Intracellular distribution of anthracyclines in drug resistant cells

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Abstract
The unresponsiveness of multidrug resistant tumor cells to antineoplastic chemotherapy is often associated with reduced cellular drug accumulation accomplished by overexpressed transport molecules. Moreover, intracellular drug distribution in resistant cells appears to be remarkably different when compared to their wild type counterparts. In the present paper, we report observations on the intracellular accumulation and distribution of doxorubicin, an antitumoral agent widely employed in chemotherapy, in sensitive and resistant cultured tumor cells. The inherent fluorescence of doxorubicin allowed us to follow its fate in living cells by laser scanning confocal microscopy. This study included flow cytometric analysis of drug uptake and efflux and analysis of the presence of the well known drug transporter P-glycoprotein. Morphological, immunocytochemical and functional data evidentiated the Golgi apparatus as the preferential intracytoplasmic site of drug accumulation in resistant cells, capable of sequestering doxorubicin away from the nuclear target. Moreover, P-glycoprotein has been found located in the Golgi apparatus in drug induced resistant cells and in intrinsic resistant cells, such as melanoma cells. Thus, this organelle seems to play a pivotal role in the intracellular distribution of doxorubicin.

Abbreviations: DAU: daunomycin; DOX: doxorubicin; EELS: electron energy loss spectroscopy; ESI: electron spectroscopic imaging; IDX: 4'-deoxy-4'-iododoxorubicin; LRP: lung resistance protein; LSCM: laser scanning confocal microscopy; MDR: multidrug resistance; MRP: multidrug resistance related protein; Pgp: P-glycoprotein; WGA: wheat germ agglutinin.

Introduction
Most of the chemotherapeutic compounds used in cancer treatment exert their cytotoxic action by multifactorial mechanisms which involve several subcellular targets. The identification of these targets can provide new understanding of cytotoxic action mechanisms and can suggest innovative strategies in anticancer chemotherapy. In fact, the neoplastic cell sensitivity to certain anticancer agents could be enhanced by modifying the subcellular targets and then the cell response to the treatment.

In this context, studies on the intracellular localization of antitumoral drugs in cancer cells may provide useful contributions. Moreover, multidrug resistant (MDR) cells are generally characterized by reduced cellular drug accumulation and different intracellular drug distribution compared with their parental sensitive counterpart. Thus, a detailed knowledge of the drug disposition in various cell types exhibiting different degrees of resistance may provide an insight into the mechanisms underlying multidrug resistance and suggest pharmacological strategies for its therapeutic circumvention.

To this aim, several methodologies have been developed and applied in order to study subcellular drug localization with improved resolution. One approach is to separate the various cellular compartments and to measure the relative drug contents. This method, however, might introduce artifacts due to the possible dislocations of the drug molecules during the fractioning procedures.
Figure 1. Effect of treatment with 100 μM DOX on the intramembrane particle (IMP) distribution of the plasma membrane of human erythrocytes. (a) Protoplasmic fracture face of the plasma membrane of untreated erythrocyte. The IMPs appear to be randomly distributed. (b) In DOX treated cells, the fracture face is characterized by the presence of numerous particle-free domains with different geometry.