In situ gentamicin concentrations in cortical bone

An experimental study using microdialysis in bone

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ABSTRACT We used microdialysis to study gentamicin in cortical bone and compared these results to values obtained in bone specimens. 10 healthy pigs were given an intravenous bolus of 160 mg of gentamicin. We measured reproducibility by inserting two microdialysis catheters into the cortex of tibial diaphyses and by taking samples of serum, dialysates and bone specimens during 6 hours and measuring the concentrations of this antibiotic.

The peak concentrations in the two microdialysis probes and bone specimens were 3 mg/L, 2.9 mg/L and 2.6 mg/L (Anova, p = 0.3). Similarly, the areas under the curve from 0 to 6 hours (AUC6h) were 704 mg/min/L, 661 mg/min/L and 569 mg/min/L (Anova, p = 0.07). The reproducibility of the measurements of the microdialysates was evaluated with the mean $AUC_{6h/catheter\ no.1}$/ $AUC_{6h/\ catheter\ no.\ 2}$ ratio, which was 1.12. It seems that microdialysis is a suitable method for making dynamic and quantitative measurements of gentamicin in bone.

In orthopedics, products containing gentamicin are used to treat soft tissue infections and osteomyelitis (Stemberger et al. 1997). Analyses of bone biopsies and homogenates have several limitations—i.e., they represent a static condition and harvesting is invasive (Heimdal et al. 1988, Riegels-Nielsen et al. 1995).

Microdialysis, a new method, was first developed to study the metabolism of subcutaneous tissue and neurobiology. It is performed by flushing a fluid through a probe with a pump and thereby obtaining diffusive exchange of endogenous or exogenous drugs across a selective permeable membrane. Thus dynamic measurements can be made of the concentrations in the interstitial fluid (Benveniste and Huttemeier 1990, Stahle 1992).

We evaluated the use of this method for making in situ measurements of gentamicin in cortical bone and compared the gentamicin levels with those in bone specimens.

Methods and material

After inserting two microdialysis catheters into the diaphysis of the right tibia, we studied the relative recovery of gentamicin in cortical bone. All animals were then given an intravenous injection of 160 mg gentamicin and the tissue concentrations in cortical bone were measured with microdialysis and compared to the values in bone specimens.

Animals and anesthesia

10 pigs (females, Danish Landrace breed, Aarhus University animal breeding facility, Aarhus, Denmark), weighing 39 (36–41) kg and with a creatinine level of 150 (107–199) μM, were included in the study. All animals underwent surgery under general anesthesia.

After an overnight fast, they were sedated with 0.5 mg/kg midazolam, and anesthesia was induced with ketamine 12.5 mg/kg, midazolam 0.5 mg/kg and atropine 0.05 mg/kg. The pigs were intubated endotracheally and ventilated by a respirator with a volumetric mixture of nitrous oxide in oxygen (2: 1) and 1–2% isoflurane. Additional fentanyl (50 μg/mL) 10 mL/h was given. The jugular vein and carotid artery were exposed and sheaths placed for taking blood samples and recording blood pressure. Normohydration was maintained by giving continuous infusion of saline (150 mL/h). A catheter was inserted to monitor urinary output. Arterial blood gases were followed intermittently. Rectal temperature was kept at 37–39 °C. The animals were anesthetized for 14–16 hours. After the study, they were killed with an overdose of pentobarbitone.

Surgical technique

After disinfecting the skin, we exposed the diaphysis of the right tibia by a longitudinal anteromedial skin incision 4 cm in length. Two incisions of 0.5 cm were made in the periosteum 1 cm apart. Guided by a protractor, two holes having a diameter of 1.1 mm. and depth of 15 mm were drilled into the cortical bone at an angle of 10 degrees at each periosteal incision (Figure 1). To ensure that the bone channel was inside the cortical bone, an x-ray was taken. The microdialysis catheters were introduced, as shown in Figure 1. At the distal end of the probe, a 1/2 × 1/2 cm piece of sleek was placed and fixed to the periosteum with a Vicryl 5-0 suture. A suture was placed in the subcutaneous tissue and skin. No postoperative exudation was seen 2 hours after this. To allow the tissue time to recover from the mild trauma of the insertion, we started the experiment 1 h later. After the animal was killed, the position of the catheters was checked by surgical exposure.

Drugs

All pigs were given 160 mg gentamicin intravenously (4 mg/kg body weight), by means of two 2-mL injection bottles with a gentamicin concentration of 40 mg/mL (Garamycin, Schering-Plough). The microdialysis inlet solution contained Garamycin without preservatives and antioxidants (Garamycin for intrathecal use, 2 mg/mL, 2 mL, Schering-Plough).

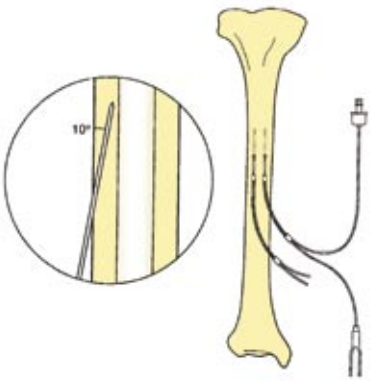

Figure 1. Use of microdialysis in cortical bone.

Microdialysis (Figure 1)

Microdialysis is based on sampling analysates from the interstitial space with a semipermeable membrane at the tip of a microdialysis probe. This probe is constantly perfused with a perfusate at a known flow rate. When the probe is implanted into the tissue, exogenous substances in the interstitial space diffuse through the membrane and can be collected inside vials for analysis (Muller 2000). The CMA 70 microdialysis brain catheters (CMA/ Microdialysis SE-171 18 Solna, Sweden) and the CMA 107 microdialysis infusion pumps (CMA/ Microdialysis SE-171 18 Solna, Sweden) were used for the vivo and in vitro studies.

Assessment of relative recovery and tissue values

To characterize the transfer rate of the probes, we assessed the in vitro and in vivo recoveries of gentamicin. For in vitro relative recovery, the microdialysis catheter was placed in glass beakers containing various concentrations of premanufactured gentamicin (range 1–27 mg/L). This experiment was done at 35–37 °C and 350 rotations per min (Buch & Holm, BCT BASIC IKA Werke). The probes were perfused at a flow of 2 μL/min and with different perfusates (saline ± 1% albumin).

The in vitro relative recovery was calculated as follows: Relative recovery (RR%) = $(C_{out} \times C_{glass}^{-1}) \times 100$, where C_{out} is the outlet concen-tration and C_{glass} is the concentration in the glass beaker. Since the in vitro recovery can differ substantially from the in vivo relative recovery, we assessed the in vivo recovery rates with the retrodialysis principle

(Muller et al. 1995, Chaurasia 1999), which relies on the assumption that the process of diffusion is quantitatively equal in both directions through the semipermeable membrane. Therefore, the compound of interest can be included in the perfusion medium at various concentrations and the disappearance rate through the membrane can be calculated. 7 premanufactured concentrations of gentamicin (ranging from 1.2 mg/L to 7.7 mg/L) and 1% albumin were added to the perfusate for calibration and the probe was perfused at a flow of 2 μL/min. The in vivo relative recovery was calculated as: RR = 1 – (C_{out}/C_{in}), where C_{out} is the outlet concentration (mg/L) and C_{in} the inlet concentration (mg/L) of a drug. By plotting the inlet concentration against the outlet concentration, the linearity of the line was checked by linear regression and for reliability of the calibration (Lorentzen et al. 1996). For calibrations that did not fulfil the requirement of linearity, we calculated the mean relative recovery (Muller et al. 1995, 1997). The value of recovery obtained was used to calculate the tissue concentration of gentamicin, using the formula $C_{tissue} = 100 \times C_{out} \times RR^{-1}$.

Analysis

Afferent and efferent fluid solutions of the in vivo and in vitro studies and serum samples were analyzed with an Abbott Drug Analyzer by a homogeneous spectrophotometric immunoassay technique, requiring samples of at least 100 μL. The detection limit was 0.27 mg/L.

Bone specimens

6 bone specimens were taken from the left tibia after injecting 160 mg gentamicin with a Coombs Bone Drill (outer diameter 5.5 mm). This drill was placed perpendicular to the bone, and standardized samples were take measuring 3–4 mm in diameter and 4–5 mm in height. We removed periosteum and bone marrow from all specimens or a small amount of corticocancellous bone. The specimens weighed 33.1 (SD 14.4) mg and were immediately frozen at –80 °C, pending bioassay.

Assays of bone specimens with a direct agar diffusion method

The concentrations of gentamicin in bone biopsies were measured by bioassays, using *S. aureus* ATCC 29213. A direct agar diffusion technique was used to determine gentamicin in bone (Dornbusch 1978). Standard curves were constructed by adding known amounts of gentamicin to weighed freeze-dried bone biopsies, dry weight 26.3 (SD 12.8) mg, which contained no antibiotics.

Tissue and serum samples

To determine the concentrations of gentamicin, blood samples were collected, bone specimens harvested from the left tibia and dialysates were taken from the microdialysis catheters in the right tibia at 1 h intervals (1–6 h).

Pharmacokinetic data and statistics

All values are means ± standard deviation (SD). The values from the two catheters were compared with a paired t-test. One way analysis of variance (ANOVA) was used to compare the microdialysates, bone samples and serum values of gentamicin. A p-value below 0.05 was considered significant.

The pharmacokinetic measures were peak concentration (C_{peak}), time to peak concentration (T_{peak}) and the area under the curve from 0 to 6 hours (AUC_{6h}), which describes the total amount of gentamicin passing through the tissue. Reproducibility was evaluated as intraanimal variability, expressed with the $AUC_{6h/medial\ probe}/AUC_{6h/lateral\ probe}$ ratio.

Reproducibility

To quantify the intraindividual variability of the microdialysis measurements, two probes were inserted into cortical bone for simultaneous measurement of analytes at the two sites.

Ethics

The surgical procedures were performed in accordance with the guidelines of the Danish Ministry of Justice, Animal Experimentation Inspectorate.

Results

In vitro recovery measurements

The recovery of gentamicin at a flow of 2 μL/min and a perfusate of saline was 30 (SD 3.6)%. The addition of 1% albumin increased the value to 41

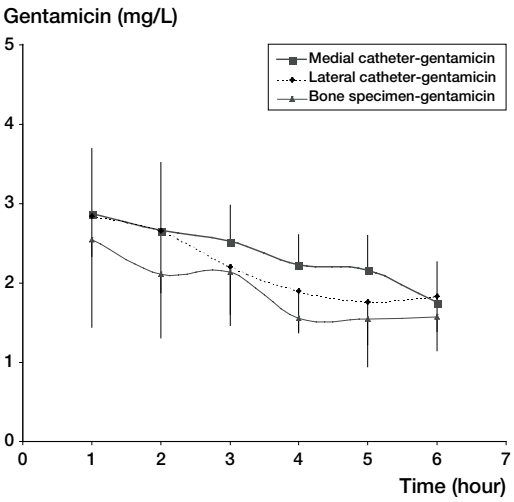

Figure 2. In situ concentration of gentamicin in cortical bone evaluated in microdialysates and bone specimens. All values are mean ± standard deviation.

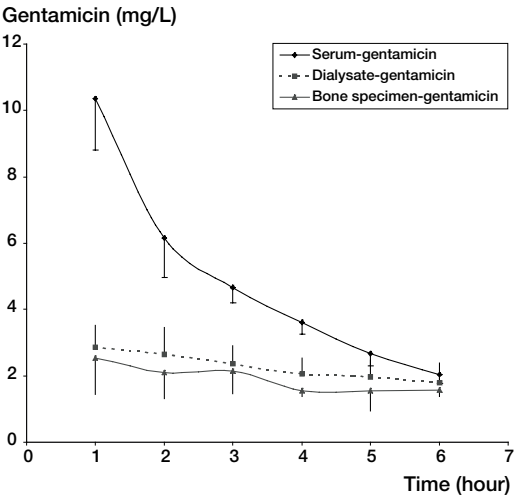

Figure 3. Concentrations of gentamicin in serum and cortical bone evaluated in bone specimens and microdialysates. All values are mean ± standard deviation.

(SD 6.8)% (p = 0.01). The linear regression values were $r_{saline} = 0.95$ (p = 0.01) and $r_{saline+1\% albumin} = 0.96$ (p = 0.001), respectively.

In vivo recovery measurements

The ideal of 7 measurements per catheter in each animal (total 70) was not realized mainly because the probe was damaged. 2 medial and 6 lateral catheters measurements were unsatisfactory. The total mean RR of the medial and lateral catheters were 33 (SD 11.9)% and 35 (SD 13.7)% (p = 0.4), and ranged from 24% to 45% and from 19% to 49%, respectively. The linear regression values with the medial catheter showed a coefficient of correlation > 0.7, and with the lateral catheter of > 0.8. Of the 20 catheters inserted, only 5 did not satisfy the criteria of linearity, mainly because of damage to the probe.

Gentamicin concentrations in microdialysates

The peak concentrations were 3.0 (SD 0.8) mg/L and 2.9 (SD 0.6) mg/L (p = 0.8) with the medial and lateral catheters (Figure 2). C_{6h} was 1.8 (SD 0.5) mg/L and 1.6 (SD 0.7) mg/L (p = 0.8), respectively. The time to peak concentration with the medial catheter was 1 hour in 6 animals and 2 hours in 4. 5 animals peaked at 1 hour and 5 at 2 hours with the lateral catheter. The AUC_{6h} with the medial and lateral catheters were 704 (SD 117) mg/min/L and 661 (SD 171) mg/min/L (p = 0.5). The peak ratio of tissue/serum was 0.30 (SD 0.1) and 0.29 (SD 0.1), respectively (p = 0.9).

We evaluated the reproducibility of the bone measurements using the microdialysis technique with the mean $AUC_{6h/medial\ probe}/AUC_{6h/lateral\ probe}$ ratio, which was 1.12 (SD 0.2).

Gentamicin concentrations in bone specimens

The peak concentration in bone specimens was 2.6 (SD 1.1) mg/L (Figure 2). The time to peak concentration was 1 hour in 6 animals, 2 hours in 3 animals and 3 hours in 1. The C_{6h} was 1.6 (SD 0.2) mg/L and the AUC_{6h} was 569 (SD 68) mg/min/L. The ratio of the mean peak concentration in tissue/peak concentration in serum was 0.27 (SD 0.12). The AUC_{6h} values obtained with the two probes of microdialysis using the technique and in bone specimens were similar (p = 0.07).

Serum concentrations of gentamicin

The peak concentration of gentamicin in serum was 10.4 (SD 1.5) mg/L (Figure 3). The time to peak concentration in serum was 1 hour in all animals. 6 hours after injecting gentamicin, the serum value was 2.0 (SD 0.3) mg/L. The AUC_{6h} in serum was 1394 (SD 116) mg/min/L.The peak concentration of gentamicin in serum and the AUC_{6h} were higher than in the microdialysates and bone specimens (p = 0.001).

Discussion

Microdialysis has been used in soft tissue to evaluate pharmacokinetic and pharmacodynamic substitutes (Muller et al. 1996, Hollenstein et al. 2000). In this study, the antimicrobial agent, gentamicin, was selected because of its clinical importance (Berger et al. 1981, Ipsen et al. 1991).

We inserted the microdialysis catheters in the diaphysis of the tibia in pigs we chose because of its easy access and relatively thick cortical bone at this side. We found no significant differences between the concentrations obtained with microdialysis and analysis of the bone specimens.

Our standard procedure consisted in placing the microdialysis catheter in the cortical bone and trying to avoid any contact with soft tissue. Since the catheters could not be fixed, we preferred to study the animals under general anesthesia because any movement might have changed the position or damaged the fragile probe. Analyses in bone specimens served as the controls because they have previously been used to evaluate antimicrobial agents in bone. We chose the direct agar diffusion method because of its high reproducibility (Dornbusch 1978, Heimdahl et al. 1988).

In all studies concerning microdialysis, it is important to assess the probe in vitro. One then obtains information about the recovery of the drug of choice, and factors affecting the collection of substances, like perfusion media, flow and type of probe, can be changed (Benveniste and Huttemeier 1990). We found that a flow of 2 μL/min^{-1} and perfusion medium containing 1% albumin gave a sufficient amount and recovery of gentamicin for analysis. The in vivo recovery of gentamicin in cortical bone tissue is relatively high, as compared to the 10–38% insubcutaneous and muscle tissue (Muller et al. 1995, Lorentzen et al. 1996). It is difficult to compare the relative recovery of antimicrobial agents from different species, and tissues, with different microdialysis catheters, flow of microdialysis and perfusates.

As regards the penetration of gentamicin in bone, Lorian (1978) reported a peak bone/serum ratio close to 30%, while we found one of 30%, 29% and 27% with the two probes and bone specimens, respectively. Moreover, we noted no significant difference between the AUC_{6h} of gentamicin, using the two probes and bone specimens.

Microdialysis seems to give reproducible results in bone as we found similar values with the medial and lateral probes. This observation is in agreement with measurements in muscle (Muller et al. 1995). In the present study, with therapeutic doses of gentamicin, the peak concentration exceeded the MIC values of surgically relevant bacteria, such as *P. aeruginosa* (MIC 0.9 mg/L), *S. aureus* (MIC 0.2 mg/L) and *Klebsiella* spp. (MIC 1.4 mg/L), which are representative values for members of the family of *Enterobacteriaceae* (Stemberger et al. 1997). With aminoglycosides and quinolones, a high peak concentration relative to the MIC value for the infecting organism is a major determinant of clinical response (Moore et al. 1987, Hyatt et al. 1995).

It is hard to evaluate antimicrobial agents from bone specimens and they vary greatly. Factors such as age, type of bone, antimicrobial agent used and types of assay performed are important (Heimdahl et al.1988). Moreover, biopsies are invasive. Microdialysis has the advantage of little trauma from the insertion, continuous sampling in the same individual and the ability to measure the free, unbound and active concentration of an antimicrobial agent (Chaurasia 1999). In conclusion, microdialysis seems ideal for dynamic in vivo measurements in cortical bone.

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No competing interests declared.

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