MECHANISMS OF IMMUNOSUPPRESSION BY ORGANOTINS – APOPTOSIS vs PROLIFERATIVE ARREST.

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The organotin compounds di-n-butylin dichloride (DBTC) and tri-n-butylin chloride (TBTC), used as stabilizers and biocides respectively, induce thymus atrophy inhibiting immature thymocyte proliferation. The aims of the study were to examine whether apoptosis has a role in this atrophy and whether DBTC, like TBTC, induces apoptosis in *in vitro* Thym from rats treated with a dose (15 mg/kg) of organotin known to induce thymocyte proliferation, did not show DNA fragmentation, indicating that apoptosis is not evident in organotin-induced thymus atrophy at low doses. *In vitro*, data showed that 3-5 μM of DBTC or TBTC significantly increased the percentage of apoptotic nuclei in rat thymocytes. Further mechanistic studies indicated a relation between the cytotoxic effects of the compounds and their capacity to induce apoptosis. At lower concentrations than required to induce apoptosis, both organotins inhibited protein and DNA synthesis and increased RNA and heat shock proteins synthesis. We demonstrated that the increase of RNA synthesis occurred in small thymocytes, which comprised the same subset of cells sensitive to apoptosis by organotins. Moreover, co-exposure to DNA or protein synthesis inhibitors protected cells from apoptosis by DBTC or TBTC, indicating that macromolecular synthesis is required for the initiation of the process. Besides effects on macromolecular synthesis, organotins disrupt energy metabolism and affect mitochondria. Previously, TBTC has been shown to increase intracellular calcium level, to produce reactive oxygen species (ROS) and to release pro-apoptotic factors. We showed similar changes in case of DBTC, i.e., increase of calcium, ROS production, release of cytochrome c and activation of caspase 3. Thus, induction of apoptosis is a relevant mechanism at relatively high concentrations/doses of organotin compounds, while lower concentrations/doses cause a proliferative arrest without signs of apoptosis.

EXPOSURE TO PESTICIDES INDUCES APOPTOSIS IN SPLENOCYTES.

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Alachlor and fenuron are extensively used as pesticide mixtures throughout the world and present a potential health risk to human and the environment. The immunotoxic potential of these pesticides and pesticide mixtures is currently unknown. We examined the effects of pesticide mixtures in inducing apoptosis in splenic immune cells of C57BL/6 male mice *in vitro*. Splenocytes were subjected to minimal pesticide concentrations and exposure time necessary to cause detectable cell damage to parameters often associated with apoptosis. The results of TUNEL assays and DNA ladder assays indicated an approximate 20% increase in degradation of high molecular weight genomic DNA after 4 hours exposure in cells treated with either 100μM fenuron, 100 μM alachlor or 100μM each of these pesticides in mixture. The annexin assays show a 40–50% increase in cell membrane damage in cells treated with either 100 μM alachlor, 100μM fenuron or 100 μM each of pesticide mixture for 4 hours versus that seen in untreated cells (p ≤ 0.01). LDH assays also show an increase of 15% (p ≤ 0.01) cell membrane damage treated cells that was first detected after a 4 hours exposure to either 100 μM alachlor or 100 μM fenuron. These data indicate that immune cells exposed to alachlor, fenuron, and alachlor/fenuron mixtures sustain cell damage that can be associated with apoptosis. Both DNA and membrane damage continues to increase in a dose (10-250 μM) and time (0-16 h) dependent manner. There was no significant increase in either DNA damage or cell membrane damage when cells were exposed to mixed pesticides versus that observed when cells were exposed to individual pesticides.

APOTOPSIS IN TESTES INDUCED BY CO-EXPOSURE OF RATS TO DEET, PERMETHRIN AND PYRIDOSTIGMINE BROMIDE ALONE, AND IN COMBINATION WITH STRESS.

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Male Sprague-Dawley rats were treated with a combination of DEET (40mg/kg dermal), permethrin (0.13mg/kg dermal), and PB (1.3mg/kg oral) with and without stress for 28 days. The animals were subjected to stress by putting them in...
sensitive to TNF-α-induced cytotoxicity which could involve a mitochondrial electron transport chain-dependent protective mechanism against apoptosis. (Supported by NIH grant ES09047).

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TRANSLATION REPRRESSOR 4E-BP1 ACTIVATED
APOPTOSIS DEPENDS ON ITS PHOSPHORYLATION
STATUS.

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Translation rates generally increase by growth factors, cytokines, hormones and mitogens. Regulation of translation mainly occurs at the level of initiation. Eukaryotic translation initiation factor 4E (eIF4E) is the mRNA cap binding protein which functions during translation of cellular mRNAs possessing the 5' cap structure. Overexpressed eIF4E suppresses oncogene-dependent apoptosis, causes malignant transformation and leads to multi-drug resistance. The function of eIF4E is negatively regulated in part by members of the family of translational repressors, eIF4E-binding proteins 4E-BPs. When hypophosphorylated, 4E-BPs block cap-dependent translation by sequestering eIF4E in a translationally inactive complex. Upon hyperphosphorylation in response to hormones or growth factors, 4E-BPs dissociate from the complex with eIF4E allowing it to form an active translation initiation complex. Previously, we found that overexpression of eIF4E blocks Myc-induced apoptosis whereas enforced expression of 4E-BP1 promotes both spontaneous and drug-induced apoptosis in Ras-transformed fibroblasts in vivo and diminishes tumorigenicity of oncogenic Ras in vivo. Here we show that ectopic expression of 4E-BP1 activates apoptosis. Rapamycin, an inhibitor of the signaling pathway leading to phosphorylation of 4E-BP1, augments its pro-apoptotic function. Furthermore, we demonstrate that mutations of 4E-BP1 decreasing or eliminating its phosphorylation significantly potentiate spontaneous and drug induced apoptosis in normal and Ras-transformed fibroblasts and dramatically reduce their colony forming efficiency. These data suggest that phosphorylation of 4E-BP1 through a rapamycin-sensitive kinase cascade promotes cell viability and increases resistance to anti-cancer therapy.

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CYTOCHROME C RELEASE AND SUBSEQUENT
ACTIVATION OF CASPASE-3 IS INVOLVED
IN CYANIDE-INDUCED APOPTOSIS.

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Both in vitro and in vivo studies suggest that cyanide induces apoptosis in cortical neurons. To identify the mechanistic pathways leading to apoptosis, primary cultured cortical cells were used to study cyanide cytotoxicity. Caspase activation is a characteristic criterion of apoptosis. After cyanide treatment (100-300 µM) for 24 hrs, caspase-3 was cleaved to its active form as detected by western blot analysis. Poly(ADP-ribose) polynucleotide (PARP), an important substrate of caspase-3, was activated after cyanide treatment, further confirming that caspases were activated during cyanide-induced apoptosis. Cytochrome c release from mitochondria is one pathway that has been identified to activate the caspase cascade. After 300 µM cyanide treatment for 3 hr, cytochrome c was released into cytosol and it remained there for up to 24 hrs as detected by western blot analysis. NMDA receptor activation and reactive oxidative species (ROS) generation were upstream events of cytochrome c release, since the selective NMDA receptor antagonist MK801 and the antioxidant PBN (N-t-butyl-phenylnitrone) partly blocked cytochrome c release from the mitochondria. Also mitochondrial depolarization plays an important role in cytochrome c release, since immediately after cyanide treatment, the mitochondria membrane was depolarized by cytofluorometric analysis of cells stained with rhodamine 123 and blockade of mitochondria depolarization by cyclosporin A partly blocked cytochrome c release. Z-VAD, a caspase inhibitor, had little effect on cytochrome c release, further confirming that caspase activation was downstream of cytochrome c release. These results show that cytochrome c release from mitochondria plays an important role in cyanide-induced apoptosis, and mitochondria depolarization contributes in part to the release of cytochrome c. (Supported by NIH grant ES01410).

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INCREASED SENSITIVITY TO TNF-α-INDUCED
APOPTOSIS IN CELLS LACKING MITOCHONDRIAL DNA.

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Mitochondria play pivotal roles in apoptosis. Our previous results showed that cells lacking mitochondrial DNA (p0 cells) retain complete apoptotic machinery and have similar response to staurosporine-induced apoptosis as compared to p+ cells. In this study, we looked how p0 cells respond to tumor necrosis factor-α-induced apoptosis. 143B osteosarcoma p+ and p0 cells were treated with 20 ng/ml TNF-α, and apoptosis was measured by Annexin V staining followed by flow cytometry. Caspase activity was measured with fluorogenic substrates. Results showed that p0 cells had a higher percentage of apoptotic cells after 12 hr treatment and they had a much more rapid and potent activation of effector caspases. Intracellular GSH/GSSG redox potential in p0 cells was largely unchanged with an 8 hr treatment, while p+ cells were considerably oxidized. We conclude that p0 cells are more sensitive to TNF-α-induced cytotoxicity which could involve a mitochondrial electron transport chain-dependent protective mechanism against apoptosis. (Supported by NIH grant ES09047).

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ACTIVATION OF OXIDATIVE STRESS-DEPENDENT
CELL SIGNALING PATHWAYS IN MERCURIC-CYCLOPENTADIENYL MANGANESE TRICARBONYL (MMT)-INDUCED APOPTOSIS: DOWNSTREAM EVENTS AND REGULATORY
MECHANISMS.

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It is well known that chronic manganese intoxication can lead to Manganism, a neurological condition similar to Parkinson’s disease. Despite the known neurotoxic effect of manganese on the dopaminergic system, MMT has recently been localized...