Effects of fluoroquinolones on the locomotor activity in rats

Abstract During therapy with fluoroquinolones adverse CNS reactions such as dizziness, light-headedness, insomnia or sleepiness are observed in up to 20% of patients. Using a device developed at our institute for the simultaneous registration of the activity of rats housed in single cages, we have investigated the effects of trovafloxacin, fleroxacin or ofloxacin on the locomotor activity of juvenile and adult rats (11 per group) after oral administration of 600 mg/kg for 5 consecutive days. The effects were most pronounced after fleroxacin, which induced a reduction in activity to 36 ± 9% (mean ± SD) of the values measured in juvenile rats before treatment and to 60 ± 21% (mean ± SD) in adult rats. HPLC analysis of the plasma concentrations in juvenile rats showed that the concentrations of trovafloxacin were considerably lower than those of the other fluoroquinolones that had been studied previously in our laboratory: the peak concentration of trovafloxacin was 14 ± 2.9 mg/l (mean ± SD) after a single dose of 600 mg/kg in juvenile rats. Overall, we showed that the locomotor activities of juvenile and adult rats were significantly depressed during treatment with fluoroquinolones. The effects were more pronounced in juveniles. Monitoring of the locomotor activity of rats is a suitable approach to study CNS effects of fluoroquinolones in animals, but pharmacokinetics have to be taken into account.

Key words Fluoroquinolones · Neurotoxicity · Behaviour · Pharmacokinetics · Rats

Introduction Adverse reactions of the central nervous system (CNS) are a well-known complication during therapy with quinolones. Neurotoxic reactions to fluoroquinolones are numerous and include headache, dizziness, tiredness or sleeplessness, abnormal vision, restlessness, bad dreams. The adverse reactions occur dose-dependently and the patterns of neurotoxic effects of the various quinolones seem to differ, but clinical data are often complicated by the unclear nature of the cause-effect relationship (Lipsky and Baker 1999; Stahlmann and Lode 1999). For example, severe insomnia was seen in 8% of patients treated with 400 mg fleroxacin daily, but in approximately 60% of those receiving 800 mg once daily, a regimen which is not licensed or recommended for therapy (Bowie et al. 1989). During treatment with trovafloxacin, variable frequencies of CNS symptoms such as dizziness and light-headedness have been observed. In some reports the incidence of such effects was seen in more than 10% of patients. Symptoms usually resolved with continued dosing (Lipsky and Baker 1999).

We have investigated the effects of three fluoroquinolones in rats by recording their locomotor activity. Our intention was to establish a model that would allow us to study the effects of quinolones on the behaviour of the animals and thus allow a comparison of the effects of various quinolones in an objective manner.

Because major differences exist between the kinetics of fluoroquinolones in rats and man, we have also assessed the plasma levels of trovafloxacin in juvenile rats. Corresponding data for the other two drugs had been established previously in our laboratory (Stahlmann et al. 1990; Förster et al. 1996).
Materials and methods

Animals and housing conditions

Wistar rats (Hsd Cpb:WU), weighing 180 to 270 g, were purchased from Harlan Winkelmann (Borchen, Germany). They were acclimatized to the conditions of our animal quarters for 3 weeks before mating. The females were kept single-caged from the beginning of the third week after mating and allowed to deliver. The pups remained in their peer groups until commencing the experiment.

In the first part of the study 5-week-old rats (hereafter called juvenile rats) of either sex (six females, five males) were used. Their body weight at commencement of the study was 64.1 ± 10.6 g (mean ± SD). They were allocated to the different treatment groups (11 rats per group) at random. For the second part of the study, the same rats now as adults (3-month-old) weighing 219 ± 49 g, were used.

Juvenile and adult rats were kept under specified pathogen-free conditions in Macrolon cages at a constant day/night cycle (light from 900 to 2100 hours) at 21 ± 1°C and 50 ± 5% relative humidity, and they received standard pellet feed (Altromin 1324; Altromin, Lage, Germany) and water ad libitum.

Fluoroquinolones

Commercially available fluoroquinolone-containing tablets were suspended in 2% starch solution. The drugs investigated were: ofloxacin (Tarivid200, Hoechst, Frankfurt am Main, Germany), fleroxacin (Quinodis200, Hoffmann-La Roche, Grenzach-Wyhlen, Germany) and trovafloxacin (Trovan, Pfizer, Karlsruhe, Germany).

Treatment

In both parts of the study the rats were treated by gastric intubation for 5 consecutive days with 600 mg/kg body weight of ofloxacin, trovafloxacin, or fleroxacin. Controls received only a starch solution (2%) (10 ml/kg body weight).

Kinetics

Additional groups of juvenile rats (n=6 per time point) were treated with a single oral dose of trovafloxacin (600 mg/kg body weight). Blood samples were obtained by decapitation, and plasma, separated by centrifugation, was stored at −25°C until analysis by high-performance liquid chromatography (HPLC). We studied the kinetics of trovafloxacin only, because corresponding data for the two other drugs have been established previously in our laboratory.

HPLC analysis

Concentrations of trovafloxacin in rat plasma were determined using HPLC with fluorescence detection as described by Borner et al. (1986). Briefly, plasma samples and standard solutions spiked with plasma were deproteinized with acetonitrile, lyophilized, and dissolved in the mobile phase consisting of 4 parts of acetonitrile (Merck, Darmstadt, Germany) and 86 parts of an aqueous solution of 5 mM tetrabutylammonium phosphate (Sigma, St. Louis, Mo., USA), pH 2.0. The samples were measured at the appropriate excitation and emission wave-lengths (275 and 405 nm). Further equipment used consisted of an HPLC pump (Knauer 64, Berlin, Germany), Nucleosil 5C18 column (Macherey and Nagel, Düren, Germany) and Shimadzu RF 535 fluorescence detector (Shimadzu Europe, Duisberg, Germany). The standards were prepared using trovafloxacin hydrochloride (obtained as a generous gift from Pfizer GmbH, Karlsruhe, Germany). Kinetic data for ofloxacin and fleroxacin had been established under identical conditions in earlier experiments (Stahlmann et al. 1990; Förster et al. 1996).

Locomotor activity

The rats were housed in single Macrolon cages type III. Prior to the commencement of the experiment, the animals were kept in the test device for 3 days (juvenile rats) or 1 day (adult rats) of habituation. Locomotor activity was measured by light beam interruptions (LBI) using three infra-red photocells per cage that monitored locomotion of the animal at 5-min intervals. The method has been described in detail previously (Thiel et al. 1989). The activity was recorded for all rats (n=44) of one age group simultaneously over 24-h cycles for 10 days in juveniles (3 days before treatment, 5 days of treatment, and 2 days after the treatment period) or 8 days in adults (1 day before treatment, 5 days of treatment, and 2 days after the treatment period). In addition to the total LBI counts, the duration (h) of daily activity was determined.

Statistical analysis

Values for concentrations and body weight gain were expressed as group means ± SD. Statistical comparison of two means was performed by the unpaired Student’s t-test. Values for activity (LBI counts per day and hours per day) were standardized by comparing the value of each animal obtained on the day before treatment with that from all other days of the study. Since there was no statistical difference in activity between sexes, the data from female and male rats were pooled. Values are given as group means ± SD. For statistical evaluation, analysis of variance (Generalized Linear Model) followed by Dunnett’s post hoc test was performed. Calculations were carried out using SAS statistical software (v6.12; SAS Institute, Cary, N.C.). A probability level P < 0.05 was chosen as statistical significance for all tests.

Results

Body weight in juvenile rats

The relative weight gain (%) during the treatment period was significantly lower (P < 0.05) in rats treated with trovafloxacin (10.3 ± 5.0) and fleroxacin (13.0 ± 2.9) than in those treated with ofloxacin (20.6 ± 7.6) or with the vehicle (21.4 ± 2.2).

Kinetics

We used the same dose of 600 mg/kg for all three fluoroquinolones, but we observed pronounced differences in the pharmacokinetics of trovafloxacin compared with the other quinolones (Table 1 and Fig. 1). Trovafloxacin was rapidly absorbed from the gastrointestinal tract; peak concentrations were measured after 60 min. They were approximately 7 times higher than the peak concentrations observed in patients during treatment with this drug. As reported before, plasma levels of fleroxacin were significantly higher than those of the other two quinolones; the levels of fleroxacin did not change significantly between 15 min and 6 h after application (Stahlmann et al. 1990; Förster et al. 1996).

Locomotor activity in juvenile rats

With the exception of a slightly higher activity on the first and fourth day of treatment, the locomotor activity