Mechanistic Characterization of Adverse Events of Voriconazole and Telithromycin

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ABSTRACT

Background: Mechanistic characterization of the side effects of drugs helps to optimize the side-effect profiles of drugs in development. We have previously described the inhibition of neuronal nicotinic acetylcholine receptors (nAChR) by telithromycin, which contains a pyridine in its side chain. In this report we have characterized the reason for the "curare-like" effect at the neuromuscular junction seen in myasthenia gravis patients treated with telithromycin. Similarities in the visual effects of voriconazole to those observed with telithromycin and the presence of a heterocyclic N in the pyrimidine side chain of voriconazole led us to test the effects of voriconazole in nAChR assays.

Methods: Electrophysiological studies were conducted using expression of human nAChRs in *Xenopus* oocytes.

Results: Telithromycin inhibits the presynaptic α3β2 nAChR and its major metabolite, telithromycin-N-oxide, caused > 80% sustained inhibition of the ganglionic α3β2 nAChR. It also inhibited the post-synaptic neuromuscular junction receptors (NMJ) (50% inhibition). This structure-adverse event relationship correlated with the insignificant inhibition by another telithromycin metabolite lacking the pyridine moiety. The α3β4 receptor found in the ciliary ganglion of the eye was strongly inhibited by telithromycin-N-oxide (80% reduction) and also by voriconazole (63% reduction). Fluconazole, which has had no visual side effects, caused no significant inhibition of nAChRs.

Conclusion: The cumulative effects of telithromycin and telithromycin-N-oxide can explain the curare-like neuromuscular effects seen after telithromycin administration. The visual effects resulting from the inhibition α3β4 and α7 nAChRs by telithromycin is further enhanced by the inhibition of the α3β4 nAChR by the N-oxide metabolite of telithromycin. The heterocyclic N in the pyrimidine of the side chain of voriconazole possesses nAChR inhibitory activity, which could be the origin of the adverse events observed with voriconazole.

INTRODUCTION

The ketolide antibiotic telithromycin has been linked to many unusual side effects including visual disturbances, loss of consciousness, and severe aggravation of myasthenia gravis suggesting that this macrolide or its metabolites might perturb neurotransmission at ganglia and the neuromuscular junction. A recent report demonstrated that some of these side effects are related to the profound inhibition of nicotinic acetylcholine receptors (nAChR) by telithromycin **(1)**.

Telithromycin inhibits nAChRs in the low micromolar range, whereas the fluoroketolide solithromycin (CEM-101) does not **(Fig. 1) (1)**. This suggests a correlation between the chemical structure of telithromycin and receptor inhibition. A closer analysis of the telithromycin structure compared to other macrolides/ketolides indicates that the inhibition might correlate with the presence of the pyridine moiety **(Fig. 2)**. 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. 3)**. There have been reports of a strong association between blurred vision and altered color perception and usage of voriconazole, but not fluconazole **(2, 3)**. In view of the similarities in visual effects observed between telithromycin and 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. Inhibition of ganglionic and central nAChRs by telithromycin. Concentration-inhibition curves for α3β4 **(A)**, α7 **(B)**, and α4β2 **(C)** with ketolide telithromycin (closed circles) and novel fluoroketolide solithromycin (CEM-101; open circles). *Adapted from Bertrand et al. 2001. AAC 54:5399-402.*

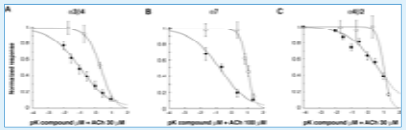


Fig 2. Chemical structures of the ketolide telithromycin, its metabolites telithromycin-N-oxide and RU76363, and the fluoroketolide solithromycin (CEM-101)

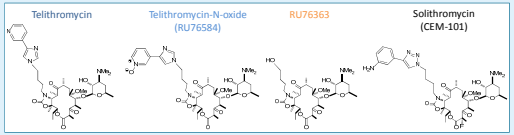
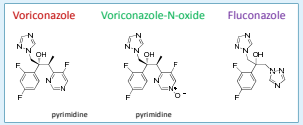


Fig 3. Chemical structures of voriconazole, its major metabolite voriconazole-N-oxide, and fluconazole



METHODS

Oocytes preparation and injection. *Xenopus* oocytes were prepared and injected with cDNAs encoding for the human αβδε, α3β4, α7 and α3β2 using standard procedures **(4)**.

Electrophysiological recordings. Brief acetylcholine (Ach) test pulses were applied at regular intervals to assess the effect of the test compounds on the receptor activity. Currents evoked by Ach in the presence and absence of the test compounds were recorded.

Experimental protocol. During an initial control period, the stability of the receptor response to Ach pulses was recorded. Next, cells were challenged for 20 minutes (10 responses) in the presence of 2 μM telithromycin, 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.

RESULTS

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

- Telithromycin-N-oxide **(B)** caused a marked inhibition of the ACh-evoked current at the neuromuscular junction (αβδε) that was significantly larger than that caused by RU76363 **(C)**.
- The inhibition of 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 pre-synaptic α3β2 receptors by telithromycin **(D)** could explain the "curare-like" effect experienced by myasthenia gravis patients who received telithromycin **(E) (1, 5)**.

Effects of telithromycin-N-oxide and RU76363 at the human α7 and α3β4 receptors are shown in **Figure 4**.

- The telithromycin metabolites had no significant effect at the α7 receptor **(A, B)**. These 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 **(B)**.
- RU76363, which lacks the pyridine moiety, caused less inhibition than telithromycin-N-oxide at the α3β4 receptor, and moreover, recovery was observed following removal of RU76363 indicating that this metabolite inhibits α3β4 to a lesser extent **(C)**.

RESULTS (CONT.)

Fig 3. Inhibition of both pre- and post-synaptic neuromuscular nAChRs by telithromycin and its N-oxide metabolite may combine with defects in ACh transmission due to myasthenia gravis to produce a "curare-like" effect.

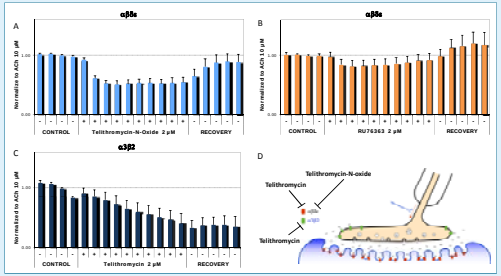
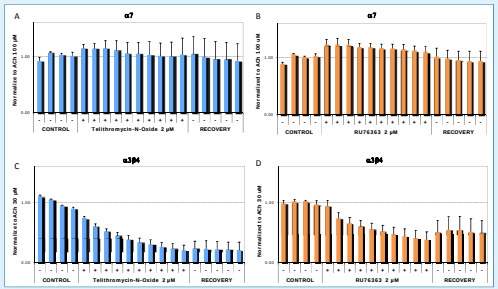


Fig 4. Repression of nAChR activity by telithromycin metabolites. Telithromycin-N-oxide, which retains the pyridine ring, displays a more potent inhibition of the α3β4 receptor than RU76363.

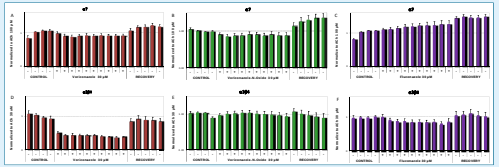


Effects of voriconazole, voriconazole-N-oxide and fluconazole at the ciliary ganglionic α7 and α3β4 receptors are shown in **Figure 5**.

- None of the triazoles tested produced substantial inhibition of α7 receptor activity **(A, B, C).**
- Voriconazole caused profound inhibition at the α3β4 receptor, with up to 63% reduction of the ACh-evoked current after 20 minutes of exposure **(D)**.
- Neither fluconazole or voriconazole-N-oxide led to substantial inhibition at the α3β4 receptor, illustrating the specificity of the molecular structure and supporting the hypothesis of a direct interaction of voriconazole with the receptors **(E, F)**.

RESULTS (CONT.)

Fig 5. Differential inhibition of ciliary ganglionic nAChRs by the triazoles voriconazole and fluconazole. Voriconazole, which contains a pyrimidine moiety, is a strong inhibitor of the α3β4 receptor.



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.

Some interactions are highly specific to the molecular structure, as indicated by the findings that while telithromycin is a potent inhibitor of α7, its metabolites telithromycin-N-oxide and RU76363 have no effect. Similarly, although voriconazole leads to profound repression of α3β4, its metabolite voriconazole-N-oxide does not.

The inhibition of αβδε by telithromycin-N-oxide may augment the activities of the parent drug telithromycin at this receptor. The concomitant effect of telithromycin and its metabolites at the neuromuscular junction pre-synaptic (α3β2) and post-synaptic (αβδε) receptors could exacerbate the inherent defect in acetylcholine transmission seen with myasthenia gravis and could explain the "curare-like" effect experienced by myasthenia gravis patients who received telithromycin **(see Fig. 3) (1, 5)**.

The inhibition of nAChRs by telithromycin and voriconazole may lead to impairment of the cholinergic transmission at the ciliary ganglion in patients receiving these drugs and is potentially the origin of the visual disturbances reported by patients.

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.

References

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