DISTRIBUTION, METABOLISM AND TOXICITY OF DOXORUBICIN IN CARDIOMYOCYTES : ROLE OF MYOGLOBIN

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Antitumor therapy with the anthracycline doxorubicin (DOX) is limited by a dose-related cardiotoxicity that correlates with the cardiac levels of DOX and its metabolic conversion to Reactive Oxygen Species (ROS). We reported that peroxidase-like intermediates of myoglobin were able to oxidize and degrade DOX in cell-free systems (1). Here, we characterized how this degradation process influenced the pharmacokinetics and toxicity of DOX in cardiomyocytes. By having screened a library of potential compounds, we identified *tert*-butoxycarbonyl-alanine (*t*-boc-Ala) as a pharmacologic tool for inhibiting DOX degradation. When assessed in H9c2 cardiomyocytes *t*-boc-Ala neither caused toxicity *per se* nor affected DOX uptake or efflux; however, *t*-boc-Ala was highly specific at inhibiting DOX degradation induced by peroxidase-like intermediates of oxyferrous myoglobin ($Mb^{II}O_2$). By this mechanism, 1 to 10 mM *t*-boc-Ala concentration-dependently increased the cellular levels of DOX and its conversion to ROS, measured by a quantitative HPLC assay for dichlorofluorescein-detectable H_2O_2 (2). The *t*-boc-Ala-induced accumulation of DOX and ROS was accompanied by mitochondrial dysfunction and loss of viability, measured by thiazolyl blue tetrazolium reduction under basal or Complex III-inhibited conditions. The effects of *t*-boc-Ala were highly specific to DOX; in fact, *t*-boc-Ala did not aggravate the toxicity of ROS produced by anthracycline-independent stimuli like bolus H_2O_2, aminotriazole, paraquat, streptonigrin, and heme or non-heme iron. These studies uncover that an $Mb^{II}O_2$-dependent degradation of DOX serves as a salvage pathway for diminishing the cardiac levels of DOX and its bioactivation to ROS. Also of note is that the toxic synergism of *t*-boc-Ala with DOX was most evident at 0.1 to 1 μM DOX, but much less evident at >1 μM DOX. This suggests that the salvage function of myoglobin may contribute to the better cardiac tolerability of treatment schedules that reduce the plasma explosure to DOX (like e.g., substitution of slow DOX infusion for bolus DOX) (Supported by AIRC and MIUR Cofin 2004).

1) Cartoni A., Menna P., Salvatorelli E., Braghiroli D., Giampietro R., Animati F., Urbani A., Del Boccio P. and Minotti G. (2004) *J Biol Chem* 279: 5088-5099.
2) Salvatorelli E., Guarnieri S., Menna P., Liberi G., Calafiore A.M., Mariggiò M.A., Mordente A., Gianni L. and Minotti G. (2006) *J Biol Chem* 281: 10990-11001.