Synthesis and Antibacterial Property of An Encapsulated Sulfonamide Nanoparticle in a Multidisciplinary Approach

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Abstract

Multigram quantity of a novel Sulfa Drug complex -poly(amido)amine-sulfonamide or PAMAM-Sulfa- was synthesized, from commercially available materials. It was characterized with spectroscopic methods such as nuclear magnetic resonance (NMR). The Kirby-Bauer test was used to test it against gram positive and/or gram negative bacteria using different concentrations of an ethanol solution of the PAMAM-Sulfa complex. The goal of this experiment was to synthesize and study the effect of water soluble encapsulated sulfonamides on common bacteria by undergraduate students engaging in research involving more than one STEM discipline. Students synthesized a dendrimer-sulfonamide complex before evaluating its antibiotic properties. In doing so, students employed research methods that are common to chemistry, biology and nanoscience while also learning about mechanism of infectious diseases, drugs and drug resistance. This project allowed students to combine aspects of scientific research that are usually done separately, and an opportunity to observe the seamlessness of multidisciplinary science.

Keywords: Nanoparticle, Multidisciplinary, Dendrimer, Antibiotic, Sulfonamide.

Introduction

Infectious diseases, and drug resistance to common antibiotic drugs continue to represent a major threat to human health.[1-4] In developing countries, millions of children die each year due to infections that are otherwise treatable.[5-6] Sulfa drugs or sulfonamides are inexpensive, yet effective antibacterial drugs that have been known for more than a century.[7] Several types of sulfa drugs are marketed for treatment of common bacterial infections in addition to being effective against malaria. Sulfonamides control the growth of bacteria by inhibiting folic acid synthesis needed for bacterial growth. Sulfonamide is structurally similar to *p*-aminobenzoic acid (PABA) which is part of dihydrofolate. Bacterial growth is hindered by the progressive replacement of PABA by sulfonamide during dihydrofolate biosynthesis in bacteria. Therefore sulfonamides are considered inhibitors of dihydropteroate synthetase.[8-9] Several analogs of sulfonamide have been synthesized and described in textbooks. Metal complexes of sulfonamides are water soluble but have also been linked to higher toxicity and increasingly linked to clinical resistance to sulfonamides.[10] Over the past few decades dendrimers have emerged as versatile drug delivery and targeting vehicles with increasing market share owing to markedly improved outcomes and efficiency when administered with Doxorubicin and paclitaxel as first-line treatments in various cancer types. Dendrimers are macromolecules

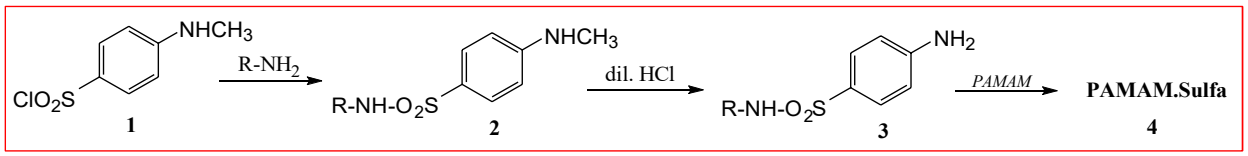
which behave as monodispersed nano-reactors with ligand sites on both their surface and inside suitable for conjugation/entrapment with drugs. Dendrimers host their guest-molecules with a molecular level of dispersion to the latter that increases the bio-availability of the nanocomposites thus formed. Highly branched, tree-like dendrimers such as polyamidoamine (PAMAM) are structurally well-defined. For instance, PAMAM-paclitaxel conjugates have demonstrated good stability under physiological conditions and greater permeability across endothelial cells monolayers than paclitaxel alone, making it a choice nano-carrier for poorly water-soluble drugs. We hypothesized that improved pharmacokinetics, bio-distribution and controlled release of a PAMAM-Sulfa drug can markedly improve the antibacterial properties of sulfonamides to overcome drug resistance. We herein report the enhanced antibacterial activity of a novel PAMAM-Sulfa drug relatively to commercial Sulfonamide towards the microorganisms *E.Coli* and *Luteus Mycrococcus*.

Material and Method

1. Chemistry: Synthesis of the PAMAM Sulfa Drug Complex

Ready to use PAMAM dendrimer was purchased from Dendritech. The PAMAM-Sulfa drug complex was prepared by a simple procedure as described in the scheme below. Equimolar amounts of an aqueous solution of the poly(amido)amine (PAMAM) dendrimer was added to a solution of sulfanilamide (or sulfonamide) **3**, before stirring the mixture which formed a water soluble PAMAM-Sulfa drug complex.

Scheme 1: Synthesis of a library of PAMAM.Sulfa conjugates.



Sulfanilamide was synthesized in three steps from commercially available acetanilide.[11,12] Acetanilide (1mol) was added to ice-cold chlorosulfonic acid (2mol) with stirring. The mixture was gradually brought to room temperature, and the precipitate was vacuum filtered to afford acetamido-benzenesulfanil chloride **1** in quantitative yield. Compound **2** was suspended in .5M aqueous ammonium hydroxide, and refluxed for 1hr. The product precipitated and was vacuum filtered. The acetamido-benzenesulfanilamide **3** was then added to a dilute hydrochloric acid solution (.5M) and refluxed to afford sulfanilamide which was identical to the commercial drug. The PAMAM-Sulfa drug complex **4** was prepared by mixing an equimolar amount of an aqueous solution of the dendrimer (PAMAM) to that of the sulfanilamide, and subsequent stirring formed a water soluble complex which was used in the subsequent antibacterial tests.

2. In vitro Testing of the PAMAM Sulfa Drug and Sulfonamide Against Bacteria

Bacteria cultures was purchased from ATCC, and used to validate antibacterial activity of the dendrimer-encapsulated synthetic Sulfa drug. Students used the Kirby-Bauer diffusion antibiotic sensitivity testing to measure the Zone of Complete Inhibition of the PAMAM-Sulfa drug complex for comparison with commercial Sulfonamide.[13-15] Using four simple steps, students were to: 1) swab the entire surface of a Petri dish with the organism to be tested (culture broth of *E.coli*, and *Luteus Mycrococcus*) after preparing the Mueller Hinton II agar medium which is, then, poured to a uniform thickness of 4 mm in the Petri dish.

2) Students were to place four evenly spaced high-potency disks impregnated with solutions of defined amounts of the various compounds to be tested (Sulfonamide, and the PAMAM-Sulfa drug complex) on the agar using flame-sterilized forceps to press them down onto the agar. 3) After inverting the plate, student were to incubate it for 18 hours at 37 °C, and then evaluate the zone of complete inhibition by measuring the diameter of diffusion (i.e. inhibition) in millimeters using a ruler and record their data on a chart. 4) Students were then to determine the nature of the action of the PAMAM-Sulfa drug towards the microorganism being studied by comparing the significance of zones of inhibition in the KB testing between sulfonamide and the nanometer-sized PAMAM-Sulfa drug.[16]

Results and Discussion

We used Gram-positive bacteria (*E.Coli* and *M. Luteus*) in this study owing to their availability and known resistance to common antibiotics and sulfonamides in particular.[17-19] Agar diffusion assays for the qualitative detection of growth inhibition halo around the satellite disks were used to demonstrate that the PAMAM-containing disks displayed strong antibiotic property. The first plates (Fig.1) contained four equal quadrants for ethanol the control (C), and solutions in increasing concentration of 5[redacted]mL, 40[redacted]mL and 50[redacted]mL of an ethanol solution of the PAMAM Sulfa drug (SP). Round halos have developed around the disks with 40 and 50 while nothing noticeable formed around disks on both petri dishes.

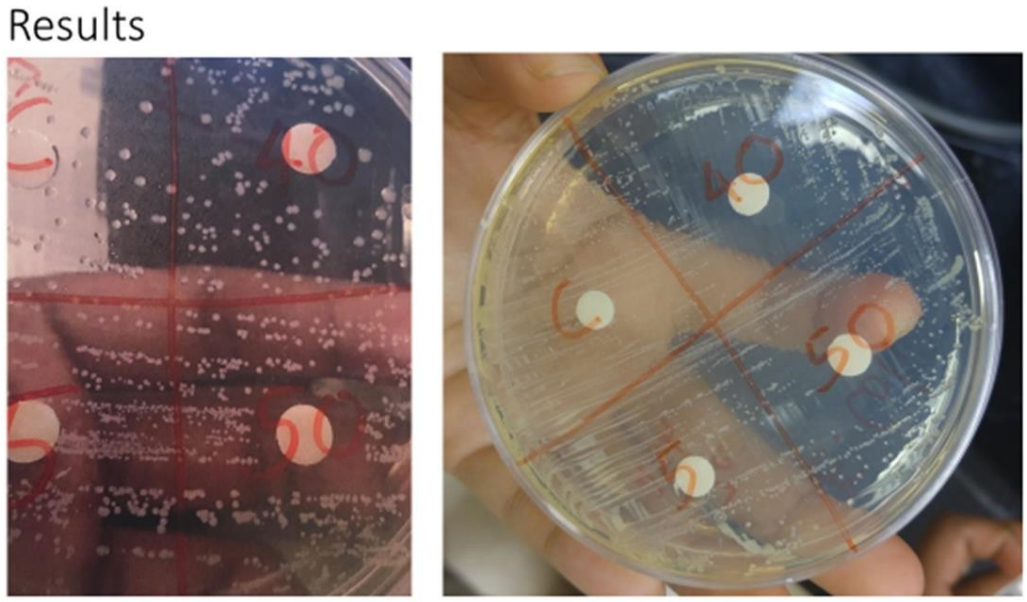


Fig. 1. Agar diffusion assay for *E.Coli* and *M.Luteus* with PAMAM-Sulfa drug

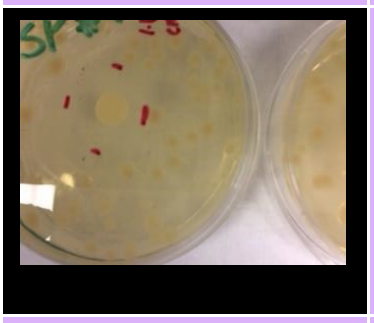
Using these preliminary results as background, we compared zones of inhibition of sulfanilamide and PAMAM Sulfa drug against *E.coli* and *M.luteus* with the goal of quantifying the extent of bacterial growth inhibition. The drug susceptibility was detected by the disc diffusion method with bacterial liquids (*E.coli* and *M.luteus*) uniformly spread onto agar medium (Sigma-Aldrich) before the PAMAM Sulfa drug-soaked disks, in different concentrations, were placed on the medium. After inverting the plates, they were incubated for 18 hours at 37 °C. Students were then able to measure the zone of complete inhibition or the

diameter of diffusion (i.e. inhibition) in millimeters using a ruler and they recorded their data on the chart below. Students used 10 mg/mL as the standard concentration of both sulfonamide and PAMAM Sulfa drug. Plates used for *E.coli* testing were labelled with a green-colored pen while plates used for *M.luteus* testing were labelled with an orange pen. The halo of diffusion in each of the plates was measured (in red marking) from the disk outward around it where the compound cleared the growth of the microorganisms. In entry 1, the zone of inhibition of *M.luteus* by PAMAM Sulfa drug was measured to be 55 mm while commercial sulfonamide (entry 2) produced a zone of inhibition that's measured at 10 mm. The nanoparticle formulation of sulfonamide with PAMAM displayed an increase of inhibition that's more than five times the potency of the commercial drug in 18 h. It's conceivable that with longer exposure, the zone of inhibition could potentially get larger. Entry 3 depicts the inhibition of the Gram-positive *E.coli* by PAMAM-encapsulated sulfonamide, measured at 25 mm after 18 h of incubation. The commercial drug (entry 4) inhibited the growth of the bacterium much slower during the same time as measured by a zone of inhibition of 19 mm. The impact of dendrimers in enhancing therapeutic properties of several drugs has been pinned down to an improvement of their bio-availability owing to increased solubility of the dendrimer-encapsulated drug. Indeed, dendrimers such PAMAM form hydrogen bonds with water which help to solubilize the drug complex faster than the pure drug.[20] Dendrimers have found a lot of interest in drug-delivery applications because they offer markedly improved therapeutic potency and enhanced drug properties. The reasons are to be found mainly in their water solubility (bio-availability), but also in their nanometer size shown to afford superior pharmacological properties. Moreover, Dendrimers can form internal hydrophobic cavities capable of conjugating or encapsulating dispersed drug's molecules, hence, improving their solubility in those micro-environments.[21-22] Drugs with inadequate bioavailability have been conjugated to, or encapsulated in, dendrimers to enhanced their solubility in water. This makes dendritic molecules such as PAMAM ideal drug carriers with adequate properties to overcome issues with cell membrane permeability for a host of synthetic drugs and therapeutics. For all these reasons, PAMAM is also used in gene-delivery (transfection) to cells and tissues as well as in photodynamic therapy. [23-24] Site-specific internalization of macromolecules containing porphyrins within cells, also known as photochemical internalization (PCI), allows for *in vivo* surface modification of PAMAM dendrimers by radiotherapy to fine-tune site-specific cytotoxicity targeting cancer cells.[25]

Conclusion

Engaging students in this multidisciplinary exercise of synthesizing, characterizing and testing the antibiotic property of a dendrimer-encapsulated sulfonamide provided an opportunity to expose students to several techniques. More importantly, this student-led exercise allowed them to observe the seamless links existing between the STEM disciplines that are taught by different professors that are, often, from different departments. The practicality of students handling experiments involving disease-causing bacteria such as *E.coli* and *M.Luteus* could, we hope, excite their interest for further multidisciplinary scientific investigation and/or internship *en route* to a rewarding scientific career.

Chart 1. Zones of Inhibitions of *E.coli* and *M.luteus*

Compound	Zone of Inhibition (plates)	Zone of Inhibition of *E. Coli*	Zone of Inhibition of *Myc. Luteus*
SP.C 10 □g/mL of PAMAM Sulfa drug Zone of inhibition in *M.luteus*		X	55mm
SP A 10 □g/mL of Sulfonamide Zone of inhibition in *M.luteus*		X	10mm
SP #3 10 □g/mL of PAMAM Sulfa drug Zone of inhibition in *E.coli*		25mm	X
SP #1 10 □g/mL of Sulfonamide Zone of inhibition in *E.coli*		19mm	X

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