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Retention time of gentamicin in the vitreous of horses after an intravitreal injection

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Summary: An intravitreal gentamicin injection (IVGI) was first described as an alternative treatment option for equine recurrent uveitis (ERU) in 2005. The aim of the present study is to determine the retention time of gentamicin in the vitreous of horses after an IVGI. Accordingly, an IVGI was performed on a total of 15 horses and vitreous samples were taken from 3 horses each after 0, 7, 10, 14 and 21 days and tested for their gentamicin concentration. The measurements showed that gentamicin can be detected in all horses sampled with concentrations above the minimal inhibitory concentration in the vitreous up to 10 days after an IVGI. After a sampling period of 14 days, only one of the three horses still showed a detectable concentration of gentamicin in the vitreous (2.87 µg/ml). No gentamicin above the detection limit of 0.4 µg/ml could be measured in any of the three vitreous humours 21 days after an IVGI. Blood samples taken one hour after an IVGI from three healthy horses and three horses suffering from ERU showed no measurable gentamicin concentrations. Because of this, a systemic effect with potential nephro- and ototoxicity of gentamicin after an IVGI of 4 mg can be neglected. A gentamicin concentration of 138.0–147.0 µg/ml was measured in vitreous samples taken at the end of a vitrectomy in three additional horses. The measurements of three additional vitreous samples without a prior IVGI as a control group showed no evidence of gentamicin. Further research in this area is necessary in the future to clarify the long-lasting efficacy of gentamicin against recurrences of ERU with a relatively short retention time in the vitreous.

Keywords: equine recurrent uveitis, ERU, intravitreal injection, gentamicin

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Introduction

The aim of treating equine recurrent uveitis (ERU) is to avoid recurring inflammatory episodes in the inner, vascularised structures of the eye (iris, ciliary body, choroidea) and, thus, counteract blindness of the eye and further pain in the horse. [1–3] An intravitreal injection of 4 mg gentamicin was first described as a treatment against ERU in horses in 2005. [4] Compared to vitrectomy as the gold standard method, an intravitreal injection of gentamicin (IVGI) offers several advantages: it is cost-effective, can be performed at the home stable under sedation, requires little equipment and, in the absence of success, a subsequent vitrectomy is possible. [1,5,6] Six studies about the long-time prognosis after a low dose IVGI in horses with ERU have been published since 2005 [4,5,7–10] and the success rates achieved differ between 26 [10] and 98.6 %. [9] It was counted as a success if after one IVGI, no recurrences of uveitic symptoms were observed between 1 [5,7] and 96 [8] months.

Gentamicin is an aminoglykoside antibiotic which was isolated from the Micromonaspora species. It inhibits the protein synthesis of bacteria because of a binding at the 30S ribosomal subunit and, thus, has a bactericidal effect. Gentamicin is used to treat severe infections with extracellular, Gram-negative bacteria. The minimum inhibitory concentration (MIC) is 1–4 µg/ml. Excretion is unchanged via the kidneys, [11,12] where it is actively absorbed into the proximal tubule and leads to the release of lysosomal enzymes. This can result in necrosis and autolysis in the tubule cells. [13] In addition to nephrotoxicity, gentamicin is ototoxic due to its accumulation in the perilymph of the inner ear and a particular affinity for the sensory cells located there. [12,14] Further accumulation in the pigment epithelium of the retina leads to a functional disorder of the lysosomes with increased storage of lipids, which makes gentamicin retinotoxic. [6,15] Toxicity depends on the concentration in the front of the retina and is influenced by the technique of injection. [16] More than 0.71 mg/ml vitreous intravitreal injected gentamicin (1 mg per eye) caused cataract formation, retinal degeneration, necrosis and signs of inflammation in studies in the rabbit eye, which has a vitreous volume of 1.4 ml. [17] At up to 0.36 mg gentamicin per ml vitreous (0.5 mg per eye), no histological or electroretinographic changes were observed in the rabbit eye. [15,17] Assuming a vitreous body volume of 28 ml in a horse eye, [18] an IVGI of 4 mg corresponds to a concentration of 0.14 mg/ml vitreous body, which is higher than the MIC. An antibiotic effect against Gram-negative leptospires, which are often detectable in the eye with ERU, [19–21] could be suspected. [22] Studies have shown that the elimination of gentamicin from the vitreous body is much slower with a half-life of approximately 20 h than that of penicillin with a half-life of 2–3 h. [15,17] The slower excretion could be due to the binding of gentamicin to vitreous proteins and melanin. The binding is pH-depen-

dent and, therefore, depends on the severity of the inflammation. Infected cell material has many binding sites due to free amino acids.[20] When gentamicin is injected intravitreally into horses suffering from ERU, a high and long-lasting effective concentration can be achieved with low doses.[8] An immunosuppressive effect of gentamicin besides the antibiotic effect[7,23] is controversially discussed in the literature.[8] While *Fischer* et al.[7] assume a suppression of specific T-cells, *Launois* et al.[9] support the theory that gentamicin inhibits protein synthesis in the vitreous body and, thus, prevents further inflammatory episodes. Findings in a recent study rebuke previous propositions that T-lymphocyte suppression is the mechanism of action of an IVGI, because no alteration of cell viability and no suppression in CD3+ T-lymphocyte proliferation could be observed.[24] There is currently no clear explanation for the mode of action of gentamicin in the vitreous body in the treatment of ERU.[7] Medication can leave the vitreous body in two ways: via the anterior chamber of the eye (anterior route) and the retinal surface (posterior route). Based on a study in aphakic rabbit eyes and an increase in the concentration of gentamicin in the anterior chamber of the eye and the cornea, it can be assumed that elimination of gentamicin from the vitreous body occurs predominantly via the anterior route.[25,26] Basically, it depends on the molecular weight, the diffusion rate and the permeability of the retina with which kinetic drugs leave the vitreous body.[27] Due to the permeability of the blood-ocular barrier in horses with ERU,[28] the possibility of the passage of intravitreally administered gentamicin into the blood and the associated risks of a systemic effect with nephro- and ototoxicity should not be neglected.[27] The aim of the current study was to determine the retention time of gentamicin in the vitreous body of horses after an IVGI and, accordingly, come closer to the mechanism of action of gentamicin against ERU.

Materials and methods

Study population

The study population consisted of a total of 18 horses, which were divided into groups of 3 horses each (Table 1). The study was authorised by the ethics committee within the University of Veterinary Medicine, Hannover, and the State Office for Consumer Protection and Food Safety in accordance with the German Animal Welfare Law (LAVES – Reference number: AZ 33.19-42502-04-19/3137). The main research comprised 15 healthy eyes of 15 horses (no. 1–15) which were each treated with an IVGI of 4 mg (technique described below). After various time intervals, a sample was taken from the vitreous treated and tested for their concentration of gentamicin (technique described below). Additionally, three healthy eyes of three euthanized horses (no. 16–18) were sampled and tested for their gentamicin concentration without a prior IVGI as a control group. An ophthalmologic examination was performed in all eyes by the first author, a veterinarian trained by a German board certified veterinary ophthalmologist. The ophthalmologic examination included tonometry, induction of mydriasis by tropicamid, slit lamp biomicroscopy and direct ophthalmoscopy. An ultrasonographic examination was performed on all eyes included in the study to determine the longitudinal and transversal diameter of the eyes.

Intravitreal injection

An IVGI of horse no. 1–3 took place in the euthanised horse as described below without surgical preparation and analgesia. They were euthanised due to other diseases unrelated to the study. The remaining 12 horses (no. 4–15) were stabled one day prior to the IVGI and underwent a blood control, physical and ophthalmological examination. On the day of injection, horses were given meloxicam[2] through an intravenous catheter in a dosage of 0.6 mg per kg body weight 1 h prior to the intervention. The horses were sedated with detomidin[3] and butorphanol[4]. After flushing the selected eye with 1 % povidone-iodine solution and saline solution[5], a topical anaesthesia of conjunctiva and cornea was induced with Oxybuprocain[6] ophthalmic drops. A nerve block anaesthesia was performed subcutaneously with mepivacain at the nervus auriculopalpebralis caudal of the arcus zygomaticus and the nervus frontalis located above the foramen supraorbital[7]. The dorsal sclera was presented to the treating veterinarian by using a single eyelid lifter dorsal, a rotator in the conjunctival sac ventral and by rotation of the head of the horse in the longitudinal axis with the selected eye above. The IVGI of 4 mg gentamicin[8] in 0.1 ml mixed with 0.5 ml saline solution[5] was performed approximately 10–12 mm above the dorsal limbus in the pars plana at nearly 12 o'clock. The puncture is performed while avoiding the vessels, and the gentamicin

Table 1 Division of study population and gentamicin concentration in µg/ml at the respective sampling periods after intravitreal injection of 4 mg gentamicin in healthy eyes (detection limit < 0.4 µg/ml.) | *Unterteilung der Studienpopulation und Gentamicin-Konzentration in µg/ml zu den jeweiligen Beprobungszeiträumen nach intravitrealer Injektion von 4 mg Gentamicin bei gesunden Augen (Nachweisgrenze < 0.4 µg/ml).*

Horse number	Sampling period	Utilisation in study	µg/ml
1	0	main research, euthanised	38.3
2			18.2
3			38
4	7	main research, blood sample	4.26
5			5.4
6			11.94
7	10	main research	3.9
8			3.2
9			3
10	14	main research	< 0.4
11			2.87
12			< 0.4
13	21	main research	< 0.4
14			< 0.4
15			< 0.4
16	0 (control group)	sample without gentamicin injection, euthanised	< 0.4
17			< 0.4
18			< 0.4

was slowly and steadily injected into the vitreous using a 1 ml syringe and a 25 gauge needle. The needle was aimed at the optic disc to prevent damage to the retina and lens. After removing the needle, the puncture was compressed by forceps to avoid any leakage of vitreal material and haemorrhage of the conjunctiva. A subconjunctival injection of 10 mg triamcinolone[9] was performed at 10 or 2 o'clock in the same eye after the intravitreal injection. Finally, all eyes were nursed with vitamin A[10] ophthalmic ointment. An ophthalmologic examination with a slit lamp and direct ophthalmoscopy was performed about 10 h after the injection and on each of the following two days. Meloxicam[11] in a dosage of 0.6 mg per kg body weight was given once daily for a further two days. Intra- and postinjection complications were documented.

Sampling

Sampling of horses no. 1–3 took place within 1 min via a separate puncture after the IVGI without surgical preparation or analgesia. The sampling was performed after briefly mixing the vitreous body using a rotator. The remaining samples of the main research were taken from three horses each (no. 4–6, 10–15) after 7, 14 and 21 days. After the gentamicin concentrations were obtained from these groups, the last group of three horses (no. 7–9) was sampled after a period of 10 days. All 12 horses were stabled one day prior to sampling and underwent a blood control, physical and ophthalmological examination. Horses were given meloxicam[2] through an intravenous catheter in a dosage of 0.6 mg per kg body weight 1 h prior to sampling. Regarding sampling, the horses were put under general anaesthesia with a triple drip (guaifenesin[12], xylazin[13], ketamin[14]) and placed in lateral recumbency with the eye treated previously above. The eye was flushed with a 1 % povidone-iodine solution and saline solution and a topical anaesthesia of conjunctiva and cornea was induced with Oxybuprocain[6] ophthalmic drops. After draping surgically, an eye speculum was used to open the eyelid. The dorsal sclera was presented by utilising a rotator in the ventral conjunctival sac. An intrascleral cruciate suture using coated vicryl (4/0 USP) was preplaced approximately 10–12 mm above the dorsal limbus in the pars plana at nearly 12 o'clock to allow a fast closure after the removal of the needle (Figure 1a). The puncture into the vitreous body was made at a 45 ° angle with an 18 gauge needle. After removing this needle, dilatation was performed using a dilator. An amount of 0.5 ml of vitreous material was aspirated with a new 18-gauge needle and placed in an Eppendorf cap (Figure 1b). A quantity of 0.5 ml of saline solution[5] was refilled into the vitreous through the same needle for pressure equalisation. The sclera was closed immediately with the preplaced intrascleral cruciate sutures after the removal of the needle. The conjunctiva was closed with as many single stitches as was needed using vicryl (4/0 USP). At least 20 mg gentamicin[8] and 2 mg dexamethasone[15] were injected subconjunctivally (Figure 1c) and the eye was nursed with vitamin A[10] ophthalmic ointment. An ophthalmologic examination with a slit lamp and direct ophthalmoscopy was performed about 10 h after sampling and on each of the following two days. Meloxicam[11] in a dosage of 0.6 mg per kg body weight was given once daily for a further two days. Intra- and post-sampling complications were documented. Horses no. 19–21, which were euthanised due to other diseases unrelated to the study, were sampled as described above without surgical preparation and analgesia without previous IVGI.

Additional samples

Vitrectomy in the clinic for horses in Hannover was performed similar to that described in the literature[29,30] using buffered saline solution[16] containing gentamicin[8] (80 mg in 480 ml). Vitreous samples were taken from three horses suffering from ERU at the end of the vitrectomy and tested for their gentamicin concentration. Blood samples of three horses with healthy eyes (no. 4–6) and of three horses which were affected by ERU were taken 1 h after an IVGI and tested for serum gentamicin concentration.

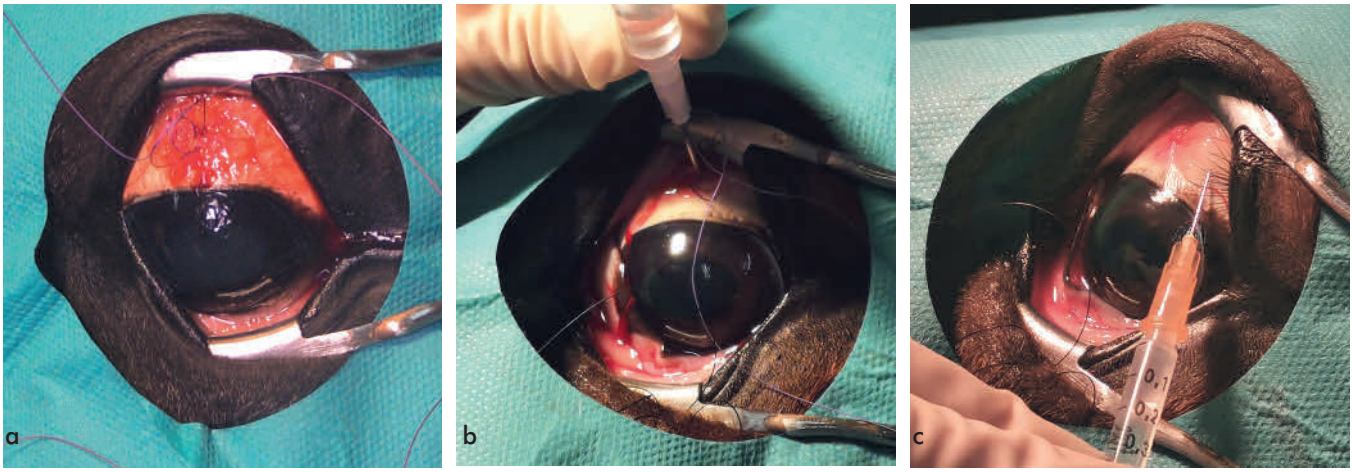

Fig. 1a-c Surgical technique of sampling of 0.5 ml vitreous material. | *Operationstechnik der Probenentnahme von 0.5 ml Glaskörpermaterial.*
a) An intrascleral cruciate suture was preplaced approximately 10–12 mm above the dorsal limbus in the pars plana at nearly 12 o'clock. | *Ungefähr 10–12 mm oberhalb des dorsalen Limbus im Bereich der Pars plana wird bei etwa 12 Uhr intraskleral ein Sultan'sches Diagonalheft vorgelegt.*
b) With a 18-gauge needle 0.5 ml of vitreous material were aspirated after dilatation the puncture. | *Mit einer 18 G Nadel wurden 0,5 ml Glaskörpermaterial aspiriert, nachdem die Punktionsstelle dilatiert wurde.*
c) 20 mg gentamicin and 2 mg dexamethasone were injected subconjunctival after surgery. | *Nach der Injektion wurden 20 mg Gentamicin und 2 mg Dexamethason subkonjunktival injiziert.*

Sample testing

The vitreous samples were taken to the Institute for Clinical Chemistry from the Center for Laboratory Medicine of the Hannover Medical School and tested for gentamicin concentration using the cobas® 8000[17] immunoassay. The lower detection limit is 0.4 µg/ml.

Statistical evaluation

The pharmacokinetic parameters for gentamicin were determined at R 4.3.1.[31] Values below the limit of detection of the gentamicin assay (0.4 mg/l) were randomly generated with the 'msm' R package[32] for the interval 0–0.4 mg/l from a truncated log-normal distribution whose parameters were estimated with the 'NADA' R package.[33] A self-starting nls asymptotic regression model corresponding to the equation $C(t) = C_f + (C_0 - C_f)^{-k_e t}$ was fitted with R's 'nls' function using the 'nl2sol' algorithm. C(t) describes the gentamicin concentration at time t, C_0 the initial gentamicin concentration, C_f the final gentamicin concentration with a lower bound of 0, and k_e the elimination rate constant. The elimination half-life was calculated as $\ln 2/k_e$. The 95% confidence interval for the regression line was obtained using Taylor series approximations, as implemented in the 'predFit' function from the investR R package.[34]

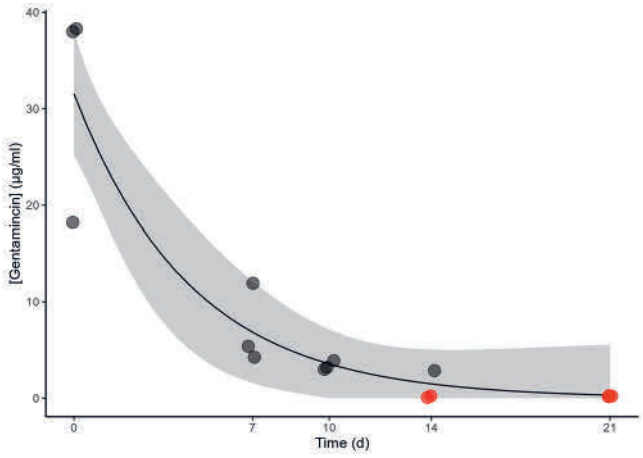

Fig. 2 Gentamicin concentration in µg/ml in the vitreous humour during the sampling period. Each point corresponds to one individual. The red points indicate values below the limit of detection of the gentamicin assay. The regression line was obtained from the pharmacokinetic analysis and is shown with its 95% confidence interval. *Gentamicin-Konzentration in µg/ml im Glaskörper während des Beprobungszeitraums. Jeder Punkt entspricht einem einzelnen Pferd. Rote Punkte stellen Werte unterhalb der Nachweisgrenze von Gentamicin mit der verwendeten Messmethode. Die Zerfallskurve und das entsprechende 95%ige Konfidenzintervall wurden mittels pharmakokinetischer Analyse bestimmt.*

Table 2 Pharmacokinetic parameters of intravitreal gentamicin (given in µg/ml. | *Pharmakokinetische Parameter von intravitrealem Gentamicin (in µg/ml).*

Parameter	Estimate	Std. Error	t value	P value
C_f	0.00	2.88	0.00	1.00
C_0	31.53	2.91	10.82	< 0.001
ln k_e	-1.52	0.38	-3.99	0.002

Results

Intravitreal Injection

All 18 horses of the study population were Warmbloods and had no known pre-existing eye diseases or signs of ERU. There were 10 mares and 5 geldings with a median age of 14.4 years (ranged from 5–23 years). Twelve right and three left eyes were treated with an IVGI. The longitudinal diameter was an average of 2.23 ± 0.23 cm long, and the transversal axis was 2.1 ± 0.22 cm long. No intra-injection complications were observed. Post injection, 8 of 12 horses treated (no. 4–15) showed a slight scleral haemorrhage. One horse showed blindness due to the IVGI on the eye treated. It showed a positive dazzle reaction and a negative menace response, which regressed in the first 10 days after the intervention. At the timepoint of sampling 14 days after the IVGI, the horse was able to see again unrestrictedly. No complications occurred while sampling. After sampling, 9 out of 12 horses (no. 4–15) showed a slight scleral haemorrhage. A bloody vitreous deposit was observed in one horse the day after surgery, which was no longer visible after two days. None of the horses in the main research showed a change in the intraocular pressure after sampling. Table 1 gives a review of the results of the main research.

The highest results of up to 38.3 µg/ml were measured during immediate sampling (no. 1–3). Ten days after IVGI is the last time point at which gentamicin could be detected in all three eyes sampled (no. 7–9). There was only one horse after a sampling period of 14 days with a measurement result above the detection limit (no. 11). After a sampling period of 21 days, gentamicin is no longer detectable in the vitreous body of any horse (no. 13–15). The intravitreal gentamicin concentrations over time are shown in Figure 2.

With C_f estimated to be 0, the regression equation simplifies to a classical first order elimination $C(t) = C_0\, e^{-k_e t}$ (Table 2). The elimination half-life is equal to 3.18 days (95% confidence interval: 1.50–6.72 days). Gentamicin persists in the vitreous for approximately 10–14 days, and at day 10, the gentamicin content is still above the MIC. Control horses (no. 16–18) were tested for gentamicin without treatment, and showed a gentamicin concentration below the detection limit.

Additional samples

A gentamicin concentration from 138 to 147 µg/ml was measured in the vitreous body samples obtained from three additional horses at the end of a vitrectomy. nNo gentamicin concentration above the detection limit could be determined in the serum in blood samples taken 1 h after an IVGI from horses number 4–6 of the main research group and from three additional horses suffering from ERU.

Discussion

An IVGI has been described in various studies on the treatment of ERU.[4,5,7–10] In these studies, however, there is no

information on the fate of the gentamicin after injection. In the present study, a controlled examination of the retention time of 4 mg gentamicin in the vitreous of horses was carried out as part of an authorised animal experiment.

Discussion of materials and methods

In order to be able to observe the course of the concentration over time, it would be optimal to collect vitreal samples from several horses at close time intervals until the detection limit of 0.4 µg/ml had been reached. This has not been done in this study for animal welfare reasons. In order to limit the number of study horses and the exposure, vitreous samples were obtained from three horses each at different times after the IVGI. In addition, for this reason, vitreous samples were obtained from the control horses and the horses after injection (timepoint 0) on dead horses immediately after euthanasia. Sampling in the other horses was, in contrast to the IVGI, not possible on the standing, sedated horse, but required general anaesthesia. This was probably due to the structure of the vitreous body. Because the time until the concentration fell below the detection limit was initially unknown, the increasing intervals between the injection and sampling during the study were determined on the basis of the concentration detected previously. This made it possible to determine that 21 days after injection, gentamicin was no longer detectable in the vitreous body of any horse. After 14 days, gentamicin was still detectable in one of three horses. Further statements on the elimination of gentamicin from the vitreous body would have been possible with a larger number of horses in the study population in the period between 14 and 21 days after the IVGI. With the limitation that the progression of concentration was only determined in small test groups, the study provides an initial overview of the retention time of gentamicin in the vitreous of horses. Therefore, this study shows a relatively short detectability compared to a long-lasting effect of gentamicin against ERU after an IVGI of up to 96 or 55 months.[5,8]

The immunoassay used is normally utilised in human medicine and is not specific to horses. The detection limit is 0.4 µg/ml and is classified as "no detection" in the study. None of the measurements of the untreated vitreous body samples of the control horses showed any detection of gentamicin, which means that false positive results can be ruled out.

Statistical analysis

The null hypothesis is that the coefficient is 0. Since the final concentration obviously approaches 0 asymptotically, the null hypothesis cannot be rejected. Accordingly, the P value of C_f does not mean that the determined value of $C_f = 0$ is incorrect or unreliable (Table 2).

Based on an intravitreal injection of gentamicin into rabbit eyes, a half-life of 20 h was determined. The rabbit eyes were removed intact after euthanasia and the gentamicin concentration was measured by a standard curve disk-plate assay using Staphylococcus aureus as the test organism.[17] By contrast, a half-life of 3.18 days was determined in the vitreous of horses in this study. The differences could be explained by the different animal species, different sampling techniques and different detection methods.

Antibiotic effect against leptospires (IVGI and systemic)

One theory of the effect of gentamicin against ERU is an antibiotic effect against Gram-negative leptospires because of special characteristics of binding to proteins, free amino acids and melanin and the resulting high and long-lasting concentration in the vitreous.[8] On the other hand, Gram-negative leptospires form biofilm[35,36] and are more difficult to eliminate by antibiotics than their planktonic counterparts.[37] This study shows a gentamicin concentration in the vitreous above or within the MIC over a period of about 10–14 days, which also makes an antibiotic effect possible. In a study, intravitreal injected rapamycin could still be detected in therapeutic dosages in the vitreous 21 days after injection.[38] However, studies on the efficacy of rapamycin in the treatment of ERU are not yet available. Additionally, vitreous concentrations above the MIC of leptospires were achieved with a systemic administration of enrofloxacin,[3,39–41] but 30.1 % of samples continued to show positive culture results.[41] The blood-ocular barrier may be impaired in horses suffering from ERU[28] and, therefore, different distributions may occur than in measurements on healthy horses, which needs to be considered regarding the results. As an alternative treatment method, oral doxycycline administration is described in the literature anecdotally. But no sufficiently effective concentrations against leptospiral ERU could be detected in the vitreous.[3,42]

Non-antibiotic effects of an IVGI

Furthermore, there could be an immunosuppressive effect of gentamicin, which prevents further bouts of inflammation,[7,9,23] even without the presence of gentamicin in the vitreous. However, gentamicin causes no alteration in cell viability and no suppression of the proliferation of T-lymphocytes.[24] But there were two retinal autoantigens identified (interphotoreceptor retinoid-binding protein and cellular retinaldehyde-binding protein), which have the ability to induce experimental uveitis in the horse. These retinal binding proteins have an autoreactivity with T-lymphocytes and epitope spreading is probably involved leading to bouts of ERU.[43,44] *Smith* et al.[24] see the retinal toxicity as an appealing explanation for an IVGI's mechanism of action, because this could explain both the positive clinical response and retinal complications. The binding of gentamicin to vitreous proteins and melanin is pH-dependent and, therefore, depends on the severity of the inflammation, because infected cell material has many binding sites due to free amino acids[20] Because of this, it is questionable whether the vitreous of healthy eyes are representative for the retention time of gentamicin in ERU eyes. On the other hand, the blood-ocular barrier is damaged in eyes with ERU, which could lead to a faster elimination of gentamicin from the vitreous, whereby the main excretory way is via the anterior route.[25]

Retinotoxic effects in IVGI under consideration of the concentration curve

Even if it is rather unlikely based on data collected on rabbit eyes in the literature,[15,17] retinotoxicity of 4 mg gentamicin cannot be completely ruled out, because it is not known if

horses may be more sensitive than rabbits to the toxic intravitreal effects of gentamicin.[10] One horse in a recent study about the long-term prognosis after intravitreal injection of gentamicin of eyes with ERU[5] also suffered blindness due to the intervention, but no reason for the reversible blindness could be found in an ophthalmologic examination. In another study, retinal degeneration was observed in 7/32 eyes (22 %) after IVGI. There a horizontal area of diffuse tapetal hyperreflectivity in the area of the visual streak with most distinct abnormalities in the area centralis developed in all eyes diagnosed with retinal degeneration.[10] Therefore, it would be very interesting to perform histopathologic studies of equine eyes after IVGI. In addition, the immunosuppressive antibiotic rapamycin was injected intravitreally in horses in order to avoid the potential retinotoxicity of gentamicin. Up to 10 mg intravitreal injected rapamycin did not trigger any electroretinographic or histopathological changes in the retina.[38] But, as has already been mentioned above, an effect against ERU has not yet been researched.

Additional sampling

The jelly-like structure of the vitreous and the resulting poor distribution of gentamicin after a short time could be an explanation for the different results at timepoint 0, although the attempt of a short mixing of the eye was performed after the IVGI. In addition, the measurements at the timepoint 0 are below the values calculated. With an injection of 4 mg gentamicin per vitreous, a concentration of 142.86 µg/ml could be expected arithmetically with an assumed vitreous volume of 28 ml.[18] The binding to vitreous proteins[8] and less free gentamicin could also explain the low values and play a role in follow-up measurements. The samples taken at the end of vitrectomy show similar concentrations of gentamicin of 138.0–147.0 µg/ml due to the exchange and complete mixing of the vitreous which ensured an even distribution.

Gentamicin could not be detected in blood samples after intravitreal injection, irrespective of a destroyed blood-ocular barrier, either in healthy horses or in horses suffering from ERU. Therefore, the risk of a systemic effect with nephro- and ototoxicity[27] can be neglected with the amount of 4 mg per eye used.

Limitations

Limitations to this study included a small sample size and a large age range of horses included (5–23 years). The metabolism and the vitreous body structure change over the years and could have had an influence on the degradation processes and distribution of gentamicin. Furthermore, the immunoassay used has a high minimum detection level compared to the MIC of gentamicin. Exact results around the MIC cannot be considered or exact statements about when the concentration falls below the MIC cannot be made.

Conclusion

Due to the detectability of gentamicin in the vitreous humour of horses for 10–14 days, an antibiotic effect against leptospires is conceivable. However, the long-term success remains astonishing due to the short detectability. Only one horse out of ten horses that were retreated for recurrences in a recently published study showed retinal degeneration following the second injection. Six other horses showed retinal degeneration already after the first injection.[10] Due to this and the relatively short retention time, a second IVGI seems to be possible if there are further bouts of uveitis, as there does not appear to be any accumulation of gentamicin in the vitreous and retinal degeneration seems to occur regardless of the number of injections. There are indications in the present study that gentamicin is only detectable in the vitreous body of horses shortly after IVGI. Whether the long-term effect in ERU described is based on a permanent elimination of an intraocular leptospiral infection or on other, non-antibiotic effects of gentamicin could not be clarified and should be the subject of further research.

Manufacturer's addresses

1 Mydriatikum Stulln UD®, Pharma Stulln GmbH, Stulln Germany
2 Melosolute® 20 mg/ml, CP-Pharma, Burgdorf, Germany
3 Cepesedan® 10 mg/ml, CP-Pharma, Burgdorf, Germany
4 Butorgesic® 10 mg/ml, CP-Pharma, Burgdorf, Germany
5 Natriumchlorid-Lösung 0.9 % ad us. vet. WDT, Wirtschaftsgenossenschaft deutscher Tierärzte eG, Garbsen, Germany
6 Novesine® 0.4 % Augentropfen, OmniVision GmbH, Puchheim, Germany
7 Mepidor® 20 mg/ml, Richter-Pharma AG, Wels, Austria
8 Gentamicin-ratiopharm® 80 mg/2 ml SF, ratiopharm GmbH, Ulm, Germany
9 Triam 10 mg Lichtenstein, Zentiva Pharma GmbH, Frankfurt am Main, Germany
10 Vitamycin® Augensalbe, CP-Pharma, Burgdorf, Germany
11 Melosus® Pferd 15 mg/ml, CP-Pharma, Burgdorf, Germany
12 Myorelax® 100 mg/ml, Dechra, Aulendorf, Germany
13 Xylavet®, 20 mg/ml, CP-Pharma, Burgdorf, Germany
14 Narketan® 100 mg/ml, vetoquinol, Ismaning, Germany
15 Dexa-ratiopharm®, Ratiopharm GmbH, Ulm, Germany
16 BSS PLUS®, Alcon Laboratories, Inc., Texas, USA
17 cobas® 8000 modular analyzer series, Roche Deutschland Holding GmbH, Grenzach-Wyhlen, Germany

Abbreviations

ERU: Equine Recurrent Uveitis
IVGI: Intravitreal gentamicin injection
MIC: Minimum Inhibitory Concentration

Animal Welfare statement

Information was obtained from clinic-owned horses during the study which has been approved by the ethics committee within the University of Veterinary Medicine, Hannover, and the State Office for Consumer Protection and Food Safety in accordance with the German Animal Welfare Law (LAVES – Reference number: AZ 33.19-42502-04-19/3137)

Statement of informed consent

All owners provided informed consent that information can be included in the study.

Conflict of interest statement

The authors declare that they have no competing interests.

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