ANTIMICROBIAL ACTIVITY OF CDTE QDS MODIFIED WITH LANTHANIDES ON *PSEUDOMONAS AERUGINOSA*

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Abstract: The aim of this study is to obtain data based on an experimental procedure and check the effectiveness of some antibacterial agents against pathogenic bacteria. In the present experiment, two different nanoparticles (Cadmium Telluride Quantum dots with Lanthanides: Gadolinium and Terbium) were used to check their antibacterial properties on *Pseudomonas aeruginosa*. The Cadmium Telluride Quantum dots without the Lanthanides, Gadolinium nitrate and Terbium nitrate were also tested on those bacteria as control. In the present experiment, the following methods were employed: disc-diffusion test, the determination of the growth properties of the bacteria and comparison of absorbance after treatment with antimicrobial agent. From the results it has been found that the tested Cadmium Telluride Quantum dots with Lanthanides (Gadolinium and Terbium) have good antimicrobial effects. Additionally, GdQDs show stronger antibacterial effect than Tb QD and other tested compounds.

Key Words: Pseudomonas aeruginosa, antimicrobial activity, lanthanides, quantum dots

INTRODUCTION

Bacterial infections are one of the major threatening for human health. A serious problem is the treatment of diseases causing resistant bacteria and bacteria forming a biofilm (*Pseudomonas aeruginosa*). More than 70% of nosocomial pathogens have become resistant to the drugs considered to be their first line treatment (Muto et al. 2003).

In hospitals antibiotic resistance is an important issue (Cook et al. 2004). In the last 50 years, the number of bacterial strains resistant to antibiotics has increased almost uniformly around the world. The bacteria started to be resistant to antimicrobial agents by changing their chromosomes and exchanging their genetic materials through plasmids (Lutsar et al. 1997). Due to excessive use of these drugs, antibiotic resistance increases rapidly (Andersson and Levin 1999).

Pseudomonas aeruginosa is one of the important nosocomial pathogens worldwide. Nosocomial infections caused by this organism are often hard to treat. *Pseudomonas aeruginosa* has intrinsic resistance and remarkable ability to acquire further resistance mechanisms to multiple groups of antimicrobial agents (Strateva and Yordanov 2009).

Nanoparticles with unique chemical and physical properties have shown an increasing importance in biomedical and pharmaceutical applications. Inorganic nanomaterials are regarded as good candidates to replace traditional organic antimicrobial agents, because they have large specific surface area and high bioactivity. A number of nanoparticles with antimicrobial activities have been reported recently. QD are crystalline clusters synthesized from semiconductor materials. Due to their wide potential, the study of antimicrobial activity of QDs go up logically (Lu et al. 2008). Lanthanide complexes are of increasing importance in diagnosis and therapy, due to the versatile chemical and magnetic properties (Teo et al. 2016).

Due to the increasing antibiotic resistance CDTE QDs modified with Lanthanides have been manufactured and their antimicrobial activity on *Pseudomonas aeruginosa* was investigated in this study.

MATERIAL AND METHODS

Chemical compounds

All the reagents for quantum dots synthesis, standards, and other chemicals were purchased from Sigma-Aldrich (St. Louis, MO, USA) in ACS purity, unless noted otherwise. Media for cultivation of microorganisms were purchased from OXOID CZ (ThermoFisher Scientific - CZ).

Preparation of CDTE QDS modified by a Gadolinium-schiff base complex (GDQDs) and Terbium-schiff base complex (TBQDs)

The Schiff base, [(2-[(E)-2-pyridylmethyleneamino]-N-[2-[(E)-2-pyridylmethylene-amino]ethyl]ethanamine)] was prepared according to previous experiment (Kopel et al. 2014). In a separate beaker, methanol was mixed with an aqueous solution of gadolinium nitrate, which was subsequently added to the Schiff base solution. The prepared Gd-SB solution was stored at 25 °C until use. Microwave preparation of the CdTe QDs was carried out according to our previous study (Moulick et al. 2015) with necessary modifications. Next, the Gd-SB solution was added to the prepared CdTe QD solution, followed by heating using 300 W under microwave irradiation to prepare the GdQDs. The sample and control particles were filtered through 0.22 μm membranes and subsequently dialysed against deionized water several times to remove the unreacted initiators. Then, the particles were dispersed in deionized water for further characterization and use. TBQDS were produced in a similar way like GDQDs where Terbium nitrate was used in place of gadolinium nitrate.

Collection of wound swabs from the patients with bacterial infections

The smears, collected from infected wounds with the agreement of patients from Trauma Hospital in Brno, were sampled by rolling motion at the wound using a sterile swab sampler. All patients were divided into two subgroups, on the grounds of infection severity: deep and superficial wound. A detailed description of comorbidities and duration of treatment was obtained. Patients were classified according to the Classification of surgical wounds – SSI (surgical site infections). Infected wounds were sampled by using disposable tampon swabs maximizing collection of representative microflora. Tampons were subsequently stored in transport medium (inorganic salts, sodium thioglycolate, 1% agar, activated charcoal). The important part of our work-flow process comprised sampling in duplicates with further transport in both aerobic and anaerobic conditions to preserve bacterial viability (Chudobova et al. 2015).

Cultivation of clinical specimens

Four types of selective nutrient media (blood agar enriched by 10% NaCl, Endo agar, blood agar without any other component, and blood agar with amikacin) we employed for further microbiological selection. Petri dishes, containing the above mentioned media were subsequently incubated according to conventional protocols, as described elsewhere, to maintain suitable conditions for growth of all types of bacteria. These Petri dishes were incubated for 24–48 h at 37 °C supplemented by TGY medium (1 g L−1glucose, 5 g L−1tryptone, 2.5 g L−1yeast extract). Subsequently, individual colonies were collected from each Petri dish and stored in 1 μL of enriched media. These samples were processed and utilized for both – MALDI-TOF MS identification and PCR with subsequent sequencing. The glycerol stocks were prepared from bacterial cultures and 80% glycerol for long-term storage and further use (Chudobova et al. 2015).

Cultivation of bacterial strains

The pathogenic bacterial strain *Pseudomonas aeruginosa* from the infected wounds of the patients were cultivated. The composition of cultivation medium was as follows: Mueller Hinton broth 21 g and 1000 mL distilled water with 18 MΩ. Mueller Hinton agar 38 g into the 1 000 mL of distilled water. The pH of the cultivation medium was adjusted to pH 7.4. Prior experiments, the cultures were diluted by Phosphate-buffered saline (PBS) to OD_{600} nm = 0.5 McF standard

(Chudobova et al. 2015). PBS was prepared with NaCl 8 g, KCl 0.2 g, Na_2HPO_4 1.44 g, KH_2PO_4 0.2 g and 1000 mL of miliQ water (Chudobova et al. 2015).

Analysis of the inhibition zones using agar microdilution method

To determine the antimicrobial effect of the compounds on the bacterial culture of *Pseudomonas aeruginosa* the measurement of the inhibition zones was performed. Agar surface in Petri dish was covered with a mixture of 0.5 McF standard bacterial cultures (100 µL of 24 h bacterial cultures in the exponential phase of growth, and 3 mL of MH broth). Discs (Ø 0.6 cm) were filled with 10 µL of 2 mM antimicrobial agents. Soaked discs were then laid on a Petri dish. Petri dishes were insulated against possible external contamination and placed in a thermostat (Tuttnauer 2450EL, Israel) at 37 °C for 24 h. After 24 hours of incubation, the inhibition zones were measured and photographed in each Petri dish (Chudobova et al. 2014).

Determination of growth properties

The second procedure for the evaluation of an antimicrobial effect of antimicrobial agents employed apparatus Multiskan EX (Thermo Fisher Scientific, Germany) for analysis of bacterial growth curves. The diluted cultures (OD_{600nm} = 0.5 McF and next dilution 1:100 with MH medium) were pipetted into a microplate (total volume of 200 µL) alone as a control variant, or with various concentrations of antimicrobial agents. The concentrations of compounds were 0; 15.6; 31.25; 62.5; 125; 250; 500; 1000 µM. Measurements were carried out at time 0, then each 30 min for 24 hours at 37 °C, at a wavelength of 600 nm (Chudobova et al. 2014).

Statistical analysis

Software STATISTICA (data analysis software system), version 10.0 (Tulsa, Oklahoma, USA) was used for data processing. The general regression model was used to analyse differences between the measured values. To reveal differences between the cell lines, Tukey's post hoc test within homogenous groups was employed. Unless noted otherwise, $p < 0.05$ was considered significant (Berney et al. 2007, Milosavljevic et al. 2017).

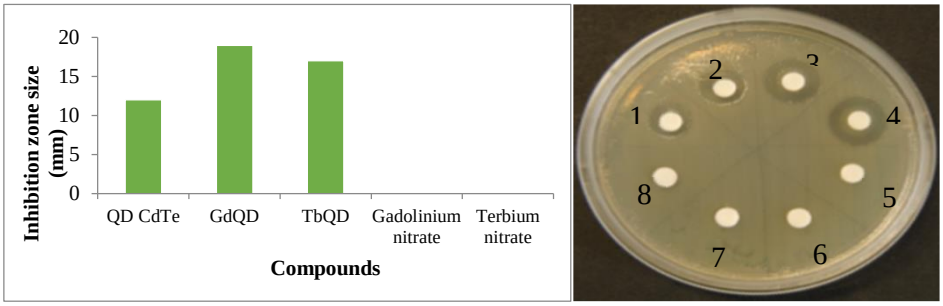
RESULTS AND DISCUSSION

The antibacterial activity of CdTe QDs modified with Lanthanides has been determined using disc-diffusion method and diameter size of the inhibition zone (mm) was showed (Figure 1). CdTe QDs modified with Lanthanides were applied on *Pseudomonas aeruginosa*. The influence of CdTe QDs modified with Lanthanides on pathogenic bacteria has been shown in Figure 1, 2 and 3. The highest inhibitory effect after 24 hours of incubation can be seen after the application of GdQD on *P. aeruginosa*.

Disc-diffusion method

The graphics and pictures below show the results of inhibition zones after the application of circular discs with antibacterial compounds against the pathogenic bacterial strain. Bacterial pathogen (OD_{600} = 0.5 McF) was exposed to a 2mM antibacterial agent and the incubation was carried out at 37 °C for 24 hours.

Figure 1 Results of the disc-diffusion test on Pseudomonas aeruginosa



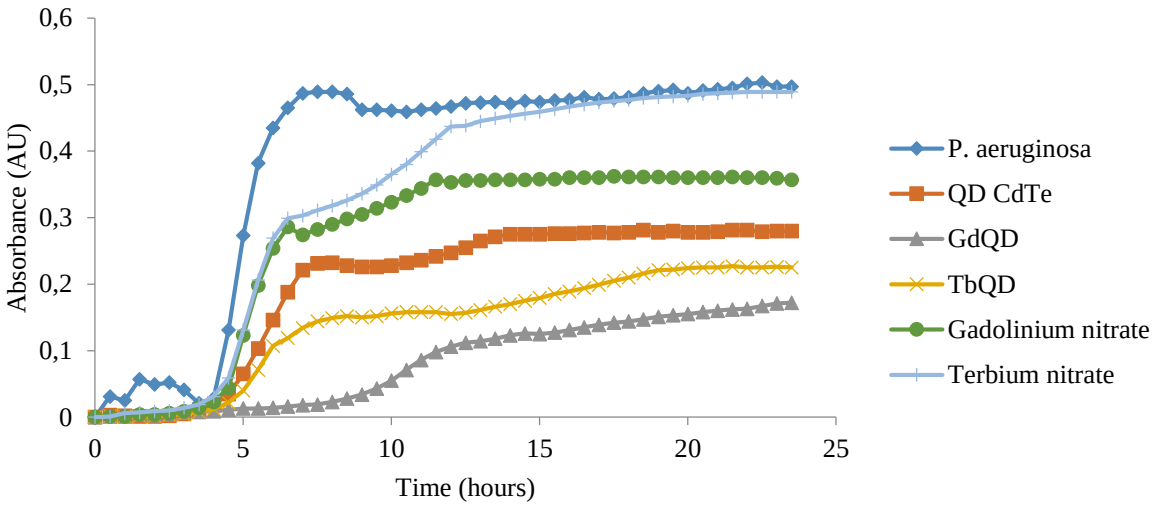
Legend: The discs on the plate: 1. QD CdTe; 2. TbQD; 3. TbQD; 4. GdQd; 5. Gadolinium nitrate; 6. Gadolinium nitrate; 7. Terbium nitrate; 8. Terbium nitrate

It can be noticed with the graphic and picture above, that the two compounds with lanthanides have the best results, with a significant difference between the QD CdTe and them. Although GdQD is working better than TbQD against *Pseudomonas aeruginosa.*

Determination of the growth properties of bacteria

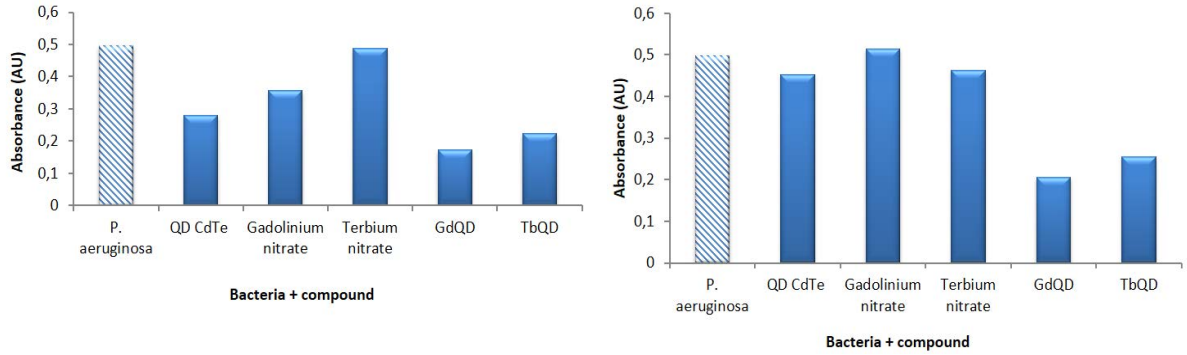
The growth curves of bacteria alone and after application with antimicrobial agent show that GdQD is the compound with higher effectiveness against *P. aeruginosa* in measurement by using Multiscan EX.

Figure 2 Growth curves after application of different compounds on Pseudomonas aeruginosa (concentration of the compound is 250 µM)



Comparison of the absorbance after 24 hours by Multiscan EX. The pathogenic bacteria were treated with and without different antimicrobial agents. In the Figure 3 it can be seen that each antimicrobial compound inhibited bacterial growth. The most effective in this case was GdQD on *Pseudomonas aeruginosa*.

Figure 3 Measurement of absorbance of Pseudomonas aeruginosa after 24 hours



Legend: The concentration of compounds, which was used in the experiment of measurement of Absorbance: the figure left 250 µM for each compound; the figure right 500 µM for each compound

CONCLUSION

The aim of this experiment was to study the antimicrobial effects of Cadmium Telluride Quantum dots with Lanthanides (Gadolinium and Terbium) and the chemicals themselves without Quantum dots against pathogenic bacterial strain (*Pseudomonas aeruginosa*). The antimicrobial activity of prepared CdTe QD with Lanthanides was measured by a disc-diffusion method, measurement of growth properties and comparison of absorbance measured after 24 hours by Multiscan EX. In view of the results obtained with the experiments carried out, it can be established as a conclusion that the QDs with Lanthanides are working well against the pathogenic

bacteria (*Pseudomonas aeruginosa*). Additionally, GdQDs showed stronger antibacterial effect than Tb QD and other tested compounds. The use of CdTe QD in combination with Lanthanides (mostly Gadolinium) appears to be a good way for the reduction of bacterial infection.

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