CIRCUITRY VARIATIONS OF THE ACUTE TOXICITIES OF AMITRIPTYLINE AND MIANSEMIN IN MICE

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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 LD50 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 2300, and minimum at 1100. Mortality rates for Mianserin did not show significant differences, although a 80% mortality was observed at 2300 and a minimum of 30% at 1500 and 1900. By determining the acute LD50 values for both drugs at these two different times, i.e. 1100 and 2300, we demonstrated that Amitriptyline is 29% more toxic when applied to mice at 2300 than 1100, and Mianserin is 30% more toxic at 1100 than 2300. 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 perazinepexine, 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 0600 - 2000) 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 (LD50 values at 90) determined in a preliminary experiment (100 mg/kg Amitriptyline or 90.7 mg/kg Mianserin) at one of the following hours : 0300, 0700, 1100, 1500, 1900 and 2300. 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 LD50's were determined at two circadian times, i.e. at 1100 and 2300 (the times of low and high susceptibilities for Amitriptyline). The method of Miller and Tainter (12) was used to evaluate the acute LD50's. Potency ratios were determined as the ratio of higher LD50 to lower LD50 at 1100 and 2300 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 lethals, 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 1100, and the highest (100%) at 2300. A second peak of mortality (80%) was also observed with Amitriptyline at 0700. The difference between the highest and lowest values were found to be significant (p<0.01). Mean light and dark lethals 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 0800 to 2000 hr. 24-hr acute toxicities were determined by applying a predetermined LD50 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 2300 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\textsuperscript{th} and 19\textsuperscript{th}. 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\textsubscript{50} values obtained by using the method of Miller and Tainter (12) appear in Table 1. A maximum LD\textsubscript{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\textsuperscript{rd} hour for Amitriptyline. On the other hand, at the time of maximum susceptibility to Amitriptyline, i.e. at 23\textsuperscript{rd} hour, animals were found to be more susceptible to Mianserin with a LD\textsubscript{50} of 118.2 mg/kg than 11\textsuperscript{th}. The potency ratios, which was calculated as the ratio of higher LD\textsubscript{50} to lower LD\textsubscript{50} at these two times, were found to be similar for both drugs as about 1.3, but they were staged inversely.

![Table 1](attachment:image.png)

All values are expressed as Mean ± S.E.M. Potency ratios were calculated as the ratio of higher LD\textsubscript{50} to lower LD\textsubscript{50} for each drug.

**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\textsubscript{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\textsuperscript{th} (3 hours after light onset), but the same dose caused 100% mortality when applied at 23\textsuperscript{rd} (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\textsubscript{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 1.29 times more LD\textsubscript{50} for Amitriptyline at 11\textsuperscript{th} than 23\textsuperscript{rd}, and inversely 1.30 times more LD\textsubscript{50} for Mianserin at 23\textsuperscript{rd} than 11\textsuperscript{th}. 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\textsubscript{50} to minimum LD\textsubscript{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,
susceptibility between 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.

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