Polymyxin/Non-Antibiotic Combinations

Subjects: Infectious Diseases

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The polymyxin/non-antibiotic combinations covered function synergistically by augmenting penetrative damage against the outer membrane causing bacterial lysis. Alternatively, the outer membrane may be permeabilized sufficiently (either by the polymyxin or the adjuvant) for the combination to access the inner membrane, leading to either perforation of the inner membrane (and lysis) or diffusion across the membrane, substantially disrupting vital metabolic pathways (i.e., respiration, DNA replication, cell envelope maintenance) and/or repressing plasmid-mediated polymyxin resistance.

antimicrobial resistance polymyxins drug repurposing non-antibiotic agents

1. Antineoplastic Drugs

The researcher's group firstly reported the antibacterial synergetic activity of three mainstream selective estrogen receptor modulators (SERMs), tamoxifen, raloxifene, and toremifene in combination with polymyxin B [1][2]. While conventionally used for breast cancer treatment, some SERMs were reported to exhibit direct antimicrobial properties via interference with cell wall synthesis (e.g., inhibition of wall teichoic acid synthesis in *E. faecium* and *S. aureus* by clomiphene) and membrane perforation (e.g., tamoxifen) [3][4]. Based on time-kill assays, polymyxin-resistant *P. aeruginosa*, *Klebsiella pneumoniae*, and *Acinetobacter baumannii* strains were considerably re-sensitized to polymyxin B, in the presence of each SERM (≥2.0-$\log_{10}$ decrease in bacterial viability relative to either monotherapy at 24 h) [2]. Metabolomics analysis of the tamoxifen-polymyxin B combination against an MDR polymyxin-resistant cystic fibrosis *P. aeruginosa* (FADDI-PA006) strain revealed extensive perturbations in the fatty acid and glycerophospholipid synthetic pathways involved in membrane biogenesis; thus implying the combination exerts synergy via concerted damaging effect against the outer membrane [1].

Mitotane, an antineoplastic drug used for the treatment of adrenal cancers [5], displayed synergy with polymyxin B against polymyxin-resistant MDR *A. baumannii*, *P. aeruginosa*, and *K. pneumoniae* in vitro. It also prevented the development of polymyxin-resistance (i.e., regrowth) in the polymyxin-susceptible isolates, compared to monotherapy from time-kill assays [6]. The in vitro synergy was further confirmed in a mouse burn wound model, in which the combination treatment decreased wound infection by a polymyxin-resistant *A. baumannii* isolate, compared to either polymyxin B or mitotane monotherapy. Scanning electron microscopy and transmission electron microscopy (SEM/TEM) imaging of *A. baumannii* isolates ATCC™ 17978 and FADDI-AB225 treated with the combination, revealed the formation of aberrant cell clusters suggesting the combination disrupts binary fission. While outer membrane damage was observed in the polymyxin-susceptible ATCC™ 17978 following polymyxin monotherapy, the polymyxin-resistant FADDI-AB225 strain displayed membrane blebbing; thus, it was inferred that

polymyxin B perforates the outer membrane sufficiently for the hydrophobic mitotane to diffuse across the outer membrane and exerts antibacterial activity against intracellular targets. Untargeted metabolomics analysis revealed significant disruptions in the TCA cycle and nucleotide metabolism in the metabolome of four *A. baumannii* isolates treated with the combination [7].

2. Antipsychotic and Antidepressant Agents

Phenothiazines are heterocyclic compounds clinically utilized as antipsychotics, that function as dopamine antagonists [8][9]. Over time, the antimicrobial characteristics of the phenothiazines were serendipitously discovered, such as chlorpromazine which was observed to exert anti-mycobacterial activity and thioridazine which potentiates first-line anti-tuberculosis antibiotics enabling rapid clearance of MDR/XDR (extensively drug-resistant) tuberculosis infections [8][10][11][12]. The proposed antibacterial mechanisms exerted by phenothiazines include augmenting complete phagocytosis of bacteria by macrophages, inducing abnormalities in binary fission, and inhibiting antibiotic efflux pumps [8][9][10].

Selective serotonin reuptake inhibitors (SSRI) function by blocking presynaptic axon terminal serotonin transporters, and are largely prescribed for major depressive and anxiety disorders [13]. Interestingly, various research groups have reported antimicrobial activity from SSRIs, such as femoxetine and paroxetine, that purportedly exert direct antibacterial activity via efflux pump inhibition [14]. One SSRI in particular, sertraline, was reported by Ayaz et. al., to exert concentration-dependent reversal of resistance towards ciprofloxacin, levofloxacin, norfloxacin, gentamicin, and moxifloxacin in the clinical isolates of *S. aureus*, *E. coli*, and *P. aeruginosa* [13]. Sertraline was observed to readily synergize with polymyxin B at lower concentrations in time-kill assays (>3.0-$\log_{10}$ CFU/mL decrease in bacterial viability) against polymyxin-resistant *P. aeruginosa*, *A. baumannii*, and *K. pneumoniae* strains [15]. Further SEM/TEM and metabolomics studies indicated the combination synergistic killing activity primarily involves the inhibition of amino-sugar, sugar-nucleotide metabolism, glycerophospholipid, and fatty acid metabolism causing disruption of the outer membrane integrity, cell wall synthesis, and respiration [15].

3. Antifungal Drugs

Caspofungin is an antifungal drug that operates by inhibiting the enzyme (1→3)-β-D-glucan synthase, destabilizing fungal cell wall integrity. Caspofungin serves as a therapeutic agent against *Aspergillus* and *Candida* fungal infections [16]. Intriguingly, caspofungin was observed to exert antibacterial activity by inhibiting biofilms [17]. Further untargeted metabolomics analysis by Hussein et. al. [18] revealed the synergistic combination of polymyxin B/caspofungin against *K. pneumoniae* acts by inhibiting multiple interconnected metabolic pathways, including bacterial envelope biosynthesis, the phosphotransferase system (involved in biofilm formation), ATP-binding cassette (ABC) transporter production. Another antifungal, miconazole, possesses broad spectrum fungicidal activity by penetrating the chitin fungal cell wall and permeabilizing the cell membrane to external noxious substances [19]. This membrane-disrupting ability of miconazole allows it to interfere with bacterial lipid membranes and accounts for its direct antibacterial activity [20]. The combination of polymyxin B/miconazole was reported to

exert synergistic bacterial killing, purportedly through permeabilization of the outer membrane by polymyxin B, which in turn allows miconazole to access the periplasmic space and disrupt the inner cytoplasmic membrane, causing bacterial lysis [21].

4. Antiparasitic Drugs

Closantel exerts an anti-parasitic mechanisms of action via the disruption of oxidative phosphorylation and the inhibition of chitinase activity [22]. As monotherapy, closantel had no antibacterial effect (minimal inhibitory concentration, MIC ≥ 16 mg/L); however, in combination with polymyxin B, synergy and inhibition of polymyxin resistance against *A. baumannii* were achieved at therapeutic concentrations [23]. In the presence of closantel, the polymyxin-resistant *A. baumannii* strains (polymyxin MIC ≥ 4 mg/L) were considerably re-sensitized to polymyxin B concentrations at 2 mg/L and below, which coincides with the polymyxin MIC susceptibility breakpoints (≤2 mg/L) [24]. In addition, Domalaon et. al., investigated the combination of colistin/closantel, and with other related anthelmintic salicylanilides oxyclozanide and rafoxanide. The combinations displayed synergy against MDR *P. aeruginosa*, *A. baumannii*, *K. pneumoniae*, *E. coli*, and *E. cloacae*, and reversed resistance to colistin apparently via potentiating damage to the outer membrane [25].

5. Natural Products

Cannabinoids are secondary metabolites isolated from the plant *Cannabis sativa*, the same plant from which the illicit drug marijuana is derived [26]. Several cannabinoids are behind the psychotropic effects of marijuana; these are synthesized via the alkylation of olivetolic acid, producing cannabigerolic acid, a precursor to most cannabinoids [27]. Of all the cannabinoids discovered, cannabidiol is the main non-psychoactive ingredient of *Cannabis sativa* [28]. Reports of antibacterial activity exerted by cannabidiol date back to the 1950s, with an investigation conducted by Van Klingeren and Ten Ham [29], noting MICs of cannabidiol within the ranges of 1–5 µg/mL for Gram-positive *Staphylococci* and *Streptococci*. Additional investigations carried out by Blaskovich et. al. [28] revealed consistent MICs (1–4 µg/mL) of cannabidiol against many Gram-positive MDR *Staphylococci* (methicillin-resistant *S. aureus*, vancomycin-intermediate *S. aureus*, vancomycin-resistant *S. aureus*), vancomycin-resistant *Enterococci* (*E. faecalis*, *E. faecium*), *Clostridioides* (*C. difficile*), and *Streptococci* (*S. pyogenes*, *S. pneumoniae*) strains. Radiolabeled macromolecular synthesis assays in *S. aureus* RN42200 and bacterial cytological profiling, indicated cannabidiol exerts antibacterial activity via membrane permeabilization, as evident from the marked perturbations in lipid synthesis at sub-MIC levels.

However, cannabidiol was inactive against Gram-negative species tested, with the exceptions of *Neisseria* and *Legionella* isolates. Further investigations involving exposure of efflux pump *E. coli* and *P. aeruginosa* mutants revealed the inactivity of cannabidiol nonetheless, ruling out efflux pumps as a possible explanation for the cannabinoid's general ineffectiveness against Gram-negative bacteria. This was also confirmed by Abichabki et. al., who noted the inactivity of cannabidiol against *K. pneumoniae*, *A. baumannii*, *P. aeruginosa*, and *E. cloacae* even, in the presence of efflux pump inhibitors such as curcumin [30]. However, cannabidiol was active against an

E. coli lpxC cell membrane mutant, indicating that outer membrane LPS deficiency enables penetration of cannabidiol. This conclusion was further supported by the increased susceptibility of lipid A-deficient *A. baumannii* (ATCC™ 19606R) to cannabidiol (MIC >128 to 0.25 µg/mL), which in turn has an polymyxin MIC (>128 µg/mL) relative to its parent strain (0.25 µg/mL) [31]. The researcher's group investigated polymyxin B and cannabidiol as another possible antibiotic/adjuvant combination for treating polymyxin-resistant Gram-negative infections [32]. Broth microdilution tests involving polymyxin B combined with cannabidiol (fixed at 256 µg/mL) against 13 different Gram-negative pathogens (52 strains in total), yielded observable antibacterial activity of the combination against 47 of the 52 strains. Subsequent checkerboard assays combining polymyxin B/cannabidiol verified the synergistic activity, as evident from the low polymyxin B concentrations (≤2 µg/mL, within the EUCAST “susceptible” breakpoint) enabling cannabidiol to exert antibacterial activity at a minimal effective antibiotic concentration (MEAC) ≤4 µg/mL. Moreover, the polymyxin B-cannabidiol combination substantially decreased the viability of four *K. pneumoniae* strains (>2.0-$\log_{10}$ CFU/mL decline) relative to polymyxin B monotherapy at several time points, verifying the combination’s synergistic bactericidal activity. Similarly, checkerboard and time-kill assays confirmed synergy against *A. baumannii*, *P. aeruginosa*, and *K. pneumoniae*, both polymyxin-resistant and polymyxin-susceptible isolates alike. Moreover, it was inferred from metabolomics analysis of *A. baumannii* ATCC™ 19606 that the polymyxin B/cannabidiol combination substantially disrupts cell envelope biogenesis through a time-dependent perturbation of amino–sugar and nucleotide–sugar metabolism, and the pentose phosphate pathway. Consequently, peptidoglycan and LPS synthesis are disrupted owing to decreased levels of core membrane lipid components, phosphatidylethanolamine, phosphatidic acid, *sn*-glycero-3-phosphoethanolanime, and *sn*-glycerol 3-phosphate.

Curcumin, a phenolic compound isolated from the plant *Curcuma longa*, has been traditionally used for gastrointestinal treatments owing to its antioxidant and anti-inflammatory activities [33]. Intriguingly, this natural product was also reported to exhibit antimicrobial activity; various reported antibacterial mechanisms exerted by curcumin include membrane damage, efflux inhibition, biofilm disruption, and growth inhibition [34][35]. When combined with polymyxin B, considerable synergy was observed against polymyxin-resistant strains (as well as expanding the polymyxin activity spectrum coverage to Gram-positive bacteria) at reduced polymyxin MICs; the mechanisms purported to account for synergy involve outer membrane permeabilization by curcumin, allowing access to the inner cytoplasmic membrane by polymyxin B [36]. Furthermore, colistin in combination with curcumin was observed to reverse polymyxin-resistance in *A. baumannii* through outer membrane permeabilization by colistin, facilitating the entry of curcumin which triggers increased ROS production, severely impacting bacterial viability [37].

Tetrandrine is a bis-benzylisoquinoline alkaloid isolated from the herb *Stephania tetrandra* [38]. Although tetrandrine functions pharmacologically as a calcium channel blocker, it was also found to possess anti-inflammatory and antineoplastic properties through the inhibition of pro-inflammatory cytokine production and the induction of apoptosis in tumor cells, respectively [39]. Interestingly, tetrandrine exhibits antibacterial properties as observed from its ability to inhibit antibiotic efflux from MRSA and *Mycobacterium tuberculosis* [40][41]. Hence, when tested in combination with colistin against *mcr-1*-positive colistin-resistant *Salmonella*, tetrandrine was noted to enhance colistin activity by enhancing the permeability of the outer membrane through efflux inhibition sufficiently for colistin

to act, as well as by disrupting oxidative phosphorylation (ATP production) and down-regulating *mcr-1* expression [42].

6. Other Non-Antibiotic Drugs

The cystic fibrosis transmembrane conductance regulator (CFTR) potentiator ivacaftor, commonly used to treat cystic fibrosis patients, exerts apparent antimicrobial activity via weak inhibition of DNA gyrase and topoisomerase IV enzymes, owing to certain structural similarities with quinolones. When combined with polymyxin B against *P. aeruginosa*, augmented damage against the outer membrane was observed via nitrocefin assay, SEM and TEM [43]. This was further substantiated by metabolomics analysis which revealed extensive phospholipid and LPS biosynthesis perturbations, following exposure to the polymyxin/ivacaftor combination [44]. Auranofin, an anti-rheumatic drug for treating arthritis, displays negligible anti-Gram-negative activity [45]; however, in combining with colistin, it results in extensive cell shrinkage and lysis as observed from SEM/TEM imaging, validating auranofin as a prospective adjuvant. In addition, the colistin/auranofin combination extensively counters colistin resistance in Gram-negative pathogens and substantially improves the survival rate in mouse peritoneal infection models [46].

Zidovudine is a nucleoside/nucleotide reverse transcriptase inhibitor that acts by incorporating into the elongating viral DNA strand, and slowing down the progression of HIV infection [47]. Intriguingly, antibacterial activity was also observed from zidovudine, likewise by acting as a DNA-chain terminator and arresting bacterial DNA replication [48]. When combined with polymyxin B, zidovudine extensively potentiated polymyxin activity against *K. pneumoniae*, ≥4.0-$\log_{10}$ CFU/mL in time-kill assay; ≥3.0-$\log_{10}$ CFU/mL in murine thigh infection model [49]. Similarly, the colistin/zidovudine combination was observed to reverse colistin resistance in time-kill assay (>3.0-$\log_{10}$ CFU/mL) with the absence of regrowth in the combination 24 h post-exposure, and displayed in vivo synergy against NDM-1 *K. pneumoniae* and *mcr-1*-positive *E. coli* in murine peritoneal infection models [50][51].

Melatonin, a hormone secreted by the pineal gland in the brain, functions to regulate circadian rhythms and blood pressure, thus is used medically to treat sleep disorders. Astonishingly, melatonin was reported to exert bacteriostatic (growth-inhibiting) activity, via the perturbation of key metabolic pathways and the expression of genes involved in cell division. Furthermore, the combination of colistin with melatonin substantially permeabilizes the outer membrane and inhibits efflux pump activity, hence re-sensitizing polymyxin-resistant Gram-negative pathogens to polymyxins [52]. Additional transcriptomics analysis involving *mcr-1*-positive *E. coli* reveals significant disruptions in the expression of genes involved in LPS modifications and efflux pump production, in the presence of the colistin–melatonin combination [53].

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