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Impaired Accumulation of Drugs in Multidrug-Resistant Cells. hat are the Respective Contributionsinfluencing the Kinetics of Uptake and of Transporter-Mediated Efflux of Drugs?

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In most metastatic forms of cancer, multidrug resistance (MDR), which can be an intrinsic resistance or an acquired resistance against a broad spectrum of chemotherapeutic agents, is observed. This MDR is characterised as the resistance of tumour cells against a variety of functionally and structurally dissimilar drugs involving several biochemical mechanisms. The current hypotheses include alteration of topoisomerase II and the development of an active efflux system, already recognised in MDR tumour cells, that reduces the intracellular drug accumulation.

The expression of two ATP-binding cassette transporter proteins, the 170 kD glycoprotein (Pgp) [1] and the 190 kD multidrug-resistance-associated protein (MRP_1) [2], confers MDR to mammalian cells in reducing the cellular accumulation of the drugs due to an increased active efflux. The first one, Pgp can be inhibited by chemosensitizer agents such as calcium-channel blockers, cyclosporins, *etc.* Mechanistic hypotheses include the 'aqueous pore', the 'vacuum cleaner' and the 'flippase' theory. Regarding MRP_1, those hypotheses are proposing a co-transport of the drug and glutathione (GSH) and a conjugation of the drug with GSH [2]. The biological mechanisms of those two transporters are still unknown. The activity of a drug depends upon its intracellular concentration, the kinetics of its transport being of crucial importance. Reversing the actively mediated efflux using chemosensitizer agents occurs in systemic toxicity, and designing new semisynthetic antitumour analogues circumventing MDR transporters is one of the strategies followed to decrease MDR.

The presented work proposes a new concept with regard to kinetics and uptake efficiency of those two different active efflux systems (Pgp and MRP_1) monitored by intracellular anthracyclin fluorescence. Anthracyclin analogues have been studied aiming to increase the antitumour activity and to reduce the toxicity of drugs [3][4]. They are weak bases having inherent fluorescence properties and the ability to passively diffuse through the plasma membrane. Their rate of influx increases as the lipophilicity of the drug increases and as the pK_a of the drug decreases [5–8]. Their fluorescence is quenched upon intercalation into nuclear DNA, thus allowing to follow the accumulation inside living cells and to determine the kinetics of uptake and active efflux mediated by the pumps [5][9–14]. The resulting data show that the kinetics of passive uptake vary over a very large range depending on the structure of the tested compound. In contrast, the drugs are all extruded at comparable rates, and for both transporters, the active transport follows the *Hill* equation with the cooperative transport of two molecules of anthracyclin per transporter [14][15]. The resistance factor (RF) can be expressed as the IC_{50} in the presence of a pumping system compared with the IC_{50} in its absence. The RF depends strongly on the permeability of the anthracyclin in the absence of a pumping system (diffusion constant k), but very little on its permeability in the presence of a pumping system ($k+k_a$, where k_a is the rate constant for the outward transport of the drug). It is possible to describe the RF of resistant cells expressing Pgp or MRP_1 transporter as follows: $RF = 1 + k_a/k$. If diffusion (k) increases while active transport (k_a) decreases, the resistance factor RF falls down to a value of 1. This leads to the insight that one should not try to block those actively extruding transporters. It is easier to develop drugs which diffuse easily through the membrane. Provided the rate of diffusion is large enough, the active extruding systems will not keep the pace and the inner concentration of the drug will increase while the RF decreases.

This description of the kinetics of anthracyclin transport in a MDR tumour cell allows to predict how modifications in the anthracyclin molecule affect its transport characteristics and cell-killing activity. The same considerations apply for chemosensitizer agents (at least in case of P-glycoprotein) since drugs and chemosensitizers are handled by Pgp in exactly the same way, they are transported by hydrolysis of ATP (verapamil, cyclosporin A, *etc.*) However, MDR cells are not resistant to these compounds because the rate of membrane equilibration is so rapid that pumping *via* Pgp cannot keep pace with it.

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