Effect of NO and H2S releasing doxorubicins on an *in vitro* model of colon cancer with MDR phenotype

L.Cinci[1], K. Chegaev[2], C. Riganti[3], E. Bigagli[1], C. Luceri[1]

[1]Dept. NEUROFARBA, University of Florence, Florence, Italy
[2]Dept. of Drug Science and Technology, University of Turin, Turin, Italy
[3]Dept of Oncology, University of Turin, Turin, Italy

The major cause of treatment failure in cancer chemotherapy is the development of multidrug resistance (MDR). The MDR phenotype is multifactorial and partially unknown; it primarily involves the overexpression of efflux pumps such as P-glycoprotein (P-gp) and MDR-associated proteins (MRPs), which results in decreased drug accumulation and cellular death. Doxorubicin is a substrate of Pgp and it has been suggested that its ability to induce synthesis of nitric oxide (NO) could explain some of its cytotoxic effects (Riganti et al., 2005; Kopecka et al. 2011). Nitric oxide (NO) donors might represent a promising strategy to re-establish both chemosensitivity and immunosensitivity to doxorubicin in MDR cells (Tufi et al., 2008; De Boo et al., 2009). Hydrogen sulfide (H2S) also plays a key role in P-gp modulation and seems to exert protective effects on doxorubicin-induced cardiotoxicity (El-Seweidy et al., 2011). We developed several semisynthetic doxorubicins modified with moieties containing NO-releasing groups, which displayed more cytotoxic activity than doxorubicin alone against MDR cells (Riganti et al., 2013). We also developed a new series of synthetic doxorubicins, containing H2S-releasing groups that in a preliminary screening, demonstrated a significant toxicity in several cancer cell lines.
The aim of this study is to assess the toxicity of synthetic NO and H2S releasing-doxorubicins (DR6 and CC2790A respectively) in a MDR *in vitro* model of colorectal cancer (HCT-8) over-expressing the P-gp protein.
A doxorubicin resistant HCT-8/R clone was selected from sensitive parental cells HCT-8 and the P-gp status was studied at RNA, protein and functional level. The toxicity of DR6 and CC2790A was evaluated on HCT-8/Rafter 24, 48 and 72 hours after administration. After 24 hours, DR6 and CC2790A showed a four-fold higher toxicity than doxorubicin. This difference was maintained, even if reduced, after 48 hours. After 72 hours, the toxicity of DR6 and CC2790A was similar to that of doxorubicin; toxicity after 72 hours did not change even after repeated administration every 24 hours. These data showed that in a P-gp overexpressing cell model, DR6 and CC2790A were able to decrease the P-gp functionality. The significant effect of DR6 and CC2790A after 24 hours showed that these compounds are able to quickly inhibit P-gp. Conversely, doxorubicin reached effective intracellular concentrations only after 72 hours. This result is probably due to a saturation phenomenon of the pumps.
In conclusion, these compounds represent a promising therapeutic strategy to revert the MDR phenotype.

This work was funded by FIRB 2012 'Optimization of oncology therapy: novel drugs affecting multi drug resistance' cod RBFR12SOQ1.

Riganti et al.(2005) Cancer Res.65(2):516-25.
Kopecka et al. (2011) J Cell Mol Med.15(7):1492-504.
Tufi et al. (2008) Cell Death Differ.15(2):274-82.
De Boo et al. (2009) Mol Cancer.19;8:108.
El-Seweidy et al. (2011) Arch Biochem Biophys.1;506(1):48-57
Chen et al. (2010) Clin Exp Pharmacol Physiol.37(3):316-21