Corallopyronin A Specifically Targets and Depletes Essential Obligate *Wolbachia* Endobacteria From Filarial Nematodes In Vivo

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Doxycycline and rifampicin deplete essential *Wolbachia* from filarial nematodes that cause lymphatic filariasis or onchocerciasis, resulting in blocked worm development and death. However, doxycycline is contraindicated for children and pregnant/breastfeeding women, as is rifampicin in the latter group with the additional specter of possible resistance development in *Mycobacterium spp*. Novel antibiotics with a narrower spectrum would aid in eliminating filarial diseases. *Corallococcus coralloides* synthesizes corallopyronin A, a noncompetitive inhibitor of RNA polymerase ineffective against *Mycobacterium spp*. Corallopyronin A depleted *Wolbachia* from infected insect cells (1.89 µM vs 7.8 µM of doxycycline). In vivo, corallopyronin A depleted *Wolbachia* by 4.7-logs, resulting in impeded worm development. Thus the antibiotic is effective against intracellular bacteria despite the many intervening surfaces (blood vessels, pleura, worm cuticle) and membranes (worm cell, vesicle, *Wolbachia* inner and outer membranes). Corallopyronin A is an antibiotic to develop further for filariasis elimination without concern for cross-resistance development in tuberculosis.

Wuchereria bancrofti, *Brugia malayi*, and *Onchocerca volvulus* parasitic filarial nematodes infect >150 million people in >80 developing countries of the tropics and subtropics, and 1.3 billion people are at risk of infection. All 3 species can cause severe pathologies resulting in high morbidity and increased mortality [1]. *Wuchereria bancrofti* and *B. malayi* infections can develop into lymphatic filariasis (LF): hydrocele in men and/or lymphedema that can develop into elephantiasis [1–3]. *Onchocerca volvulus* infections can cause severe dermatitis and/or visual impairment (onchocerciasis) [1, 3]. Combined, LF and onchocerciasis are responsible for the loss of 6.3 million disability-adjusted life years, making these diseases leading causes of morbidity in developing countries [4].

Diethylcarbamazine and ivermectin, both given in combination with albendazole, are used in the mass drug administration programs designed to control and eliminate these infections. The greatest effect of all 3 drugs is the killing of first-stage larvae found in the blood stream (LF) or in the dermis (onchocerciasis), with few adult effects [1]. Because adult worms are long lived and fecund for most of their lifespan, populations in endemic regions must be treated with high coverage (at least 65%) for many years to break transmission of the disease to uninfected persons [5, 6]. This puts financial and logistical stress on the developing countries. Additionally, the presence of larvae in the skin or blood of individuals, especially children, after many rounds of treatment indicates possible suboptimal responders to the current antifilarial drugs, leading some researchers to suspect possible resistance development in the worms may be occurring [7, 8]. Diethylcarbamazine treatment of *O. volvulus*–infected

Received 17 October 2011; accepted 6 January 2012; electronically published 14 May 2012.

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The Journal of Infectious Diseases 2012;206:249–57

DOI: 10.1093/infdis/jis341

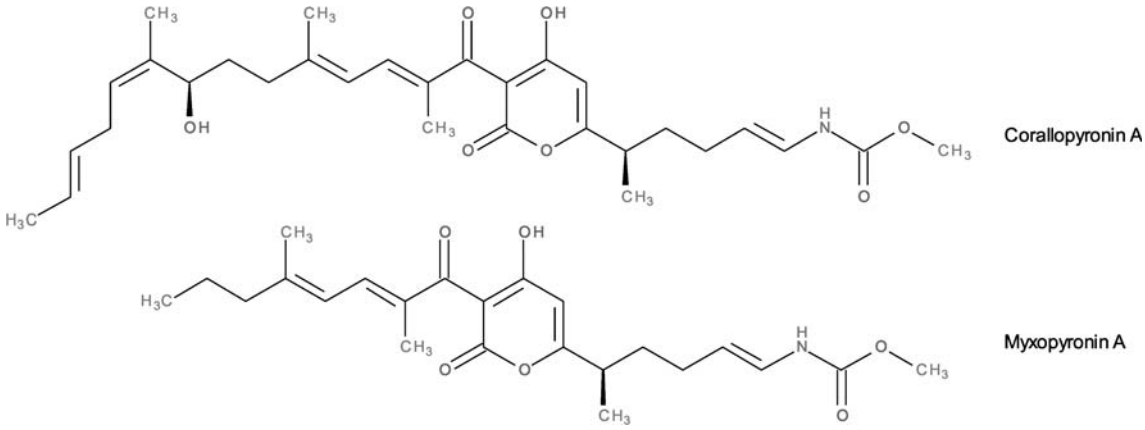

Figure 1. Structures of corallopyronin A and myxopyronin A. Structures were constructed using Symyx Draw, version 4.0, Symyx Solutions, Inc.

persons can lead to severe adverse reactions, making ivermectin the only drug available for controlling the disease. A drug that can be given to all members of an endemic population and that has macrofilaricidal activity is desired.

Wolbachia obligate endosymbionts, Gram-negative proteobacteria in the order Rickettsiales [9, 10], of filarial nematodes are a novel target for controlling filarial infections because they are susceptible to antiricketssial treatment [11–13]. Depletion of *Wolbachia* from filarial nematodes results in strong phenotypes in the filarial worms. The first phenotype seen was a block/delay in molting, resulting in shorter larvae [11, 14, 15]. Examination of treated adult worms showed that without the endobacteria, embryogenesis was blocked and resulted in sterility [14, 16]. Antiwolbachial pretreatment also reduced the number and severity of adverse reactions, thought to be due to the rapid release of *Wolbachia* by dying worms, to diethylcarbamazine or ivermectin treatment [17, 18]. *Wolbachia* depletion from filarial nematodes also resulted in a reduction in LF pathology [19, 20], offering the hope of recovery in persons with early stages of pathology. More importantly, antiwolbachial therapy has macrofilaricidal activity, resulting in the loss of 70% of *O. volvulus* and 90% of *W. bancrofti* adult worms [21–26]. The ability to reverse early LF pathology and to sterilize and kill adult worms could hasten the elimination of these diseases by increasing participation in elimination programs and shortening the time required to treat endemic populations. Recently, antiwolbachial therapy with doxycycline has been recommended for treating individuals under the supervision of their physician or local health official [1, 27]. However, despite these positive factors, the long treatment regimes of 3–4 weeks of daily administration [21, 24] and the contraindications for children aged ≤9 years and pregnant/breastfeeding women are an impediment to the use of doxycycline in the mass drug control programs aimed at eliminating lymphatic filariasis and onchocerciasis [1, 28].

Natural products have been among the best antibiotics for controlling infections [29, 30]. Gliding myxobacteria synthesize the structurally related antibiotics myxopyronin A (Myx) and corallopyronin A (Cor) (Molecular Weight = 527.65) (Figure 1) [31, 32]. Recently the putative biosynthesis of Cor, produced by *Corallococcus coralloides* B035, was elucidated [33]. Corallopyronin A is active against Gram-positive bacteria, including methicillin-resistant *Staphylococcus aureus* and shows no toxicity against eukaryotic cells [32]. Corallopyronin A is a noncompetitive inhibitor of bacterial DNA-dependent RNA polymerase (RNAP). Recent x-ray analysis of RNAP complexed with Myx or desmethyl-myxopyronin B (dMyx) and biochemical data showed that the molecule interacts in a unique way with the target molecule by binding inside the RNAP clamp head domain, preventing the correct interaction of RNAP with the DNA template [34, 35]. Rifampicin binds RNAP at a nonoverlapping region near the active center, preventing extension of the RNA molecule [35]. Point mutations resulting in an amino acid change lead to rifampicin resistance. Due to their different binding sites and modes of action, there is no cross-resistance between rifampicin and Cor [36], thus Cor is effective against rifampicin-resistant *S. aureus* [37].

The genome of *Wolbachia* of filarial nematodes encodes a complete RNAP and rifampicin depletes the endobacteria [38, 39]. Rifampicin, with physician monitoring, can be given to children even younger than the accepted 9 years of age. However, as it is 1 of the few low-cost antibiotics available for treating tuberculosis, its use in a mass drug administration program for other infections is problematic. By a disc diffusion experiment, Irschik et al showed that *Mycobacterium phlei* was resistant to Cor [32]. Minimum inhibitory concentration (MIC) tests required 64 μg/mL of Cor in Müller-

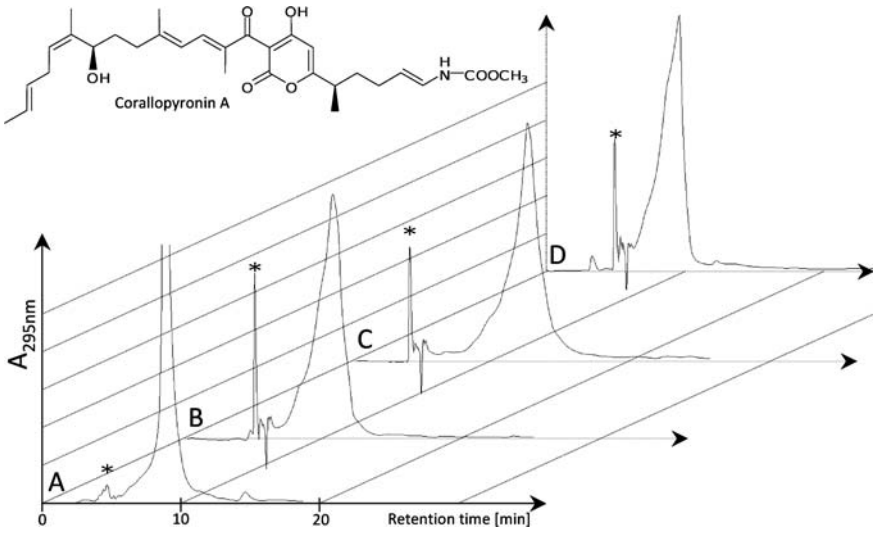

Figure 2. High Pressure Liquid Chromatography chromatograms of purified corallopyronin A (Cor) and of 3 samples used for the in vivo experiment. *A*, Purified Cor in methanol. *B–D*, Corallopyronin A samples diluted in phosphate-buffered saline, 10% dimethyl sulfoxide used for injections on days 1 (B), 14 (C), and 28 (D). Corallopyronin A had a peak elution at 9 minutes for all samples measured, indicating no degradation of Cor over the time of storage. * marks the peak of the solvent used.

Hinton medium and 128 µg/mL of Cor in Lysogeny Broth medium to inhibit *Mycobacterium smegmatis*. An MIC of 16 µg/mL of Cor was determined for *Mycobacterium bovis* bacille Calmette-Guérin (BCG) (G. Bierbaum, University Hospital Bonn, and T. Schneider, University of Bonn, personal communication). Thus, Cor has low efficacy against *Mycobacterium* spp. We present in silico, in vitro, and in vivo results that demonstrate Cor is effective against *Wolbachia* of arthropods and filarial nematodes and has the potential to be developed for antiwolbachial therapy.

MATERIALS AND METHODS

Cultivation and Purification of Cor

Cultivation and purification were performed as described with some modifications [33] (see Supplementary Material and Methods). Corallopyronin A was confirmed to be stable in phosphate-buffered saline (PBS) over the whole period of the in vivo experiment by High Pressure Liquid Chromatography analysis [33] (Figure 2*A–D*).

Antibiotic Susceptibility of *Wolbachia* In Vitro

The susceptibility of *Wolbachia* toward Cor was investigated as described with some modifications [40]. One $\times 10^4$ C6/36 cells (*Aedes albopictus* cell line infected with *Wolbachia* from *A. albopictus* B) were cultured for 9 days in 96-well plates at 26°C in L15 Leibovitz's medium (Invitrogen) supplemented with 5% Fetal Calf Serum, 1% nonessential amino acids (PAA Laboratories), 2% tryptose phosphate broth (Sigma), and 1% penicillin/streptomycin (PAA Laboratories) with and without the different antibiotics (4 µg/mL of doxycycline hyclate [Merck]; 1, 0.1 and 0.01 µg/mL of Cor or rifampicin [Sigma]) in duplicate. The medium was replaced every third day. The treated cells were harvested on day 9.

Extraction of genomic DNA was performed with the QIAamp Kit (Qiagen) according to the manufacturer's instructions. Depletion of *Wolbachia* was monitored by quantitative real-time polymerase chain reaction (qPCR) using primers targeting the *16S-rRNA* gene (GenBank Accession No.: X61767) of *Wolbachia* [41] and the *A. albopictus* B *actin* gene (GenBank Accession No.: DQ657949) as published [40] (see Supplementary Material and Methods).

Antibiotic Susceptibility of *Wolbachia* In Vivo

Protocol approval and ethical clearance for animal handling was obtained from the Landesamt für Natur Umwelt und Verbraucherschutz Nordrhein Westfalen in Recklinghausen, Germany (AZ 8.87-50.10.35.08.024). German and European Union guidelines to minimize animal suffering were followed.

The *Litomosoides sigmodontis* life cycle was maintained at the Institute for Medical Microbiology, Immunology and Parasitology. Female BALB/c mice aged 6–8 weeks were purchased from Charles River and infected with *L. sigmodontis* as described [42]. Beginning the day after the infection, the mice were untreated or given intraperitoneal injections of 10% dimethyl sulfoxide (DMSO) (vehicle control), 50 mg/kg/day of doxycycline (Merck), or 35 mg/kg/day of Cor. Doxycycline was given for 14 days, whereas Cor and vehicle control were

given for 28 days. Using a 14-day regimen of doxycycline at 50 mg/kg/day, we have standardized markers for comparing new antiwolbachial antibiotics. For substances that are limited in quantity or with little pharmacokinetic information available, a 28-day treatment is performed to first determine if there is antiwolbachial activity. As a follow-up experiment to a positive result with the 28-day regimen, a short course is used to assay potency compared with doxycycline (can include a higher dosage). Due to the limiting amounts of Cor, it was administered using the 28-day regimen. All substances were diluted in PBS, 10% DMSO. Five weeks postinfection, worms were recovered from the pleural cavity by PBS lavage. The worms were sorted by sex, their lengths were measured, and they were individually frozen for DNA extraction.

Genomic DNA was extracted from individual worms using the reagents from a QIAamp mini kit (Qiagen). The Qiagen protocol was used with the following changes: the worms were incubated with proteinase K overnight at 56°C; and Wizard SV96 DNA binding plates (Promega) and vacuum manifold instead of DNA columns were used to bind, wash, and elute the DNA in 50 µL of 10-mM Tris, 0.5-mM ethylenediaminetetraacetic acid, pH 9. Elution plates were sealed and stored at −20°C.

Depletion of *Wolbachia* was monitored by qPCR using primers for *Wolbachia ftsZ* (GenBank Accession No.: AJ010271), a single copy number gene, normalized to the *L. sigmodontis actin* gene (GenBank Accession No.: GU971367) as described [43, 44] (see Supplementary Material and Methods).

Sequence Alignment of the RNAP Subunits β and β′ Required for Cor Binding

The β and β′ subunits (RpoB and RpoC) shown to be required for Myx/Cor binding to RNAP were aligned using ClustalW2 [45]. Sequences and accession numbers are as follows: RpoBC *Wolbachia* endosymbionts of *Drosophila melanogaster* (*w*Mel) AE017196, locus tag WD0024; *Wolbachia* endosymbionts of *Brugia malayi* (*w*Bm) AE017321.1, locus tag Wbm0647; RpoB/C *Escherichia coli* U00096, locus tags b3987 and b3988; RpoB/C *Thermus thermophilus* NC_006461, locus tags TTHA1813 and TTH1812; RpoB/C *S. aureus* NC_009632, locus tags SaurJH1_0579 and SaurJH1_0580; RpoB/C *Mycobacterium tuberculosis* BX842574, locus tags RV0667 and RV0668; RpoB/C *M. bovis* AP010918, locus tags JTY_0686 and JTY_0687; and RpoB/C *M. smegmatis* CP000480, locus tags MSMEG_1367 and MSMEG_1368.

Modeling Cor Binding to the *Wolbachia* RNAP

The experimental structure of the *T. thermophilus* RNAP/dMyx complex was used as a reference for modeling of Cor bound to *Wolbachia* RNAP (wRNAP) [34]. Modeling was carried out in 2 steps using the O program [46]. First, the homology model of wRNAP was constructed in vicinity to the dMyx/Cor binding site in which several *T. thermophilus* RNAP residues were mutated to match the wRNAP sequence. Second, the experimental dMyx model was modified to generate the Cor structure, and the additional bulky Cor group was fit into the enzyme binding pocket using the allowed torsion rotations to avoid unfavorable close contacts. The resulting model possessed no van der Waals distances >3.5Å between the Cor atoms and RNAP residues. Notably, the model building required no structural alterations of the conserved RNAP residues or the portion of the Cor structure that is identical to that of dMyx.

Statistics

Normal distribution of the data was calculated using the D'Agostino and Pearson omnibus normality test. For comparing the level of *Wolbachia* depletion in worms, the Kruskal–Wallis test with Dunn's multiple comparison test was performed. For comparing *Wolbachia* depletion from the C6/36 cells and worm length between the treatment groups, 1-way analysis of variance with Bonferroni's multiple comparison test was performed. All statistics were calculated using GraphPad Prism version 5.02 for Windows.

RESULTS

Wolbachia Are Susceptible to Cor In Vitro

Corallopyronin A activity was tested using the *A. albopictus* C6/36 cell line infected with *A. albopictus Wolbachia*. Rifampicin also inhibits RNAP via a different mechanism than Cor [47]. Therefore, rifampicin was included in the assay. After 9 days of treatment, both Cor and rifampicin had depleted *Wolbachia* from the C6/36 cell line (Figure 3*A*). All concentrations of rifampicin were effective at depleting *Wolbachia* to levels equivalent to the gold standard of 4 µg/mL (7.8 µM) of doxycycline. Corallopyronin A depleted >50% of the *Wolbachia* at 0.1 µg/mL (189.5 nM), whereas 1 µg/mL (1.895 µM) of Cor had depleted *Wolbachia* to levels equivalent to doxycycline (Figure 3*A*). To control for possible toxicity of the antibiotics to the C6/36 cells, the *actin* copy numbers were compared. All treatments that depleted *Wolbachia* did not affect cell growth (Figure 3*B*).

Corallopyronin A Has Antifilarial Activity

Tetracycline therapy of rodents infected with *L. sigmodontis* results in depletion of *Wolbachia* from the filarial worm and, when the antibiotic is administered concomitant with the infection, in a blockage/delay of larval development seen as shorter worms [11, 15]. Using the endobacterial single copy *ftsZ* gene normalized to worm *actin*, we monitored the *Wolbachia* load per worm. *FtsZ* was detected in all untreated, DMSO, and doxycycline control samples, but *ftsZ* could only be detected in 19 of 31 worms from the Cor-treated mouse.

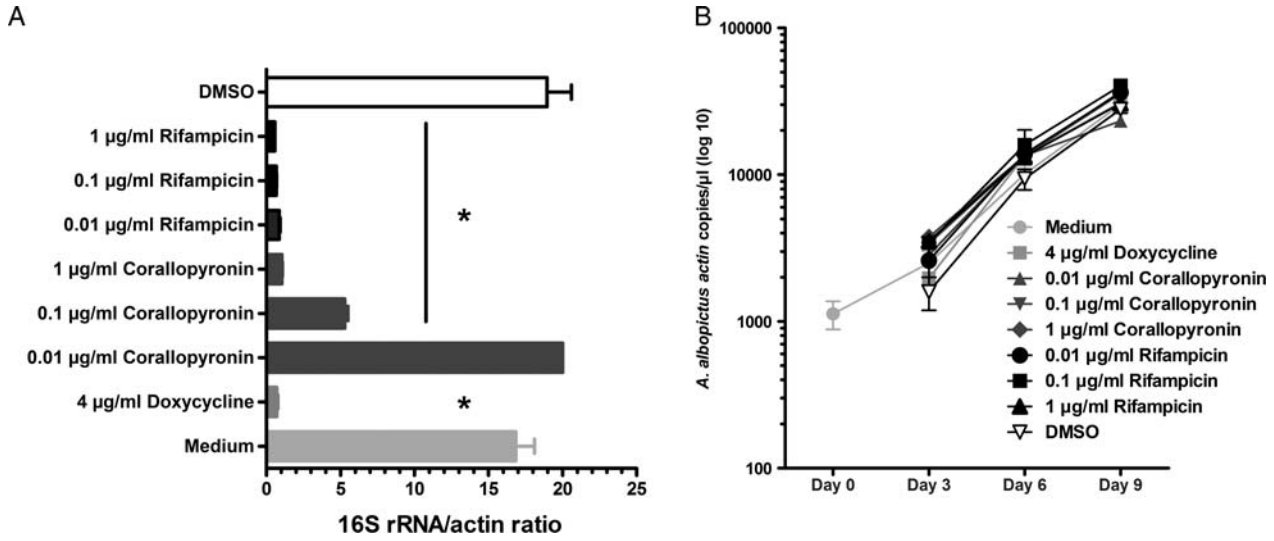

Figure 3. Corallopyronin A (Cor) depletes *Wolbachia* from C6/36 insect cells. *A*, Inclusion of Cor in the medium resulted in a loss of *Wolbachia* equivalent to doxycycline (the gold standard) and rifampicin, another antibiotic effective against the endobacteria that inhibits the same RNA polymerase. *B*, Analysis of the *actin* copies/µL of the samples demonstrated that Cor does not negatively affect the C6/36 cells over the range of concentrations tested. C6/36 cells were treated in duplicate with the antibiotics or medium at the concentrations shown for 9 days with medium changed every 3 days. Genomic DNA was extracted and real-time polymerase chain reaction (qPCR) performed on the DNA to measure *Wolbachia 16S-rRNA* copies/µL normalized to C6/36 *actin* copies/µL as determined by qPCR of genomic DNA. Graph is representative of 3 experiments. $^*P < .05$; analysis of variance with Bonferroni's multiple comparison test. Abbreviation: DMSO, dimethyl sulfoxide.

Treating infected BALB/c mice for 28 days with 35 mg/kg/day of Cor resulted in a 4.7-log reduction (>99.9%) in the *Wolbachia* load compared with the control (Figure 4*A*). The median level of endobacteria was also lower than that of the gold standard doxycycline given for 14 days at 50 mg/kg/day, which produced a 3.9-log drop. The vehicle control had no effect on the *Wolbachia* content of the worms. As a result of the *Wolbachia* depletion by doxycycline treatment for 14 days, the worms were significantly shorter (median, 8.3 mm) than the untreated worms (38 mm) or vehicle controls (34 mm) (Figure 4*B*). Corallopyronin A given for 28 days at 35 mg/kg/day also resulted in significantly shorter worms compared with the control worms (9.0 mm vs 38 mm, respectively).

Wolbachia RNAP Shares the Myx/Cor Binding Pocket

Recently, the mode of action of Myx and dMyx, both similar in structure to Cor, was investigated. X-ray analysis of bacterial (*T. thermophilus*) RNAP complexed with Myx or dMyx and biochemical data showed that these compounds interact with the target enzyme in a way that is different from that of known RNAP inhibitors like rifampicin [34, 35]. The binding of Myx to a pocket deep inside the RNAP clamp head domain, which interacts with the DNA template in the transcription bubble, hinders messenger RNA synthesis by preventing entrance of the DNA template [34]. Based on the structural similarities of Cor and Myx, it was concluded that Cor interacts with RNAP in the same pocket.

To see how transferable the results would be to other bacteria, an alignment of the RNAP amino acid sequences of *T. thermophilus*, 2 *Wolbachia* strains, *E. coli*, *M. tuberculosis, M. bovis, M. smegmatis*, and *S. aureus* was made. The sequences were highly conserved across the 8 species, especially the residues forming the binding pocket (Figure 5). Nevertheless there were 3 amino acid exchanges that could have an effect on Cor binding: (1) a valine residue is replaced by a cysteine in *Wolbachia* and *M. tuberculosis*, *M. bovis*, and *M. smegmatis* (C1365 *W.*, βC1067 *M.t./M.b.*, βC1064 *M.s.*, βV1037 *T.t.*); (2) a glutamic acid involved in hydrogen-bond formation with the antibiotic is replaced by glutamine in *Wolbachia, M. tuberculosis, M. bovis, and M. smegmatis* (Q1369 *W.*, βQ1071 *M.t./M.b*, βQ1068 *M.s.*, βE1041 *T.t.*); and (3) the *T. thermophilus* histidine at position 1103 (β′H1103) is replaced by glutamine in all other species.

To analyze if these changes might affect the binding of Cor to the wRNAP, Cor was modeled into the binding pocket based on the structure models of *T. thermophilus*, *E. coli*, and *M. tuberculosis* RNAP (Figure 6*A*) [34, 36]. Corallopyronin A has a longer hydrophobic tail than Myx/dMyx. This tail was tightly packed and filled the remaining space of the (adjacent) hydrophobic pocket (Figure 6*B*). The amino acid exchanges mentioned in the previous section would not affect the

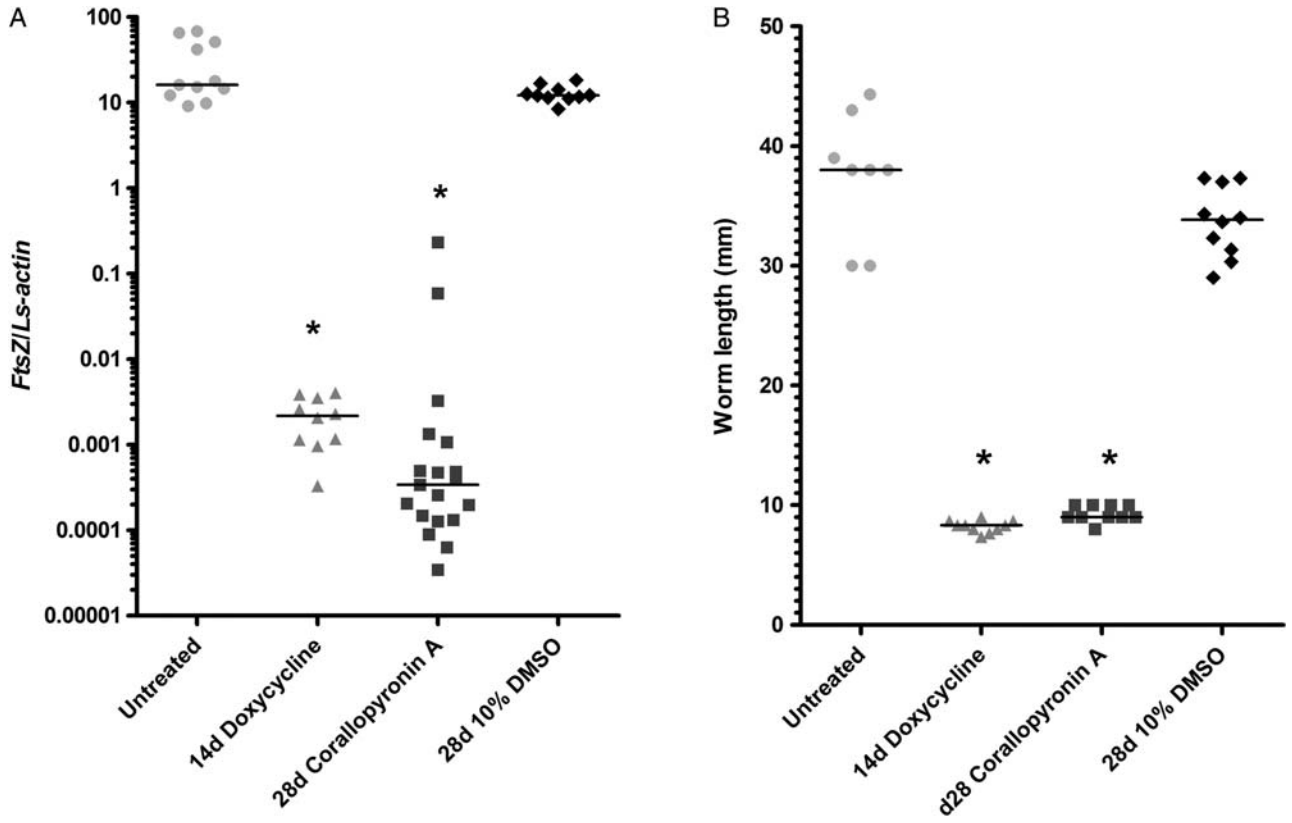

Figure 4. Corallopyronin A (Cor) depletes *Wolbachia* from the filarial worm *Litomosoides sigmodontis*, resulting in impeded worm development. Beginning on day 1 after the infection *L. sigmodontis*–infected mice were treated in vivo with doxycycline (50 mg/kg/day) for 14 days, Cor (35 mg/kg/day) for 28 days, or 10% dimethyl sulfoxide (DMSO) vehicle for 28 days, or left untreated. Worms were recovered 5 weeks postinfection, sorted by sex and length, measured, and processed for DNA extraction. *Wolbachia ftsZ*/worm *actin* was generated by real-time polymerase chain reaction. *A*, More than 99% (4.7 log drop) of *Wolbachia* were depleted from *L. sigmodontis* worms (n = 19 treated, n = 11 untreated, n = 10 doxycycline, n = 10 10% DMSO control) after Cor treatment. **P* < .0001 compared with untreated; Kruskal–Wallis with Dunn's multiple comparison test. *B*, Depletion of *Wolbachia* from *L. sigmodontis* by 28 days of Cor treatment during larval development resulted in shorter worms, equivalent to the gold standard of doxycycline for 14 days (n = 10 treated, n = 8 untreated, n = 10 doxycycline, n = 10 10% DMSO). Graphs are representative of 2 experiments. **P* < .0001 compared with control; analysis of variance with Bonferroni's multiple comparison test.

binding because van der Waals interactions and hydrogen-bond formation would not be diminished. Two further substitutions in the wRNAP compared with *T. thermophilus* RNAP that may have an effect on Cor binding were W1434F and L1435I (*T. thermophilus* numbering of β′). However, both substitutions opened additional space for the Cor atoms without interfering with binding.

DISCUSSION

Despite enormous efforts to identify new antifilarial drugs, few have made it to phase 2 testing [48]. Natural products are a source of new antibiotics against known and novel bacterial targets [29, 30]. The natural product Cor, synthesized by the gliding bacterium *C. coralloides* [33], and the structurally related Myx bind to the switch region of RNAP, interfering with the clamp region of the holoenzyme and preventing interaction of RNAP with the DNA template [34, 35]. Because the site and mode of action of Cor is different from that of rifampicin, Cor has activity against rifampicin-resistant *S. aureus* [37]. Eukaryotic RNAP is resistant to Cor, making this antibiotic attractive for further study [32].

We characterized Cor activity against the RNAP of the alpha-proteobacteria *Wolbachia* of filarial nematodes (*w*Bm) as an antifilarial chemotherapeutic. Alignment of the *w*Bm RNAP β and β′ subunit sequences with *T. thermophilus* and *S. aureus* predicted that *w*Bm RNAP would form the requisite binding pocket, and molecular modeling confirmed that Cor could bind the wRNAP and therefore inhibit the holoenzyme [35].

As a method to quickly screen for antibiotic activity, we used the C6/36 cell line infected with *Wolbachia pipientis*

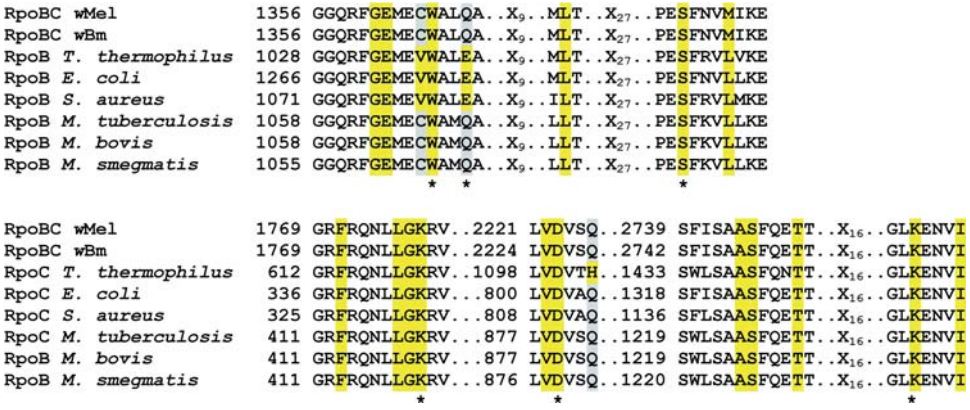

Figure 5. The *Wolbachia* DNA-dependent RNA polymerase forms a corallopyronin A (Cor) binding pocket. The *Wolbachia* RpoBC protein was aligned with the RpoB/C regions shown to be necessary for myxopyronin A (Myx)/Cor binding by crystallography studies in *Thermus thermophilus*. The amino acids forming the binding pocket for Cor are highlighted. Gray highlight indicates an amino acid change from *T. thermophilus*. GenBank accession numbers for the corresponding sequences were: RpoBC *Wolbachia* endosymbionts of *Drosophila melanogaster* (*w*Mel) AE017196, locus tags WD0024; RpoBC *Wolbachia* endosymbionts of *Brugia malayi* (*w*Bm) AE017321.1, locus tag Wbm0647; RpoB/C *Escherichia coli* U00096, locus tags b3987 and b3988; RpoB/C *T. thermophilus* NC_006461, locus tags TTHA1813 and TTH1812; RpoB/C *Staphylococcus aureus* NC_009632, locus tags SaurJH1_0579 and SaurJH1_0580; RpoB/C *Mycobacterium tuberculosis* BX842574, locus tags RV0667 and RV0668; RpoB/C *Mycobacterium bovis* AP010918, locus tags JTY_0686 and JTY_0687; and RpoB/C *Mycobacterium smegmatis* CP000480, locus tags MSMEG_1367 and MSMEG_1368. * indicates involved in hydrogen bond.

from *A. albopictus*. In the 9-day assay, Cor depleted *Wolbachia* in a dose-dependent manner, and 1 μg/mL (1.895 μM) of Cor depleted the endobacteria from the cells to levels equivalent to those of 4 μg/mL (7.8 μM) of doxycycline and 0.1 μg/mL (121.5 nM) of rifampicin.

Corallopyronin A activity was tested in vivo against filarial *Wolbachia* in the rodent filarial nematode *L. sigmodontis*, a well-established model for filarial worms that helped to establish doxycycline as a antifilarial chemotherapeutic [11, 15]. In this model, administration of antiwolbachial drugs concomitant with

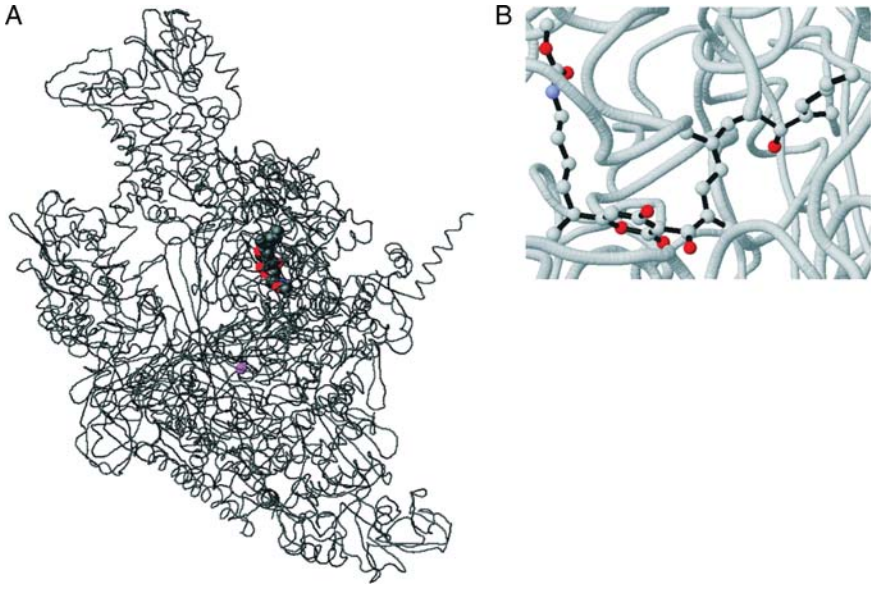

Figure 6. Structure of the *Wolbachia* RNA polymerase (wRNAP)–corallopyronin A (Cor) complex. *A*, Overall view of the complex. Corallopyronin A and the magnesium ion in the active site (violet) are shown as spheres. *B*, Ball and stick model focused on Cor bound to the wRNAP. Oxygen in red, nitrogen in blue.

L. sigmodontis infection allows for a rapid assessment of activity on the day the worms are retrieved from the infected animals. If the therapy is effective, larval development will be blocked, and the worms from treated animals will be significantly shorter in length, a phenotype visible to the naked eye [11, 15]. Corallopyronin A at 35 mg/kg/day for 28 days was well tolerated by the mice, with no visually apparent toxic effects. All worms were depleted of >98% of their *Wolbachia* (a 4.7-log drop). A larger reduction than with doxycycline (3.9-log drop) was seen, indicating that this dosage regimen was equivalent to treatment with a higher dose of doxycycline for a shorter time, and Cor is able to transit the many barriers (worm cuticle, host cell membrane, vesicle membranes, endobacterial membrane) that separate the *Wolbachia* from the pleural cavity where the larvae are located in the mice. As with doxycycline and rifampicin treatment, Cor treatment of infected mice concomitant with the infection resulted in significantly shorter worms. Once a larger amount is available, in vivo experiments with a higher dosage given for 14 days will be done.

Based on these results, we concluded that wRNAP is sensitive to Cor treatment, and in silico sequence alignment and molecular modeling predicted that the wRNAP contains the binding pocket formed by the holoenzyme, indicating that Cor probably inhibits via the same mechanism as has been described for Myx [34, 35]. The in vivo results confirmed that the bioavailability of Cor in the mouse is sufficient to reach the endobacterial target despite the many physical barriers between the fluid of the pleural cavity (the site of *L. sigmodontis* adult worms) and the *Wolbachia*, which are contained within intracellular vesicles [1]. The latter point is biologically important because the in vivo results have demonstrated antibacterial activity of Cor against an intracellular bacterium.

The sequence alignment of the RNAP β and β′ subunits of *Mycobacterium* spp. indicated that Cor might also bind to the mycobacterial RNAPs. However, *M. phlei* was resistant to Cor by disc diffusion assay [32], and the MIC against *M. smegmatis* was ≥64 μg/mL, depending on the medium used, and was 16 μg/mL of Cor for *M. bovis* BCG (G. Bierbaum, University Hospital Bonn, and T. Schneider, University of Bonn, personal communication). The exact reason(s) (ie, permeability issues, expression of a pump with affinity for Cor, etc.) for this resistance to Cor is not yet known.

Normally Gram-negative bacteria are resistant to Cor. Irschik et al demonstrated weak Cor activity against an *E. coli* strain that was mutated to have greater outer membrane permeability [32], suggesting that the outer membrane of some Gram-negative bacteria can be an effective barrier to Cor uptake. We hypothesize that Cor is effective against *Wolbachia* because they cannot synthesize lipopolysaccharide [49].

The Cor target is specific to prokaryotes, and the mode and site of action is different and specific from that of rifampicin and will therefore not lead to cross-resistance [32, 34, 35] (rifampicin being one of a few inexpensive antibiotics widely available in developing countries to combat tuberculosis). Therefore, with further development and study, Cor is an antibiotic in the developmental pipeline with the potential to be used in countries endemic for filarial infections without selecting for rifampicin-resistant *M. tuberculosis*. Because of its narrow spectrum of activity, Cor could provide a much-needed chemotherapeutic for onchocerciasis should there be an increase in the number of foci with ivermectin suboptimal responders. It could also provide an acceptable replacement for doxycycline to treat areas coendemic for *O. volvulus* and *Loa loa*.

Supplementary Data

Supplementary materials are available at *The Journal of Infectious Diseases* online (http://jid.oxfordjournals.org/). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

Notes

Acknowledgments. The technical assistance of Ms Helene Neufeld with maintenance of the C6/36 cells and DNA extraction is greatly appreciated. We would like to thank Dr Gabriele Bierbaum (University Hospital Bonn) and Dr Tanja Schneider (University of Bonn) for sharing their results for corallopyronin A activity against *Mycobacteria* spp.

Financial support. This work was directly supported by the German Research Foundation (PF 673/3-1 to K. P. and A. H.; KO 902/5-1 to G. M. K.); University Hospital Bonn intramural grant (BONFOR to K. P. and A. H.); and the National Institutes of Health (GM074840 to D. G. V.).

Potential conflicts of interest. A. S., A. S., T. F. S., S. S., G. M. K., A. H., and K. P. hold a patent for corallopyronin A (patent EP 11164963.8: Compounds for use in the treatment of filariasis). All other authors declare no potential conflicts.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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