A STUDY OF PENICILLIN CONCENTRATIONS IN BOVINE CONJUNCTIVAL SAC FLUID AS IT PERTAINS TO THE TREATMENT OF *MORAXELLA BOVIS* INFECTION

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ABSTRACT

Infectious bovine keratoconjunctivitis is one of the commonest eye diseases of cattle. A specialised organism *Moraxella bovis*, is generally held to be responsible for the often serious damage to the cornea and the conjunctiva. Temporary blindness is common but even without treatment, most cattle eventually regain their vision. Although the disease has been recognised for more than 30 years in most cattle-farming areas of the world, only in the past 8-10 years has it become evident in New Zealand.

A wide range of antibacterial products has been used for treatment but there has been very little definitive work undertaken which would form a sound basis for any schedule of medication. In view of the information lacking in this respect, it was decided to study the pharmacokinetics of an antimicrobial drug in the conjunctival sac fluid after different formulations of the drug had been administered by different routes. Penicillin was chosen as the model antibiotic because it is remarkably free of side effects, effective against *M. bovis* and available in a range of products suitable for administration by various means. The overall aim of the work was to determine a concentration-time profile for penicillin in conjunctival sac fluid (CF) and it was reasoned that this data could then be used to establish a schedule of treatment that would produce an optimum effect against *M. bovis*.

The preliminary requirement of the research programme was to

investigate a suitable method of collecting unchanged samples of CF over a number of days (i.e. 1-7 days). As soon as the project was initiated, it became clear that any substantial distress to the animals caused lachrymation, and the possibility of the CF then containing endogenous antibacterial substances could not be discounted. Of the three methods of collection tested, the use of blunted capillary tubes was found to be best for the purpose because the method avoided any local tissue irritation and the cattle soon learned to tolerate any associated handling and minor restraint. Special safeguards were built into the experiment to confirm the absence of antibacterial substances other than penicillin. The specificity of the inhibitory substance in CF, namely penicillin, was regularly confirmed by testing for parallelism against standard dilutions of penicillin, and periodically by neutralizing all antibacterial activity in a sample, using penicillinase.

Estimations of the penicillin concentration in CF samples were carried out by means of the agar-well diffusion technique. Minor modifications to the basic assay system were required to ensure that the sensitivity would cover the range of penicillin concentrations expected to appear in CF. After a series of titrations involving the size of the inoculum of the indicator organism, the depth and volume of the agar medium, the volume of the test solution for each well and the incubation schedule, each variable was standardized for all subsequent assays. A large plate (28 x 28 cm, containing 64 wells 4.5 mm in diameter,

2.5 cm apart) permitted the assay of 12 CF samples alongside four standard dilutions of penicillin under uniform conditions. Using *Bacillus subtilis* as the indicator organism, the assay system was sensitive in the range between 10 and 0.07 iu/ml penicillin, with a standard error of predicted values of 0.04-0.17.

In order to nominate a penicillin concentration in CF that would be effective against *M. bovis*, the penicillin sensitivity of several strains of the organism were measured in terms of their minimum inhibitory concentration (MIC). The four New Zealand isolates tested were highly sensitive; most having a MIC of penicillin of 0.03 iu/ml, and this was identical for the bactericidal concentration. After making allowance for an *in vivo* safety factor of 5, the minimum therapeutic concentration (MTC) of penicillin against *M. bovis* was defined for this series of experiments to be 0.15 iu/ml. The length of time that the penicillin concentration in CF remained equal to or above the MTC following treatment with any particular product, was considered as the duration of therapeutic concentration (DTC; hours) for that particular treatment.

The major experiments using clinically normal cows involved the estimation of penicillin concentration in CF following systemic, subconjunctival or topical administration. Every treatment was repeated in five or six animals but without exception any variation in the DTC between eyes and animals was found to be not significant. In all experiments, the decline in penicillin

concentration in CF followed an exponential pattern, irrespective of the route of administration.

A series of systemic injections was carried out by the appropriate route using three different products of penicillin at a standard dose-rate of 20,000 iu/kg. Penicillin concentrations observed in CF (± SEM) following the intravenous injection of sodium benzyl penicillin (peak 2.0 iu/ml and DTC 5.5 ± 0.25 hours) and the intramuscular injection of procaine penicillin (peak 1.0 iu/ml and DTC 16.5 ± 1.25), were considered inadequate for the treatment of IBK.

Penethamate hydriodide, administered by either the intramuscular or the subcutaneous route, achieved an approximate peak concentration of 3.0 iu/ml and produced a minimum therapeutic duration of 61 ± 5.57 hours. Such a difference between the kinetics of penethamate hydriodide and benzyl penicillin was attributed to the greater lipid solubility of the diethylamino-ethyl ester of penicillin. Although the profile of penicillin in CF following penethamate administration seems favourable, the cost of the product would probably prohibit its regular use. In a further experiment in which half the dose was used, the DTC was reduced to 23.5 ± 4 hours.

A subconjunctival injection of procaine penicillin at a dosage of 6×10^5 iu in 2 ml , administered either through the skin or through the conjunctiva, achieved an approximate peak of 8 iu/ml

for both routes and DTCs of 67.6 ± 5.0 hours and 40 ± 2.6 hours respectively. The faster rate of penicillin decay following an injection given through the conjunctiva, is possibly attributable to the back diffusion of the drug through the needle puncture. In general, the penicillin profile in CF following a subconjunctival injection is conducive to an extended bactericidal effect and the trial results tend to confirm the clinical impressions of its usefulness in the field. Treatment by this means is relatively cheap and easily undertaken. If a more prolonged effect is desirable, another dose might be administered two days after the first.

Topical application of sodium benzyl penicillin in aqueous solution at a concentration isotonic with 0.9% saline, produced a DTC in CF for 12.6 ± 1.5 hours. This duration is considered long for a water soluble salt in an aqueous base. When this salt and other less water soluble ones were formulated in an ointment base, the time of effect was significantly prolonged. Sodium benzyl penicillin and procaine penicillin in the ointment base, produced DTCs of 39.8 ± 2 hours and 37 ± 4 hours respectively, while the ointment formulation of benethamine penicillin produced a DTC of 56 ± 4.5 hours. The prolonged decline observed for all eye ointments can be partly accounted for by the viscous nature of the base but other differences may be dependent on the relative water solubility of each penicillin salt. In addition, the various structures of the surface mucosae such as crypts and specialised cells, are likely to retard free diffusion

and therefore retain penicillin, in CF. The extent of dissociation of a substance depending on its pKa may also influence the overall rate of decline.

Regular examination of the treated eyes and cell counts undertaken on CF samples, did not indicate any inflammatory reaction even after repeated application of ointments.

The various penicillin profiles observed in CF now provide a sound basis for establishing treatment schedules. Optimum treatment schedules can be advocated for different penicillin products, based on concentrations and durations that could be expected to control *M. bovis* infection in superficial tissues of the eye. However, in order to confirm the therapeutic effectiveness of such schedules, controlled clinical trials using infected animals are obligatory.

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PREFACE

Infectious bovine keratoconjunctivitis (IBK) is well known as an economically important eye condition in cattle and it occurs in most farming areas of the world. Outbreaks of the disease were first reported in New Zealand only recently (Anon,1975), but it quickly became established and the condition is now prevalent in cattle throught the country.

The disease is caused by the bacterium *Moraxella bovis* often in association with certain predisposing factors which have been incriminated in spontaneous outbreaks. While the disease is not responsible for a high rate of mortality, and indeed outbreaks are eventually self-limiting, its economic importance stems from unthriftiness in diseased animals and consequent losses in production. In addition, the disease causes disruption to the normal farm routine as blind animals warrant extra attention.

In the long term the use of vaccines seems the most promising approach to control of IBK but products giving a high protection rate are not yet available. Fortunately *M. bovis* is sensitive to a range of commonly used antibacterial drugs and treatment, particularly of early cases, is usually successful.

Effective chemotherapy of IBK demands the maintenance of therapeutic concentrations of the administered drug in fluids covering the bovine eye for sufficient time to eliminate the

infective organism , *M. bovis*. In spite of the variety of chemicals that have been used over the years for that purpose, there is very little information in the literature on drug concentrations obtainable in conjunctival fluids after administration of products by any route.

Accordingly it was decided to undertake investigations in this area; to determine if the route of administration and any specific features of the drug product would influence either the peak concentration obtainable in conjunctival sac fluid or the length of time effective levels could be sustained.

Penicillin may not necessarily be the drug of choice to treat IBK under field conditions, but it was chosen as the model antibiotic in this study for special reasons. These were, its overall safety, its effectiveness against *M. bovis* and in particular, the range of products available containing different salts of penicillin in a form suitable for administration by a number of routes.

In broad terms, this study set out to establish basic information on the pharmacokinetics of penicillin in conjunctival sac fluids. It was hoped that the investigations would lead to a greater understanding of the drug's distribution and such knowledge would enable more rational schedules of treatment to be devised.