Mitochondria-targeting doxorubicin: a new therapeutic strategy against doxorubicin-resistant osteosarcoma

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The standard therapy for osteosarcoma (OS) is based on chemotherapy regimens that include doxorubicin (DOX), but the success rate is not satisfactory. The main reasons of DOX's failure are the onset of cardiotoxicity and drug resistance, due to the overexpression of the drug efflux transporter P-glycoprotein (Pgp).

Aim of this work to is overcome the main reasons of DOX failure, by using a chemically modified DOX targeting mitochondria (mDox).

Human DOX-sensitive/Pgp-negative U2OS and SaOS cells and the corresponding clones (DX30, DX100, DX580), which have been selected in culture medium containing 30, 100, 580 ng/ml DOX, and have increasing degree of DOX-resistance and Pgp-positivity, were treated with saline solution, DOX or mDOX (both 5 µM in saline solution, 24 h), then tested for: DOX accumulation (fluorimetric assay), necrosis (extracellular LDH release activity), immunogenic cell death (HMGB1 release by Western blotting, ATP release by chemiluminescence-based assay, calreticulin translocation by flow cytometry), gene expression profile, mitochondria biogenesis (ELISA), tricarboxylic acid cycle (TCA) and fatty acids β-oxidation (metabolic radiolabeling), mitochondrial respiratory chain activity (spectrophotometric assay), ATP synthesis (chemiluminescence-based assay), reactive oxygen species (ROS) levels, mitochondrial electric potential measurement, caspases activity (fluorimetric assays). 1 x 10^6 DOX-resistant K7M2 cells were implanted s.c. in syngenic 6-weeks old female BALB/c mice, treated once a week for 4 weeks with 0.1 mL saline solution i.v., 5 mg/kg DOX or mDOX i.v., re-suspended in 0.1 mL saline solution (10 mice/group). Tumors growth was monitored by caliper measurement. After mice sacrifice (day 35), tumors were immunostained for Ki67 as index of proliferation and calreticulin as index of immunogenic death (immunostchemistry); blood was collected and analyzed for LDH, AST and ALT (as parameters of liver toxicity), creatinine (as parameter of kidney toxicity), CPK as parameter of cardiotoxicity.

Differently from DOX, mDox was more retained within whole cell and mitochondria (p<0.002), increased the LDH release (p<0.005) and all the parameters of immunogenic cell death in in all the resistant clones. DOX resistant clones upregulated at least 2-fold 111 genes related to mitochondrial functions. mDOX, but not DOX, down-regulated more than 2-fold 59 genes related to mitochondrial functions in resistant cells. mDOX decreased mitochondria biogenesis (p<0.001), mitochondrial DNA (p<0.02), TCA cycle (p<0.001), β-oxidation (p<0.001), electron transport (p<0.001) and ATP synthesis (p<0.005), while it increased ROS levels (p<0.001), mitochondrial depolarization (p<0.005) and caspase 9/3 activation (p<0.001) in resistant OS, where doxorubicin was ineffective. mDOX, but not DOX, reduced the growth of DOX-resistant OS in vivo (p<0.002) and Ki-67-positive cells (p<0.01), and increased calreticulin-positive cells (p<0.001). DOX increased CPK in treated-animals (p<0.005), whereas mDOX treated-animals had the CPK levels of mice treated with saline solution. Our work proposes a new and effective chemotherapeutic strategy for DOX-resistant OS, by using a mitochondria targeting DOX (or mDOX) that exploited the metabolic signature typical of resistant cells, i.e. the hyperactive mitochondrial functions, and hit the energy pathways crucial for DOX-resistant OS. Our results show that mDOX was effective independently from the levels of Pgp an was not cardiotoxic, overcoming the main limitations of DOX in OS therapy and paving the way to the potential use of mDox in clinical settings.