Alastair H. Kyle, Lynsey A. Huxham,
& Andrew I. Minchinton

In vitro screening of anticancer drug penetration into tumour tissue

British Columbia
Cancer Research Centre
Vancouver, Canada

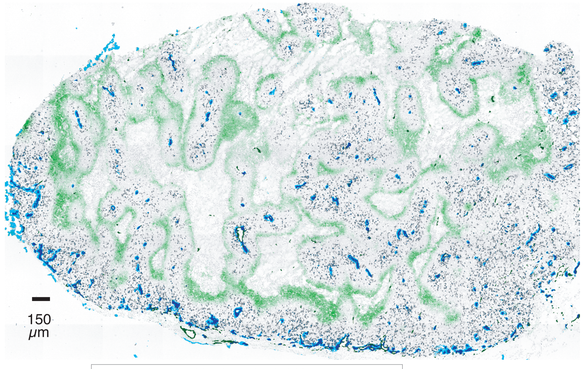
Tumour biology & drug penetration

Tumour biology

Solid tumours contain a network of blood vessels supplying oxygen and nutrients. Tumour growth is limited by the balance struck between the consumption of nutrients & oxygen by the cells comprising the tumour and their supply from an expanding vasculature. Cells within tumours can be located up to ~15-20 cell layers (~150-250 μm) from a blood vessel, tissue that becomes located beyond that distance usually becomes necrotic.

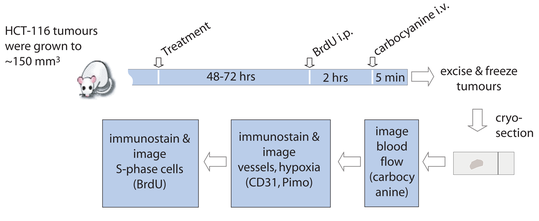
Tissue mapping to assess anticancer drug activity

In this study we assess the effect of drugs within tumours by staining for BrdUrd incorporation assuming that drugged damaged cells cease cycling.



staining key: blood vessels - dark blue (CD31 +ve); blood flow -light blue (carbocyanine); S-phase cells -black (BrdU); hypoxic areas -green (Pimonidazole)

In vivo protocol



Acknowledgements

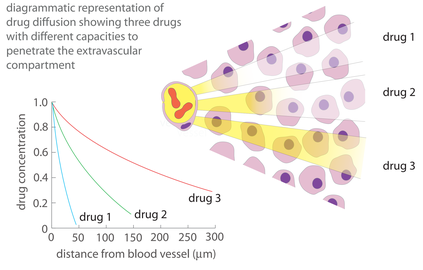
This work was funded by the National Cancer Institute of Canada with funds from the Canadian Cancer Society, the Canadian Institutes of Health Research and the Breat Cancer Research Foundation, (BC & Yukon division).
AHK is a Canadian Institutes of Health Research & Michael Smith foundation for Health Research Scholar.

Extra-vascular delivery of drugs

Diffusion limited penetration

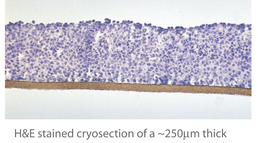
The penetration of drugs into solid tumours is limited by the balance between drug diffusion and tissue consumption in a fashion similar to that of oxygen. Drug binding, metabolism and sequesterization within tissue all act to limit penetration.

Drug penetration may be a key factor that limits the effectiveness of many anticancer drugs including DNA alkylating agents, hypoxic cytotoxins, antibodies, antibiotics, etc.



Multilayered cell culture

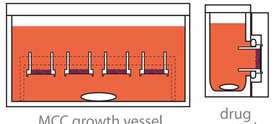
MCCs are three-dimensional tissue cultures, which mimic the tumour extravascular environment and can be used to study drug penetration in a controlled environment.



H&E stained cryosection of a ~250μm thick multilayered cell culture grown on a permeable support membrane

Multilayered cell culture protocol

Diagrammatic representation of apparatus used to grow MCCs and to perform assays of drug penetration



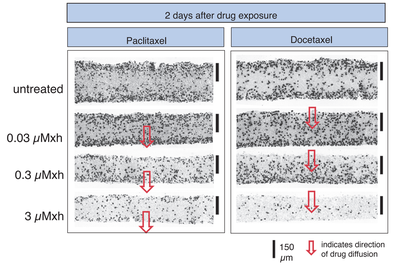
Taxanes

Using the MCC model we examined the penetration of paclitaxel and docetaxel based on their effect within MCCs.

Effect within MCCs

2 days after exposure to the Taxanes from one side MCCs were immunostained for S-phase cells using BrdUrd.

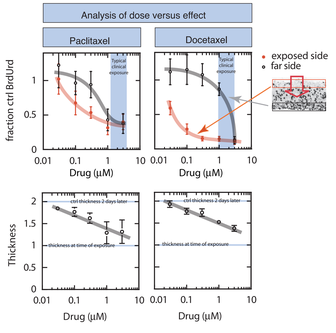
For Docetaxel cells on the unexposed side are significantly affected only after a 3 μM•h exposure.



Effect on exposed versus far side of MCCs

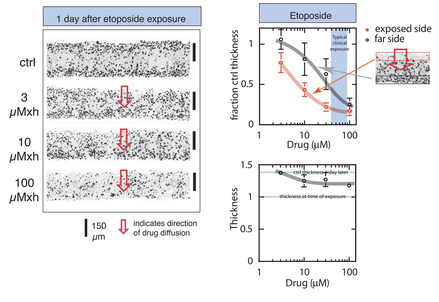
Drug effect on the first 30 μm of tissue on either side of the MCCs was analysed to determine the fraction of BrdUrd labelling relative to untreated MCCs.

Results indicated that paclitaxel exhibited a more uniform effect on the two sides in comparison with docetaxel. However the overall effect on MCC thickness was similar for the two agents. At typical human exposures, docetaxel may not be able to affect tumour cells existing in tissue distant from blood vessels.



Etoposide

MCC studies indicated that the far side of the cultures received up to a ~3-fold smaller drug 'dose' relative to the exposed side. Despite this, at clinical exposures, the effect on both sides of the cultures appeared similar.

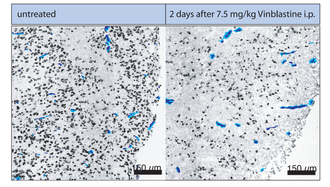


Vinca alkaloids

Tissue mapping of the vinblastine effect

HCT-116 tumour xenografts showing the effect of vinblastine 2 days after treatment.

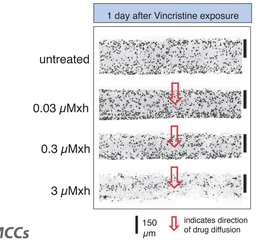
Proliferation is preferentially halted in cells near blood vessels; due either to inadequate drug penetration or differing intrinsic sensitivity of cells located distal to vasculature.



staining: blood vessels - dark blue (CD31 +ve); blood flow -light blue (carbocyanine); S-phase cells -black (BrdU)

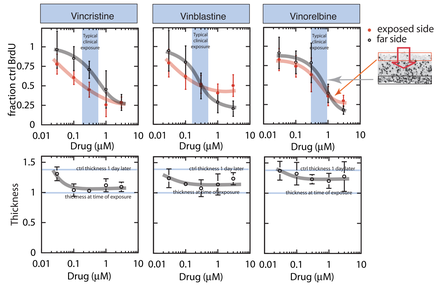
Effect within MCCs

MCCs were employed to study the effect of drug penetration independently of potential changes in intrinsic cell sensitivity with depth into tissue. Typical data for vincristine is shown. After 1h of 3 μM dosing proliferation is significantly reduced on the two edges but continues in the central region even though drug exposure occurs only from one side.



Analysis of the effect on exposed versus far sides of MCCs

Of the three vinca alkaloids studied vincristine showed the greatest difference between effect on exposed and far sides of MCCs. The improved growth control of MCC by vincristine may be related to its greater effect on the exposed side relative to the other drugs. Interestingly at high exposures vinblastine showed a greater effect on the far side of MCCs, potentially related to the short 1h exposure time and subsequent wash out from the tissue.



Cisplatin

Following 1-h drug exposure to cisplatin a 3-day incubation period was required to detect a reduction in proliferation within MCCs but only at levels greatly exceeding typical clinical exposures. At the drug exposures studied here, no indication of a drug gradient within the tissue was detected