NCX 4016, A NITRIC OXIDE-RELEASING ASPIRIN DERIVATIVE, EXHIBITS A SIGNIFICANT ANTIPROLIFERATIVE EFFECT AND ALTERS CELL CYCLE PROGRESSION IN LOVO COLON ADENOCARCINOMA CELL LINE

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Purpose. Numerous studies demonstrated that non-steroidal anti-inflammatory drugs (NSAIDs) are useful agents for prevention and cure of cancers, especially colon and rectal cancers, but side effects are a major obstacle to their assuntion. Nitric oxide releasing non-steroidal anti-inflammatory drugs (NO-NSAIDs) are reported safer than NSAIDs by their ability to decrease gastric toxicity. In our study we assessed *in vitro* the cytotoxic activity of a new aspirin derivative, NCX 4016, after different exposure schedules in LoVo colon adenocarcinoma cell line .

Experimental design. COX-1 and COX-2 expression was evaluated as protein expression and mRNA content by Western blot and RT-PCR respectively, cytotoxic activity was evaluated by sulforhodamine B assay and the data elaborated according to Monk's model, cell cycle perturbations and apoptosis were evaluated by flow cytometry, mitotic index was evaluated by at microscope on hematoxylin-eosin stained cytospin.

Results. LoVo cell line resulted positive for the presence of protein and mRNA of the two isoenzymatic form of cyclooxygenase COX-1 and COX-2. Important anti-proliferative effects were induced by NCX 4016 and GI_{50} value, ranging from 174-to 200 mM, was already reached after 24-h drug exposure. A significant cell killing was observed only at the highest concentrations and LC_{50} values were reached only after longer time exposures. NO-aspirin compound also induced an accumulation of cells in G_2-M phase in LoVo cell line with a peak after 48-h treatment which still persisted after 72-h or after 48-h exposure followed by a 24-h of wash-out. Furthermore, the block resulted be charged to G_2 phase whereas mitosis phase was not affected at all.

Conclusion. Our results indicate that NCX 4016 has an *in vitro* antiproliferative activity superior respect to parental compound aspirin that makes it a potential important tumor chemopreventive agent and the cytocidal effect to higher concentration with specific block in G_2 phase renders it a promising candidate for drug combination regimen.