Application of Microdialysis to Corticocancellous Bone Tissue for the evaluation of Gentamicin
An Experimental study

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**Introduction**: Most antimicrobial agents exert their effect inside the interstitial space, which is the site of many infections. Knowledge of the pharmacokinetic profile of antimicrobial agents is of great clinical importance, since effective treatment demands concentrations that exceed the minimum concentration required inhibiting bacterial growth [1]. For the evaluation of antimicrobial agents in bone tissue, mainly studies of bone specimens have been performed [2]. Unfortunately are bone specimens static, traumatic to harvest and represent a combination of blood, bone- and connective tissue. Bone homogenisation may be misleading since only the unbound fraction of antimicrobial agents exerts activity and different methods of homogenisation or different bioassays gave rise to great variance [3]. Microdialysis, which is minimally invasive, is a relatively new technique that allows dynamic and continuous in vivo sampling. The principle is to introduce a semipermeable membrane into the tissue and perfuse it with a liquid that equilibrates with the fluid outside of the membrane by diffusion, thus enabling dynamic measurements to be made [4]. The aim of this investigation was to introduce microdialysis to corticocancellous bone tissue for the evaluation of gentamicin and compare the measured values to values obtained from bone specimens.

**Methods**: Experimental design: After the insertion of two microdialysis catheters into the metaphysis of the right tibia, we studied the relative recovery of gentamicin in corticocancellous bone tissue. Then all animals received an intravenous injection of 240 mg gentamicin and the tissue concentration in corticocancellous bone were measured by the technique of microdialysis and compared to values obtained from bone specimens.

Eight pigs (females, weighing 46,4±0,7 kg and creatinin 144,3±7,9 µM) were included into the study and all animals underwent surgery in general anaesthesia. The right metaphysis of tibia was exposed and two holes of a diameter of 1,1 mm, and depth of 15 mm, were drilled into the corticocancellous bone in an angle of 90 degrees. The microdialysis catheters (CMA 70 catheter, CMA microdialysis/Sweden) were introduced as illustrated on fig. 1.

For calibration of the transfer rate across the semipermeable membrane, we assessed the in vivo recovery of gentamicin according to the principle of retrodialysis [4]. The in vivo relative recovery was calculated as follows: RR = 1 – (C_{in} / C_{out}) where C_{in} is the outlet concentration (mg/L) and C_{out} is the outlet concentration (mg/L) of gentamicin. The obtained value of recovery was used for the calculation of the tissue concentration of gentamicin. The tissue concentration was calculated as:

\[ C_{tissue} = 100 \times C_{out} \times RR^{-1} \]

In order to determine the concentration of gentamicin, samples of blood, bone marrow, microdialysats and bone specimens were collected at 15 minutes and from one to six hours (1-6 h). Bone specimens were harvested from the metaphysis of the left tibia. The drill was placed perpendicular to the bone and standardised samples were obtained (weighed 54,1 ±3,1 mg). All tissue samples were immediately frozen at – 80°C.

**Essential results**: The peak concentrations of the two microdialysats and bone specimens were 6,73±0,8 mg/L, 6,52±1,09 mg/L, and 5,49±1,02 mg/L (fig. 2). Similar the area under the curve from 0 to 6 hours (AUC_{6h}) were 1569±198 mg/minute/L, 1721±248 mg/minute/L and 1390±121 mg/minute/L (ANOVA, P=0,5). Serum and bone serum gentamicin peaked at 33,3±2,66 mg/L and 27,46±1,11 mg/L respectively (Student T-test, P=0,75). Reproducibility of the measurement from the microdialysats was evaluated from the mean AUC_{6h}/catheter no. /AUCh catheter no. 2 ratio. This ratio was 1,01±0,17.

**Discussion**: The evaluation of bone specimens is a difficult task and frequent sampling on the same individual is often not possible due to ethical considerations. This is the first study applying microdialysis to corticocancellous bone tissue for the evaluation of gentamicin. It seems that microdialysis is a suitable, relative non-invasive and reproducible technique for dynamic and quantitative measurements of gentamicin in corticocancellous bone tissue. By this microdialysis it contributes to the knowledge of gentamicins pharmacokinetic in bone tissue.