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CIRCADIAN VARIATION OF THE ACUTE TOXICITY AND NOCICEPTIVE ACTIVITY OF AMINOPHYLLINE IN MICE

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ABSTRACT

The circadian variation of the toxicity and nociceptive activity of aminophylline as an important methylxanthine was studied in mice. The animals were housed under controlled light-dark cycles for at least 2 weeks. Acute toxicity was determined by LD_{50}. Hot-plate test was used for determination of thermal pain threshold. Doses of 200, 250, 280 and 340 mg/kg of aminophylline were injected intraperitoneally to four separate groups of six male mice at six hour intervals (09:00, 15:00, 21:00, 03:00). Mortality was recorded at 1, 24, and 48 hours after injection and LD_{50} value was measured by logit method after 48 hours. The results showed that the lowest nociceptive effect was at the beginning of the dark phase. The lowest LD_{50} value was also at the beginning of the dark phase. This study indicated that the toxicity of aminophylline was maximum at night and had a different rhythm pattern of nociceptive activity. This indicates different mechanisms of action for these effects.

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Keywords: Chronotoxicity; Aminophylline; Nociceptive effect; Circadian variation.

INTRODUCTION

Methylxanthines block adenosine receptors[1,2] and inhibit phosphodiesterases[3] and other enzymes including 5'-nucleotidase and alkaline phosphatase.[4] They also cause the release of calcium from intracellular stores.[5,6] Adenosine receptor blockade occurs at low micromolar concentrations of methylxanthines, while other actions occur at the millimolar concentration range.[7] Aminophylline is a methylxanthine drug that is metabolized to theophylline.

Theophylline and aminophylline have a relatively narrow therapeutic index that is important in acute and chronic administration. Severe toxic symptoms mainly include neurological and cardiovascular toxic effects.[8] One branch of chronopharmacology is chronotoxicology, which shows that the toxicity of agents may vary according to the administration schedule.[9] Circadian rhythms for many neurotransmitters and drugs such as serotonin, GABA,[10] psychotropic drugs,[11] and opioid analgesics[12] have been demonstrated, but the circadian variation of toxicity and nociceptive activity of aminophylline has not been reported to date. The aim of this study was to ascertain the circadian rhythm of different doses of aminophylline via its nociceptive activity and acute toxicity, and also whether there is a similar circadian pattern for the toxicity and nociceptive effects of this drug.

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MATERIALS AND METHODS

Animals

Male albino mice weighing 25-30g were obtained from a random bred colony maintained on a normal diet in the animal house of Mashhad University of Medical Sciences. Animals were housed in a room with a 12/12 hour light/dark (07:00-19:00) cycle at 21 ±2°C. The animals had free access to food and water.

Circadian variation of nociceptive activity

Nociceptive activity was assessed using the hot-plate test. The temperature of a metal surface was maintained at 55 ±0.2°C. The latency to a discomfort reaction (licking paws or jumping) was determined before and after drug administration. The cut-off time was 25 sec. Aminophylline (from Lec Co.) was injected at 5, 10 and 20 mg/kg intraperitoneally (ip) for evaluation of nociceptive activity. For assessing the circadian variation of nociceptive activity, 10 mg/kg of aminophylline was injected (ip) at 6 hour intervals (09:00, 15:00, 21:00, and 03:00).

Circadian variation of acute toxicity

The chronotoxicity of aminophylline was measured at 6 hour intervals (09:00, 15:00, 21:00, and 03:00). Four doses of aminophylline (200, 250, 280 and 340 mg/kg) were injected intraperitoneally to four separate groups of six male mice. The death number was counted at 1, 24 and 48 hours after treatment. LD_{50} values were calculated by logit method.

Statistical analysis

The data were expressed as mean values ± S. E. M. and tested with analysis of variance followed by the multiple comparison test of Tukey.

RESULTS

Circadian variation of nociceptive activity

Except for the 5mg/kg dose, other doses of aminophylline induced nociceptive activity 1 hour after injection. 3 hours after administration of aminophylline, only 10 and 20 mg/kg doses of this drug showed nociceptive activity (Fig. 1).

After administration of 10 mg/kg of aminophylline at different times throughout the day-night period, minimum nociceptive activity was observed at 21:00 (Fig. 2).

Circadian variation of acute toxicity

Aminophylline had a variety of LD_{50} values at different times (Fig. 3). The lowest LD_{50} value was at the beginning of the dark phase. At 21:00, major mortality was one hour after administration of aminophylline.

DISCUSSION

This study indicated that the trough of the nociceptive

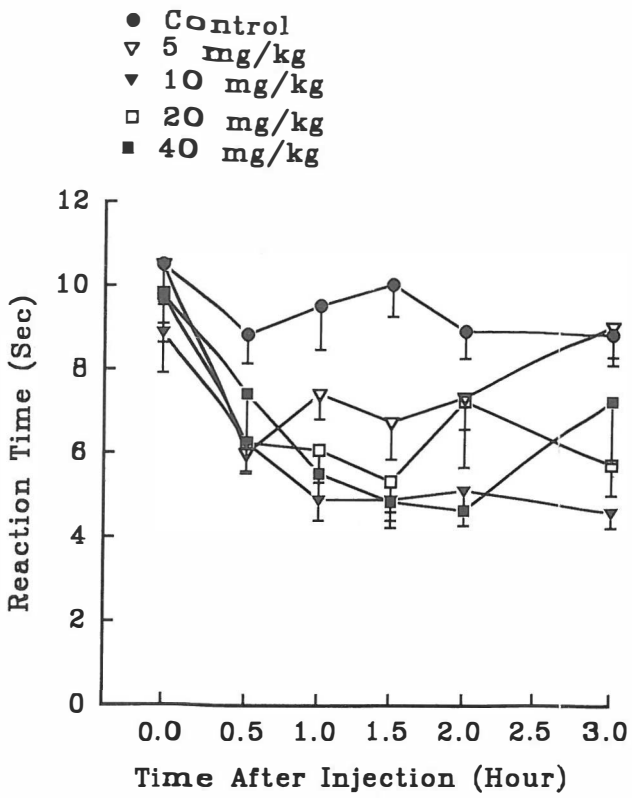

Fig. 1. Nociceptive activity of aminophylline in mice. The drug was administered intraperitoneally. Each point is the mean ± S.E.M. reaction time of 6 mice. Except for the 5 mg/kg dose, other doses of aminophylline induced nociceptive activity 1 hour after injection ($p<0.05$, Tukey test).

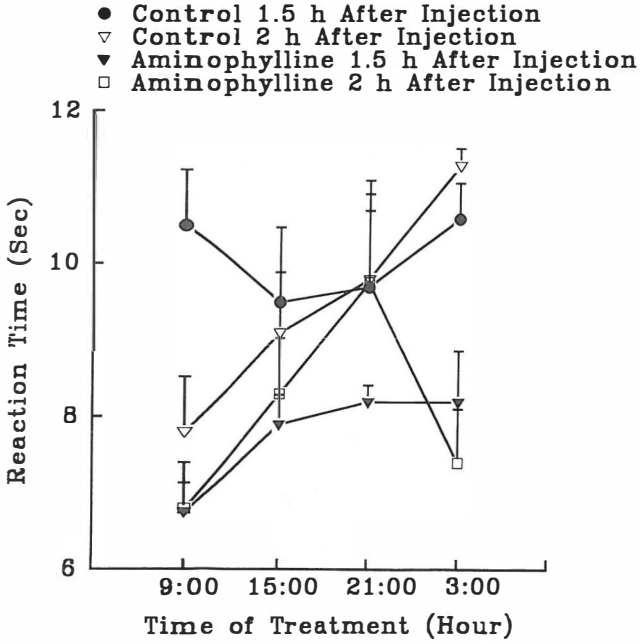

Fig. 2. Circadian variation of aminophylline (10 mg/kg, ip) nociceptive activity. Each point is the mean ±S.E.M. reaction time of 6 mice. Significant activity was seen 2 hours after injection of aminophylline which had minimum activity at 21:00 ($p<0.05$, ANOVA analysis and Tukey test).

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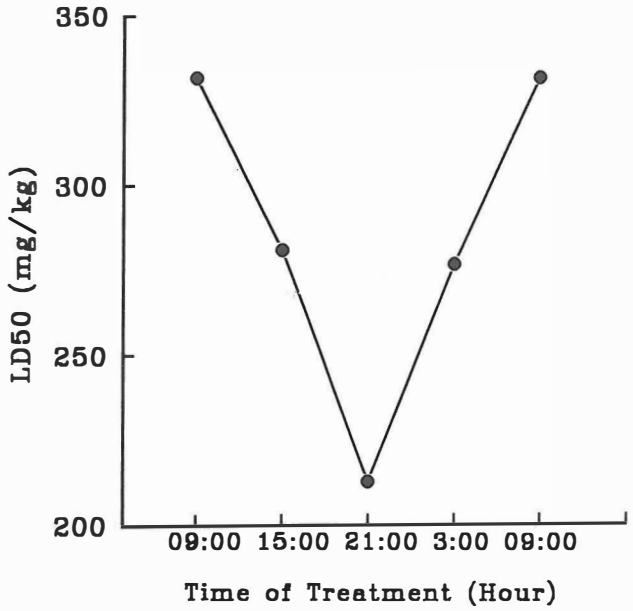

Fig. 3. Circadian variation of aminophylline acute toxicity (LD_{50}). Each point is the mean of LD_{50} for 6 mice 24 hours after

effect and LD_{50} of aminophylline, a non-specific antagonist of adenosine, is at the beginning of the dark phase.

Aminophylline induced a reduction in hot plate latency. This effect may contribute to the block of A1 adenosine receptors that have an analgesic effect.[13-15] Administration of aminophylline and theophylline induced higher blood levels of these drugs at night and the elimination of these drugs was less in the dark phase.[16,17] However, there is an increase in endogenous adenosine levels, and the low activity of metabolizing enzymes[18-20] of this neuromodulator may decrease the nociceptive effect of aminophylline at the beginning of the dark phase.

Maximum mortality or the minimum LD_{50} value of aminophylline was at the beginning of the dark phase. This effect is consistent with the fact that both the lowest elimination rate and maximum drug concentration in plasma occur at the beginning of the dark phase.[16,17] Aminophylline has a narrow therapeutic index and adverse effects such as nausea, vomiting, insomnia, nervousness, gastrointestinal bleeding, seizures, cardiac arrhythmia and cardiorespiratory arrest may occur in humans.[8] Seizures,[21,22] tachycardia and arrhythmias[23] are common causes of death in laboratory animals. In toxic or subtoxic doses, besides blocking adenosine receptors, this drug inhibits enzymes such as phosphodiesterases[3] and releases calcium from intracellular stores.[5,6] This may account for different circadian rhythm patterns in the toxicity and nociceptive effect of aminophylline.

In summary, the results of this study indicated that the trough of the nociceptive effect and LD_{50} values was at the beginning of the dark phase. These circadian variations in toxicity may be of importance in the administration of aminophylline. Measuring the plasma concentration of aminophylline at different time intervals may help to elaborate on these results precisely.

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