Effect of Cadmium Chloride (CdCl₂) on Cytoplasmic Shuttle Streaming, Structure, Growth and Migration of the Plasmodial Slime Mold Physarum polycephalum

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ABSTRACT

The effect of different concentrations of cadmium chloride on cytoplasmic shuttle streaming, growth, structure and migration of plasmodial strand of Physarum was investigated. Concentrations <5.00 mM CdCl₂ had no obvious effect on the studied parameters. Concentrations between 7.50 - 20.0 mM CdCl₂ induced disturbance in cytoplasmic streaming, depigmentation, blebbing, vacuolization, very slow growth and slow migration ability. Higher concentrations of >25.0 mM CdCl₂ induced direct and rapid stop of cytoplasmic streaming followed by complete fixation of the treated plasmodia (neither growth nor migration occurred on nutrient agar plates).

KEYWORDS: Cadmium chloride, Shuttle streaming, Growth, Migration, Lethal dose, Physarum polycephalum.

1. INTRODUCTION

The last few years gave direct attention to studies concerning the effect of toxic heavy metals found in the ecosystem. Those toxic metals came to the ecosystem from various resources of pollution such as smoking, cars' buffs, sewage, fires and variable resources that use those toxic metals in the manufacturing processes. Cadmium is one of these toxic metals that can enter our bodies with the food, water or breathing in cigarette smoke. CdCl₂ has very toxic effects, especially on human tissues and organ functions such as: kidneys (Jarup et al., 2000), respiratory system (Grasseschi et al., 2003 and Jarup et al., 2000) and aortas of smokers(Abu-Hayyeh et al., 2001). In addition, it is the cause of human pancreatic cancer (Schwartz and Reis, 2000) and prostate cancer (Achanzer et al., 2001). Cd also induces change in growth and oxidative metabolism of some plants (Sandalio et al., 2001). CdCl₂ has a chronic effect that may reach the lethal limit.

To study the cytotoxicity of chemical compounds , the migrating plasmodium stage of Physarum polycephalum is a very suitable system. This organism shows a regular reversible shuttle streaming activity. It migrates on solid substratum and exhibits a characteristic internal structural organization. The aim of this research was to study the effect of different concentrations of cadmium chloride (CdCl₂) on Physarum polycephalum and particularly on:

C- Growth. D-Migration.
E- Viability.

2. MATERIALS AND METHODS

The Study Object:
Phaneroplasmodium of Physarum polycephalum (ATCC 44912) was used in this study.

Effect of Test Solutions on Shuttle Streaming Periodicity:
A small piece of Physarum planeroplasmodium was starved on 1.5% non-nutrient agar media. Starved Planeroplasmodium was submerged in a Physiological Salt Solution (PSS) solution for 10 min. PSS consists of: 6.0 mM NaCl, 3.0 mM KCl,1.0 mM CaCl₂, 0.1 mM NaHCO₃ and 0.5 mM MgCl₂. PSS keeps the plasmodium in a good condition because it resembles the plasmodial internal environment and protects it against desiccation.

Submerged plasmodia in PSS were observed under
light microscope and shuttle streaming periods were recorded. Variable test solutions and different concentrations of CdCl$_2$ were added to the previous plasmodium in succession and observations of shuttle streaming were recorded successively. PSS recorded values (control) were compared with the test values and structural changes were recorded.

**Effect of Test Solutions on Growth of Physarum Plasmodium:**
Corn-agar plates were used for growth test. Corn-agar medium consists of: 6.0 gram rolled oats+1.0 gram D-glucose+7.5 gram agar-agar +500ml distilled water. Contents were mixed, sterilized in autoclave at 121°C for 20 min and poured in sterile plates. Petri dishes were prepared including growth media control and successive concentrations of CdCl$_2$ which were added to the growth medium. Small pieces of *Physarum* plasmodia were allowed to grow on medium surface and growth was observed and sketched at intervals of 1h.

**Effect of Test Solutions on Migration of Plasmodium:**
1.5% non-nutrient agar medium was used for migration test. Petri dishes were prepared including 1.5% non-nutrient agar media (control) and successive concentrations of CdCl$_2$ which were added to 1.5% non-nutrient agar medium (migration test). Then small pieces of *Physarum* plasmodia were added, allowed to migrate on the surface of the agar plates. Migrating plasmodia were sketched at intervals of 1h.

### 3. RESULTS AND DISCUSSION

In studying the effect of CdCl$_2$ on *Physarum* polycephalum plasmodia, four criteria were taken into consideration:
1. Microscopic responses of whole plasmodium (*In vivo*).
2. Effect on plasmodial migration.
3. Effect on plasmodial structure and shuttle streaming periodicity.
4. Effect on plasmodial growth.

**1. Microscopic Responses of Whole Plasmodium:**
Acellular slime molds or myxomycetes are strange groups of microorganisms. They show plant and animal like characteristics. A cellular slime molds live in moist cold areas of forests, dead leaves, and barks of dead and living trees. One species of myxomycetes (order physarales) was isolated from Debbein forests (Shraideh, 1988) and was described as a studying model in this research.

*Physarum* is characterized by having a phagotrophic somatic phase (Phaneroplasmodium). It is a yellowish, creeping, multinucleated mass of protoplasm enveloped by a slimy sheath and is differentiated into two main regions, a massive front and a posterior network of connected veins. The effects of different concentrations of CdCl$_2$ on whole phaneroplasmodia migrating on agar surface were summarized in table (1), which shows the sequence of events that occurred after treating whole plasmodia with CdCl$_2$.

Dissociation of endoplasmic reticulum into vacuoles and the disruption of the plasma membrane and subsequent depigmentation of the CdCl$_2$-treated plasmodium was obvious. These results are in accordance with results of Stoica *et al.* (2000).

<table>
<thead>
<tr>
<th>Concentration (mM)</th>
<th>Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.0</td>
<td>Plasmodium has good migration ability. Darkness and vacuolization of plasmodium was observed.</td>
</tr>
<tr>
<td>7.5-10.0</td>
<td>Migration ability of plasmodium was observed. Vacuolization, depigmentation and contraction of the frontal region occurred after 2 hours of treatment.</td>
</tr>
<tr>
<td>15.0-20.0</td>
<td>Very little migration ability. Condensation of the whole plasmodium. Release of pigments, decolorization of plasmodia.</td>
</tr>
<tr>
<td>25.0</td>
<td>No migration ability. Direct and complete fixation of plasmodia. Condensation, darkening and obvious depigmentation of plasmiodium was observed after 2 hours of treatment.</td>
</tr>
</tbody>
</table>
2. Effect of CdCl₂ on Plasmodial Migration:

The effect of different concentrations of CdCl₂ on migration ability of Physarum plasmodia on non-nutrient agar was investigated. The migration of the plasmodia was followed overnight and sketched at intervals of 1 h. Results are shown in Figure 1. Plasmodia transferred into CdCl₂-media were compared to those on control media. Results showed that in B1 (Plasmodium transferred into 0.50 mM CdCl₂ media) there were no obvious changes on migration, in B2, B3 and B4 (Plasmodium transferred into [0.75, 1.00 and 1.50 mM CdCl₂] media). Results showed gradient decrease in migration ability, in B5 (Plasmodium transferred into sublethal dose [2.00 mM CdCl₂] media). Results showed very short distance migration ability, while in B6 (Plasmodium transferred into lethal dose [2.50 mM CdCl₂] media). Results showed no migration ability at all. The inhibitory effect of CdCl₂ on cell migration can be explained by the blocking effect of Cd on ATPase discussed by Abu-Hayyeh et al. (2001).

3. Effect of CdCl₂ on Plasmodial Structure and Shuttle Streaming Periodicity:

Protoplasm in the veins of Physarum exhibits reversible or regular shuttle streaming with period duration of approximately 1.3-3.0 min. (Shraidheh, 1988). The protoplasm moves in one direction inside the endoplasm for a short while, stops and then turns back for a while and so on. Streaming protoplasm includes vacuoles, nuclei, vesicles and some other organelles.

Table 2 summarizes the effect of CdCl₂ on shuttle streaming periodicity. The effects of sublethal dose (20 mM) of CdCl₂ were also investigated on the structure and behaviour of treated phaneroplasmodia (Fig.2). The ectoplasm consists of plasmalemma which represents about 80% of volume from which labyrinth of invagination forming vesicles are pinched off to the inside. Contractile vacuoles and other kinds of vacuoles are present. The endoplasm, which is the central part is less viscous and includes streaming nuclei, mitochondria, ribosomes, pigment granules, vacuoles and other organelles. After half an hour of the addition of the lethal doses, only vacuolization occurred. After 1 hour darkening and vacuolization occurred. After 1.25 hour of addition of lethal doses high vacuolization, blebbing and paused shuttle streaming. Vacuolization, disruption and blebbing of plasma membrane and irregularity of shuttle streaming caused by Cd can be explained by results obtained by Sandalio et al. (2001). They found that Cd induced changes in lipid composition and affected the enzymatic actives associated with membranes i.e H⁺-ATPase. By comparing results with the control [A], it was found that the lethal dose of CdCl₂ caused complete fixation and disruption of the plasmodium after 1.5 hours of treatment.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Period Mean ± SEM</th>
<th>Percentage</th>
<th>n</th>
<th>N</th>
<th>Observations</th>
</tr>
</thead>
<tbody>
<tr>
<td>PSS</td>
<td>2.77±0.02 min</td>
<td>100.0%</td>
<td>30</td>
<td>3</td>
<td>Regular.</td>
</tr>
<tr>
<td>5.0mM</td>
<td>2.78±0.03 min</td>
<td>100.36%</td>
<td>30</td>
<td>3</td>
<td>Very small effect that is not obvious in shuttle streaming period.</td>
</tr>
<tr>
<td>7.5mM</td>
<td>3.06±0.05 min</td>
<td>110.5%</td>
<td>20</td>
<td>2</td>
<td>Increase in shuttle streaming period. Little vacuolization.</td>
</tr>
<tr>
<td>10mM</td>
<td>3.18±0.21 min</td>
<td>114.8%</td>
<td>10</td>
<td>2</td>
<td>Obvious increase in shuttle streaming period and vacuolization.</td>
</tr>
<tr>
<td>15mM</td>
<td>3.75±0.28 min</td>
<td>135.4%</td>
<td>6</td>
<td>2</td>
<td>Blebbing and disturbance occurred in large veins. Difficulty to monitor the shuttle-streaming period.</td>
</tr>
<tr>
<td>20mM Sublethal dose</td>
<td>-----------</td>
<td>-----------</td>
<td>---</td>
<td>5</td>
<td>Small veins were disrupted. Irregular streaming with blebbing in large veins.</td>
</tr>
<tr>
<td>25mM Lethal dose</td>
<td>-----------</td>
<td>-----------</td>
<td>---</td>
<td>5</td>
<td>Complete fixation of plasmodium and decolorization. Viability test showed no growth (irreversible effect).</td>
</tr>
</tbody>
</table>

n: number of periods analyzed.
N: number of plasmodia used.
Percentage: (Mean of the treated / Mean of the control) x 100%
SEM: Standard error of the mean.
4. Effect of CdCl₂ on Plasmodial Growth:

The effect of different concentrations of CdCl₂ on growth of phaneroplasmodia on corn-agar medium was investigated and reported as sketches (Fig 3). Growth of plasmodial pieces transferred onto CdCl₂-containing media were compared to the growth of control (grown on control media). Results showed that in B1 (Plasmodium transferred into 5 mM CdCl₂ growth media), there were no obvious changes in growth, in B2, B3, B4 (Plasmodium transferred into [7.5-15mM CdCl₂], growth media) results showed gradient decrease in growth ability, in B5 (Plasmodium transferred into sublethal dose [20mM CdCl₂] growth media) results showed a very little growth ability, while in B6 (Plasmodium transferred into lethal dose [25mM CdCl₂] growth media), the result showed no growth ability at all. The inhibitory effect of CdCl₂ on growth of Physarum plasmodia is in accordance with the results obtained by Sandalio et al. (2001) who discussed cadmium-induced change in the growth and oxidative metabolism of pea plant.

Acknowledgment

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Fig 1. Sketches showing migration ability of plasmodia treated with CdCl₂.

P₀: Codes for plasmodium at zero time.
P₂: Codes for plasmodium after 2 hours.
P₃: Codes for plasmodium after 3 hours.
P₅: Codes for plasmodium after 5 hours.
P₈: Codes for plasmodium after 8 hours.
Y: Yellow color caused by depigmentation.

A- Plasmodium transferred into 1.5% agar media (Control).

B- Plasmodium transferred into media containing gradient concentrations of CdCl₂:
   B₁- Plasmodium transferred into 0.50 mM CdCl₂ media.
   B₂- Plasmodium transferred into 0.75mM CdCl₂ media.
   B₃- Plasmodium transferred into 1.00mM CdCl₂ media.
   B₄- Plasmodium transferred into 1.50mM CdCl₂ media.
   B₅- Plasmodium transferred into 2.00mM CdCl₂ media (Sublethal dose).
   B₆- Plasmodium transferred into 2.50mM CdCl₂ media (Lethal dose).
A

B1

B2
Fig 2. Timelapse photomicrographs showing effect of adding CdCl₂ sublethal concentration (20 mM) on the shuttle streaming and structure of phaneroplasmodium.

A-Control plasmodium (PSS solution):
A1: After half an hour.
A2: After 1.00 hour.
A3: After 1.15 hour.
A4: After 1.30 hour.

B-Plasmodium treated with CdCl₂ sublethal concentration (20 mM):
B1: After half an hour.
B2: After 1.00 hour.
B3: After 1.15 hour.
B4: After 1.30 hour.
Fig 3. Sketches showing growth ability of plasmodia treated with CdCl₂.

P0: Codes for plasmodium at zero time.
P2: Codes for plasmodium after 2 hours.
P3: Codes for plasmodium after 3 hours.
P4: Codes for plasmodium after 4 hours.
P6: Codes for plasmodium after 6 hours.
P8: Codes for plasmodium after 8 hours.
Y: Yellow color caused by depigmentation.

A-Plasmodium transferred into corn agar medium (Control).

B-Plasmodium transferred into growth media containing gradient concentrations of CdCl₂:
   B1-Plasmodium transferred into 0.50 mM CdCl₂ growth media.
   B2-Plasmodium transferred into 0.75mM CdCl₂ growth media.
   B3-Plasmodium transferred into 1.00mM CdCl₂ growth media.
   B4-Plasmodium transferred into 1.50mM CdCl₂ growth media.
   B5-Plasmodium transferred into 2.00mM CdCl₂ growth media (Sublethal dose).
   B6-Plasmodium transferred into 2.50mM CdCl₂ growth media (Lethal dose).
4. REFERENCES


