In vitro study of the efficacy of chlorhexidine gluconate and chlorous acid/chlorine dioxide combination against Staphylococcus aureus and the cytotoxic effects on equine dermal fibroblast cell culture and in vivo effects of these products on the normal rabbit stifle joint

by

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EXPLANATION OF THESIS FORMAT

This thesis is submitted to partially fulfill the requirements of a Master's of Science degree. It is presented in alternate thesis format as outlined in the graduate college thesis manual of Iowa State University. The first paper is "The cytotoxic effects of chlorhexidine gluconate and a chlorous acid/chlorine dioxide combination on equine dermal fibroblast culture and efficacy against *Staphylococcus aureus*". The second paper is on "The histopathological effects of intraarticular amikacin sulfate, 0.05% chlorhexidine gluconate and chlorous acid/chlorine dioxide irrigation solution on rabbit stifle joints". Each paper contains its own abstract, introduction, materials and methods, results, discussion and bibliography. The "Review of the Literature" is designed as a review of the literature that created the ideas to pursue these projects.

REVIEW OF LITERATURE

Injury is followed by inflammation.[52] The vascular and cellular events that follow will vary according to the severity of the insult. In the normal wound there is an immediate vascular response of vasoconstriction. Leucocytes become adherent to the vascular walls and red cells become adherent to each other and tend to plug capillaries. After the relaxation of the initial vasoconstriction an active vasodilatation occurs allowing leakage of fluid from venules. This fluid is essentially plasma with its complement of proteinaceous components. Shortly after this the leucocytes move by diapedesis into the extravascular space. The predominant cell is the polymorphonuclear leucocyte but other cells migrate in proportion to the numbers found in the bloodstream. As these cells die and lyse they release their products into the wound. The products released form pus on the wound. This fact should not be misinterpreted as indicating an infected wound as pus can be found in a nonseptic wound. If tissue damage is extensive and blood circulation is poor the inflammatory insult can become destructive with more tissue being destroyed due to collagenase and other proteolytic enzymes than in the normal healing process.

When plasma is exuded into the wound following an initial

insult, a fibrin network is laid down and becomes incorporated into the hemostatic plug. This fibrin acts as a hemostatic barrier and framework for elements of repair. Fibroblasts utilize the fibrin network as a scaffolding to migrate into the wound. These fibroblasts do not destroy the fibrin but initiate the production of collagen. The capillary buds following closely behind these fibroblasts initiate fibrinolysis; and subsequently allows granulation tissue to fill the wound. The presence of necrotic tissue, hematomas, and bacteria can impede the migration of fibroblasts and therefore delay the production of granulation tissue.

Wounds do not have strength until fibrous tissue appears and bridges the wound gap. During the period of quiescence or lag phase, which is from the time of wounding until 4 and 6 days post wounding, fibroblasts must be mobilized, debris disposed of and circulation restored. This is the time the wound is most susceptible to infection. Any tensile strength gained during this time is probably due to the fibrin network.

Following the lag phase is the phase of fibroplasia or fibroblastic repair. This is the time in which fibroblasts multiply and lay down collagen. They carry on this function regardless of where they are in the wound. Increasing tensile strength in the wound correlates with increasing collagen production by the fibroblast.

The insult in a surgical wound is relatively minimal. Traumatic wounds create an inflammatory insult more intense than simple surgical wounds.[9] The inflammatory response to infection can be different than both traumatic and surgical insults. Unfortunately, surgical and traumatic wounds can frequently be complicated by infection.[9,23,26,29,43,53,70] Because this type of inflammation can dramatically prolong wound healing, many surgeons flush surgical wounds with antimicrobials to reduce the incidence and severity of infection.[2,11,15,29,70] Topical administration of antibiotics can induce hypersensitivity reactions and promote the development of resistance by bacteria; thereby eliminating their effectiveness when given systemically. Volume flushing with balanced electrolyte solutions can be sufficient, in many instances to reduce the bacterial counts to a minimum; and therefore allow the body to control infection by its own defenses.[9,53] With extensive trauma, contamination with foreign debris, or overwhelming infection, the body may be unable to battle the infection adequately. In these situations some clinicians feel the need to add antibiotics or antiseptics to the lavage solutions to further reduce the bacterial counts present in the wound. Because of the risks associated with local antibiotic administration, many clinicians prefer to utilize antiseptic solutions, and less frequently disinfectants.

The application of antiseptic and disinfectant solutions

should be restricted to wounds of limited area to reduce the possibility of toxic reactions or tissue injury.[55,68] These products can be irritating to the wound and may create an inflammatory response.[8,11,15,26,18,34,35,52,53,70]

A disinfectant or germicide is a substance used to destroy bacteria and other infective microorganisms. Disinfectants have bactericidal or germicidal activity and are usually applied to inanimate surfaces. An antiseptic, in contrast, does not kill microorganisms; but rather inhibits reproduction or rate of growth. Antiseptics are thus classified as bacteriostatic.[27,55,68] The difference between a disinfectant and an antiseptic is usually a matter of reaction time, numbers of organisms killed, drug concentration and temperature of the solution. Many disinfectants, when applied to host tissue, kill cells as well as bacteria. Antiseptics are generally applied to tissues to suppress or prevent bacterial propagation. Increasing the concentration of the antiseptic agent may severely irritate the normal host tissue; and therefore inhibit the healing time of a wound. The appropriate solution at the appropriate concentration should be utilized to prevent any undue damage to fibroblasts which might decrease collagen production and subsequently delay wound healing.

Two types of information are utilized to evaluate an antiseptic: the ability of the drug to have an inhibitory or

cidal effect on a microorganism, and the safety of the drug to the tissue. Antiseptics have been used in clinical trials to evaluate their effectiveness of "infection-prevention". These *in vivo* studies have been conducted in laboratory animal, canine and equine models to evaluate healing times of incised wounds flushed or dressed with an antiseptic. Many of these studies evaluate the ability of these antiseptics to assist in healing by reducing or eliminating infection in wounds inoculated with a specific bacteria.[1,2,11,15,26,29] The recovery rate or presence of infection is recorded and usually compared to saline controls. Disinfectants, in contrast, are tested on inanimate surfaces and given a coefficient of the germicidal capability. This coefficient is established by computing a ratio of the disinfectant to phenol. This form of *in vitro* test has been accepted by the Food and Drug Administration and is a standard form of testing for a disinfectants.

The academy of veterinary surgeons have been investigating means of surgical research without the use of live animals. The present trend is to initially evaluate antiseptics by *in vitro* methods looking at efficacy against specific bacteria, as well as effects on cell culture. Cell culture studies are significantly different than live animals and certain basic principles must be understood before extrapolating results to clinical situations. Differences exist between *in vivo* and *in vitro* studies with respect to drug exposure, rate of

change of concentration, metabolism, tissue penetration, clearance and excretion at the cellular level must be addressed. Studies utilizing *in vitro* methods must allow toxins or substrates to reach the cell culture in the same form as in *in vivo* studies in order to be accepted as an animal alternative. The nature of the response must also be considered carefully. *In vitro* responses may be measured by cell survivability or metabolism. There are several forms of assays utilized in these *in vitro* studies. The form utilized in most cell culture studies are short term or immediate response such as an alteration in membrane permeability or a perturbation of a particular pathway.[20,42] Most short term assays evaluate viability by the extrusion of dyes. In the first paper of this study, cytotoxicity was evaluated with trypan blue since the uptake of this dye is dependant on a breakdown in membrane integrity. Nonviable cells stain in contrast to viable cells which are impermeable to trypan blue. Advantages of cell culture include: maintenance of a constant physiochemical environment (such as pH, osmotic pressure, O_2 and CO_2 tension), and the presence of a homogeneous cell type in which to evaluate a response.[20] Unfortunately, cell interactions based on 3-dimensional relationships and specific characteristics typical of the histology of the tissue are lost. Energy metabolism is altered in cell culture and occurs largely by glycolysis with little energy generated by the more efficient citric acid

cycle. While many specialized functions can be expressed *in vitro*, the limits of the model must be appreciated.

While *in vitro* evaluations have become more common place in the scientific design of antimicrobial studies, the use of certain products still needs further evaluation *in vivo*. Conflicting results have been reported for antiseptics tested by both *in vitro* and *in vivo* wound healing studies. An *in vivo* response looks at a complex tissue response. This response is usually influenced by both neural and endocrine input to such insults as inflammation and is not typically reproducible in cell culture. Unfortunately, variables beyond the researchers control may alter the results of clinical studies. Preexistent disease, immunologic status of the animal and variations in the production of the model (i.e., creation of the wound) can lead to variable end results not characteristic for the model.

The antiseptics evaluated in this study are an experimental formulation of 20% aqueous chlorhexidine gluconate and a chlorous acid/chlorine dioxide combination. Chlorhexidine gluconate as a detergent for surgeon and patient preoperative preparation has been utilized in humans for many years.[17,27,39,55,58,62] The residual antimicrobial effect of chlorhexidine is prolonged compared to other conventional antiseptics like povidone-iodine and the benzalkonium chlorides with less hypersensitivity reactions.[1,10,11,26,63,68] Resistance of bacteria to chlorhexidine is reported to be of

low incidence. It is thought that chlorhexidine alters the bacterial cell wall which may make it synergistic with some systemically administered antibiotics.

Unfortunately, little research has been done on the chlorous acid/chlorine dioxide combination.[34,35,51] A gel formulation of this product was evaluated in the healing of guinea pig skin wounds and was found to decrease the amount of scar tissue formation present in the wound.[35] This same formulation was evaluated as a teat disinfectant with increased prevention of new intramammary infections due to *S. aureus*.[51] The present formulation is designed to be utilized as a contact lens disinfectant solution. The chlorous acid/chlorine dioxide disinfectant is an Environmental Protection Agency registered product which has demonstrated broad spectrum microbicidal effectiveness.

Because wounds in the horse frequently involve joints of the distal limb the second part of this study was designed to evaluate these products on the synovium and cartilage in the normal joint. The initial phase of this *in vivo* project was the evaluation of these products in the normal stifle joint of the rabbit. These products may also have application in septic arthritis as lavage solutions as discussed later.

Staphylococcus aureus is a major problem in the contamination of skin wounds and can frequently invade the synovium. Considerable damage occurs when it colonizes the synovium of the joint and elaborates toxins.[31,64,65,73,75] *S.*

aureus arthritis is also a common sequela to contaminated intraarticular injection of therapeutic substances in performance horses. Regardless of the inciting cause, the prevention of infection in the joint is very important in the return to function in all species.

Septic arthritis is a serious infectious process in many species with a guarded to poor prognosis irrespective of treatment. Prompt diagnosis is important and early intervention utilizing broad spectrum, systemic, bactericidal antibiotics and some form of joint drainage or lavage procedure are reported to give the best outcome.[3,4,30,40,44,46,47,50,56,60,72] The aim of therapy is the elimination of the offending organism, removal of nonspecific destructive enzymes and cells with a subsequent reduction in the inflammatory response. Inadequately addressing each of these problems can lead to progression of the septic process and/or destruction of the joint.

Some cases respond poorly to even the most aggressive therapy. This ability to multiply within the joint can be influenced by the hosts general and local immune response as well as the number, type, and virulence of the organism.[1,2,4,5,7] Clinical evaluation of therapeutic modalities can be difficult due to the variability in host/organism interactions.

Infection and inflammation cause thrombosis of vessels in the synovium and alter the filtering properties of the

synovial membrane. Larger molecules such as plasma proteins pass into the joint fluid decreasing the pH and activating proteolytic enzymes.[4,7,8,11,13,17] The effectiveness of systemically administered antibiotics in this environment is of concern. The tendency is to instil antibiotics into the joint following copious lavage to increase the concentration locally; however the preexistent synovitis may be potentiated by some chemically irritating intraarticular medications and lavage solutions.

Staphylococcus aureus is a common cause of septic arthritis in adult humans and horses.[1,3,4,19,30,44,50,65,72,75] This organism's virulence is associated with an increased ability to establish itself and multiply in the host's tissues.[31,37,45,65,75] *S. aureus* is thought to multiply within the host because it apparently suppresses phagocytosis by polymorphonuclear leukocytes (PMN) and vacuolar synovial cells (Type A and Type M). This organism also has the ability to survive within these cells when phagocytized. With the digestive mechanisms failing to lyse the pathogenic bacteria, lysis of the ingestive host cells results.[31] The products released from these cells and the toxins elaborated by *S. aureus* typically create extensive necrosis. A fibrinopurulent exudate frequently fills the wound or joint cavity; thereby reducing the efficacy of many antibiotics and perpetuating the proliferation of the staphylococcal organism. The severe inflammatory response created leads to

depletion of glycosaminoglycans and proteoglycans within the cartilage within the first five days. This depletion precedes the loss of collagen and is responsible for a loss of compressive stiffness of the cartilage. As a result, collagen fibers become more susceptible to mechanical forces and cartilage breakdown can. In order to prevent this cascade of events, early joint drainage and copious lavage has become an established procedure.[4,5,9,12,19,30,40,44,46,47,50,56,60,66,69,72,73,75] Typically, any delay in treatment will allow the bacteria to colonize and become established within the synovium.

A recent study of a *S. aureus* arthritis in the horse demonstrated that among the several treatment regimes tested, the most helpful treatment was parenteral antibiotics.[4] *In vitro* activity of systemically administered antibiotics may be altered by changes present in the joint such as increased acidity. Increased concentrations of bacteria within the joint may reduce the activity of some parenteral antibiotics by the "inoculum effect".[21] This effect has been documented for ampicillin, gentamicin, kanamycin, chloramphenicol and others. Intraarticular antibiotics or antiseptics would seemingly increase the effectiveness of treatment in many clinical situations where increased concentrations of organisms exist within the joint.

In septic arthritis it becomes difficult to distinguish between the inflammatory response generated by the infection

and that possibly due to the intraarticular therapy. Many antiseptics used clinically are thought to create a chemical synovitis when exposed to the synovium and cartilage. Unfortunately, many clinical studies designed to evaluate the effectiveness of intraarticular antiseptics and antibiotics are administered in the face of an established infection. More studies to evaluate the effects of intraarticular therapy on the synovium would be helpful in determining an optimum therapeutic regimen.

REFERENCES

1. Amber EI, Henderson RA, Swaim SF, Gray BW. A Comparison of antimicrobial efficacy and tissue reaction of four antiseptics on canine wounds. Vet. Surg., 12, No. 2, 63-68; 1983.

2. Bassetti C, Kallenberger A. Influence of chlorhexidine rinsing on wound healing of oral mucosa and osseous lesions. J. Clin. Periodontal., 7: 443-456; 1980.

3. Bayer AS. Nongonnococcal bacterial septic arthritis. Postgraduate Medicine; 67, No. 2, Feb. 1980.

4. Bertone AL, McIlwraith CW, Jones RL, Norridin RW, Radin J, Lebel L. Comparison of various treatments for experimentally induced equine infectious arthritis. Am. J. Vet. Res., 48, No. 3, 519-29; March 1987.

5. Bertone AL, McIlwraith CW, Powers BW, Radin MJ. Effect of four antimicrobial lavage solutions on the tarsocrural joint of horses. Vet. Surg., 15, No. 4, 305-315; July-August 1986.

6. Bhawan J, Roy S. Pathology of experimental pyogenic arthritis. Indian J. Med. Res., 61, 1603-4; 1973.

7. Bobechko WP, Mandell L. Immunology of cartilage in septic arthritis. Clin. Ortho. Related Res., No. 108, 84-89; 1975.

8. Branemark PI, Ekholm R, Albrektsson B, et al. Tissue injury caused by wound disinfectants. J. Bone Joint Surg.

(Am), 49: 48-62; 1967.

9. Brown G. Infectious arthritis and wounds of open joints. Vet. Clin. of N. Am., 8(3): 501-510; 1978.

10. Buckle RC, Seabridge CE. The effect of chlorhexidine in the peritoneal cavity. Lancet, 1: 193-5; 1963.

11. Crossfill M, Hall D, London D. The use of chlorhexidine antiseptics in contaminated surgical wounds. Br. J. Surg., 56, 906; 1969.

12. Curtiss PH. The pathophysiology of joint infections. Clin. Ortho. Related Res., No. 96: 129-35; Oct. 1973.

13. Daniel O, Akeson W, Amiel D, et al. Lavage of septic joints in rabbits: Effects on chondrolysis. J. Bone Joint Surg., 58A, 393-5; 1976.

14. Drug Information for the Health Care Professional. USP/DI. The United States Pharmacopoeial Convention, Inc., 1A, 901; 1990.

15. Edlich RF, Custer J, Madden J, et al. Studies in the management of contaminated wounds III; An assessment of the effectiveness of irrigation with antiseptic agents. Am. J. Surg., 20: 118-21; 1969.

16. Edlich RF, Smolka IR, Prusak MP, Edgerton MT. The molecular basis for toxicity of surfactants in surgical wounds. J. Surg. Res., 14; 277; 1973.

17. Facts and Comparisons. Chlorhexidine gluconate. St. Louis: J. B. Lippincott Co., Jan. 1985.

18. Faddis D, Daniels D, Boyer J. Tissue toxicity of

antiseptic solutions: A study of rabbits articular and periarticular tissues. J. Trauma, 7: 895; 1977.

19. Frankel CJ, Spear CV, Hicks JR, et al. The treatment of penicillin resistant *Staphylococcus aureus* joint infections with intraarticular broad spectrum antibiotics. Surg. Forum, 11: 453; 1960.

20. Freshney RI. Cultures of Animal Cells. Second edition. San Fransisco. Alan R. Liss, Inc.; 1987.

21. Fry DE, Garrison RN, Trachtenberg L, Polk HC. Bacterial inoculum and the activity of antimicrobial agents. Surgery Gynec. Obstet., 160: 105; 1985.

22. Gershuni DH, Steven CK. Articular deformation following experimental synovitis in the rabbit hip. J. Ortho. Res., 1: 313-18; 1984.

23. Gilmore OJA. A reappraisal of the use of antiseptics in surgical practice. Ann. R. Coll. Surg. Engl., 59: 93-103; 1977.

24. Gobelet C, Gerster JC. Synovial fluid lactate levels in septic and nonseptic arthritides. Annals Rheum. Dis., 43: 742-45; 1984.

25. Goldschmidt P, Cogen R, Taubman S. Cytopathological effects of chlorhexidine on human cells. J. Periodotol., 48: 212-15; 1977.

26. Gruber RP, Vistines L, Pardoe R. The effect of commonly used antiseptics on wound healing. Plast. Reconstr. Surg., 55: 472; 1977.

27. Harvey SC. Antimicrobial drugs. Antiseptics and disinfectants. In: Osol A, ed. Remington's pharmaceutical sciences. 16th ed. Easton, Pa: Mack Publishing Co., 1980.

28. Helgeland K, Heyden G, Rolla G. Effect of chlorhexidine on animal cells *in vitro*. Scand. J. Dent. Res., 79: 209-215; 1971.

29. Hohn DC, Granelli SG, Burton RW, Hunt TK. Antimicrobial systems of the surgical wound. II: Detection of antimicrobial protein in cell free wound fluid. Am. J. Surg., 133, 601-06; 1977.

30. Jackson RW, Parsons CJ. Distention irrigation treatment of major joint sepsis. Clin. Ortho. Relat. Res., No. 96: 160-64; Oct. 1973.

31. Johnson AH, Campbell WG, Callahan BC. Infection of rabbit knee joints after intraarticular injection of *Staphylococcus aureus*. Am. J. Pathol., 60: 165-77; 1970.

32. Kapral FA, Li IW. Virulence and coagulases of *Staphylococcus aureus*. Proc. Soc. Exp. Biol. Med., 104: 1551-53; 1960.

33. Kenny EB, Saxe SR, Bowles RD. Effects of chlorhexidine on human polymorphonuclear leucocytes. Arch. Oral Biol., 17: 1633-36; 1972.

34. Kenyon AJ, Douglas DM, Hamilton S. The effects of antimicrobials on collagen synthesis in wound repair in mice. J. Surg. Res.,12: 1004; 1980.

35. Kenyon AJ, Hamilton SG, Douglas DM. Controlled wound

repair in guinea pigs, using antimicrobials that alter fibroplasia. Am. J. Vet. Res., 47, No. 1: 96-101; Jan. 1986.

36. Kim HC, Klein S, Hirsch JR et al. Arthroscopic synovectomy in the treatment of hemophilic synovitis. Scand. J. Haematol., Suppl. 40, 33: 271-79; 1984.

37. Koenig MG, Melly MA, Rodgers DE. Factors relating to the virulence of staphylococci. J. Exp. Med., 116: 601-610; 1962.

38. Kushner JD, Somerville JA. Permeability of human synovial membrane to plasma proteins. Arth. Rheum., 4: 55-60; 1971.

39. Lawernce CA. Antimicrobial activity, *in vitro*, of chlorhexidine. Lancet, 1: 18-22; 1957.

40. Leitch M. Diagnosis and treatment of septic arthritis in the horse. J. Am. Vet. Med. Assoc., 175: 701-04; 1979.

41. Lennette EH, Balows A, Hausler WJ, eds. Manual of Clinical Microbiology. Washington DC: American Society for Microbiology, 1980.

42. Lineaweaver W, McMorris S, Howard R. Effects of topical disinfectants and antibiotics on human fibroblasts. Surg. Forum, 33: 37-39; 1982.

43. Lineaweaver W, Howard R, Soucy D, et al. Topical antimicrobial toxicity. Arch. Surg., 120: 267-70; 1985.

44. Lipponen Tulamo R, Gabel AA. Clinicopathologic evaluation of the pathophysiology of infectious arthritis in the horse. Master's thesis, The Ohio State Univ., Columbus,

Ohio, 1981.

45. Lyons C. Antibacterial immunity to *Staphylococcus pyogenes*. Br. J. Exp. Pathol., 41-42; 1937.

46. McIlwraith CW. Treatment of infectious arthritis. Vet. Clin. North Am.(Large Animal Pract.), 5: 363-79; 1983.

47. McIlwraith CW. Antibiotic use in musculoskeletal disease. Proc. 32nd Am. Assoc. Eq. Pract., 241-47; 1986.

48. Michaels EB, Hahn EC, Kenyon AJ. Effect of C31G, an antimicrobial surfactant, on healing of incised guinea pig wounds. Am. J. Vet. Res., 44, No. 7: 1378-81; July 1983.

49. Mitchell NS, Cruess RL. The effect of synovectomy on articular cartilage. J. Bone and Joint Surg., 49A, No. 6: 1099-108; Sept. 1987.

50. Nelson JD. Antibiotic concentrations in septic joint effusions. N. Eng. J. Med., 28, No. 7: 349-58; Feb. 1971.

51. Oliver SP, King SH, Torre PM, et al. Prevention of bovine mastitis by a postmilking teat disinfectant containing chlorous acid and chlorine dioxide in a soluble polymer gel. J. Dairy Science, 72: 3091-97; 1989.

52. Peacock EE, Van Winkle W. *Wound Repair*. Third Edition. Philadelphia: W. B. Saunders Co.; 1984.

53. Platt J, Bucknall RA. An experimental evaluation of antiseptic wound irrigation. J. Hosp. Infect., 5: 181-188; 1984.

54. Prince HN, Nonemaker WS, Norgard RC, Prince DL. Drug resistance studies with topical antiseptics. J. Pharm. Sci.,

67: 1629-31; 1978.

55. Reynolds EF (ed.), Prasad AB (Asst. ed.), Martindale. The extra pharmacopeia. London: Pharmaceutical Press; 1982.

56. Rhodes KH. Antibiotic management of osteomyelitis and septic arthritis in children. Ortho. Clin. N. Am. (Symp. Infections in Orthopedics), 6, No. 4; 915-21; Oct. 1975.

57. Rodeheaver G, Smith S, Thacker J, Edgerton MT, Edlich RF. Mechanical cleansing of contaminated wounds with surfactant. Am. J. Surg., 129: 241-45; 1975.

58. Rosenburg A, Alatary SD, Peterson AF. Safety and efficacy of chlorhexidine gluconate. Surg. Gynecol. Obstet., 143: 789-92; 1976.

59. Rosenberg L. Chemical basis for the histological use of safranin O in the study of articular cartilage. J. Bone and Joint Surg., 53A, No. 1, 69-82; Jan. 1971.

60. Ruedy J. Antibiotic treatment of septic arthritis. Clin. Ortho. and Related Res., No. 96: 150-52; Oct. 1973.

61. Saatman RA, Carlton WW, Hubben K, et al. A wound healing study of chlorhexidine digluconate in guinea pigs. Fund. and Appl. Toxicol., 6, 1-6; 1986.

62. Sanchez IR, Nusbaum KE, Swaim SF et al. Chlorhexidine diacetate and povidone-iodine cytotoxicity to canine embryonic fibroblast and *Staphylococcus aureus*. Vet. Surg., 17, No. 4, 182-85, July-August 1988.

63. Sapeika N, ed. Antiseptics. Actions and uses of drugs. Capetown, South Africa: A. A. Balkam Publishing Co.; 1976.

64. Schurman, DJ, Kajiyama G, Nagel DA. *Escherichia coli* infections in rabbit knee joints. J. Bone Joint Surg., 62A, No. 4, 620-27; June 1980.

65. Schurman DJ, Johnson BL, Amstutz HC. Knee joint infections with *Staphylococcus aureus* and Micrococcus species. J. Bone Joint Surg., 57A, No. 1: 40-49; Jan. 1975.

66. Siemering B. Painful joints: A review of rheumatoid arthritis, systemic lupus erythematous, infectious arthritis and degenerative joint disease. Comp. Cont. Ed., 12, 213-17; 1979.

67. Smith RL, Schurman DJ. Comparison of cartilage destruction between infectious and adjuvant arthritis. J. Ortho. Res., 1: 136-43; 1983.

68. Steward CH. Antiseptics and disinfectants. In: Goodman LS, Gilman AG, eds. Pharmacological basis of therapeutics. 6th ed. New York: Macmillan Pub.; 1980.

69. Stover SM, Pool RR. Effect of intraarticular gentamicin sulfate on normal equine synovial membrane. Am. J. Vet. Res., 46, No. 12, 2485-91; 1985.

70. Swaim SF. *Surgery of Traumatized Skin.* Philadelphia: W. B. Saunders; 1980.

71. Symen DG, Nathan P, Holder IA, et al. Infected surface wound. An experimental model and method for the quantification of bacteria in infected tissues. Appl. Micro. Biol., 23: 509-14; 1972.

72. Trotter GW, McIlwraith CW. Infectious arthritis in

horses. Proc. Am. Assoc. Equine Pract., 173-83; 1981.

73. Tscherne H, Giebel G, Muhr G, Howell C. Synovectomy as treatment for purulent joint infection. Arch. Orthop. Trauma Surg., 103: 162-64; 1984.

74. Wendelburg K, Smeak DD, Kowalski JJ. Chlorhexidine wound lavage and suture soaking for prevention of suture related infections. Vet. Comparative Ortho. and Traumatology, 3: 122-29; 1988.

75. Wolski K. *Staphylococcal* and other gram positive coccal arthritides. Clin. Rheum. Dis., 4(1): 181-96; 1982.

PART I.

THE CYTOTOXIC EFFECTS OF CHLORHEXIDINE GLUCONATE AND A CHLOROUS ACID/CHLORINE DIOXIDE COMBINATION ON EQUINE FIBROBLAST CELL CULTURES AND THEIR EFFICACY AGAINST *STAPHYLOCOCCUS AUREUS*

THE CYTOTOXIC EFFECTS OF CHLORHEXIDINE GLUCONATE AND A CHLOROUS ACID/CHLORINE DIOXIDE COMBINATION ON EQUINE FIBROBLAST CELL CULTURES AND THEIR EFFICACY AGAINST *STAPHYLOCOCCUS AUREUS*

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SUMMARY

Chlorhexidine gluconate and an experimental chlorous acid/chlorine dioxide combination were evaluated for cytotoxic effects on an established equine fibroblast cell culture and for antimicrobial efficacy against *Staphylococcus aureus*. The fibroblasts were exposed to dilutions ranging from 1.0% to .005% of chlorhexidine gluconate and a chlorous acid/chlorine dioxide irrigation solution and chlorous acid/chlorine dioxide disinfectant utilizing phosphate buffered saline (PBS) and sterile water for controls. The fibroblasts were incubated in each solution for thirty minutes. Cell viability or survival was determined by trypsinizing the cells, staining with trypan blue and counting live cells. All fibroblasts were killed when exposed to 1.0% and 0.5% concentrations of chlorhexidine. The survival rate of fibroblasts increased linearly with decreasing concentrations of chlorhexidine. The peak survival rate for chlorhexidine was at 0.005% with approximately 50% of the cells surviving when compared to PBS controls. The chlorous acid/chlorine dioxide irrigation solution was the least toxic to fibroblasts with equivalent survival rates to controls. The chlorous acid/chlorine dioxide disinfectant was severely cytotoxic with no cell survival even when diluting the active formulation 1:1 with PBS.

Growth of a stock culture of *S. aureus* was inhibited at

chlorhexidine concentrations of 1.0% and 0.5%, but greater dilutions had no significant effect when compared to sterile water controls. At 0.005% chlorhexidine, the *S. aureus* counts were elevated, but not significantly above those of the water controls. The chlorous acid/chlorine dioxide irrigation solution at stock concentration and diluted 1:1 with sterile water had no significant killing effect on the bacteria perhaps due to the presence of excessive brain-heart infusion (BHI) proteins. The chlorous acid/chlorine dioxide disinfectant inhibited growth of all *S. aureus*.

INTRODUCTION

Normal wound healing requires the stimulation of fibroblasts to migrate and proliferate. Wound healing depends on the presence of fibrinogen and specific enzymes released from tissue cells and blood which help convert fibrinogen to fibrin.[38] Fibrin helps stabilize the clot forming in the wound and latter provides a scaffolding for fibroblasts to migrate into the wound. There are a multitude of interactions between fibroblasts, collagen, fibronectin, and the extracellular matrix necessary for normal wound repair. Fibronectin can cross-link with itself, to fibrin, and to collagen to link the hemostatic plug to the wound margins. Collagen and fibronectin are chemotactic to neutrophils, monocyte/macrophages and fibroblasts. Debridement is initially carried out by polymorphonuclear cells and latter predominantly by the monocyte/macrophage cells. Fibroblasts migrating into the wound adhere particularly well to fibronectin cross-linked to fibrin by factor XIIIa transglutaminase which is also necessary for crosslinking fibroblast fibronectin to substrate proteins in the wound margins. Fibroblasts proliferate and begin to produce collagen leading to granulation tissue formation.[20,21]

Wounds are frequently treated topically with antiseptic solutions to reduce the incidence and severity of wound infections.[1,5,9,13,15,18] Fibroplasia can be delayed because of the ability of some antiseptic solutions to alter the configuration of acute phase proteins in the hemostatic plug and protein substrate in the wound margins.[4,10,15,20,21,25,26] Some antiseptics may nonspecifically alter cellular events within the reparative cells in the wound further inhibiting migration and proliferation.

The majority of the previous literature on antiseptics has dealt with these products in clinical trials. Unfortunately, clinical trials are frequently complicated by variables such as preexistent disease, drug side effects, or immunosuppression which may influence the response to therapy. Reports evaluating *in vitro* effects of antiseptics have utilized human and canine cell cultures.[14,19,24,34] *In vitro* studies attempt to reduce unwanted variables and evaluate the direct effects of antiseptics on cell culture. However, caution must be used in extrapolating the results to clinical situations. Validation of the results found in this form of study must come from controlled clinical research. *In vitro* studies involving antiseptics have not been performed using the equine cell culture.

The antiseptic products evaluated in this study were chlorhexidine gluconate, chlorous acid/chlorine dioxide irrigation solution and chlorous acid/chlorine dioxide

disinfectant. The purpose of this study was to evaluate the cytotoxic effects of these experimental products to equine dermal fibroblasts and also to document their antimicrobial efficacy against *Staphylococcus aureus*.

MATERIALS AND METHODS

Chlorhexidine gluconate[b] without surfactant in a 20% aqueous solution was diluted in sterile water or phosphate buffered saline to make test concentrations of 1.0%, 0.5%, 0.05%, 0.01%, and 0.005%. The chlorous acid/chlorine dioxide experimental irrigation solution was tested at the manufacturers[c] recommended ratio of one part irrigation solution base (consisting of a sodium chlorite solution of a proprietary composition) in ten parts sterile water with one part activator (consisting of a proprietary organic acid). The chlorous acid/chlorine dioxide disinfectant was tested at the manufacturers recommended ratio of one part disinfectant solution base (consisting of a sodium chlorite solution of proprietary composition) with one part activator (which contains lactic acid). For purposes of this study, the final active disinfectant solution was mixed at a 1:1 ratio with saline to evaluate cytotoxicity of this diluted sample. All solutions were mixed daily to insure fresh preparations.

An established equine dermal fibroblast cell line purchased from National Veterinary Disease Laboratory in Ames, Iowa was cultivated and utilized in this study. The cells were

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[b]Stuart Pharmaceuticals. Division of ICI Americas Inc., Wilmington, DE.

[c]Alcide Corporation, 99 Sherwood Ave., Farmingdale, NY 11735.

trypsinized and centrifuged at 1000 RPM for 10 minutes. The cells were resuspended in 10 mls of Eagle's minimal essential culture media, and then they were diluted with trypan blue and counted in a hemacytometer to adjust the cell concentration to 450,000 cells/ml. A 0.5 ml sample of the cell suspension was mixed with 0.5 ml of the PBS for control and 0.5 ml of the test solutions. After 30 minutes incubation at room temperature, the cells were centrifuged at low speed and washed with PBS. Discarding the supernatant, the cells were again washed and resuspended in Eagle's minimal essential culture media and incubated for 24 hours in 24 well culture plates at 37°C in 5% CO_2. The cells were examined under a light microscope to determine and record visible signs of cytotoxicity. The cells were then trypsinized and counts of viable cells were made in each well. Four samples of all concentrations with four saline controls were examined in this trial.

Cell viability was determined by mixing 0.05 ml of the cell suspension with 0.05 ml of 0.4% trypan blue before and 24 hours after incubation. The cell counts in four peripheral wells of a hemacytometer were calculated to give a cell concentration. The number of cells surviving were expressed as a percentage of the number of living cells in PBS control cultures.

A stock culture of *S. aureus* was used to test the antimicrobial efficacy of the different concentrations of the

experimental solutions. The *S. aureus* isolate was obtained from Colorado State University Veterinary Teaching Hospital originally as an isolate form a clinical case of septic arthritis in a horse. This particular strain was subsequently utilized at CSU in a project of experimentally induced equine infectious arthritis.

For this project, the isolate was placed on brain-heart infusion (BHI) plates and allowed to grow for 24 hours. The isolate was then placed in BHI broth and allowed to grow another 24 hours before being utilized in the trials. The bacterial suspension at this time contained approximately 10^9 organisms/ml. One ml aliquots of the suspension were mixed in duplicate one ml aliquots of sterile water to serve as a control. One ml aliquots of the bacterial suspension were mixed with duplicate test samples of each of the antiseptic concentrations. Each sample was incubated for 30 minutes and subsequently serially diluted to 10^{-6} with PBS. Each of these dilutions were plated on brain heart infusion agar utilizing a micro spot technique. Each plate was divided into three divisions. Each division represented one of the dilutions from 10^{-1} to 10^{-6}. Five 10 ul drops of each dilution were placed within each division. Each plate was examined before 24 hours and the colonies in each drop within each dilution within each division counted. The five micro drop counts of each dilution were averaged. The efficacy of each concentration in inhibiting growth of the *S. aureus* was

determined by analyzing count reduction produced by each concentration in comparison to the sterile water controls.

The effects of 5 concentrations of chlorhexidine gluconate and stock solutions of chlorous acid/chlorine dioxide irrigation solution and disinfectant were evaluated with respect to cytotoxic effects on equine fibroblasts and antimicrobial efficacy against *S. aureus*. The fibroblast study was conducted as two separate trials. Each trial was performed on two successive days with each trial containing duplicate samples. The *S. aureus* study was conducted with three trials each with duplicate samples for each concentration. This study was considered a randomized block arrangement with a modification in that the block was split further into plates and the samples were taken within these plates. An analysis of variance was used to study the difference among treatment groups. The error observed from having multiple observations within the plates was considered sampling error and was not used for testing purposes. The experimental error for testing treatment differences was taken from treatment by repetition interaction. A least significant difference analysis was used to compare the means of the bacterial counts for the different treatment groups to the mean counts in water.

RESULTS

All chlorhexidine concentrations were cytotoxic to equine dermal fibroblasts. Chlorhexidine concentrations of 1.0%-0.05% were considered lethal because they did not allow significant ($p<0.05$) fibroblast survival. Chlorhexidine concentrations of 0.04% allowed 25% survival of the dermal fibroblasts while concentrations of 0.01%-0.005% allowed survival of approximately 50% of the fibroblast cell culture (see Figure 1).

Chlorhexidine concentrations of 1.0% and 0.5% allowed no growth of the *S. aureus*. The 1.0% and 0.5% chlorhexidine samples formed a dense precipitate immediately upon mixing with the bacterial suspension. Bacterial counts for 0.05% chlorhexidine were significantly ($p<0.05$) less than the 0.01% and 0.005% concentrations. Bacterial counts for 0.05%, 0.01%, 0.005% chlorhexidine were not significantly different from the water control counts (see Figure 2).

The chlorous acid/chlorine dioxide irrigation solution was the least toxic to equine fibroblasts of all the products tested. There was increased survivability though not significantly ($p<0.05$) when compared to saline controls. The chlorous acid/chlorine dioxide disinfectant was lethal to fibroblast dermal cells with no survival. For purposes of the fibroblast study, the chlorous acid/chlorine dioxide disinfectant was diluted 1:1 with saline, and again, there

was no cell survival (see Figure 3).

The chlorous acid/chlorine dioxide irrigation solution at when used maximum strength, and diluted 1:1 with sterile water, was not effective against the *S. aureus* when compared to sterile water controls. The chlorous acid/chlorine dioxide disinfectant was effective against the bacteria with complete inhibition of growth (see Figure 4).

Five degrees of freedom among treatment groups were split in such a way that comparisons of the bacterial counts between chlorous acid/chlorine dioxide irrigation solution and water were performed separately from a comparison of the linear trend among the bacterial counts for the different concentrations of chlorhexidine. The near significant value ($p < 0.05$) for lack of fit suggests there appears to be more to the concentration relationship than a simple linear trend for chlorhexidine. The linear trend study suggested that the decreasing concentrations had a predictable decrease in effective bacterial kill. The near significant p value for lack of fit suggests that at the most dilute concentration of 0.005% there may be an unexpected increase in growth of bacteria above water controls.

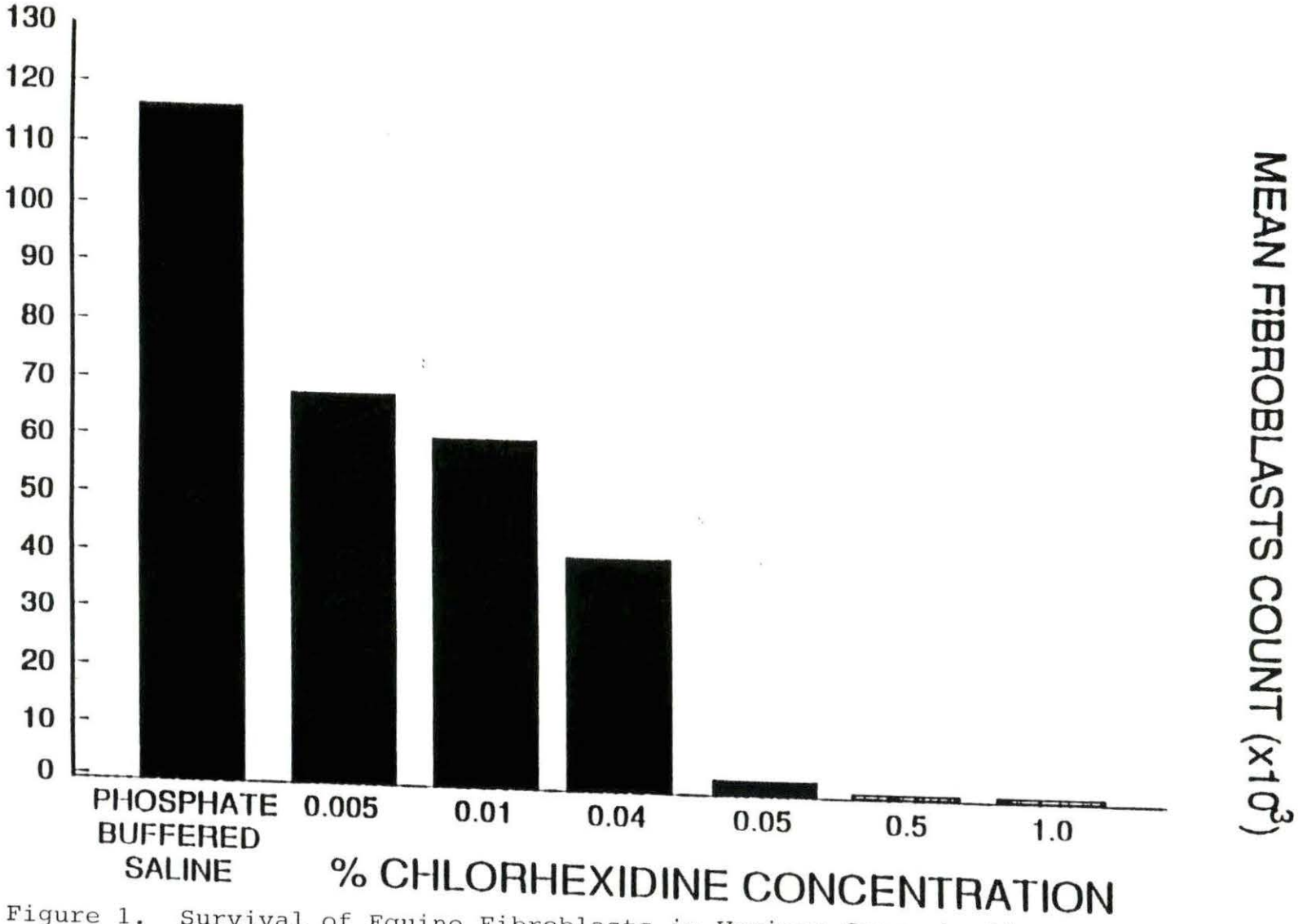

Figure 1. Survival of Equine Fibroblasts in Various Concentrations of Chlorhexidine

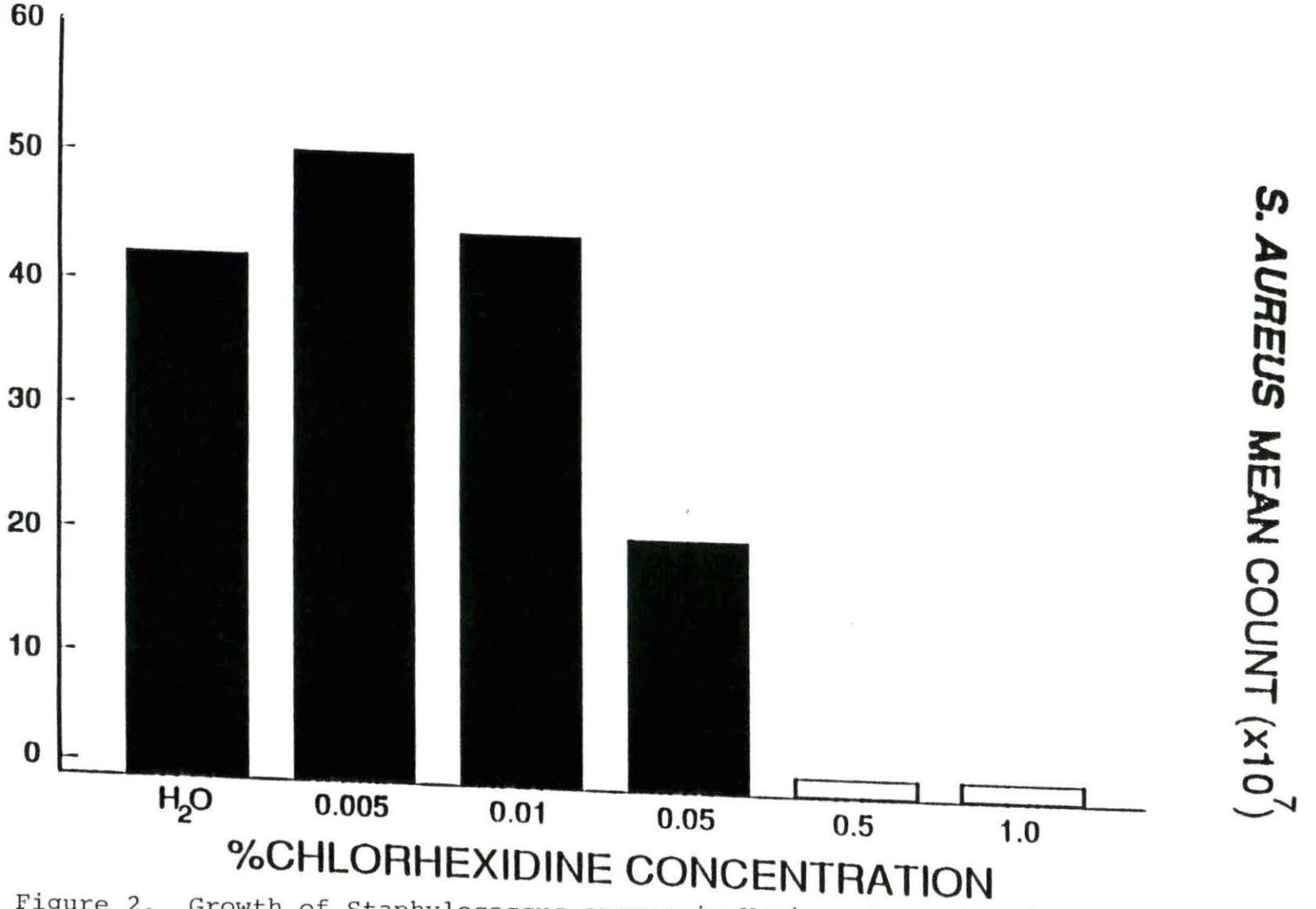

Figure 2. Growth of Staphylococcus aureus in Various Concentrations of Chlorhexidine

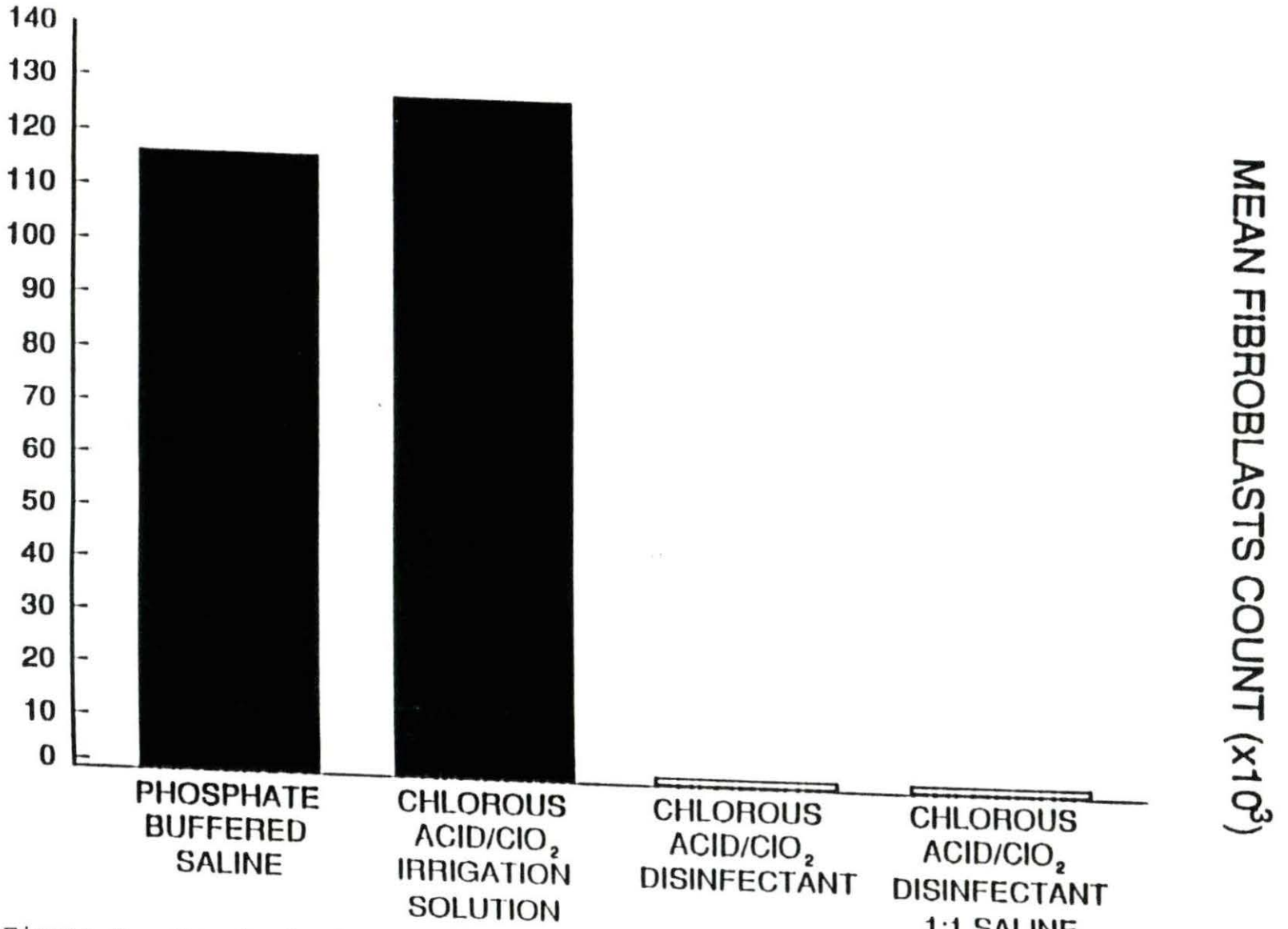

Figure 3. Survival of Equine Fibroblasts in Chlorous acid/chlorine dioxide Irrigation Solution and Disinfectant

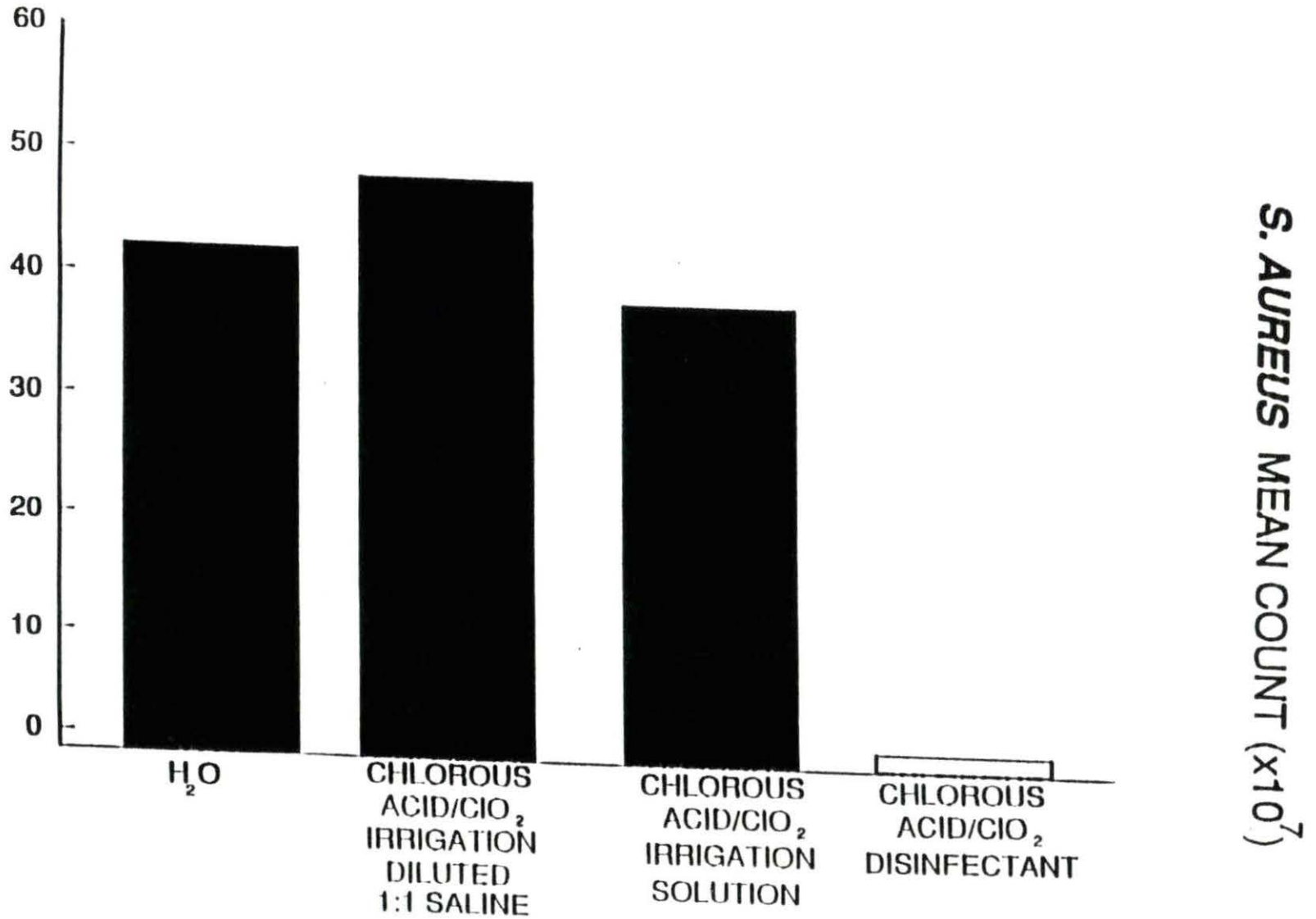

Figure 4. Growth of Staphylococcus aureus in Chlorous acid/chlorine dioxide Irrigation Solution and Disinfectant

DISCUSSION

Chlorhexidine is reported to have a persistent antimicrobial effect and a rapid bactericidal action against a wide variety of gram positive and gram negative microorganisms.[8,11,16,34,36,37] A reduced incidence of hypersensitivity reactions, and a decreased incidence of bacterial resistance make it advantageous to use this product over local antibiotics. Chlorhexidine preparations come as gluconate, acetate and hydrochloride salts. Chlorhexidine diacetate[d] is marketed as a 2.0% solution with disinfectant, bactericidal and virucidal properties. This solution requires the addition of a surfactant (triton X100) to increase the solubility of the chlorhexidine acetate. Chlorhexidine gluconate is a hexamethylenebis biguanide compound with broad spectrum bactericidal activity. Chlorhexidine gluconate was chosen for this study because it lacks alcohol, surfactants or detergents that are present in other commercial preparations; and thereby eliminates these factors as causes for any inflammatory response seen clinically.

There is some question as to the effect organic material such as blood and peritoneal fluid may have on chlorhexidine products. Chlorhexidine is known to bind with electrolytes;

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[d]Nolvasan Solution, Fort Dodge Laboratories, Inc., Fort Dodge, Iowa 50501.

and therefore precipitates from saline and balanced electrolyte solutions.[3,30] An *in vitro* study suggested that protein binding to chlorhexidine reduced its effectiveness in killing animal cells.[17] In the present study, the 1.0% and 0.5% chlorhexidine concentrations produced a dense white precipitate upon mixing with the bacterial suspension. Protein present in the BHI broth may be forming complexes with the chlorhexidine. At these increased concentrations, sufficient active chlorhexidine may remain after precipitation to allow significant bacterial kill. In contrast, the fibroblast cell culture had only nutrient media in the wells; and the cells were incubated in the various chlorhexidine concentrations. With less electrolyte and protein concentrations in the nutrient media to precipitate the chlorhexidine, a more direct effect of the antiseptic may be seen.

Chlorhexidine is believed to alter the permeability of bacterial cells by damaging the cell wall, which may make it synergistic with some systemically administered antibiotics.[3,16,40] Of particular interest to this study is the specific effects chlorhexidine gluconate has against *Staphylococcus aureus*. *S. aureus* is considered one of the least susceptible bacteria to antiseptics.[1] Drug resistance studies have shown a stock culture of *S. aureus* did not develop resistance to chlorhexidine.[38] Other strains of *S. aureus* and various other bacteria have been reported to

develop resistance to chlorhexidine gluconate.[28] In this study, the effective kill of chlorhexidine gluconate dropped dramatically at 0.05% concentration with counts not significantly ($p<0.05$) below water controls. Sanchez et al. in an *in vitro* study utilizing chlorhexidine diacetate showed no significant growth of *S. aureus* at 0.05%.[34] Efficacy was reported as percent survivability in comparison to saline controls. In contrast, the present study reported the actual counts of the bacteria and compared these counts to sterile water controls. It may be possible that the surfactant present in chlorhexidine diacetate, by improving solubility, also added to the efficacy against *S. aureus;* or it may act directly as an antimicrobial agent. Direct comparisons between these projects are difficult to make as the stock cultures for both projects were different strains of *S. aureus*. In addition, the controls were different.

Two clinical studies in the dog determined that 0.05% and 0.5% chlorhexidine diacetate solution had minimal effects on the healing of surgically created wounds.[1,35] One of these studies inoculated wounds with *S. aureus* and found chlorhexidine diacetate to be very effective in reducing the incidence of infection.[1] The other study irrigated wounds with chlorhexidine and found that wound healing was more rapid than saline irrigation at both 0.5% and 0.05%. An unrelated study in the dog showed significantly less histological evidence of inflammation in the face of high

bacterial inoculation doses with surgically placed prosthesis material in chlorhexidine diacetate (0.5%) treated limbs compared with saline controls.[40] These clinical studies seem to contradict the *in vitro* study showing the 0.5% and 0.05% concentrations to be lethal to canine fibroblasts.[34] Regardless of the direct effects chlorhexidine has on cell culture, there seems to be a generous amount of evidence supporting the clinical use of 0.5% and 0.05% chlorhexidine.[1,6,7,33,34,40]

Elemental chlorine and the hypochlorites have been used for centuries but have the disadvantage of being unstable, and therefore require preparation before each use. These chlorous acid/chlorine dioxide products used in this study are composed of two component systems that require mixing to activate. When mixed, the two components, sodium chlorite and an organic acid, combine to form an oxidative complex. This complex is purported to maintain a controlled release of active chlorine. The manufacturer has several formulations of potential interest to the veterinary market. The initial product utilized in this study is an irrigation solution designed to be used as a contact lens soaking solution. Application of this product in the veterinary field may be as a lavage solution. The other product tested is a new Environmental Protection Agency registered disinfectant which has demonstrated superior broad spectrum microbicidal effectiveness.

The bactericidal activity of chlorine products can be influenced by a variety of factors. Because it is a highly reactive element it can be easily bound by organic matter. This may be why the chlorous acid/chlorine dioxide irrigation solutions were not efficacious against *S. aureus*. The BHI broth probably contains significant amounts of organic material to effectively neutralize the active chlorine in the irrigation solution. The disinfectant, with significantly higher concentrations of active complexes than the irrigation solution demonstrated excellent efficacy against *S. aureus*.

These formulations are experimental and have yet to be thoroughly tested in animal models. Previous research in incised wounds in the guinea pig and the mouse showed chlorous acid/chlorine dioxide treated wounds to have less scar tissue formation than controls with little indication of antimicrobial toxicity histologically.[20,21] An experimental chlorous acid/chlorine dioxide teat dip was clinically tested and shown to be effective in preventing new intramammary infections with *S. aureus*.[27] The present study demonstrated the chlorous acid/chlorine dioxide irrigation solution caused minimal damage to equine dermal fibroblasts; and therefore it can be considered a nontoxic, nonirritating lavage solution. Unfortunately, the lack of efficacy against *S. aureus* is of major concern in that grossly contaminated and effusive wounds will probably require additional antimicrobials. It may be possible that volume flushing will reduce the

bacterial counts and organic material present in the wound sufficiently to allow enough active chlorine to be present in the wound to be effective as an antiseptic. Because the irrigation solution is unstable, the effects of the chlorous acid/chlorine dioxide may be short lived and require repeated application or lavage on a daily basis.

Wounds and septic joint fluid can contain high concentrations of protein, which may bind the chlorhexidine and chlorous acid/chlorine dioxide irrigation solution present in the lavage solutions tested. This may partly account for the fact that concentrations of 0.5%-0.05% chlorhexidine proven to be detrimental to fibroblasts are effectively utilized in clinical models of wound healing. The chlorous acid/chlorine dioxide disinfectant proved equivalent to 1.0% and 0.5% chlorhexidine in both cytotoxicity to fibroblasts and antimicrobial efficacy against *S. aureus*. All solutions should be evaluated in clinical models of wound healing to better define effectiveness *in vivo*.

There are several problems associated with the *in vitro* evaluations in this study. The increased protein in the BHI broth could be allowing the bacteria ample media to continue to proliferate. This may not be the most ideal environment to evaluate kill of *S. aureus*. The direct effects of these antiseptics may best be evaluated by removing the bacteria, washing them in saline and incubating them directly in the

concentrations utilized in this study.

Cell cultures have strict media requirements and conditions to allow survival of cells. While this study utilized an established line of equine dermal fibroblasts, viability studies show increased death outside the minimal essential media. This media contains essential amino acids, electrolytes and vitamins. Simply diluting this nutrient media with saline to establish controls for this study brought cell counts down to a mean of 116,250 viable cells/ml when the initial sample contained 450,000 cells/ml. Adding potentially irritating antiseptics should expectedly decrease survival rates below controls. This is particularly true of chlorhexidine, which precipitates with electrolytes and binds to protein critical to cell survival. Elucidation of further direct effects these antiseptics have on metabolism and cellular interaction may give insight into these *in vitro* effects not seen clinically.

REFERENCES

1. Amber EI, Henderson RA, Swaim SF, Gray BW. A Comparison of antimicrobial efficacy and tissue reaction of four antiseptics on canine wounds. Vet. Surg., 12, No. 2, 63-68, 1983.

2. Bassetti C, Kallenberger A. Influence of chlorhexidine rinsing on wound healing of oral mucosa and osseous lesions. J. Clin. Periodontal., 7: 443-456, 1980.

3. Bertone AL, McIlwraith CW, Powers BW, Radin MJ. Effect of four antimicrobial lavage solutions on the tarsocrural joint of horses. Vet. Surg., 15, No. 4, 305-315, July-August 1986.

4. Branemark PI, Ekholm R, Albrektsson B, et al. Tissue injury caused by wound disinfectants. J. Bone Joint Surg. (Am), 49: 48-62; 1967.

5. Brown G. Infectious arthritis and wounds of open joints. Vet. Clin. of N. Am., 8(3): 501-510, 1978.

6. Buckle RC, Seabridge CE. The effect of chlorhexidine in the peritoneal cavity. Lancet, 1: 193-5; 1963.

7. Crossfill M, Hall D, London D. The use of chlorhexidine antiseptics in contaminated surgical wounds. Br. J. Surg., 56, 906; 1969.

8. Drug Information for the Health Care Professional. USP/DI. The United States Pharmacopoeial Convention, Inc., Vol 1A, 901; 1990.

9. Edlich RF, Custer J, Madden J, et al. Studies in the management of contaminated wounds III; An assessment of the effectiveness of irrigation with antiseptic agents. Am. J. Surg., 15, 118-21; 1969.

10. Edlich RF, Smolka IR, Prusak MP, Edgerton MT. The molecular basis for toxicity of surfactants in surgical wounds. J. Surg. Res., 14; 277; 1973.

11. Facts and Comparisons. Chlorhexidine gluconate. St. Louis: J. B. Lippincott Co.; Jan. 1985.

12. Faddis D, Daniels D, Boyer J. Tissue toxicity of antiseptic solutions: a study of rabbits articular and periarticular tissues. J. Trauma, 7: 895; 1977.

13. Gilmore OJA. A reappraisal of the use of antiseptics in surgical practice. Ann. R. Coll. Surg. Engl., 59: 93-103; 1977.

14. Goldschmidt P, Cogen R, Taubman S. Cytopathological effects of chlorhexidine on human cells. J. Periodotol., 48: 212-15; 1977.

15. Gruber RP, Vistines L, Pardoe R. The effect of commonly used antiseptics on wound healing. Plast. Reconstr. Surg., 55: 472; 1977.

16. Harvey SC. Antimicrobial drugs. Antiseptics and disinfectants. In; Osol A, ed. Remington's pharmaceutical sciences. 16th ed. Easton, Pa: Mack Publishing Co.; 1980.

17. Helgeland K, Heyden G, Rolla G. Effect of chlorhexidine on animal cells *in vitro*. Scand. J. Dent. Res., 79: 209-215;

1971.

18. Hohn DC, Granelli SG, Burton RW, Hunt TK. Antimicrobial systems of the surgical wound. II: Detection of antimicrobial protein in cell free wound fluid. Am. J. Surg., 133, 601-06; 1977.

19. Kenny EB, Saxe SR, Bowles RD. Effects of chlorhexidine on human polymorphonuclear leucocytes. Arch. Oral Biol., 17: 1633-36; 1972.

20. Kenyon AJ, Douglas DM, Hamilton S. The effects of antimicrobials on collagen synthesis in wound repair in mice. J. Surg. Res., 12: 1004; 1980.

21. Kenyon AJ, Hamilton SG, Douglas DM. Controlled wound repair in guinea pigs, using antimicrobials that alter fibroplasia. Am. J. Vet. Res., 47, No. 1: 96-101; Jan. 1986.

22. Lawernce CA. Antimicrobial activity, *in vitro*, of chlorhexidine. Lancet, 1: 18-22; 1957.

23. Lennette EH, Balows A, Hausler WJ, eds. Manual of Clinical Microbiology. Washington DC: American Society for Microbiology; 1980.

24. Lineaweaver W, McMorris S, Howard R. Effects of topical disinfectants and antibiotics on human fibroblasts. Surg. Forum, 33: 37-39; 1982.

25. Lineaweaver W, Howard R, Soucy D, et al. Topical antimicrobial toxicity. Arch. Surg., 120: 267-70; 1985.

26. Michaels EB, Hahn EC, Kenyon AJ. Effect of C31G, an antimicrobial surfactant, on healing of incised guinea pig

wounds. Am. J. Vet. Res., 44, No. 7: 1378-81; July 1983.

27. Oliver SP, King SH, Torre PM, et al. Prevention of bovine mastitis by a postmilking teat disinfectant containing chlorous acid and chlorine dioxide in a soluble polymer gel. J. Dairy Science, 72: 3091-97; 1989.

28. Platt J, Bucknall RA. An experimental evaluation of antiseptic wound irrigation. J. Hosp. Infect., 5: 181-188; 1984.

29. Prince HN, Nonemaker WS, Norgard RC, Prince DL. Drug resistance studies with topical antiseptics. J. Pharm. Sci., 67: 1629-31; 1978.

30. Reynolds EF (ed.), Prasad AB (Asst ed), Martindale. The extra pharmacopeia. London: Pharmaceutical Press; 1982.

31. Rodeheaver G, Smith S, Thacker J, Edgerton MT, Edlich RF. Mechanical cleansing of contaminated wounds with surfactant. Am. J. Surg., 129: 241-45; 1975.

32. Rosenburg A, Alatary SD, Peterson AF. Safety and efficacy of chlorhexidine gluconate. Surg. Gynecol. Obstet., 143: 789-92; 1976.

33. Saatman RA, Carlton WW, Hubben K, et al. A wound healing study of chlorhexidine digluconate in guinea pigs. Fund. and Appl. Toxicol., 6, 1-6; 1986.

34. Sanchez IR, Nusbaum KE, Swaim SF, et al. Chlorhexidine diacetate and povidone-iodine cytotoxicity to canine embryonic fibroblast and *Staphylococcus aureus*. Vet. Surg., 17, No. 4: 182-85, July-August 1988.

35. Sanchez IR, Swain SF, Nusbaum KE, et al. The effects of chlorhexidine diacetate and povidone-iodine on wound healing in the dog. Vet. Surg., 17, No. 6: 291-95; Nov.-Dec. 1988.

36. Sapeika N, ed. Antiseptics. Actions and uses of drugs. Capetown, South Africa: A. A. Balkam Publishing Co.; 1976.

37. Steward CH. Antiseptics and disinfectants. In: Goodman LS, Gilman AG, eds. Pharmacological basis of therapeutics. 6th ed. New York: Macmillan Pub., 964; 1980.

38. Swaim SF. *Surgery of Traumatized Skin.* Philadelphia: WB Saunders; 1980.

39. Symen DG, Nathan P, Holder IA, et al. Infected surface wound. An experimental model and method for the quantification of bacteria in infected tissues. Appl. Micro. Biol., 23: 509-14; 1972.

40. Wendelburg K, Smeak DD, Kowalski JJ. Chlorhexidine wound lavage and suture soaking for prevention of suture related infections. Vet. Compar. Orthop. and Traumatology, 3: 122-29; 1988.

PART II.

THE HISTOPATHOLOGICAL EFFECTS OF INTRAARTICULAR AMIKACIN SULFATE, .05% CHLORHEXIDINE GLUCONATE AND CHLOROUS ACID/CHLORINE DIOXIDE IRRIGATION SOLUTION ON RABBIT STIFLE JOINTS

THE HISTOPATHOLOGICAL EFFECTS OF INTRAARTICULAR AMIKACIN SULFATE, .05% CHLORHEXIDINE GLUCONATE AND CHLOROUS ACID/CHLORINE DIOXIDE IRRIGATION SOLUTION ON RABBIT STIFLE JOINTS

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SUMMARY

This study was designed to evaluate histopathological effects of intraarticular amikacin, 0.05% chlorhexidine gluconate solution and a chlorous acid/chlorine dioxide compound in the rabbit stifle joint. Two mls of each product were injected into the suprapatellar pouch of one stifle joint, while the contralateral joint was injected with the carrier solution to serve as a control. A second injection utilizing the same protocol was given 48 hours later. The animals showed no lameness post injection or at any time throughout the study. The joints were evaluated for effusion by digital palpation at 1, 2, 5, and 10 days; for the first group and 1, 2, 5, 10 and 21 days for the second group following the first injection without any noticeable abnormality. The rabbits were killed at 10 and 21 days after the first injection for each group respectively.

Gross changes were not evident at necropsy within either 10 day or 21 day groups. Histopathological changes were evaluated by sampling the suprapatellar synovial membrane and cartilage of the medial femoral condyle. Hematoxylin and eosin stain was utilized for both sampling areas to interpret cellular inflammatory changes. Histochemical analysis by use of a safranin-O-red fast stain was utilized on the cartilage sections to determine the extent of proteoglycan depletion associated with injection of the substances into the joints.

the joints. Inflammatory changes were minimal and not significantly different between treatment groups. Histochemical analysis showed little proteoglycan depletion among all the treatment groups. There again was no significant difference between groups. These products appear to be safe when injected into the normal stifle joints of rabbits. Efficacy in the face of infection needs to be evaluated.

INTRODUCTION

Septic arthritis and wounds involving joints are frequently volume flushed with an irrigation solution. The solution typically used is a balanced electrolyte solution. These solutions, when introduced into the wound or joint under pressure, can effectively remove debris and bacteria by mechanical means. Having no antibacterial properties, these solutions provide their major benefit in volume dilution. Wounds in which gross contamination or overwhelming bacterial colonization of the synovium occur may benefit by local antimicrobial therapy in the form of an antiseptic added to the irrigation solution, or direct intraarticular injection of an antibiotic. Solutions used for irrigation should be nonirritating, have a neutral pH, and be isotonic.[31] Many antiseptics, while not directly irritating, come as formulations which contain concentrations of alcohols, detergents or surfactants which can be extremely irritating to the host's tissues.[7,12,29,30] Solutions with surfactants and detergents that create intense inflammatory responses and/or damage reparative cells in the wound should be avoided.

The products evaluated in this study were amikacin sulfate, 0.05% chlorhexidine gluconate and a chlorous acid/ chlorine dioxide irrigation solution. Each solution was injected intraarticularly to evaluate the effects these products have on synovial cells, chondrocytes and cartilage matrix of the

rabbit stifle joint.

MATERIALS AND METHODS

Tissue Toxicity of Amikacin Sulfate, Chlorhexidine Gluconate and Chlorous Acid/Chlorine Dioxide Irrigation Solution

Twenty-four New Zealand white rabbits of similar weight and housed in the same environment were used in this study. A 0.05% chlorhexidine gluconate solution[f] and chlorous acid/chlorine dioxide irrigation solutions[g] were prepared the day of injection utilizing sterile water. The amikacin solution was prepared by diluting 25 mg (4 mg/kg) amikacin sulfate[h] in balanced electrolyte solution[i] (BES) to a final volume of 2 mls. The rabbits were randomly assigned to one of three groups with eight per group. After aseptic preparation, each rabbit was injected with two mls of the appropriate solutions in the suprapatellar pouch of the stifle joints. The first group received the amikacin in BES in one stifle joint and the equivalent volume of BES without

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[f]Stuart Pharmaceuticals, Division of ICI Americas Inc., Wilmington, DE.

[g]Alcide Corporation, 99 Sherwood Ave., Farmingdale, NY 11735.

[h]Amiglyde V, Fort Dodge Laboratories, Inc., Fort Dodge, Iowa 50501.

[i]Lactated Ringer's Injection USP. Baxter Healthcare Corporation, Deerfield, Il 60015.

amikacin in the contralateral joint to serve as a control. The second group received 2 mls of a 0.05% chlorhexidine solution in one stifle joint and the equivalent volume of sterile water in the contralateral joint. The third group received 2 mls of the chlorous acid/chlorine dioxide irrigation solution and the contralateral joint received sterile water. All injections were repeated 24 hours later. The rabbits were examined for periarticular enlargement, pain on palpation and flexion of the stifle and clinical lameness on days 1, 2, 5 and 10 for the first four rabbits within each treatment group and days 1, 2, 5, 10 and 21 for the second four rabbits within the same treatment group. Attempts were made to collect synovial fluid samples both before injection and at 24 hours after the first injection. The attempts were inconsistent among all treatment groups; and therefore eliminated from the protocol. Four rabbits from each treatment group were sacrificed on day 10 using intravenous pentobarbital. The remaining rabbits from each group were sacrificed at day 21.

At necropsy, each stifle was examined for gross changes associated with the synovium and cartilage of the joint. Samples of the suprapatellar synovial membrane were collected from the proximal aspect of the suprapatellar pouch and fixed in 10% neutral buffered formalin. The medial condyle was harvested for histological and histochemical analysis. The samples were fixed in 10% neutral buffered formalin. The

medial condyle was then decalcified and sagittal sections cut to allow staining with hematoxylin and eosin (H&E) and safranin-O-red fast, an orthochromatic stain which selectively stains mucopolysaccharides. Treated and control sections were stained simultaneously to reduce differences due to staining technique. Sections were coded to eliminate bias.

Histological evaluation of the hematoxylin and eosin stained synovial membrane samples were evaluated based on 1) proliferation of synovial cells and villi, 2) presence and location of fibrin, 3) number and location of mononuclear cells, 4) number and location of neutrophils and 5) an overall score as to grade of inflammation. These parameters were scored by the authors from 0 (no inflammation present) to +++ (severe inflammation). The inflammatory response within the synovial membrane was compared between treated and control joints. Inflammation was considered significant if a ++ difference existed between the treated and control joints.

Histochemical evaluation of intercellular matrix was determined by utilizing safranin-O-red fast stain. Lack of staining in the superficial zone of articular cartilage was considered normal. The intensity of staining was graded from 0 (absent) to +++ for the intermediate and radiate zones of the articular cartilage. Within these zones the levels were further divided into upper and lower sections.

RESULTS

Clinical Assessment

All rabbits survived the study without obvious problems. Five out of eight rabbits injected with intraarticular amikacin reacted with sharp withdrawal of the limb indicating a pain response to the medication. This reaction was not present with injection of the other products. The amikacin injected rabbits showed no obvious lameness at any point in the study but three of five rabbits which responded to the intraarticular injection developed mild effusion within the joint after the last injection of amikacin. This effusion had resolved by the time the first group was necropsied at ten days. The limbs of the other rabbit groups showed no dramatic change throughout the study.

Necropsy

Gross changes were not evident in any of the stifles examined for either 10 day or 21 day groups. The joints from the amikacin treated groups when effusion was present were not grossly different from the controls or the other treatment groups necropsied. The synovial membrane samples appeared normal from treatment group to treatment group. The articular cartilage in all treatment groups appeared normal.

Histopathology

H&E sections of the synovial samples showed very little cellular infiltrates in either the acute or chronic samples. Two rabbits, one from the chlorhexidine 10 day group and one from the amikacin 21 day group, had a ++ inflammatory response but this change was consistent in both treated and control joints. The H&E sections of the medial condyle showed very mild inflammatory responses associated with all treatment groups. There was little change evident in the cartilage sections evaluated with either H&E or Safranin-O-red fast.

DISCUSSION

Joint lavage is frequently combined with broad spectrum bactericidal antibiotics in the treatment of septic arthritis. Irrigation with a balanced electrolyte solution is more effective than simple aspiration in removing inflammatory cells, protein, enzymes and fibrin from the joint.[2,14,24,25,36,38] Antibiotics, however, have decreased ability to penetrate fibrin and inflammatory lesions within joints.[2,11] There also seems to be a reduced antibiotic activity in the presence of high concentrations of bacteria termed the "inoculum effect".[2,11] Removal of the inflammatory products and bacteria within the joint is, therefore, critical to achieve maximum effect of systemically administered antibiotics.

Antiseptics and/or antibiotics are frequently added to the lavage solutions to enhance their effect. Differentiation between the inflammatory reaction created by the primary disease and that created by intraarticular therapy can be difficult. For this reason, some form of controlled research is necessary to evaluate the inflammatory response created by lavage solutions or intraarticular antibiotics in the normal joint. This study looked at 0.05% chlorhexidine gluconate, chlorous acid/chlorine dioxide irrigation solution, and amikacin sulfate as an intraarticular therapy.

Lavage solutions should be nontoxic and have a normal pH,

temperature, and osmolality. Irrigation pressure should be minimal to reduce the inflammatory response.[3] Temperature and pressure remained constant between treatment groups in this project. The pH and osmolality were quite variable between groups and may explain some of the differences in response to injection. All solutions were acidic with 0.05% chlorhexidine, chlorous acid/chlorine dioxide and amikacin in BES having mean pH values of 6.6, 4.6 and 4.7 respectively. The osmolality was even more variable between treatments in that 0.05% chlorhexidine gluconate is hypoosmolar at 1-2 mOsm/kg, the chlorous acid/chlorine dioxide solution was closer to normal at 275 mOsm/kg and the amikacin solution was hyperosmolar at 464 mOsm/kg. The hyperosmolarity of the amikacin solution may have created the irritation evident on intraarticular injection. Regardless of these factors, these products induced minimal inflammatory changes when injected into normal stifle joints.

McIlwraith at one time believed that injection of acidic solutions into a joint can create a chemical synovitis.[25] Stover and Pool showed that intraarticular injection of gentamicin (mean pH = 3.2) created minimal inflammation and suggested that factors other than pH may be involved in inducing synovitis.[36] Our study is in agreement with Stover and Pool in that all solutions, while acidic and with variable osmolality, produced no obvious histopathological inflammation. The fact that the injection of amikacin

created an instantaneous withdrawal reflex suggested joint irritation. This was short lived and may be explained by the fact that the drug has a high osmolality. The sodium bisulfite or benzethonium chloride preservative present in the solution might also be irritating to the synovial membrane. Fluid palpable as effusion may have subsequently been drawn from the interstitium into the joint by the hyperosmotic solution creating the effusion that was palpated.

Chlorhexidine comes as acetate, gluconate and hydrochloride salts. The salts have different solubilities of which the gluconate is the most soluble. The chlorhexidine veterinary product most commonly used is a diacetate form which has a surfactant added. Surfactants are frequently added to increase the solubility of the antiseptic or to increase the antimicrobial effect. Surfactants can injure tissues by: 1) occluding the microcirculation leading to tissue necrosis (cationic surfactant); 2) prolonging the inflammatory response (anionic surfactant); 3) and inhibiting collagen cross-linking (penicillin).[16,17]

A previous study demonstrated that 0.05% chlorhexidine diacetate caused a severe synovitis when used as a lavage solution in the tarsocrural joint of the horse.[3] The authors speculated that the addition of the surfactant (triton X100) may have contributed significantly to the synovitis. In our study chlorhexidine gluconate was used at a concentration of

0.05%. This product was chosen primarily because of the lack of a surfactant. The lack of an obvious inflammatory response with chlorhexidine gluconate in this study may be due to the reduction in concentration of chlorhexidine, the lack of surfactant, or both.

Considerable controversy exists regarding the incorporation of intraarticular antibiotics as a means to supplement systemic therapy. This technique is utilized with the intent of achieving high levels of antibiotics in the joint without creating systemic toxicity from parenteral administration.

Aminoglycosides are frequently utilized systemically in septic arthritis, primarily for their activity against gram negative aerobes. Previous reports have shown that intramuscular injection of amikacin quickly reached bactericidal levels in the normal rabbit stifle.[33] In the previously cited study, the doses ranged from 0.8 mg/kg to 8 mg/kg with the intermediate dose of 4 mg/kg proving to be efficacious. This same study implied that the aminoglycosides are readily inactivated at a pH less than 7. In people, a 13 fold increase in the minimum inhibitory concentration (MIC) of gentamicin for *E. coli* has been reported as the pH of synovial fluid decreased from 7 to 6.[36] Because infected joints are generally acidic, the question becomes whether parenterally administered aminoglycosides will be effective. In a previous study utilizing rabbits with a *S. aureus* arthritis, intraarticular kanamycin alone

was an efficacious treatment.[10]

The products tested in our study showed no obvious inflammation when injected intraarticularly in the normal rabbit stifle joint. This is surprising in the case of chlorhexidine because it produced severe cytotoxic effects on equine dermal fibroblasts in a previous *in vitro* study. Clinically, the 0.05% concentration appears to be a nontoxic and minimally inflammatory product to use in the rabbit stifle.

Previous reports suggested aminoglycosides were too acidic to be used intraarticularly, and some clinicians buffered the drugs with sodium bicarbonate before intraarticular injection.[25,26] The amikacin and BES combination, while moderately acidic, apparently had little effect on the synovium. This product can be administered in conjunction with or following a lavage solution and should not need to be buffered. Efficacy in the face of clinical septic arthritis needs to be evaluated.

The chlorous acid/chlorine dioxide irrigation solution proved to be nonirritating. This product created no obvious inflammation in the rabbit joints. The previous report on cytotoxicity to equine fibroblasts showed equivalent survivability to saline controls.

Safranin-O-red fast stain was utilized in this study to evaluate glycosaminoglycan and proteoglycan depletion. Many of the histological samples had quite variable uptake of the

stain by the articular cartilage of the medial condyle. It was felt that the weight bearing surfaces should be the primary area of interest because it appeared to be the area that stained more uniformly. Hexosamine quantification may be a more accurate means of evaluating depletion of these substances in the minimally inflammed joints than the safranin stains.

REFERENCES

1. Bayer AS. Nongonnococcal bacterial septic arthritis, Postgraduate Medicine; 67, No. 2, 1001-13. Feb. 1980.

2. Bertone AL, McIlwraith CW, Jones RL, Norridin RW, Radin J, Lebel L. Comparison of various treatments for experimentally induced equine infectious arthritis. Am. J. Vet. Res., 48, No. 3, 519-29; March 1987.

3. Bertone AL, McIlwraith CW, Powers BW, Radin MJ. Effect of four antimicrobial lavage solutions on the tarsocrural joint of horses. Vet. Surg., 15, No. 4, 305-315, July-August 1986.

4. Bhawan J, Roy S. Pathology of experimental pyogenic arthritis. Indian J. Med. Res., 61: 1603-4; 1973.

5. Bobechko WP, Mandell L. Immunology of cartilage in septic arthritis. Clin. Ortho. Related Res., No. 108, 84-89; 1975.

6. Buckle RC, Seabridge CE. The effect of chlorhexidine in the peritoneal cavity. Lancet, 1: 193-5; 1963.

7. Curtiss PH. The pathophysiology of joint infections. Clin. Ortho. Related Res., 96, 129-35; Oct. 1973.

8. Daniel O, Akeson W, Amiel D, et al. Lavage of septic joints in rabbits: effects on chondrolysis. J. Bone Joint Surg., 58A: 393-5; 1976.

9. Faddis D, Daniels D, Boyer J. Tissue toxicity of

antiseptic solutions: A study of rabbits articular and periarticular tissues. J. Trauma, 7: 895; 1977.

10. Frankel CJ, Spear CV, Hicks JR, et al. The treatment of penicillin resistant *Staphylococcus aureus* joint infections with intraarticular broad spectrum antibiotics. Surg. Forum, 11: 453; 1960.

11. Fry DE, Garrison RN, Trachtenberg L, Polk HC. Bacterial inoculum and the activity of antimicrobial agents. Surgery Gynec. Obstet., 160: 105; 1985.

12. Gershuni DH, Steven CK. Articular deformation following experimental synovitis in the rabbit hip. J. Ortho. Res., 1: 313-18; 1984.

13. Gobelet C, Gerster JC. Synovial fluid lactate levels in septic and nonseptic arthritides. Annals Rheum. Dis., 43: 742-45; 1984.

14. Jackson RW, Parsons CJ. Distention irrigation treatment of major joint sepsis. Clin. Ortho. Relat. Res., No. 96: 160-64; Oct. 1973.

15. Johnson AH, Campbell WG, Callahan BC. Infection of rabbit knee joints after intraarticular injection of *Staphylococcus aureus*. Am. J. Pathol., 60: 165-77; 1970.

16. Kapral FA, Li IW. Virulence and coagulases of *Staphylococcus aureus*. Proc. Soc. Exp. Biol. Med., 104: 1551-53; 1960.

17. Kenyon AJ, Douglas DM, Hamilton S. The effects of antimicrobials on collagen synthesis in wound repair in mice.

J. Surg. Res., 12: 1004; 1980.

18. Kenyon AJ, Hamilton SG, Douglas DM. Controlled wound repair in guinea pigs, using antimicrobials that alter fibroplasia. Am. J. Vet. Res., 47, 1011, No 1; Jan. 1986.

19. Kim HC, Klein S, Hirsch JR et al. Arthroscopic synovectomy in the treatment of hemophilic synovitis. Scand. J. Haematol., Suppl. 40, 33: 271-79; 1984.

20. Koenig MG, Melly MA, Rodgers DE. Factors relating to the virulence of staphylococci. J. Exp. Med., 116: 601-610; 1962.

21. Kushner JD, Somerville JA. Permeability of human synovial membrane to plasma proteins. Arth. Rheum., 4: 55-60; 1971.

22. Leitch M. Diagnosis and treatment of septic arthritis in the horse. J. Am. Vet. Med. Assoc., 175: 701-04; 1979.

23. Lipponen Tulamo R, Gabel AA. Clinicopathologic evaluation of the pathophysiology of infectious arthritis in the horse. Master's thesis. The Ohio State Univ., Columbus, Ohio, 1981.

24. Lyons C. Antibacterial immunity to *Staphylococcus pyogenes*. Br. J. Exp. Pathol., 41-42; 1937.

25. McIlwraith CW. Treatment of infectious arthritis. Vet. Clin. North Am.(Large Animal Pract.), 5: 363-79; 1983.

26. McIlwraith CW. Antibiotic use in musculoskeletal disease. Proc. 32nd Am. Assoc. Eq. Pract., 241-47; 1986.

27. Mitchell NS, Cruess RL. The effect of synovectomy on

articular cartilage. J. Bone and Joint Surg., 49A, No. 6: 1099-108; Sept. 1987.

28. Nelson JD. Antibiotic concentrations in septic joint effusions. N. Eng. J. Med., 28, No. 7: 349-58; Feb. 1971.

29. Rhodes KH. Antibiotic management of osteomyelitis and septic arthritis in children. Ortho. Clin. N. Am. (Symp. Infections in Orthopedics), 6, No. 4; 915-21; Oct. 1975.

30. Rosenberg L. Chemical basis for the histological use of safranin O in the study of articular cartilage. J. Bone and Joint Surg., 53A, No. 1, 69-82; Jan. 1971.

31. Ruedy J. Antibiotic treatment of septic arthritis. Clin. Ortho. and Related Res., No. 96: 150-52; Oct. 1973.

32. Schurman, DJ, Kajiyama G, Nagel DA. *Escherichia coli* infections in rabbit knee joints. J. Bone Joint Surg., 62A, No. 4, 620-27; June 1980.

33. Schurman DJ, Johnson BL, Amstutz HC. Knee joint infections with *Staphylococcus aureus* and Micrococcus species. J. Bone Joint Surg., 57A, No. 1: 40-49; Jan. 1975.

34. Siemering B. Painful joints: A review of rheumatoid arthritis, systemic lupus erythematous, infectious arthritis and degenerative joint disease. Comp. Cont. Ed., 12: 213-17; 1979.

35. Smith RL, Schurman DJ. Comparison of cartilage destruction between infectious and adjuvant arthritis. J. Ortho. Res., 1: 136-43; 1983.

36. Stover SM, Pool RR. Effect of intraarticular gentamicin

sulfate on normal equine synovial membrane. Am. J. Vet. Res., 46, No. 12, 2485-91; 1985.

37. Trotter GW, McIlwraith CW. Infectious arthritis in horses. Proc. Am. Assoc. Equine Pract., 173-83; 1981.

38. Tscherne H, Giebel G, Muhr G, Howell C. Synovectomy as treatment for purulent joint infection. Arch. Orthop. Trauma Surg., 103: 162-64; 1984.

39. Wolski K. *Staphylococcal* and other gram positive coccal arthritides. Clin. Rheum. Dis., 4(1): 181-96; 1982.

SUMMARY

Wounds and septic joint fluid contain high concentrations of protein which may bind chlorhexidine and chlorous acid/chlorine dioxide irrigation solution. This may partly account for the fact that concentrations of 0.05-0.5% chlorhexidine (proven to be detrimental to fibroblasts) are effectively utilized in clinical models of wound healing. The chlorous acid/chlorine dioxide disinfectant proved equivalent to 1.0% and 0.5% chlorhexidine in cytotoxicity and efficacy against *S. aureus*. All solutions should be evaluated in clinical models of wound healing and joint lavage to better define effectiveness *in vivo*.

There are several problems associated with the *in vitro* evaluations in this study. The increased protein in the BHI broth could be allowing the bacteria ample media to continue to proliferate. This may not be the most ideal environment to evaluate kill of *S. aureus*. The direct effects of these antiseptics may best be evaluated by removing the bacteria, washing them in saline and incubating directly in the concentrations utilized in this study.

Cell cultures have strict media requirements. This medium contains essential amino acids, electrolytes and vitamins. While this study utilized an established equine line of dermal fibroblasts, simply diluting this nutrient media with saline to establish controls in this study brought cell counts down to a mean of 116,250 viable cells/ml when the

initial sample contained 450,000 cells/ml. Adding potentially irritating antiseptics should expectedly decrease survival rates below controls. This is particularly true of chlorhexidine which precipitates with electrolytes and binds to protein critical to cell survival.

There our certain limitations in the utilization of cell cultures to evaluate the cytotoxic effects created by antiseptics. A decreased rate of change in the antiseptic (i.e., in the case of chlorhexidine the lack of sufficient quantities of electrolytes and protein in the essential media may reduce the amount of active chlorhexidine precipitated) presented to the cell culture may increase the cytotoxic effects on the fibroblast seen in this study. Elucidation of further direct effects these antiseptics have on metabolism and cellular interaction may give insight into these *in vitro* effects not seen clinically.

The products tested did not create any obvious inflammation when injected intraarticularly in the normal rabbit stifle joint. This is surprising in the case of chlorhexidine because of the severe cytotoxic effects seen in the first part of this thesis. The maximum cell survival rate in the presence of 0.005% chlorhexidine was only 50% survivability when compared to saline controls. Significant differences exist between *in vitro* and *in vivo* evaluations. Clinically, the 0.05% concentration appears to be a nontoxic and minimally inflammatory product to use in the rabbit stifle.

Concern exists as to the efficacy against common infectious agents found in wounds and septic arthritis, particularly *Staphylococcus aureus*.

Previous reports about aminoglycosides suggested that these products were too acidic to be used intraarticularly.[25,26] The amikacin and BES combination while moderately acidic apparently had little effect on the synovium when evaluating inflammatory response in this project. This product can be administered in conjunction with or following a lavage solution and should not need to be buffered. Efficacy in the face of clinical septic arthritis needs to be evaluated.

The chlorous acid/chlorine dioxide irrigation solution proved to be nonirritating. This product created no obvious inflammation in these rabbit joints. The previous report on cytotoxicity to equine fibroblast showed equivalent survivability to saline controls. The efficacy against *S. aureus* was the most questionable result because of the high organic material present in the BHI broth. Removal of the organic material from the culture media should improve the efficacy of this irrigation solution.

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