and colorectal. An unique feature of MX2167 includes rapid induction of apoptosis (<1 h) mediated through a cell-surface receptor. We have now identified the molecular target for MX2167 through different affinity procedures and LC/MS/MS sequencing and have validated its identification through different studies that include RNA interference and protein binding assays. We will describe the target for MX2167 and its validation as it relates to the induction of apoptosis. These results suggest the potential for MX2167 to be developed as a potential anticancer agent and MX2167 represents a molecular mechanism of action uniquely different from known cancer drugs.

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Ceramide promotes JNK translocation and Bim phosphorylation in lung cancer derived A549 cells

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The sphingolipid, ceramide, is an important second signal molecule and potent apoptotic agent. The production of ceramide is associated with virtually every known stress stimuli, and thus, generation of this sphingolipid has been suggested as a universal feature of apoptosis. Ceramide regulates diverse signaling pathways involving cell senescence, the cell cycle, and apoptosis. Ceramide is known to potently activate a number of stress-regulated enzymes including the c-Jun N-terminal kinase (JNK). Though ceramide promotes apoptosis in human lung cancer derived A549 cells, a role for JNK in this process is unknown. Here, we report that ceramide promotes apoptosis in A549 cells by a mechanism involving the translocation of JNK. A role for JNK in ceramide-induced apoptosis in A549 cells became apparent when it was found that cells pretreated with the JNK inhibitor SP600125 became resistant to killing by ceramide. A similar role for the p38 kinase is not likely since the p38 inhibitor, SB 203580, failed to effectively protect A549 cells from ceramide. To understand which JNK-mediated pathway may be involved, a number of JNK target proteins were examined including the transcription factor, c-Jun, and the apoptotic regulatory proteins Bcl2, Bcl-X_L, and Bim. A549 cells exhibited basal levels of phosphorylated c-Jun in nuclear fractions revealing active c-Jun is present in these cells. Ceramide was found to inhibit c-Jun phosphorylation suggesting that JNK-mediated phosphorylation of c-Jun is not likely involved in ceramide-induced apoptosis. Likewise, ceramide suppressed phosphorylation of Bcl-X_L, suggesting that dephosphorylation of this Bcl2 family member is not involved in the apoptotic process. Little if any Bcl2 was detected in A549 cells. Thus Bcl2 also appears not to be involved in ceramide-induced killing. On the other hand, ceramide promoted phosphorylation of Bim and promoted JNK translocation from the nucleus to the cytosol and the mitochondria. Ceramide-mediated changes in localization of JNK were consistent with the observed changes in phosphorylation status of c-Jun, Bcl-X_L, and Bim. Furthermore, ceramide promoted Bim translocation to the mitochondria. Mitochondrial localization of Bim has recently been shown to promote apoptosis. These results suggest that JNK may participate in ceramide-induced apoptosis in A549 cells by a mechanism involving Bim.

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P53-mediated apoptosis induced by NCX 4040, a nitric oxide-releasing aspirin derivative, in human colon cancer cell lines

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Background: Nitric oxide-releasing non-steroidal anti-inflammatory drugs (NO-NSAIDs) are reported to be safer than NSAIDs because of their ability to decrease gastric toxicity. In our work, we assessed the cytotoxic activity of a new aspirin derivative, NCX 4040, and of its parental compound, aspirin, in *in vitro* and *in vivo* human colon cancer models.
Material and Methods: *In vitro* cytotoxicity was evaluated on a panel of colon cancer lines (LoVo, LoVoDX, WiDr and LRWZ) by sulforhodamine B assay and data were elaborated according to Monk's model. Cell cycle perturbations and apoptosis were evaluated by flow cytometry. P rotein expression and mRNA content were detected by Western blot and RT-PCR. In the i*n vivo* experiments, tumor-bearing mice were treated with 10 mg/kg/die of NCX 4040, five times a week and treatment was repeated for six consecutive weeks. Treatment was begun on day six after tumor cell injection when the tumor mass weighed about 300 mg.
Results: In the *in vitro* studies, the parental compound, aspirin, did not induce an effect on any of the cell lines used, whereas NCX 4040 produced a marked cytostatic dose-related effect, with GI_{50} values already reached after a 24-h drug exposure in all lines. A significant cell killing was observed at the highest concentrations in all but LoVo DX cells, which showed the lowest sensitivity. NCX 4040 induced an accumulation of cells in S phase in all four cell lines. Furthermore, in LoVo and LRWZ cell lines, which basally express p53 wild type, we observed Caspase-9- and 3-mediated apoptosis with a maximal peak after 20-h and 48-h drug exposures, respectively, and also an increased level of the p53-target protein, NAG-1. Conversely, no apoptotic effect was observed after NCX 4040 exposure in WiDr or LoVoDx cell lines, which harbored p53 mutations and also expressed COX-2. In *in vivo* studies, both NCX 4040 and its parental compound were administered *per os*. At a non toxic dose of 10 mg/kg, NCX 4040 exhibited a half-life of about 6 h and induced a 40% reduction in tumor weight. This antitumor effect is important, especially if we consider that antitumor drugs widely used in clinical practice are ineffective on this colon cancer model. Conversely, aspirin did not influence tumor growth at all.
Conclusion: NCX 4040, but not its parental compound, aspirin, showed an *in vitro* and *in vivo* antiproliferative activity, indicating its potential usefulness alone or in combination with conventional cytotoxic drugs to treat colon cancer.

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Evaluation of 2 new Rhodium ferrocene complexes for cytotoxicity, and apoptotic propensity to invoke alternative cell death pathways in prostate tumour cell lines

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Background: Drug induced cytotoxicity may invoke apoptosis, necrosis, micronucleation, abnormal nuclear morphology and intermitotic failure. The operation of these cell death pathways has now become an important criterion in the mechanistic distinction of cytotoxic drugs. This investigation explores the onset of apoptosis and abnormal morphology in response to 3 drugs i.e. cisplatin, a novel ferrocene (fctfa) and a novel Rhodium-ferrocene [Rh(fctfa)(cod)] complex.
Materials and Methods: A pair of prostate cell lines from normal human prostate epithelium (1542N) and malignant human prostate epithelium (1542T) were exposed to increasing concentrations of the drugs for 24 hours, double stained with FITC-AnnexinV and with Propidium Iodide and analysed by dual parameter flow cytometry to quantitate viable cells in quadrant **I**, early apoptotic cells in quadrant IV and late apoptotic/necrotic cells in quadrant **III**. Apoptosis was also scored by microscopy after Acridine Orange staining, by Western Blots for caspase 3 induction and for caspase 8 induction using a colorimetric assay.
Results: The toxicity of cisplatin and the ferrocene and Rhodium-ferrocene complexes was found to be 0.9–1.3 μM; 4.1–4.5 μM and 10.1–13.2 μM, respectively. Apoptotic propensity scored after 24 hours was found to be dose dependent and in the range of 7–19% for cisplatin and 1–4.1% for the ferrocene and Rhodium-ferrocene complexes. Cisplatin produces a distinct apoptotic response followed by a necrotic response, whereas the ferrocene and the Rhodium-ferrocene complexes produce a massive necrotic reaction in the region of 3–19% and very little if any apoptosis. Absence of apoptosis was corroborated by lack of caspase 3 activation, absence of typical apoptotic morphology and by lack of caspase 8 activation.
Conclusions: The 3 drugs cisplatin, the novel ferrocene and the novel Rhodium-ferrocene complexes show similar toxicities in the 1–10 micromolar range in prostate cell lines. However the drugs differ significantly in the activation of death pathways. While cisplatin predominantly induces apoptosis documented by morphology, Annexin V staining and caspase 8 activation, the ferrocene and Rhodium-ferrocene complexes induce late necrosis and abnormal nuclear morphology. Unlike cisplatin-treated cells which enter apoptosis and necrosis sequentially the 2 Ferrocene drugs invoke direct entry of cells into late necrosis without first entering the early apoptotic compartment.

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Two distinct pathways regulate Bak function in apoptosis: a requirement for JNK1 in Bak 80–170 kDa complex formation but not in Bak activation

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The stress-activated protein kinase JNK1 is required for apoptosis induced by many cell death stimuli, likely by regulating Bcl-2 protein family members. Of the two Bcl-2 family members Bim and Bid, which are both known to be upstream regulators of Bak, Bim rather than Bid is here shown to be required for cisplatin-induced apoptosis, and to be a cisplatin-induced