CIRCADIAN VARIATIONS OF THE ACUTE TOXICITIES OF AMITRIPTYLINE AND MIANSERIN IN MICE

Uğur HODOĞLUGİL, M.D., Cüneyt GÜZEY, M.D., Hakan ZENGİL, Ph.D.

Gazi University, Faculty of Medicine, Department of Pharmacology, Ankara, Turkey
Gazi Medical Journal 6 : 59-62, 1995

SUMMARY : *The time dependence of the acute toxicities of two antidepressant drugs were studied. Groups of adult male mice maintained under controlled environmental conditions were injected with either Amitriptyline (100 mg/kg) or Mianserin (90.7 mg/kg) at different times of day. Acute LD_{50} values of both drugs at two different circadian times selected by their susceptibility rhythm patterns were also determined. The maximum acute toxicity of Amitriptyline occurred at 23^{00}, and minimum at 11^{00}. Mortality rates for Mianserin did not show significant differences, although a 80% mortality was observed at 23^{00} and a minimum of 30 % at 15^{00} and 19^{00}. By determining the acute LD_{50} values for both drugs at these two different times, i.e. 11^{00} and 23^{00}, we demonstrated that Amitriptyline is 29 % more toxic when applied to mice at 23^{00} than 11^{00}, and Mianserin is 30 % more toxic at 11^{00} than 23^{00}. These results indicate a significant time dependent acute toxicity for Amitriptyline but not for Mianserin in mice.*

Key Words : *Acute Toxicity, AntidePressant Drugs, Mice*

INTRODUCTION

It is clearly demonstrated that there are circadian variations in many of the biological and physiological functions of the mammalian organism. One of the expected result of this rhythmic phenomenon is the variation in the behavior of the organism to the same stimulus when applied at different times of day, i.e. the living animal corresponds alternating periods of susceptibility or resistance to the effects of drugs (17, 18, 21). In accordance with this biorhythmicity, circadian variations in toxicity have been demonstrated for several drugs, including phenylbutazone (10), diazepam (16), beta adrenoceptor blockers (5), anticancer drugs (13), aminoglycosides and heavy metals (3), and local anaesthetics (1, 11).

Amitriptyline is an important and widely used tricyclic antidepressant drug with prominent toxic effects due to its antimuscarinic and sedative properties. Mianserin, another widely used antidepressant drug which is a tetracyclic perazinoazepine, is regarded less toxic than conventional tricyclic antidepressants (4). In clinical practice, both drugs are usually given without paying any attention to the circadian rhythmic state of the patients.

The present study was undertaken to determine whether circadian variations in the acute toxicity to Amitriptyline and Mianserin occurred in mice, and if so, whether there was any difference in their

susceptibility rhythms among these agents.

MATERIALS AND METHODS

Animals

The experiments were performed in 28-34 g local bred albino male mice. They were given food and water *ad libitum* , and were synchronized by maintaining under controlled environmental conditions at least two weeks prior to and throughout the duration of experiments. The lighting regimen was 12 hours of light and 12 hours of darkness (lights on 08^{00} - 20^{00}) with a light intensity of approximately 100 lux. This standardized light-dark cycle acts as an entraining agent of the circadian rhythmicity, and after two weeks of such synchronization the biology of each animal is approximated to the biology of entire experimental group (2).

Experimental protocol

To avoid seasonal variations all the experiments were performed during the winter from November to January. Groups of 10 animals were injected intraperitoneally in a volume of 5 ml/kg with single median lethal doses (LD_{50} values at 9^{00}) determined in a preliminary experiment (100 mg/kg Amitriptyline or 90.7 mg/kg Mianserin) at one of the following hours : 03^{00}, 07^{00}, 11^{00}, 15^{00},19^{00} and 23^{00}. Both drugs were dissolved in 0.9 NaCl %. A photosafe red bulb was used to allow visualization and injection of the mice during the dark. Acute toxicities of both drugs were determined as mortality in 24 hours after injection. All deaths occurring in the observation period were assumed to be due to the treatment with Amitriptyline or Mianserin. No autopsy was performed. To verify the difference between the extreme times for chronotolerance to these drugs, the LD_{50}'s were determined at two circadian times, i.e. at 11^{00} and 23^{00} (the times of low and high susceptibilities for Amitriptyline). The method of Miller and Tainter (12) was used to evaluate the acute LD_{50}'s. Potency ratios were determined as the ratio of higher LD_{50} to lower LD_{50} at 11^{00} and 23^{00} for each drug.

Statistics

Results were analyzed statistically by Chi-square test to assess the rhythmic behaviour of drug toxicity, and Student's "t" test for the determination of difference between the highest and lowest lethalities , and also the overall light and dark responses to Amitriptyline and Mianserin.

RESULTS

The animals exhibited motor incoordination, tremor, convulsion, and respiratory irregularities after Amitriptyline or Mianserin administrations beginning in about 20 minutes after injection. The 24-hour mortality rates after a single i.p. injection of Amitriptyline (100 mg/kg) or Mianserin (90.7 mg/kg) at different times of day are shown in Figure 1. These data indicate a statistically significant ($p<0.01$) circadian variation in the acute toxicity of Amitriptyline which showed great fluctuations with the lowest (20%) mortality at 11^{00}, and the highest (100%) at 23^{00}. A second peak of mortality (80%) was also observed with Amitriptyline at 07^{00}. The difference between the highest and lowest values were found to be significant ($p<0.01$). Mean light and dark lethalities were also found to be different significantly ($p<0.05$).

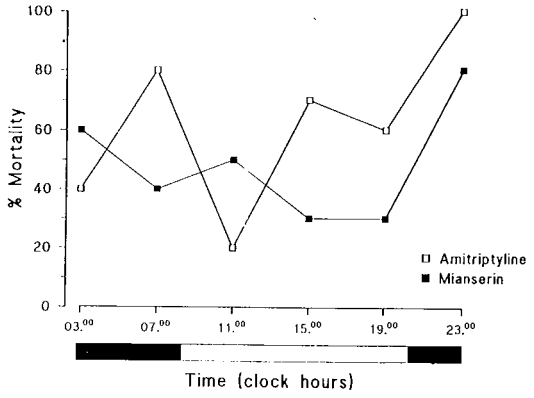

Fig - 1 : Circadian variations of Amitriptyline (100 mg/kg) and Mianserin (90.7 mg/kg) acute toxicity in mice. Animals were synchronized by maintaining under controlled environmental conditions. The photoperiod was automatically timed (12 hr light/12 hr dark) with the light period from 08^{00} to 20^{00} hr. 24-hr acute toxicities were determined by applying a predetermined LD_{50} dose of each agent at six different times of day (n = 10).

Chronotolerance to Mianserin did not show much fluctuations, and mortality rates were found to be around 30-60% at all injection times with the exception of 23^{00} hour. At that time, animals were found to be most susceptible to Mianserin with a

80% mortality. The lowest mortality (30%) was observed at 15^{00} and 19^{00}. But, there was no statistical difference in the time-dependent susceptibility to Mianserin ($p>0.05$). On the other hand, the time of maximum lethality were found to be similar for both drugs.

The LD_{50} values obtained by using the method of Miller and Tainter (12) appear in Table 1. A maximum LD_{50} of 102.1 mg/kg (i.e. minimal toxicity) was obtained at 11.00 hour, and a minimum of 79 mg/kg (i.e. maximal toxicity) was obtained at 23^{00} hour for Amitriptyline. On the other hand, at the time of maximum susceptibility to Amitriptyline , i.e. at 23^{00} hour, animals were found to be more susceptible to Mianserin with a LD_{50} of 118.2 mg/kg than 11^{00}. The potency ratios, which was calculated as the ratio of higher LD_{50} to lower LD_{50} at these two times, were found to be similar for both drugs as about 1.3, but they were staged inversely.

1.29 times more LD_{50} for Amitriptyline at 11^{00} than 23^{00} , and inversely 1.30 times more LD50 for Mianserin at 23^{00} than 11^{00}. Animals were found to be most susceptible to Mianserin while they were most resistant to Amitriptyline at that time. The potency ratios, the ratio of maximum LD_{50} to minimum LD_{50}, were found to be similar for both drugs, but it must be emphasized that they were staged inversely. It must be stressed here that, in contrast to man, rodents such as mice are active during darkness and rest during light. It is also reported that there is sufficient correlation between animal and human data of many drugs when this 12-hour difference in the activity pattern of rodents and humans is taken into consideration (19).

The variations in antidepressant toxicity observed in this study might be related to either differences in pharmacokinetics of drugs, or the changing pattern of susceptibility due to the administration time of the drugs. Although, there is no chronopharmacological data for Mianserin,

	Acute LD_{50} for each drug		
	11^{00}	23^{00}	Potency ratio
Amitriptyline	102.1 ± 5.9	79.0 ± 5.5	1.29
Mianserin	90.7 ± 9.4	118.2 ± 10.0	1.30

All values are expressed as Mean ± S.E.M. Potency ratios were calculated as the ratio of higher LD50 to lower LD50 for each drug.
Table 1 : 24-hour acute LD_{50} values for Amitriptyline or Mianserin at two circadian times

DISCUSSION

The data presented here show that there is a substantial rhythm in resistance to the lethal effect of Amitriptyline. The rhythm in mortality was determined by applying a predetermined LD_{50} dose of each drug to mice at different times of day. So, 100 mg/kg Amitriptyline resulted in only 20% mortality when applied at 11^{00} (3 hours after light onset), but the same dose caused 100% mortality when applied at 23^{00} (15 hours after light onset) (7). Although there were some differences in resistance to Mianserin at different administration times, no statistically significant rhythm in toxicity was found.

In animals fatal toxicity of a drug is expressed in terms of LD_{50}, the dose required to kill 50% of animals on test. Obviously, this term does not include any reference to dosing time. So, we found administration time-dependent changes in Amitriptyline pharmacokinetics have been reported (14). On the other hand, the variations in the effectiveness and/or toxicity of CNS drugs have been generally attributed to the changing endogenous levels of brain biogenic amines (15, 16) and also their receptor binding properties that fluctuate during the day (8). In fact, it is suggested that there was a substantial circadian rhythm of [^{3}H]-imipramine binding in rat suprachiasmatic nuclei (20) and other regions of the rat brain(9). It is also reported that, Imipramine, another tricyclic antidepressant, changed the rhythms of adrenergic, muscarinic, benzodiazepin, opiate, and dopamine receptors in rat brain (8).

The results presented here demonstrate that the acute toxicity of antidepressant drugs in mice varies with the administration time and there are also particular differences between their

susceptibility rhythms.The different pattern of toxicity between Amitriptyline and Mianserin that observed in this study might also be related to the different mode of actions of these two drugs. In fact, Mianserin is free from anticholinergic effects, and appears not to block the reuptake of monoamines (6). It will, therefore, be interesting to investigate in a future study the differential mechanisms of toxicity of these drugs.

Correspondence to : Dr.Uğur HODOĞLUGİL
Gazi Üniversitesi Tıp Fakültesi
Farmakoloji Anabilim Dalı
Beşevler
06500 ANKARA - TÜRKİYE
Phone : 312 - 214 10 00 / 6951

REFERENCES

1. Bruguerolle B, Prat M: Circadian phase-dependent pharmacokinetics and acute toxicity of mepivacaine. J Pharm Pharmacol 1988; 40: 592-594.
2. Burns ER: A critique of the practice of plotting data obtained in vivo on an hours after treatment format. Oncology 1982; 39: 250-254.
3. Cal JC, Dorian C, Catroux P, Cambar J: Nefrotoxicity of heavy metals and antibiotics. In B. Lemmer, editor. Chronopharmacology. New York: Marcel Dekker, 1989: 655-681.
4. Chrome P: Antidepressant overdosage. Drugs 1982; 23: 431-461.
5. Fujimura A, Kumagai Y, Kajiyama H, Ebihara A: Circadian variations in the acute toxicity of adrenoceptor blocking agents in spontaneously hypertensive rats. Acta pharm toxicol 1986; 59: 432-433.
6. Ghose K, Coppen A, Turner P: Autonomic actions and interactions of mianserin hydrochloride (ORG.GB94) and Amitriptyline in patients with depressive illness. Psychopharmacology 1976; 49: 201-204.
7. Güzey C, Zengil H: Circadian variations in the acute toxicity of Amitriptyline in mice. Gazi Med J 1993; 1: 1-3
8. Kafka MS, Wirz-Justice A, Naber D, Moore RY, Benedito MA: Circadian rhythms in rat brain neurotransmitter receptors. Fed Proc 1983; 42: 2796-2801.
9. Kraeuchi K, Wirz-Justice A, Morimasa T, Willener R, Feer H : Temporal distribution of [3H]-imipramine binding in rat brain regions is not changed by chronic methamphetamine. Chronobiol Int 1986; 3: 127-133.
10. Labrecque G, Belanger PM, Dore F X: Chronopharmacological studies of phenylbutazone in the rat. Can J Physiol Pharmacol 1986; 61: 649-652.
11. Lutsch EF, Morris RW X: Circadian periodicity in susceptibility to lidocaine hydrochloride. Science 1986; 156: 100-102.
12. Miller LC, Tainter ML: Estimation of the ED50 and its errors by means of logarithmic probit graph paper. Proc Soc Exp Biol Med 1944; 57: 261-264.
13. Mormont C, Boughattas N, Levi F X: Mechanisms of circadian rhythms in the toxicity and efficacy of anticancer drugs : Relevance for the development of new analogs. In B. Lemmer, editor. Chronopharmacology. New York: Marcel Dekker, 1989: 395-437.
14. Nakano S, Hollister LE: Chronopharmacology of amitriptyline. Clin Pharmacol Ther 1983; 33: 453-459.
15. Owasoyo JO, Walker CA, Whitworth UG: Diurnal variation in the dopamine level of rat brain areas: effect of sodium phenobarbital. Life Sci 1979; 25: 119-122.
16. Ross FH, Sermons AL, Owasoyo JO, Walker A: Circadian variation of diazepam acute toxicity in mice. Experientia 1981; 37: 72-73.
17. Scheving LE, Mayersbach HV, Pauly J.E: An overview of chronopharmacology. J Eur Toxicol 1974; 7: 203-207.
18. Smolensky MH, D'Alonzo GE: Biological rhythms and medicine. Am J Med 1988; 85(Suppl.18): 34-46.
19. Turek FW: Pharmacological probes of the mammalian circadian clock : use of the phase response curve approach. Trends in Pharmacological Sciences 1987; 8: 212-217.
20. Wirz-Justice A, Kraeuchi K, Morimasa T, Willener R, Feer H : Circadian rhythm of [3H]-imipramine binding in rat suprachiasmatic nuclei. Eur J Pharmacol 1983; 87: 331-333.
21. Wisser H, Breuer H: Circadian changes of clinical chemical and endocrinological parameters. J Clin Chem Clin Biochem 1981; 19: 323-337.