Survey and Comparison of Sporecidal Property of CuO and AgO Nanoparticles upon Spore of Clostridium botulinum type E

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Abstract

Aims: In this paper examined sporecidal effect of metal oxide nanoparticles of silver and copper against bacterium Clostridium botulinum type E. Nanoparticles due to high volume levels are high sporecidal and bacterial properties. Methods: This study we used the bacterium Clostridium botulinum type E. and Silver and copper oxide nanoparticles with average diameter of 20 nm. All the colors and media culture used were of Merck Company. The bacterium determined and confirmed by PCR and antibody raised against Clostridium botulinum type E. The spores were performed with the Peterson method. Ratios of MIC / MBC and phenol Coefficient were calculated by Microdilution method and Syndics’ of sporecidal obtained amount of D value were determined by pure plate method. Results: Strains of bacteria confirmed by Catalytic domain of Clostridium botulinum type E. SEM and TEM images confirm nanoparticles size was about 20 nm. Power sporiecidal the nanoparticles were compared with other chemical sporecidale, like Glutaraldehyde. Phenol coefficient obtained of about 50 and the ratio MIC / MBC good was about 1/2, respectively. D value for the critical concentration of silver nanoparticles was about 7 minutes. Conclusion: The results of this paper showed that nanoparticles studied for its high ratio of surface to volume properties have high sporeidal and It is predicted can be used of this nanoparticles that the environment to be sterilize.

Key words: Clostridium botulinum type E, Ag and Cu oxide nanoparticles, sporecidale material

Introduction

Strain Clostridium botulinum bacteria must include of gram-positive, large-bacilli, sporogenous, microaerophilic bacteria that optimal growth in aerobic condition. These bacteria during growth produce a toxin that is the most poisonous substance known. Mortality ratio of this toxin can be 1 ng per kg of body weight in mice. Bacteria is mobility and lack capsule and more seen of single, pairs or short chains in media [1-2]. By this strain of bacteria are produced four types of botulism (food, infant, wound botulism, and unknown).

This toxin often enters into the bloodstream through mucosal surfaces such as colon, lung tissue or wound. And preventing the release of acetyl-choline in nerve endings, causing muscle paralysis, which in extreme cases, is often death [3-4].

The specificity of the antigenic has been identified seven different types of bacteria that are called with the letters A to G label. Type A, B and E mainly from disease in humans, and type C and D are cause disease in most animals [5]. Epidemiologic study conducted shows that most of the poisoning is type E botulism. In Asian countries, including Iran, due to the desire to consume food products of marine type E is a high frequency. In 72% Poisoning outbreak, caused by foods prepared at home and 9% of cases, the food is prepared in Food Factory [6]. Spores are resistant
form of these Bacteria. Spores are more oval or sub terminal. Because the diameter of the spore is larger than cell, spores often bind to the cell wall [7]. Deal with spores’ Strain es of Clostridium botulinum is vital in the food industry, because it is food poisoning factor. Different Sporecidal and sporestatics Introduced, that are most of the chemicals and its impact on the structure of spores is also different [8]. Exact location effects of the sporecidal on different layers are different, as spore is made up of different layers. This layer are added spore resistance on against chemical and physical agents.

One of the great achievements of nanotechnology is used of nanoparticles, especially metal oxide nanoparticles, in various fields of medicine, various industries such as agriculture, animal husbandry, depending on classification, appliances, cosmetics and health and military. This technology is to serve humanity by control of disease-causing agents. Damages, losses and damages of bacterial pathogenesis such as Clostridium botulinum to various industries and human and animal on the one hand, and will be spent high costs for improving prevention and treatment on the other hand, Use of nanoparticles to introduce an appropriate alternative for many disinfectants [9].

The aim of this study is sporecidal Characterization properties of metallic silver and copper oxide nanoparticles on the bacterium Clostridium Botulinum. These results were investigated by usual sporecidal agent in different conditions, as temperature and pH. The nanoparticles used in future against disease bacteria in the food industry category.

Material and Methods

2.1 Strain produced and methods for its detection
Bacterium Clostridium botulinum type E was prepared by Environmental Science Research Center of IHU. Bacteria were Culture in Cook Meat Media of Sigma Co. for 72 hours in 32 °C in aerobic Jar contains 15 percent nitrogen gas and carbon dioxide. Biochemical tests such as sugars and gelatin hydrolysis was performed to confirm the strain studied. The first bacterial genome was purified with mini-preparation method to confirm the strain by PCR. Forward and reverse primers were designed against catalytic domain of Clostridium botulinum type E. Sequence of the forward and reverse primer that Shown following. Bioinformatics analysis was carried out for confirmation of the genome. All material was prepared by PCR and primers from Sinogen Co.

Upstream primer: 5’ ATGCCAAAAATTAATAGTT 3’  Tm= 52/1

Downstream primers: 5’ CATTTCCGTATTCCTTT 3’  Tm= 54/9

2.2 Purification of spores:
Spores of bacteria purified by Pterson method. Then, to achieve the high purity of the spores this method was correct. For example, to reach the final rinse increased of pure water 5 times to 10 times and the term primary cultures of bacteria also increased from 46 hours to 72 hours in Pterson method. After staining with Malachite Green color, bacterium were studied under a light microscope model (Model UNBC-11) and necessary photo were taken with Digital photographs. The t - student test was used to analyze the results (p<0/05).

2.3 Preparation of nanoparticles
Silver and zinc oxide nanoparticles were ordered Nanoshell companies of USA. XRD diagram were taken by device Philips U234 model and TEM image were taken by H987 electron microscope photographs in the Faculty of Tehran University.

2.4 Sporecidal properties of nanoparticles
Sporecidal properties of nanoparticles were performed by calculating the MIC/MBC ratio. These methods were of microdilution method. Purification of spores was cultured in the presence of concentrations of 1, 0/5, 0/01, 0/05, 0/001 and 0/005 M of both the nanoparticles for 32 °C in a aerobically Jar. To obtain the MIC/MBC, colonies from 72-hour culture in BHI medium counted with a colony counter. Also the MIC/MBC ratio was calculated before and after the addition of nanoparticles, using the optical absorption spectrum changes. The formulas used for this work are listed below:

Formula 1:

\[
\left( \frac{\text{viable CFU at 0 hours} - \text{viable CFU at 24 hours}}{\text{viable CFU at 0 hours}} \right) \times 100\%
\]

And for formulating and study of sporecidal power of nanoparticles used following formula:

Formula 2:

\[
\frac{\text{alive number in reference group} - \text{alive number in experiment group}}{\text{alive number in reference group}} \times 100\%
\]

Inhibitory and attraction power of nanoparticles were compared o with phenol and formaldehyde that a common chemical sporecidal. Phenol coefficient for silver oxide nanoparticles were calculated from the following table:
Table 1: Table of calculate the phenol coefficient.

<table>
<thead>
<tr>
<th>The disinfectant</th>
<th>Dilution</th>
<th>Time (min)</th>
<th>The standard Material</th>
<th>Dilution</th>
<th>Time (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>5</td>
<td>10</td>
<td>15</td>
<td></td>
<td>5</td>
</tr>
<tr>
<td>AgO nanoparticles</td>
<td>1:2000</td>
<td>-</td>
<td>-</td>
<td>1:120</td>
<td>-</td>
</tr>
<tr>
<td>AgO nanoparticles</td>
<td>1:4000</td>
<td>+</td>
<td>-</td>
<td>1:133</td>
<td>-</td>
</tr>
<tr>
<td>AgO nanoparticles</td>
<td>1:8000</td>
<td>+</td>
<td>-</td>
<td>1:144</td>
<td>-</td>
</tr>
<tr>
<td>AgO nanoparticles</td>
<td>1:16000</td>
<td>+</td>
<td>+</td>
<td>1:154</td>
<td>+</td>
</tr>
<tr>
<td>AgO nanoparticles</td>
<td>1:32000</td>
<td>+</td>
<td>+</td>
<td>1:173</td>
<td>+</td>
</tr>
<tr>
<td>AgO nanoparticles</td>
<td>1:64000</td>
<td>+</td>
<td>+</td>
<td>1:200</td>
<td>+</td>
</tr>
</tbody>
</table>

+ Means growth and - means No growth

Result

Strains confirmed down by PCR. Designed primers has been specific primers for the strain. PCR sequencing results are shown in Figure 1, which this Fig. confirms the strain.

Figure 1: Image of gel to confirm the bacterial strains. The temperature gradient, Columns 4 and 6 is the highest expression. Columns from left to right include:
- Column 1: PCR product at Tm 50°.
- Column 2: PCR product at Tm 52°.
- Column 3: PCR product at Tm 54°.
- Column 4: PCR product at Tm 56°.
- Column 5: PCR product at Tm 58°.
- Column 7: PCR product at Tm 60°.
- Column 7: the ladder 10000 bp.

3.1. Purification of spores

Spores were purified using Pterson method. But for better quality and purification method was changed. Finally, purified spores were painted with Malachite Green dye. An example stained spores is shown in Figure 2.

3.2. Confirmation of nanoparticles size

To confirm the size classification of nanoparticles were taken SEM and TEM images. The photos showed that silver nanoparticles with a diameter of 20±5 nm, and copper nanoparticles with a 10-30 nm.

Figure 2: Purification spore and painted by Malachite Green method.

Figure 3: SEM and TEM images of nanoparticles (right, silver oxide and left copper oxide).
3.3. Sporecidal properties of nanoparticles
3.3.1. The MIC / MBC ratio of nanoparticles

The minimum inhibitory concentration, minimum concentration to inhibit growth of bacteria and spores was to for both copper and silver nanoparticles to the bacteria. That result for both copper and silver nanoparticles show in Table 2. As specified in this table, Than MC/MBC ratio for silver nanoparticles is 0/1, but this rata not be calculated for the copper nanoparticles. Copper nanoparticles not sporecidal property But in 1 molar concentration of Cu have a sporestatic properties.

Table 2: Results of the MIC/MBC ratio.

<table>
<thead>
<tr>
<th>The type of Microbe</th>
<th>type of Nano particle</th>
<th>MBC Concentration</th>
<th>MBC Concentration</th>
<th>MIC/MBC Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Effect on Spore</td>
<td>AgO</td>
<td>0/1 M</td>
<td>0/1 M</td>
<td>0/1</td>
</tr>
<tr>
<td></td>
<td>CuO</td>
<td>1 M</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Effect on Bacteria</td>
<td>AgO</td>
<td>0/001 M</td>
<td>0/005 M</td>
<td>0/2</td>
</tr>
<tr>
<td></td>
<td>CuO</td>
<td>0/0005 M</td>
<td>0/01 M</td>
<td>0/5</td>
</tr>
</tbody>
</table>

Phenol coefficient for silver nanoparticles is 52, that this indicated AgO nanoparticle is good sporecidal property (Table 2).

Phenol coefficient for silver nanoparticles = 8000/154 = 52

The second formula shown sporecidal power of AgO nanoparticles in 0/01 M (the critical concentration) is about 79 percent. But this power for copper nanoparticles in 1 M (critical concentration) is about 49 percent. Therefore, sporecidal power of silver oxide nanoparticles is high; therefore, these nanoparticles are introduced into an appropriate sporecidal. D value we obtained for the sporecidal power of the Silver oxide nanoparticles. Concentration of nanoparticles in the specified time and temperature specified Reduced 90% Primary germ is D value. Therefore, the concentration ratio was investigated at 36 0C to 0/01 M silver nanoparticles. That was about 7 minutes.

D36 = 7 min

Discussion

The strong connection of the outer membrane of nanoparticles to prevent the transfer agent dehydrogenase, activity of periplasmic enzymes in spores, and preventing the RNA, DNA and synthesized of proteins. That ultimately leads to cell lyses. In this paper, the performance was shown for the spores of clostridium botulinum type E (native in Iran) [11]. Some factors such as the minimum time, the MIC/MBC, phenol coefficient, D value were calculated. This factor is necessary and appropriate for the treatment of infections caused by spore clostridium. Nanoparticles such as nano-particles of silver and copper can be used as a preventive of building damage, casualties and a suitable replacement for many disinfectants. This alternative is affordable also economically [12]. The many results reported of reactions between particles with biological macromolecular. The contrast between the negative charges of microorganisms with a positive charge of nano-particle is created an electromagnetic attraction between the microbe and effective levels of active nano-particle. Finally, a large number of contacts lead to oxidation of surface molecules of microbes and the immediate cause of death. Metallic silver and copper oxides to that show biodestructive effects, such as degradation of DNA [13-14]. All toxicity tests of AgO and CuO nanoparticles on Gram-positive bacteria, immune cells and bacteria in human toxicity of nanoparticles of Ag and Cu suggests that for a variety of biological systems [15].

The release of nonmaterial reacted with thiol groups (-SH) cell surface proteins in bacterial cell and perhaps in spores. Nano-materials cause of inactivation and impermeable in the membranes protein of bacteria [16-17]. Carbonic acid or phenol is used as a standard to determine the germicidal effects. The fact, that the phenol coefficient of disinfectants and disinfection with phenol how much power is dramatic. How phenol coefficient is much, the sanitizer is stronger and microorganisms are destroyed in 10 minutes earlier. In fact, the matter is diluted more antiseptic than phenol.

We MIC/MBC test results confirm the silver nanoparticles have sporecidal property stronger than copper nanoparticles. Perhaps, the high power sporecidal of silver is effect on the metabolism of bacteria, stimulated of hydrogen peroxide. Nanoparticles have a relatively large surface area and have more contact with the outdoors. That resulted is high sporecidal or sporestatic property.

Results

These results showed that copper and silver nanoparticles to inhibit the growth of spores are high Capability. It is hoped in future to prevent the nanoparticles in the treatment of diseases caused by pathogenic bacteria causing.

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References