

Appendix 1

Implantation of Microdialysis Probes (Surgery 2 on Day 5)

Anesthesia was administered as described for surgery 1. All animals had developed subcutaneous abscesses adjacent to the implant cavity. Again under sterile conditions, with the animal in the lateral decubitus position and starting on the infected side, the anteromedial side of the proximal part of the tibia was exposed via the horizontal incision from surgery 1. This led to drainage of the abscesses. At a distance of 8 mm, a drill-hole with a depth of 27 mm was made parallel to the implant cavity with the use of a 2-mm drill. The animal was then turned around, and an identical incision and drill-hole were made in the contralateral, healthy hind leg. Then, the animal was placed in supine position, and microdialysis probes (CMA 63; membrane length, 30 mm) were introduced in subcutaneous tissue parallel to and at a distance of 10 mm distal to the skin incision on both the healthy and the infected extremity, according to the guidance of the manufacturer. Microdialysis probes (CMA 63; membrane length, 20 mm) were then introduced into the drill-holes and the implant cavity. In order to access the implant cavity, the periosteum was perforated with either a thin Kirschner wire or a standard introduction cannula, which did not result in drainage of the cavity. Finally, the skin was closed, and all probes were fixed to the skin with a single suture. The correct location of the bone probes was assessed with fluoroscopy.

Appendix 2

Calibration Procedures

Following the surgical procedures, all microdialysis probes, except the one in the implant cavity, were perfused with 0.9% NaCl containing cefuroxime at a concentration of 3.75 µg/mL at a perfusion rate of 2 µL/min. The implant cavity probe was perfused with 0.9% NaCl at the same perfusion rate. After a thirty-minute tissue equilibration period, all microdialysis probes except that in the implant cavity were individually calibrated using the retrodialysis method, with a sample collected over a thirty-minute interval¹. Relative recovery (RR) was calculated using the following equation:

$$RR = 1 - \frac{C_{out}}{C_{in}}$$

where C_{in} is the cefuroxime concentration in the perfusate and C_{out} is the concentration in the dialysate. Following calibration, the perfusate was changed to 0.9% NaCl, and a 120-minute washout period was initiated. During the last sixty minutes of this period, three twenty-minute dialysates were collected in order to evaluate and quantify the effectiveness of washout. The implant cavity probe was calibrated after the experiment using a similar approach, but at a cefuroxime concentration of 50 µg/mL. The decision to calibrate the implant cavity probe at the end of the experiment was based on an

expectation of low cefuroxime concentrations in the cavity, which would easily be contaminated by spillover of cefuroxime from the calibration procedures.

The measured concentrations in the dialysates were attributed to the midpoint of the sampling interval, and they were corrected for RR using the following equation:

$$C_{tissue} = \frac{C_{out}}{RR}$$

Reference

1. Ståhle L, Arner P, Ungerstedt U. Drug distribution studies with microdialysis. III: Extracellular concentration of caffeine in adipose tissue in man. *Life Sci.* 1991;49(24):1853-8. Epub 1991 Jan 1.