of escitalopram and R- citalopram (Sanchez, 2006). Escitalopram has a 50 fold higher affinity for the human serotonin reuptake transporter compared to R-citalopram (Sanchez, 2006; Zhong et al., 2009). Previously, we have reported that citalopram and tianeptine (10mg/kg) improve serotonin dysfunction by enhancing free tryptophan uptake from periphery to the brain (Bano et al., 2010; Ara and Bano, 2012). This paper argues the comparative mechanism of action of tianeptine 20mg/kg/ml and citalopram (20mg/kg/ml) with reference to brain regional tryptophan and serotonin levels in alcohol withdrawn rats.

**MATERIAL AND METHODS**

**Animals and treatment**

All animal procedures described below were conducted in strict accordance with the national research council for the care and use of laboratory animals (1996). Ethical approval was obtained from the institutional animal ethics committee, University of Karachi. All efforts were made to minimize the number of animals and any pain/distress they might incur. Locally bred male Albino Wistar rats weighing 200-250g were housed in a quiet temperature (22±3°C) and humidity-controlled room maintained on which 12h dark-light cycle.

**Chronic ethanol administration to rats**

Eight animals/group were assigned for controls (Matched controls) and other groups (alcohol treated and alcohol withdrawal). Rats for the behavioural study were different from those used for neurochemical estimation The rats were housed in quiet room and were given alcohol-free liquid diet ad libitum for three days before introducing alcohol into the diet alcohol was administered in modified liquid diet as described earlier (Ara and Bano, 2015). Alcohol was then added to the liquid diet in the proportion of 8% (V/V). Matched control rats were fed isocaloric amounts of the alcohol-free liquid diet, in which the alcohol contribution was substituted with maltose-dextrin. Treatment of rats was continued for 28 days and the weight of the rats was recorded daily. Other relevant experimental details have already been described in detail earlier (Bano et al., 1996).

**Measurement of blood alcohol concentration**

Blood alcohol concentrations were determined by alcohol dehydrogenase (EC 1.1.1.1) based enzymatic procedure (Badawy and Aliya, 1984). in rats given a chronic treatment of ethanol liquid diet. The blood ethanol concentration was determined in rats (not-withdrawn, 0h) in mg/dL 306±43, was calculated as means ± SEM of eight rats.

**Assessment of the alcohol withdrawal behaviour**

For the assessment of the effects of alcohol withdrawal signs, the alcohol containing liquid diet was substituted with drinking water for 7h (before the rats were killed by this time signs of withdrawal were strongest. At each observation time, rats were assessed simultaneously for behavioral signs including agitation, tremors, stereotyped behaviors and wet dog shakes (Hunter et al., 1975). An experiment was also designed for studying the prevention of alcohol withdrawal induced disturbances in brain tryptophan metabolism.
In the experiment both control and test animals were treated i.p. at -1 and 3h after a withdrawal with the drug (citalopram, 20mg/kg/mL or tianeptine, 20mg/kg/mL) or an equal volume of vehicle (2mL/kg). The animals were killed (by decapitation) 4h after the last injection, i.e. 7h after withdrawal. The behavioral activity of the animals for both control and drug treated was monitored (Oretti et al., 1996). Rats were killed by decapitation and their livers were perfused in situ with ice-cold 0.9% saline and were rapidly removed and frozen in liquid nitrogen until analysis.

**Microdissection of brain**

A fresh brain was dipped in ice-cold saline and placed with its dorsal side up in the molded cavity of a brain slicer (Henry and Yashpal, 1984). A fine fishing line wire was inserted into the slots of the slicer to give slices of 1 mm thickness. The slices were transferred to a Petri dish kept on ice, moistened with ice-cold 0.9% NaCl and the desired brain regions identified with the aid of a stereotaxic atlas (Paxinos and Watson, 1982). Olfactory nucleus material was discarded from the slice containing cortex. Hippocampal material (CA1-4 fields + subiculum + dentate gyrus) was dissected out with a sharp scalpel and the hypothalamus and amygdala regions were obtained by taking punches of 2mm diameter from slices.

**Brain neurochemical analysis**

Brain tissues were weighed, homogenized and deproteinized in volumes of 0.1M Perchloric acid (1g in 4mL 0.1M perchloric acid). The homogenates were sonicated at 0-4°C at a medium setting for two 15s periods using a sonicator. After adding 0.5mL of 4M perchloric acid and mixing the samples were spun at 10,000g for 10min at 4°C and a portion of the supernatant was taken and stored at -70°C for analysis. The analytical measurements were performed by high-performance liquid chromatography with a fluorescent detector. A reverse phase chromatography was used for the analysis of TRP, 5-HT and its metabolite 5-hydroxyindolacetic acid (5-HIAA). The ratio of 5-HIAA/5-HT was used as an index of 5-HT turnover. For mobile phase 0.01 M sodium acetate was made and pH was adjusted up to 4.5 with glacial acetic acid and finally, the volume was made up to 1L with water. 15% methanol was added to filtered mobile phase and was passed through the ODS separation column (25cm in length 4.6mm in diameter) at a constant flow rate (2mL/min) with an operating pressure of 2000–3000 psi, using a 200 series pump. Fluorescence detection was performed on Shimadzu VT 03 detector at an operating potential of 0.8V. The fluorimetric detector was used with 254-nm excitation and 360nm excitation (Anderson et al., 1981).

**Drugs and chemicals**

The drug citalopram (20mg/kg/mL) as (HB; Lundbeck Pakistan private limited) and tianeptine (20mg/kg/mL), (Servier; Pakistan) were dissolved in saline. All other chemicals used were from Sigma-Aldrich, St Louis MO USA. Drugs were freshly prepared.

**Statistical analysis**

Data was analyzed by one-way ANOVA followed by Dunnett’s test and where appropriate using student’s t-test. The P value of < 0.05 was considered to be statistically significant.

**RESULTS AND DISCUSSION**

**Effects of alcohol withdrawal on brain regional tryptophan metabolic changes**

Figure 1-4 shows the effects of alcohol withdrawal on brain regional tryptophan metabolism. There was shows significant decreases in tryptophan concentration, 5-HT and 5HIAA concentrations in hypothalamus, amygdala, hippocampus regions in alcohol withdrawal rats. These results are in agreement with the findings reported earlier (Ara and Bano, 2015) with the experimental evidences that the abnormal 5-HT functions in alcoholism is associated with a low number of 5-HT transporters binding sites in the living and postmortem brains of alcoholics (Heinz et al., 2000; Kranzler et al., 2002). Generally alcohol elevates serotonin in brain regions (Ding et al., 2012) by increasing the activity of serotonin receptors 5HT3, 5HT1A, 5HT1B, and serotonin transporters therefore low serotonin turnover accounts for the negative mood states, aggressive behavior, feeling of insecure and threat that leads to the development of anxiety behavior (Sari et al., 2011; Virkunen et al., 1994; Johnson, 2004).

Therefore an AW-induced reduction in serotonin turnover in all the regions in the present results confirm the hypothesis consistent with the development of agitation, tremors, stereotype behavior, WDS and audiogenic seizures (Overstreet et al., 2004) (Table I).
Citalopram and Tianeptine

Effects of citalopram and tianeptine on brain regional tryptophan metabolic changes and on the blockade of Alcohol Withdrawal signs in AW rats

Citalopram and tianeptine effectively prevented alcohol withdrawal-induced agitation and autogenic seizures when compared to vehicle-treated alcohol–withdrawn rats (Table I). However, tianeptine was found to be more effective in preventing tremors, stereotype behavior and wet dog shakes. Earlier investigators have reported that tianeptine and fluoxetine had similar affinity to block alcohol withdrawal symptoms; however, escitalopram had lesser affinity comparatively. Anxiety-like behavior following chronic ethanol dependence has been characterized as a set of withdrawal symptoms and tianeptine has been proposed to possess anxiolytic property (Uzbay, 2008; File et al., 1993; McEwen et al., 2010).

The results of the present study are in agreement with our previous findings that the tianeptine (10mg/kg) and the citalopram (20mg/kg) increased brain tryptophan concentration (Bano et al., 2010; Ara and Bano, 2012).

Results analyzed by one way ANOVA (Table II) shows significant effects of citalopram and tianeptine on tryptophan levels in hypothalamus ($F=11.86; P<0.01$), in amygdala ($F=10.68; P < 0.01$) and in hippocampus ($F=12.18; P<0.01$). Dunne’s test shows the rise in tryptophan ($p<0.01$) by the citalopram in all regions, while, tianeptine shows insignificant effects only in the hypothalamic region.

Figure 1-4. Effect of alcohol withdrawal on brain regional (1) tryptophan (2)5-HT (3) 5hIAA and (4) 5HIAA/5HT concentration experimental details are given in material and methods section. All values are means ± S.E.M for each group of eight rats. The values obtained in control rats were compared statistically by students t-test with those obtained in group of rats withdraw from alcohol. The significance of difference is indicated as follows *$P<0.05$, **$P<0.01$, ***$P<0.001$. 

Effects of citalopram and tianeptine on brain regional tryptophan metabolic changes and on the blockade of Alcohol Withdrawal signs in AW rats

Citalopram and tianeptine effectively prevented alcohol withdrawal-induced agitation and autogenic seizures when compared to vehicle-treated alcohol–withdrawn rats (Table I). However, tianeptine was found to be more effective in preventing tremors, stereotype behavior and wet dog shakes. Earlier investigators have reported that tianeptine and fluoxetine had similar affinity to block alcohol withdrawal symptoms; however, escitalopram had lesser affinity comparatively. Anxiety-like behavior following chronic ethanol dependence has been characterized as a set of withdrawal symptoms and tianeptine has been proposed to possess anxiolytic property (Uzbay, 2008; File et al., 1993; McEwen et al., 2010).

The results of the present study are in agreement with our previous findings that the tianeptine (10mg/kg) and the citalopram (20mg/kg) increased brain tryptophan concentration (Bano et al., 2010; Ara and Bano, 2012).

Results analyzed by one way ANOVA (Table II) shows significant effects of citalopram and tianeptine on tryptophan levels in hypothalamus ($F=11.86; P<0.01$), in amygdala ($F=10.68; P < 0.01$) and in hippocampus ($F=12.18; P<0.01$). Dunne’s test shows the rise in tryptophan ($p<0.01$) by the citalopram in all regions, while, tianeptine shows insignificant effects only in the hypothalamic region.
Serotonin deficiency is caused by a decreased availability of its precursor tryptophan to the brain. This decrease is caused by accelerated Trp degradation, most likely induced by enhancement of the hepatic enzyme tryptophan 2,3-dioxygenase (TDO) by glucocorticoids and/or catecholamines. Liver TDO appears to be a target of many antidepressants; Enhancing Trp availability to the brain is thus the key to normalization of serotonin synthesis and could form the basis for future antidepressant drug development (Badawy, 2013; Bano et al., 2010; Bano and Sherkeli, 2003). Alternatively tryptophan depletion exerts serotonergic dysfunction related to depression, anxiety, and cognitive impairment (Badawy, 2002; 2013; Richard et al., 2009).

Increases in tryptophan levels in the hypothalamus were not significant, that may pinpoint the dose-dependent effects of tianeptine. Since, hypothalamus predominantly regulates the visceromotor functions by adjusting the balance between sympathetic and parasympathetic outputs to the autonomic nervous system (Flugge, 1999; Fuchs and Flugge, 2003) therefore neuroanatomical factors may add to the development of tolerance in specific brain areas in alcohol dependence. Further, serotoninergic drugs presumably downregulate and reverse the activity of receptors and transporters that are up-regulated in the presence of alcohol (Johnson, 2004; Sari et al., 2011).

Effects of drugs on brain regional tryptophan metabolism (Table II). Data analyzed by one way ANOVA shows significant effects on 5-HT in hypothalamus (F=22.06; P<0.01), amygdala (F=7.09; P<0.05) and in hippocampus (F=7.94; P<0.01). Dunnett’s test shows insignificant changes in all the three regions by tianeptine while...
Citalopram shows a significant increase (p<0.001). Citalopram, a serotonergic re-uptake inhibitor, act by enhancing synaptic 5-HT concentrations by increasing the availability of tryptophan as reported earlier (Ara and Bano, 2012). However, insignificant change in 5-HT by tianeptine may suggests its unique pharmacological profile as indirect acting drugs. Microdialysis study reported that the acute and chronic treatment of tianeptine did not alter extracellular serotonin concentration in rat frontal cortex and raphe nuclei (Malagie et al., 2002). Similarly, Fattaccini et al., (1990) reported no change in 5-HT in brain tissue with the increased concentration of 5-HIAA following 1h of acute tianeptine (10mg/kg) administration. However, chronic treatment increased serotonin uptake at the dose 20mg/kg, (i.p) in hippocampus and cortex (Mennini and Garattini, 1991).

Studies conducted earlier have reported that chronic alcohol consumption may lead to reduced binding potential of the serotonergic drugs for serotonin transporters. (Mantere et al., 2002; Stovrik et al., 2008) however, subsequent use of serotonergic drugs may overcome these effects via increasing binding affinity (Ketcherside et al., 2013; Garbutt, 1999). Immunohistochemistry studies by Jang et al., (2002) showed reduction in the expression of the tryptophan hydroxylase (the rate-limiting enzyme in serotonin synthesis) upon alcohol exposure in rats. According to another study, 4-week administration of tianeptine and fluoxetine enhanced the density of 5-HT and serotonin transporter-immunoreactive in the neocortical layer IV and certain forebrain limbic brain areas with insignificant effects on tryptophan hydroxylase 2 and serotonin transporter at mRNA level (Zhou et al., 2006). In view of the present findings, tianeptine appears to exert its effects indirectly on serotonergic neurons by causing inhibition in the release of neurotransmitter from the vesicles. Therefore, tianeptine might play significant role in attenuating the alcohol-induced aggression and withdrawal symptoms by altering serotonergic neurotransmission.

Our data (Table II) shows significant effects of citalopram and tianeptine on serotonin turnover (5-HIAA/5-HT) in hypothalamus (F=34.19; P<0.01), amygdala (F=16.73; P<0.01) and in hippocampus (F=25.8; P<0.01). These significant effects show the rise in 5-HT turnover by tianeptine in all the three regions (P<0.01), with the significant increase in hippocampus only (p<0.05) by citalopram. Since alcohol mimics serotonergic responses and withdrawal indices low serotonin turnover rate, therefore, increased 5-HT turnover rate by these antidepressants, more prominently by the tianeptine commensurate with the preventive effects on alcohol withdrawal signs. The results of the present findings are in agreement with previous studies (Uzbay, 2008). File and coworkers (1993) have found tianeptine reduced AW related anxious behavior and hypoactivity in social interaction when administered to rats (File et al., 1993), while, the anxiogenic profile was observed when administered chronically in mice for 21 days (Cutler et al., 1997; Rodgers et al., 1997). In contrast, clinical findings suggest that citalopram appears to reduce the drive to alcohol consumption only moderately to some alcoholics and does not contribute to reducing pathogenesis effectively (Naranjo et al., 2000; 1992; Martijena et al., 2005). However, other SSRIs such as fluoxetine has shown to reduce alcohol intake and craving compared to baseline only at higher doses (60mg) (Naranjo et al., 1990; 1994). It has been reported that long-term tianeptine treatment had some beneficial effects to recover depression and anxiety related symptoms in patients with alcoholism (Malka et al., 1992). Therefore, the adaptive changes in the 5-HT system may play a crucial role in the therapeutic effect of antidepressant treatments (Warner-Schmidt et al., 2006).

**CONCLUSION**

The anxiolytic characteristics of citalopram and tianeptine both provide novel findings as a useful treatment for the prevention of alcohol withdrawal behavioral syndrome. Unlike citalopram, the mechanism of action of tianeptine remain speculative for its interaction and binding affinity with intraneuronal and membrane transport protein and channels. While the notion that citalopram improves serotonin dysfunction may still be a matter of debate as higher doses in clinical trials can provide better therapeutic options and understanding the neurobiological basis of AWS. There is no doubt that a comprehensive approach to the mechanism of serotonin transporters is paving a road to better pharmacological management of alcohol withdrawal syndrome.

**ACKNOWLEDGMENT**

We thank Grant support from Higher Education Commission Pakistan for financial support.
REFERENCES


Brink CB., Harvey BH. and Brand L. 2006. Tianeptine: a novel atypical antidepressant that may provide new insights into the biomolecular basis of depression. *Recent Pat CNS Drug Discov.* 1:29-41.


Hunter BE., Riley JN., and Walker DW., 1975. Ethanol dependence in the rat: a parametric
Citalopram and Tianeptine


Oversveer DH, Knapp DJ, and Bree GR, 2004 Similar anxiety-like responses in male and female rats exposed to repeated withdrawals.

Paxinos G and Watson C., 1982. The Rat brain in stereotaxic coordinates


