Impaired Accumulation of Drugs in Multidrug-Resistant Cells. 
What are the Respective Contributions influencing 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 chemo-
therapeutic agents, is observed. This MDR is characterised as the resistance of tumour 
cells against a variety of functionally and structurally dissimilar drugs involving se-
veral biochemical mechanisms. The current hypotheses include alteration of topo-
iso merase 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) [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 
‘flipassage’ theory. Regarding MRP, 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 chemosen-
sitizer agents occurs in systemic toxicity, and designing new semisynthetic antitu-
mour analogues circumventing MDR transporters is one of the strategies fol-
lowed 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) 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 pKₐ 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 struc-
ture 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 IC₅₀ in the presence of a 
pumping system compared with the IC₅₀ in its absence. The RF depends strongly 
on the permeability of the anthracyclin in the absence of a pumping system (diffu-
sion constant kₗ), but very little on its permeability in the presence of a pumping 
system (kₗ+kₜₚ, where kₜₚ 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, transporter 
as follows: RF = 1 + kₜₚ/kₗ. If diffusion (kₗ) increases while active transport (kₜₚ) de-
creases, 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 de-
develop drugs which diffuse easily through the membrane. Provided the rate of diffu-
sion is large enough, the active extruding systems will not keep the pace and the 
intra 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 chemosen-
sitizer 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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