coated with the secondary antibody and with silver electrodes fabricated on both sides. The Pan-Ab-Ag binds to the secondary antibody, forming a Pan-Ab-Ag-Ab sandwich, and which creates a molecular bridge between the two electrodes. This molecular bridge reduces the resistance between the electrodes, and resistance measurement is taken using a multimeter. In phase I of the study the biosensor was constructed and initially tested using both type 1 and 2 BVDV grown in cell culture and diluted to different concentrations. Concentrations of the various capture antibodies and polyaniline was optimized, and the detection limit of the sensor determined. In phase 2 of the study, serum known to be free of BVDV virus and BVDV neutralizing antibodies was inoculated with known concentration of BVDV virus and then assayed using the optimized biosensor.

Results

A conductometric biosensor was successfully constructed that was capable of detecting both type 1 and type 2 BVDV viruses grown in cell culture. The minimum virus concentration in cell culture that was detectible using the current architecture was 10(-3) cell culture infective doses per milliliter (CCID/ml). When applied to BVDV spiked serum, the biosensor was capable of detecting virus at the same concentration of 10 (-3) CCID/ml. The amount of time between sample application in the biosensor to stabilization of the electrical current averaged two minutes.

Significance

Results of this preliminary study provide proof of concept that a rapid field-based biosensor can be developed that is capable of detecting BVDV in both cell culture media and blood at a concentration that is biologically relevant for identifying PI's. This biosensor architecture has the capability of being miniaturized and automated to facilitate large scale field-testing if necessary. With mass production, the cost of the biosensor has the capability of being very low. Extension of this biosensor architecture to other disease agents is of great interest, including agents of bioterrorism and foreign animal diseases.

Penetration of Ceftiofur into Sterile versus *Mannheimia Haemolytica*-Infected Tissue Chambers in Beef Calves after Subcutaneous Administration of Ceftiofur Crystalline Free Acid Sterile Suspension in the Ear Pinna

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Introduction

Ceftiofur is a third-generation cephalosporin approved in the US for the treatment of bovine respiratory disease in beef and dairy cattle. Ceftiofur crystalline free acid sterile suspension (CCFA-SS) is a single administration product developed as an extension to the ceftiofur product line. The efficacy of a single dose of CCFA-SS administered subcutaneously in the neck was established in several clinical field studies. However, this route of administration was not acceptable for registration because of extended residues at the injection site. Consequently, the middle third of the posterior aspect of the ear pinna was selected as an alternative site of administration, and pilot studies were conducted to investigate the duration of efficacy resulting from a

high concentration formulation. These studies indicated that administration of 3.0 mg CE/lb (6.6 mg CE/kg) was efficacious, suggesting that therapeutic concentrations of ceftiofur and its active metabolite, desfuroylceftiofur, were maintained in interstitial fluids at sites of infection for prolonged periods. Bacterial infections of soft tissues, such as the lung, cause interstitial fluid composition changes which may affect the distribution of antibacterials or their *in vitro* activity in the target tissue. Evaluation of drug concentrations in intersitial fluid poses significant sampling difficulties; however, several studies have employed perforated tissue chambers implanted subcutaneously to characterize interstitial fluid drug concentrations in cattle.

The objectives of this study were to evaluate the plasma and tissue chamber fluid pharmacokinetics of ceftiofur and desfuroylceftiofur after subcutaneous administration of CCFA-SS in the ear pinnae of healthy feedlot calves, and to compare the penetration of ceftiofur and related metabolites into sterile versus *Mannheimia haemolyica* infected tissue chambers.

Materials and Methods

Four sterile tissue chambers were implanted into the paralumbar fossa of fifteen calves, two on each side. Thirty days following implantation, chamber fluid samples were obtained and cultured for sterility. All chambers found to be infected at this point were subsequently removed. Calves with at least one sterile chamber on each side were included in the study. Forty-five days following implantation, available chambers on one side of each calf were randomly inoculated with *Mannheimia haemolytica* (MIC = 0.03 μg/ml), while the remaining chambers on the opposite side were inoculated with sterile saline. Twenty-four hours after inoculation of chambers, each calf was injected with 3.0 mgCE/lb (6.6 CE mg/kg) CCFA-SS subcutaneously in the middle third of the posterior aspect of the ear pinna. Chamber fluid and blood samples were collected at predetermined times for 10 days following dosing and analyzed for total protein, ceftiofur and desfuroylceftiofur metabolites by high-performance liquid chromatography. Physical parameters such as attitude, respiratory score, rectal temperature and rumen fill were monitored throughout the entire study period.

Results

Twelve calves were included in the study. Concentrations of ceftiofur and desfuroylceftiofur metabolites in plasma and tissue chamber fluid remained above a pharmacokinetic threshold of 0.2 μg/mL for at least eight days. Infected tissue chamber fluid concentrations of ceftiofur and desfuroylceftiofur metabolites were significantly higher than those in non-infected tissue chamber fluid, which correlated with significantly higher total protein concentration in infected tissue chambers. Rectal temperatures were elevated in 10 of the calves 24 hours after inoculation of chambers with *Mannheimia haemolytica*, however, the temperatures were back to normal within 24 hours of CCFA-SS administration. Attitude, respiratory score and rumen fill scores remained normal throughout the study.

Significance

These results indicate that a single subcutaneous administration of CCFA-SS in the ear pinna at 3.0CEmg/lb (6.6 CE mg/kg) can be expected to provide effective therapy of susceptible bacterial infections for a period of at least one week. In addition, interstitial fluid is a better indicator of target site ceftiofur concentrations than plasma, and protein bound ceftiofur and related metabolites at active sites of infection may serve as a reservoir for microbiologically active drug in the animal. Due to the fact that, in previous studies, information on the affect of infection on ceftiofur and related metabolite concentrations reached in interstitial fluid had not been determined, this study provides additional data to support the efficacy of CCFA-SS as a viable mode of treatment for bovine respiratory disease. The tissue chamber model represents an excellent model of soft tissue "sequestered" or "walled off" from the rest of the body. Therefore, by infecting these chambers, estimates of drug concentrations reached in infected, consolidated lung tissue can be derived.