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Molecular Characterization of Off-Target Activities of Telithromycin: a Potential Role for Nicotinic Acetylcholine Receptors▽†

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Adverse effects have limited the clinical use of telithromycin. Preferential inhibition of the nicotinic acetylcholine receptors (nAChR) at the neuromuscular junction (α3β2 and NMJ), the ciliary ganglion of the eye (α3β4 and α7), and the vagus nerve innervating the liver (α7) could account for the exacerbation of myasthenia gravis, the visual disturbance, and the liver failure seen with telithromycin use. The studies presented here enable the prediction of expected side effects of macrolides in development, such as solithromycin (CEM-101).

Although macrolides have been known to adversely affect liver function, the greater frequency and severity of liver damage by telithromycin than by other macrolides resulted in the withdrawal of its approval for use in simple infections such as sinusitis and bronchitis (9), although it remains licensed for serious and otherwise intractable pneumococcal pneumonia. Exploration and future development of new macrolides and ketolides require addressing the mechanisms underlying the telithromycin effects (5). Side effects on muscle function and vision led us to explore the possible interaction of telithromycin at nicotinic acetylcholine receptors (nAChRs) and to compare the effects caused by older, safer macrolides such as azithromycin and clarithromycin. In addition, we analyzed the uniqueness of telithromycin effects by investigating a novel ketolide, solithromycin (CEM-101). Although both telithromycin and solithromycin are ketolides, solithromycin does not share the pyridine-imidazole group of the telithromycin side chain (Fig. 1).

Compounds containing pyridine moieties are known to interact with nAChRs, and therefore, we focused on this aspect of the molecule structure. At first, we examined the neuromuscular junction receptors (NMJ). Synaptic transmission at the neuromuscular junction is mediated by acetylcholine (ACh) released from the motoneuron, causing activation of nAChRs expressed on muscle cells (1). nAChRs belong to the superfamily of ligand-gated channels and result from the assembly of five subunits around an axis of pseudosymmetry. Seventeen genes encoding nAChR subunits in humans, allowing the formation of a vast repertoire of receptor subtypes displaying distinct physiological and pharmacological properties, have been identified (2).

All experiments were carried out with human nAChRs expressed in *Xenopus laevis* oocytes by cDNA. Oocytes were prepared, injected with cDNA encoding the nAChR subunits, and recorded using standard procedures (3). Currents evoked by ACh were recorded using a two-electrode voltage clamp, and cells were maintained at −80 mV with a GeneClamp axon instrument. Drugs were applied by immersion of the oocytes into the desired compound using an *x-y-z*-axis table controlled by Matlab software (MathWorks, Inc.).

Sustained exposure of nAChRs to a fixed concentration of telithromycin, such as that encountered under therapeutic conditions (4, 8), caused a progressive inhibition of the ACh-evoked currents. The degree of inhibition varied with the function of the receptor subtype. Typical results obtained with an oocyte expressing the adult form of the human NMJ composed of the α1, β1, δ, and ε subunits are shown in Fig. 2A. These data illustrate that exposure to 2 μM telithromycin caused a progressive decline of the amplitude of the ACh-evoked current and confirm previously published data (6). Partial recovery of the ACh response was observed upon removal of telithromycin. Such partial recovery is frequently observed with open channel blockers. Reduction of the current amplitude was accompanied by a significant modification of the response time course as illustrated in Fig. 2B, which is also indicative of an open channel blocker effect. Average levels of inhibition of the ACh-evoked currents measured in several cells are represented in the histogram in Fig. 2C. These data illustrate that exposure to 2 μM telithromycin caused a profound inhibition of the ACh-evoked current ($P < 0.005$).

When tested against the ganglionic (α3β4), the brain high-affinity (α4β2), and the homomeric α7 receptors (Fig. 3 and Table 1), exposure to 2 μM telithromycin inhibited up to 90% of the α3β4 and α7 ACh-evoked currents whereas lesser inhibition was observed with clarithromycin and azithromycin. However, the comparable degrees of inhibition caused by the three macrolides at the neuromuscular junction suggest that the inhibition at these receptors alone cannot explain the previously reported risk of muscle failure in myasthenia patients receiving telithromycin (7). We therefore turned our attention to the structural differences among macrolides. A particularity of telithromycin is its pyridine-imidazole-containing moiety. In addition, a review of telithromycin metabolism revealed pyridine-imidazole

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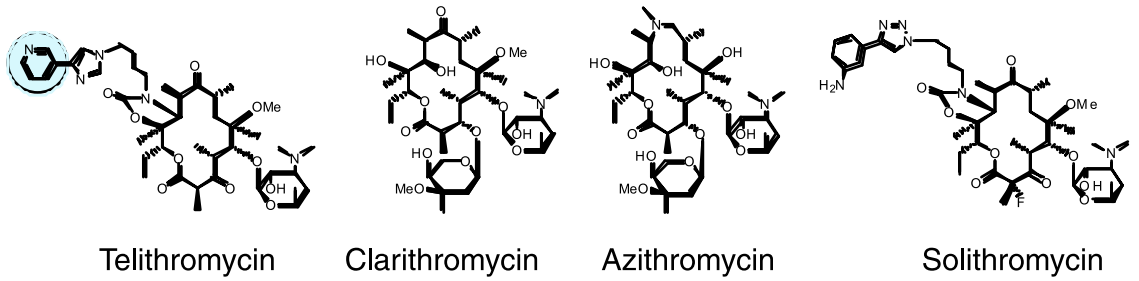

FIG. 1. Chemical structures of the macrolides tested at the human nAChRs. The pyridine moiety of telithromycin has been highlighted for clarity.

and pyridine-imidazole-*N*-oxide (10). Pyridine-*N*-oxide was tested as a control. Pyridine-imidazole and pyridine-*N*-oxide-imidazole markedly inhibited the neuromuscular junction receptors (Fig. 4 and Table 2). The greatest degree of inhibition was caused by pyridine-*N*-oxide-imidazole, which also inhibited the α7 nAChRs. Furthermore, this compound also inhibited α3β2 nAChRs, which are thought to be expressed on the presynaptic ending of the neuromuscular junction (10), by up to 25%. This finding suggests that telithromycin and its metabolites inhibit both the pre- and postsynaptic receptors and that the dual action of telithromycin and its metabolites may exacerbate myasthenia gravis symptoms. Concentration-inhibition curves for telithromycin, azithromycin, clarithromycin, and solithromycin were

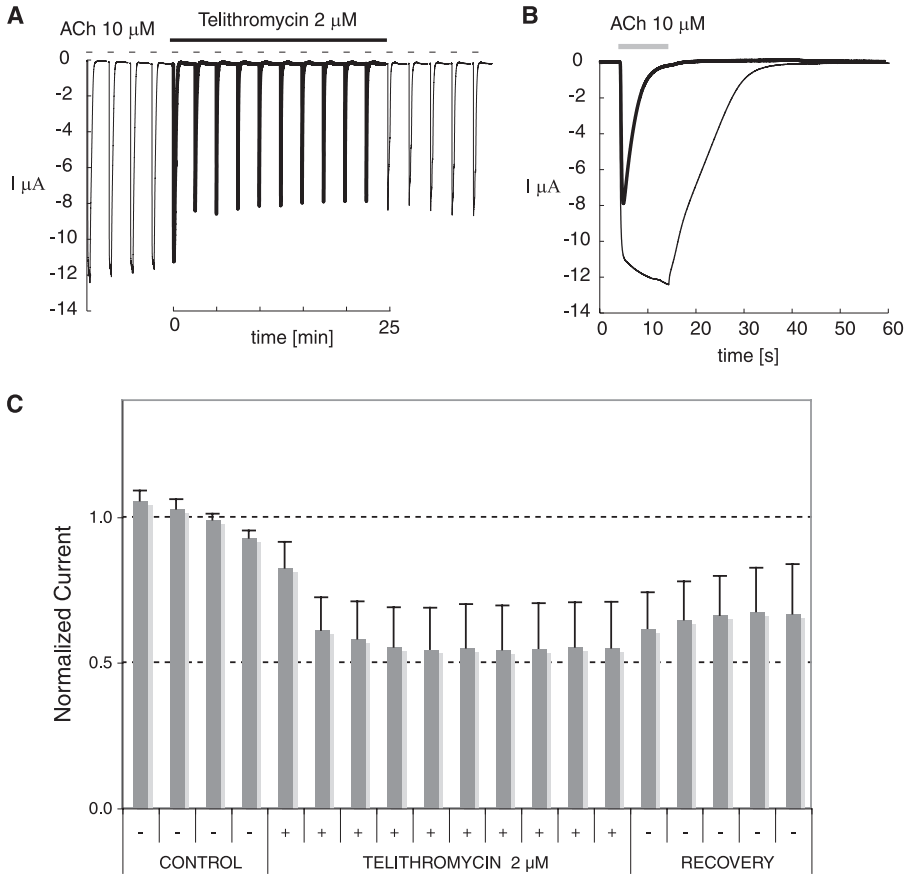

FIG. 2. Telithromycin inhibits the muscular nAChRs. (A) Experimental protocol used to assess effects of telithromycin. Currents evoked by ACh (at 10 μM for 10 s) were measured at regular intervals (2 min). Following a stabilization period, cells were exposed to a sustained concentration of telithromycin (2 μM) as indicated by the bar. The response of the cells to the same ACh test pulse was determined over 20 min. Recovery from telithromycin effects was then assessed over 10 min. (B) Effects of telithromycin on the time course of the ACh-evoked current. A typical response recorded as a control is shown in gray, and the response of the same cell after a 20-min exposure to 2 μM telithromycin is indicated in black. (C) Histogram of the peak currents measured in a series of cells (n = 3). Currents were normalized to unity versus the average responses obtained for controls using the experimental protocol depicted in panel A. Bars indicate the standard errors of means (SEM).

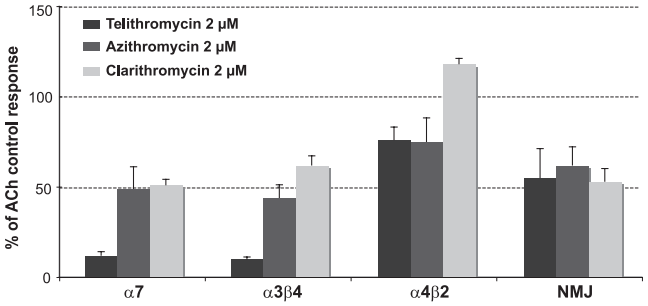

FIG. 3. Differential inhibition of nAChRs caused by three macrolides. Measurements were carried out using the protocol depicted in Fig. 2A. The bar graph represents a plot of the normalized ACh-evoked current measured after 20 min of incubation in the presence of the macrolide versus the control. Bars indicate the SEM obtained for at least 5 cells. These data illustrate the preferential inhibition caused by telithromycin at the α3β4 and α7 receptors.

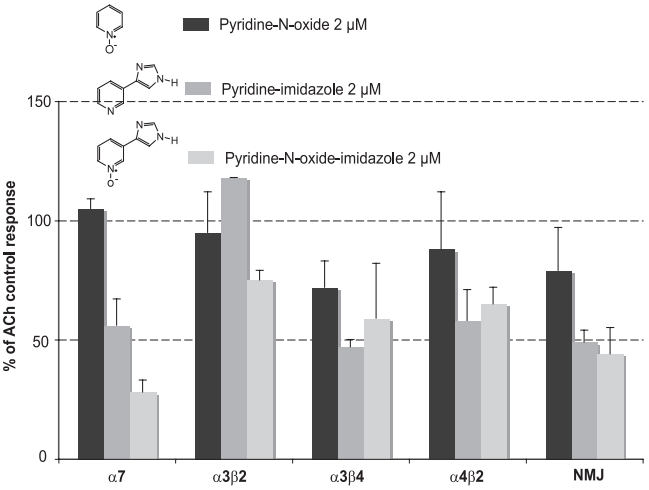

FIG. 4. Effects of three putative metabolites of telithromycin. The levels of inhibition caused by a 20-min exposure to the putative telithromycin metabolites at a 2 μM concentration were determined using the experimental protocol illustrated in Fig. 2A. The plot of the maximum inhibition observed for each of the metabolites for four nAChR subtypes clearly illustrates the differential effects observed at the receptors. Note that the greatest inhibition was observed at α7 with pyridine-*N*-oxide-imidazole.

determined to further delineate the spectrum of activity at the nAChRs. Figure 5A to D show that α3β4 receptors are preferentially inhibited by telithromycin, with a 50% inhibitory concentration (IC_{50}) of telithromycin of 0.1 μM. At the highest concentration (10 μM), telithromycin almost abolishes the ACh-evoked current at this receptor subtype. At least a 10-fold shift toward lower sensitivity was observed with azithromycin and clarithromycin. Solithromycin, which has a macrolide/ketolide core ring similar to that of telithromycin but not the pyridine moiety at its side chain, shows a clear difference in the inhibition profile. While marked inhibition was observed for solithromycin concentrations above 3 μM, the IC_{50} of solithromycin is more than 30-fold higher than that of telithromycin and at least 3-fold higher than that of azithromycin or clarithromycin. Results obtained for the α7 receptors (Fig. 5B and E) showed a similar trend, with telithromycin causing the greatest inhibition and having an IC_{50} of about 0.15 μM. This value is close to that observed for the α3β4 receptors and roughly 10-fold lower than that of azithromycin. Analysis of the IC_{50}s alone may give a false impression of the blockade caused by macrolide exposure. For example, while the α7 receptors display rather high-level sensitivity to clarithromycin, the inhibition reaches a plateau at only 30% inhibition. Thus, even at the highest concentration of clarithromycin, about 60% of the ACh response remains, whereas only 10% or less of the response was observed with telithromycin. Comparison of telithromycin and solithromycin inhibition profiles further underlines the critical role of the pyridine moiety, emphasized by the difference in IC_{50}s between these two ketolides. We also examined the concentration-inhibition curves for the four macrolides at the α4β2 nAChRs, which are the high-nicotine-affinity receptors in the central nervous system (CNS). Concentration-inhibition curves obtained for this receptor subtype are shown in Fig. 5C and F. Interestingly, while these receptors also displayed the greatest sensitivity to telithromycin, the IC_{50} was about 4 μM, which is significantly higher than that observed for α3β4 or α7 nAChRs. Moreover, azithromycin, clarithromycin, and solithromycin displayed even higher IC_{50}s for α3β4 and α7 nAChRs.

Altogether, these data reveal an otherwise unexpected action of telithromycin at the nAChRs with a blockade of the ACh-evoked currents at clinically relevant concentrations. Both the sensitivity to telithromycin and the degree of blockage indicate that these mechanisms can readily account for the observed side effects. Effects at the ganglionic receptors α3β4 and α7 explain the blockade of the ciliary ganglion neurotransmission and the resulting vision blur. These data provide for

TABLE 1. Macrolides inhibit nAChR responses

Receptor type	% Inhibition of ACh-evoked current by sustained (20-min) exposure[a] to:		
	Telithromycin	Azithromycin	Clarithromycin
αa7	88 ± 2	51 ± 12	49 ± 3
α3β4	90 ± 1	56 ± 7	38 ± 5
α4β2	24 ± 7	25 ± 13	0
NMJ	45 ± 16	38 ± 10	47 ± 7

[a] Compounds were tested at 2 μM concentrations. Results are means ± SEM (n = 3 to 5).

TABLE 2. Putative metabolites of telithromycin inhibit nAChR responses

Receptor type	% Inhibition of ACh-evoked current by sustained (20-min) exposure[a] to:		
	Pyridine-*N*-oxide	Pyridine-imidazole	Pyridine-*N*-oxide-imidazole
α7	0	44 ± 11	72 ± 5
α3β2	5 ± 17	0	25 ± 4
α3β4	28 ± 11	53 ± 3	41 ± 23
α4β2	12 ± 24	42 ± 13	35 ± 7
NMJ	21 ± 18	51 ± 5	56 ± 11

[a] Compounds were tested at 2 μM concentrations. Results are means ± SEM (n = 3 to 5).

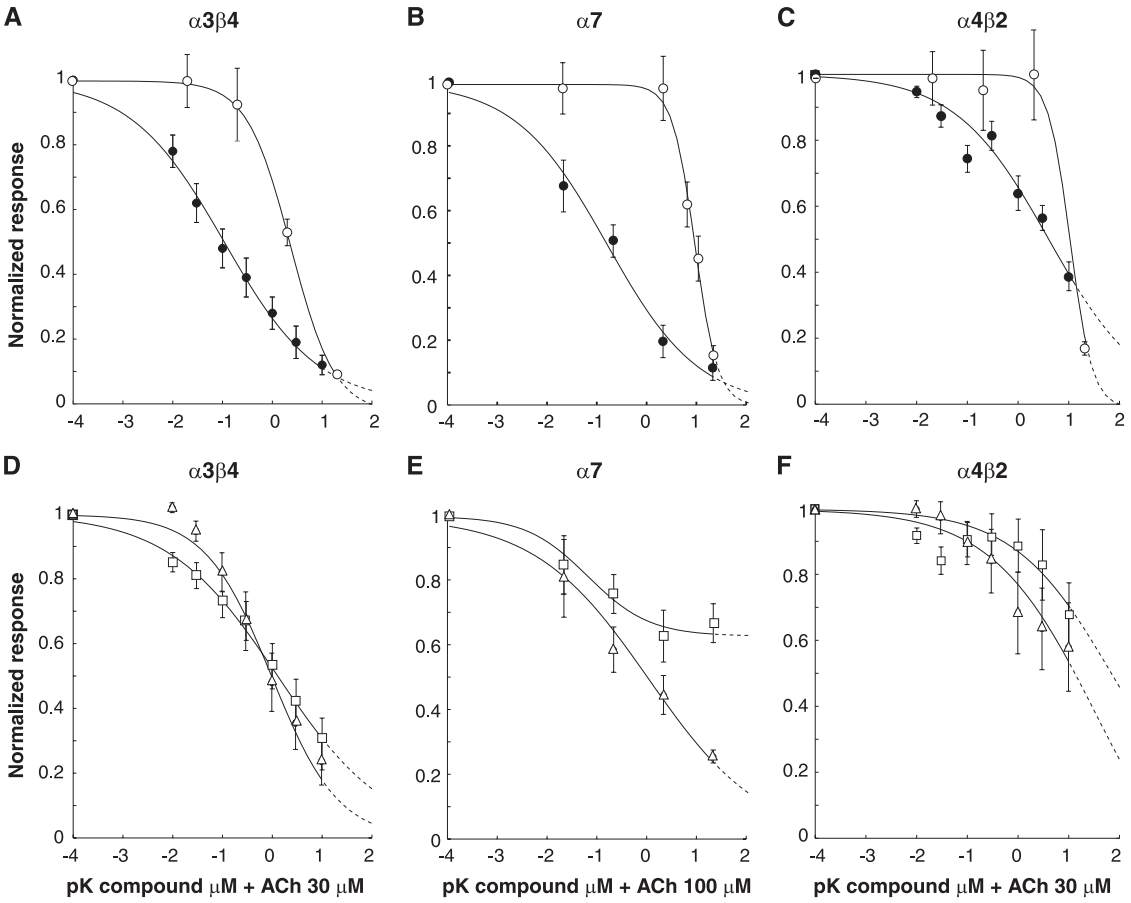

FIG. 5. Inhibition of ganglionic and central nAChRs by four macrolides. (A to C) Concentration-inhibition curves for α3β4, α7, and α4β2 with telithromycin (closed circles) and the novel ketolide CEM-101 (open circles). (D to F) Concentration-inhibition curves for α3β4, α7, and α4β2 with azithromycin (open triangles) and clarithromycin (open squares). Responses obtained from three to seven cells were normalized versus the ACh-evoked current measured as a control and plotted as a function of the logarithm of the macrolide concentration. Bars indicate the standard errors of means. Continuous curves through the data points are the best fits obtained with the empirical Hill equation.

the first time a predictor measure of the unwanted side effects of telithromycin.

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Daniel Bertrand conceived the experiments, analyzed the results, and wrote the manuscript in collaboration with Prabhavathi Fernandes. Sonia Bertrand and Estelle Neveu effectuated the experiments and analyzed the results, and Prabhavathi Fernandes brought an important contribution in the understanding of the macrolides and the relationship of their effects to their chemical structures.

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