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Proposed Pathway For Distribution of Nepafenac and Amfenac to Posterior Segment Tissues of the Eye

JE Chastain, ME Sanders, M Curtis, NV Chemuturi, MA Kapin, DC Dahlin

The authors of this presentation are employees* of Alcon Research, Ltd., Fort Worth, TX

(*NV Chemuturi and DC Dahlin are former employees)

Background

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- Nepafenac ophthalmic suspension is a topical ocular NSAID used to treat pain and inflammation associated with cataract surgery[1-3]
- *In vitro* and *ex vivo* studies have shown active nepafenac to rapidly permeate the cornea and sclera, and to be converted to its active metabolite amfenac, primarily in the ICB and retina/choroid[4]
  - The bioconversion of nepafenac is targeted to the ICB and retina, which have the highest prostaglandin concentration and COX activity[5]
- Published preclinical and clinical data suggest that nepafenac and amfenac reach the posterior segment of the eye following topical administration of nepafenac[6–8]
  - The distribution pathway(s) and extent of local delivery of nepafenac and amfenac are, however, not well characterized

NSAID, nonsteroidal anti-inflammatory drug; ICB, iris-ciliary body.

1. Flach AJ. *Int Ophthamol Clin*. 2002;42:1–11; 2. Lane SS, et al. *Cataract Refract Surg.* 2007;33:53–58; 3. O'Brien TP. *Curr Med Res Opin*. 2005;21:1131–1137. Erratum, 21:1431–1432; 4. Ke TL, et al. *Inflammation*. 2000;24;371–384; 5. Kulkarni PS, Srinivasan BD. *Prog Clin Biol Res*. 1989;312:39–52; 6. Kapin MA, et al. *Inflammation*. 2003;27:281–291; 7. Singh R, et al. *Clin Ophthalmol.* 2012;6:1259–1269; 8. Lindstrom R, Kim T. *Curr Med Res Opin*. 2006;22:397–404.

Objectives

- Following topical ocular instillation of nepafenac ophthalmic suspension 0.1% or 0.3% in animal models, this study aimed to:
  - Evaluate the bioavailability of nepafenac and amfenac in the posterior segments of the eye, and the contribution of local ocular delivery to the distribution of nepafenac and amfenac from the anterior segments
  - Expand the understanding of distribution pathways of these molecules to the posterior segments of the eye

Methods

- Nepafenac ophthalmic suspension was delivered topically into the right eye of:
  - 36 New Zealand White rabbits (a single dose of one 30-µL drop of 0.1% ophthalmic suspension)
  - 64 New Zealand White rabbits (4 q24hr or 10 q8hr evenly spaced multiple doses):
    - One 30-µL drop of 0.3% ophthalmic suspension instilled QD for 4 days; OR
    - One 30-µL drop of 0.1% ophthalmic suspension instilled TID for 3 days, plus one additional drop on Day 4
  - 10 cynomolgus monkeys (22 q8hr evenly spaced multiple doses):
    - One 30-µL drop of 0.3% ophthalmic suspension instilled TID for 7 days, plus one additional drop on Day 8
- The animals were euthanized at various times after the last dose, and ocular tissues were harvested from both the dosed and undosed eyes
- High-performance liquid chromatography coupled with tandem mass spectrometry was used to measure nepafenac and amfenac concentrations
- The difference in levels between dosed and undosed eyes in the same animal was used to determine locally-distributed concentrations of both compounds

q24hr, once every 24 hours; q8hr, once every 8 hours; QD, once daily; TID, three times daily.

Nepafenac Distributes Locally to the Posterior Segments and Peaks More Rapidly Than Amfenac in Rabbits – Single Dose

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- Mean maximum nepafenac concentrations between 2.72–55.1 nM were observed in the posterior segments of the eye within 0.25 hours following unilateral instillation
- Mean maximum amfenac concentrations between 0.768–41.8 nM were achieved in the same posterior segments approximately 1–4 hours following unilateral instillation

Tissue	Nepafenac OD (LD*)			Amfenac OD (LD*)		
	C_{max} (nM)		T_{max} (hr)	C_{max} (nM)		T_{max} (hr)
	Mean	SD		Mean	SD	
Anterior Sclera	714	108	0.25	469	157	1.00
Posterior Sclera	**55.1**	**15.5**	**0.25**	**41.8**	**11.3**	**1.00**
Anterior Choroid	92.1	60.3	0.25	47.2	24.4	1.00
Posterior Choroid	**4.02**	**3.26**	**0.50**	**3.10**	**0.28**	**4.00**
Anterior Retina	110	67.3	0.25	10.9	5.3	0.25
Posterior Retina	**2.72**	**2.06**	**0.25**	**0.768**	**0.588**	**1.00**
Vitreous Humor	3.67	6.48	0.25	0.398	0.439	0.25

C_{max}= maximum observed concentration; hr = hour; OD = right eye (dosed eye in this study); T_{max} = time at which maximum concentration was observed; SD, standard deviation.

* Locally-distributed (LD) nepafenac or amfenac concentrations, determined as the difference between dosed and undosed fellow eye concentrations. The percent of local distribution (versus systemically-derived) ranged from 49.9–99.8%. Vitreous humor concentrations represent total OD levels.

Distribution of Nepafenac and Amfenac to the Posterior Segment Occurs Predominantly via the Local Ocular Versus Systemic Route in Rabbit Eyes

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- In the undosed fellow eyes, the mean maximum concentration of both nepafenac and amfenac in the anterior and posterior segments (combined) were substantially lower than those for the corresponding regions of the dosed eye
  - **The percent of total drug in the dosed eye attributed to local distribution* ranged from about 50% to nearly 100%**
- The pattern of local distribution* of nepafenac and amfenac to the posterior segments in the dosed eye was similar to that observed for the overall concentration of each molecule in these tissues
  - **This suggests that distribution to the posterior segments of the dosed eye occurs predominantly through local ocular routes rather than systemic circulation**

*Locally-distributed nepafenac or amfenac concentrations, determined as the difference between dosed and undosed fellow eye concentrations

Concentration Gradients of Nepafenac and Amfenac Indicate a Mass Transfer From Anterior to Posterior Ocular Tissues in Rabbit Eyes

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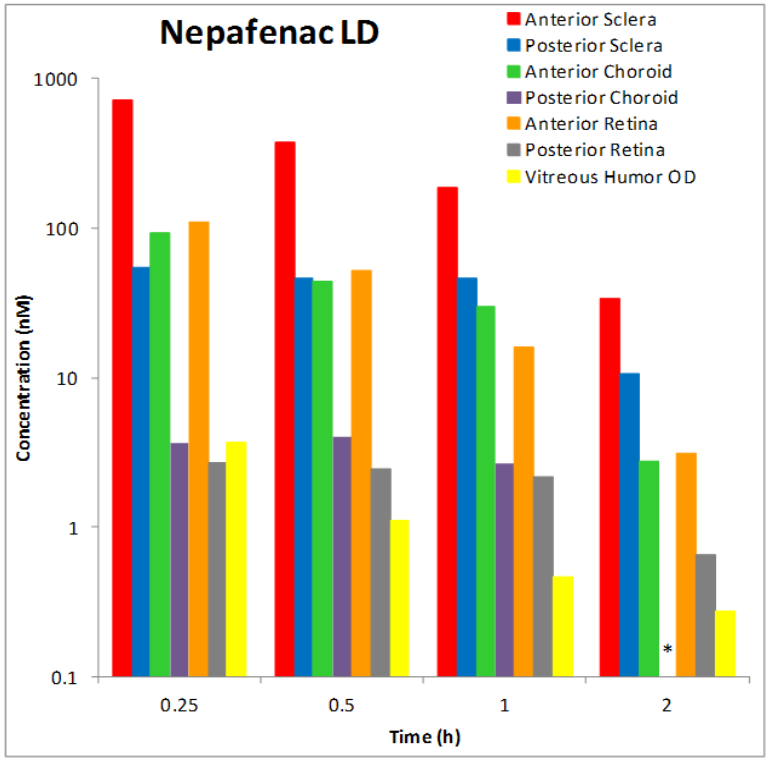
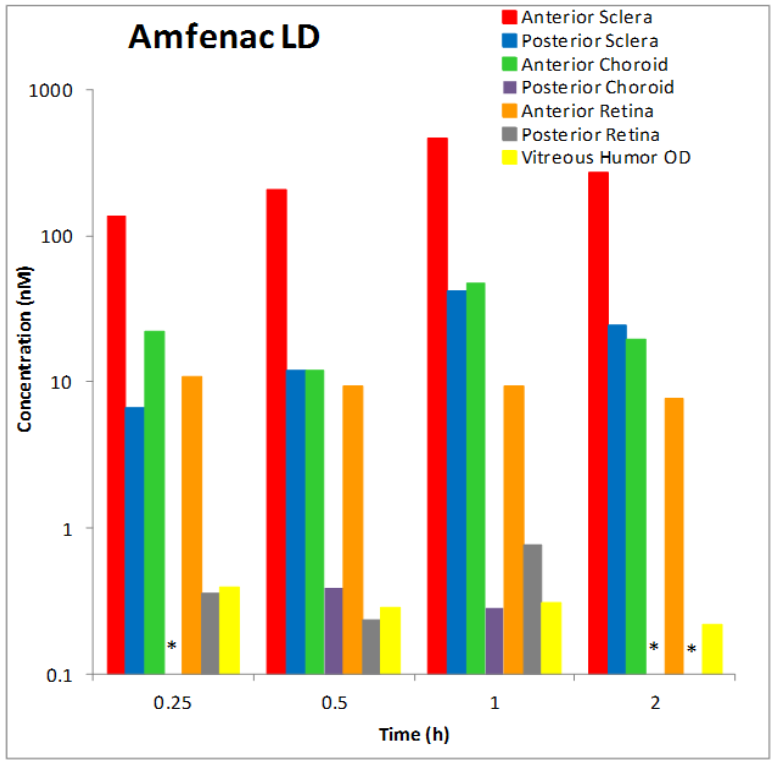
Concentrations of locally-distributed nepafenac (714, 92.1 and 110 nM, respectively) and amfenac (469, 47.2 and 10.9 nM, respectively) in the anterior sclera, choroid, and retina, and in the corresponding posterior segments (respectively: 55.1, 4.02 and 2.72 nM nepafenac; 41.8, 3.10 and 0.768 nM amfenac), after instillation of a single topical dose of nepafenac 0.1% ophthalmic suspension into the right eye of New Zealand White rabbits (n=36).





LD concentrations obtained by subtracting the concentrations for the undosed eye from those for the dosed eye. Graphs present mean concentrations at each time point. * Data not available; LD, local distribution.

Nepafenac and Amfenac Distribute Locally to the Retina in Rabbits – Multiple Dose Administration

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- Peak concentrations of nepafenac and amfenac were higher following 0.3% QD (88.1–645 nM and 25.5–80.3 nM, respectively) compared with of 0.1% TID (36.8–227 nM and 13.6–40.7 nM, respectively), and were higher in anterior retina versus posterior retina
- Nepafenac cumulative 24-hour AUCs following 0.1% TID and 0.3% QD were similar in anterior retina (920 vs 790 nM, accounting for SDs) and posterior retina (162 vs 169 nM)
- Amfenac cumulative 24-hour AUCs following 0.1% TID and 0.3% QD were similar in anterior retina (240 vs 243 nM). In posterior retina, 0.3% QD cumulative 24-hour AUC was 51% of 0.1% TID AUC

Tissue	Nepafenac Treatment	Nepafenac C_{max} (nM)		Nepafenac $AUC_{24hrCum}$ (nM*hr)		Nepafenac T_{max} (hr)	Amfenac C_{max} (nM)		Amfenac $AUC_{24hrCum}$ (nM*hr)		Amfenac T_{max} (hr)
		Mean	SD	Mean	SD		Mean	SD	Mean	SD	
Anterior Retina	0.1% TID	227	123	920	109	0.5	40.7	18.8	240	12.2	0.5
	0.3% QD	645	340	790	99.5	0.5	80.3	41.1	243	16.5	0.5
Posterior Retina	0.1% TID	36.8	15.8	162	19.3	0.5	13.6	6.93	140	4.94	0.5
	0.3% QD	88.1	57.8	169	28.5	0.5	25.5	12.5	70.9	9.05	1

C_{max}= maximum observed concentration; OD = right eye (dosed eye in this study); T_{max} = time at which maximum concentration was observed; SD = standard deviation. $AUC_{24hrCum}$ = area under the curve cumulative from 0 to 24 hours., with TID total AUC derived by simulating full day concentration-time curves, based on post-last dose data. * Locally-distributed nepafenac or amfenac concentrations, determined as the difference between dosed and undosed fellow eye concentrations.

AUC, area under curve; QD, once daily; TID, three times daily.

Nepafenac and Amfenac Rapidly Distribute to the Posterior Segment of the eye in Cynomolgus Monkeys

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- Peak concentrations of both nepafenac (306–1630 nM) and amfenac (3.8–26.7 nM) were achieved in the posterior segments of the eye within 1 to 2 hours (T_{max}) following drug instillation (samples were collected on the last day of the instillation schedule [Day 8] at 0, 0.25, 1, 2 and 3 hours after the last of 22 total doses)
- Similar to the rabbit study, the pattern of local distribution (LD) in the posterior sclera, choroid and retina was similar to that observed for the overall concentration of each molecule in these tissues, and distribution occurred predominantly via the local ocular versus systemic circulation

Tissue	Nepafenac OD		Nepafenac LD		Amfenac OD		Amfenac LD	
	C_{max} (nM)	T_{max} (hr)	C_{max}* (nM)	% **	C_{max} (nM)	T_{max} (hr)	C_{max}* (nM)	% **
Anterior Sclera	7170	0.25	7140	99.5	890	0.25	887	99.7
Middle Sclera	2260	0.25	2240	98.9	392	1.00	387	98.7
Posterior Sclera	**1630**	**2.00**	**1590**	**97.4**	**26.7**	**2.00**	**21.3**	**79.8**
Anterior Choroid	1920	2.00	1830	94.9	89.1	1.00	78.5	88.1
Posterior Choroid	**514**	**2.00**	**386**	**75.1**	**22.8**	**1.00**	**11.8**	**51.8**
Anterior Retina	376	1.00	364	94.4	19.9	1.00	17.6	88.4
Posterior Retina	**306**	**1.00**	**292**	**99.4**	**3.80**	**1.00**	**2.58**	**67.9**
Vitreous Humor	15.2	2.00	13.8	90.8	3.43	2.00	2.82	82.3

C_{max} = maximum observed concentration; hr = hour; OD = right eye (dosed eye); LD = local distribution; T_{max} = time at which maximum concentration was observed. *For each animal, the C_{max} of nepafenac or amfenac obtained for the undosed eye was subtracted from that obtained for the fellow dosed eye, thereby providing a measure of LD of the compounds in the dosed eye through intraocular pathways other than systemic delivery. The individual C_{max} values were then averaged to provide the mean LD C_{max} concentrations. **The percentage contribution of local distribution of nepafenac or amfenac to posterior segments was obtained by dividing the LD C_{max} by the OD C_{max} of the respective matrix.

An Anterior-to-Posterior Gradient of Nepafenac and Amfenac Occurs in the Monkey Eyes

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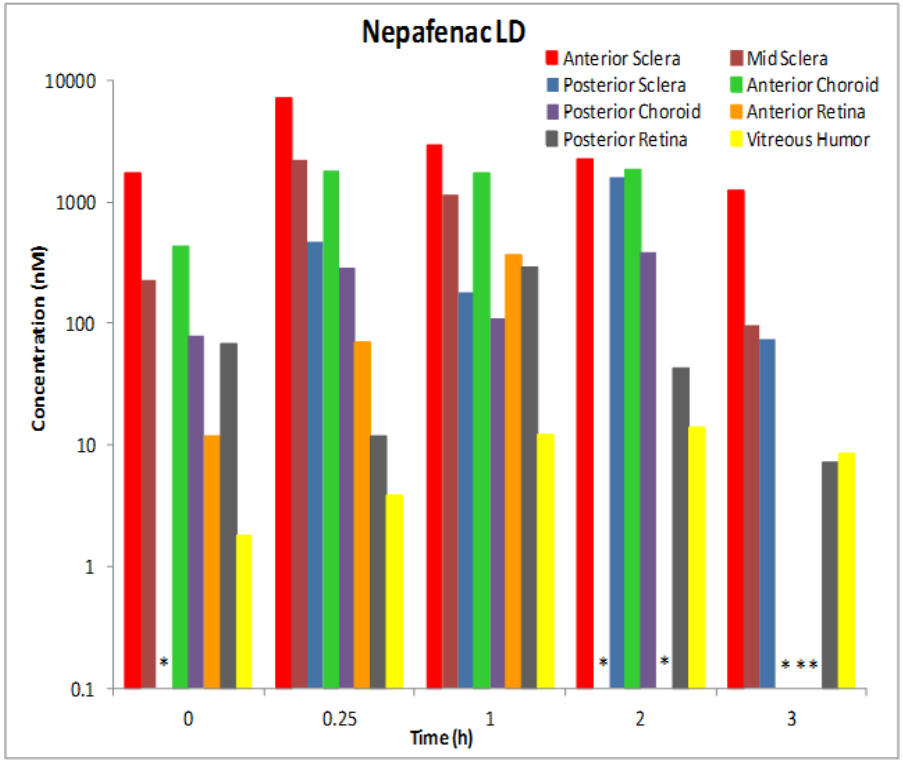
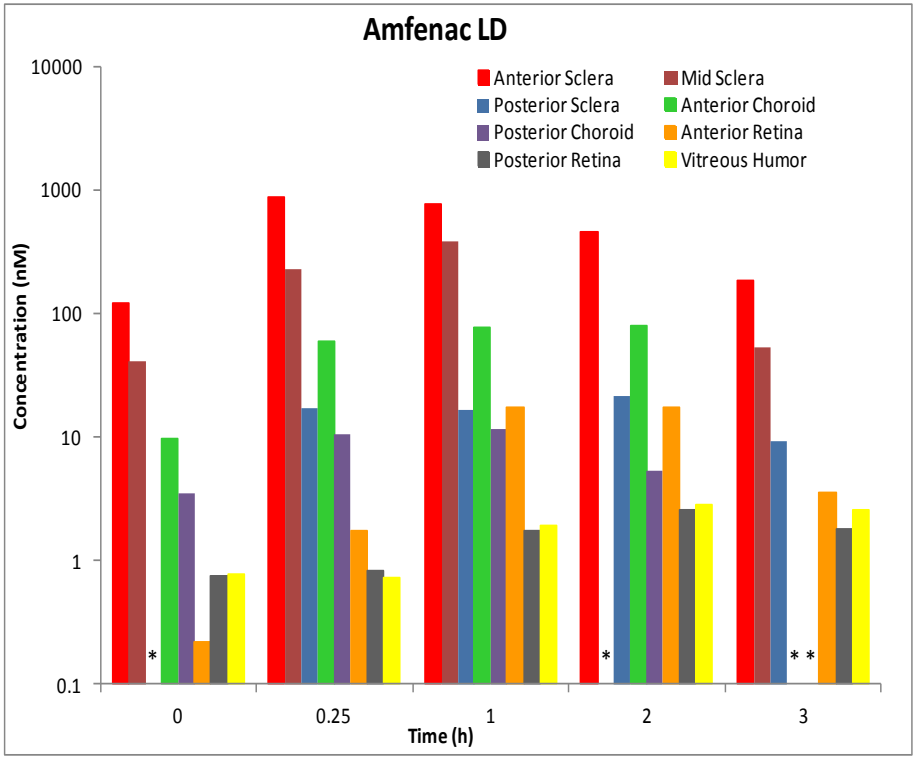
Concentrations of locally-distributed nepafenac (7170, 1920 and 376 nM, respectively) and amfenac (890, 89.1 and 19.9 nM, respectively) in the anterior sclera, choroid, and retina, and in the corresponding posterior segments (respectively: 1630, 514 and 306 nM nepafenac; 26.7, 22.8 and 3.8 nM amfenac), as well as vitreous humor, after repeated topical instillations of nepafenac 0.3% ophthalmic suspension into the right eye of cynomolgus monkeys (n=10).





LD concentrations obtained by subtracting the concentrations for the undosed eye from those for the dosed eye.
Graphs present mean concentrations at each time point. * Data not available; LD, local distribution.

Conclusions

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- Following the unilateral, ocular instillation of nepafenac 0.1% or nepafenac 0.3% ophthalmic suspension:
  - Nepafenac and amfenac were found to be rapidly distributed into the posterior segment tissues in rabbit and monkey eyes
  - The delivery of both nepafenac and amfenac to the posterior segment tissues occurred predominantly through local ocular routes
  - A sustained concentration gradient was observed between the anterior and posterior sections of the sclera, choroid, and retina, with minimal involvement of the vitreous humor
  - The low and short-lived concentrations of both nepafenac and amfenac in the vitreous humor indicate that this is unlikely to serve as a drug reservoir or pathway for drug distribution
- Dosing of nepafenac 0.3% QD for 4 days yielded cumulative 24-hour locally-distributed exposure in the retina similar to that following repeated dosing of 0.1% TID
  - The only exception to this was the amfenac exposure in the posterior retina of 0.3% QD-treated rabbits, which was approximately half that in 0.1% TID-treated rabbits.

QD, once daily; TID, three times daily.

Nepafenac and Amfenac Rapidly Distribute to the Posterior Segment of the Eye in New Zealand White Rabbits and Cynomolgus Monkeys

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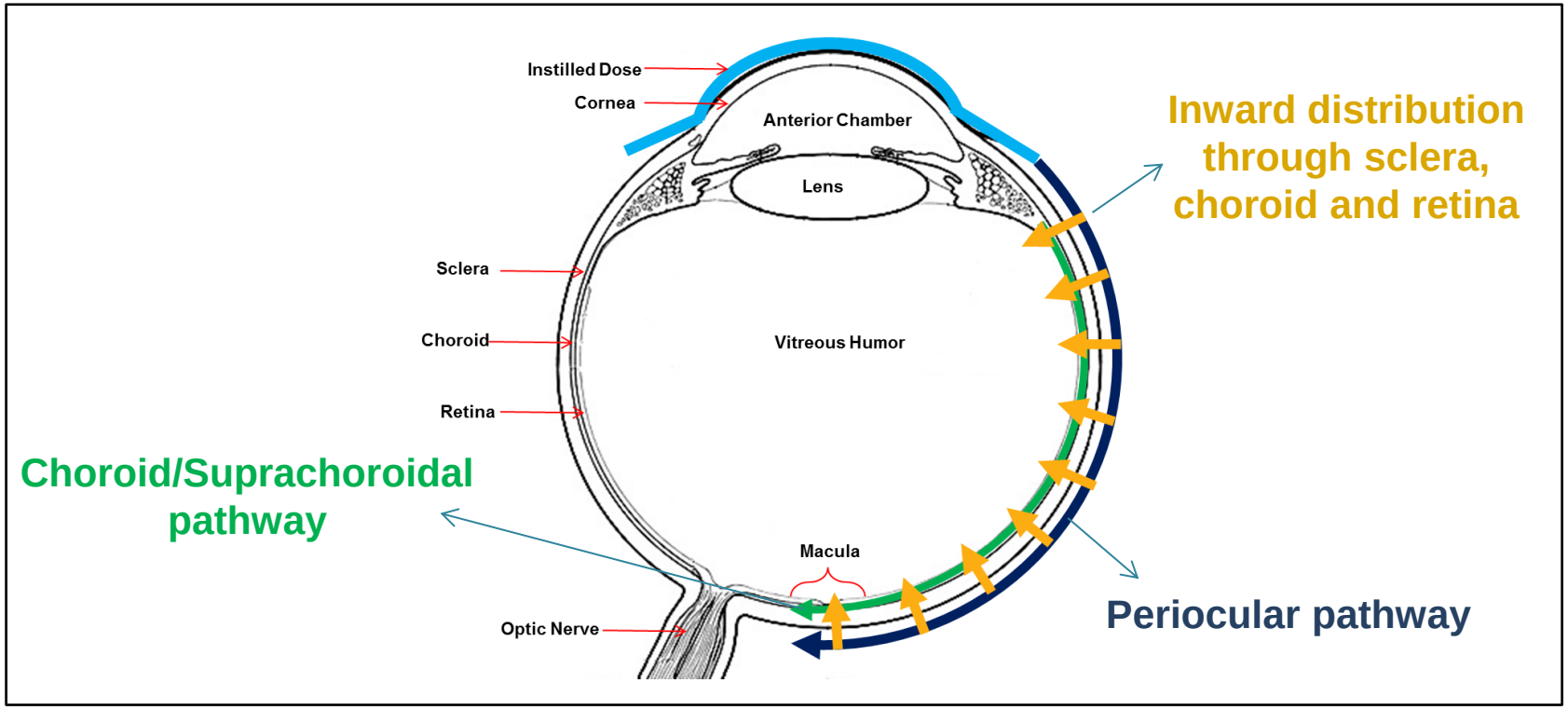

Distribution is only shown on one side of the eye for illustration purposes

The results of this study suggest a route for the distribution of nepafenac and amfenac to the posterior segment tissues *via* a local transconjuctival/scleral absorption route, with subsequent anterior-to-posterior and inward movement, possibly involving a choroidal, suprachoroidal or periocular pathway.