Effects of Long Term Implantation on *in vivo* Chemical Sampling Calibration into Microdialysis Probes

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Statement of Purpose

The *in vivo* sensing of biochemical alterations is of great importance to the diagnosis and management of many diseases. In this work, the foreign body response and its affect on the calibration of microdialysis sampling probes was studied. The bi-directional diffusion nature of micodialysis sampling allows simultaneous delivery and collection of chemicals at the implant site, which is not possible with specific sensors.

The calibration of the microdialysis probe is obtained through its extraction efficiency (*EE*), which is a function (equation 1) of the analyte outlet concentration (C_{outlet}), inlet concentration (C_{inlet}), and the concentration far from the probe (C_{sample}). As a tracer to determine glucose uptake variation at the implant site, 2-deoxyglucose (2-DG) was delivered to the implant site through implanted probes with the other two internal standards, vitamin B_{12} (VB_{12}) and antipyrine, which were used to reflect the permeability of probe membrane and surrounding capillaries. The *EE*s of these three analytes are highly sensitive to the kinetic processes during the sampling (equation 2), which are relevant to the change in the rate constants of metabolism (k_m) and exchange between plasma and extracellular fluid (k_{ep} & k_{pe}).

$$EE = \frac{C_{inlet} - C_{outlet}}{C_{inlet} - C_{sample}} \times 100 \qquad \textbf{Eq. 1}$$

$$\frac{dC_e}{dt} = D_e \frac{1}{r}\frac{d}{dr}\left(r\frac{dC}{dr}\right) + k_{ep}C_p - k_{ep}C_e - k_m C_e \qquad \textbf{Eq. 2}$$

Methods

The polycarbonate (PC, 10 mm with 20 kDa MWCO) microdialysis probes were implanted into the dorsal subcutis of male Sprague-Dawley rats (200-300 grams) for seven days (2 per animal) using aseptic technique and an approved protocol from the Albany Medical College IACUC committee. On the acute day and everyday from day 3 to day 7, VB_{12} (100 µM), 2-DG (5 mM) and antipyrine (100 µM) were delivered (1 µL/min) to the implant site in awake and freely moving rats. Samples were collected every 30 minutes for 3 hours. Glucose and 2-DG were quantified from diluted microdialysates (500 fold) using anion-exchange chromatography coupled with pulsed amperometric detection (IC-PAD). Vitamin B12 and antipyrine dialysate samples were analyzed by LC-UV without further pretreatment or dilution. The explanted probes were subjected to Eosin staining.

Results and Discussion

Six animals were used for collection of the analytes glucose, 2-DG and VB_{12}. A separate set of six animals were used for antipyrine studies. For each animal, one probe (daily-perfused probe) was perfused with internal standards on the day of implantation (Day 0), days 3, 4, 5, 6 and 7. The second probe (control probe) was only perfused on day 0, day 3 and day 7.

Table 1 shows the *EE*s for the infused internal standards and the glucose concentrations collected. The delivery (*EE*%) of VB_{12} from the perfusate into the tissue space significantly decreased from 26 ± 4% on the acute day to 10 ± 5% ($p<0.005$, $n=6$) on day 7 after implantation through the daily-perfused probes (DPP). The same trend was observed from the control probes (CP). The *EE*s of antipyrine and 2-DG varied within the range of 50-60%, which showed no significant decline or increase during the implantion.

Table1. Internal Standard delivery through Implanted PC Probes

	VB_{12} (*EE* %)		Antipyrine (*EE* %)		2-DG (*EE* %)		Glucose (mM)	
Probe	DPP	CP	DPP	CP	DPP	CP	DPP	CP
Day 0	26±14	29±6	56±9	59±6	53±12	53±12	4.8±0.9	4.4±1.0
Day 3	21±3	19±3	61±7	57±6	54±9	51±10	3.6±0.9	3.8±0.9
Day 4	18±7		62±6		52±7		3.1±1.0	
Day 5	17±7		59±4		53±13		2.9±1.0	
Day 6	13±9		58±7		52±11		2.0±1.3	
Day 7	10±5	9±5	58±6	54±5	49±9	48±11	1.3±0.6	1.4±1.2

Interestingly, the glucose concentration in the dialysates significantly decreased from 4.8 ± 0.9 mM to 1.3 ± 0.6 mM ($p<0.005$, $n=6$) during the long-term implantation from the daily-perfused probes. The control probes also gave the same declining concentration of glucose into the probe suggesting a decreased recovery.

Upon explanting the 7-day implanted probe, the device was surrounded by a fibrotic capsule. A layer of compact cells and collagen was detected by the Eosin histological staining. Some small groups of red blood cells were present in the surrounding normal tissue close to the encapsulation layer.

Conclusions

The calibration with antipyrine (MW 188) remains constant over the 7-day implantation. However vitamin B_{12} with a higher molecular weight (MW 1355) showed a reduction in *EE*%. This suggests that the encapsulation tissue limited the movement of the higher molecular weight material. The metabolism variation by active cells, like macrophages, appeared to be minimal as compared to the hindrance of diffusion pathways. The histological analysis showed the encapsulation layer outside the probe and angiogenesis within the surrounding tissue. However, more specific staining methods, such as trichrome and immunohistochemical staining, combined with mRNA analysis will be used to identify the cell types in the encapsulation tissue in the future.

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