

**ALKYLATED METAL COMPOUNDS MIMIC SIGNAL MOLECULES:
THE INDUCTION OF PROGRAMMED CELL DEATH VIA
MEMBRANE RECEPTORS**

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Organometal compounds are widely used chemicals. They can be found in antifouling paint, wood preservatives and they were utilised for chemical syntheses. Moreover, trialkylated tin is a contaminant in plastics. Tributyltin (TBT) causes severe toxicity in mammals like thymus atrophy and immunosuppression, *in vitro* it induces apoptosis very potently (1). Nevertheless, the mechanism of cytotoxicity is obscure. We could demonstrate that TBT induces cell death in a variety of cell lines by triggering the intracellular signalling cascade via membrane receptors leading to apoptosis. This effect appears already at concentrations as low as 100 nM up to 2 μ M, depending on the cell type and the incubation time. Exposure of phosphatidylserine on the surface of apoptotic cells is a caspase-dependent process and frequently used as apoptotic endpoint. TBT induced a rapid exposure of phosphatidylserine as determined by Annexin V binding and this early onset of apoptosis could be prevented completely in a BJAB cell line (BJAB-FADD-dn) which overexpressed the dominant-negative form of FADD, an adapter protein which is recruited by the activated receptor. Under these conditions, the inactive form of the proteolytic enzyme caspase 8 (FLICE/Mach) is not processed and all subsequent events, like activation of caspase 3 (CPP32/Apopain) or PARP-cleavage, were inhibited. These experiments demonstrate that TBT triggers programmed cell death and the functional adapter protein FADD is mandatory for this process. Prompted by this finding we proved whether TBT activates the 'death-inducing signalling complex' (DISC) (2), a protein complex composed of the receptor CD95 (APO-1/Fas), the adapter-protein FADD and the effector enzyme caspase 8. Co-precipitation experiments revealed that TBT activates the DISC: BJAB cells were incubated with the CD95 ligand and cells were lysed immediately. Cell lysates were precipitated with an antibody directed against CD95, followed by immunoblotting using a FLICE-specific antiserum. TBT mediated the processing of caspase 8 in BJAB-wt, but not in BJAB-FADD-dn cells. In summary, TBT induces apoptosis by activation of the DISC and the CD95 receptor could be the primary target.

REFERENCES

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