DR. SIMMONS: Thank you, Dr. Furrow.

Our next presenter this morning will be Dr. Jerry Ling. Dr. Ling received his D.V.M. degree from the University of California in 1965. From there he went to Boston for an internship at Angel Memorial Animal Hospital where he also served on the staff until 1968. In 1968, Dr. Ling returned to the University of California where he is presently a Professor in the Department of Medicine. Dr. Ling's clinical interests are internal medicine and urology and his primary research interest is in canine urology. Dr. Ling...

DR. LING: Thank you, Robert. The model I'm going to present to you today is a canine acute model for urinary tract infection.

USE OF AN ACUTE URINARY TRACT INFECTION MODEL FOR ANTIMICROBIAL DOSE DETERMINATION IN DOGS

Gerald V. Ling, D.V.M.

MATERIALS AND METHODS

Preparation of the Bacterial Inoculum

A suitable strain of bacteria, previously isolated from a canine patient with urinary tract infection (UTI) is selected to be the test organism. Selection of a certain genus and species as well as selection of certain strain characteristics such as antimicrobial susceptibility or certain uroepithelial binding capabilities may be considered prior to final selection of an infecting strain. If the strain has been stored in an organism bank, a small amount of bacteria from the storage receptacle is streaked for colony isolation onto a blood agar plate and incubated in air at 37°C overnight.

If the organism is a member of the family Enterobacteriaceae (Escherichia coli, Klebsiella spp., Proteus spp., Enterobacter spp., Salmonella spp., etc.), approximately 10 well isolated colonies from the blood agar plate are used to inoculate a flask containing 250 ml of brain-heart infusion (BHI) broth. Serial passages of the organism are made on each of the next 2 days by pipetting 1 ml of broth culture from the flask inoculated the previous day to a new flask containing 250 ml of BHI broth. Four hours before inoculation of dogs, 1 ml of broth culture from the third 250 ml flask is transferred to a flask containing 50 ml of BHI broth. This flask and all previous flasks are incubated in air overnight at 37°C. Dogs are inoculated with material from the 50 ml flask which contains about 10^8 bacteria per ml after 4 hours of incubation.

If the organism is Gram-positive (Staphylococcus aureus, or Streptococcus spp.), a 1/1,000 ml calibrated loop of well isolated colonies are used to inoculate a 50 ml flask of BHI broth. Dogs are inoculated from this flask after 6 hours of incubation in air at 37°C.

Preparation and Inoculation of Dogs

Dogs are anesthetized and the hair clipped from a 30 x 20-cm area over the left paralumbar fossa; the skin is surgically prepared. Each anesthetized dog is placed in dorsal

recumbency on a movable fluoroscopic table. After administration of (IV) meglumine and sodium diatrizoate USP (1 ml/kg), an abdominal compression band is positioned over the caudal part of the abdomen to dilate the renal pelves and proximal ureters by obstructing urine flow. The fluoroscopic tube is positioned over the left kidney so that its image is in the center of the fluoroscopic screen and the cross-hairs of the collimeter light mark the center of the kidney in the cranial-caudal plane. After a sterile disposable drape is applied, a disposable arteriole needle (20-gauge 7.5 cm, Model 8274[a]) is introduced through the skin and body wall. The inner stylet is partially withdrawn and the blunt outer cannula is used to probe for the kidney. When the tip of the cannula is seen to contact the convex surface of the kidney midway between the cranial and caudal poles at the edge of the fluoroscopic image, the stylet is repositioned and the needle is inserted through the parenchyma into the renal pelvis and proximal 0.5 cm of ureter. Care must be taken to position the needle parallel to the table surface during the entire procedure. After the needle is in position, the stylet is withdrawn and urine is seen to flow from the cannula. A sterile syringe containing about 10^8 bacteria in BHI broth (about 1 ml) is attached to the cannula and the broth culture is injected into the renal pelvis and ureter. The cannula is removed, the compression band is removed, and the dog is allowed to recover from anesthesia.

Forty-eight to 72 hours after inoculation, a specimen of urine is obtained by cystocentesis and is cultured in order to verify the presence of infection of the urinary tract by the inoculated organism. Treatment with the test drug may begin following verification of UTI.

DISCUSSION

The therapeutic half-life of the test drug, and the percentage of the dose that may be found in active form in the urine (or the attainable concentration in urine of a range of dosages) must be established for dogs prior to effective use of this model. It is preferable to establish the % of the dose to be found in urine within a certain time period following dosing (6 or 8 hours) after steady state has been achieved, since pet dogs do not usually refrain from urinating much longer than 8 hours under normal conditions.

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[a]Becton-Dickinson Division of Becton-Dickinson and Company, Rutherford, N.J.

The model yields virtually 100% incidence of UTI with the inoculated organism without the need to induce pre-infection physical or chemical trauma to the urinary tract. Approximately 1 in 20 dogs (varies depending on the organism used) die acutely (within 24 hours) after inoculation from septicemia presumably due to inadvertent entry of the bacteria into the renal vasculature. Formation of a large perirenal abscess around the inoculated kidney has occurred rarely within 2-3 weeks after inoculation and is a cause of "poor-doer" animals.