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

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Abstract

Background: 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, including reversible blurred vision, muscle weakness and liver failure were related to the profound inhibition of the α7 and the ganglionic α3β4 nicotinic acetylcholine receptor (nAChR) (Antimicrob. Agents Chemother. 54: 5399-5402, 2010). The interaction of telithromycin metabolites with nAChRs are now reported. In view of the similarity of some of the side effects reported for patients treated with the antifungal voriconazole, but not fluconazole, experiments probing the effects of these compounds at the neuronal nAChR were conducted.

Methods: Human nAChr receptors expressed in *Xenopus* oocytes were used for electrophysiolgical studies.

Results: The telithromycin metabolite (telithromycin-N-oxide), which includes the pyridine moiety, inhibits the ganglionic α3β4 nAChR 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 a7 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 nAChRs by telithromycin. Voriconazole, which possesses a pyrimidine moiety, inhibits the ganglionic α3β4 nAChR, 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 nAChR resulting in clinical side effects.

Introduction

Clinical observations that the antibiotic telithromycin displayed unusual side effects with visual disturbances, loss of consciousness, severe aggravation of myasthenia gravis, and rare instances of hepatotoxicity suggested that this macrolide or its metabolites perturb 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 putative telithromycin 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 (RU76584), which retains the pyridine moiety, and RU76363, 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 triazoles known to be effective against human fungal pathogens and similar in structure with the notable exception of a pyrimidine residue in voriconazole that is absent from fluconazole (Fig. 2). There have been reports of a strong association between blurred vision and altered color perception and usage of the voriconazole, but not fluconazole **(2, 3)**. In view of the molecular structure of voriconazole and the presence of a receptor accessible pyrimidine moiety, we hypothesized that the side effects associated with this compound might arise from an interaction with neuronal nAChRs.

Fig 1. Chemical structures of telithromycin, and its metabolites telithromycin-N-oxide and RU76363

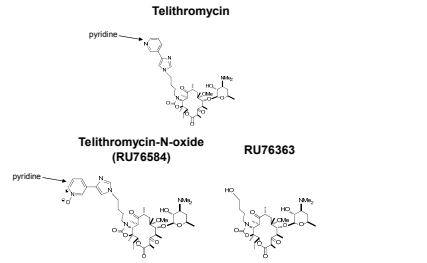
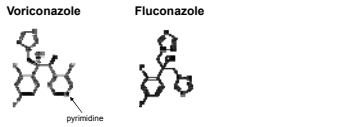


Fig 2. Chemical structures of the triazoles voriconazole and fluconazole



Materials and Methods

Oocytes preparation and injection

- *Xenopus* oocytes were prepared and injected with cDNAs encoding for the human α3β4, α7 and αβδε using standard procedures **(4)**. Injections of cDNAs were performed in 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 RU76363, 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

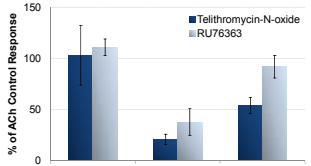
- Currents evoked by Ach in the presence and absence of the test compounds were recorded using an automated process equipped with standard two-electrode voltage-clamp configuration. Unless indicated, cells were held at –70 mV. Data were captured and analyzed using a HiQScreen proprietary data acquisition and analysis software running under Matlab (Mathworks Inc.).

Results

Effects of telithromycin-N-oxide and RU76363 at the human α7, α3β4, and αβδε receptors are shown in Figure 3.

- The telithromycin metabolites had no significant effect at the α7 receptor. This results are in contrast to those obtained with telithromycin itself, which profoundly inhibits α7 **(1)**.
- Similar to what was observed with the parent compound **(1)**, telithromycin-N-oxide strongly inhibited the ganglionic α3β4 receptor and no recovery was observed during the washout period.
- RU76363 caused less inhibition than telithromycin-N-oxide at the α3β4 receptor and, moreover, a significant recovery was observed with RU76363 indicating that this metabolite inhibits α3β4 to a lesser extent.
- Telithromycin-N-oxide caused a marked inhibition of the ACh-evoked current at the neuromuscular junction (αβδε) that was significantly larger than that caused by RU76363.

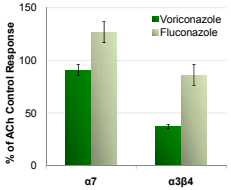
Fig 3. Differential repression of nAChRs by telithromycin metabolites. Telithromycin-N-oxide, which retains the pyridine ring, displays a more potent inhibition of α3β4 and αβδε than RU76363.



Effects of voriconazole and fluconazole at the ciliary ganglionic α7 and α3β4 receptors are shown in Figure 4.

- Exposure to voriconazole significantly inhibits the α7 receptor response to Ach, whereas exposure to fluconazole had no impact on α7 cholinergic transmission.
- Voriconazole caused a profound inhibition at the α3β4 receptor, with up to 63% reduction of the ACh-evoked current.
- Fluconazole, which lacks a pyrimidine, caused significantly less inhibition at the α3β4 receptor compared to voriconazole.

Fig 4. Differential effects at ciliary ganglionic nAChRs by the triazoles voriconazole and fluconazole. Voriconazole, which contains a pyrimidine moiety, is a strong inhibitor of α3β4.

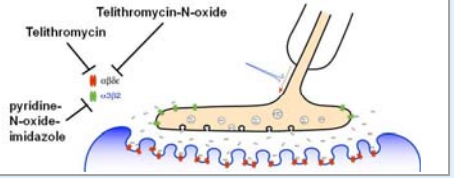


Conclusions

- 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 ciliary ganglion.
- The inhibition of α3β4 and αβδε by telithromycin-N-oxide could augment the activities of the parent drug telithromycin at this receptor. These results, together with the antagonism of the α3β2 receptors by the putative telithromycin metabolite pyridine-N-oxide-imidazole could explain the "curare-like" effect experienced by myasthenia gravis patients who received telithromycin (see Fig. 5) **(1, 5)**.

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 preclinical 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.

Fig 5. In individuals with myasthenia gravis, inhibition of both pre- and post-synaptic neuromuscular nAChRs by telithromycin and its metabolites may combine with the disruption of αβδε and blockage of ACh transmission due to myasthenia gravis to produce a "curare-like" effect.



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