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Intraocular distribution of melanin in human, monkey, rabbit, minipig and dog eyes

Chandrasekar Durairaj¹, James E. Chastain², and Uday B. Kompella^{1,3,4}

¹Department of Pharmaceutical Sciences, University of Colorado Anschutz Medical Campus, Aurora, CO 80045

²Drug Metabolism and Pharmacokinetics, Alcon Research Ltd., Fort Worth, TX 76134

³Department of Ophthalmology, University of Colorado Anschutz Medical Campus, Aurora, CO 80045

⁴Department of Bioengineering, University of Colorado Anschutz Medical Campus, Aurora, CO 80045

Abstract

The purpose of this study was to quantify the melanin pigment content in sclera, choroid-RPE, and retina, three tissues encountered during transscleral drug delivery to the vitreous, in human, rabbit, monkey, minipig, and dog models. Strain differences were assessed in NZW X NZR F1 and Dutch-belted rabbits and Yucatan and Gottingen minipigs. The choroid-RPE and retina tissues were divided into central (posterior pole area) and peripheral (away from posterior pole) regions while the sclera was analyzed without such division. Melanin content in the tissues was analyzed using a colorimetric assay. In all species the rank order for pigment content was: choroid-RPE > retina > sclera, except in humans, where scleral melanin levels were higher than retina and central choroid. The melanin content in a given tissue differed between species. Further, while the peripheral tissue pigment levels tended to be generally higher compared to the central regions, these differences were significant in human in the case of choroid-RPE and in human, monkey, and dogs in the case of retina. Strain difference was observed only in the central choroid-RPE region of rabbits (NZWxNZR F1 > Dutch Belted). Species, strain, and regional differences exist in the melanin pigment content in the tissues of the posterior segment of the eye, with Gottingen minipig being closest to humans among the animals assessed. These differences in melanin content might contribute to differences in drug binding, delivery, and toxicity.

Keywords

Melanin content; retina; sclera; choroid; human; rabbit; dog; minipig

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Corresponding author: Uday B. Kompella, PhD, 12850 East Montview Blvd, C238-V20, Department of Pharmaceutical Sciences, University of Colorado Denver, Aurora, CO 80045. Phone: (303) 724-4028; Fax: (303) 724-4666; Uday.Kompella@ucdenver.edu.

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Introduction

In the eye, melanin is an important pigment, distributed primarily in the iris, ciliary body, choroid and retinal pigment epithelium (RPE). Chemically, melanin is a polyanionic polymer consisting of various repeating units of 5,6-dihydroxy indole-2-carboxylic acid and 5,6-dihydroxy indole (Nofsinger et al., 2000). Many drugs and chemicals are known to bind to ocular melanin either reversibly or irreversibly (Rubin, 1992). Binding of drugs to melanin is known to affect the pharmacologic and pharmacokinetic properties of drug molecules. The importance of drug melanin binding in pharmacology was demonstrated decades earlier (Potts, 1962; Salazar et al., 1976). Literature supports altered pharmacokinetics and/or pharmacodynamics due to the presence of melanin pigment for drugs including gentamicin (Kane et al., 1981), ofloxacin (Perkins et al., 1995), pefloxacin (Cochereau-Massin et al., 1991), grepafloxacin (Solans et al., 2004), beta-blockers (Araie et al., 1982), celecoxib (Amrite et al., 2010; Cheruvu et al., 2008), ganciclovir, and acyclovir (Durairaj et al., 2009b; Hughes et al., 1996). Since ocular drug product development relies heavily on preclinical studies, choosing an animal model with melanin pigmentation similar to humans is most desirable in obtaining valid preclinical data. Alternatively, understanding the differences in melanin pigment between species might allow for appropriate assumptions in extrapolating preclinical data to humans.

Melanin content differs in various ocular tissues (e.g., cornea and lens are non-pigmented, whereas iris, ciliary body, choroid, and RPE are pigmented) and also between pigmentation strains or phenotypes (e.g., albino vs. pigmented). Also, regional difference in the distribution of RPE melanin has been reported by Schmidt and Peisch in human eyes (Schmidt and Peisch, 1986). The distribution of melanin within a tissue is also an important factor to be considered since such differences might affect drug delivery to a specific region within the target tissue. We have previously demonstrated differences in melanin content in the eye tissues of Sprague-Dawley and Brown-Norway rats and observed significant decrease in the transscleral delivery of celecoxib in Brown-Norway rats (Cheruvu et al., 2008). This is consistent with our theory that melanin binding impeded transscleral drug delivery to the retina. However, the results in a preclinical study should be interpreted based on the melanin content differences between humans and the preclinical animal models. With this objective, we investigated the melanin content levels in various ocular tissues (retina, choroid-RPE and sclera) in rabbit, dog, minipig, and monkey models that can potentially be employed in ocular pharmacokinetic studies supporting drug product development. Further, we compared these levels to human eye tissues. Additionally, we investigated the melanin content level in different regions (central and peripheral) for retina and choroid-RPE since local differences in pigment content may influence drug delivery.

Materials and methods

Materials

Sodium hydroxide, glacial acetic acid, synthetic melanin (Cat no. M0418-100 mg, Lot no. 046K12461), and dimethyl sulfoxide were obtained from Sigma-Aldrich (St. Louis, MO).

Animal and human tissues

All the ocular tissues and isolated regions of tissues were received from Alcon Laboratories, Inc. (Fort Worth, TX) and maintained at 4 °C. Experiments were conducted in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. The study used tissues from freshly excised eyes of Dutch-belted (DB) and New Zealand White (NZW) x New Zealand Red (NZR) F1 rabbits (Myrtle's Rabbitry, Thompsons Station, TN); Yucatan minipig, Gottingen minipig and Beagle dog eyes (enucleated at Covance Research

Products, Denver, PA); and Cynomolgus monkey eyes (enucleated at Covance Research Products, Alice, TX). Human cadaver eyes were obtained from anonymous donors (Tampa Eye Bank, Tampa, Florida) within 48 hours of death in accordance with the protocol approved by the Institutional Review Board, with procedures in compliance with the Declaration of Helsinki for research involving human tissues. The choroid-RPE and retina tissues were divided into central (3 mm around macular area or, in animals lacking a defined macula, posterior pole area) and peripheral (away from macula or posterior pole) regions.

Melanin content estimation

Melanin content was estimated in the central and peripheral retina, central and peripheral choroid-RPE, and in sclera by a previously described method (Cheruvu et al., 2008). Briefly, weighed amount of tissue was placed in Eppendorf tubes containing 100 μ l of 1M sodium hydroxide solution followed by addition of 10 μ l of dimethyl sulfoxide. Tubes were kept in a water bath and boiled for 45 minutes to solubilize melanin. Volume of the contents in the tube were brought up to 500 μ l with distilled water and then neutralized with 1M diluted acetic acid (500 μ l). Immediately after melanin solubilization, the absorbance of the samples was measured at 476 nm against the blank solubilization buffer using a spectrophotometer (Hewlett Packard 8452A Diode Array Spectrophotometer). Melanin content in the tissues was quantified using a standard curve for synthetic melanin (Sigma-Aldrich, St. Louis, MO) prepared just before each analysis.

Statistical data analysis

All statistical analysis were performed using GraphPad Prism (Version 5.01, GraphPad Software, Inc., La Jolla, CA). Melanin content in each tissue of every species and strain was compared with the human levels using the nonparametric Mann-Whitney U test. Also comparison was made between the central and peripheral regions of choroid-RPE and retina to investigate the regional differences. For estimating the strain differences in rabbits and minipigs, melanin pigment levels in each tissue of one strain was compared with the other (Rabbits: NZW X NZR F1 vs. Dutch-belted, Minipigs: Yucatan vs Gottingen).

Results

Melanin content in the various ocular tissues was estimated by the sodium hydroxide solubilization method. With synthetic melanin, standard curve signal linearity was observed in the range of 1.9 to 248.3 μ g/mL. The melanin content estimated in the various ocular tissues from different species and strains of animals is summarized in Table 1. The mean melanin content in various ocular tissues was in the following general order in different species:

NHP monkey: Peripheral choroid-RPE > central choroid-RPE > peripheral retina > central retina > sclera

Human: Peripheral choroid-RPE > sclera > central choroid-RPE ~ peripheral retina > central retina

Dog: Peripheral choroid-RPE > central choroid-RPE > peripheral retina > central retina > sclera

F1-Rabbit: Peripheral choroid-RPE > central choroid-RPE > peripheral retina > central retina > sclera

DB-Rabbit: Peripheral choroid-RPE > peripheral retina ~ central retina > central choroid-RPE > sclera *Yucatan Minipig*: Peripheral choroid-RPE > central choroid-RPE > peripheral retina > central retina ~ sclera

Gottingen Minipig: Central choroid-RPE > peripheral choroid-RPE > peripheral retina > central retina > sclera

The rank order of melanin pigment content among various species was in the following general order:

Central choroid-RPE: Monkey > F1 rabbit > dog > Yucatan minipig > Gottingen minipig > DB rabbit > human

Peripheral choroid-RPE: Monkey > F1 rabbit > dog > Yucatan minipig > DB rabbit > Gottingen minipig ~ human

Central retina: F1 rabbit > DB rabbit > Gottingen minipig > dog > human = Yucatan minipig > monkey

Peripheral retina: F1 rabbit > DB rabbit > Yucatan minipig > monkey > Gottingen minipig ~ dog > human

Sclera: Human > Yucatan minipig > DB rabbit ~ dog > monkey = F1 rabbit > Gottingen minipig

Statistical significance of the above relationships has been indicated in Table 1.

In case of monkey, melanin pigment content in all the tissues except central retina was significantly different from that of human levels (Table 1). Melanin levels in the sclera were lower than in the human tissue, while higher melanin levels were observed in other tissues except central retina. Dog model exhibited trends similar to monkey, in comparison to human tissues, and the melanin pigment levels were significantly different from that of human in all tissues except central retina. Both F1 and DB rabbits had significantly different melanin levels (higher in retina and choroid-RPE, lower in sclera) in all the tissues when compared with the human levels. Yucatan minipig had significantly higher melanin levels in the peripheral retina and entire choroid-RPE, while Gottingen minipig had significantly different melanin levels in central choroid-RPE and sclera when compared to human tissue levels.

Across the tissues, in the central retina, melanin content of all the species except rabbits was similar to that of humans. In the peripheral retina, melanin pigment levels of all species except Gottingen minipig were significantly higher than the human levels. In the central and peripheral choroid-RPE, all species had higher melanin levels except Gottingen minipigs. Human scleral melanin pigment levels were higher than any other species.

Regional difference in the melanin content was observed in the central and peripheral retina of monkey, human and dog ($p < 0.05$), where melanin content in the central retina was lower than the peripheral region (Fig. 1a). In other species, no significant difference was observed in the regional distribution of melanin in the retina. However, in case of choroid-RPE, only human exhibited significantly different ($p < 0.05$) melanin distribution among the central and peripheral regions, with melanin levels being higher in the peripheral region of choroid-RPE (Fig. 1b).

No significant difference was observed in the retina and sclera pigment levels among the different pigment strains of rabbits (F1 and DB) and minipigs (Yucatan and Gottingen). However, melanin levels in the central choroid-RPE were significantly higher in the F1 rabbits when compared with the DB rabbits. Although mean values for peripheral choroid-RPE were higher in Yucatan minipig as compared to Gottingen minipig, the difference was not statistically significant.

Discussion

In this paper we report for the first time that a) melanin content is the highest in the choroid-RPE followed by retina and sclera in all the species except human, where scleral melanin levels were higher than those of the retina and the central choroid; b) significant differences exist in the melanin content between species; c) significant regional differences exist in the choroid-RPE and retinal melanin pigment levels in some species; d) strain differences exist in the central choroid-RPE region of rabbits (F1 > DB). Collectively, these findings might impact preclinical model selection in transscleral drug product development.

Successful treatment of anterior and posterior segment diseases of eye depends on the extent of delivery of therapeutics to the target tissues. Delivery of molecules to the target site is hindered by the presence of multiple biological barriers. Melanin, an ocular pigment, is known to bind drugs with varying affinities, thereby altering pharmacokinetics (Araie et al., 1982; Cochereau-Massin et al., 1991; Durairaj et al., 2009b; Hughes et al., 1996). Preclinical (animal) studies are vital during drug discovery and development process in order to assess the potential safety and efficacy of a therapeutic agent. However, selection of an appropriate animal model that is comparable to humans plays an important role in obtaining translationally relevant results. Alternatively, an understanding of species differences would be helpful in making interpretations relevant to clinical medicine. In this study, melanin content was estimated in various ocular tissues from monkey, rabbit, dog, and minipig models and compared with the human model. Melanin content was estimated in central retina, peripheral retina, central choroid-RPE, peripheral choroid-RPE, and sclera. These tissues were selected since they are the key barriers encountered during drug delivery to the vitreous humor during transscleral delivery (Kadam et al., 2011; Kompella, 2007).

The central retina includes the macula and is the target for vision threatening posterior segment diseases like age-related macular degeneration and macular edema. Melanin content in this region for monkey, dog and minipig models were close to that of human model. Both F1 and Dutch belted rabbits had ~4 fold higher melanin levels than human model in the central retina. However, in the peripheral retina, both dog and Gottingen minipig, melanin levels were closer to the human. Similar to the central region, melanin levels in the peripheral retina of Dutch belted and F1 rabbit were 3 to 4 fold higher than the human model. Peripheral retinal levels of the monkey model were 1.7 fold higher compared to the human model.

Sclera and choroid-RPE are two barriers encountered prior to retinal delivery of a therapeutic agent via the transscleral or periocular routes (Amrite et al., 2008; Ayalasomayajula and Kompella, 2004; Kim et al., 2009). In the central choroid-RPE region, among the different species, melanin levels were lowest in the human. All other species had a 2 to 17 fold higher melanin content when compared with the human model. However, in the peripheral choroid-RPE region, Gottingen minipig melanin levels were similar to that of the human levels while other species had 2.8 to 10 fold higher melanin levels. In contrast to the retina and choroid-RPE tissues, melanin content of sclera in humans was higher than the other species investigated in this study. All other species had 1.9 to 5 fold lower melanin levels in sclera. It is noteworthy to mention here that the melanin content observed in sclera could be an artifact of tissue matrix. In our previous study with albino rabbits, which are devoid of melanin pigment in the sclera, measurable melanin levels were observed at 476 nm (Kadam et al., 2011). This artifact could also be due to the tissue dissection where the suprachoroidal layer which is rich in melanocytes often remains attached to the sclera (Csillag, 2005). All these results collectively indicated that no single animal model is close to the human melanin levels in all these tissues. However, the Gottingen minipig levels were closest to the human levels except in central choroid-RPE (2.9 fold higher) and sclera (5.3

fold lower). Although Gottingen minipig melanin levels were closer to that of human in most of the ocular tissues studied here, there is a paucity of pharmacokinetic data from minipigs.

Although significant difference was observed between the central and peripheral retina in humans, the melanin content was mere 1.3 fold higher in the peripheral region. Similarly, the peripheral retina tended to have higher melanin content than central retina in several preclinical models, with the differences being significant in the human, dog, and monkey models. The melanin content in the peripheral choroid-RPE region was 2 fold higher when compared with the central choroid-RPE in human tissues. Similar observation was reported in an earlier study where higher melanin concentrations were detected in the mid-peripheral and far-peripheral region of human retinal pigment epithelium when compared with the macula region (Schmidt and Peisch, 1986). Although similar trend in choroid-RPE melanin distribution was observed in most of the animals except Gottingen minipig, none of them were statistically significant.

Due to the limited bioavailability of drugs in the posterior segment after topical administration, alternative routes like subconjunctival, sub-tenon, and suprachoroidal injections are being increasingly explored for drug delivery to the back of the eye (Kompella, 2007). In order to exhibit its therapeutic action in the retina, a molecule has to cross sclera and/or choroid-RPE (Amrite et al., 2008). Depending on the melanin content in the barriers encountered before reaching the target tissue, the pharmacokinetics of drugs that bind to melanin may be altered in the target tissue. For instance, melanin content in the sclera of human is higher than any other species investigated in this study, indicating that there might be higher binding of drugs (with high melanin binding affinity) to human sclera. This, in turn, will have implications in assessing transscleral drug delivery in animal models, where the increased binding to scleral melanin pigment of humans will be undermined.

Intravitreal injections of drug suspensions (Baranano et al., 2009; Durairaj et al., 2009a), delivery systems and implants (Durairaj et al., 2009b) are being increasingly employed in the delivery of drugs to the posterior segment diseases where the target site is the macular region. In addition to other factors (Kadam and Kompella, 2010), distribution and elimination of intravitreally injected drugs depends on the position of injection (anterior or posterior) (Kompella U. B. , 2009). Drugs with higher binding affinity to melanin will be cleared slowly from the retina depending on the melanin content. Rabbits are one of the most commonly used animal model for assessing the delivery of intravitreally injected delivery systems. However, the retinal melanin content in Dutch belted rabbits is about 3 fold higher than the human model assessed in this study, indicating that clearance of drugs that bind to melanin will be slower from the retina of pigmented rabbits when compared with the humans.

Our results collectively indicate that melanin content varies between different species and even between regions of the same tissue. These variations have to be taken into consideration in selecting an appropriate animal model and in interpreting the ocular pharmacokinetic differences observed between humans and animal species for drugs that bind to melanin. Previous studies have shown regional variation and age-related reduction in the melanin content of normal human RPE (Schmidt and Peisch, 1986) and differences in the melanin content of iris, ciliary body and choroid-RPE between human blue and brown eyes (Menon et al., 1992). In addition to this, melanin levels may differ depending on the race. This age-related alteration in the human melanin content has to be considered while interpreting the outcomes of the present study. Another limitation to the current study is the absence of information on the race, gender and age of the human eye source and the use of a small number of eyes. These eyes were collected from anonymous donors, and no more

subject information was available. Also, we could not detect any melanin granule in the histology slides of sclera and retina from a Caucasian human eye (not shown). Besides these limitations, the current study compares the regional melanin differences in various species that could be considered for assessing the variations in drug delivery. In our previous study, we have shown the difference in melanin content between Sprague Dawley and Brown Norway rats and its role in the transscleral delivery of celecoxib (Cheruvu et al., 2008). To the best of our knowledge, this is the first study to compare the eye tissue melanin content in various animal species that are used in the pre-clinical drug development stage, with the human eye tissues.

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References

- Amrite A, Pugazhenth V, Cheruvu N, Kompella U. Delivery of celecoxib for treating diseases of the eye: influence of pigment and diabetes. *Expert Opin Drug Deliv*. 2010; 7:631–645. [PubMed: 20205602]
- Amrite AC, Edelhauser HF, Kompella UB. Modeling of corneal and retinal pharmacokinetics after periocular drug administration. *Invest Ophthalmol Vis Sci*. 2008; 49:320–332. [PubMed: 18172109]
- Araie M, Takase M, Sakai Y, Ishii Y, Yokoyama Y, Kitagawa M. Beta-adrenergic blockers: ocular penetration and binding to the uveal pigment. *Jpn J Ophthalmol*. 1982; 26:248–263. [PubMed: 6130180]
- Ayalasomayajula SP, Kompella UB. Retinal delivery of celecoxib is severalfold higher following subconjunctival administration compared to systemic administration. *Pharm Res*. 2004; 21:1797–1804. [PubMed: 15553225]
- Baranano DE, Kim SJ, Edelhauser HF, Durairaj C, Kompella U, Handa JT. Efficacy and Pharmacokinetics of Intravitreal Nonsteroidal Anti-Inflammatory Drugs for Intraocular Inflammation. *Br J Ophthalmol*. 2009
- Cheruvu NP, Amrite AC, Kompella UB. Effect of eye pigmentation on transscleral drug delivery. *Invest Ophthalmol Vis Sci*. 2008; 49:333–341. [PubMed: 18172110]
- Cochereau-Massin I, Bauchet J, Faurisson F, Vallois JM, Lacombe P, Pocidal JJ. Ocular kinetics of pefloxacin after intramuscular administration in albino and pigmented rabbits. *Antimicrob Agents Chemother*. 1991; 35:1112–1115. [PubMed: 1929251]
- Csillag, AL. The Organ of Vision. In: Csillag, AL., editor. *Atlas of the Sensory Organs: Functional and Clinical Anatomy*. Humana Press Inc; New Jersey: 2005. p. 89
- Durairaj C, Kim SJ, Edelhauser HF, Shah JC, Kompella UB. Influence of Dosage Form on the Intravitreal Pharmacokinetics of Diclofenac. *Invest Ophthalmol Vis Sci*. 2009a
- Durairaj C, Shah JC, Senapati S, Kompella UB. Prediction of vitreal half-life based on drug physicochemical properties: quantitative structure-pharmacokinetic relationships (QSPKR). *Pharm Res*. 2009b; 26:1236–1260. [PubMed: 18841448]
- Hughes PM, Krishnamoorthy R, Mitra AK. Vitreous disposition of two acycloguanosine antivirals in the albino and pigmented rabbit models: a novel ocular microdialysis technique. *J Ocul Pharmacol Ther*. 1996; 12:209–224. [PubMed: 8773936]
- Kadam RS, Cheruvu NP, Edelhauser HF, Kompella UB. Sclera-choroid-RPE Transport of Eight Beta-blockers in Human, Bovine, Porcine, Rabbit, and Rat Models. *Invest Ophthalmol Vis Sci*. 2011
- Kadam RS, Kompella UB. Influence of lipophilicity on drug partitioning into sclera, choroid-retinal pigment epithelium, retina, trabecular meshwork, and optic nerve. *J Pharmacol Exp Ther*. 2010; 332:1107–1120. [PubMed: 19926800]
- Kane A, Barza M, Baum J. Intravitreal injection of gentamicin in rabbits. Effect of inflammation and pigmentation on half-life and ocular distribution. *Invest Ophthalmol Vis Sci*. 1981; 20:593–597. [PubMed: 6971281]

- Kim ES, Durairaj C, Kadam RS, Lee SJ, Mo Y, Geroski DH, Kompella UB, Edelhauser HF. Human scleral diffusion of anticancer drugs from solution and nanoparticle formulation. *Pharm Res.* 2009; 26:1155–1161. [PubMed: 19194787]
- Kompella UBLX, Zhan G, Lee SS. Influence of Intravitreal Implant Location on Ocular Disposition of Fluocinolone Acetonide. *Invest Ophthalmol Vis Sci.* 2009:E-Abstract 5971.
- Kompella UB. Drug delivery to the back of the eye. *Arch Soc Esp Oftalmol.* 2007; 82:667–668. 669–670. [PubMed: 17979032]
- Menon IA, Wakeham DC, Persad SD, Avaria M, Trope GE, Basu PK. Quantitative determination of the melanin contents in ocular tissues from human blue and brown eyes. *J Ocul Pharmacol.* 1992; 8:35–42. [PubMed: 1402293]
- Nofsinger JB, Forest SE, Eibest LM, Gold KA, Simon JD. Probing the building blocks of eumelanins using scanning electron microscopy. *Pigment Cell Res.* 2000; 13:179–184. [PubMed: 10885677]
- Perkins RJ, Liu W, Drusano G, Madu A, Mayers M, Madu C, Miller MH. Pharmacokinetics of ofloxacin in serum and vitreous humor of albino and pigmented rabbits. *Antimicrob Agents Chemother.* 1995; 39:1493–1498. [PubMed: 7492092]
- Potts AM. The concentration of phenothiazines in the eye of experimental animals. *Invest Ophthalmol.* 1962; 1:522–530. [PubMed: 14488312]
- Rubin, LF.; Weisse, I. Species differences relevant for ocular toxicity studies. In: Hockwin, O.; KG; Rubin, LF., editors. *Manual of Oculotoxicity Testing of Drugs.* Gustav Fischer Verlag; Stuttgart: 1992. p. 177-191.
- Salazar M, Shimada K, Patil PN. Iris pigmentation and atropine mydriasis. *J Pharmacol Exp Ther.* 1976; 197:79–88. [PubMed: 1263134]
- Schmidt SY, Peisch RD. Melanin concentration in normal human retinal pigment epithelium. Regional variation and age-related reduction. *Invest Ophthalmol Vis Sci.* 1986; 27:1063–1067. [PubMed: 3721785]
- Solans C, Bregante MA, Garcia MA, Perez S. Ocular penetration of grepafloxacin after intravitreal administration in albino and pigmented rabbits. *Chemotherapy.* 2004; 50:133–137. [PubMed: 15282440]

- Binding to melanin is known to alter the distribution of drugs
- Melanin content was measured in posterior segment tissues of various species
- Species, strain and regional differences exist in melanin content

Fig. 1. Regional differences in the melanin pigment content of (a) retina and (b) choroid-RPE of human, Gottingen minipig, Yucatan minipig, Dutch belted (DB) rabbit, F1 rabbit, dog, and monkey models. Melanin content is expressed as arithmetic mean $\pm$ SD of $n = 3$ to 6 eyes. *Denotes significant difference ($p < 0.05$) between the central and peripheral regions of a given tissue.

Table 1

Melanin pigment content in sclera, choroid-RPE, and retina in human, Gottingen minipig, Yucatan minipig, Dutch belted rabbit, F1 rabbit, dog, and monkey models. The pigment content is expressed as arithmetic mean $\pm$ S.D. The number of subjects/repetitions are indicated in the table.

Tissue	Melanin content ($\mu\text{g}/\text{mg}$ tissue)						
	Human (N=6)	Gottingen minipig (N=3)	Yucatan minipig (N=3)	Dutch belted rabbit (N=6)	F1 rabbit(N=3)	Dog (N=6)	Monkey (N=4)
Central Retina	8.05 $\pm$ 1.51	10.42 $\pm$ 4.60	7.88 $\pm$ 2.29	29.51 $\pm$ 3.11 *	33.20 $\pm$ 4.57 *	9.53 $\pm$ 3.64	6.89 $\pm$ 3.41
Peripheral Retina	10.53 $\pm$ 1.69	14.02 $\pm$ 2.16	18.15 $\pm$ 4.41 *	29.79 $\pm$ 5.02 *	40.87 $\pm$ 10.12 *	13.64 $\pm$ 2.69 *	17.56 $\pm$ 2.69 *
Central Choroid-RPE	11.05 $\pm$ 3.43	32.16 $\pm$ 15.91 *	56.58 $\pm$ 30.33 *	24.29 $\pm$ 4.88 *	80.32 $\pm$ 27.26 *	67.46 $\pm$ 17.31 *	191.22 $\pm$ 21.51 *
Peripheral Choroid-RPE	22.26 $\pm$ 4.18	22.29 $\pm$ 6.67	74.19 $\pm$ 29.73 *	62.26 $\pm$ 27.57 *	116.94 $\pm$ 32.51 *	94.45 $\pm$ 29.35 *	233.99 $\pm$ 60.25 *
Sclera	14.95 $\pm$ 4.69	2.81 $\pm$ 0.65 *	7.91 $\pm$ 5.78	5.69 $\pm$ 1.23 *	4.34 $\pm$ 0.74 *	5.61 $\pm$ 2.65 *	4.86 $\pm$ 0.66 *

* Significantly different from human melanin content ($p < 0.05$)