

SOME FACTORS INFLUENCING THE PROGNOSIS OF SOME COMMON BOVINE NEUROLOGICAL DISEASES WITH PARTICULAR REFERENCE TO THE CONCENTRATION OF PENICILLIN G REACHED IN CEREBROSPINAL FLUID

Scott, P.R.⁽¹⁾, Sutton, D.⁽²⁾

1. Royal (Dick) School of Veterinary Studies
University of Edinburgh EH25 9RG, Scotland.
2. Mycofarm Ltd., Cambridge CB4 4FP, England.

Summary

Treatment of nine cases of acute bacterial meningitis with high dose penicillin G therapy was unsuccessful in all cases. Cerebrospinal fluid (CSF) analysis demonstrated a markedly elevated protein concentration indicating disruption of the blood brain barrier. Two of five listeriosis cases, with slight elevation of CSF protein, and both cases of vestibular abscess responded well to antibiotic therapy.

An enzyme-linked, immunosorbent assay (ELISA) method has been developed for the determination of penicillin G in ruminant cerebrospinal fluid. Analysis of cerebrospinal fluid from eleven clinically normal bovines, collected two hours after the intramuscular injection of penicillin G at 44,000 iu/kg body weight, revealed concentrations of 0.006 mcg/ml penicillin G in five animals, less than 0.006 mcg/ml in three animals and between 0.023 to 0.056 in three animals.

Penicillin G concentration in cerebrospinal fluid two hours after the intramuscular injection of 44,000 iu/kg penicillin G in one case each of brain abscess and listeriosis and two cases of acute bacterial meningitis were less than 0.006 mcg/ml in each case. In one case of listeriosis the CSF penicillin G concentration was 0.038 mcg/ml two hours after injection.

Cerebrospinal fluid samples collected from three animals with acute bacterial meningitis 48 hours after initiation of high dose penicillin G treatment revealed penicillin G concentrations of 0.09, 1.02 and 1.70 mcg/ml.

It is concluded that despite high dose treatment, penicillin G does not penetrate well into the CSF of healthy bovines nor within two hours of treatment of certain neurological diseases. By 48 hours of treatment of bacterial meningitis with high doses of penicillin G, the cerebrospinal fluid penicillin G concentration had reached effective minimum inhibitory concentrations against some of the common central nervous system bacterial pathogens but not against E. coli.

Introduction

The first report of bovine spongiform encephalopathy⁽¹⁾ in the United Kingdom during 1987, and subsequent statutory reporting of suspected cases of bovine spongiform encephalopathy, demanded that the practising veterinarian perform a thorough neurological examination of suspicious cases. Despite detailed case reports⁽²⁾⁽³⁾, between 10-12% of suspected cases of bovine spongiform encephalopathy failed to reveal pathognomonic histopathological changes at necropsy. Listeriosis is the most important differential diagnosis of bovine spongiform encephalopathy in the United Kingdom. In the first six months of 1990⁽⁴⁾ for example, it has been estimated that from over 600 histopathologically negative bovine spongiform encephalopathy animals, between 90 and 180 were probably listeriosis cases. This is a costly exercise, both in terms of indemnity and destruction of valuable animals.

A recent report⁽⁵⁾ describes a good prognosis for listeriosis cases which are still ambulatory at the commencement of penicillin G treatment at 44,000 iu/kg bodyweight b.i.d. This study investigated some factors which may affect the penetration of

penicillin G across the blood brain barrier with particular reference to the prognosis of bovine neurological disease cases.

Materials and Methods

All animals were subjected to a thorough clinical examination with particular reference made to the neurological examination⁽⁶⁾. An attempt was made to determine the duration of neurological dysfunction as precisely as possible. The bodyweight of the animal was estimated and penicillin G (Depocillin^(R), Mycofarm UK) injected intramuscularly at a dose rate of 44,000 iu/kg b.i.d.

Cerebrospinal fluid samples were collected under local anaesthesia from the lumbosacral site⁽⁷⁾. An aliquot of cerebrospinal fluid was frozen immediately after collection and stored at -20°C until analysed for penicillin G concentration. Penicillin G concentration in cerebrospinal fluid was determined using a direct, competitive, enzyme-linked, immunosorbent assay (ELISA)⁽⁸⁾.

Specific gravity of cerebrospinal fluid was determined with a hygrometer. Total white blood cell counts of cerebrospinal fluid were performed on a haemocytometer. Aliquots of cerebrospinal fluid were concentrated by cytospin within 2 hours of collection, air-dried and stained with Leishman stain. Protein concentration was determined by the pyrogallol method (Randox Laboratories Ltd.)

Confirmation of diagnosis was obtained at necropsy for those animals which were destroyed on humane grounds due to deterioration of the clinical condition.

Results

Treatment of acute bacterial infections of the bovine central nervous system was undertaken with high dose penicillin G therapy at 44,000 iu/kg bodyweight b.i.d. All nine cases of acute bacterial meningitis affecting three to ten day old calves failed to respond to therapy. Analysis of CSF revealed a markedly elevated CSF protein concentration in all animals at the start of treatment (Table 1). Two of five cases of listeriosis and both cases of peripheral vestibular abscess responded to high dose penicillin G therapy. Cerebrospinal fluid results from three listeriosis cases and both vestibular abscess cases are presented in Table 1. There was no difference in CSF composition from one of the listeriosis cases which responded to treatment and two animals which did not. In the three listeriosis cases that were destroyed on humane grounds due to recumbency and deterioration of the clinical condition 1 to 10 days after initiation of therapy, the mean lumbar CSF protein concentration had increased from 0.8 to 1.4 g/l.

Cerebrospinal fluid collected from eleven clinically normal adult bovines two hours after the intramuscular injection of penicillin G at 44,000iu/kg bodyweight revealed penicillin G concentrations of <0.006 mcg/ml in three animals, 0.006 mcg/ml in five animals and between 0.023 to 0.056 in three animals. Gross analysis of cerebrospinal fluid revealed a normal protein concentration of 0.3 g/l and normal white blood cell count and cytology.

Penicillin G concentration in cerebrospinal fluid two hours after the intramuscular injection of 44,000 iu/kg penicillin G in one case each of brain abscess and listeriosis and two cases of acute bacterial meningitis were less than 0.006 mcg/ml in each case. In one case of listeriosis the CSF penicillin G concentration was 0.038 mcg/ml two hours after injection.

In three animals with acute bacterial meningitis, with considerable disruption of the blood brain barrier, indicated by a mean CSF protein concentration of 2.5 g/l at the start of antibiotic therapy, the CSF penicillin G concentrations 48 hours later were 0.094, 1.02 and 1.70 mcg/ml.

Protein electrophoresis of cerebrospinal fluid in ovine⁽⁸⁾ and bovine⁽⁷⁾ neurological diseases has revealed a consistent albumin percentage value irrespective of the CSF protein concentration. This has been interpreted as indicating a lack of

intrathecal immunoglobulin production in those neurological diseases studied. In this study an elevated CSF protein concentration has been interpreted as indicating the extent of disruption of the blood brain barrier. The small variation in the serum albumin concentration of between 26 to 32 g/l was considered to have little influence on the interpretation of CSF total protein concentration which ranged from 0.2 g/l to greater than 6.4 g/l.

TABLE 1
Treatment response and cerebrospinal fluid analysis
of some common bovine neurological diseases

	Bacterial meningitis	Listeriosis	Vestibular abscess	Normal
Number	9	5	2	11
Treatment response	0	2	2	
CSF analysis				
Protein (g/l) median range	2.6 0.95-6.4	0.83 0.8-0.87	0.24	0.3
Cells ($\times 10^6/l$)	0.3 0.012-5.4	0.3 0.12-0.7	0.05	0.012
Neutrophil %	79 4-100	72 45-98	occ.	<5
Lymphocyte %	0 0-20	10 2-15		>95
Histocyte %	20 0-94	8 0-25		

occ. - occasional cell seen

Discussion

In this study cerebrospinal fluid analysis in the neonate, which revealed a marked elevated protein concentration, increase in total white blood cell count and predominance of neutrophils, was an accurate indicator of acute bacterial infection of the central nervous system. None of these animals survived despite treatment with high dose of penicillin G.

In five cases of listeriosis affecting grass silage-fed cows, clinical signs of facial nerve paralysis were observed in the early stages in only one cow. In the other four cows the main presenting clinical signs were depression, anorexia, a marked drop in milk yield, weight loss, ataxia with the trunk leaning towards the affected side and constant pushing forward. Some of these clinical signs are not dissimilar to those observed with bovine spongiform encephalopathy and this may explain why some cases of listeriosis have been mistaken for bovine spongiform encephalopathy⁽⁴⁾.

In listeriosis there was no difference between the CSF analysis of the animal that responded to treatment and the two cows that did not. The small increase in CSF

protein concentration that occurred between recognition of clinical signs of listeriosis and destruction due to recumbency (1-10 days) would indicate limited meningeal reaction during that period. While insufficient numbers of cows have been studied to interpret the prognostic index of CSF analysis in bovine listeriosis, the major application of this technique is the accurate differentiation of listeriosis or other inflammatory condition of the CNS from bovine spongiform encephalopathy⁽⁷⁾.

The results from this study demonstrate that penicillin G does not penetrate the intact blood brain barrier of healthy animals well, even at high dose rates. Two hours after injection of penicillin G there was no increase in CSF penicillin G concentration in those animals with early neurological disease compared to control animals.

The higher penicillin G concentrations recorded in three calves with bacterial meningitis 48 hours after treatment commenced may indicate passage of penicillin G across the disrupted blood brain or a cumulative effect from repeated injections.

The lack of treatment response in neonatal meningitis cases is probably due to the extent of infection when clinical signs are observed. A reasonable treatment response was achieved in those cases of listeriosis and vestibular abscess where there is only moderate disruption of the blood brain barrier. The minimum inhibitory concentration (MIC₉₀) of penicillin G for Listeria monocytogenes has been quoted as 0.02 mcg/ml⁽¹⁰⁾. While such concentrations of penicillin G were achieved in all three cases of bacterial meningitis 48 hours after the commencement of treatment, the mean CSF protein concentration in these animals was 2.5 g/l compared to 0.8 g/l in listeriosis cases. The range of penicillin G concentrations achieved from 0.09 to 1.02 are well above quoted minimum inhibitory concentration figures⁽¹⁰⁾ for L. monocytogenes and it is possible that the CSF penicillin G concentrations achieved in listeriosis cases 48 hours after treatment commenced would be above the minimum inhibitory concentration. More work is obviously necessary before firm conclusions can be drawn from such a small survey.

Provisional results would indicate that penicillin G therapy of neonatal bacterial meningitis cases affords a hopeless prognosis. The elevated CSF protein concentration would indicate that considerable damage to the meninges has occurred before clinical signs are reported. Efforts would best be directed towards preventive measures ensuring adequate passive transfer of immunoglobulin in colostrum and reducing environmental bacterial challenge as bacterial meningitis probably results from entero-invasion in hypogammaglobulinaemic neonates kept in dirty conditions.

High dose penicillin G treatment of listeriosis cases was recommended by Professors Rebhun and de Lahunta in 1982. These authors⁽⁵⁾ describe a good prognosis for listeriosis cases provided the animals are still ambulatory when treatment commences. Four cases in the present study were ambulatory at the start of treatment, two of which made a complete recovery.

If the minimum inhibitory concentration of common bovine isolates of L. monocytogenes is 0.02 mcg/ml⁽¹⁰⁾ and a linear relationship exists between CSF protein concentration and CSF penicillin G concentration, it is possible that minimum bactericidal concentrations of penicillin G are reached within two days of high dose penicillin G treatment. If this is so it may be possible to reduce the subsequent dose of penicillin G after two days. In agricultural veterinary practice the cost of treatment is critical to the farming client and a prolonged period of high dose penicillin G is prohibitively expensive apart from the valuable dairy cow. Work is currently underway to investigate the CSF penicillin G concentrations and treatment response of listeriosis cases to a treatment regimen of 44,000 u/kg penicillin G q.i.d. for three days followed by 22,000 u/kg b.i.d. for seven days.

Initial studies with sheep, a species more susceptible to listeriosis, are also in progress and five of nineteen (26%) have responded to this treatment regimen. This positive treatment response rate is higher than that reported in the literature⁽¹¹⁾.

The results presented in this study illustrate that in neonatal calves cerebrospinal fluid analysis can be usefully employed to confirm the provisional diagnosis of acute bacterial meningitis. Penicillin G is not recommended for the

treatment of such cases. Cerebrospinal fluid analysis of bovine spongiform encephalopathy cases⁽⁷⁾ revealed no significant departure from normal and as such these findings would permit differentiation from the bovine listeriosis cases as presented in this study and by Rebhun and de Lahunta⁽⁵⁾.

Cases of listeriosis and brain abscess afforded the best prognosis but these results can be improved upon. Work is continuing on factors which influence the CSF penicillin concentration in ruminant neurological disease conditions and the authors would be pleased to hear from colleagues interested in this work. In particular, these investigations are concentrating on the dose of penicillin G that is required to achieve 10-30 times the minimum bactericidal concentration of penicillin G in CSF⁽¹⁰⁾ on the first day of treatment of listeriosis cases.

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ABSTRACT

Cerebrospinal fluid analysis from nine calves with acute bacterial meningitis revealed an elevated protein concentration, indicating disruption of the blood brain barrier, and increased white blood cell concentrations with a predominance of neutrophils. The concentrations of penicillin G 48 hours after treatment with 44,000 u/kg bodyweight b.i.d. in three of these calves were 0.09, 1.02 and 1.70 mcg/ml. Despite this intensive antibiotic therapy regimen all the calves died.

In five cows with listeriosis the marginal increase in cerebrospinal fluid protein concentration indicated only mild disruption of blood brain barrier function. Two of these five listeriosis cases and two cases of vestibular abscess responded well to high dose penicillin G therapy despite low cerebrospinal fluid penicillin G concentrations two hours after a single intramuscular injection.

It is concluded that penicillin G does not penetrate the intact blood brain barrier well within two hours of injection in healthy bovines. Effective minimum inhibitory concentrations of penicillin G were achieved against some common CNS pathogens including *L. monocytogenes* where there was disruption of the blood brain barrier.

ZUSAMMENFASSUNG

Die Analyse der Hirnrückenmarkflussigkeit von neun Kälbern mit akuter bakterieller Meningitis zeigte eine erhöhte Eiweisskonzentration, welches auf eine Störung der Blut-Hirn-Schranke und eine erhöhte Anzahl von weissen Blutkörperchen mit überwiegend neutrophilen granulozyten hindeutere. Die Konzentration von Penicillin G, 48 Stunden nach der Behandlung mit 44,000 u/kg Körpergewicht b.i.d. betrug in drei dieser Kälber 0.09, 1.02 und 1.70 mcg/ml. Trotz dieser intensiven Antibiotikumbehandlung starben alle Kälber.

Bei fünf Kühen mit Listeriose, die geringfügige Hirnrückenmarkflussigkeitskonzentration zeigte eine nur geringfügige Störung der Blut-Hirn-Schranke. Zwei dieser fünf Listeriose-Fälle und zwei Fälle von vestibularen Abszessen zeigten guten Erfolg bei einer hochdosierten Penicillin G. Behandlung, trotz niedriger Hirnrückenmarkflussigkeitskonzentration Stunden nach einer einzigen intramuskulären Injektion.

Daraus lässt sich schliessen, dass Penicillin G die intakte Blut-Hirn-Schranke bei gesunden Rindern nicht vor Ablauf von gut zwei Stunden nach der Injektion nicht durchdringt. Wirksame minimale inhibitorische Konzentration von Penicillin G wurden gegen einige verbreitete ZNS. Pathogene erzielt, einschliesslich L. monocytogene, wohin eine Störung der Blut-Hirn-Schranke auftrat.

ABSTRACTO

El análisis del líquido cefalorraquídeo, tomado de nueve terneras con meningitis bacterial aguda, reveló una elevada concentración de proteínas, lo que indica una alteración en la barrera hemato cerebral. También se vió una subida en la concentración de glóbulos blancos, predominando los neutrófilos. La concentración de penicilina G, 48 horas después del tratamiento con 44,000u/kg peso vivo b.i.d., en tres de las terneras fueron, 0.09, 1.02, 0.70 mcg/ml, y a pesar de una intensa terapia con antibióticos, murieron todas las terneras.

En 5 vacas con listeriosis el incremento marginal de la concentración de proteínas del líquido cefalorraquídeo indico una ligera alteración en el funcionamiento de la barrera hemato cerebral. Dos de estos cinco casos y otros dos con absceso vestibular respondieron bien a una terapia con altas dosis de penicilina G, a pesar de la baja concentración de penicilina G encontrada en el líquido cefalorraquídeo dos horas después de una sola inyección intramuscular.

La conclusión sacada, es que la penicilina G no penetra bien a través de una barrera hematocerebral intacta, durante las dos horas siguientes de inyectar bóvidos en buen estado de salud.

Mínimas concentraciones de Penicilina G fueron efectivas contra algunos patógenos CNS, L. monocitogena incluida, donde se encontró alteración de la barrera hemato-cerebral.