Side Effects of Telithromycin and Voriconazole Are Attributable to Nicotinic Acetylcholine Receptor Interactions

D. Bertrand, S. Bertrand*, D. Pereira, P. Fernandes* 1 Faculty of Science, USCB, Box 305, Edisto Beach, SC 29438 2 Cempra Pharmaceuticals, Chapel Hill, NC, USA

Abstract

Backgroun: Some side effects caused by drug exposure can be understood by their molecular interactions with biochemical pathways while others remain obscure. An example of a now well understood side effect is the interaction of compounds with the cardiac potassium channel (hERG). Side effects caused by the ketolide, telithromycin, comprising reversible blunted vision, muscle weakness and brief failure were related to the hERG channel. In contrast, a ketolide metabolite, Telithromycin-N-oxide (RU76383) (Antimicrob. Agents Chemother. 44:5394-5402, 2010). The interaction of telithromycin metabolites with hERG is important. Telithromycin metabolites and the ketolide may inhibit hERG. Certain groups of the side effects reported for patients treated with the arylalkylacetone, but not fluorocarbons. The experiments probing the effects of these compounds at the neuronal hERG were conducted.

Methods: Human nACh receptors expressed in Xenopus oocytes were used for electrophysiological studies.

Results: The telithromycin metabolite (telithromycin-N-oxide), which includes the pyridine moiety, inhibits the ganglionic α3β3 hAChR and the post-synaptic neuromuscular junction receptor (NMJE) whereas another metabolite without the pyridine moiety did not show significant inhibition. Voriconazole inhibits the human α3β4 resulting in 63% reduction of the ACh-evoked current, while only 10% inhibition of the α1 receptor. Fluconazole caused no significant inhibition of the ACh-evoked currents.

Conclusions: Telithromycin-N-oxide contributes to the inhibition of the α3β4 and the post-synaptic NMJE hAChR by telithromycin. Voriconazole, which possesses a pyrimidine moiety, inhibits the ganglionic α3β3 hAChR, which could be the origin of the visual disturbances observed in voriconazole treated patients. The absence of fluconazole effects at nAChR correlates with the absence of reported side effects. These data suggest that compounds containing pyridine or pyrimidine residues could interact with the hAChR resulting in clinical side effects.

Introduction

Clinical observations that the antibiotic ketolide displayed unusual side effects with visual disturbances, loss of consciousness, severe aggravation of myasthenia gravis, and rare instances of hallucinations suggested that the metabolite or its metabolites perturbed physiological functions. A recent report demonstrated that some of these effects are related to the profound inhibition of nicotinic acetylcholine receptors (nAChR) by telithromycin (1). This same report showed that potentiating metabolites, including a pyridine-N-oxide imidazole, also inhibit nAChRs. Telithromycin inhibits nAChRs in the low micromolar range, whereas other macrolides such as azithromycin, clarithromycin, and solithromycin (CEM-101) do not. This suggests a correlation between the chemical structure of telithromycin and receptor inhibition (1). A closer analysis of the telithromycin structure compared to other macrolides indicates that the inhibition might correlate with the presence of the pyridine moiety (Fig. 1). Two metabolites of telithromycin, telithromycin-N-oxide (RU76504), which retains the pyridine moiety, and RU76383, which does not, permit a means to further probe this structure side effect hypothesis. Voriconazole and fluconazole are two molecules of the broader family of imidazoles known to be effective against human fungal pathogens and similar in structure to the notable exception of a pyridinium residue in voriconazole that is absent from fluconazole (Fig. 2). There have been reports of a strong association between blurred vision and altered color vision associated with the presence of the pyridine moiety. In view of the molecular structure of voriconazole and the presence of a receptor accessible pyridinium moiety, we hypothesized that the side effects associated with this compound might arise from an interaction with neuronal nAChRs.

Materials and Methods

Oocytes preparation and injection: Xenopus oocytes were prepared and injected with cDNAs encoding for the human α3β4, α7 and α11 using standard procedures (6). Injections of cRNAs were performed at least one hundred oocytes using a proprietary automated injection device and receptor expression was examined at least 2 days later.

Experimental protocol: Brief acetylcholine (Ach) test pulses were applied at regular intervals to assess the effect of the test compounds on the receptor activity. Cells were treated for 20 minutes (10 responses) in the presence of 2 µM telithromycin-N-oxide or RU76383, or 30 µM voriconazole or fluconazole. Following compound exposure, cells were washed during a 10 minute period and recovery of the ACh-evoked currents was monitored.

Electrophysiological recordings: Currents evoked by Ach in the presence and absence of the test compounds were recorded using an automated patch equipment equipped with standard high-precision current-clamp configuration. Unless indicated, cells were held at -70 mV. Data were captured and analyzed using a TIDScyon proprietary data acquisition and analysis software running under MetaCl (Meditek Inc.).

Results

Effects of telithromycin-N-oxide and RU76383 at the human α7, α3β4, and α11 receptors are shown in Figure 3.

• The telithromycin metabolite had no significant effect at the α7 receptor. These results are in contrast to those obtained with telithromycin itself, which profoundly inhibits of (1).

• Telithromycin-N-oxide, RU76504, strongly inhibited the ganglionic α3β4 receptor and no recovery was observed during the washout period.

• RU76383 caused less inhibition than telithromycin-N-oxide at the α3β4 receptor and, moreover, a significant recovery was observed with RU76383 indicating that this metabolite inhibits α3β4 is a lesser extent.

• Telithromycin-N-oxide caused a marked inhibition of the ACh-evoked current at the neuromuscular junction (α11) that was significantly larger than that caused by RU76383.

In contrast, voriconazole, but not fluconazole, experiments probing the effects of these compounds at the neuronal nAChR were conducted.

• Fluconazole, which lacks a pyrimidine, caused significantly less inhibition at the α11 receptor compared to voriconazole.

• Voriconazole caused a profound inhibition at the α3β4 receptor, with up to 63% reduction of the ACh-evoked current.

• Fluconazole, which lacks a pyridine, caused significantly less inhibition at the α3β4 receptor compared to voriconazole.

Conclusions

• The data presented here illustrate that molecules containing a pyridine or pyrimidine moiety are prone to interact with the neuronal nAChRs causing inhibition of the ACh-evoked currents.

• Such inhibition is probably the origin of the visual disturbances reported by patients treated with telithromycin and voriconazole that can be mediated by impairment of the cholinergic transmission at the ganglionic junction.

• The inhibition of α3β4 and α11 by telithromycin-N-oxide could augment the activities of the parent drug telithromycin at this receptor. These results, together with the antagonism of the α2β2 receptors by the positive telithromycin metabolite pyridine-N-oxide-imidazole could explain the “cece-like” effect experienced by myasthenia gravis patients who received telithromycin (see Fig. 5) (1, 9).

Visual effects are not easily discernible in animal models and therefore are often not identified until an investigational compound is in clinical trials. Checking for activity against nAChRs as a method for early detection of visual side effects, or other nAChR mediated side effects, could be a useful predictive tool. Drugs that contain heterocyclic nitrogen groups, especially those administered in large amounts like antibacterial and antifungal agents, should be evaluated for activity against nAChRs before entering the clinic.

References


