In vitro screening of drugs based on their ability to diffuse through tissue

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Overview

The failure of many anticancer drugs to control growth of solid cancers may stem in part from inadequate delivery to cells distant from vasculature. However, extricating the effect of tissue penetration from the many other changes that affect a drug's efficacy in tumours with distance from vasculature is generally not possible.

We introduce a new method to assess drug penetration based on multilayered cell culture (MCC).

Drugs are exposed to the MCCs from one side and the effects then assessed throughout the culture using immunohistochemical techniques. Since the biochemical status of cells on opposite sides of the disc are similar, comparing the effect of drugs after exposure from one side facilitates direct assessment of drug penetration.

Using this method the tissue penetration of a selection of representative anthracyclines (doxorubicin, epirubicin, daunorubicin and mitoxantrone) and the pyrimidine analogue gemcitabine were assessed.

Conclusions

1. At their respective clinical exposures, none of the anthracyclines penetrated completely, suggesting only those cells close to blood vessels are affected.
2. Of the four, epirubicin penetrated most efficiently, followed by doxorubicin, daunorubicin with mitoxantrone penetrating least efficiently.
3. In contrast gemcitabine, a pyrimidine analogue, showed more uniform activity throughout the tissue.
4. This model could be applied as a screening system for the discovery of biologically active drugs that exhibit desirable penetration properties.

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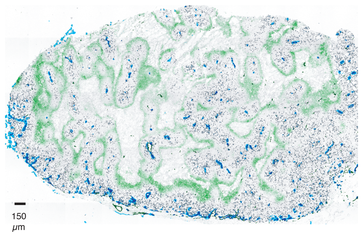
Tumour biology & drug penetration

The structure of solid tumours

— a supply & demand problem

Solid tumours contain a network of blood vessels supplying oxygen and nutrients. Growth of tumours 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.



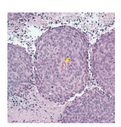
tumour xenograft exhibiting a corded architecture - cords of viable cells surrounding functional micro vessels
staining: blood vessels - dark blue (CD31 +ve)
blood flow -light blue (carbocyanine)
S-phase cells -black (BrdU)
hypoxic areas -green (Pimonidazole)

Extra-vascular delivery of drugs

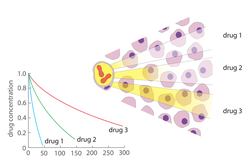
— diffusion limited penetration

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.



H&E stained tumour section showing a sheath of cells surrounding a tumour blood vessel



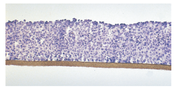
diagrammatic representation of drug diffusion showing three drugs with different capacities to penetrate the extravascular compartment

Multilayered Cell Culture

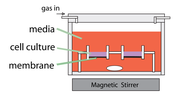
Culture

MCCs are three-dimensional tissue cultures, which mimic the tumour extravascular environment. They exhibit cell-cell contact and possess an extra-cellular space similar to tumour tissue. They display diffusion-limited penetration of oxygen and nutrients similar to that found in solid tumours.

MCCs have been employed to assess tissue drug penetration using two either drug effect or drug flux based methods.



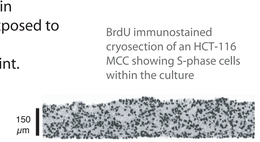
H&E stained cryosection of a ~250µm thick multilayered cell culture grown on a permeable support membrane



growth chamber - MCCs are grown immersed in stirred media using standard cell culture inserts

Drug Effect Experiments

The distribution of effect of drugs within MCCs was assessed in cryosections from MCCs exposed to drugs from one side using proliferation as the end point.



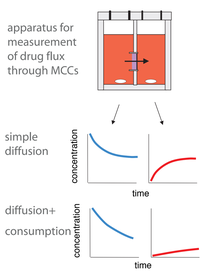
BrdU immunostained cryosection of an HCT-116 MCC showing S-phase cells within the culture

Protocol

Grow MCCs	Drug Exposure	Incubate	Detect Effect
2-4 days	1-5 h	1-3 days	

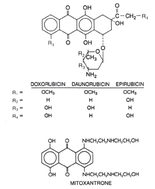
Drug Flux Experiments

MCCs were oriented so as to separate two reservoirs of a diffusion apparatus. By adding a drug to one reservoir, the flux through the MCC and into the second reservoir was then monitored over time via HPLC analysis.



Anthracycline Studies

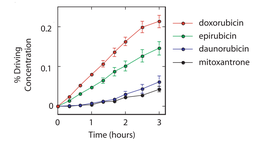
Using the MCC model we examined the penetration of 4 anthracyclines based on their flux through the cultures and their effect on cell proliferation.



Flux through MCCs

Flux data showing the rate of appearance of anthracyclines in the receiving reservoir following diffusion through SiHa MCCs.

All experiments were carried out using 100 µM drug in the donating reservoir.



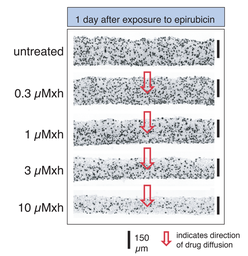
Data shown are averages from several experiments (dox : MCC thickness 180±15 µm, n=7; epi: 165±15 µm, n=8; dau: 165±15 µm, n=7; mtx: 175±5 µm, n=5).

Effect within MCCs

2-day-old MCCs exposed to epirubicin from one side.

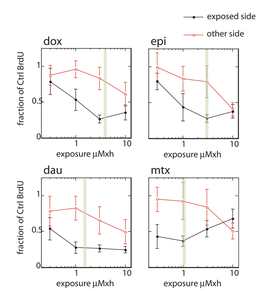
Cells on the unexposed side are only affected after a 10 µMxh exposure.

Experimental conditions:
- 1 h drug exposure
- wait 24 h
- 4 h BrdU exposure.



Comparing the effect of the 4 anthracyclines on the first 3 cells layers of each side of the MCCs as a function of drug exposure.

Green lines show typical clinical AUC exposures following following bolus injection.



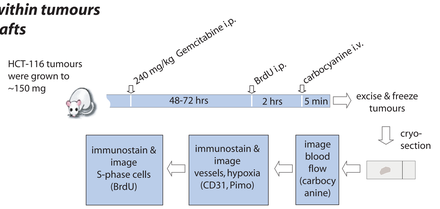
Each data point shows average from 4-8 experiments, error bars show standard deviation. Average thickness 150 ± 30 µm at time of exposure.

Pyrimidine Analogue Studies

We assessed the tissue penetration of gemcitabine based on its effect within tumour xenografts and compared it with the effect observed within MCCs

gemcitabine

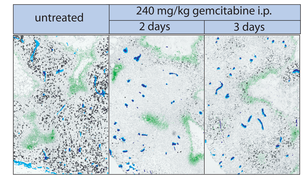
Effect within tumours xenografts



HCT-116 tumour xenografts showing the effect of gemcitabine 2 & 3 days after treatment.

Proliferation appears to recur in cells distant from vasculature; due either to inadequate penetration or differing intrinsic sensitivity.

AUC for a 240 mg/kg dose >100µMxh.



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

Effect within MCCs

3-day-old MCCs exposed to gemcitabine from one side.

Results indicate that drug penetration in not likely to be a limiting factor at exposures greater than 30 µMxh

Experimental conditions: 1 h drug exposure - wait 1 & 3 days - 4h BrdU exposure.