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Changes in brain interleukin-1β following the coadministration of norfloxacin with biphenylacetic acid in rats

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Abstract

We sought to determine the changes in brain interleukin-1β (IL-1β) following the coadministration of norfloxacin (25 mg/kg, i.p.) with biphenylacetic acid (100 mg/kg, p.o.) in rats. Norfloxacin provoked clonic convulsions in rats treated concomitantly with biphenylacetic acid, a major metabolite of the nonsteroidal anti-inflammatory drug fenbufen. Seizure activity was analyzed by EEG monitoring. Behavioral changes were also monitored. IL-1β expressions in the prefrontal cortex and hippocampus at different time intervals were studied by reverse transcriptase–polymerase chain reaction (RT-PCR) and enzyme-linked immunosorbent assay (ELISA). The epileptiform discharges appeared in all the rats, accompanied with limb twitching and clonic–tonic seizures after administration of norfloxacin coadministered with biphenylacetic acid. Norfloxacin plus biphenylacetic acid-induced convulsions rapidly and transiently enhanced IL-1β mRNA in the prefrontal cortex and hippocampus. IL-1β mRNA expression in the prefrontal cortex and hippocampus was detected as soon as 30 min after norfloxacin injection, and decayed to control levels by 6 h. ELISA analysis revealed significant increase of the IL-1β protein in the prefrontal cortex and hippocampus at 2 h and 6 h. Administration of either norfloxacin or biphenylacetic acid alone did not elicit convulsions and increase in IL-1β mRNA and protein expressions. The results suggest that the increased IL-1β expressions in the prefrontal cortex and hippocampus induced by norfloxacin with biphenylacetic acid relate to seizure activities, and that these brain regions play pivotal roles in norfloxacin-induced convulsions.

Keywords: Norfloxacin; Biphenylacetic acid; Convulsion; Interleukin-1β; (Rats)

1. Introduction

Fluoroquinolones are among the most frequently used antimicrobial agents because of their wide spectra of antibacterial activities and excellent tissue permeability (Andriole, 1993). However, they have been shown to possess adverse effects related to the central nervous system (CNS), such as headache, dizziness and paroxysmal convulsions. Although the incidence of these adverse CNS effects are quite low (1–4%) (Christ, 1990), convulsive seizures have been observed more frequently after the coadministration of some fluoroquinolones and an acidic nonsteroidal anti-inflammatory drug in both humans and experimental animals (Anastasio et al., 1988; Yoshino et al., 2005).

Several published studies showed that some quinolones, such as enoxacin, norfloxacin and lomefloxacin, are weak inhibitors of [^{3}H]GABA and [^{3}H]muscimol binding to brain membranes (Segev et al., 1988; Tsuji et al., 1988; Akahane et al., 1989), and GABA-induced Cl^- currents in hippocampal neurons (Akaike et al., 1991; Halliwell et al., 1995). Furthermore, these $GABA_A$ receptor antagonistic actions were potentiated dramatically when these quinolones were administered in combination with biphenylacetic acid, an active metabolite of fenbufen, and consequently, elicited convulsions (Akahane et al., 1989; Halliwell et al., 1995; Akahane et al., 1994; Ito et al., 1996). Two types of hybrid molecule of norfloxacin and biphenylacetic acid were synthesized and their inhibitory effects on $GABA_A$ receptors were investigated. Inhibition curves of

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muscimol-stimulated $^{36}Cl^-$ uptake and GABA-induced Cl^- currents revealed that a hybrid with a $-CONH(CH_2)_3-$ chain between norfloxacin and biphenylacetic acid (flexible structure) was a more potent inhibitor of both $^{36}Cl^-$ uptake (Ito et al., 1996) and Cl^- currents (Imanishi et al., 1996) than the combination of norfloxacin and biphenylacetic acid. In contrast, another hybrid linked by —CONH— (stretched structure), at relatively high concentrations (>10 μM) exhibited weak inhibitory effects. These results suggest that quinolones and biphenylacetic acid interact with $GABA_A$ receptors at nearby sites and that the binding affinities and antagonistic potencies of quinolones at the $GABA_A$ receptors are enhanced mainly as a result of intermolecular interactions with biphenylacetic acid (Akahane et al., 1994; Ito et al., 1996; Imanishi et al., 1996). Although the $GABA_A$ antagonistic activities of quinolones in combination with fenbufen or biphenylacetic acid in the CNS are considered to play a role in eliciting convulsions in humans and experimental animals, the characteristics of these convulsions have not been carefully investigated.

Recently, the administration of convulsant doses of kainic acid, pentylenetetrazole or soman was found to result in the expression of interleukin-1β (IL-1β) in the brain (Jankowsky and Patterson, 2001; Svensson et al., 2001). IL-1β is one of several cytokines involved in inflammation both outside and inside the CNS. It also plays a role in non-inflammatory processes of CNS, e.g. downmodulation in pain and stimulation of hypothalamic hormone release. Effects of elevated levels of IL-1β have been associated with injury or illness, for example Alzheimer's disease, Down's syndrome, and stroke (Mrak and Griffin, 2000). Relton and Rothwell (1992) found that IL-1β receptor antagonist inhibits ischemic, and excitotoxic neuronal damage in the rat, thus indicating that IL-1β may have toxic properties in brain. Yamasaki et al. (1992) suggested the possible involvement of IL-1β in ischemic brain edema formation.

To be able to increase knowledge of the molecular mechanisms of convulsions induced by quinolones, we have investigated the effects of norfloxacin plus biphenylacetic acid on inflammatory response, indicated as IL-1β mRNA and protein production, at different time intervals following norfloxacin in combination with biphenylacetic acid.

2. Materials and methods

2.1. Chemicals

Norfloxacin (white, purity >99%) was provided by the Henan Institute for Drug Control, and dissolved in 0.1 mol/L NaOH and sterile saline. Solution was prepared immediately before injection. 4-biphenylacetic acid was purchased from Sigma Chemical Co (USA). Biphenylacetic acid was suspended in a solution of 1% methylcellulose.

2.2. Animals/tissue

A total of 86 male Sprague–Dawley rats (10–14 weeks old, 200–250 g, Grade II, Certificate No. 003) were obtained from the Laboratory Animal Centre, Fudan University. Animals were housed under controlled conditions (temperature: 21°C±1°C and lighting: 8:00–20:00) with food and water ad libitum. The project was approved by the animal ethical committee.

The rats were injected with norfloxacin (25 mg/kg, i.p.) or saline 30 min after pretreatment with biphenylacetic acid (100 mg/kg, p.o.) or saline, and decapitated at the time intervals of 30, 60, 120 and 360 min. Two different brain regions were dissected, hippocampus and prefrontal cortex, due to earlier studies (Minami et al., 1990), and kept frozen (−80°C) until the day of analysis.

2.3. General epileptic behavior

Motor seizures induced by norfloxacin plus biphenylacetic acid in rats were visually observed by two independent investigators unaware of the identity of the experimental groups. They were quantified in experimental and matched control rat using the following parameters: (a) the time to onset of the first seizure (either clonic or tonic); (b) the duration of the clonic and tonic components of seizures; (c) the number of rats showing motor seizures; and (d) the number of rats showing death.

Clonic seizures consisted of a rhythmic contraction of forelimbs and/or hindlimbs and/or the back muscles. A tonic seizure consisted of a rigid extension of the fore- and/or hindlimbs with or without loss of posture. The time of observation was of 360 min, according to a previously published study (Zhang et al., 2003).

2.4. EEG recording

Implantation of electroencephalogram (EEG) recording electrodes was carried out as described previously (Zhang et al., 2003). All recordings were carried out on unanesthetized and freely moving rats at least 5 days after surgery. Every day in the post-operative period each rat was habituated for 1 h to the recording box (Plexiglas, 40 cm×40 cm×30 cm). The EEG signals were digitized using an EEG recording system, as described previously (Zhang et al., 2003).

The EEG recording of each rat was analyzed visually to detect any activity different from baseline. Seizures were defined by the occurrence of discrete episodes consisting of the simultaneous occurrence of at least two of the following alterations: high-frequency and/or multispike complexes and/or high-voltage synchronized spikes. The quantitative parameters chosen to quantify seizure activity after norfloxacin were the latency to the first seizure (onset) and the total time spent in seizures, which was determined by adding together the duration of all ictal episodes during the EEG recording period.

2.5. Reverse transcription polymerase chain reaction (RT-PCR)

Total RNA was extracted from homogenized brain tissues using the TRIZOL (Invitrogen) method. The amounts of isolated RNA were determined by analysis of absorbance at 260 nm. Purity of mRNA was calculated by the ratio 260:280, and for all used samples this ratio was 1.7–2.0. Levels of IL-1β mRNA were examined following reverse transcription (RT) by using Oligo(dT) 15 primers and amplification of the cDNA transcripts. Briefly, 1 μg of total RNA in 1 μl was mixed with Oligo(dT)15 primers (50 μM,