

Meeting abstract

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Involvement of ethylene, oxidative stress and lipid-derived signals in cadmium-induced programmed cell death in tomato suspension cells

Elena Iakimova*¹, Veneta Kapchina-Toteva², Anke de Jong³,
Atanas Atanassov¹ and Ernst Woltering³

Address: ¹AgroBioInstitute, Sofia, Bulgaria, ²Faculty of Biology, University of Sofia, Bulgaria and ³Agrotechnology and Food Innovations, Wageningen University and Research Center, Wageningen, The Netherlands

Email: Elena Iakimova* - elena_iakimova@abi.bg

* Corresponding author

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Background

Extensive research is ongoing looking for the characterisation of programmed cell death (PCD) in plants involving pathogen attack, chemical elicitation and abiotic inducers, but there are still limited reports on the role of heavy metals in PCD induction and little is known about cadmium-triggered signal transduction in plant systems. Contamination of biosphere with heavy metals has hazardous effect on agricultural crops and human health. In animal models, cadmium intoxication occurs through apoptosis appearing by apoptotic phenotype and an oxidative stress is involved in the mechanism of Cd action. The goal of this present work was to investigate if programmed cell death occurs in cadmium-treated tomato suspension cells; to identify some of the biochemical processes contributing to the signal transduction pathway(s) involved in cadmium toxicity; to investigate the role of oxidative stress (hydrogen peroxide), ethylene and lipid-derived signals and to look for similarities between cadmium- and camptothecin-induced cell death.

Materials and methods

The experiments were undertaken with tomato suspension cells, line Msk8. Specific inhibitors of different biochemical steps were administrated simultaneously with either CdSO₄ or topoisomerase-1 inhibitor camptothecin (CPT). Cell viability (FDA staining of the viable cells) was determined after 24 hours and the dynamics of H₂O₂ production was measured by chemiluminescence in a ferricyanide-catalysed oxidation of luminol. Specific caspase peptide inhibitors, antioxidants, NADPH oxidase inhibi-

tors, calcium channel blockers, inhibitors of phospholipid cycle, protein kinase inhibitor and ethylene blockers were tested. Ethylene was applied during 24 h in concentrations up to 100 ppm in the head space. For details on methodology see [1,2].

Results

The human caspase-1 inhibitor Ac-YVAD-CMK and the broad range caspase inhibitor Z-Asp-CH₂-DCB, abolished the cell death of Cd-treated and CPT-treated cells (See Table 1). This strongly suggests that the cell death pathway that is induced by cadmium employs caspase-like proteases and gives a reason to assume that in tomato suspension cells Cd-triggered cell death most probably resembles features of programmed cell death. The amount of hydrogen peroxide increased in response to Cd and CPT. Efficient inhibition of cell death occurred at the application of antioxidants and calcium channel blocker (See Table 1). The inhibition of NADPH oxidase by imidazole, quercetin and kaempferol significantly reduced the percentage of dead cells.

Treatments with ethylene further decreased both Cd- and CPT-reduced cell viability. Comparative experiments with Cd- or CPT-treated cells revealed an analogy in cell response to the ethylene inhibitor AVG (See Table 2). AVG greatly reduced the cell death that was enhanced in response to Cd or CPT. An increase of endogenous ethylene production (measured by laser photoacoustics) occurred in cadmium-treated cells. The data are a clear demonstration of ethylene involvement in Cd- and CPT-

Table 1: Effect of caspase peptide inhibitors, antioxidants (ascorbic acid, catalase, spermine) and calcium channel blocker LaCl₃ on viability of CPT- or Cd-treated tomato suspension cells

Chemicals	Cell viability (%)
Control	97.5
CPT 5 μ M	72.5
CdSO ₄ 100 μ M	65.0
CPT μ M + Ac-YVAD-CMK 100 μ M	92.5
CdSO ₄ 100 μ M + Ac-YVAD-CMK 100 μ M	94.0
CdSO ₄ 100 μ M + Z-asp-CH2-DCB 100 μ M	91.5
CPT 5 μ M + ascorbic acid 100 μ M	93.5
CPT 5 μ M + catalase 10 Units/ml	91.5
CPT 5 μ M + spermine 100 μ M	88.5
CdSO ₄ 100 μ M + ascorbic acid 100 μ M	92.5
CdSO ₄ 100 μ M + catalase 10 Units/ml	91.5
CdSO ₄ 100 μ M + spermine 100 μ M	88.2
CPT + 5 μ M + LaCl ₃ 100 μ M	95.0
CdSO ₄ 100 μ M + LaCl ₃ 100 μ M	94.5

Table 2: Effect of ethylene and ethylene inhibitor AVG on cell viability of CPT and cadmium treated tomato cell suspension

Chemicals	Cell viability (%)
Control	95
CPT 5 μ M	72
CdSO ₄ 100 μ M	68
AVG 10 μ M	98
Ethylene (Eth) 100 μ L/L	95
CPT 5 μ M + Eth 100 μ L/L	50
CdSO ₄ 100 μ M + Eth 100 μ L/L	48
CPT 5 μ M + AVG 10 μ M	90
CdSO ₄ 100 μ M + AVG 10 μ M	86
CPT 5 μ M + AVG 10 μ M + Eth 100 μ L/L	38
CdSO ₄ 100 μ M + AVG 10 μ M + Eth 100 μ L/L	49

triggered cell death. Administration of IP₃ cycle inhibitors showed a strong inhibition to Cd-induced cell death.

Conclusion

Evidence is accumulating that caspase-like cysteine proteases showing functional similarity to animal caspases, participate in the programmed cell death in plants. In addition to discoveries that caspase-like proteases are involved in cell death in response to pathogen invasion, abiotic stresses and chemical elicitation, our data show that cell death induced by cadmium is also a form of programmed cell death mediated by caspase-like proteases. We have established a key role of hydrogen peroxide and calcium in cadmium-induced apoptotic cell death and have demonstrated that oxidative stress is associated with both cadmium and camptothecin-triggered cell death. We

have also shown that polyamine spermine can effectively preserve the cell viability at conditions of chemical stress.

Ethylene was found to be an important mediator of plant cell death. The finding that ethylene greatly stimulated cadmium-induced cell death and that cadmium treatment enhanced endogenous ethylene production indicated that ethylene participates in cadmium-induced cell death in tomato suspension cells. The application of specific inhibitors of phospholipase C, phospholipase D, inositolphosphate monophosphatase, inositol-3-phosphate kinase and phosphatidic acid caused considerable decrease of Cd-stimulated cell death and are the first more detailed evidence that Cd-triggered cell death in plants involves the phospholipid pathway.

Collectively, the cell response to cadmium elicitation and the inhibitors indicate that Cd-triggered cell death is analogous to cell death in response to CPT treatment [1-4] and involves caspase-like proteases, oxidative stress and ethylene. Cd-induced cell death in plant cells exhibits similarities to HR [5] and cell death induced by known apoptosis inducing chemicals and to its effect in animal systems.

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