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In vitro and in vivo antitumor efficacy of NCX-4040, a nitric oxide-releasing non-steroidal anti-inflammatory drug, in combination with antineoplastic drugs on human colon cancer lines

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Background: Non-steroidal antinflammatory drugs (NSAIDs) are the most widely studied agents for the prevention of colon cancer, but the mechanisms by which such agents are active are not fully understood. Nitric Oxide (NO) is a small free radical molecule that exhibits a wide spectrum of effects on different biological systems and is active in the control of inflammatory processes. It also plays an important role in gastric cytoprotection. NO-NSAIDs consist of traditional NSAID compounds in which an NO-releasing molecule is covalently linked to the parental molecule via a chemical spacer. In the present study we evaluated the antitumor activity of NCX 4040, a novel NO-NSAID, in combination with antineoplastic drugs on a panel of human colon cancer lines *in vitro* and on xenografted immunosuppressed mice.
Materials and Methods: *In vitro* cytotoxicity was evaluated by sulforhodamine B assay and data were elaborated according to Monk's model. Cell cycle perturbations and apoptosis were evaluated by flow cytometry. Drug interactions were evaluated according to Kern's method modified by Romanelli. In the *in vivo* studies, based on toxicological and pharmacokinetics information available, NCX 4040 was administered *per os* in tumor-bearing mice at a dose of 10 mg/kg/die five times a week and treatment was repeated for 6 consecutive weeks. Oxaliplatin was administered by single parenteral injection of 10 mg/kg followed by a second administration after 7 days.
Results: The cytotoxic activity of the NCX 4040 and oxaliplatin combination was evaluated *in vitro* following simultaneous exposure or different drug sequences. Simultaneous exposure to the two drugs or a 24-h exposure to oxaliplatin followed by a 24-h treatment with NCX 4040 did not significantly increase additive cell kill caused by single drugs. Conversely, a synergistic interaction was produced in all cell lines after the sequence NCX 4040 (24 h) $\rightarrow$ oxaliplatin (24 h). Similarly, in xenografted colon cancer-bearing mice, whereas oxaliplatin and NCX 4040 used alone produced about a 20% and 40% tumor growth reduction, respectively, the combination of the two agents produced a 60% tumor weight inhibition which was significantly higher than that induced by oxaliplatin ($p<0.01$) or NCX 4040 ($p<0.05$) used alone.
Conclusion: Our data demonstrate the ability of NCX 4040 to sensitize *in vitro* and *in vivo* human colon cancer to the effect of chemotherapeutic agents and suggest that the combination of this novel NO-NSAID with antineoplastic drugs could be potentially useful for the clinical management of this tumor.
Supported in part by grants from Ministero della Salute and AIRC

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Activation of NFkB pathway in diffuse large B-cell lymphoma

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Background: NF-κB plays a critical role in regulating proliferation, apoptosis and immune responses although the precise mechanism, frequency, relevance and extent of NF-κB activation in human cancer remains to be fully elucidated.
The aim of this study was to study the expression of NF-κB in diffuse large B-cell lymphoma (DLBCL). DLBCL, the commonest type of lymphoma in adults, whose strikingly variable clinical presentation, morphology and treatment response, posibly reflects the presence of complex and diverse molecular alterations.
Material and Methods: Expression of NF-κB was analyzed in both reactive lymphoid tissue and in a series of 250 DLBCL, using immunofluorescence (IF) and immunohistochemistry (IHC). The IF determination was evaluated by confocal microscopy in order to identify nuclear translocation of NF-κB, consistent with activation of NF-κB pathway. The IHQ results were correlated with the confocal analysis. This series of 250 tumours has been previously IHC studied for 52 proteins related with cell cycle, apoptosis and B-cell differentiation control. The relation between deregulation of this critical cellular function and NF-κB activation in DLBCL was analyzed.
Results: Almost 50% of DLBCL expressed NF-κB. These tumours had a expression profile characterized by profound changes in molecules regulating apoptosis and survival signalling (bcl-2, survivin, etcetera). Patients identified with this profile did not represent a specific clinical subset.
Conclusions: The identification of NF-κB pathway and its signatures reveals the molecular heterogeneity of DLBCL.

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Induction of cell death by imidazoacridinone derivative C-1311 in T lymphoblastoid leukemia MOLT4 cells

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The imidazoacridinone derivative C-1311 is a highly potent antitumor agent with a broad spectrum of activity against different experimental tumors. C-1311 has been synthesized in Gdansk University of Technology and is currently undergoing phase I clinical trials. This compound has previously been shown to display DNA-binding properties and to inhibit activity of topoisomerase II. One of the early biological effects induced by pharmacologically relevant doses of C-1311 (EC_{90} concentrations) was arrest in the G2M phase of the cell cycle. C-1311 induced apoptosis of L1210 murine leukemia cells and to much lesser extent in ostegogenic sarcoma cells as well as abortive mitosis followed by cell death of HT29 human colon adenocarcinoma cells. The aim of this work was to investigate the ability of C-1311 to induce apoptosis of T lymphoblastoid leukemia MOLT4 cells. DAPI staining of cytospin preparations was performed to analyze the cellular morphology. Mitochondrial membrane potential was measured by flow cytometry using JC-1 fluorochrome. Caspase-3 activity and phosphatydilserine externalization was evaluated using commercially available assay kit on flow cytometry. DNA fragmentation was analyzed by 1,8% agarose gel electrophoresis. Cell death studies were carried out over continuous exposure times varying from 3 to 72 h at EC_{90} concentration of the drug.
Microscopic examination of MOLT4 cells showed alterations of the nuclear morphology after 24 h but cells with more condensed chromatin and apoptotic body-like structures appeared after 48 and 72 h. Percentage of Annexin-V positive cells increased gradually upon treatment starting from 12 h and after 72 h it reached about 80%. However, by this latter time all cells were also stained red (due to PI) which indicated late stages of apoptosis and/or necrosis. Disruption of mitochondrial transmembrane potential was observed in the cells after 30 h of incubation with C-1311. Induction of caspase-3 activity was detectable in 30% of cell population after 39 h of treatment and after 72 h this percentage increased to 80%. Characteristic internucleosomal DNA fragmentation was observed only after 48 and 72 h.
These results indicate that C-1311 induced apoptosis of MOLT4 cells in a time-dependent manner and both mitochondria and caspase-3 are involved in this process.

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Rapid activation of caspases involving microtubule stabilization and p53 upregulation by D501036, a novel selenophene derivative active against human renal cancer cells

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D501036, 2,5-bis(5-hydroxymethyl-2-selenienyl)-3-hydroxymethyl-N-methyl-pyrrole, was identified in our laboratory as a novel antineoplastic agent with a broad spectrum of antitumor activity against many human cancer cells with IC50 in nanomolar range. Single-dose I.P. administration of D501036 to Balb/C nude mice at 50 mg/kg resulted in a complete abrogate the growth of xenografted human renal carcinoma cell A 498 tumor in vivo. In this study, we investigated the cellular and molecular events underlying the antitumoral function of this compound in A498, focusing on the early cytotoxic effect. Treatment of A498 cells with D501036 induced S phase arrest followed by sub-G1 phase accumulation. The DNA fragmentation assay further indicated that D501036 induced cell death proceeded through an apoptotic pathway as opposed to via necrosis. This compound produced a time–dependent activation of caspase-3 and -8, however, another caspase-3 initiator, caspase-9, was only marginally activated at later time point. Furthermore, up-regulation of p53 in A498 cells was found after D501036 exposure. We further demonstrated that D501036 prevented microtubules de-polymerization similar to Taxol treatment. Notably, no cross-resistance with D501036 was observed in Taxol–resistant cell lines. Overall, our findings suggest that D501036 can induce a rapid apoptosis involving microtubule stabilization and p53 activation. The compound may have broad therapeutic value.