

斑蝥素对草地贪夜蛾Sf9细胞膜完整性和膜电位的影响

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Effect of cantharidin on cell membrane integrity and potential in *Spodoptera frugiperda* Sf9 cells

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- 摘要
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摘要 为明确斑蝥素对昆虫细胞膜的作用及其机理, 本研究利用草地贪夜蛾*Spodoptera frugiperda*的卵巢细胞系Sf9细胞作为实验材料, 采用透射电子显微技术(transmission electron microscope, TEM)、激光共聚焦显微镜(laser scanning confocal microscope, LSCM)结合荧光探针FDA/PI及DiBAC4(3)技术研究斑蝥素(cantharidin, CTD)对Sf9细胞膜完整性及膜电位(membrane potential, MP)的影响。结果表明: 32 $\mu\text{mol/L}$ CTD处理6 h和12 h后, 电镜观察均未发现细胞膜结构破损; FDA/PI染色后, 32 $\mu\text{mol/L}$ CTD处理0.5 h后细胞FDA荧光强度比对照显著降低($P<0.05$), 碘化丙啶(propidium iodide, PI)染色的细胞比例与对照无显著性差异($P\geq 0.05$)。32 $\mu\text{mol/L}$ CTD处理140 s后即引起MP发生显著性去极化($P<0.05$); 64 $\mu\text{mol/L}$ CTD处理瞬时MP发生显著性去极化($P<0.05$); 32 $\mu\text{mol/L}$ CTD处理3 h内及64 $\mu\text{mol/L}$ CTD处理2 h内MP仍保持显著性去极化($P<0.05$), 之后去极化程度降低; 32 $\mu\text{mol/L}$ CTD处理6 h及64 $\mu\text{mol/L}$ CTD处理3 h时MP去极化与对照组相比已无显著性差异($P\geq 0.05$)。结果说明, CTD处理短时间内可引起Sf9细胞膜电位去极化并维持一段时间, 同时导致细胞活性发生不可逆下降, 但未对细胞膜结构完整性产生破坏。

关键词: 草地贪夜蛾 斑蝥素 Sf9细胞 膜完整性 膜电位 去极化

Abstract: To investigate the effect of cantharidin (CTD) on insect cell membrane and its mechanism, the cell membrane integrity and potential in *Spodoptera frugiperda* Sf9 cells treated with CTD were tested by transmission electron microscope (TEM) and laser scanning confocal microscope (LSCM) combined with fluorescent dyes FDA/PI and DiBAC4(3). The results showed that after the Sf9 cells were treated with 32 $\mu\text{mol/L}$ CTD, their membrane structure was not disrupted at 6 h and 12 h after treatment, respectively. The FDA fluorescence intensities of the treated cells evidently declined compared with the control ($P<0.05$) while the proportion of the PI-staining cells was not significantly different from that of the control ($P\geq 0.05$) at 0.5 h after treatment under TEM observations. The membrane potential (MP) in Sf9 cells was depolarized significantly both at 140 s after treatment with 32 $\mu\text{mol/L}$ CTD and in the instantaneous treatment with 64 $\mu\text{mol/L}$ CTD ($P<0.05$). The MP in Sf9 cells depolarized obviously within 3 h after treatment with 32 $\mu\text{mol/L}$ CTD or 2 h with 64 $\mu\text{mol/L}$ CTD ($P<0.05$), and then the degree of depolarization declined. At 6 h after treatment with 32 $\mu\text{mol/L}$ CTD or at 3 h with 64 $\mu\text{mol/L}$ CTD, MP depolarization in treated cells was not significantly different from that in the control ($P\geq 0.05$). The results suggest that CTD causes the depolarization of cell membrane potential in Sf9 cells, which will maintain for a period of time, and induces an irreversible decline in cell activity, but not destroys the integrity of the cell membrane structure.

Key words: *Spodoptera frugiperda* cantharidin Sf9 cells membrane integrity membrane potential; depolarization

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