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Possible interaction of fluoroquinolones with the benzodiazepine-$GABA_A$-receptor complex

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1 The possible involvement of the benzodiazepine (BZD)-$GABA_A$-receptor complex in mediating CNS stimulatory effects of fluoroquinolones was tested *in vitro*, in a binding inhibition assay and *in vivo*, in a clinical drug interaction study using electro-encephalogram (EEG) monitoring.
2 The specific binding of [^{3}H]-flunitrazepam to rat synaptic brain membranes was inhibited by various fluoroquinolones in a concentration-dependent manner.
3 Ofloxacin had CNS-stimulating effects as revealed by the EEG which were slightly augmented by flumazenil but reversed by coadministration of midazolam.
4 In conclusion, our findings suggest that clinically observed CNS adverse effects of fluoroquinolones could be mediated at least in part through interaction with the BZD-$GABA_A$-receptor complex and may be controlled by BZD agonist administration.

Keywords fluoroquinolones benzodiazepines adverse drug reactions pharmaco-EEG benzodiazepine-$GABA_A$-receptor complex

Introduction

There are accumulating clinical reports describing the occurrence of different CNS-related adverse drug effects after administration of fluoroquinolone carboxylic acid derivatives (Fass, 1987; Holmes *et al.*, 1985; Jüngst & Mohr, 1987; Schacht *et al.*, 1988). The overall incidence of described neurological effects varies between 0.9–3.3% depending upon the substance and these are more common with advancing age (Ball, 1989; Gonzalez & Henwood, 1989). The most frequently observed symptoms are dizziness, headache, insomnia, restlessness, visual impairment and more severe effects comprising hallucinations, confusion and other psychotic reactions. However, reports about neurological disturbances such as convulsions are extremely rare, and are seen more often in combination with either theophylline or non-steroidal anti-inflammatory drugs (NSAID; Arcieri *et al.*, 1987). So far only little is known about the possible underlying mechanism(s) of these neurotoxic effects. It has been shown by different investigators (Hori *et al.*, 1985, 1986; Tsuji *et al.*, 1988a) that fluoroquinolones caused concentration-dependent inhibition of GABA post-synaptic binding. Such effects were demonstrated after simultaneous administration of theophylline and fluoroquinolones (Segev *et al.*, 1988). In addition an increased inhibition was also found after coadministration of several NSAID (Hori *et al.*, 1987; Tsuji *et al.*, 1988b).

We therefore further investigated the interaction of ciprofloxacin, norfloxacin and ofloxacin with the benzodiazepine (BZD)-$GABA_A$-receptor complex in a radio-receptor assay (RRA) and evaluated *in vivo* by EEG monitoring the excitatory effects of ofloxacin and their possible attenuation after administration by

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either the short-acting BZD-agonist midazolam (Allonen *et al.*, 1981) or the specific BZD-antagonist flumazenil (Klotz & Kanto, 1988).

Methods

In vitro *study*

Chemicals Norfloxacin was a kind gift of Merck Sharp & Dohme, Rahway, USA; ciprofloxacin of Bayer AG, Wuppertal, FRG; ofloxacin of Hoechst AG, Frankfurt, FRG. GABA and trimethoprim were purchased from Sigma Chem. Comp., St Louis, USA; [^{3}H]-flunitrazepam (spec. act. 90 Ci mmol^{-1}) was supplied by New England Nuclear, Boston, USA. Diazepam, clonazepam and flumazenil were gifts of Hoffmann-La Roche, Grenzach/Wyhlen, FRG. Ivermectin was supplied by MSD, Munich, FRG. All other reagents were commercial products of analytical grade.

Preparation of rat brain synaptic membranes Whole brains of male Sprague-Dawley rats (150–200 g) were pooled and homogenised in 10 vol. ice-cold sucrose buffer (0.3 M) and centrifuged for 5 min at 5,000 *g*; the supernatant was further centrifuged at 47,000 *g* for 20 min at 4° C. The pellet was suspended in 10 vol. Tris-HCl buffer (50 mM, pH 7.4) and centrifuged again at 47,000 *g*. This procedure was repeated five times to remove endogenous GABA. For the binding assay this stock solution was diluted ten times to give a final protein concentration of 0.28 mg ml^{-1} (measured by the method of Lowry *et al.*, 1951). Aliquots of the stock solution were stored at −20° C prior to analysis.

Effect of fluoroquinolones on the binding of [^{3}H]-flunitrazepam to central BZD-receptors For the binding assay, to aliquots of 400 µl diluted synaptosomal preparations containing 20 µl of [^{3}H]-flunitrazepam (0.4 nM final concentration), 50 µl of different concentrations (1, 2, 5, 10, 50, 100, 500, 800 and 1000 µM) of ciprofloxacin, norfloxacin, ofloxacin and 50 µl Tris-HCl buffer were added to give a final volume of 500 µl per assay tube. Non-specific binding was determined in the presence of excess of unlabelled diazepam (10 µM per assay tube). In a control experiment, trimethoprim and ivermectin (an antihelmethic agent reported to bind to the $GABA_A$ receptor), were tested in the same concentration range as above. Test tubes were incubated at 37° C for 60 min and placed on ice for 5 min prior to filtration. Bound and free radioactivities were separated in the cold by rapid vacuum filtration through Whatman GF/B glassfibre filters (Whatman Intern., Maidstone, USA) and two subsequent washes with 5 ml ice-cold Tris-HCl buffer. Membrane-bound [^{3}H]-flunitrazepam was extracted from filters with 4 ml scintillation liquid (Quickszint 212, Zinsser Anal., Frankfurt, FRG) and counted by conventional scintillation counting. In parallel experiments, 300 µM GABA was added prior to the addition of labelled BZD and assays were conducted as described above. All measurements were performed at least in triplicate.

For data analysis, specific binding was calculated by subtracting non-specific from total and test bindings. The results were expressed as percentage inhibition of specific total binding by the different ligands and plotted in the form of displacement curves. IC_{50}-values were extrapolated based on a log-logit transformation of the original data.

In vivo *study*

Drugs Ofloxacin (Tarivid®, 200 mg 100 ml^{-1}) for clinical trials was provided by Hoechst AG, Frankfurt, FRG and flumazenil (Anexate®, 1 mg 10 ml^{-1}) by Hoffmann-La Roche, Grenzach/Wyhlen, FRG. Midazolam (Dormicum®, 15 mg 3 ml^{-1} ampoule) was purchased from Hoffmann-La Roche.

Subjects/Study design After obtaining written informed consent, 12 drug-free healthy volunteers (three females, nine males) between 23 and 47 years old participated in the study, which was approved by the Ethics committee of our hospital. Inclusion criteria were normal values in the haematological, physical and clinical safety checkups and a stable alpha-rhythm with an alpha proportion > 40%, evaluated in an EEG pre-screening. Prior to testing, subjects were adapted to protocol and test facilities until a stable baseline was obtained.

The study was performed under placebo-controlled, double-blind, randomized cross-over conditions. The wash-out periods between the different medications were at least 4 days.

Ofloxacin (400 mg) or placebo (0.9% NaCl solution) was infused constantly over a period of 30 min; 20 min later placebo or either midazolam (0.075 mg kg^{-1} body weight) or flumazenil (0.01 mg kg^{-1}) was injected intravenously over 1 min. EEG and psychometric data were recorded before starting the infusion (pre-baseline), (t_R 1) 10 min before the end of infusion, (t_R 2) immediately after administration of placebo/midazolam/flumazenil, (t_R 3) 40 min after this comedication and (t_R 4) 1 h later, at the end of the test period (see Figure 1).

Table 1 Inhibition of [^{3}H]-flunitrazepam (0.4 nM) binding by different fluoroquinolones

	% inhibition of total binding *Concentration (μM)*						*Extrapolated*
	10	*50*	*100*	*500*	*800*	*1000*	*IC_{50}*
Ciprofloxacin	11	29	35	41	54	62	717
(+GABA, 300 μM)	15	24	25	32	35	38	1450
Norfloxacin	14	16	28	41	46	55	844
(+GABA, 300 μM)	4	20	24	24	33	38	1248
Ofloxacin	11	17	31	38	n.m.	49	921
(+GABA, 300 μM)	14	16	18	31	n.m.	46	1043

n.m.: not measured

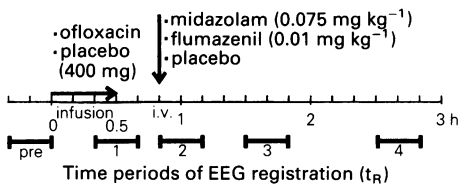

Figure 1 Time periods of EEG registration (t_R).

Measurements EEG electrodes were placed in frontal, central and occipital locations on the non-dominant hemisphere according to the 10–20 system and signals monitored in recumbent, resting position with eyes closed for 20 min. The data were evaluated after analogue to digital conversion, data tapering and transformation to frequency domain by Fourier algorithm. Subsequently log power values were calculated and integrated in 9 epochs of 15 s. Different EEG wave band intervals (between 1.5 to 32 Hz) were selected for analysis, according to common classification (see Herrmann & Schärer, 1987) and made complete with the dominant frequency value which is different from the peak frequency, a parameter for the major frequency in the power spectrum (see Figures 2 and 3). Artifacts were controlled by visual inspection. Digitalisation was conducted by an ADC-card, HP (100 Hz) and processed over HP 9000, model 220 T with a floating point processor. The data were further pulse-code modulated and tape stored.

Data handling was performed using a 'Stat Library' program (Hewlett Packard c/o Colorado State University, USA) and other statistical software.

The mean values of the single drug conditions at each time point were compared with each other using the least significant difference test (LSD) for multiple comparisons (Winer, 1971) with a level of significance at $P < 0.05$.

Other criteria Psychometric tests included a subjective sedation index formed from visual analogue scales (VAS), a self-rating mood scale, and a complaint inventory (see van Zerssen, 1976 a,b).

Additionally, subjective side effects as experienced during the whole test periods, were recorded by the subjects.

Blood pressure (including mean arterial blood pressure, MBP) and heart rate were monitored automatically (Dinamap®, Critikon).

Results

In vitro *study*

The effects of ciprofloxacin, norfloxacin, and ofloxacin on [^{3}H]-flunitrazepam binding to specific BZD receptors are summarised in Table 1. All three fluoroquinolones showed a concentration-dependent inhibition of specific [^{3}H]-flunitrazepam binding in the range of 10–1000 μM. IC_{50}-values averaged 717 μM for ciprofloxacin, 844 μM for norfloxacin and 921 μM for ofloxacin, respectively. After addition of 300 μM exogenous GABA, the inhibition curves were shifted to higher values with corresponding IC_{50}-values of 1450, 1248 and 1043 μM. In the lower concentration range (1–10 μM), less than 10% inhibition was observed with great variability between triplicate measurements.

Neither trimethoprim nor ivermectin reduced binding of [^{3}H]-flunitrazepam to a significant extent in the same concentration range as tested for the fluoroquinolones.

In vivo *study*

EEG data The results presented are based upon evaluation of nine registration epochs during the last 5 min of each 20 min recording period

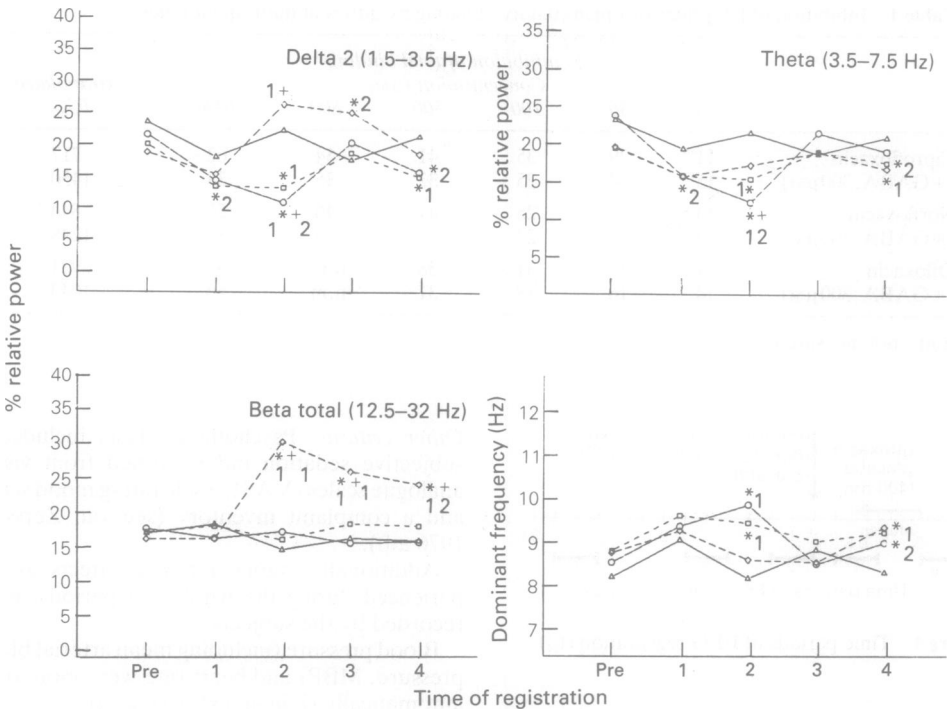

Figure 2 Time-dependent EEG wave band patterns (Delta 2, Theta and Beta) and dominant frequency, measured for different infusions and coadministered drugs: △—△ placebo-placebo, □—□ ofloxacin-placebo, ◇—◇ ofloxacin-midazolam, ○—○ ofloxacin-flumazenil. *1: $P < 0.05$ for placebo-placebo *vs* ofloxacin-placebo or *vs* ofloxacin-midazolam, ofloxacin-flumazenil, *2: $P < 0.1$ for placebo-placebo *vs* ofloxacin-placebo or *vs* ofloxacin-midazolam, ofloxacin-flumazenil, $^{+}$1: $P < 0.05$ for ofloxacin-placebo *vs* ofloxacin-midazolam or *vs* ofloxacin-flumazenil, $^{+}$2: $P < 0.1$ for ofloxacin-placebo *vs* ofloxacin-midazolam or *vs* ofloxacin-flumazenil.

('vigilance-controlled' EEG). The most pronounced effects were observed in recordings of the central region, although frontal and occipital measurements showed similar tendencies. All changes occurred most significantly at the time of registration (t_R) 2, a period when ofloxacin as well as flumazenil or midazolam had reached sufficient concentrations to produce CNS effects.

Figures 2 and 3 illustrate the central EEG-profiles over the selected time periods of registration, expressed as percentage of relative power, obtained for different frequency intervals.

As expected, no changes occurred in the placebo EEG-profile and in the prevalue recordings, preceding the different treatment regimens.

Compared with placebo conditions, an activating effect on the EEG recordings was observed after ofloxacin treatment, as visualised in a pronounced decrease in delta 2 and theta wave bands and an augmentation of alpha 1, alpha total power and alpha/slow wave-index (ASI). Consequently, a shift in the dominant frequency to higher values was seen. The most marked effects could be observed 30 min following completion of the infusion and lasted in a diminishing manner, over the whole time period of recording.

Following administration of flumazenil after ofloxacin similar profiles were recorded and the effects observed under pure ofloxacin treatment became intensified by flumazenil; this action disappeared after t_R 3. Administration of midazolam after ofloxacin revealed a significant increase in both delta 2 and theta as well as in beta 1 and beta total wave band intervals compared with placebo. An effect, which was even more pronounced in comparison to ofloxacin or flumazenil dosing. On the other hand, all alpha frequencies and the dominant frequency were markedly decreased and became significant compared with the other treatments. Additionally, a highly significant increase ($P < 0.0001$)

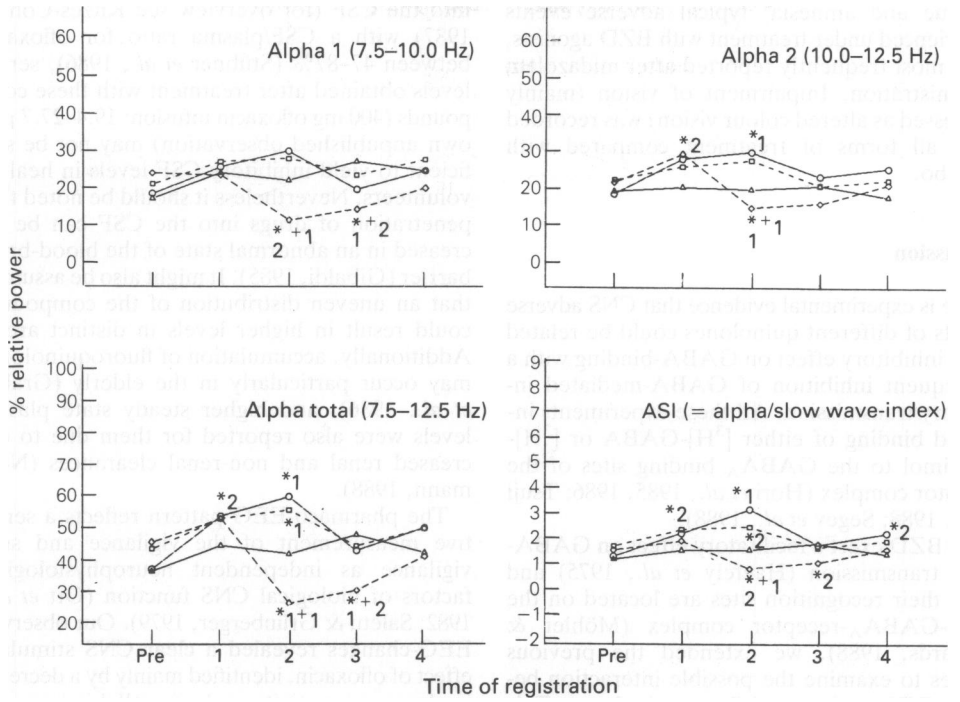

Figure 3 Time-dependent EEG wave band patterns (Alpha 1, Alpha 2 and Alpha Total) and ASI (alpha/slow wave-index) for different infusions and coadministered drugs: △—△ placebo-placebo, □—□ ofloxacin-placebo, ◇—◇ ofloxacin-midazolam, ○—○ ofloxacin-flumazenil. *1: $P < 0.05$ for placebo-placebo *vs* ofloxacin-placebo or *vs* ofloxacin-midazolam, ofloxacin-flumazenil, *2: $P < 0.1$ for placebo-placebo *vs* ofloxacin-placebo or *vs* ofloxacin-midazolam, ofloxacin-flumazenil, $^{+}1$: $P < 0.05$ for ofloxacin-placebo *vs* ofloxacin-midazolam or *vs* ofloxacin-flumazenil, $^{+}2$: $P < 0.1$ for ofloxacin-placebo *vs* ofloxacin-midazolam or *vs* ofloxacin-flumazenil.

Table 2 Frequency of reported adverse drug events

Symptoms	*Placebo + placebo*	*Ofloxacin + placebo*	*Ofloxacin + flumazenil*	*Ofloxacin + midazolam*
Dizziness	1	—	4	—
Vertigo	—	1	4	1
Fatigue	—	1	—	11
Impairment of vision	—	1	3	4
Amnesia	—	—	—	12
Heat sensation	—	—	5	—
Irritation of veins	—	2	2	2

was found in the sigma wave portion (not shown), which could neither be recorded after ofloxacin nor flumazenil administration.

Other tests No significant effects were seen in any of the psychometric self-rating scales except for a strong sedation (evaluated from the VAS) following coadministration of midazolam.

No clinically relevant changes could be observed in the blood pressure and heart rate measurements.

Side effects All side effects observed during the whole study are shown in Table 2. No severe complaints were reported by any of the subjects.

Fatigue and amnesia, typical adverse events experienced under treatment with BZD agonists, were most frequently reported after midazolam administration. Impairment of vision (mainly expressed as altered colour vision) was recorded after all forms of treatment compared with placebo.

Discussion

There is experimental evidence that CNS adverse effects of different quinolones could be related to an inhibitory effect on GABA-binding with a subsequent inhibition of GABA-mediated inhibitory transmission. All these experiments included binding of either [^{3}H]-GABA or [^{3}H]-muscimol to the $GABA_A$ binding sites of the receptor complex (Hori *et al.*, 1985, 1986; Tsuji *et al.*, 1988; Segev *et al.*, 1988).

As BZD exert a facilitatory effect on GABAergic transmission (Haefely *et al.*, 1975) and since their recognition sites are located on the BZD-$GABA_A$-receptor complex (Möhler & Richards, 1988), we extended the previous studies to examine the possible interaction between BZD and several fluoroquinolones. This was also prompted by an experimental observation showing that diazepam reduced in a dose-dependent manner the stimulant effect of oxolinic acid (a quinolone analogue), which produced insomnia in humans and a stereotyped behaviour with an increased locomotor activity in mice and rats. Conversely oxolinic acid blocked the anti-conflict activity of diazepam in rats (Thiébot *et al.*, 1980).

In a recent case report of ofloxacin-induced psychosis it was described that the observed panic attacks could be successfully treated with BZD (Zaudig *et al.*, 1989). On the other hand, in two cases of fluoroquinolone-provoked delirium (Altés *et al.*, 1989; Zaudig *et al.*, 1989), hallucinations and delusions could not be improved by treatment with clomethiazole, a potent CNS-depressant agent with GABA enhancing pharmacological properties, indicating a different mechanism of action of clomethiazole (Zaudig *et al.*, 1989).

Our *in vitro* results demonstrate an interaction between the BZD agonist [^{3}H]-flunitrazepam and the examined fluoroquinolones which was modulated by exogenous GABA. The specificity of fluoroquinolone inhibition of [^{3}H]-flunitrazepam binding was further characterised by using two other agents, namely trimethoprim and ivermectin (a GABA-mimetic agent, Sieghart *et al.*, 1985), which did not exert similar effects.

Although fluoroquinolones penetrate well into the CSF (for overview see Kitzes-Cohen, 1987) with a CSF/plasma ratio for ofloxacin between 47–87% (Stübner *et al.*, 1986), serum levels obtained after treatment with these compounds (400 mg ofloxacin infusion: 19.4–27.7 μM, own unpublished observation) may not be sufficient to yield inhibitory CSF levels in healthy volunteers. Nevertheless it should be noted that penetration of drugs into the CSF can be increased in an abnormal state of the blood-brain barrier (Gibaldi, 1985). It might also be assumed that an uneven distribution of the compounds could result in higher levels in distinct areas. Additionally, accumulation of fluoroquinolones may occur particularly in the elderly (Graber *et al.*, 1986), and higher steady state plasma levels were also reported for them due to decreased renal and non-renal clearances (Neumann, 1988).

The pharmaco-EEG pattern reflects a sensitive measurement of the vigilance and subvigilance as independent neurophysiological factors of biological CNS function (Ott *et al.*, 1982; Saletu & Grünberger, 1979). Our observed EEG-changes revealed a clear CNS stimulant effect of ofloxacin, identified mainly by a decrease in slow wave activity and a parallel increase of alpha power. This is an important index of increased vigilance under drug treatment and points to a partial inverse agonist profile of the compound. After administration of flumazenil following ofloxacin the effects were even more significant in some distinct wave band intervals (delta 2, theta, alpha 2 and ASI), indicating an increased CNS activity.

So far little is known about the EEG pattern following different doses of the BZD-antagonist dosing. Whereas in some studies (Doenicke *et al.*, 1984, Gath *et al.*, 1984; Noderer *et al.*, 1988) no significant changes compared with placebo were reported, Schöpf *et al.* (1984) found with 5 mg flumazenil a similar central stimulant action to that described above. Furthermore, Ziegler *et al.* (1985) could demonstrate that flumazenil (10 mg i.v.) had some CNS-activating (wakening) effects in sleep which is consistent with some experimental data showing that the compound can exert dose dependent inverse agonist properties (File & Pellow, 1986). To clarify whether the increased activity under flumazenil reflects an intrinsic action of the compound itself or is due to an enhancement of CNS-activating effect of the fluoroquinolone an additional double-blind, randomized placebo-controlled trial (Ziegler *et al.*, in preparation) was performed recently in 10 of the above 12 healthy individuals by administration of flumazenil (0.01 mg kg^{-1} i.v., 20 min

after the end of a 30 min placebo infusion). In this study it could be observed that a slight CNS-stimulant effect was provoked by flumazenil, expressed mainly as a minor increase of alpha 2 relative power and decrease of beta and sigma wave bands. As no other changes were registered this effect can only partially explain the additional augmentation in CNS-activity recorded under flumazenil following ofloxacin administration.

The BZD-agonist midazolam was able to reverse ofloxacin-induced effects, which could be mainly attributed to its opposite effects on the EEG. This pattern was known from different studies conducted with midazolam (Handel *et al.*, 1988; Klotz *et al.*, 1985), as well as other BZD (Sittig *et al.*, 1982). Additionally, the increased sedation index and reported side effects such as fatigue and amnesia, indicate the typical agonist BZD action.

It is concluded that CNS-adverse effects of fluoroquinolones might be due, at least in part, to an involvement of the BZD-$GABA_A$-receptor complex as demonstrated by *in vitro* and *in vivo* interactions between these compounds and BZD-receptor ligands. The ofloxacin-induced increased CNS activity, as measured by EEG monitoring, became more pronounced after subsequent administration of a specific BZD-antagonist and could be completely reversed after midazolam coadministration. It is suggested that administration of BZD-agonists might be useful in the treatment of fluoroquinolone-induced neurotoxic events.

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