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PHARMACOKINETIC EVALUATION OF CEFTIOFUR SODIUM IN SERUM, TISSUE CHAMBER FLUID, AND BRONCHIAL SECRETIONS FROM BEEF-BREED CALVES

By

Steven Leroy Halstead

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ABSTRACT

PHARMACOKINETIC EVALUATION OF CEFTIOFUR SODIUM IN SERUM, TISSUE CHAMBER FLUID, AND BRONCHIAL SECRETIONS FROM BEEF-BREED CALVES

By

Steven Leroy Halstead

Ceftiofur sodium (Naxcel[R], Upjohn), is a 3rd generation cephalosporin intended for treating infections caused by Pasteurella haemolytica, Pasteurella multocida, and Hemophilus somnus; organisms commonly associated with bovine respiratory disease (BRD). To determine the pharmacokinetic (PK) behavior of this drug in healthy beef-breed calves, Naxcel[R] was administered by daily intramuscular injection for 4 days at dosages of 1.1, 2.2, and 4.4 mg/kg of body weight, with 4 week intervals between dosing regimens. Serum, tissue chamber fluid, and bronchial secretions concentrations of Naxcel[R] were evaluated by microbiological assay and microcomputer-based PK software. Susceptibility of BRD-associated bacterial pathogens to Naxcel[R] was evaluated by microdilution techniques. Our findings suggest that Naxcel[R] is well absorbed from the injection site, produces linear increases in serum concentrations with increasing dosage, penetrates tissue cage fluid and bronchial secretions well, has a long elimination half-life, and exceeds the minimum inhibitory concentrations of organisms commonly associated with BRD.

DEDICATION

This accomplishment is dedicated to Shelly, Benjamin, and Gresham, my family; for the support and encouragement they have provided, and the patience they have shown in the completion of this work. Their hands, hearts, and souls have been one with mine from the development of the hypothesis to the typing of the last period in this manuscript, a gift of sacrifice and love which defies measure.

And to my parents, for unfailing faith and assistance.

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An effort such as this is in no way a solo accomplishment. John Baker, Robert Walker, Gary Stein and Robert Holland, my graduate committee, provided encouragement, support and occasional (and necessary) prodding beyond their official obligations, as well as the knowledge, inspiration and committment to make ideas reality.

Kathleen MacDonald deserves more than a few lines of type for her contribution to this project. Her technological knowledge was exceeded only by her friendship and healthy perspective. All were vital to the completion of this graduate program.

Joseph Hauptman provided invaluable experience and knowledge in the statistical modeling and evaluation of this project, again, an effort that contributed much more than is visible in the simple facts and data in this report.

Although not officially involved in this project, Michelle Kopcha and Kent Ames were vital as providers of support and encouragement, deserving much credit for the completion of this undertaking.

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INTRODUCTION

Ceftiofur sodium is a 3rd generation cephalosporin antimicrobial agent manufactured by The Upjohn Company of Kalamazoo, Michigan, and marketed under the trade name Naxcel®. The product is labeled for use in beef cattle as treatment for bovine respiratory disease caused by Pasteurella haemolytica, Pasteurella multocida, or Haemophilus somnus, at a once daily intramuscular dose of 0.5 mg/lb (1.1 mg/kg)[a]. Ceftiofur sodium is reported to possess broad spectrum antimicrobial activity (Yancey et al., 1987), low toxicity at doses 10 to 25 times in excess of the recommended dose, serum protein binding of approximately 90%, and the ability to produce high blood concentrations with a relatively long serum elimination half-life[b]. While serum pharmacokinetic parameters such as peak concentration and elimination half-life have been used as indicators of drug-host interaction, serum pharmacokinetic data alone may not completely describe the behavior of a drug in various tissues and tissue fluids (Bergan, 1981; Weinstein et al., 1951). Understanding the disposition of a drug at specific tissue locations is important in that most bacterial infections occur at locations other than vascular beds (Wise, 1986), and serum pharmacokinetics may not reflect target tissues values (Clarke et al., 1989a; Schentag, 1988). Knowledge of drug pharmacokinetic parameters in samples that most closely approximate target tissue fluids, therefore, allows for more accuracy in predicting potential treatment efficacy (Prescott and Baggot, 1988).

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[a] Naxcel® package insert, The Upjohn Company, Kalamazoo, Michigan.

[b] Miller CC. Ceftiofur research and development. Personal communication.

BOVINE RESPIRATORY DISEASE COMPLEX

Bovine respiratory disease complex (BRDC) is a term inclusive for many etiologies and syndromes of bovine respiratory disease (BRD). Like most syndromes, it is poorly understood and, therefore, difficult to define. The interaction of numerous infectious agents, environmental factors and host factors result in disease incurring greater economic loss than all other feedlot diseases combined (Jensen and Mackey, 1979). Bovine respiratory disease complex is considered by both the National Cattleman's Association and the National Livestock Feeders Association to be the number one problem in commercial beef production (Jensen and Mackey, 1979). Costs to the producer and ultimately the consumer include death loss, treatment supplies and labor, loss of production efficiency, decreased feed conversion, replacement animals, preventive measures, and other expenses (Yates, 1982). Bovine respiratory disease resulted in estimated losses of 25 million dollars for the United States cattle industry in 1954, and increased to at least five hundred million dollars for the entire North American cattle industry in 1984 (Loan, 1984).

The multifactorial nature of BRD makes it a complex subject for study, as indicated by the volume of literature generated in attempts to understand and recreate the disease. Examination of each of the factors mentioned above helps to illustrate the complexity of this disease syndrome, and allows better understanding of certain critical components.

Host Factors

The bovine respiratory system is typical of the mammalian design in that it is composed of an upper portion primarily responsible for conducting and conditioning inspired air, and a lower portion that is active with both respiratory and non-respiratory functions (Lillie, 1974; Ryan and Grantham, 1989). The exchange areas are those portions of the lung where pulmonary capillaries are exposed to inspired gasses: the respiratory bronchioles and alveoli. These areas are lined primarily with a monolayer of thin, non-ciliated cells called type 1 pneumocytes that are in direct contact with the pulmonary vascular system, across which the respiratory gasses diffuse (Murray, 1986). Non-respiratory functions of the lung include water, electrolyte and pH balance, temperature regulation, elimination of volatile materials, synthesis of essential compounds, and maintenance of blood reserves (Lillie, 1974; Ryan and Grantham, 1989). Injury to the lung results in varying levels of loss of both respiratory and non-respiratory activity.

Passage of inspired air from the nostril to the thoracic trachea warms the air to near body temperature and adds moisture to approximately 95% relative humidity (Breeze, 1985). The air is also filtered of almost all inspired particles. Particulates suspended in the inspired air are removed by impaction, sedimentation, brownian motion, turbulent diffusion and other means, depending on the size, shape, mass and respiratory rate (Clarke, 1983). Particles greater than 2 microns in diameter will not penetrate to the level of the alveoli, while those smaller than 0.5 microns will be exhaled without settling on the mucosa (Veit and Farrell, 1978). Particles within these extremes, including those containing bacteria and viruses, will be capable of settling on the alveolar epithelial surfaces (Veit and Farrell, 1978; Thomson and Gilka, 1974).

The airways from the upper respiratory passages to the terminal bronchioles are lined with ciliated epithelium and coated with a mucous layer that is approximately 7 microns thick. This mucous blanket is made up of 2 distinct zones: a superficial, water impermeable gel layer that is approximately 2 microns thick, and the remaining deep sol layer that surrounds and

suspends the epithelial cilia, providing the proper environment for ciliary propulsive activity (Jubb et al., 1985; Liggitt, 1985). The tips of the cilia extend beyond the sol layer to contact the gel layer. The ciliary propulsive activity results in gel layer movement toward the oropharynx at 10-15 mm/min (Jubb et al., 1985; Veit and Farrell, 1978). Aerosol particles that have reached the pulmonary parenchyma are transported to the oropharynx by this mucociliary escalator mechanism, and subsequently swallowed (Liggitt, 1985). Particles reaching the non-ciliated areas of the respiratory mucosa (areas beyond the terminal bronchioles) are phagocytized by pulmonary alveolar macrophages or removed by the slow anterior migration of the surfactant layer (Dyer, 1982).

In addition to ciliated epithelial cells, the tracheo-bronchial mucosa contains serous cells, goblet cells, and submucosal mucous glands that produce the gel and sol layers. Brush cells are scattered among the ciliated cells and are thought to regulate the volume of the secretions (Murray, 1986). Additional types of epithelial cells that have been identified are the basal cells that are capable of differentiation into other cell types, and neuroepithelial cells which may be important as chemoreceptors, modulating pulmonary vasoconstriction and smooth muscle activity (Breeze, 1985). Two types of mesenchymal cells: lymphocytes and globule leukocytes, have also been identified in tracheo-bronchial epithelium. Globule leukocytes appear to be derived from subepithelial mast cells that have altered histochemical and morphologic properties resulting from the loss of some of their granular contents. The function of these cells is unknown, but they are frequently seen during histopathological examination of bovine lung with or without active parasitic infection, a condition which is known to increase mast cell population due to antigenic stimulation, and are therefore felt to be involved in lung defense through antigen recognition and processing (Breeze, 1985).

Corresponding mesenchymal cells in the alveoli and respiratory bronchioles are the pulmonary-alveolar macrophages (PAMs), lymphocytes (primarily T-lymphocytes), plasma cells, mast cells,

eosinophils and neutrophils. These cells are responsible for phagocytosis and immune processing of particulates, and are the primary defense of the non-ciliated respiratory epithelium, either by the mechanisms mentioned above or by chemical signaling and triggering of other host defenses (Dyer, 1982; Liggitt, 1985).

The respiratory system is also protected by humoral immune mechanisms. Secretory IgA is predominant in the upper respiratory tract (except in calves less than 6 weeks of age, in which IgG_1 predominates) while IgG (IgG_1 being the predominant subclass) is the major opsonizing immunoglobulin in the lower respiratory tract (Berggren, 1981; Dyer, 1982; Liggitt, 1985). Immunoglobulin G also activates complement, agglutinates bacteria, neutralizes toxins, and inactivates viruses (Dyer, 1982). Although IgA also contributes to the total immunoglobulin content in the lower respiratory tract, concentrations decrease in fluids closer to the alveoli (Dyer, 1982). Pulmonary IgA appears to be most effective in toxin neutralization and blockage of viral and bacterial adherence, as it has not been shown to be a significant opsonin (Walker et al., 1980). Immunoglobulin A may also reduce immune recognition of antigen and by this, moderate immune inflammatory response, blocking phagocytosis (Liggitt, 1985).

Interferon is present in the respiratory secretions, being produced by a variety of cell types including epithelial cells, lymphocytes, neutrophils, and macrophages, in response to active viral infection (Babiuk et al., 1987; Bielefeldt et al., 1987). Interferon is active as an inhibitor of viral, bacterial, fungal, and protozoal organsims, and tumor cells (Ligitt, 1985; Rosenquist and Allen, 1990). Interferon also serves in regulation of both humoral and cellular immunity by enhancing macrophage-mediated phagocytic activity (Ligitt, 1985).

Complement content of the bovine lung has not been accurately measured (Liggitt, 1985). It is likely that complement plays a role in chemotaxis and opsonization, and may prolong inflammation in the lung, resulting in further lung injury (Liggitt, 1985).

The alveolar lining material is also active in protection of the respiratory system, primarily as a physical barrier to attachment of invading organisms (Liggitt, 1985). The predominant component of this material is surfactant, which, when concentrated from lung lavage fluid and tested in vitro, also appears to enhance phagocytosis and intracellular killing by alveolar macrophages (O'Neill, 1984).

Ruminant lungs differ from other mammalian lungs in that the pulmonary pleura is continuous with the septa between lobules (Dellman and Brown 1976). These septa are dense and fibrous, creating a highly compartmentalized lung (Jubb et al., 1985; Blood et al., 1983). Bovine lungs possess the greatest amount of compartmentalization, carnivores the least, with swine and sheep being intermediate (Dellman and Brown, 1976). Cattle also have fewer interalveolar connections known as Pores of Kohn, to provide collateral routes of air passage (Dellman and Brown, 1976). With lung injury, this high degree of compartmentalization and low degree of collateral ventilation leads to airway and alveolar hypoxia, decreased clearance and decreased phagocytosis with injury to the lung (Blood et al., 1983). These changes result in an environment favorable to bacterial survival and growth (Blood et al. 1983).

In summary, the defense of the respiratory epithelium and parenchyma depends upon removal of infectious and irritating substances by mucociliary clearance, phagocytosis, and immune processing and clearance. Epithelial cell, secretory cell, or leukocyte damage, and anatomic properties intrinsic to the ruminant respiratory system may allow pathogenic organisms to accumulate, elaborating toxins or other virulence factors in concentrations that cause further damage, resulting in pneumonia.

Environmental Factors

Normal, healthy, immunologically competent, non-stressed calves are quite resistant to primary bacterial respiratory disease as shown by experimental challenges with common respiratory pathogens such as P. haemolytica or P. multocida (Hjerpe ,1983; Potgieter et al., 1984; Yates, 1982). Stressing these same calves, however, can alter host resistance and result in clinical BRD (Confer et al., 1988; Filion et al., 1984). Stress is a poorly defined neuroendocrinologic reaction that cattle experience when exposed to new environmental and social conditions (Dyer, 1982). These factors are often production and management related, and include weaning, shipping, crowding, exhaustion, dehydration, chilling or overheating, starvation, exposure to diseased animals or various infectious agents, vaccination, tagging, dehorning, deworming, delousing, antibiotic treatment, etc. (Lillie, 1974; Slocombe et al., 1984). These influences combine to reduce the effectiveness of the mucociliary and cellular clearance mechanisms by raising endogenous steroid levels (Filion et al., 1984; Roth, 1984). High corticosteroid concentrations, endogenous or exogenous, suppress neutrophil function and antibody concentrations, depress the bactericidal activity of phagocytes (Dyer, 1982; Roth, 1984), may alter the composition of the mucous blanket by increasing goblet cell mucous production, and may depress ciliary activity (Hjerpe, 1983) coincident with primary exposure to specific pathogens, increasing the likelihood of development of pneumonia (Hjerpe, 1983; Roth, 1984).

Infectious Agents

Many infectious agents have been associated with BRD. Some appear to be capable of inducing severe natural disease as solo agents, although most outbreaks of respiratory disease involve combinations of agents, host factors and environmental factors. Of the viral agents implicated in BRD, only bovine herpesvirus 1 (BHV-1, also known as infectious bovine rhinotracheitis virus, or IBR virus), bovine herpesvirus 4 (BHV-4), bovine virus diarrhea virus (BVDV), bovine respiratory syncytial virus (BRSV) and malignant catarrhal fever virus (MCF) have been shown to cause severe primary disease (Hjerpe, 1983; Mosier, 1989). Parainfluenza

type 3 virus (PI_3) can also cause primary respiratory disease of limited severity, the demonstration of clinical signs depending on the severity of the infection (Dyer, 1981). Both PI_3 and BRSV attack and replicate in the epithelium of the upper and lower respiratory system, causing cellular necrosis and death (Bryson, 1983a; Bryson, 1983b). Destruction of these ciliated cells results in mucociliary clearance failure, and leads to replacement with immature epithelial cells which may favor subsequent bacterial colonization (Babiuk, 1987). Parainfluenza type 3 and BRSV also infect and disturb the function of alveolar macrophages, reducing phagocytosis and destruction of engulfed particles (Dyer, 1982; Bryson, 1985). Bovine viral diarrhea virus has been shown to induce mild interstitial pneumonia following experimental endobronchial inoculation, and may stimulate the development of host antibodies in cases of naturally occurring pneumonia (Yates, 1982). Bovine herpes virus type 1 causes acute necrotic rhinotracheitis, pharyngitis, and laryngotracheobronchitis in natural and experimental infections, and pneumonic lesions in experimental infections (Yates, 1982). Again, ciliated tracheal epithelial cell destruction occurs and macrophages are infected, although replication within macrophages has not been demonstrated (Yates, 1982).

In addition to the previously discussed viruses that are classically associated with BRD; adenoviruses, coronaviruses, rhinoviruses, reoviruses and enteroviruses have also been recovered from outbreaks, and many are capable of causing airway as well as interstitial lesions (Bryson, 1985; Dyer, 1981). Only PI_3, BHV-1, BHV-4, BRSV and BVDV, however, have been experimentally shown to reduce respiratory tract resistance to bacteria (Al-Darraji et al., 1982; Dyer, 1981; Dyer, 1982; Hjerpe, 1983; Trigo et al., 1984).

Numerous bacterial organisms have been associated with BRD. Pasteurella haemolytica, P. multocida, H. somnus, Actinomyces pyogenes, Salmonella spp., Mycoplasma spp., Escherichia coli, Staphyloccus aureus, Listeria monocytogenes, and Streptococcus spp. have all been isolated in pure culture from feedlot cattle suffering from pneumonia, with or without concurrent

systemic infections (Wikse, 1985). Of these, P. haemolytica, P. multocida, H. somnus and A. pyogenes are considered the most important bacterial causes of pneumonia.

Pasteurella haemolytica biotype A serotype 1 is the bacterial organism most frequently isolated from cases of BRD in North America, followed in frequency of isolation by P. multocida (Frank, et al., 1989; Wikse, 1985). The lesions attributed to P. haemolytica are described as fibrinous pleuropneumonia with interstitial vascular and lymphatic thrombosis. Bronchitis or bronchiolitis is less commonly seen (Friend et al., 1977). Fibrinous bronchopneumonia is the lesion most frequently described for P. multocida-associated pneumonia, with much less pleuritis than is seen with P. haemolytica (Rehmtulla and Thomson, 1981).

Both P. haemolytica and P. multocida exist as commensals on the nasopharyngeal mucosa of calves, but are isolated infrequently and in low numbers from tracheal or bronchial samples (Frank and Smith, 1983; Gray and Thomson, 1971; Yates, 1982). Of the 12 recognized serotypes of P. haemolytica, serotype 2 has been isolated most frequently from feeder cattle at the farm of origin, while serotype 1 was predominant in these same cattle after shipping through an auction barn to a feedlot (Frank and Smith, 1983). This shift in predominant isolate indicates that the stresses associated with feeder cattle operations create changes in the calf that favor P. haemolytica serotype 1 (Frank and Smith, 1983). The ability of these organisms to produce disease without predisposing lung damage or defense compromise is questionable, however, as demonstrated by the need for massive experimental dosing (on the order of 2×10^9 colony forming units of P. haemolytica in logarithmic growth phase) to non-immune or systemically stressed animals (Walker et al., 1981; Shoo, 1989; Yates, 1982). Variation in virulence between strains of P. haemolytica may also determine the ability of a particular strain to cause primary infection (Chang, 1987; Shoo, 1989). The importance of P. haemolytica and P. multocida in BRDC is beyond question, regardless of primary or secondary involvement, based on the frequency of isolation and observation of characteristic lesions in naturally or

experimentally occurring disease (Allen, 1985; Allen, 1984; Potgieter et al., 1984; Lillie, 1979). In addition, although management-related stress and viral pathogens are as common in Australia as in the United States, P. spp. organisms are much less likely to be isolated. Correspondingly, Australian cattle experience very little BRDC (Wiksie, 1985).

Haemophilus somnus is commonly associated with acute septicemia, but also causes acute or chronic respiratory disease, and can be cultured from cattle with bronchopneumonia and fibrinous pleuritis (Harris, 1989; Stephans et al., 1981). Although this organism is rarely isolated from nasal swab samples collected from healthy cattle (Corbeil and Gogolewski, 1985) it is reported to exist as a commensal in the bovine nasopharynx (Corstvet et al., 1973; Dyer, 1981). Pneumonic lesions associated with the suppurative bronchopneumonia caused by H. somnus include hemorrhage, thrombosis, and infarction, and may also include laryngitis and hemorrhagic tracheitis (Harris, 1989; Hjerpe, 1983). The respiratory system appears to be the portal of entry for the systemic spread of this organism, leading to septicemia (Harris, 1989). This organism is capable of survival within bovine neutrophils and macrophages, and replicates within monocytes (Harris, 1989). Haemophilus somnus causes thrombotic lesions in small blood vessels, leading to interruption in blood flow and some of the signs associated with clinical manifestations of infection with this organism. Thrombolic meningoencephalomyelitis, myocarditis, myelitis, mastitis, and polyarthritis have all been reported due to H. somnus (Harris, 1989).

Actinomyces pyogenes is frequently isolated from purulent lesions in cattle, and is commonly isolated from chronic lung lesions and abscesses along with Bacteroides melaninogenicus (Wikse, 1985). Hematogenous spread is suspected in many of these cases, especially when A. pyogenes has been cultured from the blood or from lesions associated with bacterial endocarditis.

To summarize in a quote from C.A. Hjerpe, "Bovine respiratory disease complex refers to bacterial infections of the lower respiratory tract with organisms that are normal saprophytes in the upper respiratory tract and to which the normal lower tract is constantly exposed. Clinical disease results when mucociliary and/or pulmonary macrophage clearance is depressed by the combined effects of respiratory viruses and stress, permitting initiation of a bacterial bronchopneumonia and/or fibrinous pneumonia."

Pathogenesis

The pathogenesis of BRDC, then, is thought to involve primary viral infection and/or stress-associated vascular, humoral and immune system changes that alter the respiratory system such that bacterial colonization can occur. Bacterial localization and colonization of the lower respiratory tract may occur by aerosol inhalation, gravitational flow, progressive colonization (Babiuk et al. 1987), or possibly by hematogenous spread from the upper respiratory tract to the lower respiratory tract (Thomas et al., 1989). Virus-induced epithelial and mucus layer injury, or stress-related suppression of normal defense and clearance mechanisms allow increased bacterial adherence, focal replication and microcolonization (Frank et al., 1986). In pneumonia caused by P. haemolytica, elaboration of toxins by developing bacterial colonies, rather than invasion by the organism, further damages the host. The exotoxin known as leukotoxin has been shown at low concentrations to reduce the immunocompetency of pulmonary-alveolar macrophages, lymphocytes, and neutrophils (Styrt et al., 1990). At higher leukotoxin concentrations the phagocytes are killed, releasing lysosomal enzymes that damage the host and attract additional phagocytes that will respond in like fashion (Markham and Wilkie, 1980; Mosier et al., 1986; Shewen and Wilkie, 1982; Styrt et al., 1990; Wilkie et al., 1990). The leukotoxin is produced in bacteria grown in vitro only during the early logarithmic phase of growth in enriched medium (Markham and Wilkie, 1980; Wilkie and Shewen, 1989), is heat and pH labile, oxygen stable, and non-hemolytic to bovine and ovine erythrocytes (Baluyut et al., 1981), is specific for ruminant leukocytes (Moore et al., 1985; Mosier et al.,

1986), is immunogenic in cattle (Moore et al., 1985; Vega et al., 1987), and is reduced in effect by antibody directed against antigens on the bacterial surface (Styrt et al., 1990). Pasteurella haemolytica is also capable of injuring the host by neutrophil independant means, as demonstrated in studies with neutrophil deficient calves (Brieder et al., 1988, Slocombe et al., 1985) Another P. haemolytica toxin of primary importance is the lipopolysaccharide component of the bacterial cell wall known as endotoxin. This variably toxic surface antigen is produced by all gram negative bacteria, and is responsible for many systemic responses, including hyperthermia, pulmonary hypertension, systemic hypotension, and vascular thrombosis seen with this and other gram negative bacterial infections (Babiuk et al., 1987; Dyer, 1981; Breider et al., 1988; Slocombe et al., 1985; Volk et al., 1986a). The current understanding of pneumonic pasteurellosis suggests that elaboration of these virulence factors subsequent to patent infection with this organism leads to severe pneumonia as accumulations of dead host and bacterial cells, blood and plasma products, and devitalized tissues produce an ideal environment for bacterial growth: complete with nutrients and protected from endogenous or xenobiotic antimicrobial agents (Babiuk et al., 1987).

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CONTROL OF BRDC

Vaccination

Prevention of BRDC is an obvious management goal. Controlling stress factors and exposure to clinically ill animals can do much to prevent bovine respiratory disease. Because this is not always possible or practical, and because some incidence of disease will occur regardless of the level of management intervention, an effective vaccination method has been sought for some time. Many products intended to stimulate active immunity to BRDC pathogens have been produced and marketed. Vaccines and bacterins in various combinations of IBR, PI_3, BVDV, BRSV, P. haemolytica, P. multocida, and H. somnus are currently available (Dyer, 1982; Yilma and Breeze, 1985; Confer et al., 1988). Numerous studies have shown that vaccination against viral components of BRDC can reduce incidence or severity of both natural disease and experimental disease, and many authors offer suggestions for vaccine programs (Stockdale et al., 1979; Woods et al., 1973; Hjerpe, 1983; Mosier et al., 1989).

Systemic vaccination with P. haemolytica bacterins cannot be recommended as the incidence and severity of the disease is not affected, or is actually enhanced, by these products (Confer et al. 1988; Mosier et al., 1989; Purdy et al., 1986; Wilkie et al., 1980). Bacterin-induced enhancement of pneumonic pasteurellosis may be a direct result of high endotoxin content that results from culture procedures intended to optimize cell yield rather than other specific virulence factors, or by increased immune recognition of the bacterial organism, resulting in leukocyte influx and phagocytosis (Wilkie and Shewen, 1989). Immune-mediated toxin neutralization is not produced with these vaccines, however, and leukotoxin induced phagocyte damage, degranulation, and host tissue damage results (Amstutz et al., 1981; Confer et al.

1988; Friend et al., 1977; Mosier et al., 1989; Wilkie et al., 1980; Wilkie and Shewen, 1989). Vaccines consisting of live Pasteurella organisms delivered by aerosol or trans-tracheal route may offer protection but are not practical on a commercial scale and may in fact result in problems similar to those encountered with the bacterins (Walker et al., 1980). Vaccines directed against P. haemolytica toxins should be possible in that the toxins are immunologically active. These products may offer protection and avoid the problems of enhanced bacterial surface antigen recognition without cytolysis as seen with bacterins (Confer et al., 1989; Moore et al., 1985; Mosier et al., 1989; Panciera et al., 1984), but are as yet unproven. Aerosol vaccination depends on stimulation of mucosal IgA production, with subsequent IgA-induced inhibition of bacterial multiplication on mucosal surfaces. While this may indeed occur, local mucosal factors responsible for inhibiting or enhancing colonization by P. haemolytica have not been specifically identified (Confer et al., 1988). One report of an intramuscular (IM) vaccine containing P. haemolytica A-1 leukotoxin and serotype specific antigens, however, does suggest significant protection (Wilkie and Shewen, 1989). From this report and other reviews, it appears that as specific antigen components (subunits) are isolated and characterized, their inclusion in subunit and toxin vaccines will offer the most potential for effective, safe vaccines for use against BRDC (Confer et al., 1988; Mosier et al., 1989).

Treatment

Treatment of BRDC has classically involved a reliance on suppression of the bacterial component using antibiotics to control the population of organisms, thereby reducing the toxins that are produced as metabolic by-products or cell constituents (Mechor et al., 1988; Hjerpe, 1984). Anti-inflammatory drugs are also frequently used to reduce the toxin-mediated destructive inflammatory reaction that accompanies bacterial pneumonia (Emau et al., 1985). Other treatment modalities such as fluid, antihistamine, and bronchodilator administration, and nutritional support are also common (Bryson, 1985). The key to treatment of the disease, however, appears to be the appropriate use of antimicrobial agents. While bacterial culture

and antimicrobial susceptibility determination of the isolated pathogens is the most accurate method for defining specific antimicrobial treatment in a given case, several investigators have looked at trends of antimicrobial susceptibility for pneumonic pathogens in an effort to predict susceptibility patterns and treatment response (Biberstein and Kirkham, 1979; Fales et al., 1982; Mechor et al., 1988). Of drugs approved for use in food animals, erythromycin, sulfachlorpyridazine and tetracycline were declared the most appropriate based on in vitro sensitivity studies with P. haemolytica, P. multocida and undifferentiated P. spp. collected from animals that died of naturally occurring pneumonia (Fales et al., 1982). Of non-approved drugs, gentamicin was most effective. Cephaloridine, a first generation cephalosporin, demonstrated excellent activity against non-specified pasteurella species, but was not tested against typed P. haemolytica or P. multocida (Fales et al., 1982). Cephalothin, another first generation cephalsoporin, was found to be effective against both A and T biotypes of Pasteurella haemolytica (Biberstein and Kirkham, 1979). Other investigators found oxytetracycline to be less effective than penicillins, but did not look at erythromycin (Mechor et al., 1988). This study also indicated that first generation cephalosporins might be appropriate for antimicrobial treatment of BRDC based on the susceptibility of these pathogens to cephalothin.

Of these drugs, cephalothin was active against various strains of P. haemolytica and P. multocida regardless of plasmid content, implying that transmissible plasmids do not code for resistance to this drug class. This was not true for penicillins and oxytetracyclines, where resistant strains possessed at least one plasmid genetically coding for resistance (Yung-fu et al., 1987).

ANTIMICROBIAL THERAPY

Introduction

Knowledge of in vitro susceptibility and resistance patterns are important when considering antimicrobial therapy, but this understanding only addresses one aspect of the interaction between host, antimicrobial agent, and microorganism. To understand this interaction, characterization of the absorption, distribution, metabolism and excretion of the drug in the host is necessary. That is: quantification of the host's response to the drug, such that dose, route of administration and frequency of administration are optimal for the specific infectious disease process (Welling, 1986). This quantification involves measurement of parameters such as the half-life of distribution ($T_{1/2\alpha}$), half-life of elimination ($T_{1/2\beta}$), maximum attainable concentration (C_{max}), time from drug administration to C_{max} (T_{max}), and total drug present over time (area under the curve, or AUC) for each target tissue location (Welling, 1986; Greenblatt and Shader, 1985). Additionally, computation of the therapeutic margin from the ratio of therapeutic concentration to toxic concentration is important for obvious reasons (Schentag, 1989; Volk et al., 1986b). Finally, these values, and therefore the dosing parameters, may not be consistent from animal to animal, being influenced by age, body weight, nutritional status, diet, and specific disease state (Kirkwood and Widdowson, 1990; Welling, 1986; Greenblatt and Shader, 1985).

Basic Principles Of Pharmacokinetics

DISTRIBUTION Distribution of a drug throughout the body of the host is dependant upon such physiochemical properties of the drug as molecular size, degree of ionization, pK value, lipid solubility, and protein binding (Greenblatt and Shader, 1985; Wise, 1986). When distributed via

the vascular system, the size of a tissue component and volume of blood flow through that tissue are also important (Greenblatt and Shader, 1985). These factors can be applied mathematically to create pharmacologic "compartments" in which a drug is rapidly and homogeneously distributed upon introduction (Kinabo and McKellar, 1989). The vascular system or a specific tissue, such as lung or skeletal muscle may be considered examples of compartments (Wise, 1985; Kinabo and McKellar, 1989).

When a drug is administered by intravenous route it is assumed to be rapidly and homogeneously distributed throughout the vascular compartment (referred to as the central or primary compartment). Equilibrium is then reached with peripheral compartments (also known as secondary compartments), at which time the distribution phase is completed (Welling, 1986). At no time is the concentration of the drug in the vascular compartment higher than immediately after administration when given by the intravenous route (Greenblatt and Shader, 1985; Welling, 1986). Distribution to peripheral compartments, metabolism, and elimination, result in lower drug concentrations from that time on.

Administration by intramuscular or oral routes results in a time delay between administration and the earliest measurable concentration within the vascular compartment. This time period is known as the lag time (Greenblatt and Shader, 1985). From this time to the time of peak concentration, the drug is being absorbed, distributed, and eliminated simultaneously. This period is characterized by the absorption half-life, $T_{1/2\alpha}$ (Greenblatt and Shader, 1985; Welling, 1986).

After C_{max} has been reached, concurrent elimination of the drug by excretion or metabolism results in decreasing concentrations. The rate of decrease is expressed by the elimination half-life, $T_{1/2\beta}$ (Greenblatt and Shader, 1985; Welling, 1986). Half-life values depend on adherence to first order kinetics models where the change of drug concentration over time varies

continuously relative to the concentration itself (Greenblatt and Shader, 1985). This relationship is expressed as:

$$\frac{\text{The change in Concentration } (\Delta C)}{\text{The change in Time } (\Delta T)} = -k\,C$$

Where the relationship:

$$\frac{(\Delta C)}{(\Delta T)}$$

is the rate of change of drug concentration with time, and "-k" is the rate constant (with units of reciprocal time).

This expression indicates that, for a given $T_{1/2\beta}$, when the concentration of a drug is high, the amount of drug eliminated in a given time period will be large, and when the concentration is low, the amount eliminated will be correspondingly small. The rate of change (-k), however, remains constant (Greenblatt and Shader, 1985). All first order processes will be expressed as straight lines when plotted semi-logarthmically (Welling, 1986). Small half-life values indicate rapid processes, while large or "long" values indicate a relatively slow process (Greenblatt and Shader, 1985). This expression is important in evaluating the frequency at which a drug must be administered to maintain concentrations within certain limits (the therapeutic range) (Drusano, 1988).

CONCENTRATION The concepts of C_{max} and T_{max} are straight forward and have previously been explained. Maximum concentration in serum after intramuscular injections is achieved at the time the drug is being absorbed at the maximum rate exceeding elimination, and before equilibrium of distribution is reached (Greenblatt and Shader, 1985).

Values for C_{max} and T_{max} in secondary compartments are reached at the point of distribution equilibrium, decreasing from that time point due to irreversible elimination (Welling, 1986;

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Greenblatt and Shader, 1985). Irreversible elimination occurs primarily, but not solely, from the central compartment (Greenblatt and Shader, 1985; Mayer et al., 1980). Elimination from secondary compartments occurs by compound metabolism, while elimination from the central compartment results from excretion of unchanged compound or by drug metabolism followed by excretion of metabolites (Mayer et al., 1980). Elimination by excretion or metabolism in the liver or kidney is considered to be a central compartment process as these organs are kinetically equivalent to blood (Kinabo and McKeller, 1989). As the compound is eliminated it redistributes across the compartments, again, dependant on the factors that affect distribution. While $T_{1/2\alpha}$ represents the time needed for drug distribution across the central and all peripheral (secondary) compartments, elimination from these compartments may occur at different rates, generating different values for $T_{1/2\beta}$ for each compartment (Greenblatt and Shader, 1985). Similarly, the concentration of the drug may vary considerably between compartments or tissue types, depending on protein binding, inflammation, active transport, and drug-receptor affinity, establishing concentration gradients and tissue reservoirs of the compound (Greenblatt and Shader, 1985; Mayer et al., 1980; Wise, 1986).

AREA UNDER THE CURVE Half-life data and concentration data can be combined to provide a value for the total drug presence over a given time period. By plotting these parameters as a time versus concentration curve, the area under the curve (AUC) can be derived (Welling, 1986). The area under the curve is also used as an indicator of drug penetration (Vogelman, 1988; Welling, 1986). Comparison of AUC values for different tissues at similar doses, and the same tissues at different doses provides insight into the relative penetration of drug into the tissues, and the influence of dose on this penetration (Welling, 1986). Another way of looking at these variations in penetration is to use ratios of AUCs, such as tissue AUC values/serum AUC values (Greenblatt and Shader, 1985; Welling, 1986). These comparisons are essentially concentration gradients across the central compartment and the secondary compartment of

interest, and, again, provide comparisons for relative drug penetration and persistence within peripheral tissues (Greenblatt and Shader, 1985; Vogelman et al., 1988).

MINIMUM INHIBITORY CONCENTRATION Information about concentrations and duration of drug in a "compartment" or tissue is indispensable when providing and evaluating antimicrobial therapy, but additional information is required as well. Without data providing a point of reference to clinical efficacy, choice of drug and dose are based on personal bias and clinical experience, and the clinician has no way of determining validity of treatment other than observation of clinical response[c]. The point of reference commonly used is the minimum concentration of that particular drug required to inhibit the growth of each target organism[c] (Jones et al., 1985; Thrupp, 1986; Vogelman, 1988). This value is known as the minimum inhibitory concentration, or MIC[c] (Jones et al., 1985; Thrupp, 1986). Determination of MIC may be applied to a single isolate, such as when treating an infection in a clinical setting[c], or as a concentration at which a specified percentage of clinical isolates of the same organism are inhibited, as with bacterial population and drug spectrum studies (Fales et al., 1982; Walker et al., 1989). Determination of endpoint (inhibition) can be by visual or photometric means (Carlberg, 1986). The reported MIC value is the lowest concentration at which growth is suppressed, but actual growth suppression occurs at a concentration between this value and the next lower concentration in the two-fold dilution series[c] (Thrupp, 1986).

Other uses for MIC data include comparison of various organisms or strains for typing and identification, or for monitoring over time as the organisms respond to selective pressure (Thrupp, 1986). Techniques for evaluating inhibitory concentrations can also be used to study interactions such as protein binding effects on drug activity (Craig and Suh, 1986).

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[c] National committee for clinical laboratory standards Tentative standard: M7-T2. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. 2nd ed. Villanova, Pa. 1988.

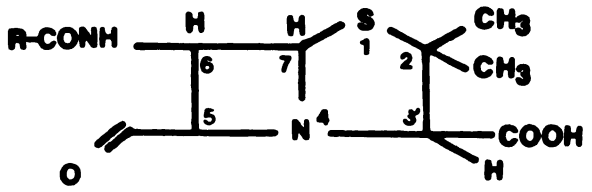
CEPHALOSPORINS

Cephalosporin antimicrobial agents are natural, semi-synthetic, or wholly synthetic beta-lactam compounds modeled after the natural product of the widely distributed, non-pathogenic fungal organism Acremonium chrysogenium brotzu. This designation is used to distinguish them from the related phytopathogen Cephalosporum acremonium (Nash et al., 1985). The antimicrobially active byproduct was first discovered in 1948 in organisms collected from seawater near a sewage outlet in Sardinia (Nash et al., 1985). The prototype commercial product (cephalothin) was marketed in 1962 by Eli Lilly and Company, Indianapolis, Indiana, under the trade name Keflin. The basic compound is a 7-aminocephalosporanic acid, which is composed of a beta-lactam ring fused to a dihydrothiazine ring. Functional groups at the third and seventh positions can be modified to alter the pharmacokinetic and antimicrobial properties of these drugs (Figure 1). Alterations of the R_2 group at the third position will effect the overall pharmacokinetic behavior, including absorption and metabolism (Balant et al., 1985). Position seven modifications will produce variations in the antimicrobial spectrum of the compound (Balant et al., 1985; Nash et al., 1985).

Cephalosporins are closely related to penicillins by the common beta-lactam structural component, and function similarly. Peptidoglycan, a compound found only in bacteria, is the major component of the cell walls of both Gram negative and Gram positive bacterial organisms (Volk et al., 1986a). The beta-lactam rings of penicillins and cephalosporins are structurally analogous to D-alanyl-D-alanine, a terminal group on the peptidoglycan molecule (Volk et al., 1986a). The carboxyl group on the alanine side chain of peptidoglycan cross-links with a free amino group on a diaminopimelic acid side chain of an adjacent peptidoglycan molecule during

the final stages of bacterial cell wall synthesis (Volk et al., 1986a). These cross-links are responsible for the final structure and integrity of the bacterial cell wall and are catalyzed by proteins known as transpeptidase enzymes (Spratt and Cromie, 1988). The beta-lactam ring covalently binds and inhibits transpeptidase enzyme (one type of penicillin binding protein, or PBP, of which several exist) by nature of it's structural similarity to the natural substrate of this enzyme, the alanine molecule. With PBP's inhibited, cell wall structure is compromised. While PBP's may vary slightly between bacterial organisms, homologous structures exist and function similarly (Spratt and Cromie, 1988). Depending on which specific PBP is inhibited, filamentous or spherical cell wall deformities may occur, resulting in lysis of the cell as differences between intracellular and extracellular osmotic pressures overcome the weakened cell wall (Spratt and Cromie, 1988). Beta-lactam compounds are considered bactericidal for this reason (Papich, 1984).

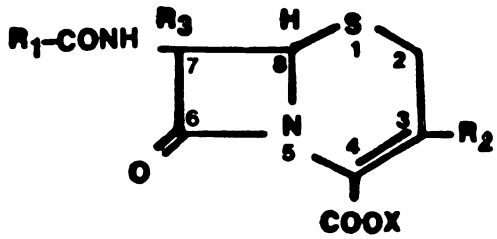
While the target structures (PBP's) are the same in both gram positive and gram negative organisms, gram-negative PBP's are protected by the multilayered cell surface (Livermore, 1988; Volk et al., 1986a). The outermost surface of both gram positive and gram negative bacteria is a variably thick polysaccharide capsule (glycocalyx) that functions as a physical barrier to deeper penetration of harmful substances (Livermore, 1988; Volk et al., 1986a). Deep to the capsule of Gram positive bacteria is the thick peptidoglycan cell wall, which is in direct contact with the cytoplasmic membrane on the inner surface (Volk et al., 1986a). Beneath the capsule of gram negative bacteria is an outer membrane, composed of lipopolysaccharides and phospholipids arranged similarly to other biomembranes, with hydrophilic surfaces surrounding the hydrophobic core. The outer membrane also contains proteins, some of which are structural and others which form aqueous pores, or porins, through the membrane (Livermore, 1988). Deep to the outer membrane lies a thin layer of peptidoglycan within a viscous region called the periplasmic space (Livermore, 1988, Volk et al., 1986a). Deep to this region is the cytoplasmic membrane itself. For beta-lactam antimicrobial agents to attack Gram



Beta-lactam Ring

Thia-zolidine Ring

Penicillins



Beta-lactam Ring

Dihydro-thiazine Ring

Cephalosporins

Figure 1. Molecular structures of penicillins and cephalosporins.

negative cells such as P. haemolytica, P. multocida, and H. somnus the agent must be able to penetrate the capsule where drug binding may occur, pass through outer membrane porins where molecular size and charge restrictions may prohibit entry, survive degradation or binding by enzymes produced by the bacteria, and attach to the active site of the PBP molecule in sufficient concentration to produce the cytolytic effect (Livermore, 1988, Volk et al., 1986a).

Bacterial organisms are protected from the effects of beta-lactam compounds by enzymes known as beta-lactamases. These enzymes are capable of hydrolysing the antimicrobial beta-lactam ring of penicillins and cephalosporins, depending on their specificity (Bush, 1989; Philippon et al., 1989). Certain gram positive organisms produce extracellular beta-lactamases (penicillinases) that act primarily against penicillins and are plasmid-encoded, with one plasmid containing the genetic code for as many as three enzymes (Bush, 1989a). Some, and possibly all gram negative organisms produce beta-lactamases that are chromosomally encoded, with a single strain of organism capable of coding for several enzymes (Bush, 1989a). Gram negative organisms also produce plasmid-mediated B-lactamases (Bush, 1989a; Phillipon et al., 1989; Vuye et al., 1989). Transmission of beta-lactamase induced resistance between strains of bacteria can occur by plasmid exchange as has been demonstrated in strains of Klebsiella pneumoniae, Serratia marcescens, and Escherichia coli (Gutmann et al., 1989; Philippon et al., 1989; Vuye et al., 1989), or by transposon activity of chromosomes encoded for beta-lactamase production (Bush, 1989a; Curtis, 1988; Volk et al., 1986c).

Gram negative beta-lactamase enzymes attach to the cell wall in the periplasmic space, and are broader in spectrum than those from Gram positive organisms, being active against both penicillins and cephalosporins (Balant et al., 1985). Classification schemes have been developed for B-lactamases, (Sykes, 1982; Bush, 1989b; Bush, 1989c) but are beyond the scope of this review. Resistance to various beta-lactamases is one of the properties that is

influenced by modification of the R_3 group at position seven on the B-lactam molecule (Figure 1) (Balant et al., 1985).

Classification of Cephalosporins

Modifications of cephalosporin functional groups to alter pharmacokinetic and physiologic behavior has resulted in the evolution of broadly associated groups of cephalosporin antimicrobial agents with similar properties. Classification schemes for these drugs have been based on various properties including potency, B-lactamase resistance, pharmacological properties, chemical structure, and antimicrobial spectrum (Balant et al., 1985). This discussion will follow the generation system of grouping that is loosely based on the latter.

FIRST GENERATION The first generation cephalosporins include the original naturally-produced formulations (Balant et al., 1985). These compounds exhibit activity primarily against Gram positive cocci, as well as against many enterobacteriaceae, including E. coli, Klebsiella spp., Proteus mirabilis, Salmonella spp., and Shigella spp. (Balant et al., 1985). Activity against anaerobes is minimal. First generation cephalosporins are uniformly resistant to extracellular beta-lactamase, but vary in their susceptibility to the cell wall-associated beta-lactamases produced by Gram negative organisms (Balant et al., 1985). These compounds have short half-lives of elimination, penetrate poorly into the cerebrospinal fluid, and are metabolized to less active or inactive compounds by desacetylation. The class-representative drug is Cephalothin. (Balant et al., 1985)

SECOND GENERATION Second generation cephalosporins, represented by cefamandole, have antimicrobial spectra similar to first generation agents, with the addition of activity against some Gram negative and anaerobic organisms due to greater beta-lactamase resistance (Balant et al., 1985). They penetrate cerebrospinal fluid to a greater degree, but have similar half-lives to the first generation compounds (Balant et al., 1985).

THIRD GENERATION Most third generation cephalosporins are semi-synthetic derivatives with greater beta-lactamase resistance than first or second generation cephalosporins. These agents have greater activity against Gram negative and anaerobic organisms, but are less active against Gram positive bacteria including Staphylococci (Balant et al., 1985; Papich, 1984). Some third generation cephalosporins (ceftazidime, cefsulodin and cefoperazone) are quite effective against Pseudomonas spp., including Pseudomonas aerugenosa, although this activity is relatively specific and these drugs tend to be limited in activity against other pathogens (Balant et al., 1985). Third generation compounds penetrate cerebrospinal fluid and bile well, and most have longer half-life values than first or second generation drugs, primarily by virtue of increased protein binding (Balant et al., 1985; Schentag, 1989). Most of these compounds are eliminated as active desacetyl metabolites by renal filtration and tubular excretion, although some are metabolized in secondary compartments by other means (Balant et al., 1985).

Third generation compounds appear to be quite safe in respect to adverse reactions. Rare Coombs positive reactions associated with red cell membrane damage or sensitization have been reported in the human literature (Balant et al., 1985). These reactions rarely result in hemolysis. Immediate hypersensitivity reactions also occur, but rarely, and are similar to the IgE-mediated leukocyte degranulation reaction (type I hypersensitivity) seen with penicillin compounds (Balant et al., 1985; Hurvitz, 1975). Intolerance reactions have been known to occur in humans when alcohol is consumed during treatment with cephalosporins having thiotetrazole substitutions at position three. This reaction is similar to the acetaldehyde toxicity reaction seen when alcohol is consumed after disulfiram, an aldehyde dehydrogenase inhibitor (Balant et al., 1985). The reaction is characterized by tremors, hypertension or hypotension, nausea, diarrhea, and occasional severe vomiting (Schuckit, 1987), but has not been reported in animals. Cephalosporin-induced hypoprothrombinemia has also been reported (Kerremans et al., 1985). This effect is the result of inhibition of gamma carboxylation of glutamic acid by a thiol by-product of cephalosporin metabolism. Gamma carboxylic glutamic acid is important

in the formation of active clotting factors and is vitamin K dependent. This side-effect was initially believed to be due to suppression of vitamin K producing bacteria in the gut, but has now been shown to be the result of this specific metabolite, and is seen only with cephalosporins that are metabolized to this compound (Kerrimans et al., 1985).

Currently 34 cephalosporin compounds are listed in The Merck Index[d]. Nineteen registered trade (proprietary) formulations are included in the 43rd edition of the Physicians Desk Reference[•]. Of the 34 compounds, only 4 are formulated as proprietary compounds (specific trade products) approved for veterinary use. With this many compounds available, the volume of literature generated about these drugs is also large, as might be expected.

Pharmacokinetics of cephalosporins

The kill rates for cephalosporins (expressed as log-reductions in bacterial population per unit time) are not as rapid as those of aminoglycosides, and are preceded by a lag period between drug administration and the onset of bacterial killing (Drusano, 1988). Bactericidal effect is also less dependent on dose once bactericidal concentrations are reached (Drusano, 1988). Dosages above those which produce bactericidal concentrations in the tissues would not increase bacterial kill rates, but would result in the prolongation of drug presence in tissues (Drusano, 1988). These high single dosages would generate larger concentration gradients across the central and peripheral compartments, increasing the likelihood of generating bacterial inhibitory concentrations in the peripheral tissues (Wise, 1986). Any improvement in control of the bacterial population by increased dosage would be from maintaining drug concentrations above bactericidal levels for the longer elimination periods that result from

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[d] The Merck Index, 10th edition. Martha Windholz, ed. Merck and Co., Inc., Rahway, New Jersey 1983; ci59-ci60.

[•] Physicians Desk Reference. Barnhart ER, ed. Physicians Desk Reference 43rd edition. Medical Economics Co., Inc. Oradell, New Jersey.

increasing the peak concentration (Vogelman et al., 1988). Consequently, it has been suggested that beta-lactam agents be administered as constant infusions or at frequent dosing intervals (Drusano, 1988). This concept is supported by data generated in 5 foals on oral and intravenous administration of the first generation cephalosporin, Cefadroxil (Duffee et al., 1989). Intravenous administration of a single dose at 23 mg/kg produced a elimination half-life of 1.37 hours with a short distribution half-life of 0.26 hours. Single dose oral administration of 100-108 mg/kg prolonged these values to 3.75 hours and 3.5 hours, respectively. Intramuscular administration would result in values intermediate to these extremes. Microbial inhibitory concentration data was not generated in this study, but with over 90% of this drug eliminated after 4 half-lives, or slightly more than 6 hours, maintaining concentrations in the blood above the MIC values for expected pathogens would require frequent dosing. Data from intravenous and intramuscular administration of the third generation agent cefoperazone to calves produced similar results. Intravenous dosing at 20 mg/kg resulted in elimination half-life values of 2.13 +/- 0.47 hours, while intramuscular administration of the same dose generated values of 2.28 +/- 0.33 hours (Soback and Ziv, 1989). These relatively short elimination half-life values support the conclusion by Drusano. Continuous infusion was not, however, shown to be superior to intermittent dosing at 6 hour intervals in a study by Roosendaal et al (1986). Normal and leukopenic rats were challenged with K. pneumonia and treated with ceftazidime intravenously. The dose required to protect 50% of the rats (PD_{50}) was 0.36 mg/kg/day and 0.35 mg/kg/day for continuous infusion and interval dosing, respectively. Pharmacokinetic data was not given, but values would be expected to be similar to those for other cephalosporins and thus, intermittent administration at appropriate dosages rather than high single dosage administration or constant infusion appears to be satisfactory, so long as the minimum concentration remains continuously above the MIC_{90} for the infecting pathogen.

Another property of cephalosporin compounds that influences their activity and effectiveness is protein binding (Wise, 1986; Schentag, 1989; Drusano, 1988). Third generation

cephalosporin compounds tend to be more highly protein bound than second generation compounds, and second generation compounds more than first (Hoffstedt and Walder, 1981; Balant et al., 1985). For example, the first generation drug cephradine is approximately 10% protein bound in human serum, while the third generation agent cefoperazone is at least 90% bound in humans (Hoffstedt and Walder, 1981). Increased protein binding prolongs the half-life values, but also decreases the ability of the drug to penetrate into secondary compartments, and reduces the amount of free, or biologically active, drug (Hoffstedt and Walder, 1981). In a study by Lam et al., (1988) cefoperazone total concentration exceeded total ceftazidime concentration in six healthy human volunteers, but unbound ceftazidime exceeded unbound cefoperazone. Serum biological activity of ceftazidime exceeded that for cefoperazone, even though both drugs exceeded MIC concentrations for the test organism (Lam et al., 1988). Thus, while protein binding may prolong the presence of cephalosporin compounds in tissues, it may also adversely affect the antimicrobial activity, both in vivo and in vitro.

Ceftiofur Sodium

Ceftiofur sodium is a third generation cephalosporin manufactured as a sodium salt by Upjohn Company of Kalamazoo, Michigan, and Smith Kline French of Philadelphia, Pennsylvania, on license from Sanofi of France. The commercial product, Naxcel[R], (Figure 2) was released in February, 1988, labeled for use in beef cattle with respiratory disease associated with P. haemolytica, P. multocida, and H. somnus. This drug is reported[f] to be active primarily against Gram negative bacterial organisms, with excellent in vivo and in vitro activity against bacterial organisms known to infect and cause respiratory disease in cattle. It also displays in vitro activity against A. pyogenes, E. coli, Salmonella choleraesuis, Salmonella typhimurium, and H. pleuropneumonia, and the Gram positive organisms S. aureus, Streptococcus agalactia, Streptococcus dysgalactia, Streptococcus uberis, Streptococcus bovis, and Streptococcus suis.

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[f] Miller CC. Ceftiofur research and development. Personal communication.

Ceftiofur is not active against Pseudomonas spp. This antimicrobial spectrum is reported to be quite similar to that of cefotaxime, a drug possessing typical third generation cephalosporin antimicrobial activity (Balant et al., 1985).

Ceftiofur sodium is metabolized by plasma esterase transformation and splitting of the thioester bond at R_2 to produce desfuroyl ceftiofur (Figure 3). The metabolite and the parent compound are both eliminated by glomerular filtration and renal tubular secretion. Both compounds have antimicrobial activity, although the metabolite is less active, resulting in higher MIC values for most organisms for which data exists[f]. Bovine pharmacokinetic data generated in research and development of ceftiofur sodium[g] indicate a serum Cmax of 5-10 μg/ml at 0.5-1 hour (Tmax), $T_{1/2\beta}$ of 5-8 hours, and concentrations of 0.5-0.8 μg/ml 24 hours after once daily intramuscular dosing at 1.0 mg/lb (2.2 mg/kg) body weight. Lung tissue concentrations of 0.9-1.4 μg/ml have been measured at 8 hours after a single dose. Ceftiofur is reported to be approximately 90% serum protein bound[g]. Safety and tolerance studies conducted with 4 calves dosed with ceftiofur at 25 mg/lb (55 mg/kg) for 15 days indicated no systemic toxic effects, adverse reactions being limited to local pain and swelling at injection sites after 5 doses, with associated aspartate transaminase (AST) and creatine phosphokinase (CPK) elevations. Further studies conducted in 10 calves at doses up to 10 mg/lb (22 mg/kg) support these findings, with the additional histopathologic evidence of mild muscle inflammation[g]. While the ceftiofur molecule does contain sulfur, heterocyclic thiol compounds are not formed as metabolites, and hypoprothrombinemia has not been reported as a risk with ceftiofur administration.

MIC_{90} data for BRD associated pathogens indicate that these organisms, with the exception of Mycoplasma bovis, are quite sensitive in vitro to this drug[g] (Yancey et al., 1987) (Table 1).

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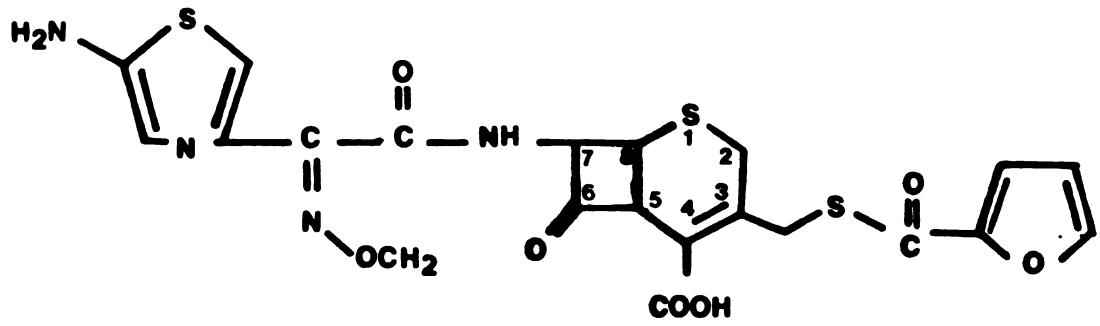
[g] Miller CC. Ceftiofur research and development. Personal communication.



Ceftiofur

Figure 2. Molecular structure of ceftiofur sodium.

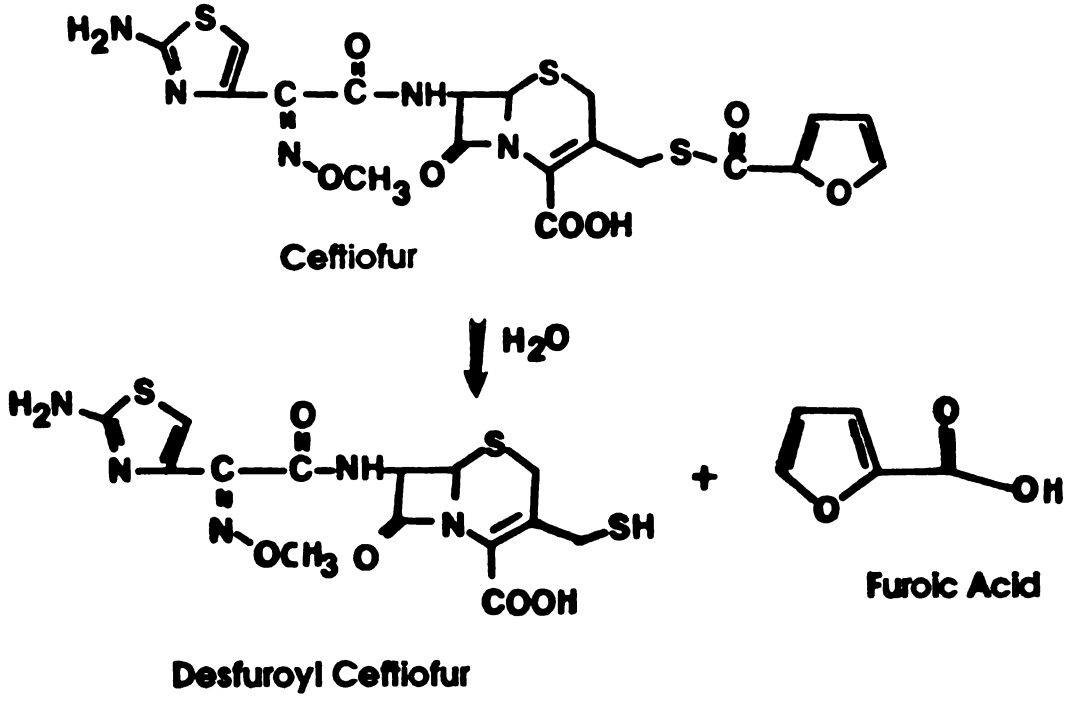


Figure 3. Metabolism of ceftiofur sodium in the bovine

Bacterial organisms associated with sporadic or opportunistic bovine pneumonia (non-BRD associated organisms) are more variable in their susceptibility to ceftiofur (Yancey et al., 1987) (Table 1).

Table 1. MIC_{90} data for bovine respiratory disease (BRD) and non-BRD associated pathogens

BRD associated organisms	No. isolates	MIC_{90} (ug/ml)
Pasteurella haemolytica	114	≤ 0.06
Pasteurella multocida	27	≤ 0.06
Haemophilus somnus	29	≤ 0.06
Actinomyces pyogenes	1	0.06
Mycoplasma bovis	7	> 128.0

Non-BRD assoc. organisms	No. isolates	MIC_{90} (ug/ml)
Escherichia coli	10	0.25
Salmonella spp	6	2.0
Staphylococcus aureus	7	32.0
Streptococcus spp	17	0.06-2.0

This product is currently approved for use at 1.1 mg/kg body weight, given intramuscularly. Treatment of calves with twice this dose produces serum and lung tissue homogenate concentrations in excess of the in vitro MIC concentration for target organisms[h]. Equivalent data for the label-recommended, FDA approved dose has not been generated, nor has data for serum or tissues by means other than tissue homogenation (tissue fluid data) for various doses been generated or published.

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[h] Miller CC. Ceftiofur research and development. Personal communication.

QUANTIFICATION OF DRUG ACTIVITY

As stated earlier, understanding the disposition of a drug at secondary compartment locations, the most common location of pathogenesis by infective organisms, is critical to the making of valid therapeutic decisions. To quote Weinstein et al: "While information concerning the concentration of drug in the blood is of great importance in studying it's absorption and excretion, it may be of little or no value in ascertaining the actual quantity of antibacterial substance at the site of infection. There is little doubt that the tissue level of penicillin is related to the blood concentration, but the degree of relationship, particularly as it involves the duration of antibacterial effect, is not settled." (Weinstein et al., 1951).

Measurement of kinetics at these peripheral locations is rarely as straightforward as in the central compartment. Collection of samples that are representative of conditions in situ and that can be quantitatively assessed without bias is simple when blood is the tissue, but becomes much more difficult for other tissues.

Serum Sampling Techniques

Serum pharmacokinetic parameters for many drugs have been reported. Whether this data is the main subject or associated with data for other tissues, the technique of sample collection in most cases involved simple percutaneous aspiration of whole blood via vascular puncture, separation of the fluid portion from the solid portion by centrifugation, filtration, or innate clot formation, followed by processing the fluid portion in a specific biologic or chromatographic assay.

Tissue Sampling Techniques

Collection of representative samples from other locations may be as straightforward as blood or serum collection; cerebrospinal fluid, bile, and urine are all easily collected as means of evaluation of the central nervous system, liver, or urinary system, respectively. Fluids from most other tissues, however, are not easily collected in volumes sufficient for pharmacokinetic analysis by current techniques. The fluids providing nutrition, lubrication and protection for these tissues exist in small quantities dispersed across large surface areas or potential spaces that are currently impossible to access directly. The challenge, then, is to collect these fluids as pure, representative aloquots of sufficient quantity. To this end, many techniques have been reported.

Homogenation of tissue is one commonly used technique. Tissue homogenates contain blood, lymph, interstitial fluid, cytoplasm, and possibly other fluid components such as synovial fluid or glandular secretions (Bergan, 1981). Any of these may be present in varying quantity depending on the time and technique of collection and the processing methodology, and may contain substantial amounts of the compound of interest. Such non-quantified sources may contribute significantly to the total sample compound measurement, leading to false interpretations of true tissue fluid concentration of that compound. The possibility also exists that enzymes may be present in homogenates that are capable of deactivating the component being assayed, reducing the measured concentration only when the tissue has been unnaturally altered by the homogenation procedure. Alternatively, protease enzymes may be released by homogenation, altering quantification accuracy by releasing drug that was protein bound , and therefore not biologically active in the intact tissue (Bergan, 1981). Quantification of drug concentration cannot be performed accurately, unless the contribution of drug from each fluid component can be determined without alteration by other substances. Methods exist for adjusting the measured values relative to blood contributions, based on measured serum concentration, tissue water volume, and blood volume; radiolabeled erythrocytes; hemoglobin

content (Bergan, 1981), or hematocrit (Wise, 1986). Each of these methods is subject to miscalculation, which, combined with error from failing to account for unmeasurable drug contributions or alteration due to technique (Parsons et al., 1976) may result in significant misrepresentation of drug concentration.

Measurement of drug concentration in lymph collected from common, central lymphatics or from regional, specific tissue lymph ducts has been reported (Bergan, 1981). Central and thoracic duct lymph are poor choices if isolated target tissue data is required because they collect drainage from many tissue beds. Differences exist between thoracic duct, central, and peripheral lymph for common substances such as protein and lipids, making the report of drug concentrations based on any one of these sources suspect to much scrutiny because of the variability each displays in what it transports, and also due to effects on drug concentration of these transported molecules (Bergan, 1981). Lymphatic vascular endothelium is not passively permeable to all interstitial fluid components, and antibiotics, especially those bound to albumin, may not pass predictably or reliably into the lymphatic fluid (Bergan, 1981). Therefore, even though lymph might in some cases be collected easily and without contamination by blood, intracellular fluid or specific secretions, it is not an ideal fluid for tissue-drug concentration evaluation (Bergan, 1981).

Another method of tissue fluid collection is the skin blister, induced by suction or contact with an irritant such as cantharidin (Bergan, 1981; Wise, 1980). The fluid enclosed by blisters is in communication with intravascular fluid across the non-inflamed vascular membranes, and is assumed to represent tissue fluid (Bergan, 1981). The irritant-induced blister is less consistent than suction blisters in size and level of deep inflammation, and thus results in less consistent drug measurements. This technique is not readily applicable in studies of pharmacokinetics on large animals due to the potential for damage and rupture of the blisters during routine handling.

Other methods of tissue fluid collection such as subcutaneously implanted threads or fibrin clots have been used (Hoffstedt and Walder, 1981; Bergan, 1981) but are not favored by many investigators because they lack a counterpart in natural disease (Bergan and Weinstein, 1974).

Sterile chambers (tissue chambers) are used frequently as sources of tissue fluid. These chambers have been constructed from teflon, wire mesh, steel spring, polypropylene, thermoplastic and silicone rubber (Chisholm et al., 1973; Clarke et al., 1989a; Higgins and Lees, 1984; Walker et al., 1989; Walker et al., 1990). Surgically implanted in the subcutaneous space, they are believed to be continuous with interstitial space and interstitial fluid. Studies comparing acid-base, pH and solute concentration between tissue chamber fluid, tissues and local lymph (Bergan, 1981), and between tissue chamber fluid and blood (Clarke et al., 1989b) support this claim, and radioisotope-labelled solutes has shown that marker penetration into chambers occurs rapidly, producing identical concentrations as those in lymph (Bergan, 1981).

Regardless of the structural material composing the tissue chamber, the principal of operation is the same. After implantation the cage fills with blood, serum and tissue fluid. As organization of this fluid progresses, the chamber becomes lined and surrounded with fibroblasts, capillaries and other interstitial tissue components. Proliferation of these tissues will eventually fill the chamber lumen completely, as the perforations that allow passage of fluid also expose the lumen to the ingrowth of this new interstitial tissue. The rate of fluid diffusion and therefore the time until equilibrium is reached between tissue fluid and chamber fluid is determined by the proportion of perforated surface area to total surface area. Diffusion rates vary for specific solutes, larger molecules such as albumin requiring longer time periods than smaller molecules (Chisholm, 1973). This will be discussed further below. Use of the chambers should be delayed until at least 4 weeks after implantation to allow the acute inflammatory phase to subside. This can be demonstrated by stabilization of tissue chamber

fluid erythrocyte and leucocyte numbers and solute content at 4 weeks compared to fluid collected shortly after chamber implantation (Clarke et al., 1989b).

Some tissue fluids for which pharmacokinetic data is desired can be accessed directly, but the convenient collection of these fluids in sufficient quantity is difficult. This is frequently the situation with serosal or mucosal surface fluids, or surgical wound fluids. Absorption of the fluid into a suitable inert media that can be used directly in the quantification assay, or from which the agent of interest can be extracted for assay, has been found to be acceptable (Wise et al., 1981; Hajer et al., 1988). This technique involves placing a pre-weighed, desiccated absorbent device on the epithelial or wound surface for fluid collection. The common medium for absorption are 6.35 mm (0.25 inch) diameter sterile filter paper antibiotic sensitivity assay discs. After collection, the discs are re-weighed to measure the fluid volume collected, then used directly in the biological assay. This technique requires adjustment of the final assay-determined antimicrobial concentration for variation in the amount of fluid absorbed, as volumes larger or smaller than the volume of the standard from which the concentration is derived will result in correspondingly larger or smaller sample concentrations. Depending on the interests of the investigators, samples may be rejected if blood contamination occurs or exceeds some arbitrary value.

Swabs have been used as an alternative to the disc as the medium of secretion collection in this technique (Hajer et al. 1988). Similar adjustments for fluid volume variation were made as are required with disc absorption. These investigators also chose to bypass the upper airway and oropharynx entirely by installing tracheostomy devices, a technique that might produce alterations in the characteristics of the respiratory mucosa due to bypass of the normal hydration and warming mechanisms (Murray, 1986).

Other investigators have used fluids as the collection media (Mair, 1987; Slocombe et al., 1985). Washing the pulmonary mucosa with a physiologically benign solution and measuring the amount of drug in the effluent produces acceptable results (Slocombe et al., 1985) but requires adjustment for dilution based on the differential of some endogenous compound in the animal's serum and in the collected effluent. This technique may not be accepted well by normal, alert subjects, requiring tranquilization that could alter cardiovascular function as well as other physiologic parameters which in turn may affect the final results.

Quantification Techniques

Samples, once collected, require processing and quantification of drug content; in the interest of toxicity, metabolic activity, or oral dosing compliance, for example, or for pharmacokinetic evaluation; as is the focus of this discussion. One of two basic assay techniques are commonly used: high pressure liquid chromatography (HPLC) or microbiological assay (Bioassay). High pressure liquid chromatography techniques require technological expertise and sophisticated equipment, but are extremely accurate, reliable, and relatively rapid, delivering results in 30-60 minutes. Chromotagraphic systems can measure original compounds, metabolites, or both, and they report all drug present in the sample.

Bioassays are labor and materials intensive, sensitive to day to day variation, highly subject to technical error, less sensitive, and less specific than HPLC (Edberg, 1986). Bioassays, however, measure only biologically active compounds, whether those compounds are parent compound or metabolites. For this reason, bioassay techniques are still in common usage in pharmacokinetic studies (Toothaker et al. 1987).

HIGH PRESSURE LIQUID CHROMATOGRAPHY Analysis of many water soluble compounds, including penicillins and cephalosporins, is frequently carried out by HPLC, and is reported to

be used preferentially in the analysis of third generation cephalosporin in secondary compartments (Toothaker et al., 1987). The technique requires extraction of the drug with a specific solvent, separation of the drug onto solid phase by HPLC, detection of the effluent off the solid phase by spectrometry, and quantification of the amount of antimicrobial present by peak height or peak area analysis. HPLC techniques are commonly used in labs to measure anti-convulsant and anti-arrhythmic drugs, or any drug that has a narrow toxic/therapeutic ratio and requires close monitoring, as in the case of antimicrobials that have high potential for nephrotoxicity. While these concerns exist for a small number of antimicrobials, the short period of time required for processing and the competitive cost relative to other techniques makes HPLC analysis attractive (Edberg, 1986).

MICROBIOLOGICAL ASSAY Ten years ago, 75-85% of clinical laboratory assays were microbiological in design. By 1986, only 30-40% of the antimicrobial studies conducted were microbiological assays, with the difference being made up by non-isotopic studies such as immuno-assays and HPLCs (Edberg, 1986). Nonetheless, microbiological assays are the cornerstone method as techniques for pharmaceutical assessment, with the advantages of low cost, simplicity and biological significance in that actual antimicrobial activity is the basis for the measured result (Edberg, 1986).

Bioassays can take the form of agar diffusion techniques, which will be discussed further, or turbidimetric techniques, where the concentration of an antimicrobial agent is determined in comparison to microbial inhibition by known concentrations, either by direct photometric measurement or indirectly by pH change as the indicator of substrate metabolism by growing bacteria (Barry, 1986).

Agar diffusion assays utilize the development of zones of bacterial growth inhibition to determine the amount of antimicrobial agent present. This zone is formed when the amount

of diffusing antimicrobial drug just capable of inhibiting microbial growth (the critical concentration) reaches a density of organisms larger than can be suppressed. When bacteria are seeded into or streaked onto agar media and placed in a favorable environment they begin to utilize the nutrients in that media for growth and reproduction. Agar media is a complex and not fully understood gel of polysaccharides and cations (calcium, magnesium, copper, zinc, and iron) dissolved in water (Barry, 1986). As these nutrients are used by the bacteria, a concentration gradient develops between the depleted area of growth and surrounding areas of the gel. Nutrients move across this gradient by the laws of diffusion, as do bacterial waste products, some of which may have a protective effect for the organism (Barry, 1986). If a spot source of antimicrobial is placed in this system it behaves similarly, diffusing along a concentration gradient in accordance with these same diffusion principles. Thus, a dynamic system develops with bacteria colonies appearing in regions where nutrient needs are met and antimicrobial concentration is below levels that inhibit their growth. In regions of sufficient antimicrobial concentration, the growth of the organisms will be suppressed, and the agar will remain clear of visible bacterial colonies.

Zone formation depends on the properties of the system, and varies as those conditions change. These properties include such antimicrobial properties as molecular concentration, size, shape, and charge; and media properties such as viscosity, temperature, moisture content, and ion content. The critical concentration of drug is given by the formula (Barry, 1986):

$$\ln m' = \ln m_0 \, (x^2/4DT_0)$$

Where:

$\ln m'$ = natural log of the critical concentration

$\ln m_0$ = natural log of concentration of drug applied to agar surface (point source)

x^2 = square of distance between edge of point source (antimicrobial reservoir) and edge of the zone of inhibition

D = diffusion coefficient of antimicrobial for the particular test system conditions

T_0 = critical time at which zone position is determined

A straight line relationship should develop if ln m_0 is plotted against x^2 with different initial concentrations run on identical test plates under identical conditions (all other factors are held constant).

The antimicrobial agent may be introduced to the system in various ways. Wells may be cut into the agar and the drug instilled with pipettes, or stainless steel cylinders may be placed on the surface and filled with the agent. Another method involves using filter paper discs, placed in full contact on the media surface, as reservoirs. All serve as point sources of antimicrobial drug, from which diffusion freely occurs, although one source reports the well technique to be 5-6 times more sensitive than the filter paper disc technique (Sabath et al., 1969).

The zone of inhibition will form at a distance (x) after a certain time period. This period is known as the critical time (T_0) after addition of antimicrobial agent to the system and initiation of incubation. It is independent of the concentration of the drug in the system but does reflect the rate of microbial growth at the time when the antimicrobial compound is diffusing most rapidly through the media (Barry, 1986). Therefore, it is the time required for the indicator organism to reach a critical cell mass that exceeds the inhibitory potential of the antimicrobial agent at the concentration present some distance x from the drug reservoir (Barry, 1986). It is expressed by the relationship:

$$T_0 - h = x^2/4D \ln(m_0/m')$$

Where:

T_0 = critical time

h = hours of pre-incubation

x^2 = zone size squared

D = diffusion coefficient of antimicrobial agent

$\ln(m_0/m')$ = natural log of concentration of drug in reservoir divided by the critical concentration

From the critical population formula:

$$T_0 = L + G \log_2(N'/N_0)$$

where:

T_o = critical time

L = lag time

G = generation time

N' = critical population at critical time (t_0)

N_0 = number of viable cells at time = 0 (inoculum density)

It can be seen that the initial inoculum and the generation time are most important in determining the critical time, and therefore the size of the zone of inhibition. For example, longer generation times or more dilute initial inoculates will give longer time periods until critical population is reached, and correspondingly larger zone sizes because of longer periods for antimicrobial diffusion before critical populations are encountered. Generation times are intrinsic properties of the organism that are dependant on nutrient availability and incubation temperature. Identical growth conditions will produce identical generation times, an important factor in reproducibility of bioassay data.

MINIMUM INHIBITORY CONCENTRATION Pharmacokinetic information is useful only if it can be compared to some objective reference. With antiarrthymic drugs, for example, this comparison parameter might be the concentration of drug required at a particular receptor to block spontaneous excitation, thus preventing arrthymias. Antimicrobial pharmacokinetic data is reported relative to the minimum concentration of antimicrobial drug required to inhibit growth of target microorganisms (MIC), or the concentration required to irreversibly inhibit (kill) an inoculum after a defined incubation period (Minimum Bactericidal Concentration, MBC) (Thrupp, 1986; Vogelman et al., 1988). While this comparison is most appropriate for beta-lactam compounds, antimicrobial efficiency of other classes of compounds, aminoglycosides, for example, are best defined by comparison of log area under the curve (Vogelman et al., 1988). The efficiency by which a beta-lactam compound exceeds the MIC is determined by a combination of the rate at which it penetrates the bacterial outer membrane, the resistance of the compound to various B-lactamase enzymes, and the kinetic parameters of the interaction of the compound with penicillin-binding proteins (Spratt and Cromie, 1988). Knowing the concentration of drug required to inhibit growth or kill a known or suspected pathogen allows calculation of the therapeutic ratio, which is equal to serum or tissue fluid concentration divided by the MIC (Sande and Mandell, 1985). This value provides an index value for efficacy in clinical cases of bacterial infection, where antimicrobial drugs that produce therapeutic ratios of 4 or greater are considered to be appropriate choices (Sande and Mandell, 1985). Techniques for determination of MIC's involve 2-fold dilutions of the antimicrobial agent either in suspensions of broth or solid (agar) media (Thrupp, 1986). Standardized inoculates are exposed to the media and incubated. The endpoint is read as the least concentration of drug by which the organism's growth is inhibited. The actual inhibitory concentration is between this concentration and the next lower concentration in the dilution series (Thrupp, 1986). Standards for procedures have been published[1].

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[1] National committee for clinical laboratory standards Tentative standard: M7-T2. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. 2nd ed. Villanova, Pa. 1988.

OBJECTIVES OF STUDY

The objectives of this study were twofold. First, to determine the pharmacokinetics of ceftiofur sodium in serum, tissue chamber fluid (TCF), and bronchial secretions (BRS) collected from feeder-age, beef-breed calves following the first and the fourth doses of once-daily intramuscular injections at 1.1, 2.2, and 4.4 mg/kg. Second, to measure in vitro the minimum concentration of ceftiofur sodium required to inhibit the growth of various bacterial isolates recovered from cattle. A comparison of these MIC values and pharmacokinetic parameters at the different tissue locations could provide guidelines for ceftiofur sodium antimicrobial therapy.

MATERIALS AND METHODS

Experimental design

This study involved trials with three dosages of the antimicrobial agent ceftiofur sodium. Each trial consisted of 4 days of single daily intramuscular drug injections, with at least 4 weeks between dosage trials. Serum, tissue chamber and bronchial secretion samples were collected from each calf at time intervals after the first dose and after the fourth dose. All 4 calves were treated with the first and fourth dose immediately after time zero samples were collected, for all 3 dosages. Drug administration to individual calves was staggered by 10 minutes on sampling days to allow time for sample collection. Samples were then processed by bioassay for pharmacokinetic evaluation.

Animals

Four beef-breed (Angus cross) calves, 2 male and 2 female (all sexually intact), were used in this study. The calves were purchased from a local commercial beef herd at approximately 4 months of age, and transported to the Veterinary Research Farm at Michigan State University, East Lansing, Michigan. The calves were maintained under natural environmental conditions on pasture grass (timothy, orchard grass, and other common grasses in natural growth), supplemental alfalfa hay, ground corn, and fresh water from an automatic waterer.

Tissue Chambers

Six cm lengths of silastic tubing[j] (1 cm outside diameter, 8.5 mm inside diameter) was used to make tissue chambers as described by Chisholm (1973). Ten circular holes of 2 mm diameter were cut in the tubing near each end, and the ends closed using silicone sealant[j]. The perforations resulted in approximately 20% of the surface area of the cylinder being open. The chambers were gas sterilized with ethylene oxide[k] and aerated for 24 hours prior to surgical implantation.

Surgical Implantation Of Tissue Chambers

The calves were in the range of 300-350 pounds (136-160 kg) body weight when the silastic tubing tissue chambers were implanted. The calves were placed in a restraint chute, sedated with approximately 0.10 mg/kg xylazine IV[l], both sides of the neck were prepared for surgery, and anesthetized locally with 5 ml infusions of 2% lidocaine[m] in the area of the skin incisions. Four horizontal incisions were made on each side of the neck, approximately 5 cm in length and located midway between the jugular furrow and the dorsal extent of the neck (ligamentum nuchae). The incisions were spaced evenly on a line drawn from the poll of the head to the glenoid area of the scapula. Subcutaneous pockets were made through these incisions in dorsal and ventral directions by introducing scissors and bluntly dissecting the subcutaneous tissue. Sterile tissue chambers were placed in these pockets, one dorsal and one ventral, with their long axis aligned dorsoventrally. After tissue chamber placement the skin was closed with number 2 vetafil in a simple continuous pattern. Seven chambers were implanted on each side of the neck. The calves were then placed on ceftiofur at 1.1 mg/kg, once daily, IM, for five

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[j] Dow Corning, Midland, Michigan.

[k] Steri-Vac 400C, 3M Corp. St. Paul, Minnesota.

[l] Rompun[R], Haver, Bayvet div., Shawnee, Kansas.

[m] Butler Co., Columbus, Ohio.

days to prevent post-operative infections. Sutures were removed after ten days, and the chambers left undisturbed for 4 weeks from the day of implantation. The course of the experiment ran for the next eight months, during which time the calves gained approximately 400 pounds (182 kg) each, and reached approximately 11 months of age.

Samples

The samples were collected according to the following schedule: Serum and tissue chamber fluid (TCF) samples collected at time (T) = 0, 0.5, 1.0, 1.5, 2, 3, 4, 5, 6, 8, 10, and 12 hours after drug administration. Bronchial secretion samples (BRS) collected at T = 0, 1, 2, 3, 4, 5, 6, 8, 10, and 12 hours. Sample collection and processing was conducted 2 times for each dosage level: after the first dose and after the fourth dose, to generate both single and multiple dose data. This investigation involved once-daily injections of Naxcel[R] at three dosage levels: 1.1 mg/kg (label recommended dose), 2.2 mg/kg, and 4.4 mg/kg, administered by deep injection into the gluteal muscles using 18 gauge, 3.81 cm long sterile needles. Successive daily injections were alternated between left and right sides of the calves. The drug used for this experiment was the commercially available Upjohn product, reconstituted and stored according to the label recommendations.

Sample Collection

The calves were brought to the Veterinary Clinical Center at Michigan State University and housed in box stalls 48 hours prior to sample collection to allow adjustment to the indoor environment and for drug trial preparation. The hair was clipped over the neck and jugular veins, the area was scrubbed with an iodine-based detergent[n], and the animals were weighed. During the sample collection period the calves were tied loosely with rope halters, from which were released periodically for access to food and water.

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[n] Betadine surgical scrub, Purdue Fredrick Co., Norwalk, Connecticut.

SERUM In the first sample collection period (1.1 mg/kg dosage) the venous blood samples were collected by 20 gauge, 3.81 cm needle venipuncture with aspiration into 10 ml vacutainer clot tubes[o]. For the second and third collection periods (2.2 and 4.4 mg/kg dosages), jugular venous blood samples were collected through 5-1/4 inch, 16 gauge catheters[p] that were placed the day before the sampling period began. This change was instituted to reduce discomfort and resistance in the calves. The catheters were sutured to the skin and wrapped in elasticon[p] for protection, and flushed with 5 mls of heparin[q] prior to use. During collection periods the catheters were flushed with heparinized saline (10 mls heparin at 1000 units per ml in 1 liter of commercial sterile saline solution) between sample collections to prevent occlusion by clotted blood. The heparinized saline and first 5 mls of blood were discarded before blood was collected to be saved for assay. Blood was pulled from the catheter by 12 ml syringe, then transferred to 10 ml vacutainer clot tubes. (5 to 8 mls of blood were collected regardless of technique.) Five mls of undiluted heparin (1000 units/ml) were placed in the catheters for overnight periods.

TISSUE CHAMBER FLUID Tissue chamber fluids for all sampling periods were collected into 3 ml syringes by direct percutaneous puncture of the tissue chambers with 22 gauge, 1 inch needles. Tissue chamber fluid was then transferred to 3 ml vacutainer clot tubes. Tissue chambers were only used once per sampling period (dosage level), with the most cranial chamber on the calf's left side being used first, progressing ventrally then caudally, while alternating sides. This pattern was followed throughout all sampling periods.

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[o] Becton-Dickinson, Rutherford, New Jersey.

[p] Johnson and Johnson, New Brunswick, New Jersey.

[q] Lympho med, inc., Melrose Park, Illinois. 1,000 units/ml.

BRONCHIAL SECRETIONS The bronchial secretion samples for the first sampling period were collected by passing a 1.2 cm outside diameter equine nasogastric tube through the calf's nostril, ventral nasal meatus, larynx, trachea and mainstem bronchi until it lodged in the mainstem bronchi to either of the caudal (diaphragmatic) lung lobes. No attempt was made to determine the specific location at which the tube lodged within the lung. The tube lumen was then swabbed with a small plug of absorbent cotton held in the jaws of endoscopic biopsy forceps[r]. The swab was withdrawn, and a preweighed sterile disc[s] was clamped in the biopsy jaws. The disc was passed through the tube, beyond the lodged end until it encountered resistance to further passage. The disc was left in contact with the mucosa for 20 seconds, withdrawn, and placed in a sterile 10 ml vacutainer clot tube. This procedure was repeated for a total of 3 discs per sampling time. This technique were modified slightly for the 2.2 mg/kg and 4.4 mg/kg sampling periods to minimize discomfort experienced by the calves. Instead of passing the tube through the nostril and nasal passage, a wooden mouth gag was placed and a Cole endotracheal tube[t] was passed through the gag and into the larynx. The equine nasogastric tube was passed through the Cole tube and samples collected as previously described.

Sample Processing

Bronchial secretion and tissue chamber fluid samples were placed in an ice bath immediately after collection, where they remained until further processing. Jugular blood was allowed to clot at room temperature for 1 hour, then held in the ice bath until processing.

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[r] Olympus Medical Instruments Div., Lake Success, New York.

[s] No. 1599-35, Difco Laboratories, Inc., Detroit, Michigan.

[t] Lane Mfg., Denver, Colorado.

Serum samples and tissue chamber fluid samples were centrifuged at 1,000g for 10 minutes at 4 C. Serum samples were separated with assistance from plastic centrifugation beads[u]. After centrifugation the serum and tissue chamber fluid samples were transferred by glass Pasteur pipette to 1.2 ml Nunc freezer vials[v]. Bronchial secretion samples were weighed as a composite of the 3 discs collected per sampling time for determination of the volume of absorbed secretions. Any grossly visible mucous or other debris were removed prior to weighing. All samples were kept at 4 C until assayed, then transferred to -70 C for long term storage.

Bioassay

PLATE PREPARATION The bioassay utilized two layers of media in standard 150 x 15 mm petri plates[w]. The top, or seed layer, contained the indicator organism in a thin layer of media. The bottom, or base layer, served as a buffer for diffusion of the samples after they had passed through the seed layer. This prevented the sample fluid from being trapped by the bottom of the plate and reflected to the seed layer, potentially affecting the size of the zone of inhibition. The base plates were prepared by pumping[x] a base layer of 30 mls of antibiotic test media number 11[y] into the petri plates. The media was prepared as per manufacturer's rehydration and autoclaving recommendations, with 1 drop of Pourite[Rz] per liter of media to reduce bubble formation on the plates. The plates were allowed to cool before moving or stacking to prevent disturbance of the base layer. Once all base plates had cooled sufficiently to solidify, they

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[u] OTI Specialties, Santa Monica, California.

[v] Intermed, Denmark.

[w] American Scientific Products, McGraw Park, Illinois.

[x] Technomara media pump, Zurich, Switzerland.

[y] Difco Laboratories Inc., Detroit, Michigan.

[z] Analytical Products, Inc., Belmont, California.

were moved to a large walk-in refrigerator for storage. Base plates that were not used within two weeks were discarded.

The seed layers were prepared the day prior to sample collection using the same precautions as used with the base layers to prevent irregularities in the media. The seed layer consisted of 20 mls of Antibiotic Test Media 1 with Providencia alcalifaciens ATCC 9886[aa] as the indicator organism. The P. alcalifaciens inoculum was prepared by growing the organism, taken from minus 70 C storage, overnight on blood agar plates at 37 C in a 5% CO_2 incubator. The blood agar plates consisted of 5% defibrinated sheep's blood[bb], 1% Yeast extract[cc], and 1% heat inactivated horse serum[dd] in Tripticase Soy Agar[cc]. Three to five colonies of the organism were then transferred to 200 mls Brain-Heart Infusion broth[ee] and incubated in a shaking water bath at 80 rpm for 5 hours at 37 C. After incubation the inoculate was diluted to produce a standard optical density of 0.042 at a wavelength of 650 nm[ff], which corresponded to a McFarland density of 0.5 (10^8 colony forming units/ml). Three mls of this inoculum was added per liter of seed media (0.3% inoculum) after the antibiotic test media 1 had been held at 120 C for 15 minutes and allowed to cool to 42 C. Pourite^R was also included in the seed media. Dispensation of the seed layers began after allowing 2-3 minutes for media and inoculum to mix by rotating paddle agitation in the media preparator[gg]. The seed media was pumped[hh]

[aa] American Type Culture Collection, Rockville, Maryland.

[bb] Cleveland Scientific, Bath, Ohio.

[cc] BBL division of Becton-Dickinson, Cockeysville, Maryland.

[dd] Gibco Laboratories, Lawrence, Massachusetts.

[ee] Difco Laboratories Inc., Detroit, Michigan.

[ff] Model J-A spectrophotometer, Coleman Scientific.

[gg] Model SH110 Jouan, Winchester, Virginia.

[hh] Technomara media pump, Zurich, Switzerland.

onto the base layers after they had been pre-warmed for 2-4 hours at 37 C. This pre-warming was used to prevent rapid gelling and possible seed layer irregularities. The plates were spread out and allowed approximately 1 hour to cool to room temperature after pouring, then stored at 4-5 C until use. Fresh plates were prepared for each sample day, as they were found to be unreliable if stored for more than 48 hours.

STANDARD CURVE GENERATION The standard curve (reference curve) was generated for this bioassay system using dilutions of 5.0, 2.5, 1.25, 0.625 (midpoint concentration), 0.3125, 0.156, and 0.078 μg/ml of Ceftiofur sodium standard[ii]. The standard was assayed by The Upjohn Company at 895 micrograms ceftiofur per milligram of total compound, or 1,000 μg in 1.12 mg of the standard. Thus, the standard curve dilutions were made by dissolving 11.2 mg of standard in 10 mls bovine serum[jj] (heat inactivated, filter sterilized) to make a concentration of 1,000 μg/ml, from which further dilutions in the same bovine serum were made to reach the range listed above. These dilutions were plated in triplicate on the prepared bioassay plates, alternated with the midpoint dilution of 0.625 μg/ml, and each plate was reproduced to create 3 plates, or 9 standard curve zones and 9 midpoint zones. The lower limit of sensitivity of this bioassay system was 0.156 μg/ml, as determined by the straight line portion of the semi-log plot of inhibitory zone size against drug concentration (Figure 4).

EXPERIMENTAL SAMPLES Serum and tissue chamber fluid were assayed by methods previously described (Walker et al., 1989). Briefly, serum and tissue chamber fluid were added to the plates as 20 μl aloquots on sterile blanks[kk]. Each sample for each animal was tested in triplicate on each plate, and each plate was replicated for a total of three plates. The fluid

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[ii] The Upjohn Company, Kalamazoo, Michigan.

[jj] Collected from MSU Veterinary Clinical Center blood donor cows.

[kk] No. 1599-35, Difco Laboratories Inc., Detroit, Michigan.

samples were alternated with similar discs containing ceftiofur in a standard concentration that corresponded with the midpoint dilution of the standard curve series. Thus, nine sample discs and nine standard midpoint discs with corresponding zones of inhibition were generated for each serum and tissue chamber fluid sample.

The bronchial secretion samples, being absorbed directly into the sterile blanks from the mucosa, were placed directly on the seed media in the same arrangement with the standards as the fluid samples. Only one plate of three bronchial secretion samples and three standard discs was set up for each animal at each sample time.

All plates were incubated overnight at 37 C under aerobic conditions. The plates were placed in stacks of no more than two for the first 2 hours, after which they were stacked to the shelf space capacity of the incubator.

All plates were read manually, measuring zone sizes for the test animal samples first. The plates generated for the first dosing period (1.1 mg/kg) were read on an overhead projector with a millimeter scale overlay. The plates for the 2.2 mg/kg and 4.4 mg/kg dosing periods were read on a light box using an electronic caliper device[11]. All zones were read to the nearest millimeter size and recorded individually.

The initial bioassay data processing was done on microcomputer using custom software from Micromath Inc.[mm]. Initial data analysis involved averaging zone sizes to reduce the data to one value for each sample type from each animal at each sample time. These averages were then adjusted for plate to plate variation using the difference of the standard midpoint values (also

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[11] Mitutoyo Corp. Tokyo, Japan.

[mm] Micromath Inc., Salt Lake City, Utah.

averaged) for each plate and the average value for all the standard midpoint samples on all plates. Adjusted averages were then converted to microgram per milliliter (μg/ml) values based on the standard curve. These μg/ml values were then plotted on time versus concentration curves by RSTRIP[R].

Bronchial secretion samples required adjustment for fluid volume differences before analysis and valid comparisons could be made. This was done by converting the zone size to an absolute volume of drug contained on the disc, rather than a concentration, as it was the amount of drug and not the volume of fluid in which it was dissolved that determined the bacterial inhibitory potential of the compound. Using this data the standard curve for each sample day could be adjusted for absolute drug content per disc regardless of fluid volume (see results). The drug contained in each bronchial secretion sample disc was then read from the linear regression plot of drug vs. zone size using a hand held computer[nn]. These new values were then adjusted for plate to plate variation in the same manner as the fluid samples.

Day 1 and 4 samples of BRS and TCF at 1.1 mg/kg were not analyzed by bioassay, but by HPLC at The Upjohn Company, Kalamazoo, Michigan, using reported techniques (Jaglan et al., 1990). Adjustment of this HPLC data was required in order to compare the values from these data sets to all others which had been evaluated by bioassay. Regression analysis of data for which both HPLC and bioassay values were available yielded a mathematical statement from which bioassay equivalent values could be calculated for those sets where only HPLC data had been generated. This calculation yielded data consisting of a mean value rather than individual values for each animal. Therefore, no standard error values are available for this data, and the random factor of individual calves could not be incorporated in the statistical model.

[nn] Model HP 11C, Hewlett-Packard, Corvallis, Oregon.

Pharmacokinetic Analysis

Pharmacokinetic analysis was performed by microcomputer using RSTRIP[R] software[oo]. The data entered for analysis was the average of the 4 animals for each sample time and type. All data sets were processed as binomial expressions during curve fitting to the 2 compartment open model (Kinabo and McKellar, 1989), without the variable of weighting factors. This analysis generated the pharmacokinetic values $T_{1/2B}$, C_{max}, T_{max}, and AUC_{0-12hr}, and the statistical values of standard deviation, coefficient of determination and correlation coefficient. The statistical values are useful for determination of how well the Least Squares Regression curve fitting approximates the actual data points.

Statistical Analysis

Four factors influenced the variables of C_{max}, T_{max}, $T_{1/2B}$, and AUC: dosage (1.1, 2.2, or 4.4 mg/kg); dose number (single vs multiple); sample location (serum vs TCF vs BRS); and individual calves. The random factor of the individual calves was removed from the model by the use of mean values, therefore three-factor analysis of variance (ANOVA) was done by microcomputer[pp] according to the general linear model (GLM):

$$Y_{ijk} = \text{mean} + A_i + B_j + AB_{ij} + C_k + AC_{ik} + BC_{jk} + ABC_{ijk}(\text{Error})$$

Where Y is the individual variable, A, B, and C were the fixed effects of dose number, dosage level, and sample location, and i, j, and k are levels of each of these factors. The interaction term ABC was used as the systematic error term. Ratios of AUC_{0-12hr} values were analyzed using factors A and B in a two-factor analysis of variance. Differences in data values were considered significant where the probability of type 1 error (p) was less than 0.05.

[oo] Micromath Inc., Salt Lake City, Utah.

[pp] Number Cruncher Statistical System, Kaysville, Utah.

Where GLM ANOVA indicated significant differences existed, comparisons between specific means was done using Tukey's T test. Again, significance was set at $p < 0.05$.

Minimum Inhibitory Concentration

Determination of the minimum concentration of ceftiofur required to inhibit growth of 207 clinical isolates[qq] was done using a microtitration, broth-dilution technique (Thrupp, 1986). Ninty six well, round bottom microtiter plates[rr] were prepared with two-fold decreasing concentrations of the drug, from 10 μg/ml to 0.078 μg/ml, in vertical columns 1 through 12. The dilutions were prepared from 100 mls of 1,000 μg/ml solution, each well being charged with 50 μls of the appropriate concentration. All dilutions were made in sterile french square bottles using double distilled water that then passed through a carbon filter canister, an ion exchange canister, and a 0.22 micron filter[ss] The 8th row (bottom row) of the plate was used as the positive growth control, containing no antimicrobial solution.

Inoculates of all organisms except H. somnus were prepared by growing overnight on blood agar at 37 C, then transferring two to five colonies to Mueller-Hinton broth (MHB)[tt] for three to five hours of incubation at 37 C in a shaking water bath[uu] at 50 rpm. After incubation the broth cultures were adjusted by addition of fresh MHB to a density of approximately 10^8 colony forming units (CFU) per ml by comparison with a 0.5 McFarland standard. This suspension

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[qq] Isolates were obtained from: Animal Health Diagnostic Laboratory, Michigan State University; South Dakota State University, Animal Disease Research and Diagnostic Laboratory, Brookings, South Dakota; Texas Veterinary Medical Diagnostic Laboratory, Amarillo, Texas.

[rr] No. 25850, Corning Glass Works, Corning, New York.

[ss] Millipore Corporation, Bedford, Massachusetts.

[tt] Difco Laboratories, Inc, Detroit, Michigan.

[uu] Model 224, Fisher Scientific.

was further diluted with MHB by a factor of 1:1000, first by addition of 100 µls of the 0.5 McFarland equivalent to 900 µls of fresh broth, a 1:10 dilution, then by addition of 10 µls of the 1:10 dilution to 990 µls of MHB to produce a 1:100 dilution. The final broth suspension contained approximately 10^5 CFU per ml. Haemophilus somnus isolates were prepared by incubating overnight in 5% CO_2 on PPLO[vv] agar supplimented with 1% IsoVitaleX™. Approximately five colonies were then transferred to PPLO broth with 1% IsoVitaleX™[ww] and incubated for 5 hours in 5% CO_2. Dilution to 0.5 McFarland equivalent was also done in PPLO broth with 1% IsoVitaleX™ by the same method as with other organisms. Fifty µls of the final dilutions of all organisms were added to the 50 µls of antimicrobial solution already in each well of columns 1-9, resulting in a final concentration in the microtiter plate wells of 10^4 CFU/ml (1:2 dilutions). The last well in each row (Column 12) received 50 µls of broth media, serving as the negative growth control. Control organisms K. pneumoniae ATCC 10031 and Staphylococcus aureus ATCC 25923 were processed in the same manner and included in columns 10 and 11, respectively, on each plate. Klebsiella pneumoniae is a Gram negative organism that is sensitive to most cephalosporins, while this strain of S. aureus is a Gram positive organism that is quite resistant to ceftiofur sodium. These bacterial organisms serve as positive inhibition and negative inhibition controls between plates. All dilutions were made with Rainin semi-automatic pipetters[xx].

The plates were incubated overnight at 37 C under aerobic conditions, and read visually with a magnifying mirror. The MIC was recorded as the lowest concentration in which no visible growth could be observed.

[vv] Difco Laboratories, Inc, Detroit, Michigan.

[ww] BBL Microbiology Systems, Cockeysville, Maryland.

[xx] Rainin Instrument Co. Inc., Woburn Massachusetts.

RESULTS

The procedure for adjusting bronchial secretion samples for fluid volume was first performed on the standard curve data. Each standard curve sample disc contained 20 μls of a microgram-per-milliliter dilution, therefore the amount of drug per disc was 0.02 times the concentration value. The absolute (abs) amount of drug for each standard curve dilution is given in the table below.

Conc.(μg/ml):	5.0	2.5	1.25	0.625	0.3125
Abs. (μg/disc):	0.1	0.05	0.025	0.125	0.0063

The adjusted bronchial secretion concentrations were calculated from these standard curve values, then used to generate the pharmacokinetic data reported in table 1.

The statement used to convert HPLC data to bioassay equivalent date was:

$Y = 0.937x - 0.789 \quad (r^2 = 0.85)$

Where:

Y is the bioassay equivalent value

0.937 is the slope of the regression plot

x is the HPLC determined value

0.789 is the Y axis intercept of the regression plot

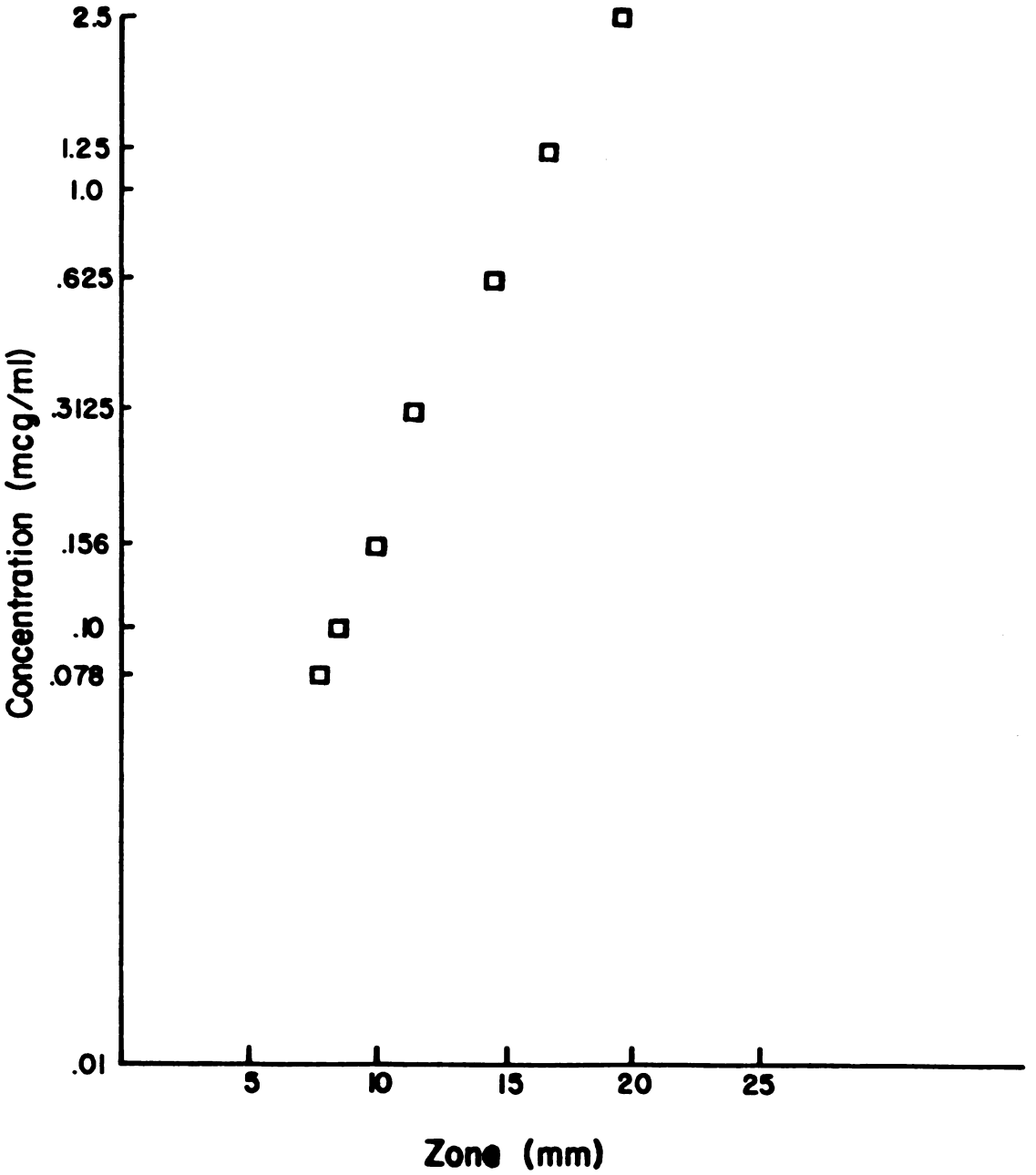

Figure 4. Semi-log plot of bacterial growth inhibition zone size against ceftiofur sodium concentration. Lowest point of plot linearity indicates assay sensitivity

Ceftiofur sodium was well absorbed from the injection site in the gluteal muscles of the calves as indicated by peak serum concentrations observed within 0.42 hours to 2.48 hours after injection, depending on dosage and treatment day (Tables 2 and 3, Figures 5-10). The serum T_{max} values differed significantly ($p<0.05$) when comparing dosages for the same treatment day, but not between day 1 and day 4 for the same dosage level (Table 2, Figure 11). Maximum serum concentrations (C_{max}) showed significant linear increases with doubling of the dose ($p<0.05$), (Tables 2 and 3, Figure 11).

Distribution into the tissue chamber fluid occurred slowly, with peak concentration occurring at between 7.13 and 11.26 hours, depending on dosage level and treatment day (Tables 2 and 3, Figures 5-10, 12). Significant TCF T_{max} differences were not detected between treatment days for the same dosage, but were observed between dosages on the same treatment day ($p<0.05$) (Tables 2 and 3, Figure 12). Values for C_{max} were significantly less in the TCF than in the serum at all dosages and treatment days ($p<0.05$) (Tables 2 and 3, Figures 5-10). Values for C_{max} in the TCF increased significantly from low dosage to higher dosage ($p=0.01$) (Tables 2 and 3, Figure 12).

Bronchial secretion concentrations of ceftiofur sodium were significantly higher than TCF, but significantly lower than serum concentrations ($p<0.05$) (Tables 2 and 3, Figures 5-10). Peak concentrations in BRS occurred much earlier (T_{max}) than in TCF, with T_{max} values much nearer the serum values, although significant differences existed between all sample types ($p<0.05$) (Tables 2 and 3, figures 5-10). As with TCF, C_{max} values for BRS samples increased significantly from low to higher dosage ($p<0.05$) (Tables 2 and 3, Figure 12).

Comparisons between the 3 sample types showed significant increases in C_{max} from TCF to BRS to Serum ($p<0.05$), with T_{max} values increasing in the opposite order: Serum to BRS to TCF ($p<0.05$) (Tables 2 and 3, Figures 5-10).

Ceftiofur elimination half-lives ($T_{1/2\beta}$) showed no significant differences for any comparisons between day, dosage, or sample type. Serum elimination half-life values varied from 1.93 to 3.56 hours, resulting in removal of over 90% of the drug by 8 to 16 hours after dosing. Elimination from BRS was slower, and from TCF slower still, resulting in prolonged drug concentrations in these peripheral compartments (Tables 2 and 3).

The AUC_{0-12hr} values expressed as a percentage of serum values showed no statistical differences for either peripheral compartment, although penetration of ceftiofur sodium into TCF increased with higher dosages, while penetration into BRS decreased, for both treatment days (Table 2 and Table 3).

Minimum inhibitory concentration results (Table 4, Figures 11, 12, 13) indicate that the concentration of Ceftiofur attained in all sample types when administered once daily at 2.2 or 4.4 mg/kg is well above the inhibitory concentration for all isolates of common bovine respiratory pathogens, regardless of geographic origin.

Table 2. Day 1 pharmacokinetic values for serum, tissue chamber fluid, and bronchial secretions (± standard error of the mean) collected from 4 calves after once daily intramuscular ceftiofur sodium injections.

DOSAGE LEVEL (mg/kg)	DOSE NUMBER (day)	SERUM[a,b,c]	TCF[a,b,c]	BRS[a,b,c]
C_{max} (μg/ml)[a]				
1.1	1	3.60±0.75	0.28[d]	2.26[d]
2.2	1	8.78±2.47	0.98±0.16	3.95±0.81
4.4	1	17.25±9.35	2.95±2.70	7.82±2.38
T_{max} (hrs)[b]				
1.1	1	1.44±0.31	8.62[d]	1.29[d]
2.2	1	1.82±0.89	10.06±1.57	2.47±1.38
4.4	1	2.48±0.42	11.26±5.38	2.20±3.80
$T_{1/2\beta}$ (hrs)				
1.1	1	1.93±0.31	8.10[d]	1.76[d]
2.2	1	3.56±0.70	11.29±5.55	6.28±1.57
4.4	1	3.30±0.89	19.36±2.27	5.15±1.18
$AUC_{0-12hrs}$ (μg•hr/ml)[c]				
1.1	1	16.46±2.13	4.87[d]	19.76[d]
2.2	1	66.17±21.63	29.20±11.98	47.04±5.16
4.4	1	131.94±47.02	111.87±67.53	78.12±37.85
AUC(% of serum value)				
1.1	1		29.59[d]	120.04[d]
2.2	1		44.13	71.09
4.4	1		84.79	59.21

a = C_{max} values differ significantly between all dosages and types ($p < 0.05$).
b = T_{max} values differ significantly between all dosages and types ($p < 0.05$).
c = AUC values differ significantly between day, dosage and types ($p < 0.05$).
d = Indicated TCF and BRS values derived by regression analysis of HPLC data against bioassay data.

Table 3. Day 4 pharmacokinetic values for serum, tissue chamber fluid, and bronchial secretions (± standard error of the mean) collected from 4 calves after once daily intramuscular ceftiofur sodium injections.

	DOSAGE LEVEL (mg/kg)	DOSE NUMBER (day)	SERUM[a,b,c]	TCF[a,b,c]	BRS[a,b,c]
C_{max}(μg/ml)[a]					
	1.1	4	5.17±0.55	0.28[d]	1.80[d]
	2.2	4	13.13±2.56	1.95±0.58	2.90±1.37
	4.4	4	24.08±7.45	3.94±1.49	5.36±0.97
T_{max}(hrs)[b]					
	1.1	4	0.42±0.25	5.77[d]	2.59[d]
	2.2	4	1.72±0.68	10.00±1.49	3.27±1.89
	4.4	4	2.31±0.68	8.21±2.02	2.52±1.18
$T_{½\beta}$(hrs)					
	1.1	4	3.55±0.30	2.07[d]	5.62[d]
	2.2	4	3.10±0.53	14.12±2.61	13.30±1.75
	4.4	4	3.31±0.99	10.10±2.13	4.84±1.17
$AUC_{0-12hrs}$(μg•hr/ml)[c]					
	1.1	4	29.68±4.82	6.48[d]	20.10[d]
	2.2	4	88.65±16.04	70.61±22.73	68.18±5.26
	4.4	4	178.35±44.63	132.20±44.57	55.41±3.34
AUC(% of serum value)					
	1.1	4		21.83[d]	67.72[d]
	2.2	4		79.65	76.91
	4.4	4		74.12	31.07

a = C_{max} values differ significantly between all dosages and types ($p < 0.05$).
b = T_{max} values differ significantly between all dosages and types ($p < 0.05$).
c = AUC values differ significantly between day, dosage and types ($p < 0.05$).
d = Indicated TCF and BRS values derived by regression analysis of HPLC data against bioassay data.

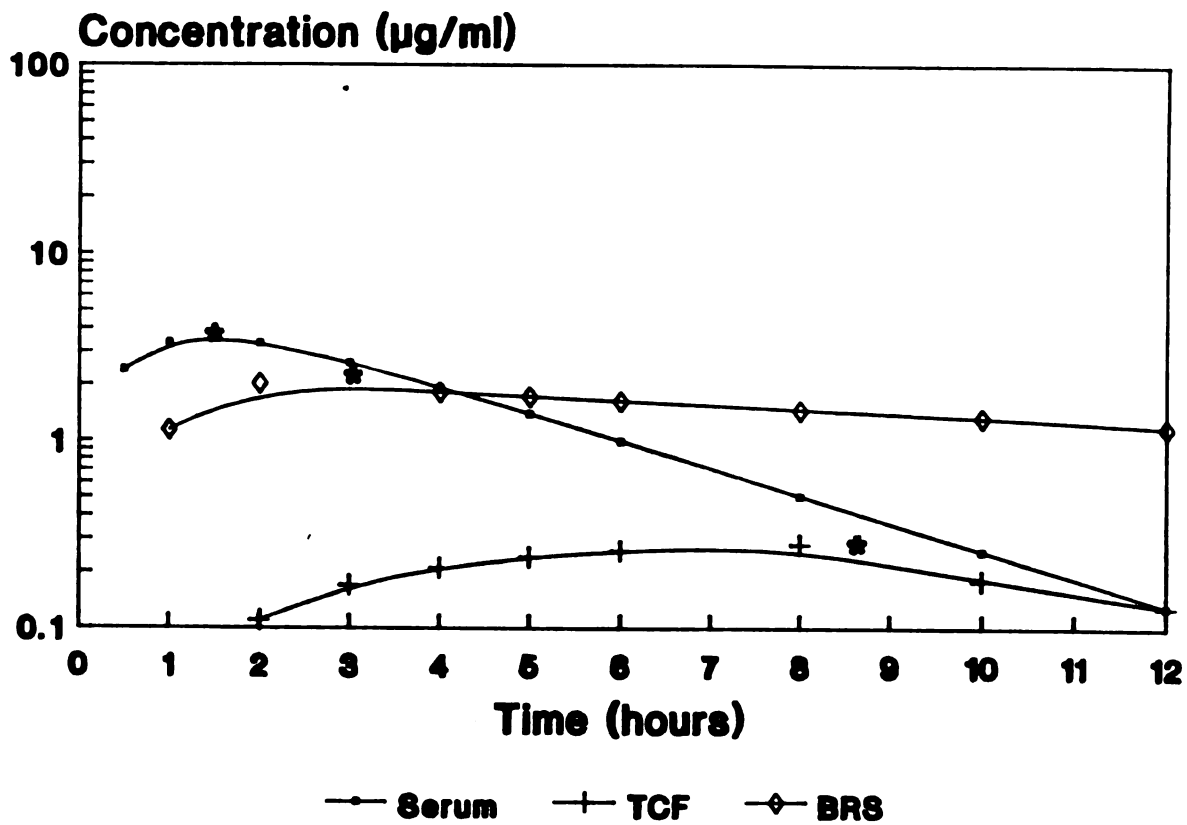

Figure 5. Calculated serum, tissue chamber fluid (TCF), and bronchial secretion (BRS) concentrations of ceftiofur sodium in 4 calves after 1 dose at 1.1 mg/kg. * = significantly different maximum concentration (C_{max}) and time of occurance (T_{max}).

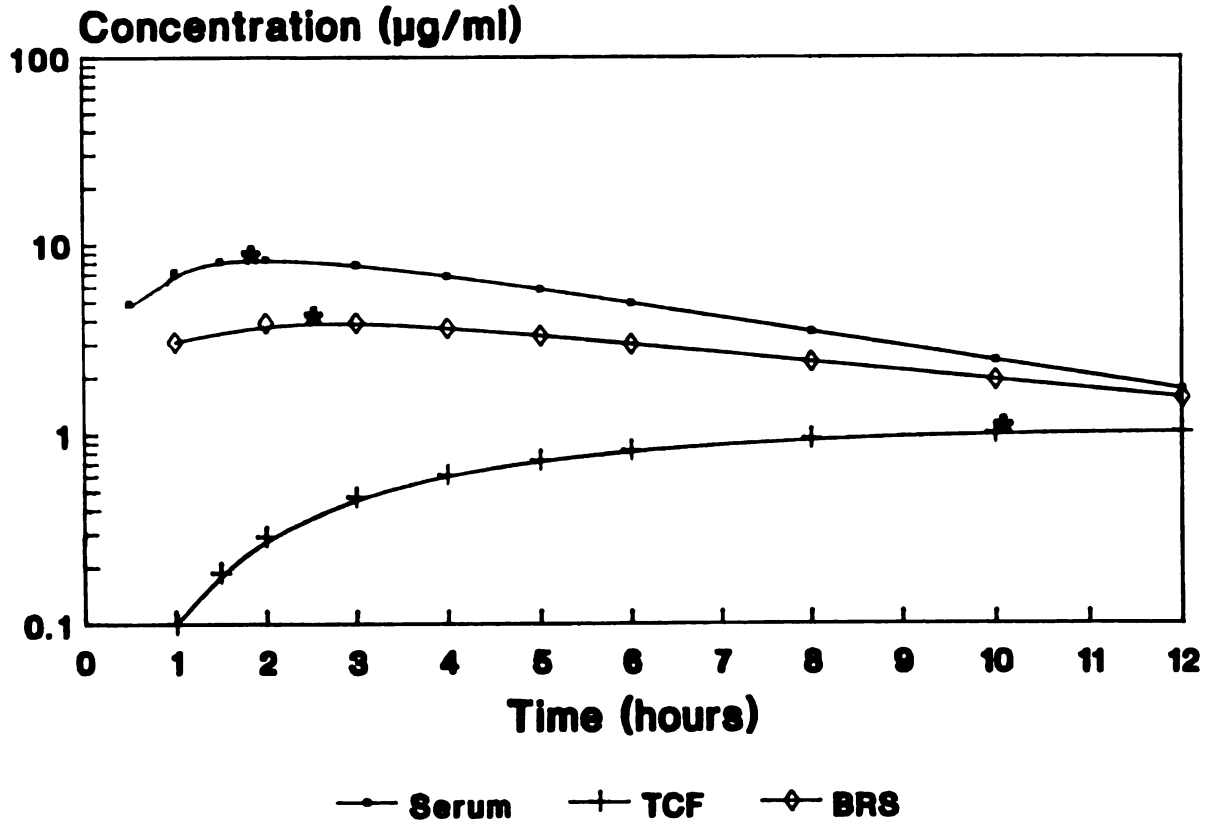

Figure 6. Calculated serum, tissue chamber fluid (TCF), and bronchial secretion (BRS) concentrations of ceftiofur sodium in 4 calves after 1 dose at 2.2 mg/kg. * = significantly different maximum concentration (C_{max}) and time of occurance (T_{max}).

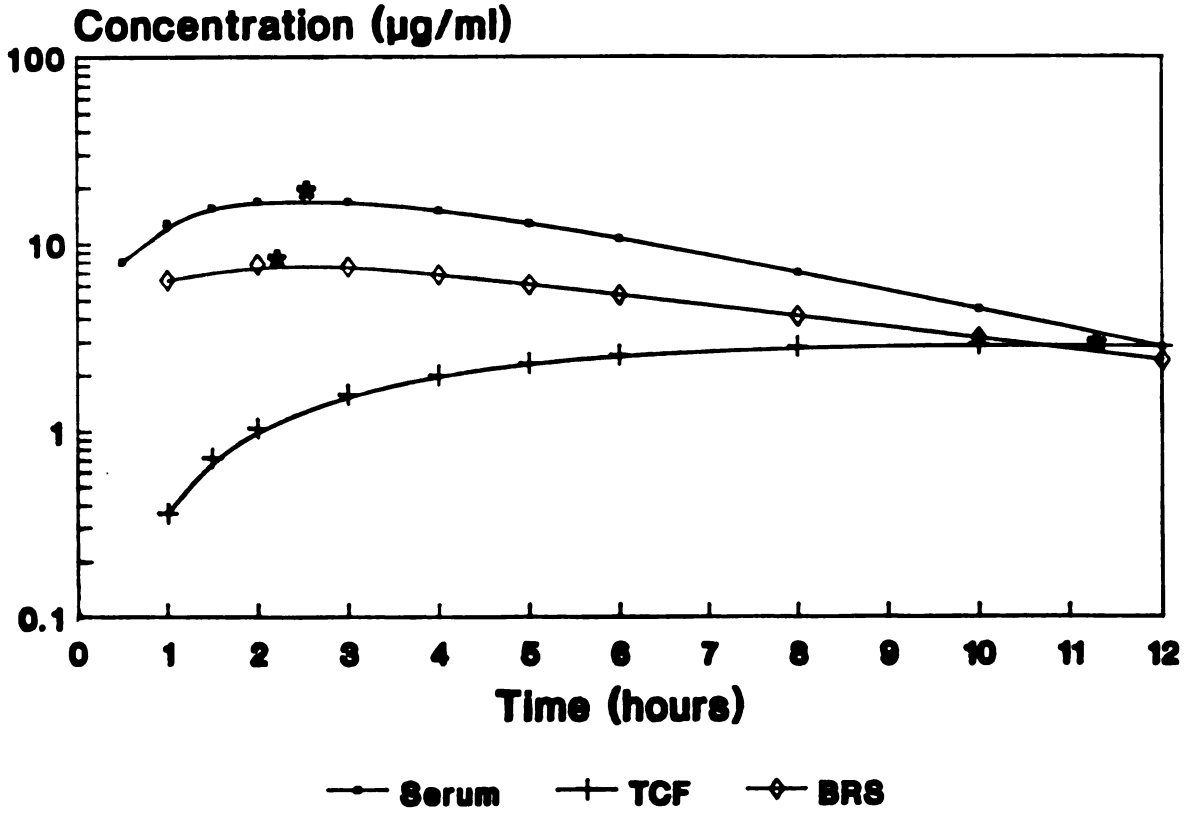

Figure 7. Calculated serum, tissue chamber fluid (TCF), and bronchial secretion (BRS) concentrations of ceftiofur sodium in 4 calves after 1 dose at 4.4 mg/kg. * = significantly different maximum concentation (C_{max}) and time of occurance (T_{max}).

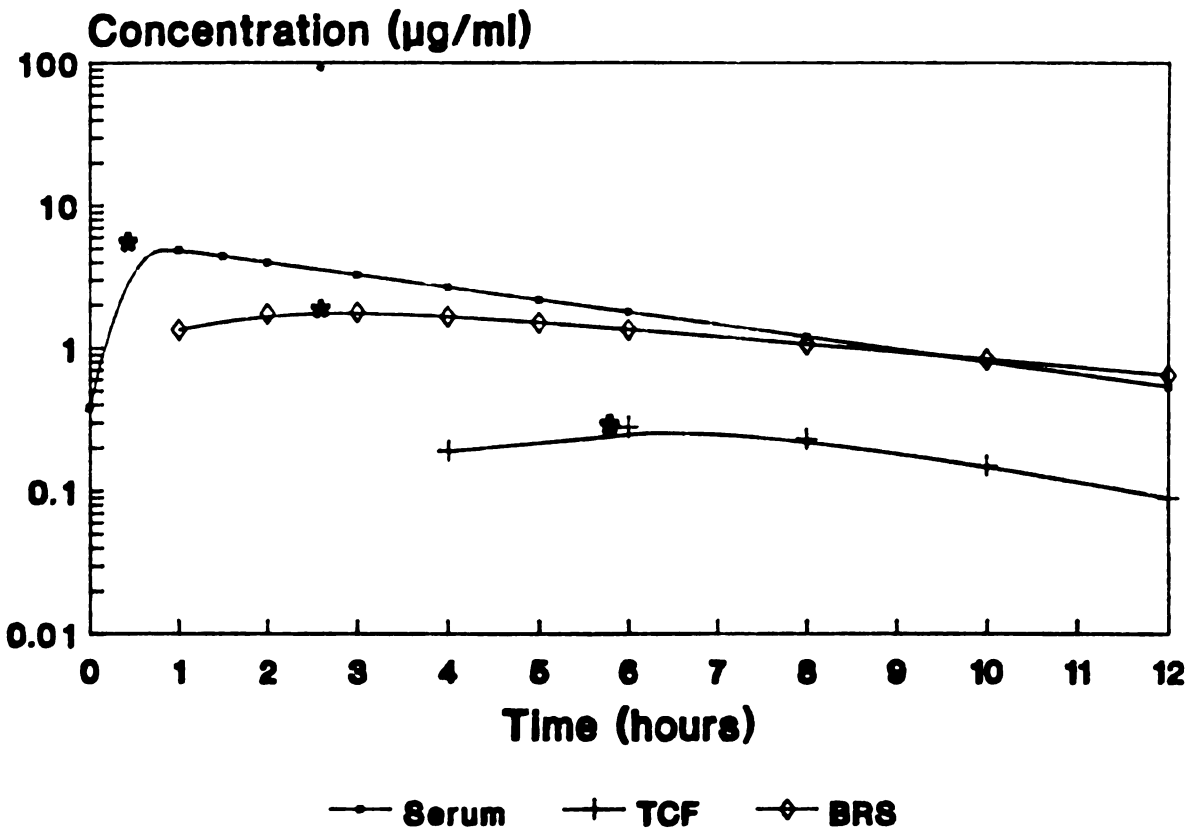

Figure 8. Calculated serum, tissue chamber fluid (TCF), and bronchial secretion (BRS) concentrations of ceftiofur sodium in 4 calves after 4 doses at 1.1 mg/kg. * = significantly different maximum concentration (C_{max}) and time of occurance (T_{max}).

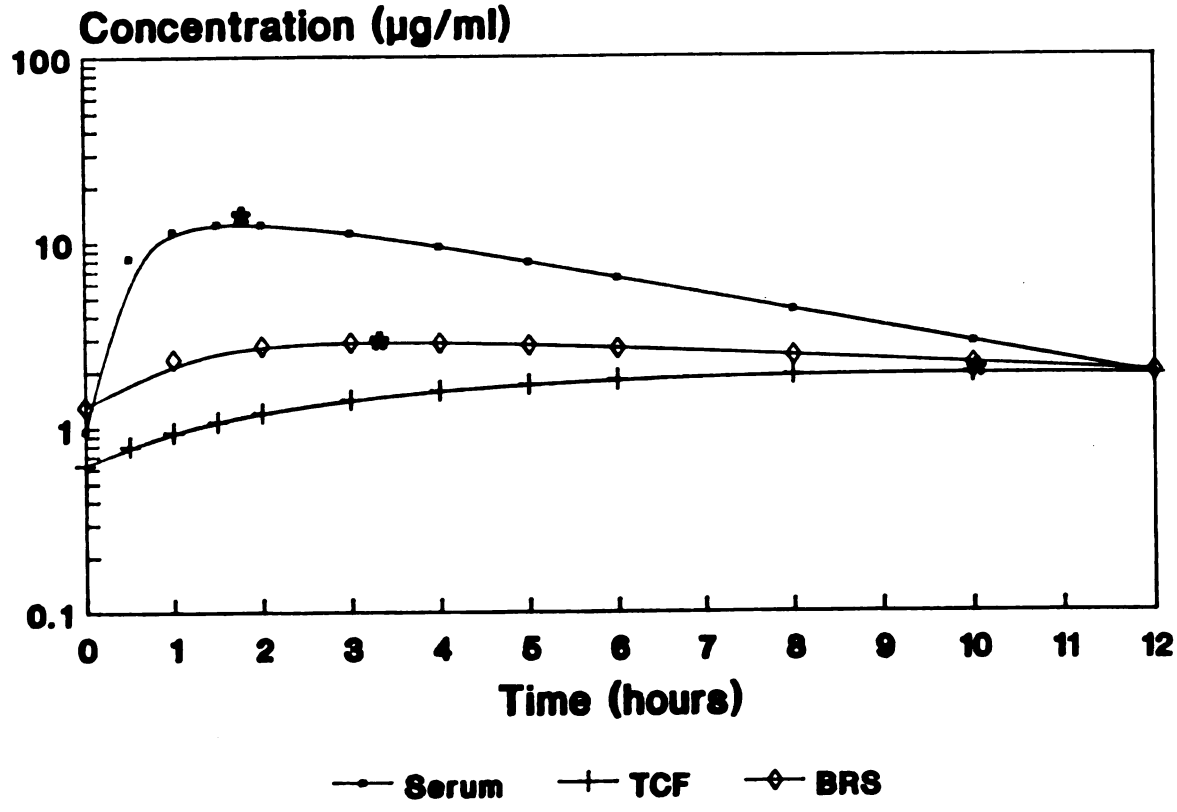

Figure 9. Calculated serum, tissue chamber fluid (TCF), and bronchial secretion (BRS) concentrations of ceftiofur sodium in 4 calves after 4 doses at 2.2 mg/kg. * = significantly different maximum concentation (C_{max}) and time of occurance (T_{max}).

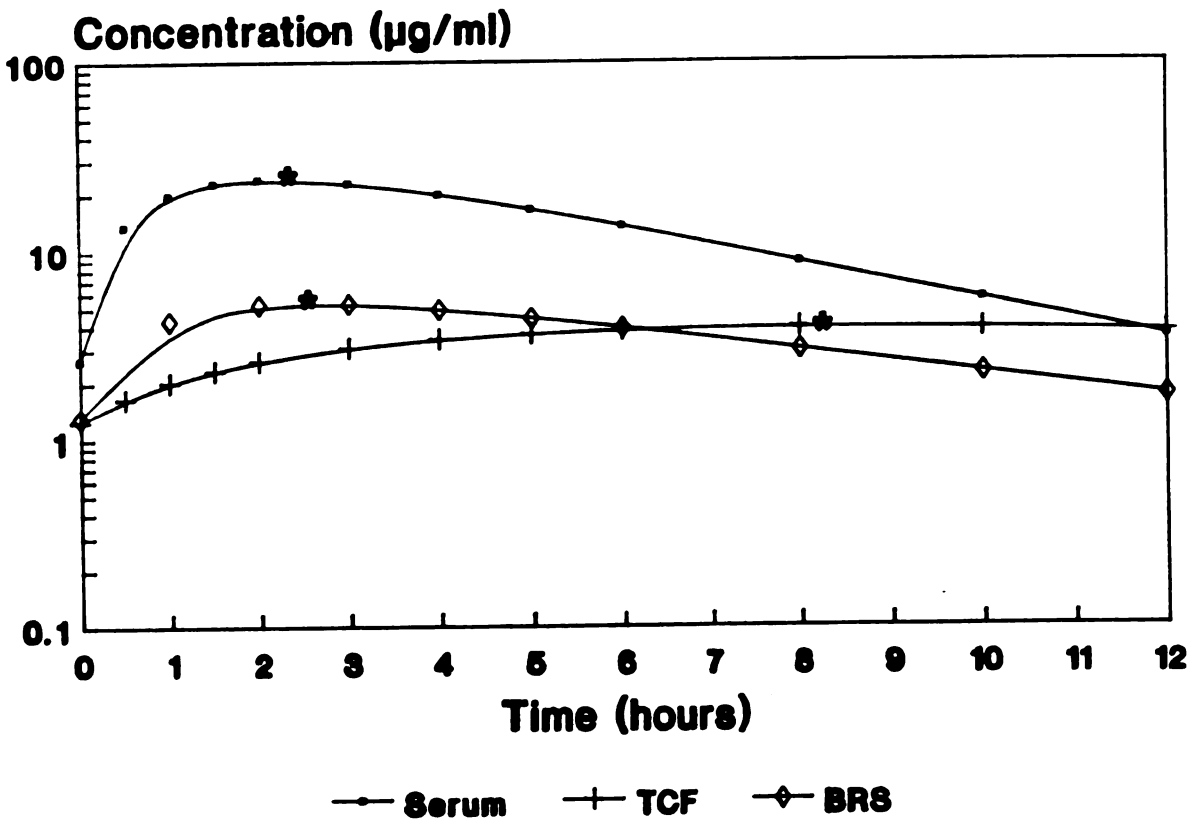

Figure 10. Calculated serum, tissue chamber fluid (TCF), and bronchial secretion (BRS) concentrations of ceftiofur sodium in 4 calves after 4 doses at 4.4 mg/kg. * = significantly different maximum concentration (C_{max}) and time of occurance (T_{max}).

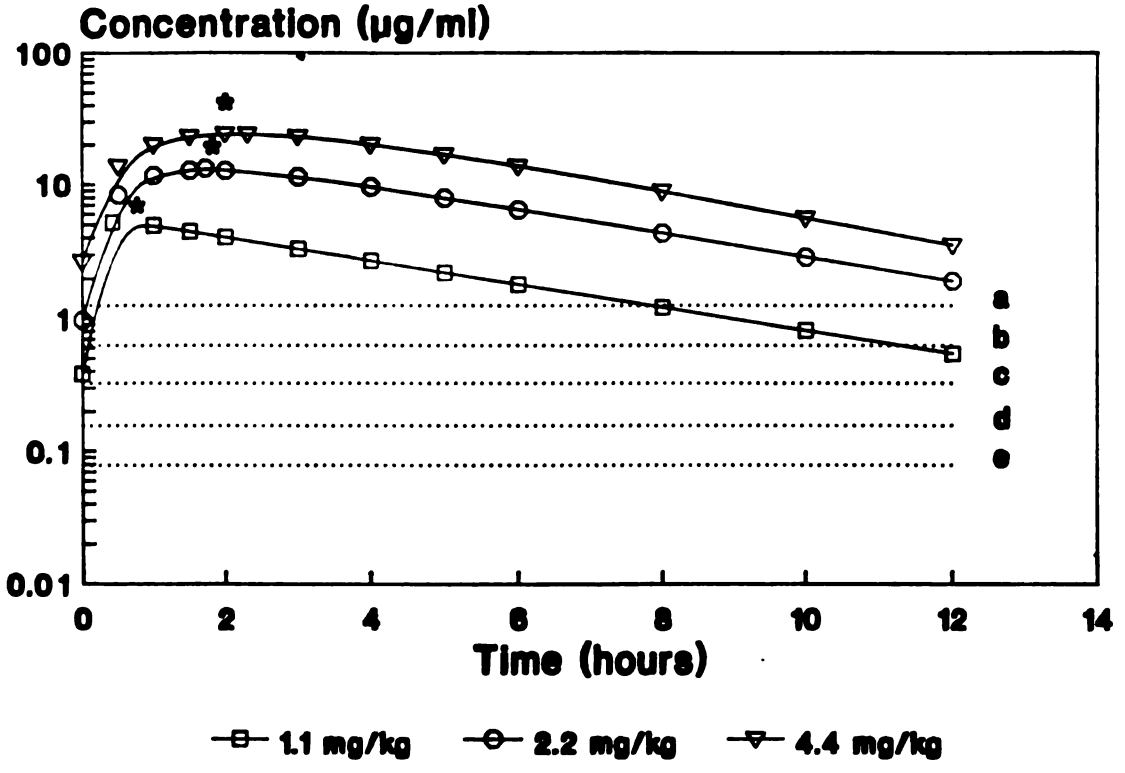

Figure 11. Calculated serum concentrations of ceftiofur for 4 calves after 4 doses, compared to minimum inhibitory concentration values for 90% of clinical bacterial isolates (MIC_{90}).

Legend:
- a = Serratia marcescens, Staphylococcus aureus
- b = Klebsiella spp., Escherichia coli, Enterobacter spp.
- c = Streptococcus agalactia
- d = Streptococcus dysgalactia
- e = Pasteurella haemolytica, Pasteurella multocida, Haemophilus somnus
- * = Significantly different maximum concentration (C_{max}) and time of occurance (T_{max}).

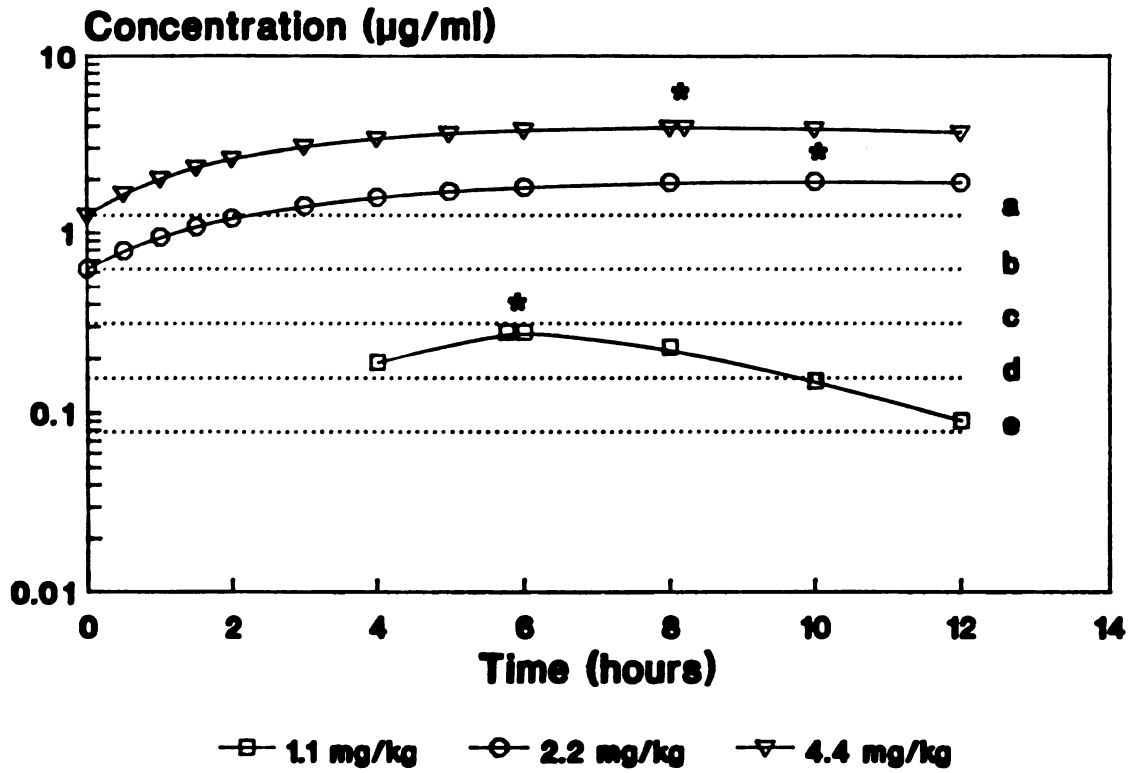

Figure 12. Calculated TCF concentrations of ceftiofur for 4 calves after 4 doses, compared to minimum inhibitory concentration values for 90% of clinical bacterial isolates (MIC_{90}).

Legend:
- a = Serratia marcescens, Staphylococcus aureus
- b = Klebsiella spp., Escherichia coli, Enterobacter spp.
- c = Streptococcus agalactia
- d = Streptococcus dysgalactia
- e = Pasteurella haemolytica, Pasteurella multocida, Haemophilus somnus
- * = Significantly different maximum concentration (C_{max}) and time of occurance (T_{max}).

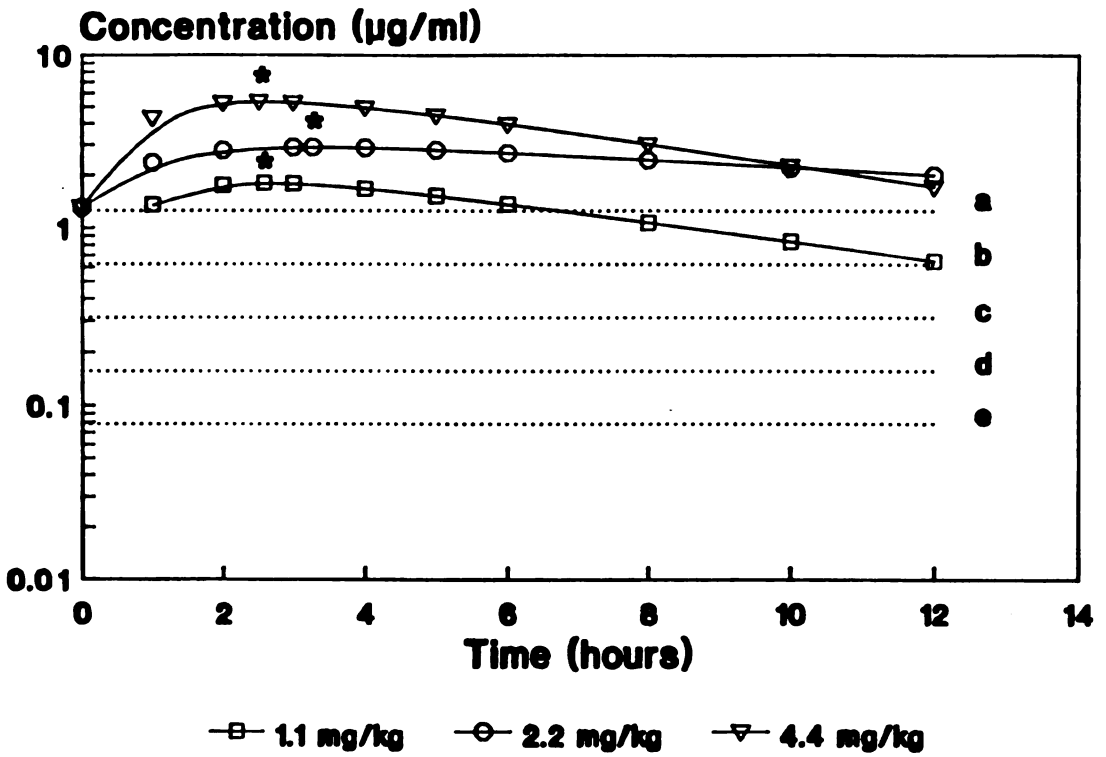

Figure 13. Calculated BRS concentrations of ceftiofur for 4 calves after 4 doses, compared to minimum inhibitory concentration values for 90% of clinical bacterial isolates (MIC_{90}).

Legend:
- a = Serratia marcescens, Staphylococcus aureus
- b = Klebsiella spp., Escherichia coli, Enterobacter spp.
- c = Streptococcus agalactia
- d = Streptococcus dysgalactia
- e = Pasteurella haemolytica, Pasteurella multocida, Haemophilus somnus
- * = Significantly different maximum concentration (C_{max}) and time of occurance (T_{max}).

Table 4. Cumulative and (percentage of total) in vitro minimum inhibitory concentrations of ceftiofur sodium for bacterial isolates recovered from cattle.

		Ceftiofur concentration (μg/ml)				
ORGANISM	NUMBER TESTED	≤0.078	0.158	0.3125	0.625	1.25
Pasteurella haemolytica	64	64(100)				
Pasteurella multocida	42	42(100)				
Escherichia coli	30		8(27)	24(80)	27(90)	30(100)
Haemophilus somnus	25	25(100)				
Staphylococcus aureus	14			2(14)	11(79)	14(100)
Actinomyces pyogenes	10	10(100)				
Streptococcus uberis	6	6(100)				
Klebsiella spp.	6			2(33)	6(100)	
Streptococcus agalactia	2	1(50)				2(100)
Serratia marcescens	2				1(50)	2(100)
Enterobacter cloacae	2			1(50)	2(100)	
Enterobacter fecalis	2					≥ 2.5
Listeria monocytogenes	2					= 2.5

DISCUSSION

This study applied tested and accepted methods in two novel ways. One was in the pharmacokinetic evaluation of a new antimicrobial drug, the other was in evaluation of a new respiratory secretion sampling technique.

Pharmacokinetic evaluation of Ceftiofur sodium (Tables 2 and 3) indicates that the drug is well absorbed and reaches appreciable concentrations in systemic circulation after intramuscular injection. The values measured in this study also compare closely with those generated in the research and development of this compound for the same sample types and dosage levels, with the exception of respiratory samples. This difference will be discussed further below.

Although no reports of pharmacokinetic analysis of this drug exist, other third generation cephalosporins have been studied in animal and human subjects (Faulkner et al., 1987; Nakashima et al., 1989; Soback and Ziv, 1988; Soback and Ziv, 1989; Hoffstedt and Walder, 1981). Most studies with human subjects involve oral or intravenous administration of antimicrobial agents, with pharmacokinetic measurement of the central compartment only. Oral and IM pharmacokinetic data differ from IV data in that no lag time exists when drugs are administered by IV routes. Bolus intravenous administration also results in the entire dose of compound being placed directly and immediately into the systemic circulation (Prescott and Baggot, 1988a). In contrast, oral and IM administration result in delays before measurable concentrations of drug appear in serum (Greenblatt and Shader, 1985; Prescott and Baggot, 1988a). Orally administered drugs may also be subject to variations in stability to gastrointestinal contents such as protozoa, bacteria, and enzymes, and are variably absorbed

into the system dependant primarily on the lipid solubility of the drug (Prescott and Baggot, 1988a). These factors may result in substantially poorer drug absorbtion when administered by oral compared to IM routes. Orally administered compounds are also subject to metabolism by gut mucosa or liver before reaching the systemic circulation, again, influencing the amount of active compound present in the systemic circulation (Prescott and Baggot, 1988a). Intramuscular admininstration, however, does not assure uniform absorbtion. Vascular perfusion and capillary blood flow, the primary determinants of absorbtion, vary between muscle groups, resulting in different drug absorbtion rates (Prescott and Baggot, 1988a). These factors affect the pharmacokinetics of the drug, making comparisons between drugs difficult or meaningless if the technique and route of administration are not identical. For these reasons, comparison of ceftiofur sodium data from this study to data from human studies of other cephalosporins cannot be made. However, most investigations of cephalosporins in animals involve similar techniques and therefore yield useful comparisons.

In studies of cephalosporins involving animals, ceftriaxone has been shown to reach peak serum concentrations of 25 μg/ml and 35 μg/ml within 30 minutes of a single intramuscular dose to calves at 10 mg/kg and 20 mg/kg, respectively (Soback and Ziv, 1988). Cefoperazone reached a concentration of 16 μg/ml of calf serum within 30 minutes of single dose intramuscular administration at 20 mg/kg to unweaned calves (Soback and Ziv, 1989). The dosages in these investigations exceeded the maximum dosage of ceftiofur in our study, which is reflected by larger C_{max} values. Serum C_{max} values for ceftriaxone and cefoperazone occured earlier than our serum C_{max} values (indicated by lower T_{max} values), suggesting that ceftiofur is absorbed more slowly than these other third generation agents. Elimination half-life values after single doses of ceftriaxone at 10 mg/kg and 20 mg/kg, and cefoperazone at 20 mg/kg were 116.8 minutes, 145 minutes, and 136.9 minutes, respectively. Serum $T_{1/2\beta}$ values for ceftiofur ranged from 115.8 minutes after a single dose of 1.1 mg/kg, to 213.6 minutes following a single dose of 2.2 mg/kg. These differences may be the result of variations in protein

binding. Ceftiofur is reported by the manufacturer to be approximately 90% bound in bovine serum. Ceftriaxone was estimated to be only 22% bound at the low dose and 18% bound at the high dose (Soback and Ziv, 1988). Increased serum protein binding delays diffusion from the vascular space to the tissue spaces (Craig and Suh, 1985. Highly protein bound drug that does reach the tissue space is also readily bound by tissue proteins (Craig and Suh, 1985). The result of these interactions is reduced tissue peak concentrations and delayed clearance (Craig and Suh, 1985), as seen in these investigations.

These observations on the effect of protein binding are supported by conclusions following the examination of cephalosporin penetration into human subcutaneous tissue fluid, using a subcutaneous cotton thread implant model (Hoffstedt and Walder, 1981). This study involved 5 cephalosporins with serum protein binding values ranging from 10% to 90%, and demonstrated that the more highly protein bound drug also had the longest elimination half-life, both in serum and interstitial fluid, irrespective of whether the cephalosporin was administered by intravenous bolus or infusion.

This study showed that while ceftiofur penetrates into TCF at concentrations that seem adequate for inhibition of susceptible bacteria that might invade this environment, it does so slowly and at concentrations substantially lower than those in serum, as indicated by the longer T_{max} and smaller C_{max} and AUC values. This is consistent with the results reported by Hoffstedt and Walder where peak tissue fluid/peak serum levels were 41% for low protein bound (10%) cephradine, compared to 5% for highly serum protein-bound (90%) cefoperazone.

Even with a relatively low serum protein binding value, cephradine (a first generation cephalosporin) was only able to achieve 41% of the serum concentration in the extravascular fluid. While it has been consistently observed (Chisholm et al., 1973; Joiner et al., 1981; Short et al., 1987) that the extravascular fluid evaluated in tissue chamber studies has lower peak

concentrations that occur simultaneously with or later than serum peaks, and that such fluids have longer $T_{1/2\beta}$ values consistant with those generated in this investigation, this phenomenon has not been completely explained. Possible explainations may involve tissue protein binding, molecular size, pH, or ionic restrictions to diffusion.

The tissue chamber model has met with acceptance as a method of tissue fluid collection, as is indicated by the number of published reports using this system in various related forms (Clarke et al., 1989a; Peterson et al., 1989; Higgins and ees, 1984; Short CR et al., 1987; Walker et al., 1989; Walker et al., 1990). Although fluid collected from these chambers appears to be quite similar to natural, non-inflamed interstitial fluid (Chisholm et al., 1973), it is collected as a result of an artificial tissue space and may be influenced by the presence of the device or the space (Bergan, 1981).

Factors other than protein binding may also influence tissue penetration of antimicrobial agents. Tissue fluid pH, for example, has been shown to effect antimicrobial concentration in tissue chambers implanted in the prostate glands of dogs (Bergan, 1981). Canine prostatic fluid is acidic; acidic compounds such as penicillins and cephalosporins penetrated less well into prostatic fluid than into the subcutaneous tissue chamber fluid, while the alkaline compounds erythromycin and trimethoprim produced concentrations that exceeded tissue fluid levels (Bergan, 1981). These differences suggest that specialized tissues such as glandular or secretory tissues may have properties that require separate consideration from such generalized tissues as skeletal muscle or subcutaneous interstitial tissue. Even though subcutaneous tissue is considered a generalized tissue, precise characterization of TCF for pH, lipid, protein and ion content, coupled with investigation of the behavior of the assayed compound under similar conditions, would allow the most accurate interpretation of the data (Clarke et al., 1989). These factors were not considered in the current study, and may have played a role in the observed low TCF ceftiofur sodium concentration, delayed T_{max}, and long $T_{1/2\beta}$.

Another factor which may influence these parameters is the perforated surface area of the tissue chambers. The chambers described by Chisholm et al. (1973) had approximately 40% of the surface area opened by perforations, those used by Walker et al. (1989, 1990) were approximately 40% open. The chambers in this study had approximately 20% perforated surface area. Since the surface area of a cylinder varies as the square root of the volume, larger chambers will have smaller area:volume ratios relative to small chambers, yet the larger cylinder will have greater volume. If these chambers function as reservoirs (Bergan, 1981), delays in equilibrium will occur between chamber lumen contents and the surrounding fluid or interstitial gel, with larger chambers or smaller perforated surface area values resulting in longer delays. While these chambers are felt to be in direct communication with interstitial space, the distances between capillaries, lymphatics, interstitial tissue, and interstitial spaces; the dynamic components that are responsible for the composition of interstitial fluid, are minute compared to the distances between those components and the tissue chamber lumen. This delay in equilibrium may be responsible for the prolonged tissue chamber T_{max} and $T_{1/2\beta}$ seen in this study (Tables 2 and 3, Figures 5-10, 12).

Bioassay-equivalent values for the low dosage (1.1 mg/kg) were generated mathematically from HPLC data and may be subject to some question in comparison to the actual bioassay data generated for other dosages, although the importance of this information makes these comparisons necessary. The HPLC analysis was conducted on pooled samples, therefore the effect of one animal's sample exerting an unusually large effect on the total measurement cannot be evaluated. The influence of a single sample on the pharmacokinetic values for the pooled sample measurement may have been responsible for some inconsistency in the values for this data set.

Ceftiofur sodium is marketed as an antimicrobial agent for the treatment of bacterially-associated respiratory disease in cattle. The importance of understanding the pharmacokinetics of this

drug in the infected tissues has been discussed. Our results show that ceftiofur sodium is capable of attaining high concentrations in the bronchial secretions soon after administration. The values shown in this study appear higher than the those measured in the ceftiofur sodium researach and development, but may be explained by the differences in experimental methods. Peak BRS concentration of 3.95 μg/ml occured at 2.47 hrs for the 2.2 mg/lb dosage (Table 2) in this study. Lung tissues sampled at 8 hours only in the development of this compound yielded concentrations of 0.9 - 1.4 μg/ml. Examination of Figure 6 shows BRS concentrations declining to near this value by 8 hours, with remaining differences falling within the error range reported in this study. Differences in sampling techniques (BRS swab vs. tissue plugs) make further comparisons difficult.

Bronchial secretion C_{max} values were significantly higher than the corresponding TCF samples, and showed significant increases from low dosage to higher dosage. Interestingly, values for sample day 1 exceeded day 4 values for all dosage levels, although the differences were not statistically significant. The bronchial secretion sampling technique, like the tissue chamber technique, may influence the composition of the sample and perturb the results by some unexplained mechanism. Alternative sample methods, however, are likely to influence the outcome as well. Biopsy and homogenation techniques have been discussed, and found to be unsatisfactory. Lavage techniques (Gray et al., 1989) require compensatory fluid dilution adjustments, and probably alter subsequent samples by dilution and inflammation similar to the disc absorption method. Pulmonary mechanical abnormalities have been observed following small volume (20 ml) fluid instillation into tracheas of calves (Killingsworth et al., 1987). This effect is felt to be at least partially mediated by the vagus nerve via irritant and chemoreceptors; other parasympathetic-mediated effectors in the lung include pulmonary and vascular smooth muscle, secretory cells, and inflammatory mediators (West, 1982). These autonomic responses have the potential to significantly modify the bronchial secretions (Murray, 1986).

Other respiratory secretion swab techniques involving rigid structure contact with bronchial mucosa would be just as likely to induce changes in the secretions by these same mechanisms (Hajer et al., 1988). Evaluation of other pulmonary fluids (pulmonary lymph) would not necessarily reflect drug activity at the airway surface, where P. haemolytica attacks, but would possibly provide useful information regarding pulmonary parenchymal drug concentrations, and might be collected by microprobe cannulation that would have minimal influence on sample composition. Differences in lymph, serum, and interstitial fluid have also been discussed.

As stated previously, successful antimicrobial therapy requires that concentrations of non-protein bound drug exceed the MIC of the pathogen at the site of infection for (Prescott and Baggot, 1988b; Sande and Mandell, 1985; Wise, 1985). The antimicrobial agent should be administered such that the free drug concentration at the site of infection exceeds the MIC target values for the entire treatment period, since inadequate (subinhibitory) beta-lactam antimicrobial levels have been associated with "breakthrough" of susceptible bacterial strains during the first 72 hours of therapy (Anderson et al., 1976). Inadequate dosing may also provide evolutionary pressure towards development of, or selection for resistance in previously susceptible bacterial strains (Bellido et al., 1989). The resistance that develops in this manner may not be specific for the antimicrobial agent in use, but may be more broad in spectrum based on binding site or membrane permeability modifications (Bellido et al., 1989). The risk of "breakthrough" resistance development is greater with antimicrobials that do not induce a significant post-antibiotic effect (PAE), which is defined as the persisting suppression of bacterial growth that follows limited exposure to an antimicrobial agent. Post-antibiotic effects vary dependant on specific drug, organism, and the amount the C_{max} for that drug exceeds the MIC for that organism (Vogelman and Craig, 1985; Craig and Gudmundsson, 1985). Beta-lactam antimicrobials are not considered to induce significant PAE against gram-negative organisms (Vogelman and Craig, 1985; Craig and Gudmundsson, 1985). These drugs require more careful

monitoring and observation of dosing guidelines as determined by pharmacokinetic behavior than do those inducing post-antibiotic effects.

Maintaining biologically active, non-protein bound drug concentrations above the MIC for the target organism throughout the dosing period has been shown to be the most important parameter in determining beta-lactam therapeutic efficacy (Vogelman et al., 1988). Because MIC determination is done in vitro, the actual tissue drug concentration required to inhibit the organism is unknown, being affected by pH, protein binding, ionic binding, penetration restraints, and other influences (Clarke et al., 1989), and varies with the dynamic processes occurring at the target location. Therefore, drug administeration dosages and intervals should maintain concentrations of at least four times the MIC_{90} for the pathogen throughout the treatment period (Sande and Mandell, 1985). Care must be taken with some drugs, such as aminoglycosides, to ensure that this does not place the drug concentration in the range of toxicity. Fortunately, beta-lactam drugs have a wide therapeutic margin (Balant, 1985), and this is rarely the problem. Although toxicity was not a subject of this investigation, existing data[yy] indicates that a wide margin of safety exists between therapeutic and toxic concentrations with ceftiofur sodium.

Our data suggests that ceftiofur would be an appropriate choice as treatment for soft tissue, respiratory or bacteremic infections in calves caused by P. haemolytica, P. multocida, A. pyogenes, and S. uberis. All samples, (serum, TCF, and BRS) and dosages (1.1, 2.2, and 4.4 mg/kg) yielded drug concentrations at least 4 times greater than the MIC_{90} of 0.078 μg/ml generated in this study for these bacterial agents (Tables 2, 3, and 4). The MIC data presented in this study corresponds well with the values generated in the research and development of this drug (Table 1), and are supported by a current report (Yancey et al., 1987) in which the

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[yy] Miller, C.C. Ceftiofur research and development. Personal communication.

MIC values for ceftiofur sodium and ampicillin were compared, finding that the MIC values for ceftiofur were lower than the ampicillin values. Ceftiofur was also more active than ampicillin or cefamandole, a second generation cephalosporin, against a multiple-antibiotic resistant strain of P. haemolytica (Yancey et al., 1987). The tissue concentrations achieved by once daily dosing in this investigation also persisted to 12 hours after dosing, satisfying the recommendations of maintaining drug levels of four times the MIC_{90} value throughout the dosing period (Figures 11, 12, 13).

Because of the overall broad spectrum of the cephalosporins, treatment of bacterial infection caused by pathogens other than those commonly associated with BRD might be attempted. These applications would require close evaluation and monitoring. For example, ceftiofur as treatment of S. aureus-induced cellulitis could not be recommended based on this study and stated guidelines since the MIC_{90} value generated in this study for this organism is 1.25 μg/ml. This value is close to the 2.2 mg/kg., day 4 peak value of 1.95 μg/ml, and although this MIC_{90} value is more than twice exceeded by the peak values generated by the 4.4 mg/kg dosage regimen, it does not provide the therapeutic ratio that is considered acceptable, and other drugs with more consistent and lower MIC_{90} values would be better choices. This same logic would apply for infections caused by Klebsiella spp., most E. coli strains, and Serratia marscesens, although the number of Klebsiella and Serratia isolates evaluated in this study were limited, and should be considered as general indicators only. Other organisms such as Listeria monocytogenes and Enterobacter fecalis appear resistant to the ceftiofur sodium, although, again, very few isolates were evaluated. Specific bacterial culture and antimicrobial susceptibility evaluation of these organisms would indicate alternate, and more appropriate, antimicrobial choices. Choice of specific antimicrobial agent, or scholastic comparison of similar compounds, might be facilitated by studies of serum bactericidal activity over time rather than estimating efficacy based on half-life, peak concentration and in vitro bacterial inhibition data as is the current standard (Gugleilmo and Rodondi, 1988).

The data generated in this study indicates that ceftiofur sodium is an appropriate antimicrobial agent against the bacteria associated with BRD. Increasing the dose from 1.1 mg/kg to 2.2 or 4.4 mg/kg, or dosing at 12 hour rather than 24 hour intervals, however, would provide larger therapeutic ratios, maintaining the drug concentration above the MIC_{90} value throughout the dosing interval in all tissues examined. As indicated by $T_{1/2\beta}$, tissue chamber concentrations and bronchial secretion concentrations of ceftiofur sodium will still exceed MIC levels by recommended margins after 24 hours, when dosed at 2.2 or 4.4 mg/kg, although serum concentrations would decrease to sub-inhibitory levels in this amount of time. As stated earlier, exceeding the MIC value by greater margins is likely to provide no therapeutic benefit (for drugs that induce little or no PAE) other than prolonging the length of time that drug concentrations exceed this value, again, ensuring that concentrations exceed MIC values during the entire dosing period (Vogelman et al., 1988). Lower dosages given more frequently would be as effective, and while toxicity does not appear to be a concern with this drug, reduced dosage therapy might be important with other antimicrobial agents (Vogelman et al., 1988). The manufacturer's recommended dosing interval of 24 hours for ceftiofur is, incidentally, an exception compared to the majority of the third generation cephalosporins. Intervals of 12 hours, with shorter intervals of 4-8 hours in life threatening situations, are recommended for most of the other cephalosporins (Balant et al., 1985). Therefore, treatment with 2.2 or 4.4 mg/kg at 24 hour intervals appears acceptable when the target organism is known and is susceptible at low drug concentrations, and when drug penetration into tissues can be expected to be consistant with the healthy subjects of this study. In debilitated or chronically diseased animals drug penetration would be expected to be reduced due to dehydration, circulatory debilitation, and chronic inflammatory change (fibrosis), in which case higher dosages or more frequent dosing intervals might be necessary for satisfactory results. This would also be true for more resistant organisms, where culture and susceptibility data would be indicated for most accurate therapeutic approach.

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