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Current techniques and future directions in antibiotic resistance breakers

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Abstract

A phenolic molecule found in the curry plant, curcumin, has numerous pharmacological actions, including an antibacterial impact. An environmental, nontuberculous, rapidly growing mycobacterium known as Mycobacterium abscessus is emerging as a serious human pathogen, especially in lung infections, as it possesses broad-spectrum resistance to antibiotics, possesses a high level of biofilm capability, and has a low prevalence of disease. To identify antimicrobial and antivirulence activity, the researchers wished to examine the antimicrobial and antivirulence activity of curcumin, as well as the ability to work together with medications against a clinical specimen from a lady admitted to hospital with suspected tuberculosis. While strain B developed resistance to amikacin, clarithromycin, ciprofloxacin, and linezolid, strain B exhibited synergism (fractional inhibitory concentration index <0.5) with curcumin, indicating resistance to amikacin, clarithromycin, ciprofloxacin, and linezolid. Inhibiting 4- and 8-day mature biofilms completely with 1/8 MIC curcumin was enough to greatly limit motility. When used together, curcumin and amikacin reduced microbial aggregation while also causing considerable cell death. The main effect found when curcumin was the dominating compound was the disruption of 4- and 8-day biofilms. The current findings confirm prior research that suggests that curcumin may be a resistance buster against antibiotics.

Keywords*— *antibiotic resistance breakers; efflux pump inhibitors; membrane permeabilisers; beta-lactamase inhibitors.

I. INTRODUCTION

Antibacterial medications, which have been in use for more than a decade, have become a crucial part of modern healthcare landscapes, treating life-threatening bacterial infections that previously could have been deadly. However, an ever-increasing resistance to antibiotics threatens the health advantages that have been gained as a result of their use. This global dilemma is now recognized. Resistance caused by all microorganisms to their respective medications is considered AMR. Some countries are seeing far more resistance than others, but it's clear that poorer countries see higher levels of resistance (Laws et al. 2019). Because they have more first- and second-line medicines available in First World countries, compared to Third World countries, this is due to various factors, including higher availability. An increase in the regional levels of resistance can have a global effect due to the recent emergence of faster intercontinental travel, which allows the global diffusion of bacteria with a greater resistance level. Several experts believe that people will be less willing to travel to locations where they can contract infectious bacterial diseases because of regional levels of resistance (Brown, 2015). This shows that while greater healthcare precautions have been implemented in the more economically developed countries of the world, levels of AMR are simply rising.

Bacterial resistance's consequences don't just apply to those people who become infected; the medical procedures themselves will be influenced as well. Preoperative antibiotic prophylaxis is often used to prevent infections, both for patients who are immunocompromised or undergoing chemotherapy for cancer. Efforts to protect against AMR are no longer practicable at the current rate that AMR is spreading, which could impair the availability of surgical procedures and the quality of patients' lives (Reza, et al. 2019).

Major pharmaceutical corporations have significantly reduced their spending in antibiotic research in recent years since there was no clear return on investment. Antibiotics are frequently short-term treatments, which means they generate less revenue for pharmaceutical corporations (Kant, et al. 2017). In addition, the emergence of other infectious diseases, such as acquired immune deficiency syndrome (AIDS), has created a paradigm change in the business, frequently leading in a reduction in research and development (R&D) funds available for antibiotics. To make antibiotic development more attractive, healthcare policy must take a different course (Akther, et al. 2019).

Antimicrobial Resistance

The simple methods via which bacteria can resist antibiotic effects include alterations to the target site, the antibiotic itself, antibiotic efflux and decreased membrane permeability (Marini, et al. 2018). Resistance mechanisms can be found in bacterial cells that are capable of producing numerous antibiotic resistances at the same time (Ko, et al. 2017). Bacteria naturally possess any of the above mechanisms either through lack of antibacterial selection pressure (ampicillin resistance in Klebsiella spp.) or absence of the antibiotic target (vancomycin resistance in lactobacilli) (Abiraami & Gowrie, 2019).

The bacteria developed resistance to drugs by either undergoing endogenous (vertical) evolution or by horizontally evolving (horizontal) (exogenous). Increasing resistance to a given drug by means of a spontaneous mutation in the bacterial genome is known as vertical evolution. Once antibiotic treatment selects for the initially mutated bacteria, it permits these mutants to thrive in the presence of fur- ther antibiotic treatments, resulting in a succession of new mutations that grant an even greater survival advantage (Wright, 2016).

Conjugation, trans-duction, and transformation are all possible methods. Conjugation involves the transfer of resistance (R) plasmids harboring antibiotic resistance genes across bacteria, while transformation involves the change of the bacterial genome by incorporating exogenous DNA. If it is a method of transmission, it allows for bacteria that are less problematic to get access to a mechanism gained by a more harmful strain, which could lead to fatal results (Bai, et al. 2019).

Bacteria possess a wide range of different resistance mechanisms encoded by different genes that are often found on transposons, which facilitates their easy transmission from one bacterium to another. Furthermore, some transposons contain specialized regions known as integrons that are able to encode different resistance genes, resulting in the creation of a new resistant strain (Ranjani, et al. 2020). The other way bacteria withstand antibacterial pressure is by having a physical condition. Hydrated matrices of extracellular polymeric substance in which the bacterial cells are embedded permitting adhesion to each other and external surfaces are also known as aseptic in biofilms (Hind, et al. 2019). Biofilms, which form on medical implants or in catheters implanted in the body, serve as a shield against antibiotics, which are less able to penetrate biofilms at fatal quantities (González-Bello, 2017). In addition to growth, the bacterial population that is present in the biofilm might enter a dormant state that contributes to antibiotic resistance (Andersson, et al. 2020).

Antibiotic Resistance Breakers

In order to counter the rise in the AMR phenomenon, new treatment approaches have been devised that attempt to reduce the amount of antibiotics administered while yet maintaining the current antibiotic classes for further usage in clinical settings. such an approach will be highlighted, a break in antibiotic resistance will be used (ARBs) (Dersch, et al. 2017). Compounds that are able to counteract antibiotic resistance mechanisms that are currently employed. Both direct and indirect antibacterial properties may be found in ARBs, which may either be co-administered with or attached to antibiotics that are failing. In the past, the term 'antibiotic adjuvant' has been used to describe ARBs. But in more recent usage, 'alternative treatments' includes both drugs that help promote a healthy immune response to help the clearance of bacterial infections (Wang, et al. 2019) as well as the use of ARBs. There are now a number of ARBs under investigation that change enzymes, permeabilize membranes, and block transporters.

In the past, dual antibiotic therapy (either synergistic or additive effects) has proven successful with either BLIs or BLI-related ARBs (β-lactamase inhibitors), and these therapies have been used to treat bacterial infections for prolonged periods of time (Hunter, 2020). Antibiotic resistance mechanisms deployed against antibiotics can be combated by co-administered ARBs, resulting in reduced dosages of antibiotics required. Another word you may hear is the minimum inhibitory concentration (MIC), the lowest concentration required for inhibiting visible growth of the pathogenic species under controlled conditions (Kauss, et al. 2020). Potentiation of this kind offers the distinct advantages of less antibiotic resistance development and relief of adverse side effects reported by patients on antibiotic monotherapy (Di Lodovico, et al. 2019).

DevelopingEnzyme Inhibitors

A wide range of enzymes are employed by bacteria to alter or eliminate antibiotics in order to establish a resistant phenotype. This enzyme class can be grouped by both the ways in which they influence cellular metabolism and their substrate antibiotics. Examples of detoxification methods include hydrolysis of vulnerable bonds within the antibiotic molecule, the transfer of a functional group to the antibiotic, and (less typically) redox and lyase reactions (Mengesha, et al. 2020). The creation of these antibiotics included an antibiotic that could survive the activity of the penicillinases such as the β-lactam flucloxacillin, which was created to withstand the effects of the penicillinases (Dar & Sorek, R2017). Enzyme inhibitors are methods that have proved to be more successful. These include various chemicals that target bacterial enzymes that play a role in antibiotic modification and destruction. Enzyme inhibitors that have been modified are used to reduce bacterial enzyme detoxification, allowing an antibiotic to be more effective. Two important groups of biotin-dependent enzymes are BLIs and aminoglycoside-modifying enzymes (Vuotto, et al. 2017).

II. METHODS AND MATERIALS

An RGM was isolated from lung illness patients who were admitted to the Hospital's Laboratory, Clinical Pathology Laboratory. It was provided curcumin (C7727) in pure form and solutions containing 1 and 10 mg/L in absolute ethanol for long-term storage at −20°C. In accordance with the Blairrecommendations, MICs were calculated using the microdilution method (Blair, et al. 2014). Microdilution plates were covered with adhesive seals and incubated at 30 °C for 4–5 days before visual inspection was utilized to determine whether the plates had been inoculated. For comparison, recent research found that MICs were measured using the fast p-iodonitrotetrazolium chloride colorimetric assay as reported below (Hunter, 2015). Every experiment was done three times.

An array of repeated concentrations of test substances in two dimensions, as shown earlier, was used to demonstrate synergy (Amin & Qadir, 2020). The concentrations were varied by using the MIC of the two drugs; sealed and incubated inoculation microdilution plates were used. The chequerboard test was used to determine the FIC index, which involves calculating the MICs of two compounds in the presence of one of them, with the formulas: FICA = MICA + B/MICA, FICB = MICB + A/MICB, and FIC Index = FICA + FICB. Synergy (FIC index ≤0.5), Antagonism (FIC index >4.0), and No Interaction (FIC index >0.5–4.0) were defined using Füchtbauer, et al. (2021) work on synergism, antagonism, and no interaction. Isobolograms built by graphing synergistic concentrations are also described (Mulyaningsih, Sporer, Zimmermann, Reichling, & Wink, 2010). Every experiment was repeated three times.

To test for the presence of M. abscessus 29904, 0.3% agar was added to a broth culture containing M. abscessus 29904 and incubated at 37 °C for 5 days. The inoculated M. abscessus cell's slide displacement was measured in millimeters. Repeated experiments were done.

In order to test biofilm forming abilities, M. abscessus 29904 was cultured in Middlebrook broth supplemented to include 1 × 106 colony forming units per milliliter at a standard concentration of OD600 = 0.1 for 3–4 days. Plates were inoculated (200 μL) and incubated at 37 °C for 2, 4, and 8 days. in order to assess biofilm formation, wells were first rinsed with PBS, then dried, then stained with 0.1% safranin solution (1 min). To get a baseline measurement, the biofilm stained in 30% acetic acid (v/v) was re-suspended in 30% acetic acid (v/v). OD492 was quantified using an ELISA reader (SAFAS, Principauté de Monaco).

Biomass reduction and biofilm inhibitory concentration (BIC)/biofilm eradication concentration was examined in mature biofilms in relation to biomass reduction and inhibition of biofilm formation (BEC).

Four and eight days after planktonic cells were extracted from mature biofilms, planktonic cells from the biofilms were removed. Wells were filled with curcumin (MIC from 4X to MIC) or amikacin (from MIC to MIC), and then they were filled with the combined synergistic concentrations. Wells were then cleaned with PBS and dyed as described above. To estimate the percentage of biofilm reduction, divide the optical density of the substance by the optical density of the absence of the substance (with biofilm):

As detailed by Di Giulio et al., the BIC and BEC values were calculated (2016a). In a nutshell, mature biofilms cultured for 4 and 8 days were picked, cleaned, and filled with curcumin or amikacin (varying from MICs to 4 times MIC). OD600 was measured immediately before and after incubation for 24 hours at 37°C. At BIC concentrations, no bacterial growth occurred on Middlebrook agar; at BEC concentrations, no bacterial growth happened on Middlebrook agar. Every experiment was repeated three times.

III. FINDINGS

Table 1 Synergy between curcumin and antibiotics

Chequerboard	Best combinationa (mg/L)	Best combinationa (mg/L)	Best combinationa (mg/L)
Curcumin/amikacin	16/2	16/2	16/2
Curcumin/ciprofloxacin	32/1	32/1	32/1
Curcumin/clarithromycin	16/16	16/16	16/16
Curcumin/linezolid	32/0.25	32/0.25	32/0.25

Table 2 Reduction of mature biofilms after treatment with curcumin and amikacin.

Antimicrobial concentration	% mature biofilm reduction			
	days		days	
	Curcumin	Amikacin	Curcumin	Amikacin
MIC	43.5 ±0.01	76.7±0.02	82.4±0.081	84.9±0.059
2*MIC	94.3±0.02	100±0.01	79.8±3.67	91.1±3.11
4*MIC	100±0.00	100±0.00	100±0.00	100±0.00

Curcumin was investigated for its ability to operate synergistically with all the antibiotics that were examined. synergy (FIC index ≤0.5) was seen in the chequerboard assay using amikacin, clarithromycin, linezolid, and ciprofloxacin, and the MICs of these antibiotics were reduced by a factor of 4 to 128. The experimental evidence verified the synergistic impact using isobolographic analysis. The antagonism never materialized.

Like the curcumin and amikacin MIC, the amikacin treatment was more effective on 8-day biofilms than on 4-day biofilms. Inhibit biofilm formation 100% with the use of 4x MIC curcumin and amikacin. Curcumin demonstrated the greatest potential for reducing mature biofilms when it was combined with amikacin. Results were most effective when they used the following combinations: 32 mg/L curcumin with concentrations of 1, 2, 4, and 8 mg/L amikacin; 8 mg/mL curcumin with concentrations of 4 and 8 mg/L amikacin; and 4 mg/L curcumin with concentrations of 8 mg/L amikacin. A new compound, 16 mg/L curcumin mixed with two, four, and eight milligrams of amikacin also decreased mature biofilms.

The curcumin BIC values were twice the curcumin MIC, but the amikacin MIC was identical to the curcumin MIC. With respect to the synergistic combinations, the most successful combinations occurred while using 4-day dosing with 16 mg/L curcumin with 2 mg/L amikacin, and 8-day dosing with 8 mg/L curcumin with 8 mg/L amikacin. The 8-day cures including 8 mg/L curcumin and 8 mg/L amikacin, and curing them with 4 mg/L curcumin and 8 mg/L amikacin were all winners. Amikacin BEC values were higher than 4× MIC, and curcumin BEC values were higher than 8× MIC.

IV. CONCLUSION

The association of curcumin with amikacin, with the lowest FIC index value, was tested for its antibacterial activities. Disaggregation effects were more strongly observed as a result of each show. When curcumin concentrations were greater than amikacin concentrations, combinative treatments with a considerable disruption effect on four-day mature biofilms was noted, however, a more notable killing effect was evident when amikacin concentrations were prevalent. A similar tendency was found in mature biofilms grown for eight days. Based on the in vitro data, it appears that curcumin likely interferes with cell viability through interfering with the integrity of the bacterial biofilm. Antiquorum sensing activity may decrease biofilm development and increase bacterial mobility (Evans & Bolz, 2019). A bacteriostatic impact was discovered exclusively on mature M. abscessus biofilms produced by smooth morphotypes, as stated by (Amin, et al. 2020). Also known as the "syn-ergistic effect," the syn-ergistic impact of curcumin and amikacin is associated with biofilm disaggregation by curcumin which facilitates the targeting of amikacin to its target and resulting in cell death.

A novel approach to tackle antibiotic resistance and bacterial pathogenicity is using natural bioactive compounds, such as probiotics, alone or in combination with antibiotics (Satras, 2019). Because of its diverse therapeutic effects and minimal toxicity, curcumin is an ideal therapeutic agent. Curcumin's very limited oral bioavailability is well-known, thereby limiting its use as a medicinal agent (Amin, et al. 2020). Research is in the early stages of development to solve the issues of poor bioavailability (Satras, 2019). Nanobiotechnologies are now being studied to improve bioavailability in vivo (Brown, 2016).

It appears that curcumin is a possible antibiotic resistance buster, and this current study supports this concept

(Rezzoagli, et al. 2020). It appears in this study that curcumin and amikacin have a pronounced effect on biofilm, showing the importance of their synergy.

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