Veterinary Ophthalmology (2011) **14**, 3, 180–185

Evaluation of the ocular penetration of topical alpha-luminol (Galavit®/GVT®)

Enry G. da Silva,* Juliet R. Gionfriddo,* Susan F. Hudachek,* Daniel L. Gustafson,* Francisco J. Olea-Popelka,* Virgina L. Scofield,† Cynthia C. Powell* and Ashley E. Hill*

**Department of Clinical Sciences, Colorado State University, College of Veterinary Medicine, Fort Collins, CO, USA; and †Department of Carcinogenesis, The University of Texas M.D. Anderson Cancer Center, Science Park-Research Division, Smithville, TX, USA*

Address communications to:

J. R. Gionfriddo

Tel.: (970) 297-4173
Fax: (970) 297-1275
e-mail: juliet.gionfriddo@colostate.edu

Abstract

Purpose Oxidative stress plays a major role in the pathogenesis of many neurodegenerative diseases. It has also been implicated as part of the pathogenic mechanisms in the development of glaucoma. Alpha-luminol has shown profound anti-inflammatory and antioxidant effects in both experimental animal and human clinical studies. The purpose of this pilot study was to investigate for the first time the ocular penetration of topical alpha-luminol.

Methods Nine animals were divided into three treated groups (three animals each; one drop OU/*n* = 18), each group receiving a different concentration of the eyedrop (0.5%, 1.5%, 2.5%). Aqueous humor and peripheral blood samples were obtained from each rabbit at three different timepoints (20 min, 4 h and 12 h). Samples were analyzed by means of high performance liquid chromatography and mass spectrometry; median values were compared.

Results Alpha-luminol was found in the aqueous humor in all treated groups at all timepoints. At the 2nd and 3rd timepoints (4 h and 12 h), aqueous humor levels decreased significantly ($P < 0.05$) for two of the three dosages tested and it was not detectable in some eyes. The highest aqueous humor concentration of the drug was 272 ng/mL after 20 min (0.0217% of one drop, 2.5% group). Alpha-luminol was found in the vitreous in two animals, one in the 1.5% and another in the 2.5% group (16.4 and 21.5 ng/mL, respectively), at 12 h.

Conclusions Topically administered alpha-luminol readily penetrates into the anterior chamber and can penetrate into the vitreous chamber. Further investigation is warranted to better understand the intraocular pharmacokinetics of alpha-luminol.

Key Words: alpha-luminol, eye, Galavit®, glaucoma, GVT®, oxidative stress, topical

INTRODUCTION

Oxidative stress is thought to play an important role in retinal diseases such as diabetic retinopathy[1] and glaucoma.[2–11] Reactive oxygen species (ROS) may initiate and potentiate retinal damage.[10,11] Thus, there has been much investigation into the use of antioxidant therapy to both prevent and ameliorate retinal damage in ROS-mediated retinal diseases. One of these anti-oxidants is alpha-luminol. Alpha-luminol is a refined monosodium luminol compound (monosodium 5-amino-2-3-dihydro-1-4-phthalazine dione) that was developed in the late 90s as part of a secret space research program at a military laboratory in Russia. It was originally used for human administration, supposedly to protect astronauts against radiation in space.[12] Amongst the different names of the drug are GVT®, Galavit® and MSL® (monosodium alpha-luminol).[12,13] Initial reports of the beneficial effects of alpha-luminol are found mainly in the Russian literature. Several abstracts on clinical evaluations of alpha-luminol were presented during conferences in Russia, although none of these initial studies were published in peer-reviewed journals.[13] Alpha-luminol was once thought to be a 'miracle drug' as well as a cure for cancer,[12] concepts that were later shown to be false. Further investigations on the efficacy and therapeutic properties of alpha-luminol have recently been published in more widely accessible journals.[14–22]

Alpha-luminol has been shown to have profound anti-inflammatory and immunomodulatory effects in humans and laboratory animals without any deleterious effects.[13–22]

The immunomodulatory effects of alpha-luminol have been linked to a 'reliable' increase in T-helper (CD4+) and natural killer cell (CD16+) counts in non cancer human patients[17] and mild increases in the concentrations of interleukin-1 (IL-1) and tumor necrosis factor (TNF) in mice.[19] In a study in mice experimentally infected with the cytopathic retrovirus *ts*1, alpha-luminol delayed the development of immunodeficiency, neuropathology, and the clinical signs of neuromuscular degeneration that were present in the controls.[20] Additionally, it is a potent antioxidant and one of the proposed mechanisms for its antioxidant properties is the up-regulation of cellular nuclear factor erythroid 2-related factor 2 (Nrf-2) levels.[21,22]

Nuclear factor erythroid 2-related factor 2 is a cytoplasmic transcriptor factor required for the activation of the antioxidant responsive element (ARE). The Nrf2-ARE pathway is responsible for the activation of protective genes and detoxification enzymes, which are essential in fighting oxidative stress and toxic changes.[23–26] In previous investigations into the possible medical uses for alpha-luminol it was given to test animals via parental routes (e.g. oral, intraperitoneal). In order to target intraocular structures (such as the lens, uveal tract and retina) for drug therapy, the parenteral route is often used.[27–29] However, a topical drug that is effective in reaching these structures in therapeutic concentrations would be preferable because topical drops provide for more targeted therapy, are easier to administer and lower amounts of the drug are absorbed systemically. The objective of this paper is to evaluate the ocular penetration of topical alpha-luminol and describe the possible mechanisms of action of the drug.

MATERIAL AND METHODS

Drug formulation

The eyedrops containing alpha-luminol were formulated and donated for this study by Bach Pharma, North Andover MA. The 0.5%, 1.5% and 2.5% concentration eyedrop vials were prepared from a powder of alpha-luminol and sterile NaCl. Commercially available eye droppers with a drop size of 50 μL were used to administer the drug. This drop size did not result in spill-over of tears.

Animals

Experiments were carried out in 11 purpose-bred, adult, New Zealand White rabbits (five males and six females) weighing an average of 2.9 kg. This study was performed in accordance with the Colorado State University Animal Care and Use Committee. Animals were acclimated for 1 week prior to the initiation of the study, after which an ocular exam was performed in each rabbit in order to rule out previous or ongoing ocular problems.

Rabbits were divided into four groups, three treatment groups composed of three animals each and one control group of two animals (n = 22 eyes). Treatment groups were tested using topical alpha-luminol in concentrations of 0.5%, 1.5% and 2.5% eyedrops. The control group received topical NaCl. All treated animals received a single drop simultaneously in both eyes.

Tissue collection

Aqueous humor and peripheral blood samples were taken from each animal at three different, randomly chosen timepoints: 20 min, 4 h and 12 h post treatment. At each timepoint, the examiner (EG) looked for potential signs of ocular toxicity from the topical compound (increased ocular discharge, abnormal degree of conjunctival hyperemia, blepharospasm, pain, peri-ocular pruritus, reluctance to touch, vocalization). Aqueous humor samples were obtained after a limbal paracentesis, following topical and local anesthesia with 0.5% proparacaine hydrochloride. At the last timepoint, rabbits were humanely euthanized with anesthetic overdose intravenously. Both eyes of each rabbit were enucleated. The cornea was removed at the level of the limbus, and after the iris was sectioned and removed, the lens was collected. Vitreous was carefully dissected posteriorly and removed as a whole, followed by the neuro-retina. All samples were placed in sterile tubes and stored under −80 °C for later evaluation. Quantification of alpha-luminol was done by high performance liquid chromatography (HPLC) coupled to electrospray ionization (ESI) mass spectrometry (MS).

HPLC apparatus and conditions

The HPLC system consisted of an Agilent 1200 Series binary pump SL, vacuum degasser, thermostatted column compartment SL (Agilent Technologies, Santa Clara, CA, USA) and a CTC Analytics HTC PAL System autosampler (Leap Technologies, Carrboro, NC, USA). The HPLC column was a Waters XBridge Phenyl column (4.6 × 50 mm ID, 5.0 μm bead size) (Waters Corporation, Milford, MA, USA) protected by a SecurityGuard™ C18 cartridge (4 × 2.0 mm ID) (Phenomenex, Torrance, CA, USA) and maintained at a temperature of 40 °C. The mobile phase consisted of 99% methanol and 1% 0.1% formic acid in water. The system operated at a flow-rate of 1 mL/min.

MS apparatus and conditions

Mass spectrometric detection was performed on an API 3200™ triple quadrupole instrument (Applied Biosystems Inc, Foster City, CA, USA) using multiple reaction monitoring. Ions were generated in positive ionization mode using an electrospray interface. Compound-dependent parameters were as follows: declustering potential (DP): 44 V; entrance potential (EP): 4 V; collision cell entrance potential (CEP): 15.127 V; collision-activated dissociation (CAD) gas (nitrogen): 6 psi; collision energy (CE): 30 V and collision cell exit potential (CXP): 2.5 V. Source-dependent parameters were as follows: nebulizer gas (GS1): 40 psi; auxiliary (turbo) gas (GS2): 60 psi; turbo gas temperature (TEM): 550 °C; curtain gas (CUR): 50 psi, ionspray voltage (IS): 4500 V and interface heater (IH): 100 °C. Peak areas obtained from

MRM of alpha-luminol (*m/z* 178 → 105) were used for quantification.

Sample preparation
Standard solutions containing alpha-luminol were prepared in water. The drug was extracted from rabbit serum and ocular tissues (aqueous humor, vitreous humor, lens and retina) using Waters Oasis® HLB 1cc (30 mg) solid phase extraction cartridges (Waters Corporation, Milford, MA, USA) following the manufacturer's instructions for the generic SPE method. Briefly, cartridges were conditioned with 1 mL of methanol, equilibrated with 1 mL of water, loaded with sample, washed twice with 1 mL of water and eluted with 1 mL of methanol. For serum, aqueous humor and vitreous humor, 100 μL of each liquid was loaded onto the cartridge for extraction. The lens was weighed, the appropriate volume of water was added to result in a tissue concentration of 500 mg/mL and the sample was sonicated on ice until the tissue was homogenated. For the retina, 250 μL of water was added to the entire tissue and the sample was sonicated on ice until the tissue was homogenated. For both the lens and the retina, 100 μL of the homogenate was loaded onto the cartridge for extraction. An aliquot of 50 μL of the methanol eluant was injected into the LC/MS system for analysis.

Statistical analysis
For each time point (20 min, 4 h, and 12 h post administration), the amount of drug in the aqueous humor using 0.5% drops was compared to that using 1.5% and 2.5% drops using Wilcoxon rank-sum testing. Similarly, for each dosage group (0.5%, 1.5%, and 2.5% drops), the amount of drug in the aqueous humor at 20 min was compared to that at 4 and 12 h post administration using Wilcoxon rank-sum testing. For all tests, results were considered statistically significant at $P \leq 0.05$.

RESULTS

Peripheral blood
After single-dose administration of 0.5% alpha-luminol eyedrops, the drug was not detected in the peripheral blood at any timepoint (level of detection ≥ 0.02 ng/mL) nor was it detected at 12 h in either the 1.5% or 2.5% groups. However, it was detectable at 20 min in one rabbit from the 1.5% group (18.8 ng/mL), in two rabbits from the 2.5% group (21.4 ng/mL, 22.2 ng/mL); also after 4 h in two rabbits, one from the 1.5% group (11.6 ng/mL) and one from the 2.5% group (35.8 ng/mL).

Aqueous Humor
After single-dose administration of 0.5%, 1.5% and 2.5% alpha-luminol eyedrops, the amount of drug in the aqueous humor per eye ranged from undetected levels to 272 ng/mL. At 20 min after administration, the level of alpha-luminol in the aqueous humor of rabbits administered 1.5% drops was significantly higher than rabbits administered 0.5% drops (Table 1). No other significant differences due to dosage were observed (Table 1). In rabbits administered 0.5% drops and 1.5% drops, aqueous humor levels of alpha-luminol were significantly lower at 4 and 12 h post-administration compared to 20 min post-administration (Table 2). In rabbits administered 2.5% drops, aqueous humor levels of alpha luminol were significantly lower at 4 h after administration than 20 min after administration, but were not significantly different 12 h after administration (Table 2). No drug was found in rabbits from the control group. Raw values from the peripheral blood and aqueous humor are shown in Table 3.

Vitreous humor, retina and lens
After single-dose administration of 0.5%, 1.5% and 2.5% alpha-luminol eyedrops, the vitreous humor concentrations were below the level of detection (0.02 ng/mL) in most of the animals, with exception of one rabbit in group 2 (1.5%) (16.4 ng/mL) and one rabbit in group 3 (2.5%) (21.5 ng/mL). No drug was detected in either the retinal homogenate or the lens.

DISCUSSION

In this study we aimed to characterize the penetration of topically administered alpha-luminol into various ocular compartments using a rabbit model. Our results demonstrate that the topically administered, potent antioxidant alpha-luminol readily penetrates the cornea and anterior chamber. In this formulation, it did not cause ocular irritation or local toxicity, as none of the rabbits showed signs of blepharospasm, itching or conjunctival hyperemia, during the study.

The drug concentrations in the aqueous humor were relatively low. The highest concentration achieved in any rabbit was 272 μg/mL at the 20 min timepoint. However, this was only 0.02% of the drug contained in one drop. Whether this

Table 1. Comparison of alpha-luminol in aqueous humor at specified time points in rabbits administered 1.5% or 2.5% drops vs. those administered 0.5% drops

Time point	0.5% median	1.5% median (*P*-value)	2.5% median (*P*-value)
20 min	39.8	131.5 (0.04)	166.5 (0.15)
4 h	<0.02	15 (0.10)	20.8 (0.10)
12 h	<0.02	<0.02 (0.90)	12.5 (0.15)

Table 2. Comparison of alpha-luminol in aqueous humor at specified dosages at 4 or 12 h post administration vs. 20 min post administration

Dosage (drops)	20 min median	4 h median (*P*-value)	12 h median (*P*-value)
0.5%	39.8	<0.02 (0.03)	<0.02 (<0.01)
1.5%	131.5	15 (<0.01)	<0.02 (<0.01)
2.5%	166.5	20.8 (0.05)	12.5 (0.17)

Table 3. Peripheral blood and aqueous humor alpha-luminol values in all treated rabbits evaluated in the study

		Peripheral blood (ng/mL)			Aqueous humor (ng/mL)			
Alpha-luminol drop dose	Rabbit no.	20 min	4 h	12 h	Eye	20 min	4 h	12 h
0.50%	1	<0.02	<0.02	<0.02	OS	216	<0.02	<0.02
					OD	65.1	95.8	<0.02
	2	<0.02	<0.02	<0.02	OS	37.3	<0.02	<0.02
					OD	42.3	<0.02	<0.02
	3	<0.02	<0.02	<0.02	OS	19.6	<0.02	15.28
					OD	14.2	<0.02	<0.02
	M	–	–	–	M	65.75	15.97	2.55
	M′	–	–	–	M′	39.8	<0.02	<0.02
	SD	–	–	–	SD	75.79	39.1	6.23
1.50%	4	18.8	11.6	<0.02	OS	178	28.9	<0.02
					OD	130	12.7	<0.02
	5	<0.02	<0.02	<0.02	OS	111	12.9	<0.02
					OD	133	17.1	<0.02
	6	<0.02	<0.02	<0.02	OS	265	30.1	25.4
					OD	125	<0.02	<0.02
	M	6.27	3.87	–	M	157	16.95	4.24
	M′	–	–	–	M′	131.5	15	<0.02
	SD	10.84	6.69	–	SD	57.53	11.28	10.36
2.50%	7	21.4	35.8	<0.02	OS	143	32.4	26.6
					OD	232	29.8	730*
	8	22.2	<0.02	<0.02	OS	190	19.3	<0.02
					OD	272	22.2	25
	9	<0.02	<0.02	<0.02	OS	108	12	<0.02
					OD	<0.02	<0.02	<0.02
	M	14.53	11.94	–	M	157.5	19.28	294.07*
	M′	21.4	–	–	M′	166.5	20.8	12.5
	SD	12.58	20.66	–	SD	97.13	11.96	294.07*

M, mean; M′, median, SD, standard deviation.
*Indicates an outlier due to lab error.

amount of drug is therapeutically relevant remains to be investigated. Interestingly, this rabbit with the highest drug concentration was part of the 2.5% group and was one of the few rabbits in which the drug was also found in the serum. Detectable serum concentrations in this rabbit were found only in the first serum timepoint (22.2 ng/mL).

There were important differences in the amounts of drug present in the aqueous humor in the lowest treatment concentration group (group 1, 0.5%) vs. group 2 (1.5%) (diff = +91.7 ng/mL) and group 3 (2.5%) (diff = +126.7 ng/mL) at the first timepoint. These results suggest that both 1.5% and 2.5% topical alpha-luminol drops were able to achieve aqueous humor levels well above those found in group 1(0.5%). Despite the lack of significance at the 5% level (95% confidence interval, $P < 0.05$), the results are promising considering that even with such a small sample size, a borderline significant difference at the 10–15% level (85–90% confidence interval, $P \leq 0.15$) was detected when comparing median levels of alpha-luminol in eyes from rabbits between the 0.5% group and the 1.5% and 2.5% group. This suggests that increasing the concentration of the drug in the eyedrops could potentially produce therapeutically significant intraocular levels. In rabbits administered 2.5% drops, aqueous humor levels of alpha luminol were significantly lower at 4 h after administration than 20 min after administration, but were not significantly different 12 h after administration (Table 2). This is due to an exceedingly high aqueous humor value (730 ng/mL) in group 3, at the 12 h period, which was most likely a lab error (Table 3).

Rabbits were chosen for this study because their corneal structure and aqueous humor volume are similar to that of humans.[28–30] In addition, they have a small body size, are easy to handle and have large eyes.[30,31] However, rabbits have an especially labile blood–aqueous barrier (BAB)[31] and this could have led to leakage of serum-derived alpha-luminol into the eye after the first paracentesis. Therefore, the aqueous humor drug levels obtained at the first paracentesis may represent the best indicators of the true ocular penetration properties of the drug. Moreover, since in most of the rabbits serum levels were either not detectable or lower than the aqueous humor levels, it is unlikely that the drug levels present inside the eye at the later timepoints were attributable to serum leakage.

A major limitation of this study was the small sample size (three rabbits per group, $n = 6$ eyes in each treatment group), which can also account for the high data variability present in the study. However, this was designed as a pilot study and the data was analyzed by statistical techniques that were appropriate for the small sample size.

The significant decrease in intra-ocular drug concentrations from the first timepoint is expected in any ocular pharmacological study. The fact that two of the rabbits

showed an increase in the alpha-luminol concentration with time (rab1-OD, rab8-OD; Table 3) could not be explained). The aqueous and vitreous humor turnover rate may profoundly affect the pharmacokinetics of a drug. The aqueous humor turnover rate in rabbits (half life = 46 min) [27] is similar to that of humans (half life = 52 min) [28] which is relatively slow compared to the fast turnover rate of the aqueous portion of the vitreous. From experiments in which heavy water was injected intraperitoneally and subsequently detected in the vitreous, it was calculated that 50% of the water content of the vitreous is replaced every 10–15 min and 75–87% is replaced in 30 min.[32] Consequently, the fact that we were able to find the drug in the vitreous after 12 h in two rabbits may mean that it could have been found in higher concentrations had the vitreous been sampled at earlier timepoints.

Topical alpha-luminol is largely hydrophilic. Drugs with higher lipophilicity have a greater ability to penetrate an intact cornea.[30] Future pharmacodynamic studies may consider increasing the penetration potential of the drug in order to achieve constant adequate intraocular concentration; potential periocular routes for posterior segment drug delivery should also be investigated, such as sub-conjunctival and sub-tenon routes.[33]

The importance of achieving therapeutic levels of alpha-luminol in the retina stems from its potential as a strong preventive and therapeutic drug in this structure. In the central nervous system as well as the retina, glial cells protect, up-regulate and stabilize neuronal levels of Nrf2.[24–26,34–36] The Nrf2 pathway in the neuronal cells coordinates the up-regulation of antioxidant defenses and confers protection to neighboring neurons.[37,38] This protective mechanism against oxidative stress is up-regulated in neuroimmunodegenerative processes such as in Alzheimer's, Parkinson's and Huntington's disease.[25,26,39–44] In diseased neurologic tissue, therapeutically increasing the expression of Nrf2 in glial cells significantly delays the onset of the disease and extends neuronal survival, both *in vitro* and *in vivo*.[22,25,37,38,43–45]

The biochemical composition of ocular structures, especially that of the retina (high concentration of unsaturated fatty acids in the retina), is an important factor that makes the eye more susceptible to oxidative stress, in comparison to other organs.[46] Oxidative stress-induced changes and microvascular damage occur in retinas of dogs with primary glaucoma. This was demonstrated by documenting the loss of major retinal antioxidants such as glutathione and taurine and changes in retinal glutamate in these animals.[3,47–49] In the DBA2J mouse model of glaucoma, administration of oral alpha-luminol decreased oxidative stress and reduced changes in glutamate distribution in their retinas.[50] This suggests that alpha-luminol could be used to reduce retinal oxidative stress and consequent neuronal damage in canine glaucoma as well. Further pharmacokinetic data is necessary along with the determination of therapeutic drug levels in the retina, prior to the clinical use of alpha-luminol in glaucoma. This pilot study represents the first step in this process as it investigates the use and the ocular pharmacokinetics of topical alpha-luminol for the first time.

ACKNOWLEDGMENTS

Supported by a grant from the Longevity Foundation, Austin, TX. The authors would like to thank Lauren Marchewitz for the help in acquiring the data for this study.

REFERENCES

1. Kowluru RA, Chan PS. Oxidative stress and diabetic retinopathy. *Experimental Diabetes Research* 2007; **2007**: 43603.
2. Zirofsky DS, Madl J, Gionfriddo JR. Oxidative stress in glaucomatous retinas of dogs. *Proceedings of the 39th Annual Conference of the American College of Veterinary Ophthalmologists 2008;* Boston, MA: 36.
3. Madl JE, McIlnay TR, Powell CC *et al.* Depletion of taurine and glutamate from damaged photoreceptors in the retinas of dogs with primary glaucoma. *American Journal of Veterinary Research* 2005; **66**: 791–799.
4. Quigley HA. Neuronal death in glaucoma. *Progress in Retinal and Eye Research* 1999; **18**: 39–57.
5. Jonas JB, Budde WM. Diagnosis and pathogenesis of glaucomatous optic neuropathy: morphological aspects. *Progress in Retinal and Eye Research* 2000; **19**: 1–40.
6. Quigley HA, Broman AT. The number of people with glaucoma worldwide in 2010 and 2020. *The British Journal of Ophthalmology* 2006; **90**: 262–267.
7. Emi K, Pederson JE, Toris CB. Hydrostatic pressure of the suprachoroidal space. *Investigative Ophthalmology & Visual Science* 1989; **30**: 233–238.
8. Morgan WH, Yu DY, Cooper RL *et al.* The influence of cerebrospinal fluid pressure on the lamina cribrosa tissue pressure gradient. *Investigative Ophthalmology & Visual Science* 1995; **36**: 1163–1172.
9. Tezel G, Wax MB. Increased production of tumor necrosis factor-alpha by glial cells exposed to simulated ischemia or elevated hydrostatic pressure induces apoptosis in cocultured retinal ganglion cells. *The Journal of Neuroscience* 2000; **20**: 8693–8700.
10. Wax MB, Tezel G, Kobayashi S *et al.* Responses of different cell lines from ocular tissues to elevated hydrostatic pressure. *The British Journal of Ophthalmology* 2000; **84**: 423–428.
11. Liu Q, Ju WK, Crowston JG *et al.* Oxidative Stress Is an Early Event in Hydrostatic Pressure–Induced Retinal Ganglion Cell Damage. *Investigative Ophthalmology & Visual Science* 2007; **48**: 4580–4589.
12. Moss RW. A friendly skeptic look at Galavit®. [homepage on internet; cited 03.04.2009] Cancer Decisions® Newsletter Arquives http://www.cancerdecisions.com/100503_page.html
13. Bach Pharma – Galavit Brochure [homepage on internet; cited 03.04.2009] Medisan Pharmaceuticals Ltd. *Galavit Documents.* Scientific Information. http://www.galavit.com
14. Sukhorukov AL. Galavit efficiency in prophylaxis and treatment of pyo-inflammatory complications at abdominal surgical pathology. *Voenno-meditsinskiĭ zhurnal* 2005; **326**: 31–33.
15. Shaplygin LV, Klopot AM. The efficiency of the drug "Galavit" in complex treatment for infectious-and-inflammatory diseases of urogenital system. *Voenno-meditsinskiĭ zhurnal* 2006; **327**: 29–34.
16. Butorov IV, Nikolenko IA, Butorov SI. Efficacy of Galavit in patients with duodenal ulcer. *Klinicheskaia Meditsina (Mosk)* 2005; **83**: 72–75.

17. Larina TV, Bondarenko AV, Bogomolova NS *et al.* The immune status and its correction in patients after reconstructive surgeries for cicatricial stenosis of trachea. *Anesteziologiia i Reanimatologiia* 2004; **5**: 83–85.
18. Vyshegurov I, Anikhovskaia I, Batmanov I *et al.* Intestinal endotoxin in the pathogenesis of ocular inflammatory diseases and the antiendoxin constituent of its treatment. *Patologicheskaia Fiziologiia i Èksperimental'naia Terapiia* 2007; **1**: 12–14.
19. Nelyubov MV. Cytokins in the pathogenesis of astrakhan spotted Fever and North Asian scrub typhus: problems of immunocorrection. *Bulletin of Experimental Biology and Medicine* 2002; **134**: 165–167.
20. Jiang Y, Scofield VL, Yan M *et al.* Retrovirus-induced oxidative stress with neuroimmunodegeneration is suppressed by antioxidant treatment with a refined monosodium alpha-luminol (Galavit). *Journal of Virology* 2006; **80**: 4557–4569.
21. Scofield VL, Yan M, Kuang X *et al.* The drug monosodium luminol (GVT) preserves crypt-villus epithelial organization and allows survival of intestinal T cells in mice infected with the ts1 retrovirus. *Immunology Letters* 2009; **122**: 150–158.
22. Scofield VL, Yan M, Kuang X *et al.* The drug monosodium luminol (GVT) preserves thymic epithelial cell cytoarchitecture and allows thymocyte survival in mice infected with the T cell-tropic, cytopathic retrovirus ts1. *Immunology Letters* 2009; **122**: 159–169.
23. Moi P, Chan K, Asunis I *et al.* Isolation of NF-E2-related factor 2 (Nrf2), a NF-E2-like basic leucine zipper transcriptional activator that binds to the tandem NF-E2/AP1 repeat of the beta-globin locus control region. *Proceedings of the National Academy of Sciences of the United States of America* 1994; **91**: 9926–9930.
24. Nguyen T, Nioi P, Pickett CB. The Nrf2-antioxidant response element signaling pathway and its activation by oxidative stress. *The Journal of Biological Chemistry* 2009; **284**: 13291–13295.
25. Johnson JA, Johnson DA, Kraft AD *et al.* The Nrf2-ARE pathway: an indicator and modulator of oxidative stress in neurodegeneration. *Annals of the New York Academy of Sciences* 2008; **1147**: 61–69.
26. de Vries HE, Witte M, Hondius D *et al.* Nrf2-induced antioxidant protection: a promising target to counteract ROS-mediated damage in neurodegenerative disease? *Free Radical Biology & Medicine* 2008; **45**: 1375–1383.
27. Schoenwald R. Ocular drug delivery pharmacokinetic considerations. *Clinical Pharmacokinetics* 1990; **18**: 225.
28. DeSantis LM Jr, Patil PN. Pharmacokinetics. In: *Havener's Ocular Pharmacology*, 6th edn (eds Mauger TF, Craig EL) Mosby, St.Louis, 1994; 22–52.
29. Bartlett JD. Ophthalmic Drug Delivery. In: *Clinical Ocular Pharmacology*, 4th edn (eds Bartlett JD, Jaanus SD) Elsevier Butterworth Heinemann, Maryland Heights, MO, 2001; 41–62.
30. Worakul N, Unlu N, Robinson JR. Ocular Pharmacokinetics. In: *Principles and Practive of Ophthalmology*, 2nd edn (eds Albert DM, Jakobiek FA) Saunders, Ellsworth, Oxford, 2000; 202–211.
31. Bito L. Species differences in the responses of the eye to irritation and trauma: a hypothesis of divergence in ocular defense mechanisms, and the choice of experimental animals for eye research. *Experimental Eye Research* 1984; **39**: 807–829.
32. Kinsey VE, Grant M, Cogan DG. Water movement and the eye. *Archives of Ophthalmology* 1942; **27**: 242–252.
33. Raghava S, Hammond M, Kompella UB. Periocular routes for retinal drug delivery. *Expert Opinion on Drug Delivery* 2004; **1**: 99–114.
34. Lee JM, Calkins MJ, Chan K *et al.* Identification of the NF-E2-related factor-2-dependent genes conferring protection against oxidative stress in primary cortical astrocytes using oligonucleotide microarray analysis. *The Journal of Biological Chemistry* 2003; **278**: 12029–12038.
35. Shih AY, Johnson DA, Wong G *et al.* Coordinate regulation of glutathione biosynthesis and release by Nrf2-expressing glia potently protects neurons from oxidative stress. *The Journal of Neuroscience* 2003; **23**: 3394–3406.
36. Kraft AD, Johnson DA, Johnson JA. Nuclear factor E2-related factor 2-dependent antioxidant response element activation by tertbutylhydroquinone and sulforaphane occurring preferentially in astrocytes conditions neurons against oxidative insult. *The Journal of Neuroscience* 2004; **24**: 1101–1112.
37. Vargas MR, Johnson DA, Sirkis DW *et al.* Increased glutathione biosynthesis by Nrf2 activation in astrocytes prevents p75NTR-dependent motor neuron apoptosis. *Journal of Neurochemistry* 2006; **97**: 687–696.
38. Qiang W, Kuang X, Liu J *et al.* Astrocytes survive chronic infection and cytopathic effects of the ts1 mutant of the retrovirus Moloney murine leukemia virus by upregulation of antioxidant defenses. *Journal of Virology* 2006; **80**: 3273–3284.
39. Calkins MJ, Jakel RJ, Johnson DA *et al.* Protection from mitochondrial complex II inhibition in vitro and in vivo by Nrf2-mediated transcription. *Proceedings of the National Academy of Sciences of the United States of America USA* 2005; **102**: 244–249.
40. Jakel RJ, Townsend JA, Kraft AD *et al.* Nrf2-mediated protection against 6-hydroxydopamine. *Brain Research* 2007; **1144**: 192–201.
41. Chen PC, Vargas MR, Pani AK *et al.* Nrf2-mediated neuroprotection in the MPTP mouse model of Parkinson's disease: Critical role for the astrocyte. *Proceedings of the National Academy of Sciences of the United States of America* 2009; **106**: 2933–2938.
42. Kanninen K, Malm TM, Jyrkkänen HK *et al.* Nuclear factor erythroid 2-related factor 2 protects against beta amyloid. *Molecular and Cellular Neurosciences* 2008; **39**: 302–313.
43. Di Matteo V, Esposito E. Biochemical and therapeutic effects of antioxidants in the treatment of Alzheimer's disease, Parkinson's disease, and amyotrophic lateral sclerosis. *Current Drug Targets CNS and Neurological Disorders* 2003; **2**: 95–107.
44. van Muiswinkel FL, Kuiperij HB. The Nrf2-ARE Signalling pathway: promising drug target to combat oxidative stress in neurodegenerative disorders. *Current Drug Targets CNS and Neurological Disorders* 2005; **3**: 267–281.
45. Vargas MR, Johnson DA, Sirkis DW *et al.* Nrf2 activation in astrocytes protects against neurodegeneration in mouse models of familial amyotrophic lateral sclerosis. *The Journal of Neuroscience* 2008; **28**: 13574–13581.
46. Augustin AJ. Oxidative tissue damage. *Klin Monbl Augenheilkd* 2010; **2**: 90–98.
47. Alyahya K, Chen CT, Mangan B *et al.* Microvessel loss, vascular damage and glutamate redistribution in the retinas of dogs with primary glaucoma. *Veterinary Ophthalmology* 2007; **1**: 70–77.
48. McIlnay TR, Gionfriddo JR, Dubielzig RR *et al.* Evaluation of glutamate loss from damaged retinal cells of dogs with primary glaucoma. *American Journal of Veterinary Research* 2004; **65**: 776–786.
49. Chen CT, Alyahya K, Gionfriddo JR *et al.* Loss of glutamine synthetase immunoreactivity from the retina in canine primary glaucoma. *Veterinary Ophthalmology* 2008; **11**: 150–157.
50. Gionfriddo JR, Freeman KS, Groth A *et al.* alpha-luminol prevents decreases in glutamate, glutathione and glutamine synthetase in the retinas of DBA/2J mice. *Veterinary Ophthalmology* 2009; **12**: 325–332.