Lumbar Intervertebral Disc Infection: Pathology, Prevention and Treatment

Rebecca Walters, B. Biomed. Sc.

Enrolled through the Department of Pathology, Faculty of Health Sciences, The University of Adelaide.

Research conducted at The Adelaide Centre for Spinal Research, Division of Tissue Pathology, Institute of Medical and Veterinary Science, Adelaide.

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Abstract

Discitis is a potential complication of any open or percutaneous spinal procedure which involves entry into the intervertebral disc. The infection initiates an inflammatory response which leads to endplate rupture. Although there are variations in the severity of symptoms, the main feature of discitis is severe back pain which is not relieved by rest. The infection may spontaneously resolve over time although incapacitating back pain may persist for many months. In some cases serious complications result from the spread of infection to the adjacent vertebral bodies and over time osteomyelitis will develop with resultant bone destruction and collapse. The prognosis for many patients with discitis is poor with continual disabling back pain, prolonged absence from gainful employment and inability to return to daily living activities.

Clinical and experimental evidence now supports the prophylactic use of a suitable antibiotic to prevent discitis. In South Australia cephazolin is the antibiotic of choice to prevent or treat discitis due to *Staphylococcus* spp. While cephazolin has been shown to prevent discitis after inoculation with *Staphylococcus* spp. it is not universally accepted. Uncertainty exists regarding the ability of the antibiotic to enter the disc, and if it is effective in preventing and treating discitis. This is further complicated by the lack of suitable methods for detecting and measuring the concentration of cephazolin in the disc.

An experimental ovine model was used to investigate (a) the natural progression of discitis in the growing lumbar spine; (b) a technique to detect and measure the concentration of cephazolin in the disc; (c) the effect of prophylaxis when dose and time of administration of cephazolin was varied; (d) the effect of parenteral cephazolin after discitis was established and (e) the influence of health and age of the disc on prophylactic and parenteral treatment with cephazolin. In a clinical study the concentration of cephazolin was measured in degenerate human disc tissue to determine if therapeutic concentrations were achieved.
The ovine studies showed that discitis had no significant effect on the development of the growing lumbar spine after one year although infection was associated with reduced disc area and height. Preventing discitis with cephazolin was reasonably successful, regardless of age and health of the disc. Timing of cephazolin administration was crucial to prevent discitis in immature animals.

A high-performance liquid chromatography technique was used to measure the concentration of cephazolin in the disc. The greatest concentration of cephazolin in ovine discs was achieved 15 minutes after a bolus dose of intravenous antibiotic was administered, although detectable levels were measured for a further 2 hours. The concentration of cephazolin was not uniform across the disc with greater concentrations in the outer disc compared to the inner disc. Although there were measurable levels of cephazolin in these discs, it was ineffective at treating discitis once established. In the clinical study detectable levels of cephazolin were recovered in human discs for more than 2 hours after administering a 1-g bolus dose. The concentration of cephazolin peaked in the human discs between 37 and 53 minutes, but in only half of the discs was the concentration of cephazolin considered therapeutic against *Staphylococcus aureus*.

While discitis may spontaneously resolve over time, the infected disc does not recover to its original form. Furthermore, parenteral cephazolin was ineffective at preventing endplate destruction once an intradiscal inoculum was established. While this study proved cephazolin is able to enter the disc and provide reasonable protection against infection, it appears that discitis cannot be completely abolished. The timing of prophylaxis remains a critical factor to achieve therapeutic concentrations of cephazolin in the disc. Due to the serious complications that result from discitis this study supports the use of prophylactic antibiotic administered at an optimal time before the disc is violated during any spinal procedure.
Declaration

This work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution. To the best of my knowledge and belief this work contains no material previously published or written by another person, except where due reference has been made.

I give consent for this copy of my thesis, to be made available for loan and photocopying. The author acknowledges that copyright of published works contained within this thesis resides with the copyright holders of those works.

Rebecca Walters

March 2006
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Chapter 1: Introduction

1.1 Discitis

Discitis, or disc space infection, is an inflammatory condition of the intervertebral disc that was first described clinically in 1953 by Turnbull. The inflammatory response causes local oedema, ischaemia, and necrosis, affecting the normal mechanical function of the disc and surrounding tissue. The resultant back pain is often severe, and not relieved by rest.

‘Iatrogenic’ or ‘postprocedural’ discitis occurs predominantly in adults. Historically iatrogenic discitis was attributed to a chemical or aseptic reaction in the disc because bacteria were rarely cultured. However it is now known that iatrogenic discitis can occur as a primary infection of the disc space following an invasive surgical procedure (discectomy, discography, percutaneous nucleotomy, laminectomies or lumbar puncture) of the spine. It is possible, albeit rare, for children to present with iatrogenic discitis, and while spinal surgery in this group is uncommon, inadvertent penetration of the disc during lumbar puncture can cause iatrogenic discitis.

Iatrogenic discitis occurs in up to 4% of all spinal surgeries. Infection is usually due to micro-organisms originating from the patient. Alternatively, organisms may be from the operating room environment, including theatre personnel. Staphylococci (Staphylococcus aureus) and coagulase negative-staphylococci (Staphylococcus epidermidis) are common skin organisms which cause the vast majority of infections. Aerobic gram-negative bacilli and Propionibacterium species account for most of the remainder. Other less common organisms include Streptococcus viridans and other Streptococcus species, Escherichia coli, Pseudomonas aeruginosa and Mycobacterium tuberculosis and fungus.

Organisms are inadvertently introduced into the disc space by direct implantation through surgical instruments. The organisms flourish, eventually initiating a vascular response and a sequence of inflammatory reactions. This leads to endplate rupture which in turn causes
herniation of disc material (predominantly nucleus pulposus) into the vertebral bodies. When the endplates are breached the infecting organisms are usually cleared by the body’s immune response. Once cleared, granulation tissue fills the void caused by the herniation and, over time, new bone will form.\textsuperscript{14,15} In a few cases the infection may spread to the adjacent vertebral bodies with resultant osteomyelitis causing bone destruction and collapse.\textsuperscript{15}

Spontaneous primary discitis in children most commonly affects the lumbar discs of individuals younger than 5 years of age.\textsuperscript{32-33} The condition is usually a benign and self-limiting inflammatory reaction of the disc.\textsuperscript{19,34,35} Infecting organisms from elsewhere in the body such as the genitourinary tract, skin or soft tissue and respiratory system enter the disc space through haematogenous spread.\textsuperscript{34,36} Unlike adults children have a direct blood supply to the intervertebral disc\textsuperscript{37,38} as well as numerous paravertebral and intraosseous collateral arteries. It is rare for adults to develop spontaneous primary discitis due to regression of the blood supply to the disc by the second decade.\textsuperscript{38}

Spontaneous secondary discitis usually results from infection of the vertebral body (osteomyelitis of the vertebral body) that spreads to the disc. The organisms most commonly associated with spontaneous discitis are \textit{Staphylococcus aureus}, \textit{Enterobacter} species, \textit{Escherichia coli}, \textit{Proteus} species, \textit{Pseudomonas aeruginosa} and \textit{Haemophilus influenzae}.\textsuperscript{9,16-19} The infection may be aggressive and destructive, involving one or more components of the spine,\textsuperscript{20-22} with possible further complications including nerve root compression and abscess formation in the psoas muscle.\textsuperscript{23-25} This contiguous infection is associated with the elderly (>65 years) and those with a previous infection\textsuperscript{16,21,26} or history of IV drug use, renal disease, diabetes and AIDS.\textsuperscript{16,27-31}

Whilst both forms of discitis (iatrogenic and spontaneous) are uncommon there has been a recent rise in the incidence of iatrogenic discitis due to the increase in invasive procedures to diagnose or treat back pain, and improved detection with magnetic resonance
imaging (MRI). It is likely therefore that iatrogenic discitis will become more significant in the clinical setting.

1.2 Symptoms, signs and investigations to diagnose iatrogenic discitis

The main symptoms of adults presenting with iatrogenic discitis are back pain (with any movement of the spine) and muscle spasm, but as these same symptoms may also be present without infection, treatment may be delayed due to incorrect diagnosis. The length of time until diagnosis can range from three days to 15 months. In general, back pain from iatrogenic discitis becomes gradually worse over 1 to 4 weeks and is not relieved with rest. It is only poorly relieved by narcotic analgesics. Fever and signs of sepsis are not usually present. Blood culture is rarely positive and needle biopsy may not always provide a positive bacterial finding. This may be due to unsatisfactory tissue sampling, the infection has resolved, or antibiotics were given before the biopsy was taken.

Laboratory investigations include non-specific blood markers (elevated ESR and serum CRP) and imaging (CT and MRI). Plain radiographs are not sufficiently sensitive to detect the early endplate changes which may themselves, take several weeks to become evident. Bone scans may detect osteomyelitis earlier than radiographs which are more informative for long-term changes, such as bony sclerosis, reduced disc height and fusion. MRI and CT are more sensitive and specific detecting changes in the disc space and adjacent bone marrow that are consistent with early discitis.

Follow-up studies have shown various outcomes after discitis. These range from a lack of symptoms plus unrestricted spinal mobility, to destruction of adjacent vertebral bodies, abscess formation, local kyphosis, recurrent backache, narrowing of the intervertebral
disc space and fusion of the vertebrae.\textsuperscript{18,52-54} Generally long-term morbidity is high with over
50\% of patients unable to return to work.\textsuperscript{11,39}

The detrimental outcomes and major costs of iatrogenic discitis to the patient and the
health system are due to prolonged hospitalisation, further surgery (debridement of necrotic
tissue, draining an abscess or fusion) and increased patient care. The patient suffers further
with the time lost from gainful employment,\textsuperscript{53} while potential medico-legal actions are
detrimental to the hospital or surgical staff.\textsuperscript{12}

In summary, symptoms and signs of discitis may be over looked unless the clinician
suspects infection. MRI appears to be the most sensitive tool to detect discitis early. This is
imperative as delay in diagnosis and treatment may lead to complications, increased length of
hospital stay, and further cost to the patient.

1.3 Current theories for treating discitis

Generally adults with discitis are initially treated conservatively with analgesics, antibiotics,
bed rest or immobilisation.\textsuperscript{10,39} Most authors advocate the use of antibiotics following
isolation of the organism involved, but if this is not successful, broad-spectrum antibiotics are
recommended.

The role of antibiotics in the later stages of the disease is controversial. The infection
may have concluded before diagnosis is made. As a result, general antibiotics are usually
administered to prevent subsequent infection without specific knowledge of the pathogen.\textsuperscript{40,55}
Furthermore, antibiotics may be ineffective due to resistance of the causative micro-
organism.\textsuperscript{42,55}

On the basis of clinical experience, some authors have questioned treatment regimens
and the efficacy of antibiotics for this condition.\textsuperscript{10,39,56} There is general agreement that a
minimum of four weeks of intravenous therapy should be given\textsuperscript{57} with some authors
recommending six to eight weeks.\textsuperscript{2,18} Oral antibiotics are recommended following intravenous therapy to reduce the risk of infection relapse.\textsuperscript{18}

If conservative treatment fails further surgery may be required to treat complications such as epidural abscess, debridement of necrotic tissue from the infected area or interbody fusion with bone graft may be indicated.\textsuperscript{18,39,42,58,59} In one study patients with disabling back pain from discitis who were treated surgically had a better clinical outcome than those treated with antibiotics alone.\textsuperscript{18}

\textbf{1.4 Current theories for preventing discitis}

Due to the unfavourable outcome of postoperative discitis, prophylactic antibiotics are recommended during any open or percutaneous spinal procedure that involves the disc. Together with good aseptic surgical technique, prophylactic antibiotics have been reported to reduce the incidence of iatrogenic discitis, as well as the incidence of post-operative wound infections.\textsuperscript{11,15,46,60-64}

For a prophylactic antibiotic to be effective it must be present in sufficient concentration in vulnerable tissue from the time of surgical incision and for the duration of the procedure.\textsuperscript{65-67} In addition it needs to prevent the development of postprocedural discitis without the risk of developing resistance and subsequent superinfection. An antibiotic used commonly in South Australia for this purpose is cephazolin.

Cephazolin is given prophylactically as a 1-2 g dose (depending on patient weight) 30 minutes to one hour before spinal surgery and generally repeated every four hours at half the initial dose for prolonged procedures (or following haemorrhage).\textsuperscript{13,68-70}

Although cephazolin penetrates vascular tissue well, there is disagreement in the literature regarding its ability to enter the disc in an active form.\textsuperscript{71-75} In fact the use of prophylactic antibiotic altogether is not universally accepted.\textsuperscript{76}
Because the healthy adult disc has no direct blood supply, nourishment occurs by diffusion through the cartilage endplates or, up until the fourth decade, by vessels in the outer annulus fibrosus. As is the case for all antibiotics administered intravenously, cephazolin must diffuse through the capillary beds of the cartilage endplates to enter the adult disc. The ability of the antibiotic to diffuse through all parts of the disc may be influenced by the structure of the disc (vascular supply, size and health) and properties of the drug (size, solubility, binding and charge). Particularly, charge of the antibiotic has been discussed in the literature.

The nucleus pulposus is rich in glycosaminoglycans and has a high density of negative charge. It has been postulated that positively charged antibiotics (gentamicin and vancomycin) can enter the disc, whereas negatively charged antibiotics (penicillin and cephazolin) have limited or no penetration because of mutually repellent charges.

It is questionable whether cephazolin reaches therapeutic levels in all regions of the disc. Few studies have measured and reported actual levels of cephazolin in the human spine. Although methods for detecting cephazolin are well documented, not many are applicable to the avascular intervertebral disc. Most papers describe indirect methods of measuring antibiotic concentration.

If cephazolin could not enter the disc it would fail as a prophylactic. However, cephazolin has been shown to prevent discitis after inoculation with Staphylococcus spp. in animal models. These results suggest if cephazolin is administered at an appropriate time and dose it can be successful at preventing discitis. In spite of this, conclusive studies to prove that the antibiotic can enter the disc, and is effective as a prophylaxis, are currently not evident in the literature. In fact, despite a better general understanding of the pathogenesis of disc infection, little is known about the ability of any antibiotics to penetrate the disc.
1.5 Thesis Objectives

To resolve the diversity of opinion about strategies for the treatment and prevention of discitis, and on the choice, dose and timing of antibiotic administration, this thesis aims to determine:

1. Whether discitis without antibiotic prophylaxis or treatment influences development of the immature sheep spine.
2. The concentration of cephazolin in nucleus pulposus and annulus fibrosus tissue of the ovine and human disc after administration of a bolus dose.
3. Whether cephazolin given at intervals over a four-hour period can prevent iatrogenic discitis in immature and mature ovine discs.
4. If disc degeneration in the sheep influences the tissue levels of cephazolin and its effectiveness in preventing or treating discitis.
5. If the concentration of cephazolin in human disc and blood reach the minimum inhibitory concentration (MIC) specific for *Staphylococcus aureus*. 
1.6 References


Chapter 2: Effects of intervertebral disc infection on the developing ovine spine

2.1 Statement of authorship

EFFECTS OF INTERVERTEBRAL DISC INFECTION ON THE DEVELOPING OVINE SPINE


WALTERS, R.M. (Candidate)

Assisted surgery, prepared and performed analysis on samples, interpreted data, contributed to manuscript writing.

Signed…………………………………………………………………Date…………………..

SMITH, S.H.E.

Performed analysis of the data and contributed to manuscript writing.

Signed…………………………………………………………………Date…………………..

HUTCHINSON, J.H.

Performed surgery.

Signed…………………………………………………………………Date…………………..

DOLAN, A.M.

Performed surgery.

Signed…………………………………………………………………Date…………………..

FRASER, R.D.

Performed surgery, supervised development of work, helped in data interpretation and manuscript evaluation.

Signed…………………………………………………………………Date…………………..

MOORE, R.J.

Supervised development of work, helped in data interpretation, contributed to manuscript writing and evaluation, acted as corresponding author.

Signed…………………………………………………………………Date…………………..
CHAPTER 2

EFFECTS OF INTERVERTEBRAL DISC INFECTION ON THE DEVELOPING OVINE SPINE

R.M. Walters¹,², S.H.E Smith¹, M.J. Hutchinson³, A.M. Dolan³, R.D. Fraser¹,³, R.J. Moore¹,²

¹The Adelaide Centre for Spinal Research, Institute of Medical and Veterinary Science.
²Department of Pathology, The University of Adelaide
³The Spinal Unit, Royal Adelaide Hospital, Adelaide, Australia


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Chapter 3: Therapeutic use of cephazolin to prevent complications of spine surgery

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WALTERS, R.M. (Candidate)
Collected samples, prepared samples for analysis, interpreted data, contributed to manuscript writing and acted as corresponding author.

Signed……………………………………………………..Date…………………..

VERNON-ROBERTS, B
Supervised development of work and manuscript evaluation.

Signed……………………………………………………..Date…………………..

FRASER, R.D.
Supervised development of work and manuscript evaluation.

Signed……………………………………………………..Date…………………..

MOORE, R.J.
Supervised development of work and manuscript evaluation.

Signed……………………………………………………..Date…………………..
CHAPTER 3

THERAPEUTIC USE OF CEPHAZOLIN TO PREVENT COMPLICATIONS OF SPINE SURGERY

R.M. Walters,1, 2 B. Vernon-Roberts,1 R.D. Fraser,3 R.J. Moore1, 2

1The Adelaide Centre for Spinal Research, Institute of Medical and Veterinary Science
2Department of Pathology, The University of Adelaide
3Spinal Unit, Royal Adelaide Hospital, Adelaide, South Australia

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Chapter 4: Prophylactic cephazolin to prevent discitis in an ovine model

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WALTERS, R.M. (Candidate)
Assisted surgery, prepared and performed analysis on samples, interpreted data, contributed to manuscript writing and acted as corresponding author.
Signed……………………………………………………..Date…………………..

RAHMAT, R.
Performed surgery.
Signed……………………………………………………..Date…………………..

SHIMAMURA, Y.
Performed surgery.
Signed……………………………………………………..Date…………………..

FRASER, R.D.
Performed surgery, supervised development of work, helped in data interpretation and manuscript evaluation.
Signed……………………………………………………..Date…………………..

MOORE, R.J.
Supervised development of work, helped in data interpretation, contributed to manuscript writing and evaluation.
Signed……………………………………………………..Date…………………..
CHAPTER 4

PROPHYLACTIC CEPHAZOLIN TO PREVENT DISCITIS IN AN OVINE MODEL

R.M. Walters\textsuperscript{1,2}, R. Rahmat\textsuperscript{1}, Y. Shimamura\textsuperscript{1}, R.D. Fraser\textsuperscript{1,3}, R.J. Moore\textsuperscript{1,2}

\textsuperscript{1}The Adelaide Centre for Spinal Research, Institute of Medical and Veterinary Science.
\textsuperscript{2}Department of Pathology, The University of Adelaide
\textsuperscript{3}The Spinal Unit, Royal Adelaide Hospital, Adelaide, Australia

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Chapter 5: Preventing and treating discitis: Cephazolin penetration in ovine lumbar intervertebral disc

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(Submitted)

WALTERS, R.M. (Candidate)

Assisted surgery, prepared and performed analysis on samples, interpreted data, contributed to manuscript writing and acted as corresponding author.

Signed……………………………………………………..Date…………………..

RAHMAT, R.

Performed surgery.

Signed……………………………………………………..Date…………………..

FRASER, R.D.

Performed surgery, supervised development of work, helped in data interpretation and manuscript evaluation.

Signed……………………………………………………..Date…………………..

MOORE, R.J.

Supervised development of work, helped in data interpretation, contributed to manuscript writing and evaluation.

Signed……………………………………………………..Date…………………..
CHAPTER 5

PREVENTING AND TREATING DISCITIS: CEPHAZOLIN PENETRATION IN OVINE LUMBAR INTERVERTEBRAL DISC

R.M. Walters\(^1,2\), R. Rahmat\(^1\), R.D. Fraser\(^1,3\), R.J. Moore\(^1,2\)

\(^1\)The Adelaide Centre for Spinal Research, Institute of Medical and Veterinary Science.
\(^2\)Department of Pathology, The University of Adelaide
\(^3\)The Spinal Unit, Royal Adelaide Hospital, Adelaide, Australia

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WALTERS, R.M. (Candidate)
Prepared and performed analysis on samples, interpreted data, contributed to manuscript writing and acted as corresponding author.

Signed……………………………………………………..Date…………………..

FRASER, R.D.
Performed surgery, supervised development of work, helped in data interpretation and manuscript evaluation.

Signed……………………………………………………..Date…………………..

MOORE, R.J.
Supervised development of work, helped in data interpretation, contributed to manuscript writing and evaluation.

Signed……………………………………………………..Date…………………..
Chapter 6

PENETRATION OF CEPHAZOLIN IN HUMAN LUMBAR INTERVERTEBRAL DISC

R.M. Walters\textsuperscript{1,2}, R.D. Fraser\textsuperscript{1,3}, R.J. Moore\textsuperscript{1,2}

\textsuperscript{1}The Adelaide Centre for Spinal Research, Institute of Medical and Veterinary Science.  
\textsuperscript{2}Department of Pathology, The University of Adelaide  
\textsuperscript{3}The Spinal Unit, Royal Adelaide Hospital, Adelaide, Australia

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Chapter 7: Final Discussion

7.1 Summary

With Animal and Human Ethics Committee approval, research proposals were conducted to resolve the diversity of opinion regarding the long-term effects of discitis on the growing spine, and the effectiveness of cephazolin to prevent and treat iatrogenic discitis. The significant findings from these studies are summarised.

Injecting ovine discs with *Staphylococcus* spp. initiated vascular and inflammatory reactions that led to changes typically associated with discitis. Infection with *Staphylococcus aureus* and *Staphylococcus epidermidis* initiated endplate rupture which in turn caused herniation of disc material. *S. aureus* produced a more aggressive response than *S. epidermidis*. The lesions from *S. aureus* infection were haemorrhagic, irregular, extended well into the adjacent vertebral body, and almost always changed in size and shape over time. Lesions from *S. epidermidis* infection were less invasive, with minimal erosion of the vertebral endplate and remained relatively unchanged in size and shape over 12 weeks which may explain that while inoculation of *S. epidermidis* in immature ovine discs results in retardation of disc development, it had no significant effect on vertebral body growth or growth of the entire lumbar spine. These results concur with long-term studies that discitis in children is relatively benign and the potential for bone destruction is low.¹

Spontaneous discitis in children is generally asymptomatic and typically has a benign outcome. Some authors also believe long-term outcome of childhood discitis is not dependent on treatment with antibiotics.²⁻⁴ This may be attributed to the healing capacity of the disc due to the abundant vascularity present at that age. An increased number of vascular channels in the cartilaginous endplate and disc may promote clearance of bacteria while limiting tissue damage. It was most probable that the threefold increase in blood vessels occupying the
endplate in sheep less than 6 months old assisted the clearance of the inoculum, because only a half of all inoculated discs developed discitis.

In adult iatrogenic discitis bacteria are not cleared by an immune response until the endplates are breached and blood vessels are prevalent. To prevent infection and the subsequent inflammatory response destroying the surrounding tissue, prophylactic antibiotics must enter the disc. Cephazolin, like all molecules, can only enter the disc by passive diffusion.\(^5\) The distribution of the antibiotic in the disc is controlled by the high concentration of glycosaminoglycan molecules in the nucleus pulposus which creates a high density of relative negative charge.\(^6\) As a result positively charged molecules are more likely to enter the disc from the capillary bed of the endplate and negatively charged molecules predominantly enter through the outer annulus.\(^7\) Cephazolin is a weak acid with a low pKa and an overall negative charge. Theoretically the route of entry of unbound cephazolin should be through the meagre supply of vessels in the outer annulus fibrosus but due to its negative charge high concentrations of cephazolin within the nucleus pulposus would not be expected.

The results from the high-performance liquid chromatography assay confirmed detectable levels of cephazolin in all regions of the ovine disc. Although the distribution was not uniform, it was nonetheless promising that it could be detected as early as 15 minutes after intravenous administration. Consistent with the theory that relative negative charge plays a significant role in molecular distribution there was higher concentrations of cephazolin in the annulus fibrosus compared to the nucleus pulposus. In fact, increasing the intravenous dose of cephazolin from 2 g to 4 g did not significantly increase the concentration of cephazolin in the nucleus pulposus, further supporting the charge theory.

By incising the outer annulus fibrosus and partially removing the nucleus pulposus (consisting of negatively charged glycosaminoglycan molecules) it was hypothesised that vascularity of the disc would increase and relative negative charge of the disc would decrease and therefore facilitate the penetration of antibiotic into the disc and reduce the incidence of
discitis. Despite histologic confirmation of vascular granulation tissue in the outer annulus fibrosus and partial nucleotomy, the concentration of cephalosporin was not significantly different compared to non-incised discs. It would appear that this treatment had no significant effect on the delivery of antibiotics to the ovine disc and did not influence the incidence of discitis. However, altering the timing of administration of cephalosporin significantly influenced the incidence of discitis in immature sheep. Although the immature discs had an abundant vascular supply and higher concentration of cephalosporin, discitis developed 10 times more readily in lambs than in mature sheep. However, prophylactic cephalosporin administered 30 minutes before inoculation consistently prevented discitis in both mature and immature sheep.

While the ovine model was able to replicate a human disease process and suggest what is likely to occur in the human spine, caution is required when extrapolating data to humans. Although the structure and composition of the sheep disc is remarkably similar to the human disc there are differences. Mature sheep discs are approximately half the size of human discs and contain a growth plate, whereas the human disc has a ring epiphysis. It would be reasonable to assume that cephalosporin would take longer to diffuse through a larger disc, but other factors such as health, weight and gender may confound the interpretation of the results.

After administering cephalosporin (IV) to humans, the concentration of cephalosporin was greatest in samples of discs collected between 37 and 53 minutes. Importantly therapeutic concentrations (against *Staphylococcus aureus*) were not detected in all samples over the 2 hour period and the period of detection varied considerably between individuals. Along with external factors, this variability may have been attributed to differences in vascular supply, size and maturity of the disc and may explain the differences in timing of prophylaxis between the ovine and human disc.
7.2 Future Directions

Intravenous administration of cephazolin was not the most efficient method of delivery to the disc, particularly to the nucleus pulposus. Further studies are required to determine if intradiscal injection, in combination with intravenous administration, would be a better option. Intradiscal injection would facilitate direct delivery of the antibiotic into the disc and potentially distribute it evenly throughout the disc. It would also overcome problems such as drug-binding, size exclusion and poor vascular supply. Intradiscal injections may benefit the patient during open or percutaneous procedures, but would not provide systemic prophylaxis or be appropriate for long-term antibiotic treatment of discitis.

Analytical methods that complement microbiological culture methods may provide a better understanding of the types of organisms that cause infection. DNA-based methods\textsuperscript{8,9} that identify the bacterial DNA causing discitis may become more significant. These methods are highly sensitive and specific and can identify bacterial species in patients with negative blood and disc aspirate cultures. Accurate and early diagnosis will enable organism specific antibiotics to be administered early. This may prevent endplate erosion, and the further complications of discitis such as osteomyelitis or abscess formation.

Further research involving the detection of antibiotic in the disc is required. The method described in this thesis, to detect the concentration of cephazolin in the disc, provided only one measurement at a single time point. It would have been more appropriate and accurate if multiple measurements of antibiotic concentration were taken from one disc. Potentially, multiple measurements of antibiotic concentration could be taken throughout a surgical procedure while the patient is undergoing surgery. Although such methods have not been established for the disc, tissue microdialysis and capillary electrophoresis have been used to determine antibiotic concentration in organs and fluid of the body.\textsuperscript{10-12} These applications are designed to take serial samples over time and provide an opportunity to quantify tissue drug distribution \textit{in vivo}. This would show the actual rise and decline of the
concentration of the antibiotic in the disc and would improve our understanding of the factors that influence antibiotic concentration, the individual variation that occurs across a population and the effects on the disc.

7.3 Conclusion

It is clear from these studies that iatrogenic discitis is detrimental to the disc and prophylactic antibiotics are necessary to prevent the outcome of infection. Intravenous cephazolin is a reasonable choice as a prophylactic antibiotic. Cephazolin could be detected in ovine and human disc however it was unable to penetrate all aspects of the disc uniformly. The uneven distribution of cephazolin in the disc may influence the incidence of discitis, as infection could not be completely abolished over a period of time. Timing of prophylactic administration remains critical to provide the best protection to the disc during open or percutaneous procedures.
7.4 References


Chapter 8: Amendments

8.1 Structure and composition of the intervertebral disc

The intervertebral discs are complex cartilaginous structures located between the vertebral bodies which allow the otherwise rigid spine to move in flexion, extension and rotation. The disc is comprised of a central nucleus pulposus enclosed by concentric layers of collagen which make up the annulus fibrosus. Located superiorly and inferiorly from the intervertebral disc are the cartilage endplates, adjacent to the vertebral bodies.

The major components of the disc are water, fibrillar collagen and aggrecan (proteoglycans consisting of glycosaminoglycan chains). However from birth the ratio and composition of these components in the disc changes.\(^1\) The most significant changes occur from birth up to the second decade of life and again with advancing age (> 40 years).\(^2\)

At birth the nucleus pulposus comprises half of the disc space. It is clear, gelatinous and highly hydrated with a high concentration of aggrecan but a relatively lower collagen content.\(^3\) The large aggregating proteoglycans consist of a protein core and sulphated glycosaminoglycan (GAG) chains. These side chains which have a high density of negative charges associated with them, are responsible for the distribution of molecules throughout the disc.\(^3\) Surrounding the nucleus pulposus are the abundant and firm collagen fibres of the annulus fibrosus that form concentric rings or lamellae. The lamellae of the immature annulus fibrosus contain blood vessels, which supply some nutrients to the disc. The collagen fibres of the annulus fibrosus continue to extend laterally to form the hyaline cartilage of the epiphyseal end plates. In the immature spine this functions as the growth plate for the adjacent vertebral body, containing a large blood supply that provides the majority of nutrition to the intervertebral disc.\(^3\)

With maturity, the collagen fibres of the annulus fibrosus thicken and the proportion of aggrecan and water in the nucleus pulposus decreases.\(^3,4\) The structure of the cartilage
endplate changes to form a layer of hyaline cartilage which calcifies and joins the bone, forming the ring epiphysis. By the second decade of life blood vessels in the annulus fibrosus and endplates have regressed, reducing the nutrient supply to the disc. Until the fourth decade of life there is no direct blood supply to the healthy adult disc. Nutrient delivery to the centre of the disc is predominantly by passive diffusion across the endplates.

Progressive changes continue to occur later in life. These include a loss of disc height, an increase in number of clefts and tears of the nucleus pulposus, neovascularisation of the outer annulus and endplate, disorganization, calcification and thinning of the endplate, and cellular changes leading to degenerative disc disease.

8.2 Comparison of the human and sheep disc

Although the sheep is a quadruped, the structure and composition of sheep discs is remarkably similar to human discs. Sheep discs also consist of an inner nucleus pulposus and outer annulus fibrosus containing water, fibrillar collagen and aggrecan that change in proportion throughout life. The most notable difference between the two species is size. The anterior disc height in the mature sheep lumbar spine is approximately 5mm lower than in humans. Likewise the size of the vertebral bodies is also notably different. Human vertebral bodies are wider than tall and contain a ring epiphysis compared to the sheep vertebral bodies which are taller than wide and contain a growth plate. However both species have a pronounced oval shaped vertebral body.

Immature lamb discs have a highly vascular cartilage endplate that supplies nutrients to the disc. However, by 6 months of age the blood vessels regress, resulting in a mature avascular disc. Similar to the human disc, the mature sheep relies on diffusion of nutrients through the capillary bed.
The sheep is a valid model for human spinal conditions and has been utilised over two decades to demonstrate intervertebral disc and vertebral pathology of the spine.\textsuperscript{11-14} Sheep are readily available in Australia, reasonably inexpensive and show much more homogeneity than do human specimens when selected for breed, sex, age and weight.\textsuperscript{15} Its spinal biomechanics are similar to the human spine and it is large enough and a useful model for a range of surgical procedures.\textsuperscript{10}

While the sheep model is useful to understand the pathology of disease and the likely consequences in the human, one is mindful of the need to be cautious when extrapolating data from an animal to recommend clinical decisions for the human. Despite such variations, patients will ultimately benefit from improved understanding and clinical practice derived from knowledge gained from animal models. In summary, there are proven sheep models for discitis and disc degeneration and these are used in the experimental chapters described in this thesis.

\subsection*{8.3 Dosing guidelines}

An introduction to dosing guidelines of cephazolin are described on pages 10 and 11 of the thesis and more detailed information is provided in the methods section of each paper respectively. However, a brief description of dosing guidelines is outlined below.

Prophylactic antibiotics are given as either a single intravenous dose at induction of anaesthesia or in combination with an intradiscal dose during surgery. Cephazolin is administered prophylactically as a 1-2 g dose 30 minutes to one hour before surgery and generally repeated (every four hours) at half the initial dose for prolonged procedures or following haemorrhage. Continuing antibiotics for longer than 24 hours is not advisable as this may promote resistant microbial pathogens, expose the patient to more adverse drug effects and increase medical costs.
Cephazolin is also indicated in the treatment of bone and joint infections due to *S. aureus*. In adults the usual dose for moderate to severe infections is 500 mg to 1 g every six to eight hours. In children a total daily dosage of 25 to 50 mg per kg of body weight, divided into three or four equal doses is effective for most infections (Mayne Pharma Pty Ltd., Australia).

### 8.4 Chapter Amendments

#### 8.4.1 Chapter 2 page 27, paragraph 2

American Type Culture Collection (ATCC) is a collection of bacterial cultures used as quality control organisms for research purposes to identify the culture type used.

#### 8.4.2 Chapter 2 page 27, paragraph 1

Previous work using the animal model of discitis in the mature sheep with *S. epidermidis* as the inoculum has been reported but none have looked at the long-term effects of this organism in the immature sheep spine. Although *S. aureus* is a common infecting organism, Staphylococcus species (including *S. epidermidis*) and other gram-negative bacteria are also implicated.

#### 8.4.3 Chapter 2 page 28, paragraph 1

The Sagittal Convexity Index is the central disc height (D), measured as the maximal thickness of the disc is divided by the sum of the anterior (E) and posterior disc heights (F), taken from the peripheral endplates.
8.4.4 Chapter 2 page 28, paragraph 1

The Farfan Index is a measure of the sum of the anterior and posterior disc height as a percentage of the width of the vertebral body.

8.4.5 Chapter 2 page 28, paragraph 2

The histological features of new bone formation were identified using polarised light microscopy. The collagen fibres were typically arranged in an irregular form suggesting formation of new bone (woven bone).

8.4.6 Chapter 2 page 29, paragraph 2

Chronic inflammation is due to microorganisms that are able to resist phagocytosis and incite an inflammatory response, which results in significant tissue damage. Significant tissue damage can be seen within and surrounding the intervertebral disc. Eosinophilic staining scar tissue shown in the low power view is evidence of a chronic inflammatory response. Scar tissue has developed at the site of the lesion, most likely in response to the release of cytokines by the activated macrophages (not shown).

8.4.7 Chapter 2 page 29, paragraph 2

Although chronic inflammatory cells (activated macrophages and lymphocytes) cannot be distinguished from one another in a low power view, in a high power view a cellular response is evident. An influx of polymorphonuclear cells surrounds the lesion, between the cartilage endplate and epiphyseal growth plate. Cellular detail is not shown in Figure 8, as this is a low power view to visualise the extent of the lesion.
8.4.8 Chapter 3 page 44, paragraph 3
The regression line is a straight line that passes through the origin (zero) and the values of the working (stock) standards (0, 16, 80 and 400 mg/L for plasma and 0.64, 3.2, 16 mg/L for disc tissue) to determine the accuracy of the assay using sheep plasma and disc tissue. The accuracy of the standards is determined by the deviation of actual values compared to the regression line.

8.4.9 Chapter 3 page 46, paragraph 4
The difference in tissue cephazolin concentration may be related to the extraction method because of the time taken to remove disc tissue from the sheep spine or possibly the disc level and variability in disc size.

8.4.10 Chapter 3 page 46, paragraph 4
One must be mindful that this is a method paper to describe the technique and not a research paper to understand the mechanisms responsible for antibiotic penetration into the different regions of the disc. The number of sheep in this study was two. Determining statistics for the concentration of cephazolin would not be appropriate or accurate. Sample results described in this paper are reported as evidence that the technique is suitable for sheep samples. The validity of this method is confirmed by statistics completed for the extraction technique on ovine disc tissue and plasma standards for every assay run (standard curve) and recovery.

8.4.11 Chapter 3 page 46, paragraph 3
The nucleus pulposus has a high concentration of proteoglycans which produce a high-density negative charge. Cephazolin is a weak acid with a low pKa and negative charge. It is assumed the high concentration of proteoglycans in the nucleus pulposus tissue controls the distribution of cephazolin in the disc. The diffusion of cephazolin into the central region of
the disc is restricted by the repulsion of the like charges. Therefore concentration of cephalixin in the outer disc (annulus pulposus) remains greater.

**8.4.12 Chapter 4 page 59, paragraph 2**

Discs that were infected and developed discitis were not used to determine concentration of cephalixin in the disc. Discitis causes vascular ingrowth which significantly influences the cephalixin concentration in the disc. Discitis also makes it difficult to identify the structural components of the disc thereby making it difficult to accurately separate annulus fibrosus tissue from nucleus pulposus tissue.

**8.4.13 Chapter 4 page 64, paragraph 3**

Although incision of the annulus fibrosus initiates a highly vascular granulation tissue response in the peripheral layers, this does not appear to significantly influence the diffusion of antibiotic into the disc. This may suggest the incision in the disc does not produce enough vascular tissue throughout the disc to influence diffusion of the antibiotic. It also suggests the capillary network in the endplate is the major source suppling cephalixin to the disc and not the meagre blood supply in the outer annulus. This was confirmed by the results of cephalixin concentration in the sheep disc with age (sheep Vs lamb). As the lamb matures the density of the capillary network diminishes. As this occurs the concentration of cephalixin in the disc decreases. It appears density of the capillary network is the significant factor influencing the diffusion of cephalixin into the disc not the vascular supply in the peripheral layers.

**8.4.14 Chapter 4 page 64, paragraph 1**

Perhaps the uneven distribution of cephalixin in the disc is more pronounced in the lamb disc due to the increased level of proteoglycans. Or other physiological factors (increased
proteoglycan and water content) may provide a nutrient rich environment for bacterial growth in the nucleus pulposus and may be responsible for an increased infection rate.

8.4.15 Chapter 4 page 64, paragraph 3
Although this was not statistically proven there appears to be a trend that operated lamb discs were more at risk of discitis than non-operated discs. The only exception was when cephazolin was given 30 minutes before inoculation. As a consequence of incising the outer annulus of the disc a vascular granulation tissue response occurs. This response may produce a favourable environment (increased nutrients) for the growth of bacteria. However, this does not explain why the same trend is not observed in the sheep. Perhaps the physiological differences (disc size and vascular supply) or maturity of the immune system between the sheep and lamb influences the incidence of discitis.

8.4.16 Chapter 4 page 63, paragraph 1
Investigations of discitis using an ovine model have shown that inoculation of the disc with only a few organisms reliably results in discitis within 1-2 weeks. Previous sheep studies have looked at the effects of discitis with *S. epidermidis* none have looked at the effects with *S. aureus*. In this study, a large number of bacteria were chosen to ensure the maximal effect of inoculation was achieved. If the prophylactic antibiotic was efficient in an extreme case then the results were more credible.

8.4.17 Chapter 4 page 59, paragraph 1
Not all of the organisms were used for inoculation or put into contrast medium. Therefore bacteria could be counted with or without contrast medium at the time before and after inoculation.
8.4.18 Chapter 4 page 60, paragraph 3
Cellular detail is not shown in Figure 3 as this is a low power view to visualise the extent of the lesion.

8.4.19 Chapter 4 page 65, paragraph 1
The extraction method was designed and performed by the candidate at the IMVS, Adelaide. The HPLC assay for cephazolin was only available in Perth and it was necessary that the samples were assayed there. The candidate spent one week in the Department of Biochemistry at Royal Perth Hospital and learnt the technique and conducted the antibiotic assay on some samples.

8.4.20 Chapter 4 page 61, paragraph 2
A large number of bacteria were chosen to ensure the maximal effect of inoculation was achieved.

8.4.21 Chapter 5 page 79, paragraph 3
Blood cultures at seven days were negative suggesting a large inoculum of bacteria were not spreading across adjacent discs via the vessels in the highly vascular immature disc or the vessels surrounding the mature disc.
8.5 References


