Applications and analytical aspects of microdialysis in oncology

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Acknowledgements

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<table>
<thead>
<tr>
<th>Question</th>
<th>Answer</th>
</tr>
</thead>
<tbody>
<tr>
<td>What?</td>
<td>Analysis of antineoplastic agents and their metabolites</td>
</tr>
<tr>
<td>Where?</td>
<td>Tissue: interstitial fluid</td>
</tr>
<tr>
<td>How often?</td>
<td>Continuous monitoring</td>
</tr>
<tr>
<td>Where not?</td>
<td>Protein bound drug</td>
</tr>
<tr>
<td></td>
<td>Intracellular space</td>
</tr>
</tbody>
</table>
Principle

Perfusate

Semipermeable Membrane

Dialysate

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Perfusion fluid enters inner tube

Dialysate exits the probe

Dialyses takes place

Exits at the distal end
Probe characteristics

- Geometry: rigid - flexible
- Material: metal - plastic
- Semipermeable membrane: 6 - 100 kDa
- Volume considerations: 20 µl dialysate for capillary electrophoresis, even less for HPLC
- Syringe pump and a microfraction collector: typical sampling periods between 15 and 30 min
Recovery

• Depends on the diffusion coefficient of the analyte
• Depends on the flow rate (0.5 - 1.5 µl/min)
• Depends on the matrix
• Differs *in vitro* and *in vivo*
• In clinical studies assessed by the *in vivo* delivery method ➔ mass transfer is equal in both directions
## Analytical Matrix

<table>
<thead>
<tr>
<th></th>
<th>Blood</th>
<th>Urine</th>
<th>Mikrodialysate</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Concentration of analyte</strong></td>
<td>variable</td>
<td>high</td>
<td>variable</td>
</tr>
<tr>
<td><strong>Protein content</strong></td>
<td>6.5 – 8%</td>
<td>variable</td>
<td>minimal (cut-off 20 kDa)</td>
</tr>
<tr>
<td><strong>Interferences</strong></td>
<td>low</td>
<td>high</td>
<td>minimal</td>
</tr>
<tr>
<td><strong>pH-value</strong></td>
<td>7.4</td>
<td>4.8 – 7.5</td>
<td>7.4</td>
</tr>
</tbody>
</table>
Plasma and ...

Plasma sample after ultrafiltration
Microdialysates

Mikrodialysate from tumor tissue

sample

blank

5-FU
Study design: 10 patients with advanced breast cancer

Therapy: CMF or FEC

- Cyclophosphamide 600 mg/m²
- Methotrexate 40 mg/m²
- 5-Fluorouracil 600 mg/m²
- Epirubicin 60 mg/m²

Probes: primary tumour and s.c. adipose tissue
5-Fluorouracil

• Sample clean-up: ultrafiltration for blood, no clean-up for microdialysates necessary
• Sampling: microdialysate and blood in 15 min intervals (perfusate: 1.5 µl/min)
• Analysis: Capillary electrophoresis in an uncoated fused silica capillary (56 cm x 50 µm i.d.); Detection: absorbance at 265 nm
• Background electrolyte: 30 mM sodium tetraborate buffer (pH = 9.0)

Electrophoresis 19, 2981-2985 (1998)
5-Flurouracil *in vivo*

- Rapid equilibration between plasma and tissue
- Identical kinetics after 30 min
- No difference between healthy and malignant tissue (AUC, ...)

Cancer Res 57, 2598-2601 (1997)
Methotrexate *in vivo*

- Partial equilibration between plasma and tissue
- Ratio $\text{AUC}_{\text{tissue}} / \text{AUC}_{\text{plasma}} \approx 0.4$
- No difference between healthy and malignant tissue (AUC, ...)

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*Cancer Res 58, 2982-2985 (1998)*
Melanoma

- Study design: 8 patients with advanced disease
- Dose escalation study: 200 - 800 mg Dacarbazine/m²
- Probes: cutaneous metastases from melanoma and s.c. adipose tissue
- Sample clean-up: ultrafiltration for blood, no clean-up for microdialysates necessary
- Sampling: microdialysate and blood in 15 min intervals
Dacarbazine and AIC

• Analysis: RP-HPLC (Lichrospher 100 RP18e)
• Eluens: Gradient elution in acetonitrile / 16 mM ammonium formate buffer (pH = 5.5)
• Detection: Absorbance at 330 nm (DTIC) and 273 nm (AIC)
• Protein binding
  DTIC: 26%
  AIC: 13%
Dacarbazine concentration (µg/mL)

Time [min]

Plasma
Subcutis
Tumour

Cancer 92, 2190-2196 (2001)
Melanoma

- No significant difference in the AUC between plasma and tumour
- Rapid and complete equilibration between plasma and tissue for both DTIC and AIC
- Significant correlation between AUC in plasma and malignoma
  - DTIC: \( r = 0.82 \) (\( p = 0.04 \))
  - AIC: \( r = 0.90 \) (\( p = 0.04 \))
- Resistance to therapy occurs at the cellular level
Capecitabine

- Study design: 10 patients with advanced breast cancer
- Therapy: 1250 mg Capecitabine/m² p.o. (2xd)
- Probes: skin metastases and s.c. adipose tissue
- Sampling: microdialysate and blood in 30 min intervals (perfusate: 1.5 µl/min)
- Analysis: Capillary electrophoresis using 200 mM sodium tetraborate buffer (pH = 9.0)
- Detection: absorbance at 266 nm, 282 nm, 297 nm
5'-DFCR = 5'-deoxy-5-fluorocytidine
5'-DFUR = 5'-deoxy-5-fluorouridine
5-FU = 5-fluorouracil
CE = carboxylesterase
CyD = cytidine deaminase
dThdPase = thymidine phosphorylase
## Tissue kinetics *in vivo*

### Plasma

<table>
<thead>
<tr>
<th>Time [h]</th>
<th>Concentration [mcg/ml]</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
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</tr>
<tr>
<td>1</td>
<td>2</td>
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<tr>
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<td>6</td>
</tr>
<tr>
<td>4</td>
<td>8</td>
</tr>
<tr>
<td>5</td>
<td>2</td>
</tr>
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</table>

- 5'-DFCR
- 5'-DFUR
- 5-FU

### Malignoma

<table>
<thead>
<tr>
<th>Time [h]</th>
<th>Concentration [mcg/ml]</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>1</td>
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<td>4</td>
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- 5'-DFCR
- 5'-DFUR
- 5-FU

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Repeated administration

Plasma

Malignoma

A

B

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Capecitabine

- Capecitabine and its metabolites easily penetrated healthy and malignant tissue
- Equilibration was completed within 45 min after p.o. administration
- Low concentrations of 5-FU in plasma and tissue interstitium
- Transcapillary transfer and metabolic pattern was not altered after repeated dosing

What characterises tumour interstitium?

• High interstitial pressure
• Abnormal geometry of tumour vessels
• Decreased pressure in tumour venules
• High collagen content
5-FU in breast cancer

Correlation: serum-AUC and interstitial-tumor-AUC

serum-AUC (µg min ml$^{-1}$) vs. tumor-AUC (µg min ml$^{-1}$)

$r = -0.17$
Response to 5-FU

- Plasma levels are not always predictive of intratumoural concentrations
- The penetration of 5-FU may be a rate-limiting step for the success of antineoplastic therapy
Response to MTX

- No correlation between plasma and tumour
- Tissue transfer is not a rate-limiting step

Partial remission
Stable disease

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PK-PD modeling

Interstitial concentration in vivo

≈

Concentration in cell culture in vitro

Simulation of interstitial tissue pharmacokinetics using MCF-7 as a model for breast cancer and evaluation of cytotoxicity by MTT-assay

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Results

• Significant correlation between the antitumour effect of 5-FU and the intratumoural AUC ($r = 0.82$, $p = 0.005$)
• No correlation for MTX  ($r = 0.05$, $p = 0.88$)

→ Poor tumour penetration of 5-FU may limit response, but not that of MTX!

The effect was highly dependent on the initial cell count

Exposure to 5-FU

- i.v. 5-FU
- p.o 5-FU prodrug

AUC (µg/mL)*h

plasma
tumor
No statistically significant difference between plasma and tissue pharmacokinetics after capecitabine p.o., but ...  

... there are subgroups of patients with
- Increased distribution of capecitabine from plasma to subcutaneous tissue (Ratio AUC > 2; n=4)
- Increased distribution/re-distribution of 5-FU in subcutaneous tissue ($c_{\text{max}} > 2; n = 6$)
Conclusions

- Microdialysis is a very useful tool to assess tissue distribution and/or metabolism using HPLC or CE.
- Microdialysis may help to individualize the relevant factors of drug resistance *in vivo*.
- The data obtained by microdialysis are an excellent starting point to develop PK-PD models dealing with response to therapy and related side-effects.