Original article

Inflammatory response to intramuscular implantation of polyacrylonitrile ultrafiltration probes in sheep

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Abstract – Polyacrylonitrile is used in the manufacture of dialysis membranes. These membranes are fundamental to the functioning of implantable probes for microdialysis and ultrafiltration sampling of tissue fluids. Although in vivo experimentation using polyacrylonitrile has been reported to cause little inflammatory response when implanted subcutaneously, such information is not available for intramuscular implantation in sheep. The procaine and benzathine salts of penicillin are formulated for intramuscular injection. These salts of penicillin or the formulation excipients may cause inflammatory reactions. Use of polyacrylonitrile probes to draw samples from sites at which these formulations have been injected may be compromised by inflammation or direct interaction between formulation excipients and the dialysis membrane. The aim of this project was to describe tissue responses to intramuscular implantation of polyacrylonitrile in the presence and absence of either procaine or procaine plus benzathine salts of penicillin G. Each of 20 normal sheep was implanted with two ultrafiltration probes, one at the site of an injection of procaine or benzathine plus procaine penicillin G. Similar injections were also made at remote intramuscular sites. After 8, 9, and 11 days of the experiment, sheep were killed and the injection and implantation site muscle were excised and prepared for histopathological examination. The implantation of the probe alone caused greater inflammatory response than the injection of procaine or procaine plus benzathine penicillin G at remote intramuscular sites. The histopathological lesions were greatest where the implantation site was coupled with the injection of either formulation of penicillin G. Polyacrylonitrile may not be a suitable dialysis membrane material for intramuscular implantation in sheep.

polyacrylonitrile / ultrafiltration / intramuscular implantation / inflammatory response / sheep

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Résumé – Réponse inflammatoire à l'implantation intramusculaire de sondes d'ultrafiltration en polyacrylonitrile chez le mouton. Le polyacrylonitrile est utilisé dans la fabrication de membranes de dialyse. Ces membranes sont fondamentales pour le fonctionnement des sondes implantables de microdialyse et d'échantillonnage de fluides tissulaires par ultrafiltration. Bien qu'une faible réponse inflammatoire ait été notée lors de l'expérimentation in vivo utilisant le polyacrylonitrile implanté en sous-cutané, une telle information n'est pas disponible pour les implantations intramusculaires chez le mouton. Les sels de pénicilline procaïne et benzathine sont formulés pour les injections intramusculaires. Ces sels de pénicilline, ou les excipients entrant dans leur composition, peuvent provoquer des réactions inflammatoires. L'utilisation de sondes de polyacrylonitrile pour l'extraction d'échantillons aux sites d'injection de ces composés pourrait être compromise par une inflammation ou une interaction directe entre les excipients et la membrane de dialyse. Le but de cette étude était de décrire les réponses des tissus à l'implantation intramusculaire de polyacrylonitrile, en présence et en l'absence de chacun de ces sels de pénicilline G. Vingt moutons normaux ont chacun subi l'implantation de deux sondes d'ultrafiltration, dont une au site d'injection de la pénicilline G-procaïne, ou de la pénicilline G benzathine-procaïne. Des injections similaires ont également été effectuées à des sites intramusculaires distants. Après 8, 9 et 11 jours, les moutons ont été sacrifiés, et le muscle a été excisé au niveau du site d'injection, et préparé pour un examen histopathologique. L'implantation de la sonde seule a provoqué une réponse inflammatoire plus importante que l'injection de pénicilline G-procaïne ou pénicilline G-procaïne-benzathine à des sites distants. Les lésions histopathologiques ont été plus importantes aux endroits où le site d'implantation était couplé à l'injection de l'une ou l'autre formulation de pénicilline G. Le polyacrylonitrile pourrait ne pas être un matériel adapté à l'implantation intramusculaire chez le mouton.

polyacrylonitrile / ultrafiltration / implantation intramusculaire / réponse inflammatoire / mouton

1. INTRODUCTION

Commercially available ultrafiltration (UF) probes are used widely for sample collection from extracellular fluid during studies in vivo. These probes use dialysis membranes of a variety of materials and size exclusions, to exclude cellular and proteinaceous components of the intercellular matrix from the acquired sample. Membrane materials can be divided into three classes according to their ability to activate complement factors; high, intermediate and low [3]. Polyacrylonitrile is a commonly used dialysis membrane material found in renal dialysis machines and some ultrafiltration probes; e.g., model MF-7026 (Bioanalytical Systems, West Lafayette, IN, USA). Polyacrylonitrile caused little complement activation in vivo when implanted as a dialyzer and was classified as a low complement activity material [3, 6]. However, the period of sampling in both studies lasted only 3–4 hours. Subcutaneous implantation of models MF-7026 and MF-7027 polyacrylonitrile ultrafiltration probes provided a recovery of 98% of glucose in mice [9]. The model MF-7026 ultrafiltration probe also performed well during continuous monitoring of glucose for 46 days in a diabetic cat [8]. These authors reported that there was no macroscopically apparent inflammatory response after 6 weeks subcutaneous implantation. The model MF-7028 ultrafiltration probe was reported also to provide good recovery of endogenous ions when implanted intramuscularly in horses and caused no significant local edema within 2 days after the implantation [16]. There were also no significant changes in values of sodium and potassium ion in collected interstitial fluid (ISF) sample. The ability to collect protein-free interstitial fluid over several weeks without provoking an inflammatory response suggested that model MF-7026 polyacrylonitrile ultrafiltration probes may be useful to examine the absorption pharmacokinetics of intramuscularly injected xenobiotics.

Penicillin G has been used extensively both for prevention and treatment of diseases in veterinary practice. Penicillin G is active against both Gram positive and Gram negative bacteria at appropriate doses [7] and is used most often in food-producing animals. Formulation of penicillin G as procaine or benzathine salts allows application of flip-flop pharmacokinetics to prolong the duration of effective therapeutic concentrations. Slow absorption from the injection site results from the formation of a muscle depot. These muscle depots may persist beyond the regulated with-holding time, especially with the benzathine salt, and have escaped regulatory attention. In food animals, penicillin G is almost always used as an injectable form of the procaine or benzathine salts, or of a combination of these two.

We proposed to examine the absorption of penicillin G from intramuscular injection sites using model MF-7026 ultrafiltration probes. However, inflammation at the site of probe implantation may alter an ultrafiltration probe's drug recovery characteristics, or may alter the absorption of the injected xenobiotic [14, 15]. Therefore, we studied the histopathological effects of model MF-7026 ultrafiltration probes, procaine penicillin G and procaine/benzathine penicillin G in sheep muscle, examining the hypothesis that MF-7026 ultrafiltration probes would cause minimal inflammation after intramuscular implantation in sheep.

2. MATERIALS AND METHODS

Four groups of five sheep ranging in weight from 31–49 kg and of various breeds were used in the study. The animals were acclimated to the surroundings for 4 days prior to the first probe implantation. The animals were not fed in the morning of each implantation.

2.1. Insertion of ultrafiltration probes

The animals were sedated using xylazine (Rompun® 100 mg·mL^{-1}, Bayer Corporation, Shawnee Mission, KS, USA) at 0.2 mg·kg^{-1} IM into the right *gluteus medius*. The wool was clipped and shaved on both sides of the vertebral column from the crest of the ilium to the last rib. The skin was prepared with isopropyl alcohol and povidone iodine scrub prior to surgery. The skin anterior to the right or left ilial crest was anesthetized by injecting lidocaine 2.0% parallel to vertebral column using an inverted 'L' block.

Two incisions were made; the first (2.5 cm long) just anterior to the ilial crest and the second (1 cm long) 15 cm anterior to the first incision. At the first incision, a retractor was used to gain access to the fascia of the *longissimus dorsi* muscle. Dexon # 2-0 was used to make a loop through this fascia, then a 5 cm 12 gauge needle with an ultrafiltration probe (Bioanalytical Systems, Model MF–7026, molecular weight cut off 30 000 daltons, 2 cm polyacrylonitrile fibers West Lafayette, IN, USA) and a 0.7 mm inner diameter (ID) teflon tubing (Becton Dickinson and Company, Rutherford, NJ, USA) inserted inside was placed at the center of the loop at a depth of 4 cm. The needle and teflon tubing were removed leaving the probe and teflon tubing inside the muscle. The teflon tubing was removed after any injection of test material (see below) leaving the probe in place. A purse string suture fixed the probe in place. A 15 cm long 12 gauge needle was used to make a subcutaneous tunnel from the first to the second anterior incision. The tubing of the probe was passed through the tunnel and lead to the outside through the anterior incision. This terminal portion of the ultrafiltration probe was connected to the hub assembly and fixed to the skin with No. 1 surgical silk. The needle end of the hub assembly was inserted into a 3.0 mL collecting vacutainer. Both skin incisions were then sutured with a simple interrupted

pattern using No. 1 surgical silk. An adhesive bandage (7.5 cm width) was used to wrap the hub assembly and vacutainer that was used to collect sample for pharmacokinetic study.

A probe was placed in the right *longissimus dorsi* muscle on day 1. This probe was used as a control probe and no injections were made at this site. A probe was similarly placed in the left *longissimus dorsi* muscle on day 4 (group 4) and day 7 (groups 1, 2 and 3), and an injection of test material was made at this site.

2.2. Experimental design

Each sheep received injections of test material at the left probe site, and at three distinct intramuscular sites according to the schedule in Table 1. The right probe was in all cases used as the internal control, and received no test material. In sheep groups 1 and 2, procaine penicillin G (Butler® Sterile Penicillin G Procaine, 300 000 IU·mL^{-1}, The Butler Company, Columbus, OH, USA) was administered at 20 000 IU procaine penicillin G·kg^{-1} intramuscularly (IM) daily

for 4 days beginning on day 4, then through day 6 into the right *quadriceps femoris*, left *quadriceps femoris* and right *triceps brachii* muscles in that order. On day 7 the left ultrafiltration probe was placed and an injection of test material made through the teflon tubing, prior to its removal. For sheep of group 3, procaine + benzathine penicillin G (Pen – BP 48®, containing 150 000 IU benzathine penicillin G and 150 000 IU procaine penicillin G·mL^{-1}, Pfizer Animal Health, Lee's Summit, MO, USA) was administered using the same schedule as in groups 1 and 2 at equal dosages of 20 000 IU·kg^{-1} procaine and benzathine penicillin G IM (total dosage of 40 000 IU·kg^{-1} of penicillin G). Due to the large volume of drug when procaine + benzathine penicillin G was used, the injection solution was divided into 2 doses each site and injected 2.5 cm apart. Sheep in group 4 were injected with procaine plus benzathine penicillin G at the same dose as in group 3 into the left *longissimus dorsi* (probe site) on day 4 and into the right *quadriceps femoris*, left *quadriceps femoris* and right *triceps brachii* muscles in that order from days 5 to 7.

Table I. Summary of implanting, sampling, injecting and slaughtering times of sheep.

Day	Intramuscular injection	Left probe	Right probe	Group of sheep slaughtered
1			implanted	
2				
3				
4	Penicillin G[a,c]	Implanted + Penicillin G[b]		
5	Penicillin G[b,c]			
6	Penicillin G[b,c]			
7	Penicillin G[b]	Implanted + Penicillin G[c]		
8				4
9				1
10				
11				2 and 3

[a] Procaine penicillin G 20 000 IU·kg^{-1} as 300 000 IU·mL^{-1} aqueous suspension was used in sheep groups 1 and 2. Procaine penicillin G 20 000 IU·kg^{-1} as 150 000 IU·mL^{-1} procaine penicillin G plus 150 000 IU·mL^{-1} benzathine penicillin G suspension was used in sheep groups 3 and 4.
[b] Sheep group 4.
[c] Sheep group 1, 2 and 3.

The sheep were stunned by captive bolt pistol followed immediately by exsanguination, in groups of 5 animals on days 8 (group 4), 9 (group 1) and 11 (groups 2 and 3) as shown in Table I. Six 4 cm cubes of right *quadriceps femoris*, left *quadriceps femoris*, right *triceps brachii*, right *longissimus dorsi*, left *longissimus dorsi* and left *triceps brachii* (the injection and control sites) were excised from each animal for histopathological study and penicillin G analysis.

2.3. Sample processing and evaluation

Tissue samples were fixed in 10% formalin and embedded in paraffin. Tissue was sectioned and stained with hematoxylineosin. Histopathological specimens of muscle from the following sites were examined: surrounding the left (injection site) and right (control) ultrafiltration probes, the injection sites for procaine penicillin G or procain + benzathine penicillin G, and uninjected muscle that served as control. The tissue sections were labeled and evaluated in a "blinded" fashion. The ordinal scoring system used in this study was similar to that of Ma et al. (1998) [12]. Each slide was scored using a semiquantitative method by assigning a rank score based on overall lesion severity, type of inflammatory response, and type of reparative response.

Overall lesion scores ranged from 0 to 5; 0 = no lesion, 1 = minimal lesion, 2 = mild lesion, 3 = moderate lesion, 4 = marked lesion and 5 = severe lesion. The inflammatory response scores ranged from 0 to 5; 0 = no inflammation, 1 = acute inflammation, 2 = subacute inflammation, 3 = chronic inflammation, 4 = chronic active inflammation and 5 = chronic granulomatous to pyogranulomatous inflammation. The reparative response scores ranged from 0 to 5; 0 = no reparative response, 1 = neovascularization, 2 = immature granulation, 3 = mature granulation tissue formation, 4 = mild fibrosis and 5 = moderate to severe fibrosis.

2.4. Statistics

All histopathological scores were ranked for statistical analysis [4]. Because many combinations of treatment and duration were missing, in order to permit intended comparisons between treatments at a given time, or a treatment at various times, the independent variable was defined as the treatment + duration of treatment. Analysis of variance was used to test the differences among variables. If significant overall differences were found, all pairwise combinations were examined for significance ($p = 0.05$) using Tukey's HSD test [13].

3. RESULTS

The overall, inflammatory and reparation scores of histopathological lesions found in experimental sheep are shown in Figures 1, 2 and 3.

The histopathological sections obtained from each tissue sample from the implanted ultrafiltration probe sites showed chronic granulomatous inflammation with immature and mature granulation tissue formation (Figs. 4 and 5). Longer implantation time increased magnitude of granulomatous inflammation and granulation tissue formation ($p = 0.01$ and 0.04). Intralesional multinucleate giant cells and foreign material were also observed. Myofiber degeneration and separation also were present. No evidence of infection was found in any tissue sections.

The histopathological sections obtained from intramuscular injection sites of procaine penicillin G and procaine plus benzathine penicillin G also showed chronic granulomatous inflammation with immature and mature granulation tissue formation. The magnitude of tissue inflammatory response was not significant between the intramuscular injection with procaine plus benzathine penicillin G versus the injection of procaine penicillin G alone. Both injected formulations caused less granulomatous

inflammation and granulation tissue forma-
tion than that of the implanted ultrafiltra-
tion probe site ($p \leq 0.01$).

The histopathological sections obtained
from each tissue sample from the implanted
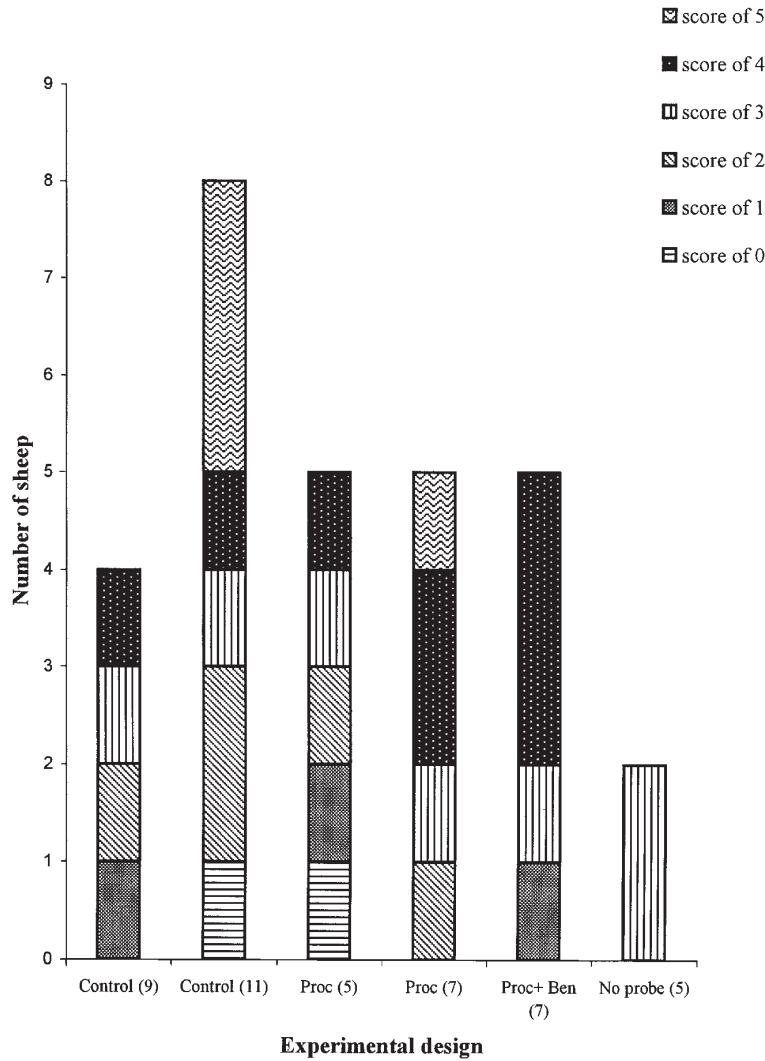ultrafiltration probe site that was injected

Figure 1. Overall score of histopathological lesions in experimental sheep. Control (9) represents
lesions at implanted probe site on day 9. Control (11) represents lesions at implanted probe site on
day 11. Proc (5) represents lesion at implanted probe site with injection of procaine penicillin G on
day 5 post-injection. Proc (7) represents lesion at implanted probe site with injection of procaine
penicillin G on day 7 post-injection. Proc + Ben (7) represents lesion at implanted probe site with injec-
tion of procaine plus benzathine penicillin G on day 7 post-injection. No probe (5) represents lesions
at injection site of procaine plus benzathine penicillin G without probe implantation on day 5 post-
injection.

with procaine or procaine plus benzathine penicillin G showed greater magnitude of chronic granulomatous tissue inflammation with immature and mature granulation tissue formation, compared to either implanted ultrafiltration probe site without injection ($p = 0.01$ and 0.03) or intramuscular injection sites ($p = 0.01$ and 0.06). The magni-
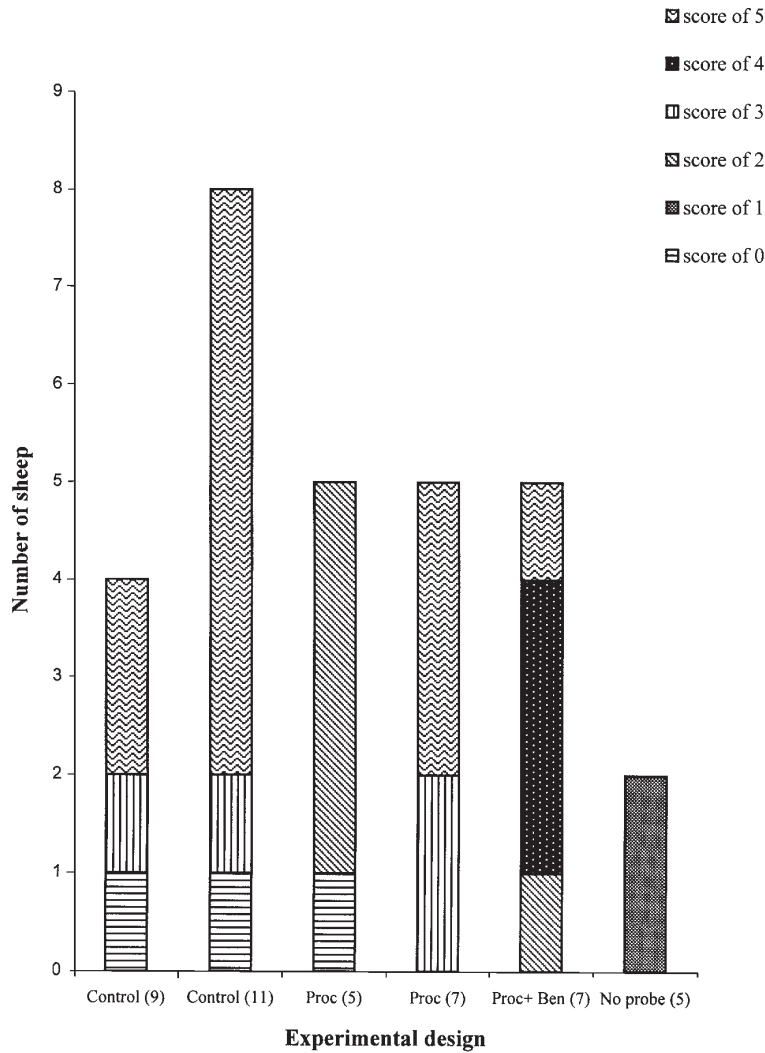

Figure 2. Inflammatory score of histopathological lesions in experimental sheep. Control (9) represents lesions at implanted probe site on day 9. Control (11) represents lesions at implanted probe site on day 11. Proc (5) represents lesion at implanted probe site with injection of procaine penicillin G on day 5 post-injection. Proc (7) represents lesion at implanted probe site with injection of procaine penicillin G on day 7 post-injection. Proc + Ben (7) represents lesion at implanted probe site with injection of procaine plus benzathine penicillin G on day 7 post-injection. No probe (5) represents lesions at injection site of procaine plus benzathine penicillin G without probe implantation on day 5 post-injection.

tude of granulomatous inflammation and granulation tissue formation did not differ where the implanted ultrafiltration probe site was injected with procaine versus procaine plus benzathine penicillin G.
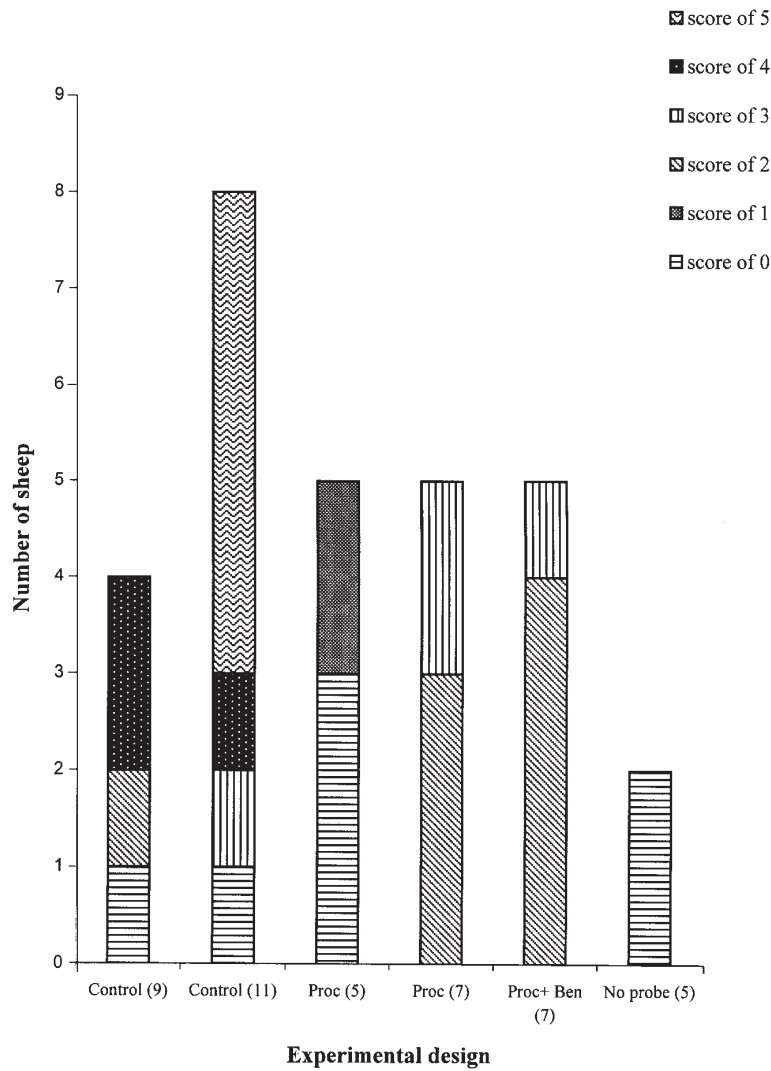

Figure 3. Reparation score of histopathological lesions in experimental sheep. Control (9) represents lesions at implanted probe site on day 9. Control (11) represents lesions at implanted probe site on day 11. Proc (5) represents lesion at implanted probe site with injection of procaine penicillin G on day 5 post-injection. Proc (7) represents lesion at implanted probe site with injection of procaine penicillin G on day 7 post-injection. Proc + Ben (7) represents lesion at implanted probe site with injection of procaine plus benzathine penicillin G on day 7 post-injection. No probe (5) represents lesions at injection site of procaine plus benzathine penicillin G without probe implantation on day 5 post-injection.
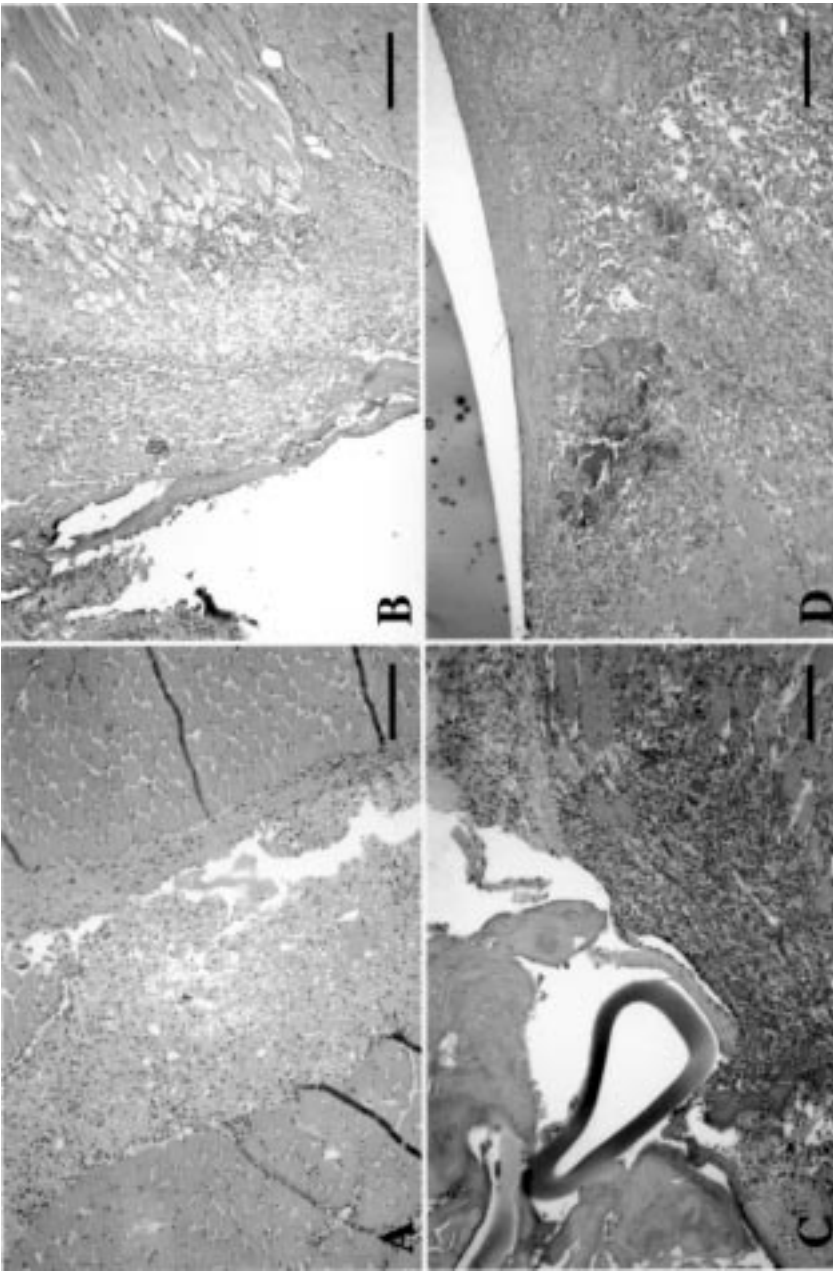
Figure 4. Histopathological lesions of tissue samples from 4 study groups (original magnification ×10: bar represents 160 µm). (**A**) Intramuscular injection site of procaine penicillin G without ultrafiltration probe implantation with an inflammatory score of 1. (**B**) Implanted ultrafiltration probe site without penicillin G injection with an inflammatory score of 5. (**C**) Implanted ultrafiltration probe site with procaine penicillin G injection with an inflammatory score of 4. (**D**) Implanted ultrafiltration probe site with procaine plus benzathine penicillin G injection with an inflammatory score of 4.
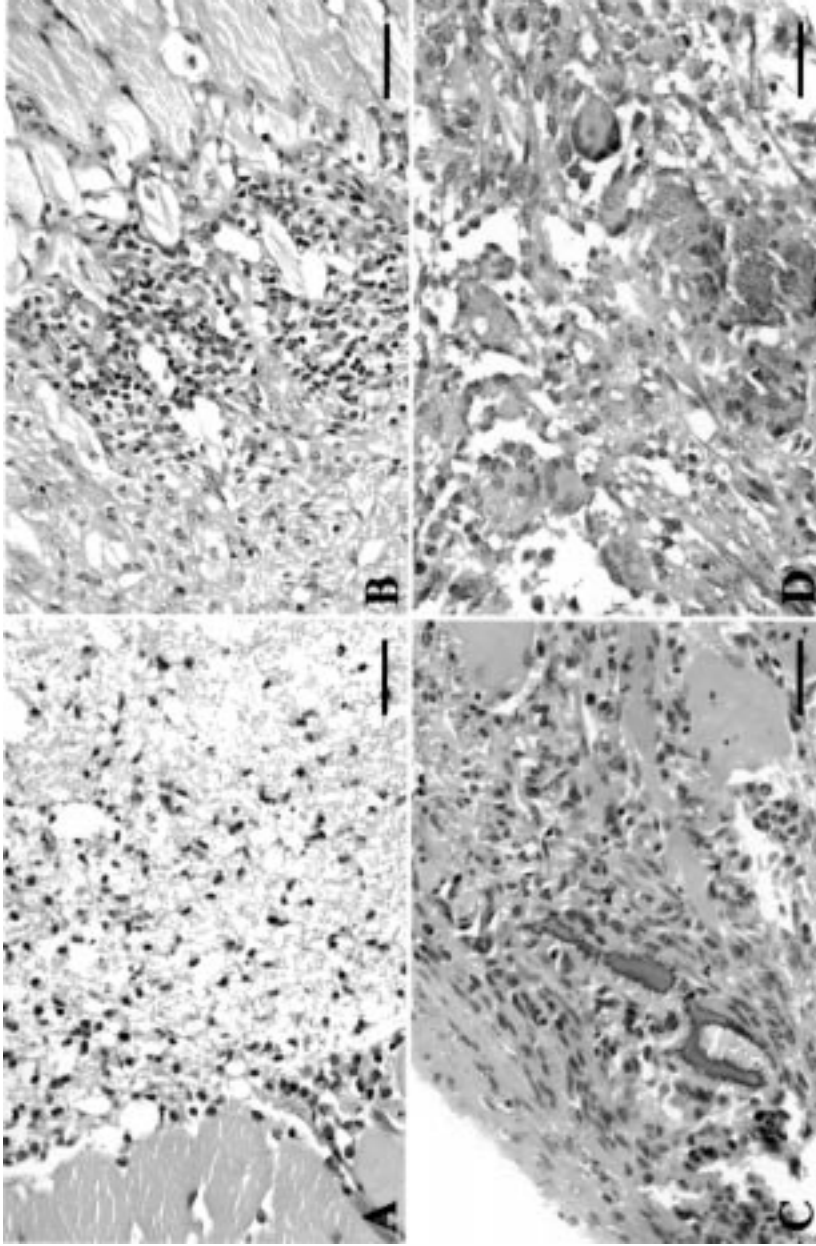
Figure 5. Higher magnification of histopathological lesion of tissue samples from Figure 1 (original magnification × 40: bar represents 40 μm). Captions indicate same tissues as Figure 1.

The histopathological sections of control muscular tissue (no injection made, no probe) showed no inflammatory response.

The magnitude of inflammatory response was dependent on both time of implantation and the time between such injection and tissue sampling. Longer periods of implantation resulted in much greater cellular infiltration and tissue granulation formation. The elapsed time between an injection and tissue sampling time which ranged from 1 to 4 days resulted in the change from acute inflammatory lesions to chronic inflammation.

4. DISCUSSION

Studies of the pharmacokinetics of drugs in interstitial fluid give further insight into drug disposition [1, 2, 5, 17]. Cooke et al. [5] successfully used microdialysis membranes with low molecular weight cut off that excluded large proteins to investigate the disposition of penicillin G in subcutaneous interstitial fluid after intravenous administration. Commercially available ultrafiltration probes have also proven useful for investigating drugs and endogenous ions in the subcutaneous space [8–11]. These workers reported that the ultrafiltration probes had good characteristics; excluding large molecules, ability to collect small volume samples, continuous sampling without causing inflammatory response, providing good recoveries and also yielding undiluted sample of tissue fluid. Based on these studies we anticipated similar suitability for intramuscular implantation in sheep.

However, the UF probe caused inflammation and degeneration of the surrounding muscle both microscopically and macroscopically. The microscopic cellular responses were those of inflammation and repair. These microscopic histopathological alterations at the probe site differed in intensity and character depending on the elapsed time after the probe was implanted.

In the 8–11 day period of this study, the longer the time of implantation, the greater the magnitude of inflammation and tissue granulation formation. The degree of inflammation and myofiber degeneration at the UF probe site were greater than at the control injection sites. The hypothesis that the UF probe would cause minimal inflammation was shown to be false. Since the probes induced tissue damage, their use to collect samples at intramuscular injection sites in sheep might not be appropriate.

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