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Alastair Kyle, Maria-Jose Gandolfo, Jennifer Baker
& Andrew Minchinton. Radiation Biology Unit,
BC Cancer Research Centre, Vancouver Canada

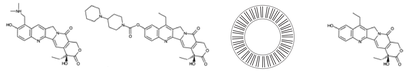
Camptothecins: tissue dynamics and implications for therapy

BC Cancer Agency
CARE & RESEARCH

Introduction

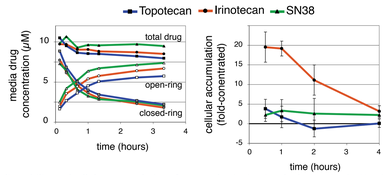
Purpose - To apply a tumour mapping technique to determine mechanisms limiting the *in vivo* biological activity of a panel of camptothecin analogues and to explore avenues for improving their activity.

Background - The Camptotheca tree extract camptothecin and its synthetic analogues represent a chemically diverse class of biologically active topoisomerase interfering agents with differing physicochemical properties, tissue dynamics and clinical use.



Topotecan **Irinotecan** **Irinophore C** (liposomal Irinotecan) **SN-38**

	Topotecan	Irinotecan	Irinophore C	SN-38
molecular weight	421	588	--	392
plasma half-life	0.3 hr	1 hr	7.5 hr	--
active metabolite	--	SN-38	SN-38	--
log $D_{(octanol:water)}$	0.8	2.85	--	2.6



Media stability and cellular accumulation of camptothecins in stirred solutions (37C, pH 7.4). The drugs undergo reversible conversion between open- and closed-ring conformations. Cellular accumulation was found to be higher in the closed-ring form.

Findings

Tissue distribution: Of the campthothecins studied topotecan exhibited a relatively uniform distribution in tissue; 100-μm distal to vessels it reached 94±5% of levels seen proximal to blood vessels while irinotecan and irinophore C reached 41±10% and 5±2% respectively.

Vessel extravasation: Topotecan extravasated equally from all vessels while irinotecan and irinophore C only emerged from certain vessels. The pattern of vessel extravastion was cell line dependent with HCT116 xenografts exhibiting a random mix of permeable and non-permeable vessels throughout the tissue while in HT29 xenografts permeable vessels were located around the periphery of the each tumour.

Caveat to drug distribution: Irinotecan is converted systemically to SN38 and it was found that SN38 exhibited a tumour distribution similar to topotecan. Hence the poor distribution of irinotecan and Irinophore C could be bypassed via systemic conversion to SN38.

Tumour drug activity: Micro-regional analysis showed that all 3 agents could initially induce a complete inhibition of proliferation. However, washout of topotecan and irinotecan (topotecan > irinotecan >> irinophore C) was linked to a rapid return to proliferation by 24-hours. Irinophore C exhibited a sustained plasma exposure and exerted much longer growth inhibition of ~1-week.

Conclusions

Tumor retention rather than drug access was the key factor limiting the activity of these camptothecins, and strategies like that used by irinophore C to extend drug exposure in plasma are promising.

Acknowledgements
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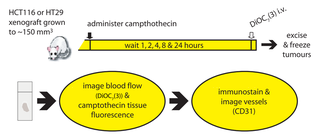
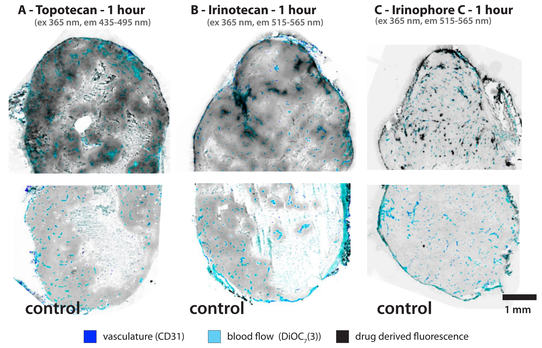
Tissue mapping I - *microregional distribution*

Methods - Using complete tissue cryosections and multiplexed immunostaining techniques drug-derived fluorescence was mapped in relation to tumour vasculature and blood flow in tissue cryosections.



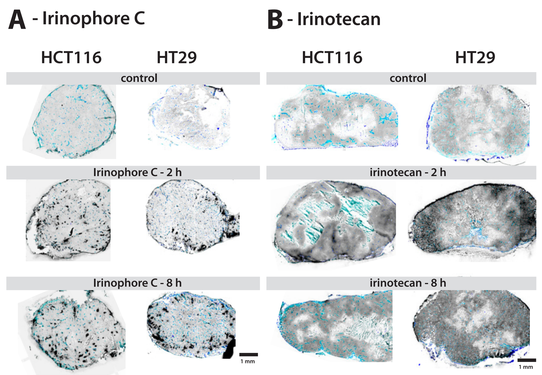
Workflow for mapping drug distribution in tissue sections

Drug-derived fluorescence in relation to vasculature & blood flow



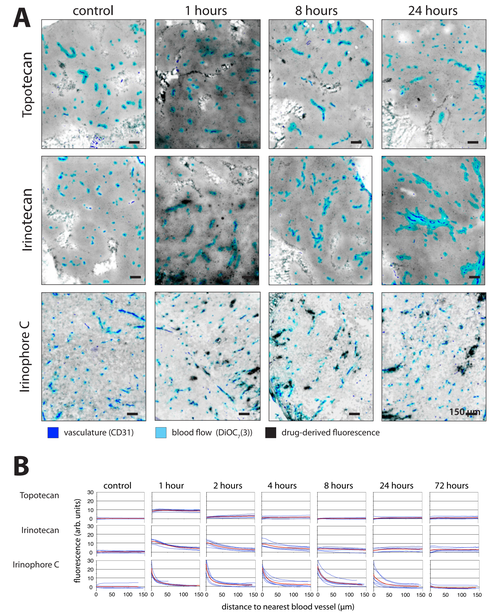
Comparison of drug distribution at 1 hour in relation to tumour vasculature and blood flow. Topotecan exhibits the most rapid tissue distribution while the extravasation and diffusion of irinotecan and irinophore C are limited to a subset of drug-permeable vessels.

Drug extravasation: HCT116 vs HT29 xenografts



Irinophore C and irinotecan extravasation is limited to a subset of drug-permeable vessels. In the HCT116 xenografts these are randomly situated throughout the tissue while in the HT29 permeable vessels are located on the periphery of the tumours.

Time course of drug build-up and wash-out

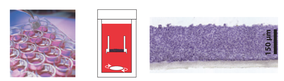


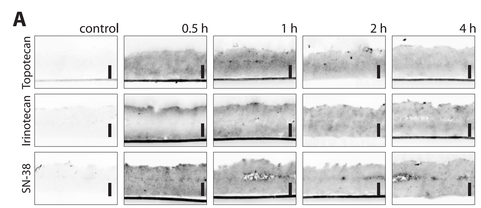
Time-course of drug build-up and wash-out in HCT116 xenografts. Topotecan exhibits the most uniform distribution at 1 h. Irinophore C is retained significantly at 24 hours but does not distribute as effectively within the tumours.

3D tissue drug kinetics under constant drug exposure

Using 3D-tissue discs a direct comparison between camptothecin diffusion was performed under matched exposure conditions.

3D tissue discs were grown using culture inserts. Once cells attach, cultures are transferred to a stirred growth apparatus and grown to 150-300 μm.

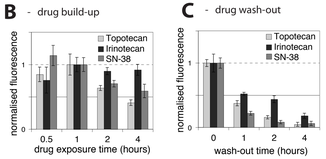




Comparison of drug-derived tissue fluorescence in 3D tissue cryosections

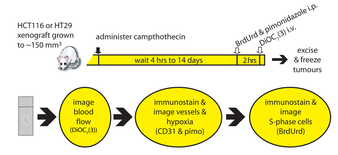
Results:

- all three camptothecins exhibited diminished tissue accumulation over time under constant exposure.
- this was consistent with their conversion from closed to open-ring configuration over time.
- irinotecan exhibited a steep gradient at 1 hour but had also longer tissue retention.

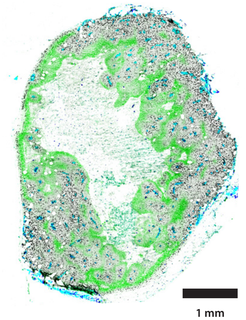


Tissue mapping II - *microregional drug activity*

Methods: Tissue sections were stained to map drug activity via proliferation & apoptosis in relation to vasculature, blood perfusion and tissue oxygenation status.

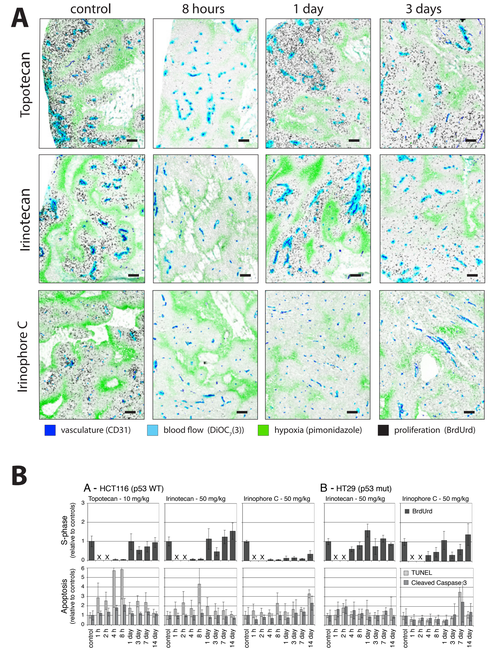


Workflow for mapping drug activity in complete tissue sections



Proliferation in relation to vasculature, blood flow & tissue hypoxia

Time course of drug activity: proliferation and apoptosis

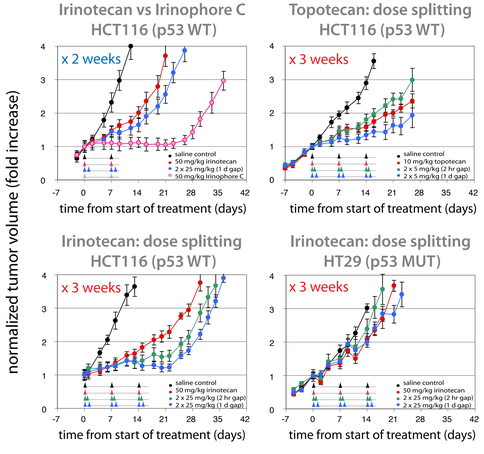


Localization and time-course of drug activity in HCT116 xenografts. All three agents uniformly inhibit proliferation at 4 & 8 hours following treatment. By 24 hours topotecan and irinotecan treated tumours return to pre-treatment levels of proliferation while irinophore C induces a much more sustained effect. Topotecan and irinotecan induce an early onset of apoptosis (1-8 hours) while Irinophore C resuts in a late induction (7-14 days)

Implications for tumour control -

Dose splitting versus extended plasma exposure

- Irinophore C outperformed irinotecan at matched doses
- Both irinotecan and topotecan benefited from a dose splitting strategy that targeted S-phase cells on two consecutive days (24-hour gap).
- Irinotecan also benefited when a single dose was split with a 2-hour gap, consistent with poor tumour distribution from a single dose.



Tumour growth delay following 2 or 3 week treatment schedules. Extended plasma exposures versus dose splitting strategies were evaluated in HCT116 (p53 WT) and HT29 (p53 MUT) tumour xenografts.