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The Effect of Ofloxacin on Pentobarbital-Induced Sleep in Mice[1]

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ENGİNAR, N., L. EROĞLU AND G. ULAK. *The effect of ofloxacin on pentobarbital-induced sleep in mice.* PHARMACOL BIOCHEM BEHAV **40**(1) 65–67, 1991.—There have been several reports that insomnia occurs in some patients who receive ofloxacin. Since almost no experimental data on ofloxacin-induced insomnia were available, this study was conducted for the evaluation of ofloxacin effects on sleep parameters in mice. In Experiment 1, mice were pretreated with ofloxacin (20 or 40 mg/kg IP) or saline 15 minutes before sodium pentobarbital (35 mg/kg IP). Experiment 2 was carried out in two days. On the first day mice were treated twice, in the morning and in the evening, with ofloxacin (20 or 80 mg/kg IP) or saline. On the second morning, mice were pretreated with the same doses of ofloxacin or saline 15 minutes before sodium pentobarbital (35 mg/kg IP). Sleep latency and sleeping time were recorded in each experiment. Results showed that ofloxacin had no apparent effect on sleep latency, but caused a shortening in sleeping time. However, this effect was significant only in the 40 and 80 mg/kg ofloxacin-treated groups.

Ofloxacin Sodium pentobarbital Sleep latency Sleeping time Mice

OFLOXACIN, a new quinolone derivative which shows a broad spectrum of antibacterial activity, is presented with a low incidence of side effects. Adverse drug events recorded in clinical trials during ofloxacin therapy were mainly related to the gastrointestinal tract and followed in order of frequency by nervous system and hypersensitivity reactions (3,9). On the other hand, the most common adverse drug event was related to the central nervous system (CNS) according to the results of the postmarketing surveillance (9). Insomnia appeared to be one of the major nervous system symptoms reported both in clinical trials and spontaneous reports. Oxolinic acid, another quinolone derivative, which has psychostimulant property, was previously observed to induce insomnia in animal experiments (13). However, there is a lack of sufficient experimental data on ofloxacin-induced insomnia. On the other hand, biochemical studies on the possible mechanisms underlying CNS symptoms of ofloxacin were performed (1, 4, 15). In this respect the investigation of ofloxacin-induced insomnia in animals may provide behavioral data to these previous findings. Thus, in the present study, the effect of ofloxacin on sleep parameters were recorded in mice.

METHOD

Animals

Inbred male albino mice, weighing 20–25 g at the time of testing, were used. The animals were housed under standard laboratory conditions for at least one week prior to experimentation.

Drugs

Ofloxacin solutions were prepared immediately before injections by dissolving the powder in distilled water. Sodium pentobarbital was prepared in saline. Injections were done by the intraperitoneal (IP) route (0.1 ml/25 g).

Procedure

Experiment 1. The animals were arranged in 3 groups. At zero time, the members of the ofloxacin groups were treated by 20 or 40 mg ofloxacin per kg body weight. Control mice received saline only. Fifteen minutes (min) after the first injections each mouse in the control and ofloxacin groups was given 35 mg/kg sodium pentobarbital. The righting reflex was used to assess whether or not the animals were "asleep." Following the pentobarbital injection, the time passed for the mouse to be placed on its back was recorded as the sleep latency. Sleeping time was measured as the time passed for the mouse to right itself.

Experiment 2. On the first day each animal was treated twice, in the morning and in the evening, with either an injection of saline (control) or ofloxacin (20 or 80 mg/kg). On the second morning, the animals in the ofloxacin and control groups received the same doses of ofloxacin or saline respectively. Fifteen minutes after these injections, each mouse was given 35 mg/kg sodium pentobarbital, and then sleep latency and sleeping time were recorded as above.

The experiments were carried out 10.00–12.00 a.m. in a quiet room in which the temperature was maintained at 22–24°C.

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TABLE 1

EFFECT OF A SINGLE TREATMENT OF OFLOXACIN ON THE SLEEP LATENCY AND SLEEPING TIME IN MICE

Groups	Sleep Latency (min)	Sleeping Time (min)
Control (28)	6 ± 0.9	72 ± 10.2
Ofloxacin (20 mg/kg) (14)	6 ± 1.3	59 ± 9.8
Ofloxacin (40 mg/kg) (28)	6 ± 0.8	51 ± 9.2*

All mice were injected (IP) with sodium pentobarbital 15 minutes after saline (control) or ofloxacin injection (IP).

The values are means ± S.E.M. with the number of animals in parentheses.

Results are analysed using Mann-Whitney's two-tailed U-test.

*$p<0.02$ significantly different from control.

TABLE 2

EFFECT OF A MULTIPLE TREATMENT OF OFLOXACIN ON THE SLEEP LATENCY AND SLEEPING TIME IN MICE

Groups	Sleep Latency (min)	Sleeping Time (min)
Control (11)	6 ± 1.4	51 ± 7.3
Ofloxacin (20 mg/kg) (8)	4 ± 0.9	40 ± 12.9
Ofloxacin (80 mg/kg) (11)	6 ± 1.0	28 ± 6.9†

All mice were injected (IP) with sodium pentobarbital 15 minutes after the last injection (IP) of saline (control) or ofloxacin.

The values are means ± S.E.M. with the number of animals in parentheses.

Results are analysed using Mann-Whitney's two-tailed U-test.

*$p<0.05$ significantly different from control.

The mice were placed on a warm table during sleeping time. No animal was tested more than once.

Data Analyses

Statistical comparisons were performed by using the Mann-Whitney's two-tailed U-test.

RESULTS

Experiment 1

The effect of a single treatment of ofloxacin on sleep parameters was shown in Table 1. Ofloxacin had no apparent effect on the sleep latency. However, both 20 and 40 mg/kg ofloxacin treatments shortened sleeping time. This effect was significant ($p<0.02$) in the 40 mg/kg ofloxacin-treated group.

Experiment 2

The effect of a multiple treatment of ofloxacin on sleep parameters was shown in Table 2. As in Experiment 1, ofloxacin, while having no apparent effect on the sleep latency, shortened sleeping time. Ofloxacin-induced shortening of sleeping time was also significant ($p<0.05$) only in the higher dose (80 mg/kg).

DISCUSSION

The most frequent CNS symptoms generally occurred within the first three days of ofloxacin therapy (9,11). This observation led us to use a single and multiple injection schedule for ofloxacin which corresponds to the first and second day of ofloxacin therapy respectively. The doses of 20 or 40 mg/kg were chosen from a mouse model of subcutaneous abcess where ofloxacin was used to prevent abscess formation (8). The higher dose, 80 mg/kg, was also used to assess whether there was a dose dependency in side effects of ofloxacin. Pentobarbital injection was done 15 minutes after ofloxacin treatment since this time is optimum to achieve high ofloxacin levels in serum after an IP injection (8).

Our results showed that ofloxacin caused a shortening in sleeping time in the first as well as in the second day of treatment. This effect of ofloxacin was more pronounced in the higher dose. This finding was, however, contrary to the suggestion that the incidence of side effects of ofloxacin has lack of correlation with dosage (2,7).

Most recently ofloxacin has been shown to inhibit the specific ^{3}H-γ-amino-butyric acid (GABA) binding in isolated rat neurons in a concentration-dependent manner (15). On the other hand, oxolinic acid (6) and amfonelic acid (17) both have structural analogies to ofloxacin and possess psychostimulant properties. Oxolinic acid was recently used therapeutically in the treatment of diurnal hypersomnia for its psychostimulant effect which leads to insomnia (10). Diazepam, a benzodiazepine which exerts a facilitatory effect on GABA-ergic transmission (12), reduced the stimulant effect of oxolinic acid (14). In accordance with these findings, in a clinical study ofloxacin has been shown to possess CNS-stimulating effects as revealed by the electroencephalogram (EEG) which were reversed by coadministration of a benzodiazepine agonist (16). In addition, in our previous study where its effects were antagonized by diazepam, ofloxacin potentiated convulsions induced by pentylenetetrazol, a GABA-lytic drug (12), in mice (5).

Although neurochemical data are inadequate a possible psychostimulant effect due to an interaction between ofloxacin and CNS via a GABA antagonism might be, at least in part, involved in the shortening of sleeping time. However, this effect of ofloxacin might not be a direct action on the CNS. Shortening in sleeping time might be due to a decrease in the blood concentration of pentobarbital, since pretreatment of ofloxacin could have altered the pharmacokinetic properties of the drug. Thereby, further experiments searching such a pharmacokinetic interaction between ofloxacin and pentobarbital, as well as the effect of ofloxacin on natural sleep behavior will be helpful to clarify this possibility.

In conclusion, although additional studies are required, present findings seem to be of value in respect to ofloxacin-induced insomnia observed in clinical trials.

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