Leading article

Antagonism of GABA<sub>A</sub> receptors by 4-quinolones

J Antimicrob Chemother 1993; 31: 457-462

Between 1 and 4% of patients administered 4-quinolone antibacterial agents may suffer an adverse drug reaction (ADR) relating to CNS function (Hooper & Wolfson, 1989; Christ, 1990; Stahlman, 1990). These ADRs, which include headaches, insomnia and, infrequently, convulsive seizures may increase in severity and/or frequency in patients concomitantly prescribed certain quinolones together with theophylline or certain non-steroidal anti-inflammatory drugs (NSAIDs) (Maesen et al., 1984; Ball, 1986; Arcieri et al., 1987; Yamamoto et al., 1988; Christ, 1990). There are several mechanisms by which quinolones may effect CNS function. These include pharmacokinetic interactions with other drugs which act on the CNS, a direct pharmacological action of the quinolone alone, and/or a pharmacodynamic interaction between quinolones and other drugs in the CNS. Inhibition of theophylline metabolism by quinolones leading to accumulation of theophylline and the resulting excitatory effects on the CNS is an example of a pharmacokinetic interaction between theophylline and quinolones (Davey, 1988). In this article we are mainly concerned with the evidence that quinolones exert a direct inhibitory effect on the GABA<sub>A</sub> receptor and that this effect is enhanced pharmacodynamically by co-administration with NSAIDs. These data do not however explain all CNS ADEs associated with quinolone treatment. Other possible mechanisms might include effects on other, as yet unidentified CNS receptors or pharmacokinetic interactions between quinolones and NSAIDs leading to a decreased metabolism or an increased CNS penetration of either drug.

In November 1986, the Japanese Ministry of Health and Welfare warned against the combined use of enoxacin and fenbufen when several patients, with no evidence of epilepsy or other underlying cerebrovascular dysfunction experienced clonic, or acute convulsive seizures after taking such a drug combination (Ministry of Health and Welfare, Japan (1986 and 1989) referenced in Hori et al., (1987) and Akaike, Shirasaki & Yakushiji. A growing body of experimental studies have indicated that some 4-quinolones alone, or in combination with certain NSAIDs, may reduce the efficacy of inhibitory (GABA<sub>A</sub>-mediated) synaptic transmission in the mammalian CNS, suggesting one potential pharmacological mechanism for the stimulant/excitotoxic effects of the 4-quinolones. This article considers these recent data in the light of what is presently known about GABA<sub>A</sub>-mediated inhibitory mechanisms. The actions of 4-quinolones on other neurotransmitter systems are also reviewed, together with the implications of these findings.

γ-Aminobutyric acid (GABA) is a major inhibitory neurotransmitter in the mammalian central nervous system. It has been estimated that about 30% of all central synapses use GABA as their neurotransmitter. There are two main types of GABA receptor, designated GABA<sub>A</sub> and GABA<sub>B</sub>. These receptors differ markedly in their structure, effector mechanisms and pharmacology (Bormann, 1988).

The GABA<sub>A</sub> receptor is a multisubunit membrane spanning protein (Olsen & Tobin, 1990). The protein complex contains both the GABA recognition site and the associated chloride conducting ion channel. Recent cloning studies have firmly established that the GABA<sub>A</sub> receptor is a member of the ligand-gated ion channel superfamily which includes glycine, 5-HT<sub>3</sub>, kainate, AMPA, NMDA and nicotinic receptors (Olsen & Tobin, 1990; Wisden & Seeburg, 1992). The binding of at least two molecules of GABA to the receptor induces a conformational change in the receptor-channel protein within microseconds such that the complex becomes selectively permeable to chloride ions (Bormann, 1988). The direction of the chloride movement through the channel is dependent upon both the cell resting membrane potential, and the chloride ion concentration gradient across the membrane. In the majority of neurons GABA<sub>A</sub> receptor activation results in an
inward flow of chloride ions, producing a small hyperpolarization of the cell membrane potential. The opening of GABA-activated ion channels, together with the concomitant drop of the neuronal cell input resistance, tends to stabilize the cell resting membrane potential and to greatly reduce the depolarizing action of excitatory transmitters such as glutamate. Hence, these effects usually result in neuronal inhibition.

In addition to the agonist recognition site, the receptor complex contains distinct binding sites for several groups of clinically important compounds including the benzodiazepines, the depressant barbiturates and certain anaesthetic pregnane steroids (Bormann, 1988). These compounds potenti ate the GABA_A-mediated response probably through different molecular mechanisms. For example, the benzodiazepine flunitrazepam, increases the probability of the GABA-gated chloride channel opening in response to GABA, whereas the barbiturate, pentobarbitone, acts to prolong the amount of time the channel remains open for, once activated by GABA (Bormann, 1988). In either case the actions of GABA at the GABA_A receptor would be potentiated.

Consistent with an important role for GABA in mediating neuronal inhibition in the central nervous system such positive allosteric modulators of the GABA_A receptor are anxiolytic, sedative and at higher doses may be hypnotic and anaesthetic (Simmonds & Turner, 1987). Furthermore, GABA_A receptor antagonists such as bicuculline (a competitive receptor antagonist) and picrotoxin (thought to bind near or close to the associated chloride channel) are proconvulsant. It is of note therefore that a number of radioligand binding experiments have indicated that certain 4-quinolones inhibit the binding of [3H]GABA or [3H]muscimol (a GABA_A receptor agonist) to a crude preparation of rat or mouse brain synaptic membranes. It should be noted that [3H]muscimol is a relatively specific ligand for the GABA_A receptor by comparison with [3H]GABA which may additionally bind to GABA_δ uptake sites and GABA_b receptors, and hence potentially complicate the interpretation of results obtained with this ligand. The IC_{50} values (i.e. the concentration of drug required to reduce the amount of bound radioligand to 50% of control) varies widely between the quinolones with all—except norfloxacin IC_{50} = 20 μM (Hori et al., 1987; Akahane et al., 1989)—being greater than 100 μM. This concentration is at least ten times higher than would be achieved in serum with therapeutic doses. However, biphenyl acetic acid (BPAA), the active metabolite of the NSAID fenbufen, dramatically potentiates the antagonist potency of certain 4-quinolones (in particular those with a piperazine or amino-pyrrolidine moiety at position 7 of the parent quinolone molecule) by several orders of magnitude. For example, the IC_{50} of enoxacin for inhibiting the binding of [3H]muscimol to rat brain synaptic membranes is 100 μM, but in the presence of BPAA (100 μM) it is 0.03 μM (Hori et al., 1987; Akahane et al., 1989).

However, such binding assays do not impart information on the drug-induced functional consequences (if any) for the neurone. For example, both GABA_A receptor agonists and antagonists would be expected to displace [3H]muscimol from the receptor and yet clearly would have opposite functional effects. In an attempt to address this issue we have utilized electrophysiological techniques to make whole cell voltage-clamp recordings of GABA-evoked responses from single neurones maintained in vitro. The main advantages of such patch clamp recordings are that the effects of drugs are determined against the primary functional response (the GABA-evoked chloride current).

In good agreement with the binding assays cited above, we have demonstrated that ciprofloxacin and ofloxacin are relatively weak inhibitors of GABA-evoked currents recorded from rat dorsal root ganglion neurones (Halliwell, Davey & Lambert, 1991). However BPAA, which alone has little effect on such responses, potentiates the inhibitory actions of these fluoroquinolones by ≥ 3000 times. Similar results were also recently found by Akaike et al. (1991) using patch clamp techniques to record from rat hippocampal neurones in vitro.

The results of both electrophysiological and radioligand binding experiments suggest that the quinolones either alone or in combination with BPAA compete with GABA for the agonist recognition site on the GABA_A receptor protein complex (Akahane et al., 1989; Akaike et al., 1991; Halliwell, 1992). In agreement with these studies, Akaike et al. (1991) have recently reported that the inhibitory actions of norfloxacin combined with BPAA on GABA-mediated responses are not influenced by the presence of a benzodiazepine antagonist RO-15–1788 (flumazenil), indicating that their actions are probably not directly mediated via the benzodiazepine receptor. Furthermore, several radioligand assays have also demonstrated that quinolones
alone or in combination with fenbufen have little or no inhibitory effects upon the binding of \[^3^H\]diazepam or \[^3^H\]flunitrazepam to rat brain synaptic membranes unless extremely high (> 500 µM) concentrations are applied (Yamamoto et al., 1988; Unseld et al., 1990), Indeed some quinolones may actually enhance GABA-stimulated binding of \[^3^H\]diazepam (see Dodd et al., 1989).

How selective for the GABA\_A receptor are the quinolones? Radioligand experiments have indicated that the quinolones do not influence the binding of ligands to excitatory (NMDA, AMPA, kainate), muscarinic, opioid \(\beta\)-adrenergic, or GABA\_B receptor sites (Segev, Rehavi & Rubenstein, 1988; Dodd et al., 1989). Using hippocampal neurones maintained in cell culture we have recently found that combinations of BPAA plus ciprofloxacin (both at 100 µM)—concentrations several thousand times greater than those required to inhibit GABA\_A-mediated responses—do not effect NMDA, AMPA or kainate-activated currents (Halliwell, 1992). In good agreement with these, Shirasaki et al. (1991a,b) have also reported that norfloxacin (10 µM) combined with BPAA (10 µM) did not antagonize NMDA-evoked whole cell currents recorded from dissociated hippocampal neurones.

Critically, the mechanism by which BPAA enhances the actions of 4-quinolones is presently unclear. Two possible mechanisms are molecular/chemical interaction between BPAA and quinolones and/or, a change in the lipid membrane permeability brought about by BPAA (Weissmann, 1991). Such an action could then result in an apparent increase in the affinity of quinolones for the GABA\_A receptor. Further experiments are clearly required to examine these possibilities.

Hence, collectively the results from a number of laboratories now reveal a consistent picture: quinolones alone specifically, and competitively, inhibit GABA\_A receptors, an action that is greatly potentiated by BPAA (and some other NSAIDs though less potently, e.g. see Shirasaki et al., (1991a,b)). Furthermore, there is a relationship between the inhibition of GABA\_A receptors by the 4-quinolones (alone or combined with BPAA) as assessed by radioligand binding and electrophysiological studies, and their potency to induce lethal neurotoxic effects in experimental animals (Table). In general, all of the studies outlined in the Table show that the fluoroquinolones ciprofloxacin, ofloxacin, enoxacin and norfloxacin antagonize the GABA\_A receptor and all are potentiated by BPAA, with ofloxacin being consistently less active than the others and also the only one which does not cause convulsions in whole animals in combination with fenbufen. The only paper which differs markedly from these results found that ofloxacin suppressed hippocampal activity in brain slices to a greater extent than ciprofloxacin (Dimpfel et al., 1991).

To what extent do the undoubtedly pharmacological effects of quinolones on the GABA\_A receptor explain the CNS ADEs associated with quinolone therapy? The effect of quinolones alone is weak, the \(IC\_50\) being 10–100 fold greater than achievable serum concentrations. Unfortunately, most data about quinolone penetration into the CNS report CSF concentrations (e.g. Gogos et al., 1991) rather than brain concentrations. Experience with \(\beta\) blockers has shown that there is a very poor correlation between CSF and brain concentrations. Concentrations of atenolol, metoprolol, oxprenolol and propranolol in CSF average 7% to 33% of plasma concentrations. Atenolol which is poorly lipid soluble, achieves brain concentrations which average 20% of plasma concentrations and are similar to CSF concentrations. In contrast, the more lipid soluble drugs metoprolol, oxprenolol and propranolol achieve brain concentrations which are 12–50-fold higher than plasma and 17–273-fold higher than CSF concentrations (McAinsh & Cruishank, 1990). In that case, lipid soluble quinolones may achieve concentrations in human brain which exceed serum concentrations, and the limited data on brain penetration support this hypothesis (Dalhoff, 1989; Branger et al., 1991).

None the less, when quinolones are administered alone there are as yet no data to show that quinolone concentrations in the human brain are anywhere near sufficient to exert clinically significant inhibitory effects on the GABA\_A receptor. Finally, although there is as yet no other pharmacological explanation of quinolone CNS ADEs we believe that inhibition of GABA\_A receptors does not explain well documented CNS ADEs associated with nalidixic acid. These differ from CNS ADEs associated with other quinolones in that visual disturbance is unusually common (Davey, McDonald & Lyndsay, 1991). None of the techniques reviewed has demonstrated any significant inhibition of the GABA\_A receptor by nalidixic acid, either alone or in combination with BPAA. It seems likely that there is another pharmacological explanation for CNS ADEs associated with nalidixic acid.

In contrast, we believe that the pharmacody-
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Table. Comparison of results of inhibition of GABA<sub>A</sub> receptors by quinolones for three in-vitro techniques with results of experiments demonstrating convulsant activity in whole animals. Allowing for grouping of data from different investigations, obtained under different experimental conditions, there is striking qualitative consistency between the results. For example, nalidixic acid is consistently inactive; ciprofloxacin, enoxacin and norfloxacin combined with BPAA are potent antagonists in vitro and cause convulsions in vivo; ofloxacin with BPAA is less potent in vitro and is not convulsant in vivo. BPAA = 100 µM unless otherwise stated. Data were obtained from: Hori et al., 1987; Segev et al., 1988; Yamamoto et al., 1988; Akahane et al., 1989; Akailte et al., 1991; Giardina, 1991; Halliwell et al., 1991; Percival, 1991; Halliwell, 1992.

<table>
<thead>
<tr>
<th>Drugs</th>
<th>Radioligand binding IC&lt;sub&gt;50&lt;/sub&gt; (µM)</th>
<th>Patch-clamp single cell electrophysiology IC&lt;sub&gt;50&lt;/sub&gt; (µM)</th>
<th>Rat vagus nerve electrophysiology IC&lt;sub&gt;50&lt;/sub&gt; (µM)</th>
<th>Convulsant activity in animals</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ciprofloxacin alone</td>
<td>76− &gt; 591:00</td>
<td>79−134:00</td>
<td>298:00</td>
<td>No</td>
</tr>
<tr>
<td>+ BPAA</td>
<td>0:03−2:10</td>
<td>0:06</td>
<td>0:07</td>
<td>Yes</td>
</tr>
<tr>
<td>Ofloxacin alone</td>
<td>&gt; 337:00− &gt; 1000:00</td>
<td>&gt; 1000:00</td>
<td>&gt; 1000:00</td>
<td>No</td>
</tr>
<tr>
<td>+ BPAA</td>
<td>0:80−7:00</td>
<td>0:50</td>
<td>172:00</td>
<td>No</td>
</tr>
<tr>
<td>(BPAA = 30/µM)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Enoxacin alone</td>
<td>&gt; 100:00− &gt; 149:00</td>
<td>297:00</td>
<td>670:00</td>
<td>No</td>
</tr>
<tr>
<td>+ BPAA</td>
<td>0:01−0:03</td>
<td>&lt; 0:30</td>
<td>0:17</td>
<td>Yes</td>
</tr>
<tr>
<td>(BPAA = 30/µM)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Norfloxacin alone</td>
<td>14:00−58:00</td>
<td>44:00</td>
<td>—</td>
<td>No</td>
</tr>
<tr>
<td>+ BPAA</td>
<td>&lt; 0:01− &lt; 0:03</td>
<td>&lt; 0:30</td>
<td></td>
<td>Yes</td>
</tr>
<tr>
<td>Nalidixic acid alone</td>
<td>&gt; 200:00</td>
<td>&gt; 100:00</td>
<td>&gt; 1000:00</td>
<td>No</td>
</tr>
<tr>
<td>+ BPAA</td>
<td>&gt; 100:0</td>
<td>&gt; 100:0</td>
<td>&gt; 1000:00</td>
<td>No</td>
</tr>
</tbody>
</table>

Dynamic interaction between quinolones and NSAIDs at the GABA<sub>A</sub> receptor is a plausible explanation for some clinical reports of CNS ADEs associated with combined therapy (Committee on Safety of Medicines, 1991). Serum concentrations of 10 µM BPAA have been measured after therapeutic dosing with fenbufen (Chiccarelli, Eisman & van Lear, 1980) and, although there are no data on BPAA concentrations in human brain, it seems likely that a pharmacodynamic interaction could occur with clinical dosing of quinolones plus NSAIDs and that this could explain some CNS excitatory ADEs, including convulsions. However, we emphasize again that nalidixic acid does not inhibit the GABA<sub>A</sub> receptor, and does not cause convulsions in mice when administered with fenbufen (Table).

In conclusion, quinolones have a weak, direct inhibiting effect on GABA induced currents at the GABA<sub>A</sub> receptor which is markedly enhanced by the presence of BPAA and, to a lesser extent other NSAIDs. Clinicians and Pharmacists should use combinations of quinolones plus NSAIDs with caution (Committee on Safety of Medicines, 1991). The search for other pharmacological effects of quinolones in the CNS should continue, in particular to explain the CNS ADEs associated with nalidixic acid. More data are required on penetration of quinolones into brain, as opposed to CSF. However, the capacity to cause ADEs must be a function of pharmacological activity as well as concentration at the target site. For example there is very little correlation between the nephrotoxic potential of aminoglycosides and their uptake by renal tissue (Davey & Harpur, 1987). Information will be required about pharmacological effect of quinolones on the GABA<sub>A</sub> receptor in order to interpret data about brain penetration.

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