All Steroids Are Equal But Some Are More Equal Than Others

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- CLINICAL RESEARCH
  - Alcon, Alimera, Allegro, Allergan, Apellis, Clearside, Genentech, GSK, Ionis, jCyte, Novartis, Regeneron, ThromboGenics

- CONSULTANT
  - Aerpio, Alcon, Alimera, Allegro, Allergan, Ampio, Catalyst, Cell Care, Dose, Eyedaptic, Genentech, Glaukos, jCyte, Novartis, Ophthotech, Regeneron, Revana, SciFluor
Not All Triamcinolone Formulations are Equal

- When a cluster of cases of sterile endophthalmitis associated with Kenalog® use appeared in 2006/2007, many retina specialists started to use compounding pharmacy preservative free triamcinolone acetonide (PFTA)
  - PFTA is micronized and lyophilized with a relatively uniform particle size
  - Kenalog is manufactured with an older methodology which varying particle size
- Clinical observation of the authors and many other retina specialists that Kenalog® appears to clear slower from the vitreous than PFTA formulations and has longer durability
  - Kenalog is usually visible one month post injection, frequently seen at two months and occasionally seen at 3 months
  - PFTA is frequently not visible at one month

*Triamcinolone and its various commercial brand name formulations (Kenalog, Trivaris, Leiter’s, and Triesence) are used off label
Background—Retina PK standard methodology

- Most animal retina/posterior segment PK work is done on young albino rabbits
  - Melanin has been shown to have significant effects on drug binding and PK
  - Young rabbits have very well formed vitreous compared to humans (particularly disease state humans)
    - 70% gel component in young rabbits
    - 50% gel component in 70 year old human
  - Vitreous liquefaction has been shown to have a significant impact on drug PK
Why experimental vitreous liquefaction?

- The vitreous becomes more liquefied with age.
  - Age 10: 83% gel
  - Age 70: 55% gel

- Vitreous liquefaction is frequently associated with vitreoretinal pathology (such as PVD, RD, ARMD, DR, RP, high myopia, congenital retinoschisis, pars planitis and Wagner’s disease, etc).

A New Animal Model of Surgically Induced Synergetic Vitreous

- 25 gauge 2 port trocar placement (no infusion line)—idea of James Burke
- 4 minute non-aspiration vitrectomy procedure (2500 cuts/min)
- Continuous swizzle stick movement in the mid-vitreous
- Creates a gel/liquid ratio of ~50% from the native ratio of ~70%
Purpose

- To compare TA dissolution and efficacy as a function of time in 4 different formulations of TA in eyes of rabbits with experimental vitreous liquefaction
  - Kenalog® (Bristol-Meyers)
  - Triesence® (Alcon)
  - Trivaris™ (Allergan)
  - Leiter’s PFTA (Leiter’s Pharmacy, San Jose)
Methods

- **TA formulations**
  - Kenalog® (Bristol-Meyers)
  - Trivaris® (Allergan)
  - Triesence® (Alcon)
  - Leiter’s PFTA (Leiter’s Pharmacy, San Jose)

- **Animals**
  - Twenty-five (25) Dutch-belted (pigmented) rabbits
  - OU vitreous received a 4 minute non-aspiration vitrectomy procedure (1500 cuts/min) to create a gel/liquid ratio of ~50% from ~70%, 4-8 weeks prior to TA dosing OU (23 animals/46 eyes TA, plus 2 animals/4 eyes control vitrectomy plus VEGF challenge below, no TA)

- **Assessments**
  - Photographic measurement of residual TA mass area q 2wks to 19wks
  - VEGF 165 challenge 50 every two weeks in half of the rabbits until inflammatory reaction noted in both eyes
  - Terminal drug concentration at 19 weeks in the non-VEGF challenged eyes
Assessment of residual TA mass area

- Wide-angle vitreous photographs were taken with the HRA system and a Staurenghi 150 deg wide-angle contact lens from conscious rabbit eyes dilated with 1% tropicamide and 10% phenylephrine on days 0, 1 and 3, weeks 1 and 2, and once every 2 weeks thereafter up to week 19.

- Outlines of the TA mass area was measured in pixels with Image Pro Plus software program.
Representative wide-angle vitreous images in an eye with Kenalog®

Day_0, 8/11/08
Day_1, 8/12/08
Week_1, 8/19/08
Week_2, 8/26/08
Week_6, 9/23/08
Week_10, 10/21/08
Week_14, 11/18/08
Week_19, 12/19/08
Assessment of efficacy

• Residual steroid efficacy was assessed once every 2 weeks by injecting rhVEGF (50 µl of 0.5 µg) into mid-vitreous of OU using a 29 gauge needle

• Retinopathy was assessed 2 days after each administration of rhVEGF by mid to late-phase fluorescein angiography after injecting 0.5 ml of 5% NaF into the ear vein
  – Severity of retinopathy assessed by the degree of blood vessel leakage was graded on a 4 point scale (0-3) by 4 masked graders
  – The time from vitreous disappearance of visible TA to development of significant (level 2) rhVEGF-induced retinopathy was calculated.
NaF leak grading standard

**Level 0.** No vascular changes

**Level 1.** Vessel dilation and tortuosity without FL leak

**Level 2.** Level 1 plus mild to moderate leak

**Level 3.** Severe leak
TA mass area up to 19 weeks follow-up: Kenalog® > Triesence® > Trivaris™ = Leiter’s*

\[ P < .05 \text{ compared to Leiter's PFTA and Trivaris.} \]
\[ b \ P < .05 \text{ compared to Leiter's PFTA, Trivaris, and Triesence.} \]

*Triamcinolone and its various commercial brand name formulations (Kenalog, Trivaris, Leiter's, and Triesence) are used off label.

Inhibition of Retinal Vasculitis Response to VEGF 165 Challenge up to 39 weeks Follow-up: Kenalog > Trivaris/Triesence/Leiter’s

Time from vitreous TA mass disappearance to rhVEGF-induced retinopathy: Kenalog® > Triescence® > Trivaris™ > Leiter’s

![Bar graph showing mean time from disappearance of TA mass to development of retinopathy with severity of ≥2 (weeks). Kenalog has the highest value of 9.2, followed by Triescence (4.0), Trivaris (3.4), and Leiter’s PFTA (1.25). There is a P < .05 compared to Leiter’s PFTA.]
**TA Particle size**

90<sup>th</sup> percentile volume distribution:

Trend: Kenalog® > Triesence® > Leiter’s = Trivaris™

<table>
<thead>
<tr>
<th>TA formulation</th>
<th>10&lt;sup&gt;th&lt;/sup&gt; Percentile of Volume Distribution</th>
<th>50&lt;sup&gt;th&lt;/sup&gt; Percentile of Volume Distribution</th>
<th>90&lt;sup&gt;th&lt;/sup&gt; Percentile of Volume Distribution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kenalog® (Lot #: 7M22628)</td>
<td>6 µm</td>
<td>19 µm</td>
<td>47 µm</td>
</tr>
<tr>
<td>Kenalog® (5 Lots Average)</td>
<td>5 µm</td>
<td>15 µm</td>
<td>42 µm</td>
</tr>
<tr>
<td>Triesence® (Lot #: 14766OF)</td>
<td>7 µm</td>
<td>14 µm</td>
<td>26 µm</td>
</tr>
<tr>
<td>Trivaris™ (Lot #: 9634X 8%)</td>
<td></td>
<td></td>
<td>22 µm</td>
</tr>
<tr>
<td>Leiters (Lot #: 05152008)</td>
<td>4 µm</td>
<td>11 µm</td>
<td>22 µm</td>
</tr>
</tbody>
</table>

Triamcinolone Formulation Differentiation: Conclusions

• Kenalog® has longer vitreous visibility and durability than PFTA formulations in this animal model
• TA particle size appears to correlate with efficacy and durability
• Possible ramifications in interpretation of DRCRnet and SCORE studies
Synthetic glucocorticoids

• Glucocorticoids potency: What do we measure?
• This potency/ specificity table is found in all pharmacology textbook: How was it built?

<table>
<thead>
<tr>
<th>Drug Name</th>
<th>Estimated Potencies</th>
<th>Anti-inflammatory effects</th>
<th>Cortisol suppression</th>
<th>Sodium retention</th>
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<tbody>
<tr>
<td>Glucocorticoid</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cortisone</td>
<td>0.8</td>
<td>0.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prednisolone</td>
<td>4</td>
<td>0.25</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prednisone</td>
<td>4</td>
<td>0.25</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Methylprednisolone</td>
<td>5</td>
<td>+/-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Triamcinolone</td>
<td>5</td>
<td>+/-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Paramethasone</td>
<td>10</td>
<td>+/-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dexamethasone</td>
<td>25</td>
<td>+/-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Betamethasone</td>
<td>25-30</td>
<td>+/-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aldosterone</td>
<td>0.3</td>
<td>400</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fluocinolone-acetonide</td>
<td>25</td>
<td></td>
<td></td>
<td>+/-</td>
</tr>
<tr>
<td>Fluocinolone</td>
<td>10</td>
<td>300</td>
<td></td>
<td>+/-</td>
</tr>
</tbody>
</table>

1. Systemic effects of corticoids

2. McKenzie skin blanching test

Skin blanching after glucocorticoid application - 6-8 hrs or 16-20 hrs

The potency of corticoids has been determined by their vasoconstriction effect

Synthetic glucocorticoids

- Is this potency table adequate when glucocorticoids are used intraocularly?

1. No or minimal systemic effects
   - No effects on sodium retention

2. Should we consider vasoconstriction as our potency marker?

Not All Steroids Suppress Cytokines Equally

Differential target potencies
- Inhibitory potency differs for each inflammatory mediator
  - VEGF is very sensitive to dexamethasone
  - Higher drug concentrations are needed for complete inhibition of all inflammatory mediators

Corticosteroids Have Different Chemical Structures
But Some Are More Equal Than Others

Chemical Structure Impacts Localization & Mobility Of Ligand/GR Complex In The Nucleus

- Differences in chemical structure affects the translocation of the steroid-receptor complex into the nucleus


Yellow fluorescent protein labeling of GRα in COS-1 cells exposed to 1 μM triamcinolone or cortisone for 3–6 hours
Corticosteroid Differential Gene Expression Indicate Different Cellular Responses

- Intravitreal corticosteroids have overlapping biological pathways
- The intravitreal corticosteroids also generate a unique set of genes

Steroid-treated TM93 cells
(35 year-old donor)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Number of Unique Genes</th>
</tr>
</thead>
<tbody>
<tr>
<td>TM93 – DEX</td>
<td>4483</td>
</tr>
<tr>
<td>TM93 – FA</td>
<td>2294</td>
</tr>
<tr>
<td>TM93 – TA</td>
<td>1150</td>
</tr>
</tbody>
</table>

Steroid-treated TM86 cells
(3 month-old donor)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Number of Unique Genes</th>
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<tbody>
<tr>
<td>TM86 – DEX</td>
<td>4562</td>
</tr>
<tr>
<td>TM86 – FA</td>
<td>3347</td>
</tr>
<tr>
<td>TM86 – TA</td>
<td>3131</td>
</tr>
</tbody>
</table>

Steroid-treated TM93 cells
(35 year-old donor)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Number of Unique Genes</th>
</tr>
</thead>
<tbody>
<tr>
<td>TM93 – DEX</td>
<td>3523</td>
</tr>
<tr>
<td>TM93 – FA</td>
<td>745</td>
</tr>
<tr>
<td>TM93 – TA</td>
<td>657</td>
</tr>
</tbody>
</table>


FA = Fluocinolone Acetonide
TA = Triamcinolone Acetonide
DEX = Dexamethasone

Unique genes regulated by steroid
Dexamethasone & Fluocinolone Exhibit Fewer Cytotoxic Effects Than Triamcinolone

Toxicity was measured by the trypan blue dye exclusion cell viability assay.

Effects of TA versus Dexamethasone on Human Lens Epithelial Cells in Vitro

Cell Viability of HLE cells in vitro After 24 hour Exposure to TA or DEX

- C-TA reduced cell viability at all doses DEX reduced cell viability only at concentrations between 0.5 (80.1 ± 0.9%, \( P < .001 \)) and 2mg/ml (14.8 ± 0.6; \( P < .001 \))

Caspase-3/7 Activity of HLE cells in vitro After 24-hour Exposure to TA or DEX

- S-TA increased Caspase-3/7 activity at all doses above 100 mcg/ml
- DEX did not increase Caspase-3/7 activity at any dose
Corticosteroids Have Different Affinities For Lens & Trabecular Meshwork

Human Lens

Bovine Lens

Bovine Trabecular Meshwork

Photoreceptor/RPE Neuroprotection by Glucocorticoids

- **Z. Smit-McBride et al., ARVO suppl 2009.** Global Gene Profiling of Early and Late Induced Changes in Gene Expression Upon Intravitreal Injection of Triamcinolone and Dexamethasone in the Mouse Retina

- **Parver LM et al. Arch Ophthalmol. 1984; 102:772.** Rhesus monkeys- “Pretreatment with subcutaneous...dexamethasone...prior to light exposure markedly decreased damage to the retinal pigment epithelium...”

- **Remé CE et al. Doc Ophthalmol. 2003; 106:25.** “Caspase-1 gene expression is distinctly upregulated after light exposure and there are several factors which completely protect against light-induced (photoreceptor) cell death, such as the anesthetic halothane, dexamethasone...”

- **Wenzel A, et al. IOVS 2003; 44:2798.** “…glucocorticoid receptor activation inhibits AP-1 and prevents (photoreceptor) apoptosis.”

- **Wenzel A, IOVS 2001; 42:1653.** “pharmacologic suppression of AP-1 activity (by dexamethasone) protected against light damage. Inhibition of AP-1 activity may have occurred by the protein-protein interaction of GR and AP-1.”
Dexamethasone Inhibits Light-Induced Photoreceptor Degeneration

Not All Steroids Are Equally Soluble
Dexamethasone Is Highly Water Soluble

<table>
<thead>
<tr>
<th>Corticosteroid</th>
<th>Aqueous Sol (mg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fluticasone propionate</td>
<td>0.14</td>
</tr>
<tr>
<td>Fluocinolone acetonide</td>
<td>5</td>
</tr>
<tr>
<td>Budesonide</td>
<td>16</td>
</tr>
<tr>
<td>Triamcinolone acetonide</td>
<td>21</td>
</tr>
<tr>
<td><strong>Dexamethasone</strong></td>
<td><strong>100</strong></td>
</tr>
<tr>
<td>Cortisol</td>
<td>&gt;200</td>
</tr>
</tbody>
</table>

*Dexamethasone requires a novel drug delivery system to maintain optimal concentrations*

# Intravitreal Corticosteroids: Ocular Hypertension Incidence

Meta-analysis of Prospective Randomized Trials, Prospective Cohort Studies, and Retrospective Studies that Reported Secondary OHT or Glaucoma Following IVT Steroid

<table>
<thead>
<tr>
<th>Medication</th>
<th>Dose</th>
<th>Number of Studies Included</th>
<th>Number of Eyes Included</th>
<th>Pooled Point Estimate for Proportion of Eyes Developing Ocular Hypertension (%)</th>
<th>95% Confidence Interval</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>8 mg/0.2 mL</td>
<td>489,133,152,153</td>
<td>319</td>
<td>31.8^a</td>
<td>20.4–45.8</td>
</tr>
<tr>
<td></td>
<td>10 mg/0.2 mL</td>
<td>2^82,197</td>
<td>53</td>
<td>30.0^a</td>
<td>17.9–45.7</td>
</tr>
<tr>
<td></td>
<td>20 mg/0.2 mL</td>
<td>5^96,105-107,182</td>
<td>396</td>
<td>39.8^a</td>
<td>35.0–44.8</td>
</tr>
<tr>
<td></td>
<td>25 mg/0.2 mL</td>
<td>3^95,101,103</td>
<td>114</td>
<td>45.9^a</td>
<td>36.9–55.3</td>
</tr>
<tr>
<td>Dexamethasone Implant</td>
<td>0.35 mg</td>
<td>4^75,77,130,141</td>
<td>650</td>
<td>10.9^b</td>
<td>6.4–17.9</td>
</tr>
<tr>
<td></td>
<td>0.7 mg</td>
<td>6^26,75-77,130,141</td>
<td>746</td>
<td>15.3^b</td>
<td>9.2–24.3</td>
</tr>
</tbody>
</table>

% = Percentage of studied eyes developing ocular hypertension (OHT).

^a Ocular hypertension defined as IOP ≥ 21 mm Hg or ≥ 10 mm Hg from baseline.

^b Ocular hypertension defined as IOP ≥ 25 mm Hg or ≥ 10 mm Hg from baseline.

Adapted from Kiddee et al. Surv Ophthalmol. 2013.
Safety and Efficacy

Dex Implant vs Triamcinolone Acetonide

Not All Steroids Are Equal: Summary

- **All Steroids Are Not Equal**
  - Steroids have different activities on cells
  - Chemical structure directly influenced steroid-receptor nuclear distribution and mobility
  - Steroids are associated with differential gene expression
  - Differences among steroids may explain varied efficacy and safety profiles

- **These Differences May Have Clinical Implications**
  - Without head-to-head study comparisons, the clinical significance of these differences is unknown
  - Physiological impact on the retina by different corticosteroids should be considered

- **Investigation of head to head steroid and steroid/anti-VEGF combination treatment regimens is warranted**
  - Steroids have a complementary mechanism of action to anti-VEGFs
  - Steroids may vary in the magnitude of their ocular side effect profile and particularly the management of those side effects may vary between steroids, as well as degree and durability of efficacy

Thank you!