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Research Article

Effect of Chronic Administration of Melatonin on Ethanol Drinking in Rat Models of Chronic Voluntary Ethanol Consumption

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Abstract

Objective: This study is planned to examine the possible beneficial effect of chronic administration of melatonin on ethanol drinking in rat models chronic voluntary ethanol consumption.

Methods: Intermittent access 10% ethanol two-bottle-choice drinking paradigm was employed in 4 groups of rats where the rats had access to ethanol on alternate days in a week and a free access to water on all day. The ethanol and water intake was recorded on each ethanol day. All rats received drug treatment (Distilled water, naltrexone, melatonin 50 mg/kg and melatonin 100 mg/kg) for 6 days continuously once they attain stable ethanol drinking pattern. The ethanol consumption on the last drinking session before the drug administration was noted as pretreatment baseline ethanol drinking value. The ethanol consumption on the first drinking session after the last dose of drug administration was noted as the post treatment value.

Results: There was no change in the amount of ethanol consumption by rats in groups receiving distilled water and melatonin 50 mg/kg body weight. There was significant reduction in the ethanol consumption in rats receiving melatonin 100 mg/kg body weight and naltrexone. Comparison among different groups showed statistically significant difference between melatonin 100 mg/kg and distilled water as well as between naltrexone and distilled water.

Conclusion: Melatonin at a higher dose and naltrexone has significantly reduced ethanol consumption in rats drinking high ethanol, indicating its potential use in the management of ethanol dependence.

Keywords: Ethanol; Melatonin; Naltrexone; Wistar rats

Introduction

Ethanol dependence is a major public health issue and the effective management of the condition is challenging. Though some progress in the therapeutic approaches is seen in the recent years, there is still a requirement for the development of new drugs for the management of ethanol dependence as the existing therapy has some limitations. Ethanol intake in rodents is reduced by opioid antagonists [1]. Naltrexone, an opioid antagonist shown to be most effective in the treatment of ethanol dependence [2]. Its mechanism in ethanol dependence is due to its effects on the dopamine reward pathway, possibly inhibiting GABAergic neurons in the ventral midbrain [3]. Naltrexone may not be effective in all patients, may be due to the genetic variations in μ opioid receptor gene [4].

Though there has been some difficulty making standard laboratory rats voluntarily consuming large amounts of ethanol, without the use of some initiation techniques, attempts were made in this direction. Now, there are reports that shows that standard laboratory rats will voluntarily consume high levels of ethanol if given intermittent-access to ethanol in a two bottle choice setting [5]. In this procedure, the animals will have access to two bottles, i.e., one containing ethanol and other one is non-ethanol drink [6]. Though the technique provides a course measure of ethanol consumption, the motivational component of dependence is difficult to measure as there is no effort required to obtain ethanol. However, the model is used to test newer pharmacological approaches in ethanol dependence [7].

Melatonin had a reversal effect on the expression of morphine-induced rewarding effect [8]. Based on this fact, we assumed that melatonin may reduce ethanol consumption by its effect on opioid system. This study is planned to examine the possible beneficial effect of chronic administration of melatonin on ethanol drinking in rat models chronic voluntary ethanol consumption.

Materials and Methods

Male Wistar rats of 5 weeks of age were procured from the central animal house, KMC, Mangalore. All animals were allowed to adapt to the laboratory conditions for 5 days before starting the study. Animals were kept in standard polycarbonate cages as five per cage initially, (during acclimatization) and after acclimatization period, each rat was housed separately. Rooms were controlled for temperature and animals were kept at 12 hours each of light and dark period. Animals had free access to food and water. To reduce the effect of circadian rhythms, tests were performed during the first half of the day. All procedures were performed in compliance with CPCSEA guidelines and all efforts were taken to reduce suffering.

Drugs and drinking solutions

The ethanol solution was prepared from 99.9% ethanol (Manufactured by: Changshu Yangyuan Chemicals China). This absolute alcohol is diluted to 10% by mixing with water.

Melatonin (M5250; Sigma- Aldrich) dissolved in distilled water and administered orally (50 mg/kg and 100 mg/kg). Selection of melatonin dose was based the previous studies of melatonin in rats [3]. Naltrexone (N3136; Sigma- Aldrich): 1 mg/kg.

Intermittent access 10% ethanol two-bottle-choice drinking paradigm

The rats are given intermittent access to 10% ethanol. After the acclimatization period, on the first day (Monday) of the experiment, animals were given access to ethanol and water bottle. After 24 hours, the ethanol bottle was replaced with a second water bottle that was

available for the next 24 hours. This pattern was repeated on the 3rd day and 5th day (i.e., Wednesdays and Fridays). The placement of the ethanol bottle is alternated daily to control for place preferences. All other days the rats had unlimited access to water. The weight of each rat was measured daily to calculate the ethanol consumption in gram per kilogram of body weight of the rat. The ethanol and water intake was documented on each ethanol day. In addition, the intake of water was recorded on the water days to enable comparison of the total fluid intake between the ethanol days (ethanol+water) and the water days.

Drug administrations began after the rats maintain stable baseline ethanol drinking levels. Once the stable drinking pattern is established, rats were divided into four groups (six rats in each group) randomly matching for baseline ethanol consumption. Drug administration schedule for these groups of rats is as shown in Table 1. All rats received drug treatment for 6 days continuously. During these days, the rats had three ethanol-drinking sessions. All drugs were given 1 hour before the rats are given access to ethanol and water. The ethanol consumed was measured on days 1, 3 and 5 in each group of rats. The ethanol consumption on the last drinking session before the drug administration was noted as pretreatment ethanol drinking value (baseline). The ethanol consumption on the first drinking session after the last dose of drug administration was noted as the post treatment value. Ethanol consumption was measured in g/kg body weight. The ratio of ethanol to total fluid intake (preference to ethanol over water) was calculated.

Statistical Analysis

Comparison of amount of ethanol consumed after the administration of the drugs with baseline value was done by using Wilcoxon signed rank test. Comparison of ethanol consumption between drug groups was done by using Mann Whitney U test. A p value less than 0.05 was considered significant.

Results

Table 2 shows the comparison of the effect of chronic administration of drugs on ethanol intake in high ethanol consuming rats. There was no change in the amount of ethanol consumption by rats in groups 1 and 2 i.e., distilled water and melatonin 50 mg/kg body weight. There was significant reduction in the ethanol consumption in rats receiving melatonin 100 mg/kg body weight and naltrexone (p value is 0.03 and 0.04 respectively). Comparison among different groups showed statistically significant difference between melatonin 100 mg/kg and distilled water (p=0.03, Mann Whitney U test) as well as between naltrexone and distilled water(p=0.03, Mann Whitney U test).

Mann whitney U test

No statistical significance was seen between the different groups. Table 3 shows the chronic administration of drugs on water intake in high ethanol consuming rats. Water consumption remained unchanged irrespective of the type of the drug administered.

Table 4 indicates the comparison of the ratio of ethanol consumption to that of total fluid consumption by rats between pre-treatment and post treatment as well as among different groups. The ratio was decreased in melatonin 100 mg/kg group as well as naltrexone group after the drug administration (pre vs post treatment value), but the significance was seen only with melatonin 100 mg where as the same ratio was significantly increased in distilled water group (p=0.028). In melatonin 50 mg/kg group, there is no change in the ratio. This indicates that there was a gradual increase in the ethanol consumption after the administration of distilled water and decrease in the ethanol consumption after the administration of melatonin and naltrexone.

Discussion

The major challenges in preclinical studies of ethanol dependence is the development of animal models showing high ethanol consumption and resembling the progressive transition from low levels to high level of ethanol consumption, eventually becoming dependent. The present study employed intermittent access two-bottle-choice ethanol drinking paradigm to study the effect of melatonin on ethanol drinking in rats. This procedure effectively showed a gradual increase in voluntary ethanol consumption as well as preference in rats, ultimately reaching pharmacologically relevant blood ethanol concentrations [9]. Standard

Groups	Drug (route-oral, dose) X 6 days
Control	Vehicle (distilled water) (0.1 ml/100 grm)
Test drug (lower dose)	Melatonin (50 mg/kg)
Test drug (higher dose)	Melatonin (100 mg/kg)
Standard drug	Naltrexone (1 mg/kg)
Control group receiving distilled water serves as a negative control and the control group receiving standard drug naltrexone serves as a positive control.	

Table 1: Treatment schedule for voluntary ethanol consumption model (Intermittent access, chronic experiment).

Groups	Drugs	Ethanol consumption (g/kg/24 hr)		Z value	p value
		Before drug administration	After drug administration		
I	Distilled water	3.27 ± 1.64	3.86 ± 1.36	-1.57	0.11
II	Melatonin 50 mg/kg	3.67 ± 1.52	3.35 ± 1.62	-1.36	0.17
III	Melatonin 100 mg/kg	4.02 ± 1.75	1.94 ± 1.43#	-2.20	0.03*
IV	Naltrexone 20 mg/kg	3.53 ± 1.37	2.27 ± 0.88$	-2.02	0.04*
Values were expressed as mean ± SD; *Wilcoxon signed rank test: Mann Whitney U test: #p=(0.03) vs Distilled water; $p=(0.02) vs Distilled water					

Table 2: Comparison of effect of chronic administration of drugs on ethanol drinking in rats.

Groups	Drugs	Water consumption (ml)		Z value	p value
		Pre-treatment	Post-treatment		
I	Distilled water (control)	16.42 ± 9.87	15.25 ± 7.85	-1.05	0.29
II	Melatonin 50 mg/kg	17.00 ± 9.20	15.58 ± 8.59	-1.58	0.11
III	Melatonin 100 mg/kg	14.50 ± 4.79	13.66 ± 4.55	-1.38	0.17
IV	Naltrexone 20 mg/kg	20.25 ± 7.12	19.00 ± 5.51	-1.26	0.21
Values were expressed as mean ± SD Wilcoxon signed rank test					

Table 3: Comparison of effect of chronic term administration of drugs on water intake in rats.

Drugs	Ethanol/Total fluid consumption (ml/24 hr)			
	Before drug administration	After drug administration	Z value	p value
Distilled water	0.48 ± 0.25	0.54 ± 0.21	-2.201	0.028*
Melatonin 50 mg/kg	0.46 ± 0.21	0.46 ± 0.20	-0.105	0.917
Melatonin 100 mg/kg	0.50 ± 0.16	0.34 ± 0.19	-1.99	0.046*
Naltrexone	0.41 ± 0.11	0.34 ± 0.07	-1.572	0.116
Values are expressed as mean ± SD; *Wilcoxon signed rank test: Mann Whitney U test: No statistical significance was seen between the different groups				

Table 4: Comparison of effect of long term administration of drugs on total fluid intake on ethanol drinking days and water drinking days in different groups of rats.

laboratory rats like Wistar rats voluntarily consumed ethanol using the intermittent-access ethanol drinking paradigm without the need for any initiation procedures like sucrose fading technique. This procedure is simple, has high validity, and reliability, making it a useful and relevant procedure for preclinical evaluation of potential therapeutic agents against alcohol dependence [9]. Our study has shown that long term administration of melatonin in higher dose (100 mg/kg) as well as naltrexone has effectively reduced ethanol intake in rats voluntarily consuming high ethanol. The volume of water consumed remained same irrespective of increase or decrease of ethanol consumption after the drug treatment in all the drug groups. Appreciable reduction in ethanol consumption was not observed at lower dose of melatonin i.e., 50 mg/kg. The ratio of ethanol to total fluid consumed is decreased significantly after the administration of melatonin 100 mg/kg, signifying that rats have consumed less ethanol after the drug administration. The same ratio was increased in control group (distilled water), showing a gradual increase in ethanol consumption, resembling transition from low to medium level of drinking to high level of ethanol drinking. Naltrexone has shown to be effective in ethanol dependence and has been approved by FDA for the same. Melatonin is a hormone secreted from the pineal gland, involved in circadian rhythm, modulation of many physiological functions and behaviours in animals and human beings. There are reports of reduced levels of melatonin and a delay in its nocturnal peak in patients with ethanol dependence as well as in rat models of ethanol dependence. A potent antagonist of melatonin receptors (MT_1 and MT_2), agomelatine has reduced relapse of ethanol consumption after its withdrawal [10]. Administration of melatonin reversed the increase in lipid peroxidation and a decline in glutathione induced by the chronic administration of ethanol in rats. This protective activity of melatonin is by scavenging free radicals and stabilizing glial activity against ethanol induced injury to the nervous system [11].

The potential efficacy of melatonin has been demonstrated in several types drug dependence in the preclinical studies. Studies have shown the possibility of melatonin being useful in some aspects of drug dependence. It has shown to inhibit the development of morphine dependence in mice. Intraperitoneal administration of melatonin was able to reverse the development of morphine tolerance and dependence in mice [12,13]. Melatonin reverses the expression of morphine induced rewarding effect [8]. It was also established that physiological doses of melatonin reduce the craving in heavy smokers during acute withdrawal from nicotine [14]. Melatonin is involved in cocaine induced reward [15]. Melatonin has reduced chronic use of benzodiazepines in patients with insomnia [16]. Our previous study also demonstrated reduction in ethanol consumption in ethanol naïve Wistar rats after the administration of single dose of melatonin [17].

Our finding suggests the possibility of melatoninergic system as a potential target to develop drugs for the treatment of ethanol dependence. The significant advantage of melatonin is its remarkable safety profile. It is well tolerated as well as not associated with abuse potential even when administered at high doses. As there are not many studies supporting the potential use of melatonin in ethanol dependence, further studies on this aspect are needed. Further studies also required to elucidate the mechanism by which melatonin reduces the ethanol consumption. One of the earlier report suggested inhibitory effect of ethanol on melatonin secretion in healthy volunteers in a randomized, double blind, crossover study [18]. This evidence suggests a link between melatonin secretion and mechanism of ethanol dependence that needs to be investigated.

Conclusion

To conclude, melatonin in higher dose (100 mg/kg) and naltrexone have significantly reduced ethanol consumption in rats drinking high ethanol indicating its potential use in the management of ethanol dependence.

References

1. Stromberg MF, Rukstalis MR, Mackler SA, Volpicelli JR, O'Brien CP (2002) A comparison of the effects of 6-beta naltrexol and naltrexone on the consumption of ethanol or sucrose using a limited-access procedure in rats. Pharmacol Biochem Behav 72: 483-490.
2. Anton RF, O'Malley SS, Ciraulo DA, Cisler RA, Couper D, et al. (2006) Combined pharmacotherapies and behavioral interventions for alcohol dependence: the combine study: a randomized controlled trial. J Am Med Assoc 295: 2003-2201.
3. Kamdar NK, Miller SA, Syed YM, Bhayana R, Gupta T, et al. (2007) Acute effects of Naltrexone and GBR 12909 on ethanol drinking-in-the-dark in C57BL/6J mice. Psychopharmacology (Berl) 192: 207-217.
4. Oslin DW, Berrettini WH, O'Brien CP (2006) Targeting treatments for alcohol dependence: the pharmacogenetics of naltrexone. Addict Biol 11: 397-403.
5. Simms JA, Steensland P, Medina B, Abernathy KE, Chandler J, et al. (2008) Intermittent Access to 20% Ethanol Induces High Ethanol Consumption in Long–Evans and Wistar Rats. Alcohol Clin Exp Res 32: 1816-1823.
6. Rhodes JS, Best K, Belknap JK, Finn DA, Crabbe JC (2005) Evaluation of a simple model of ethanol drinking to intoxication in C57BL/6J mice. Physiol Behav 84: 53-63.
7. Ripley TL, Stephens DN (2011) Critical thoughts on current rodent models for evaluating potential treatments of alcohol addiction and withdrawal. Br J Pharmacol 164: 1335-1356.
8. Han J, Xu Y, Yu CX, Shen J, Wei YM (2008) Melatonin reverses the expression of morphine-induced conditioned place preference through its receptors within central nervous system in mice. Eur J Pharmacol 594: 125-131.
9. Carnicella S, Ron D, Barak S (2014) Intermittent ethanol access schedule in rats as a preclinical model of alcohol abuse. Alcohol 48: 243-252.
10. Vengeliene V, Noori HR, Spanagel R (2015) Activation of Melatonin Receptors Reduces Relapse-Like Alcohol Consumption. Neuropsychopharmacology 40: 2897-2906.
11. Baydas G, Tuzcu M (2005) Protective effects of melatonin against ethanol-induced reactive gliosis in hippocampus and cortex of young and aged rats. Exp Neurol 194: 175-181.
12. Wei YM, Xu Y, Yu CX, Han J (2009) Melatonin enhances the expression of β-endorphin in hypothalamicarcuate nucleus of morphine-dependent mice. Acta Physiologica Sinica 61: 255-262.
13. Raghavendra V, Kulkarni SK (1991) Reversal of morphine tolerance and dependence by melatonin: possible role of central and peripheral benzodiazepine receptors. Brain Res 834: 178-181.
14. Zhdanova IV, Piotrovskaya VR (2000) Melatonin treatment attenuates symptoms of acute nicotine withdrawal in humans. Pharmacol Biochem Behav 67: 131-135.
15. Kurtuncu M, Arslan AD, Akhisaroglu M, Manev H, Uz T (2004) Involvement of the pineal gland in diurnal cocaine reward in mice. Eur J Pharmacol 489: 203-205.
16. Cardinali DP, Golombek DA, Rosenstein RE, Brusco LI, Vigo DE (2015) Assessing the efficacy of melatonin to curtail benzodiazepine/Z drug abuse. Pharmacol Res 184: S1043-S6618.
17. Rather ZA, Chowta MN, Bolumbu G, Rakesh KB (2015) Evaluation of acute effects of melatonin on ethanol drinking in ethanol naïve rats. Indian J Pharmacol 47: 383-387.
18. Ekman AC, Leppäluoto J, Huttunen P, Aranko K, Vakkuri O (1993) Ethanol inhibits melatonin secretion in healthy volunteers in a dose-dependent randomized double blind cross-over study. J Clin Endocrinol Metab 77: 780-783.