

Ivermectin concentrations in serum and cerebrospinal fluid after intravenous administration to healthy Llamas

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Abstract

The response to anthelmintic treatment in clinical cases of meningeal worm (*Parelaphostrongylus tenuis*) infestation remains unpredictable. In a previous study, ivermectin (IVM) was not detected in cerebrospinal fluid (C.S.F.) following subcutaneous administration to healthy llamas of IVM at 500µg/kg. In the present study, the same IVM dose was added to 1 L 0.9% NaCl and administered intravenously over 30 minutes to 6 healthy llamas. C.S.F and blood were collected, at baseline and 2, 4, 6, 12, 24, 48 and 60 hr after IVM administration. Serum and C.S.F. were stored at -40°C and IVM concentration was determined using high performance liquid chromatography (H.P.L.C.). No IVM was detected in the serum or C.S.F. in baseline samples. Concentrations of IVM ranging from 2291- 7742 ng/ml were present in serum at 2 hr post dosing but values decreased to between 103-615 ng/ml at the 4 hr sampling, and ranged from 11- 48 ng/ml at 60hr. Low concentrations of IVM were found in the C.S.F. of all llamas on at least one of the sampling times. Immediately after administration of IVM three of the llamas showed transient lethargy and decreased appetite for 12-24 hours. One llama developed acute neurological signs 7 days after IVM administration and was euthanased three days later. Histopathologic examination revealed diffuse myelinic oedema in the brain and spinal cord. Two other llamas developed C.S.F. changes consistent with septic inflammation. C.S.F. concentrations of IVM can be achieved following I.V. administration at 500µg/kg, however, because of the possibility of neurological damage, I.V. administration of IVM at this dose is not recommended.

Key words: blood, cerebrospinal fluid, ivermectin concentrations, llama.

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1. Introduction

White-tailed deer are the natural hosts for meningeal worm (*Parelaphostrongylus tenuis*) and do not become clinically affected. Adult worms occur in the veins and sinuses of the dura mater and cause no injury to the brain or spinal cord. Camelids and other small ruminants (sheep and goats) on the other hand are aberrant hosts. In these animals larvae migrate up the spinal nerves to the spinal cord where they can cause focal granulomatous myelitis

and chronic multifocal microinfarctions. Intralesional parasites can be demonstrated microscopically. Larvae may also migrate to the brain and cause a cerebral lymphocytic leptomeningitis (Footnote).

Ivermectin, which is frequently used in the treatment of clinical cases of meningeal worm, is a semisynthetic derivative of avermectin belonging to the macrocyclic lactones produced by fermentation of the actinomycete *Streptomyces avermitilis* (Reinemeyer, 2001). It is highly lipophilic and hydrophobic. The major liver

metabolites are avermectin B₁ derivatives with fecal excretion as the main route of elimination (Reinemeyer, 2001). From a treatment perspective, a lipophilic anthelmintic, such as ivermectin, with the potential to penetrate into the brain and spinal cord should be efficacious in cases of *P. tenuis* infestation; however, the matter is complex. A factor, which complicates the therapeutic use of IVM in clinical cases of parasite-induced encephalomyelopathy is its poor penetration through the blood brain barrier (B.B.B.). Penetration into the central nervous system depends to a large extent on whether the particular drug or anthelmintic is a substrate for the multidrug efflux transporter P-glycoprotein (P.G.P.) in the endothelial cells of the blood brain barrier. P.G.P. is responsible for pumping noxious substances from inside cells back into the blood (Kwei et al., 1999, Schinkel et al., 1994, Tamai and Tsuji, 2000, Nobmann et al., 2001). Ivermectin transport across the luminal membrane has been demonstrated in functionally intact porcine brain endothelial capillaries (Nobmann et al., 2001). Genetic deficiency of P.G.P. in mice and some inbred dogs of the collie breed results in high concentrations of ivermectin present in the central nervous system leading to neurological signs by its action as a γ -aminobutyric acid agonist, which include ataxia, blindness, coma, respiratory compromise and death (Reinemeyer, 2001, Edwards, 2003).

Anecdotally, IVM (200 – 500 μ g/kg) is administered either orally or subcutaneously to llamas with neurological disease suspected to be due to *P. tenuis* infestation, however the efficacy of IVM is unpredictable in these circumstances. Several studies regarding the pharmacokinetics of IVM. following

subcutaneous, oral or topical administration to llamas (*Lama glama*) and camels (*Camelus dromedarius*) have shown low maximum blood concentrations (<2-8ng/ml) and which may take up to 8 days to achieve (Burkholder and Jensen, 2004, Oukessou et al., 1996, Oukessou et al., 1999, Jarnivinen et al., 2002). A report on sub cutaneous (S.C.) administration of IVM in llamas at 200 μ g/kg found median peak serum concentrations of 3ng/ml after 7days (Jarvinen et al., 2002). In another study, IVM was not detected in the blood of 3 of 8 llamas 24 hours after three consecutive S.C. injections of IVM 24 hours apart at a dose rate of 500 μ g/kg bodyweight (Van Amstel and Miller, 2003). Serum concentrations in the other 5 animals ranged from 1.4 – 16.4 ng/ml (Van Amstel and Miller, 2003). In the same study, no IVM was detected in the C.S.F. (Van Amstel and Miller, 2003). However, in 2 of 4 other observed cases with neurological signs, low concentrations (2.9 – 3.5 ng/ml) of IVM were found in the C.S.F. perhaps as a result of changes in the B.B.B. and P.G.P. efflux transport (Van Amstel and Miller, 2003).

The aim of this study was to determine whether detectable concentrations of IVM can be obtained in the C.S.F. of healthy llamas following a single intravenous administration of the drug at 500 μ g/kg.

2. Materials and Methods

The experimental protocol used in this study was reviewed and approved by the Animal Care and Use Committee of the University of Tennessee.

2. 1. Llamas

Six, healthy, adult llamas weighing between 100 – 125kg were used in the study. The llamas were housed indoors in

separate stalls and were treated with moxidectin (Cydectin Pour-On for Beef and Dairy Cattle, Fort Dodge Animal Health, Ford Dodge, Iowa, 50501) 3 weeks prior to the start of the experiment and vaccinated with *Clostridium perfringens* types C and D and *Clostridium tetani* bacterin (BarVac CD/T, Boehringer Ingelheim, Vetmedica, Inc., St. Joseph, Missouri 64506).

2.2 Catheterization and ivermectin administration

Jugular and spinal catheters were placed aseptically 24 hours before IVM administration. A catheter (Mila International Inc. Long term catheter, guidewire style, 14ga x 20cm) was placed in the right jugular vein using aseptic technique and flushed every 4 hours with heparinized-saline solution. With the animal under deep sedation (intravenous administration of a mixture of xylazine (AnaSed® injection, Lloyd Laboratories, Shenandoah, Iowa, 51601) 0.25 mg/kg and ketamine (Vetalar® Fort Dodge Animal Health, Ford Dodge, Iowa, 50501, 2mg/kg) spinal catheters (Prefix® Continuous Epidural Anesthesia Set, B Braun Medical Inc., Bethlehem, PA 18018) were placed in the lumbo-sacral space. Once spinal fluid flowed freely through the needle, the catheter was inserted and advanced about 3 cm into the subarachnoid space. The catheter was then secured to the skin just distal to the point of entry. The calculated IVM (Ivomec® 1% injection for cattle and swine, Merial LTD, Duluth, Georgia. 30096) dose for each animal (500ug/kg) was added to 1 L 0.9% saline and administered intravenously over a 30 minute period.

2.3 Sampling Schedule

Cerebrospinal fluid (C.S.F.) and blood were collected (1 & 5 ml, respectively) at baseline (24 hr after catheter placement) and 2, 4, 6, 12, 24, 48 and 60hr after IVM administration. Serum and C.S.F. were stored at -40°C until IVM analysis. Spinal and jugular catheters were removed following collection of the 60hr sample. A fraction (0.5 ml) of the C.S.F. sample collected in E.D.T.A. at 60 hr. was evaluated cytologically to determine the effect of long-term spinal catheter placement.

2.4 Ivermectin Analysis

Analysis of IVM in serum, cerebral spinal fluid (C.S.F.) samples and spinal cord tissue in case of the one animal that died was conducted using reverse phase high performance liquid chromatography (H.P.L.C.). The system consisted of a 2695 separation module and a 2475 fluorescence detector (Waters Corporation, 34 Maple Street, Milford, MA 01757). Separation was attained on a Waters Symmetry C₁₈ 4.6 X 150 mm (5µm) column protected by a 5µm Symmetry guard column (Waters Corporation, 34 Maple Street, Milford, MA 01757). The mobile phase was an isocratic mixture of methanol, acetonitrile, and water (56:40:4). All solutions were prepared using double-distilled, deionized water filtered (0.22 µm) and degassed before use. The flow rate was 1.7 ml/min and the fluorescence detector was set to an excitation of 365 nm and emissions of 475 nm. The column was at ambient temperature.

Previously frozen samples were thawed and vortexed. One milliliter of sample was placed in a 7 ml screw cap test tube and mixed with 25 µl internal standard (moxidectin 1 µg/ml), and 1 ml mixture of acetonitrile:water (80:20) then placed on a

tube rocker for 20 minutes. Samples were centrifuged 15 minute at 3000 rpm, then passed through a pre-wet Oasis H.L.B. solid phase extraction cartridge (Waters Corporation, 34 Maple Street, Milford, MA 01757) and eluted with 3 mls of methanol. Samples were evaporated with N₂ in a 47°C water bath. Sample residues were derived using 100 µl imidazole: acetonitrile (1:1) and 150 µl trifluoroacetic anhydride: acetonitrile (1:2). After 30 seconds, the solutions were placed into H.P.L.C. vials, and a 20 µl volume for plasma and 60 µl volume for CSF samples was injected into the H.P.L.C. system.

Preparation of spinal tissue was done by weighing out one gram of spinal tissue into a glass 16X100 mm disposable tube. 1mL of acetonitrile water (80:20) was added to the tube and the mixture was homogenized using a Fisher Scientific brand PowerGen 125 homogenizer. The tube was then centrifuged for 20 minutes at 1700 X g. The supernatant was removed and passed through and pre-wet Oasis HLB cartridge (Waters Corporation, 34 Maple Street, Milford, MA 01757) Oasis HLB: 2ml methanol, 2ml water, 1ml of sample. Mixture washed with 2ml of 5% methanol in water then elute into a 13X100 mm glass tube with 3ml of methanol. The methanol was evaporated under nitrogen in a 47°C water bath. The sample was then derivitized the same as plasma samples with 100 µl of imidazole : Acetonitrile (1:1) and then 150 µl trifluoroacetic acid anhydride : acetonitrile (1:2). The solution was mixed and allowed to sit for 30 seconds and then loaded into an HPLC vial and injected into the HPLC system.

Standard curves for serum and C.S.F. and spinal tissue analyses were produced by spiking with IVM which produced linear concentration ranges of 1 to 9000

ng/ml for serum samples, and 0.1 to 1000 ng/ml for C.S.F. samples, respectively. Spiked standards were treated exactly as serum samples for drug determination. The average recovery for IVM was 85% for serum and C.S.F. samples but only 64% for the spinal cord sample. Intra-assay variability ranged from 2.9% to 7.6%, while inter-assay variability ranged from 0.98% to 9.16% for IVM.

3. Results

3.1 Clinical Signs

Data from only five of the llamas are reported here due to the inability to obtain C.S.F. from the remaining llama. Three of the llamas (# 2, 4 & 5) showed transient adverse signs immediately following IVM administration, which included: mild depression; drooping of the lower lip; lip and ear twitching; sternal recumbency with the head down and the neck stretched out; decreased appetite and cud chewing. No changes in rectal temperature, heart or respiratory rates were noted. The clinical signs and changes in behavior lasted for 12 - 24 hours, after which, all parameters returned to normal. Llama #3 showed no adverse signs following I.V. administration of IVM and remained bright and alert with normal food intake during the whole trial period.

The remaining llama (# 1) developed acute neurological signs seven days after receiving IVM. The llama suddenly became laterally recumbent and this was followed by a short period of extensor rigidity in all four legs. The llama was able to rise unaided shortly afterwards but was ataxic, and hypermetric. Twenty four hours later the llama was in sternal recumbency, unable to rise and developed torticollis. Ocular examination revealed mild anisocoria. No pain could be detected

during palpation of the cervical and thoraco-lumbar spine and this finding was thought to rule out traumatic injury. The neurological signs were interpreted as being multifocal involving the brainstem and forebrain. Results of a complete blood count, chemistry panel and electrolytes were unremarkable except for the presence of a stress leukogram and hyperglycemia. C.S.F. analysis indicated a non-suppurative mixed inflammation, which was attributed to the presence of the spinal catheter, which had been removed 4 days earlier at the end of the trial. Titers for Equine herpes virus and West Nile virus were negative.

Table 1. Mean concentration and range of ivermectin in the serum and C.S.F. of five llamas given ivermectin at 500 µg/kg.

Time post injection (hr)	Serum IVM ng/ml	CSF IVM ng/ml
2	4751(2291–7742)	0.258 (0–0.57)
4	491 (102–615)	0.282 (0.1–0.68)
6	298 (86–555)	0.78 (0–2.49)
12	124 (58–222)	0.145 (0–0.44) ^a
24	67 (23–208)	0.115 (0–0.22) ^a
48	38 (10–118)	0.09 (0–0.15)
60	22 (11–48)	ND ^b

^a number of llamas sampled = 4; ^b number of llamas sampled = 3; ND = none detected.

Due to lack of response to intensive anti-inflammatory, antibiotic and supportive care, the llama (# 1) was euthanased on the third day after having become recumbent and a necropsy performed approximately 3 hours after euthanasia. The significant macroscopic findings in the central nervous system included the presence of moderate focal haemorrhage at the level of the C1-C2

vertebral spinal cord and cerebellar coning with blunting of cerebral gyri, indicative of brain swelling and early herniation.

Significant histologic lesions were localised to the central nervous system and more specifically to the white matter of the entire spinal cord, with lesser involvement in the white matter tracts of the brain. Mild to moderate distension of the myelin sheaths by finely granular eosinophilic material (oedema) was noted throughout the white matter. This was subtly more prominent in the ventral tracts in the cervical and lumbar spinal cord sections but there was generalised axon involvement. Occasional acute perivascular haemorrhages were noted in all examined sections. Additionally, astrocytes were characterised by abundant eosinophilic cytoplasm (reactive gemistocytes). The final histologic diagnosis for the brain and spinal cord was moderate diffuse acute myelinic oedema with astrocytosis. The extensive oedema noted histologically would have accounted for the cerebellar herniation and cerebral gyri blunting noted grossly.

It was determined that the lesions all represented a single incident rather than multiple insults and, based on the degree of astrocytic reaction and lack of oligodendroglial cell death, were relatively acute in onset (within 3-5 days of death).

3.2 Serum ivermectin concentrations

Results of the serum concentrations of IVM are shown in Tables 1 and 2. No IVM was detected in any of the baseline samples. High concentrations of IVM were present in the 2hr samples, ranging from 2291 – 7742 ng/ml with a mean of 4751 ng/ml. The four hour sample showed on average a 90% decrease in IVM

Table 2. Concentrations of ivermectin (ng/ml) in serum and C.S.F. of individual llamas at specific sampling times.

Llama ID		Sampling times (hr)						
		2	4	6	12	24	48	60
1	Plasma	6278	102	86	58	23	10	11
	CSF	ND ^a	0.11	ND	ND	ND	ND	ND
2	Plasma	7742	565	337	66	38	17	3
	CSF	0.37	0.23	2.49	0.14	0.12	0.12	ND
3	Plasma	2291	610	555	222	208	118	48
	CSF	0.20	0.29	0.29	0.44	NS	ND	ND
4	Plasma	3563	563	272	79	42	27	15
	CSF	0.15	0.10	ND	ND	0.12	ND	ND
5	Plasma	3884	615	201	193	24	16	14
	CSF	0.57	0.68	1.13	NS	0.22	0.15	NS

ND = none detected; NS = sample not procured

concentrations with values ranging from 102 – 615 ng/ml with a mean of 491 ng/ml. Thereafter, IVM concentrations decreased by 50-60% for each of the follow up samples with final serum concentrations at 60 hr ranging from 11- 48 ng/ml with a mean of 22 ng/ml.

3.3 Cerebrospinal fluid ivermectin concentrations

C.S.F. concentrations of IVM are shown in Tables 1 and 2. IVM concentrations in the C.S.F. were low ranging from 0-2.49 ng/ml (Table 2). Except for the 4hr sampling no IVM was detected in at least one of the other samplings (Table 1). The highest mean IVM concentration in the C.S.F. was found in the 6hr samples.

3.4 Spinal cord ivermectin concentrations

Spinal cord concentration of IVM in the one animal that died was 0.1ng/gm.

3.5 Cytological examination of C.S.F.

Cytological examination of C.S.F. of all 5 llamas was done at 60hr just before catheter removal. In 2 of the llamas CSF changes consistent with septic inflammation were found. The C.S.F. in both animals had returned to normal following systemic treatment with enrofloxacin (Baytril® 100 injectable solution, Bayer Healthcare, LLC. Shawnee Mission, KS, 66201-0390). at 2.5mg/kg S.C. once a day. Cytological examination of the C.S.F. of the other 3 llamas was within normal limits at the time of catheter removal.

4. Discussion

This study showed that despite very high blood concentrations of IVM after intravenous injection only low concentrations were detected in the C.S.F. This amounted to an average of 0.25 ng/ml for all samples which is equivalent to 0.00005% of the blood value. Van Amstel and Miller (2003) found no IVM in the C.S.F. of 8 healthy llamas 24 hours after three consecutive S.C. injections of IVM 24 hours apart at a dose rate of 500µg/kg bodyweight. These findings may support the conclusion that IVM is very effectively removed by the B.B.B. (Edwards, 2003). This study also showed that IVM was rapidly cleared from the blood as serum concentrations decreased by approximately 90% between the 2 and 4 hr samples after which IVM concentrations decreased by 50-60% for each of the follow up samples.

The IVM dose in the study was based on a) A wide therapeutic index: In general, IVM has at least a tenfold safety margin in ruminants (Reinemeyer, 2001). In cattle, toxic effects generally do not appear until dosages of 30x those recommended are injected. At 40x the normal dose of 200ug/kg injected subcutaneously cattle developed ataxia, listless and occasionally death. Sheep showed ataxia and depression at 20x the normal dose (Plumb, 2005). IVM has been used intravenously in sheep at 200 ug/ml without any ill effect, however, C.S.F. concentrations of IVM were not reported in that study (Canga et al., 2007). b) Poor absorption following S.C. administration: No IVM was detected in the CSF after three consecutive S.C. injections of I.V.M. 24 hours apart at a dose rate of 500µg/kg bodyweight (Van Amstel and Miller, 2003). and c) Poor penetration of the blood brain barrier by IVM as a result of the action of drug efflux

transporters such as P.G.P. Edwards (2003).

Three of the llamas showed transient adverse signs immediately following IVM administration, which included: mild depression, drooping of the lower lip, lip and ear twitching, lying with the head down and the neck stretched out, decreased appetite and cud chewing. Ivermectin administration may not be the cause of this reaction but the vehicle propylene glycol may be implicated because vehicle-treated sheep also developed signs of lethargy, recumbency and ataxia (Reinemeyer, 2001). One llama in the study (#1 Table 2) developed neurological signs 7 days after I.V. administration despite the fact that IVM was only detected in the C.S.F. at the 4hr sampling Table 2. However samples of the spinal cord was positive for ivermectin (0.1ng/gm of tissue at 64% IVM recovery rate). This is similar to a study by Seaman et al., (1987) who found an average of 56 ug/kg avermectin B_{1a} in brain and spinal cord tissue from 5 calves showing clinical signs of toxicity. In the same study spinal cord from one affected steer contained 34 ug/kg avermectin B_{1a} but none was detected in the C.S.F. from the same animal (Seaman et al., 1987). The delay in onset of neurological signs in the present study appears to differ somewhat from other reported cases of IVM toxicity. Twenty calves out of a group of 40 developed neurological signs including ataxia and recumbency 24-36 hours after having received IVM intramuscularly at a dose rate of 330 µg/kg, two animals died and the rest recovered over a 2-6 day period (Button et al., 1988). Two other groups of calves also developed acute neurological signs including ataxia, recumbency with opisthotonus 20 hours after having received IVM at approximately 300-400 µg/kg (Seaman et

al.,1987). However in one report on IVM toxicity in a group of dogs belonging to the collie breed onset of signs varied from 3 hours to 5 days (Button et al., 1988). Nevertheless, although administration of IVM could potentially have had neurotoxic effects the delay of seven days from administration to the onset of neurologic signs remains perplexing. The neurological signs however in this case and that reported for other animals including calves with IVM toxicity are very similar. Clinical signs in a group of calves included "suddenly falling down" which is exactly what was first seen in this llama (Button et al., 1988). Other frequently reported signs of IVM toxicity also seen in the llama in this study include ataxia and hypermetria, recumbency with opisthotonos, seizure activity and comatose state (Seaman et al., 1987, Button et al., 1988). The myelinic oedema observed on histopathology from the llama that died is consistent with cytotoxic oedema that is often the result of osmotic dysregulation, either locally or systemically (Summers et al., 1995), Maxie and Youssef 2007), van der Lugt and Venter, 2007). Other causes of myelinic oedema include electrolyte abnormalities and particularly sodium and acid/base disturbances but these parameters were considered within normal limits for this llama. Cytotoxic oedema can also occur following nutritional deficiency, ischaemia/hypoxia or exposure to toxicants such as IVM (Summers et al., 1995, Maxie and Youssef 2007, van der Lugt and Venter, 2007). While neurologic signs have been reported following IVM administration in many species, histopathologic lesions associated with IVM neurotoxicosis are lacking. No histopathological lesions in the brain or spinal cord could be demonstrated in both accidental and experimental IVM toxicity

in calves (Seaman et al., 1987, Button et al.,1988).

This study has shown that low concentrations of IVM in the C.S.F. can be achieved following I.V. administration at 500µg/kg, however, it is not known whether such low levels would be effective in the treatment of clinical cases of meningeal worm. In addition because of the possibility of neurological damage, I.V. administration of IVM at this dose is not recommended.

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