분자지표를 이용한 농약의 독성평가 및 위해성평가 적용

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Carbofuran (CF) is one of the most widely used carbamate pesticide in the world applied to insect and nematode control. Due to its widespread use in agriculture and households, contamination of food, water, and air has become serious, and consequently adverse health effects are inevitable in humans, animals, wildlife and fish. It has been reported that CF alone or in combination with other carbamate insecticides influences the level of reproductive and metabolic hormones such as thyroxine and corticosterone, and results in impairment of endocrine, immune and behavioral functions. In the present study, we evaluated the effects of CF on genotoxicity, cytotoxicity and immunotoxicity in CHL cells and activated mouse splenic T cells.

CF was neither genotoxic nor cytotoxic in CHL cells at all concentrations tested. However, the N-nitroso metabolite of CF, N-nitrosocarbofuran (NOCF) induced genotoxicity determined by Ames test. NOCF inhibited the growth of Chinese hamster lung fibroblast (CHL) cells with IC50 of 12.8 M. NOCF induced apoptosis of CHL cells, which was demonstrated by morphological changes, DNA fragmentation and flow cytometric analysis. Treatment of CHL cells with NOCF induced significant G2/M cell cycle arrest. Caspase-3, an executioner of apoptosis was also activated by the treatment of CHL cells with NOCF. Caspase 8 was not activated by the treatment of NOCF. Bcl-2 protein level was decreased without any change in the Bax resulting in the overall decrease in the Bax/Bcl-2 ratio.

CF inhibited mitogen-induced IL-2 production and IL-2-induced T cell proliferation in dose-dependent manner. Interestingly, when T cells were stimulated with mitogen in the presence of CF, IFN-γ production was significantly suppressed, whereas IL-4 production was not affected. Similar effects were observed in alloantigen-stimulated T cells generated in allogeneic mixed lymphocyte reactions (MLR) in which dendritic cells (DC) were used as stimulator cells. Furthermore, when the production of bioactive form of IL-12 playing a major role in T cell skewing to Th1 subtypes was examined in LPS-stimulated dendritic cells, it was significantly decreased by CF treatment, suggesting that CF could selectively affect differentiation of T helper cells with Th1, but not Th2 phenotype.

These results suggest that NOCF is an important metabolite of CF leading to the induction of cell cycle arrest and apoptosis in CHL cells. CF might inhibit T cell-mediated immune responses through the suppression of T cell responsiveness to antigenic stimulation as well as differentiation into Th1 subtype.

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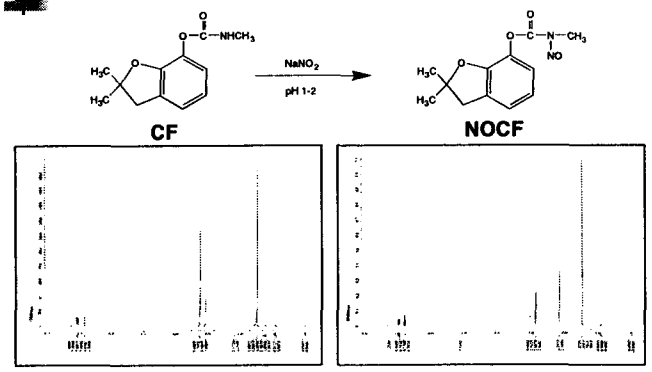
Carbofuran

- One of the most widely used carbamate pesticide in the world
- Applied to insect and nematode control
- Reversible inhibitor of AChE
- Toxic to mammals through oral & inhalation
  - LD_{50} = 2 (mice) ~ 19 (dog) mg/kg
- One of the most rapidly degradable insecticides metabolized to 3-OH CF (persistent) and 3-keto CF

Scope of the Study

- Genotoxicity & Cytotoxicity of CF
- Genotoxicity & Cytotoxicity of NOCF
- Immunotoxicity of CF
- Application to Risk Assessment

Nitrosation of Carbofuran



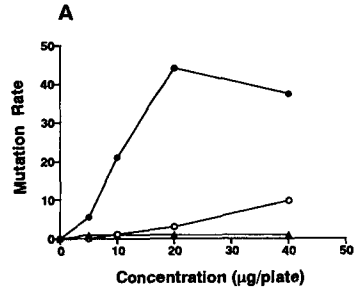
Endogenous N-Nitrosation of Carbofuran

- Chemically in acidic conditions (pH 1-2)
  - in stomach (rat < hamster)
  - in the presence of nitrate, nitrite
  - more than 2% of the dose is found as NOCF
- Biologically by intestinal bacteria
  - Nitrosation does not occur in germ-free rats

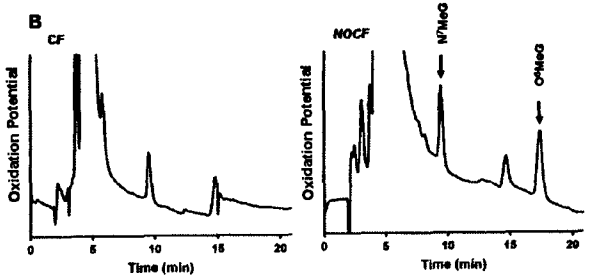
Toxicity of NOCF

- Loss of AChE inhibiting property
- Mutagenic
- Induction of CA, SCE and MN formation
- Direct acting mutagen and carcinogen
- Increase of the *hprt* locus mutation in CHL
- Induction of transition from GC to AT

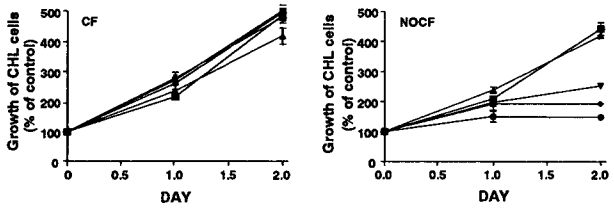
Mutagenicity of NOCF



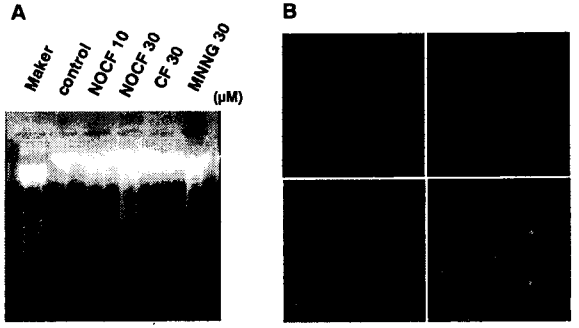
DNA Adduct Formation by NOCF

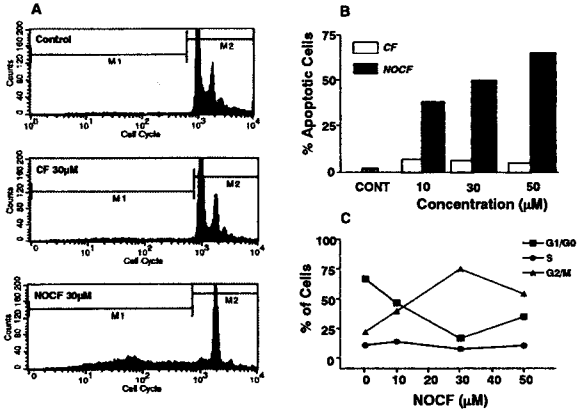


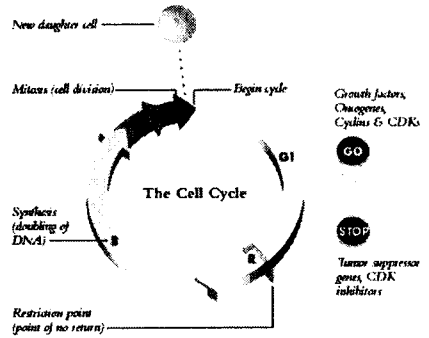
Cytotoxicity of NOCF

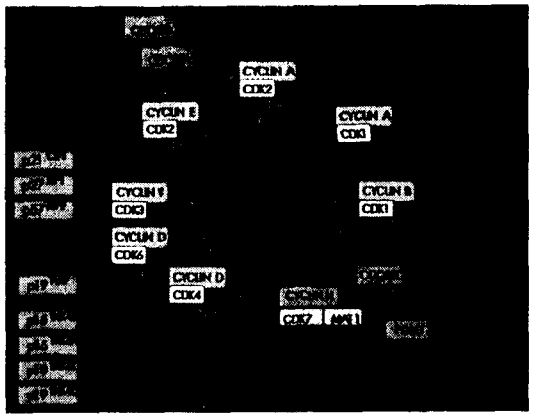


Apoptosis by NOCF

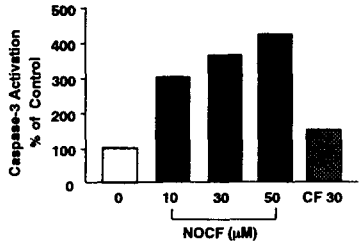








Caspase 3 Activation by NOCF



Western Blot Analysis

Conclusions

NOCF, but not CF.....

- induced the formation of O^6- and N^7-meG
- was mutagenic in *S. typhimurium* TA100
- inhibited the growth of CHL cells (IC_{50}=12.8 μM)
- induced apoptosis & G_2/M arrest of CHL cells
  - DNA ladder, flow cytometry, morphology, TUNEL
- activated caspase-3 protease
- decreased Bcl-2 expression

Immunotoxicity of Carbofuran

Immunotoxicity of Carbofuran

- Significant increase in total white blood cell count (neutrophil, basophil, lymphocyte)
- Bone marrow depression and splenic hyperplasia in mice
- Transient and marked inhibition of PFC response
- Inhibition of the IL-2 dependent proliferation of CTLL-2 cells in virto

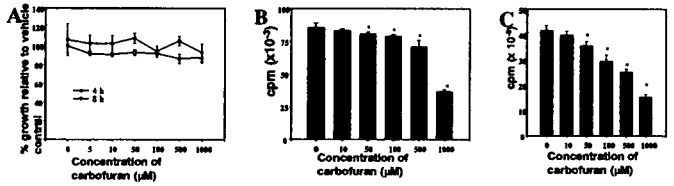
Aim of the Study

To elucidate the mechanisms of CF-induced suppression of cell-mediated immune responses (CMIR)

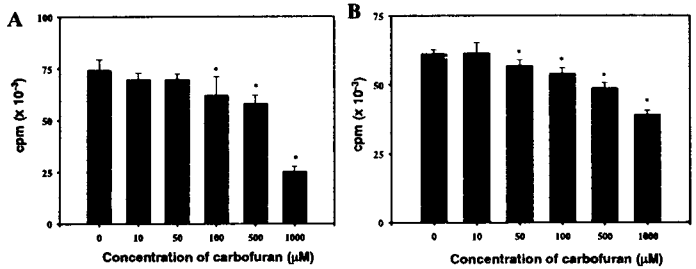
Experimental Methods

- MTT assay
- Cell proliferation assay
- Determination of IL-2, IL-4 and IFN-γ released using bioassay and ELISA
- Dendritic cell (DC) generation and determination of DC phenotypes
- T cell proliferation in mixed lymphocyte reactions
- Determination of IL-12 released by stimulated DC using ELISA

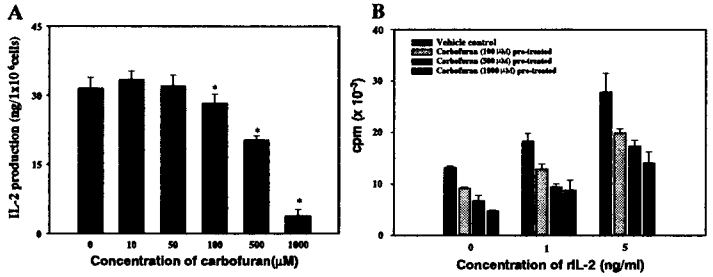
Proliferation of Splenocytes



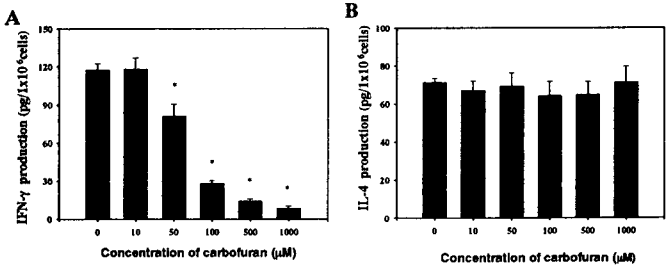
ConA or Anti-CD3 mAb Induced Proliferation of Splenic T cells



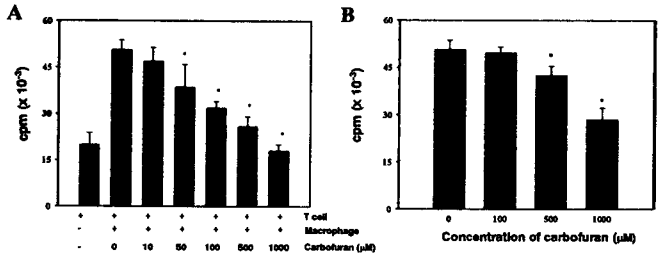
IL-2 Production & IL-2 induced DNA Synthesis



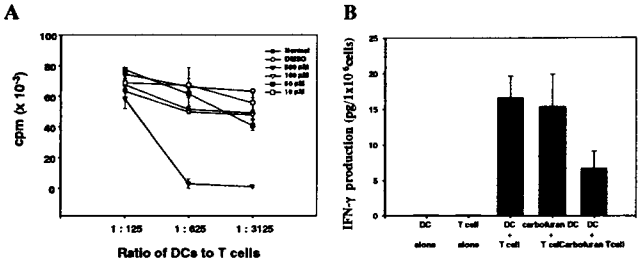
IFN-γ & IL-4 Production



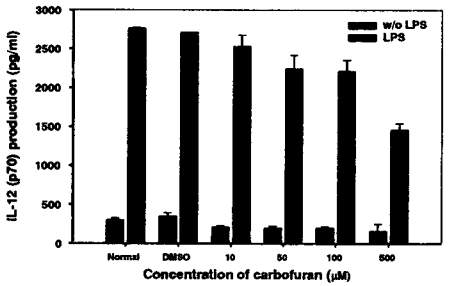
ConA-Induced Splenic T cell Proliferation



Mixed Leukocyte Reaction & Antigen Presentation



IL-12 Production by DC Stimulated with LPS



Summary

- Mitogen- or anti-CD3 antibody-induced proliferation of splenocytes was significantly suppressed
- Con A-induced IL-2 production of splenic T cells was also inhibited
- the addition of exogenous IL-2 does not restore the Con A-induced T cell proliferation
- decreased the production of IFN-g, but not IL-4, by Con A-stimulated splenic T cell
- decreased the ability of antigen-presenting cells (APC) including macrophages and dendritic cells to stimulate syngenic or allogenic T cells when pretreated
- affects Th1 cell functions stimulated by mitogens or antigens more profoundly than APC functions, although IL-12 production of LPS-stimulated dendritic cells was significantly decreased