The role of an NO-donating prodrug in producing apoptotic resistance and metal toxicity changes in cultured rat liver epithelial cells

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The liver is an important target tissue of both cadmium (Cd) and arsenic (As). The compound O_2-vinyl 1-(pyrrolidin-1-yl)diazen-1-ium-1,2 diolate (V-PYRRO/NO) is a liver-selective nitric oxide (NO) producing prodrug that is metabolized by hepatic P450 enzymes to release NO in hepatocytes. NO can compete for cellular binding sites with metals. Thus, we studied the effects of V-PYRRO/NO pretreatment on Cd (as $CdCl_2$) or As (as $NaAsO_2$) toxicity in cultured rat liver epithelial (TRL 1215) cells. Cells were pretreated with V-PYRRO/NO (at levels up to 1000 µM for up to 24 h) then exposed to Cd or As (for an additional 24 h) and cytotoxicity (by MTS assay) or apoptosis (by DNA fragmentation ELISA) was assessed. Cd was significantly less cytotoxic in V-PYRRO/NO (1000 µM) pretreated cells (LC50 = 6.1 ± 0.6 µM) compared to control cells (LC50 = 3.5 ± 0.4 µM). Likewise, the LC50 for As was 20.1 ± 1.9 µM in control cells and 30.3 ± 2.9 µM in V-PYRRO/NO pretreated cells. TRL 1215 cells acted upon the prodrug to release NO, producing nitrite levels (measured by Griess assay) in the extracellular media after 24 h of exposure to 500 or 1000 µM V-PYRRO/NO measured at 87.0 ± 4.2 µM and 324 ± 14.8 µM, respectively, compared to control levels of 7.70 ± 0.46 µM. Since both Cd and As are known to induce apoptosis, the effect of V-PYRRO/NO pretreatment on Cd- or As-induced apoptosis was studied. V-PYRRO/NO pretreatment (750 µM) markedly reduced apoptotic cell death induced by Cd (5 µM) or As (15 µM). Activation of the c-Jun N-terminal kinase (JNK) pathway can be critical to apoptotic cell death and pretreatment of cells with V-PYRRO/NO suppressed JNK activation after exposure to Cd or As. Thus, the prodrug, V-PYRRO/NO, protects against the adverse effects of Cd or As in rat liver cells in culture, including apoptotic cell death and the concurrent activation of the JNK pathway, apparently through generation of NO. The role of NO in prevention of adverse effects of Cd or As appears to increase MT and deserves further study.