Involvement of M1 and M2 subunits of ribonucleotide reductase in Ni(S-Tcitr)$_2$ resistance/sensitivity in human cell lines

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Thiosemicarbazones are molecules of considerable pharmaceutical interest due to their multiple biological properties and recent studies show that they may be used in anticancer therapy. Their effects on cells are greatly enhanced by metal ion coordination that confers augmented activity and selectivity to these compounds.

The metal complex [Ni(S-tcitr)$_2$] shows interesting antiproliferative/antimicotic characteristics. It enters the cell and induces G_2M cell cycle arrest, p53 independent-intrinsic-apoptosis by down-regulation of Bcl-2, mitochondrial membrane potential loss and caspase activation. [Ni(S-tcitr)$_2$] does not induce gene mutation or chromosomal damage, but alters DNA conformation creating knot-like structures and hairpins.

Analysis of collection of *S. cerevisiae* deletants have shown an enrichment in the classes of genes coding for components involved in nucleic acids metabolism such as ribonucleotide reductase (RNR). This enzyme is necessary for the synthesis and repair of DNA. RNR consists of one regulatory subunit (RRM1) and one catalytic subunit (RRM2). Both subunits are needed for enzymatic activity. RRM1 structure shows binding sites for ribonucleotides and RRM1 expression remains stable during the cell cycle. RRM2 contains a tyrosyl-free radical that is stabilized by a non-heme iron center, which is essential for ribonucleotide reduction and conversion of nucleotides in deoxynucleotides. Alterations in RNR genes expression have been identified in murine and human tumor cells.

Ni-S(tcitr)2

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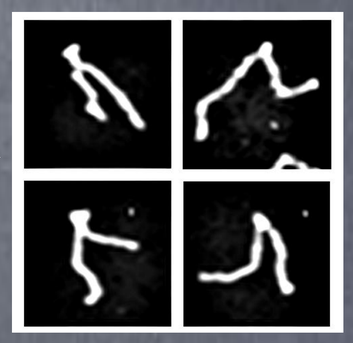
DNA damage



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Cell cycle arrest

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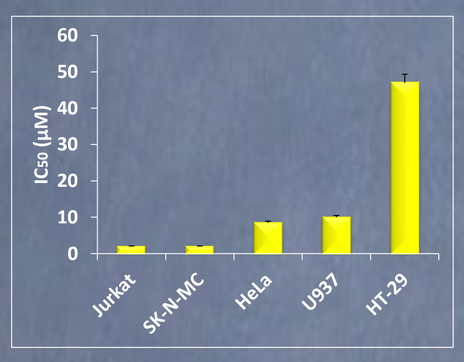
DNA repair

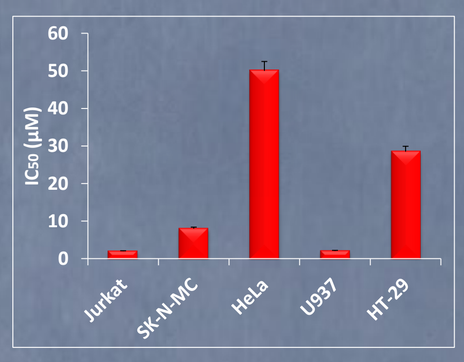
↓ Repair failure

Apoptosis

24 h treatment

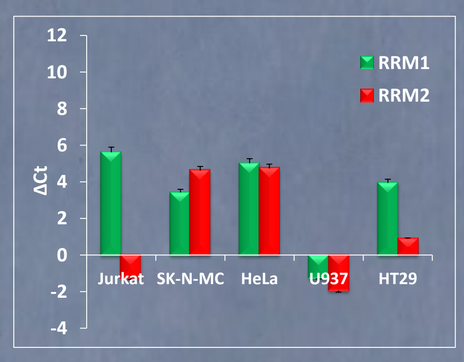


48 h treatment

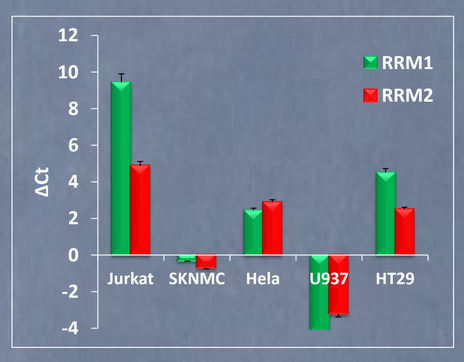


MTS cell proliferation assay was performed to assess the antiproliferative activity of Ni(S-tcitr)2 and to determine dose-response curves for each cell line. Nickel complex concentrations ranged from 0.5 to 50 µM in order to identify the value of the IC50 after 24 h and 48 h of treatment. Cell lines showed different sensitivity to the compound. Jurkat and SK-N-MC cell lines showed the same sensitivity following exposure for 24 h and 48 h. In U937 and HT-29 cell lines the IC50 value, observed after 24 h of treatment, decreases after 48 h. HeLa cell line showed sensitivity after treatment for 24 h but seemed to develop resistance after 48 h.

24 h treatment

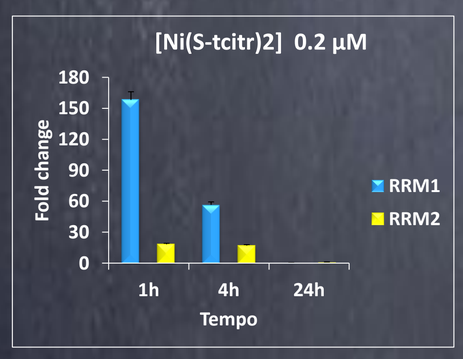


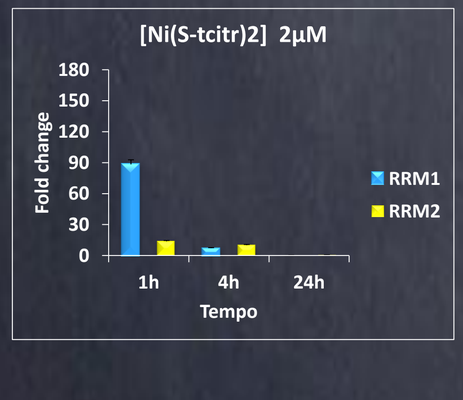
48 h treatment



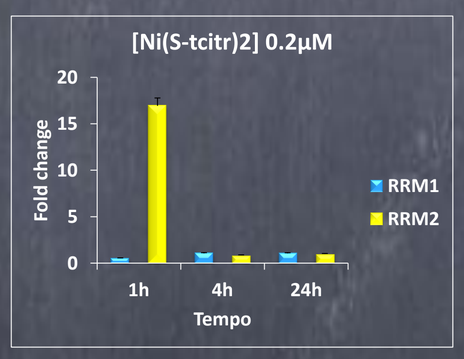
Cells were treated with two concentrations of Ni(S-tcitr)2: a toxic concentration, corresponding to the value of IC50 after 24h of treatment, and a subtoxic one with a value 10 times lower. By means of Real Time RT-PCR was possible to determine basal expression levels of ribonucleotide reductase coding genes in different tumor cell lines after 24 h and 48 h of treatment. The value of ΔCt for each gene was calculated as the difference between the Ct (threshold cycle) of the gene of interest and the Ct of the housekeeping gene GAPDH. Basal levels of transcript appears to be different for each cell line, especially for Jurkat, SK-N-MC and U937 cell lines.

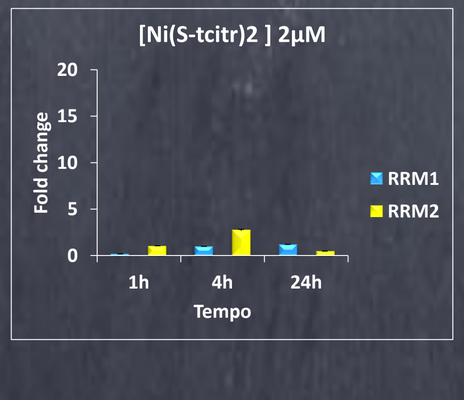
Jurkat cell line



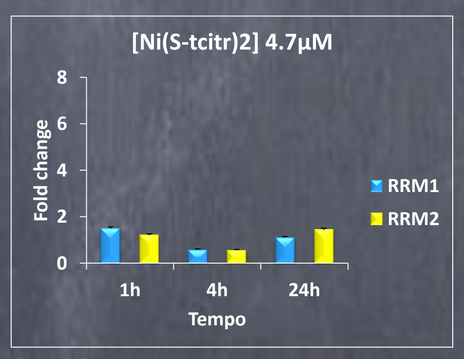


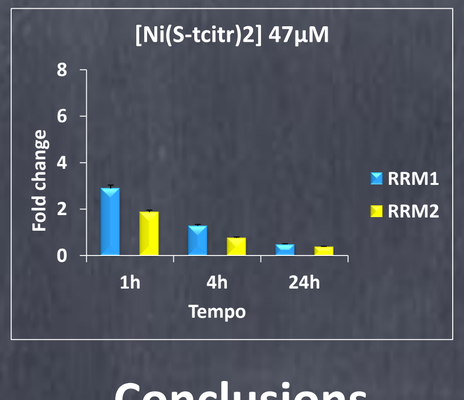
SK-N-MC cell line



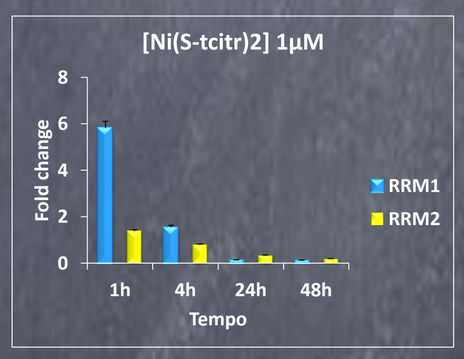


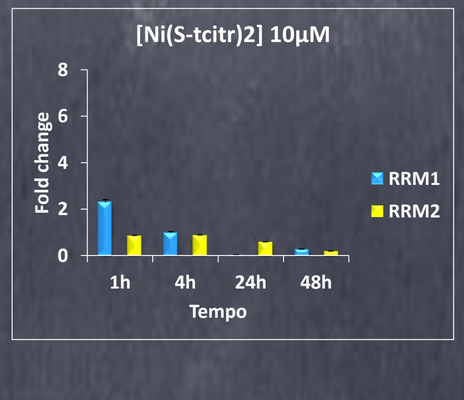
HT-29 cell line



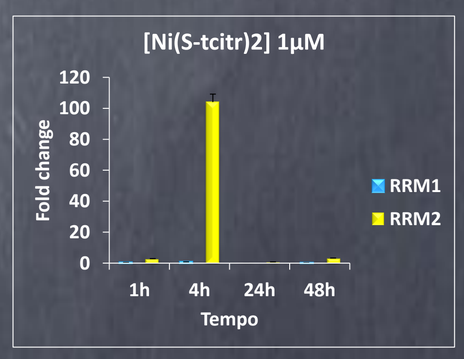


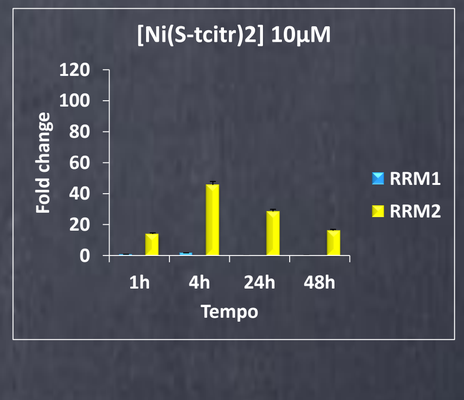
HeLa cell line





U937 cell line





Conclusions

Evaluation of the expression of the two subunits coding for ribonucleotide reductase, RRM1 and RRM2, in each cell line treated with Ni(S-tcitr)2 allowed us to observe that the treatment with the nickel complex induces a modulation of the expression of both subunits. This modulation does not appear to be related with the sensitivity of each cell line. The early over-expression of the M2 subunit could be due to the regulation mechanism of RNR. This enzyme is regulated by modulating the transcription and subsequent degradation of the M2 subunit. The modulation of the expression of the two subunits at subtoxic and toxic concentrations could indicate that the cellular response, induced by the nickel complex, could involve the intervention of RNR.

HT-29 cell line showed the lowest sensitivity to Ni(S-tcitr)2 with no evidence of a significant modulation in the transcription of the two subunit even at toxic concentrations. Assuming that the molecule enters the cell, as observed in other studies, it can be postulated that the induction of cytotoxicity could be related to a different mechanism of death.

To identify the mechanism underlying sensitivity to Ni(S-tcitr)2, we plan to investigate further the relationship between the modulation of RNR subunits and the mechanisms of response to DNA damage.

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