Following numerous enquiries about the question of the safety and efficacy profile for buffered phosphates when used in commercial tear solutions containing sodium hyaluronate, we forwarded this question to the Center for Ocular Surface Disease at the University of Waterloo School of Optometry for their review and analysis. *Clinical & Refractive Optometry* is pleased to present the following Communication by Jeffrey Lam and Dr. Faran Vafaie reflecting their findings.

Are Current Sodium Hyaluronate Solutions That Are Buffered in Phosphate Safe for Daily Administration?

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Introduction

The role of phosphates in sodium hyaluronate artificial tears is primarily to act as a buffer system to achieve a pH similar to normal tear film. Phosphate buffers are widely used by the ophthalmic preparation industry because of their high buffering capacity and high solubility in water. However, concentrations of phosphate vary widely amongst available sodium hyaluronate preparations. Recent evidence has suggested that in the presence of epithelial keratopathy, acute intensified treatment with high concentration phosphate containing sodium hyaluronate artificial tears may promote rapid corneal calcification which may lead to sight threatening complications.[1] Since most people living with dry eyes rely on chronic use of artificial tears, it is of interest to determine 1) the safety of chronic exposure phosphate buffers, and 2) the maximum toxic concentration (MTC) typical for chronic use. Knowledge of different commercial preparations' phosphate concentrations and their impacts is thus helpful in determining proper recommendations to avoid corneal complications.

Corneal Calcification

Corneal calcification due to intensified treatment with high phosphate concentration formulations was identified to be extracellular crystal deposits of hydroxyapatite $Ca_5(PO_4)_3OH$ within the cornea.[1] Unlike band keratopathy, the hydroxyapatite crystals are radially arranged around the central stroma[2] and can migrate deeper within the stroma, penetrating descemet's membrane and causingcalcareous degeneration.[3]

Hydroxyapatite has very low solubility that decreases with alkalinity. Evidence suggests that deposits occur when the epithelium and Bowman's membrane are compromised in the presence of a high concentration of phosphates. Stromal degradation and inflammation increases local free calcium and increases alkaline pH, respectively.[4] Therefore, it is important to note that factors such as increased free calcium level and alkaline pH both promote the precipitation of hydroxyapatite within the deep stroma when exposed to high amounts of phosphate buffer.

Phosphate Buffer Concentration

In 2006, Bernaurer et al took samples from different sodium hyaluronate formulations on the market and analyzed their concentration of phosphate buffers. The formulation Hylo-Comod® (URSA Pharm) or Hycosan® (Scope Healthcare) was indicated to have the highest concentration of phosphate at 50.9 mmol/L within commercially available sodium hyaluronate artificial tears. Furthermore, the authors provided evidence that the previous formulation of Hylo-Comod or Hycosan favoured the precipitation of calcium phosphate with coexisting epithelial keratopathy due to its high amount of phosphate concentration. Five patient studies showed that the use of Hylo-Comod upwards to 100 times per day with intervals as short as every 10 minutes converted to calcareous corneal degeneration.

Buffer Substitution Considerations

As a result of potential corneal degeneration secondary to contact with high levels of phosphate, phosphate containing sodium hyaluronate preparations have reformulated their solutions with other buffers such as citrate (i.e., Hylo™, CandorVision). It should be noted that although there is no evidence of corneal calcification with citrate buffers, there is evidence which suggests that high concentrations of cytosolic citrate could inhibit the glycolytic pathway.[5] Citrates inhibit phosphofructokinase, an enzyme that is required for one of the rate-limiting steps of glycolysis. As a result, energy production is halted within the cell when citrate levels increase and this may lead to cell death.[6,7]

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Furthermore, considerations should be taken when substituting buffers as it will affect pH maintenance. The physiological lacrimal film pH is 7.2 to 7.7.[8,9] Buffers that are ideal for biological tissues should have pKa ranges from 6 to 8. Citrate buffers have a pKa of 6.2 making them more ideal for lower pH maintenance, while phosphate buffers have a pKa of 7.2 which is more suitable to maintain the pH of physiological tears.

Decreased Phosphate Concentration

Although the exact critical concentration of phosphate that leads to corneal calcification in humans has not yet been determined, animal model studies involving rabbits with induced alkali burns found that a phosphate concentration of 148 mmol/L in irrigation solutions led to the development of corneal calcification.[10] In addition, we may also use the concentration of 50.9 mmol/L in Hylo-Comod as a starting reference for MTC levels of phosphate which had adverse corneal complications. All other sodium hyaluronate formulations that were compared in the 2006 Bernauer report had phosphate concentrations under 10.9 mmol/L[1] suggesting that lower concentrations of phosphate buffers with sodium hyaluronate formulations may not carry the same adverse risks. As such, the European Medicines Agency prepared a report[11] which determined that the risk of corneal complications with epithelial keratopathy treated with phosphate buffer formulations was lower than one case for every 10,000 patients treated. They concluded that the benefits of topical treatment with phosphate containing artificial tears were significantly higher than the risk of developing corneal complications.

Furthermore, low concentrations of phosphate buffers have been widely used in ophthalmic viscosurgical devices (OVD). Healon® (Abbott Medical Optics) is a sodium hyaluronic OVD that has been used for more than 35 years, becoming the most popular amongst viscoelastic substances for intraocular surgery.[12] Formulations of Healon are buffered with 2.2mmol/L of phosphate[13] which closely resembles the physiological phosphate concentration in the tear film (1.45 mmol/L).[14]

Despite there being no direct study to measure the MTC of phosphate that predisposes compromised corneas to calcification, it is assumed that low concentrations of phosphate do not cause adverse events as seen in hyaluronic artificial tears containing high concentrations of phosphate; however, more studies controlling for dosing and frequency of exposure should be established to make comparisons.

Conclusion

It is evident that high topical phosphate concentrations in sodium hyaluronate artificial tears have potential sight threatening complications due to rapid calcareous degeneration of the cornea in association with ocular surface insult. However there are many variables that determine the risk of such complications. These factors include pH, dosing frequency, and severity of epithelial defect.[15,16] Moreover, it can be assumed that low and high concentrations of phosphate carry different risk profiles for corneal calcification. In Canada, the only produced phosphate buffered hyaluronic acid formulation is the i-drop® line by I-MED Pharma. This product contains very low concentrations (<2.5 mmol/L) of phosphate buffer and has not had any reports of adverse events. Although there have been no reported cases of rapid calcareous calcification from the use low concentration phosphate formulations of sodium hyaluronate artificial tears, future studies to determine the MTC and evaluate the relative risks of corneal complications are needed to further define safe daily dose. As such, it is important to have an in-depth knowledge about the full composition of artificial tears to mitigate and identify potential adverse effects to those who are at greatest risk. ❑

References

1. Bernauer W, Thiel MA, Kurrer M, et al. Corneal calcifiction following intensified treatment with sodium hyaluronate artificial tears. Br J Ophthalmol 2006; 90(3); 285-288.
2. Daly M, Tuft SJ, Munro PMG. Acute corneal calcification following chemical injury. Cornea 2005; 24(6): 761-765.
3. Lavid, FJ, Herreras JM, Calonge M, et al. Calcareous corneal degeneration: report of two cases. Cornea 1995; 14(1): 97-102.
4. McDowell H, Gregory TM, Brown WE. Solubility of Ca5 (P04) 3OH in the System Ca (OH)2-H3PO4-H2O at 5, 15, 25, and 37°C. Journal of Research of the National Bureau of Standards–A. Physics and Chemistry 1977; 81A(2&3): 273-281.
5. Parmeggiani A, Bowman RH. Regulation of phosphofructokinase activity by citrate in normal and diabetic muscle. Biochemical and Biophysical Research Communications 1963; 12(4): 273-281.
6. Berg JM, Tymoczko JL, Stryer L. The glycolytic pathway is tightly controlled. In: Biochemistry, 5th edition. New York: W.H. Freeman, 2002: Section 16.2.
7. Denton RM, Randle PJ. Citrate and the regulation of adipose-tissue phosphofructokinase. Biochemical Journal 1966; 100(2): 420.
8. Fischer FH, Wiederholt M. Human precorneal tear film pH measured by microelectrodes. Graefes Arch Clin Exp Ophthalmol 1982; 218(3): 168-170.
9. Chen FS, Maurice DM. The pH in the precorneal tear film and under a contact lens measured with a fluorescent probe. Exp Eye Res 1990; 50(3): 251-259.
10. Schrage NF, Schlossmacher B, Aschenbernner W, Langefeld S. Phosphate buffer in alkali eye burns as an inducer of experimental corneal calcification. Burns 2001; 27(5): 459-464.
11. European Medicines Agency. Questions and answers on use of phosphates in eye drops. EMA/CHMP/753373/2012.
12. Higashide, Tomomi, and Kazuhisa Sugiyama. "Use of viscoelastic substance in ophthalmic surgery–focus on sodium hyaluronate." Clin Ophthalmol 2008; 2(1): 21-30.
13. Buratto L. Phacoemulsification: principles and techniques. Thorofare, NJ: SLACK, 2003: 228. Print.
14. Tapaszto I. Pathophysiology of human tears. Int Ophthalmology Clin 1973; 13(1): 119-147.
15. de Frutos-Lezaun M, Martinez-Soroa I, Ostra Beldarrain M, et al. Determination of phosphate concentration and pH in artificial tear drops. Arch Soc Esp Oftalmol 2016 Feb 22. pii: S0365-6691(16)00051-4. doi: 10.1016/j.oftal.2016.01. 018. [Epub ahead of print]
16. Bernauer W, Thiel MA, Langenauer UM, Rentsch KM. Phosphate concentration in artificial tears. Graefes Arch Clin Exp Ophthalmol 2006; 244(8): 1010-1014.