Photoreceptor Displaced Nuclei (PDN) in Cynomolgus Monkeys: Influence of Ocular Injection Procedures on Background Finding

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Thank you to Pamela Buie, Joel Ellis, Heather Floyd, Guangming Li, Mark Reimers and Kelly Stout for directing and/or planning the studies reviewed in this effort.
INTRODUCTION

- **Photoreceptor displaced nuclei (PDN)** are photoreceptors
  - lost almost all of their cytoplasm
  - retained their nuclei
  - occur in the layer of the photoreceptor inner and outer segments

- **Occur** in areas of
  - retinal degeneration
  - unremarkable retina

- **Most PDN** have
  - non-condensed chromatin
  - resemble nuclei in the outer nuclear layer (ONL)

- **Some have** condensed chromatin (appear pyknotic).
PURPOSE OF RETROSPECTIVE ANALYSIS

• Characterize the features of PDN in histologic sections of the globes of control cynomolgus monkeys
  – appearance
  – retinal location
  – occurrence
• Approximately half the globes received no injection
• Approximately half the globes received intravitreal injections with an innocuous control vehicle
OCULAR SECTIONS

• Control, male and female, cynomolgus monkeys
• Age: 3 to 6 years old
• Nine separate toxicology studies conducted
  – at Alcon Laboratories between 2008 and 2010
• All monkeys had prestudy funduscopic examinations
  and none had evidence of retinal degeneration.
• The globes were fixed in Davidson’s fixative followed by
  storage in 10% neutral buffered formalin until trimming.
• Sectioned globes to obtain three vertical sections along
  the following planes*
  • (1) central midsagittal plane contained optic disc
  • (2) temporal sagittal plane
  • (3) nasal sagittal plane

*Method for intravitreal injection studies used by Dr. Margarita Gruebbel, EPL
INTRAVITREAL INJECTIONS

• Left globe (OS) was not injected (NIG)

• Right globe (OD) was injected (IG)

• Various intravitreal vehicles were formulated in sterile water for injection and included well-qualified, innocuous excipients.

• Intravitreal injections varied in volume from 20 to 100 µL. When the injection volume exceeded 50 µL, an equal amount of vitreous was aspirated prior to administration of the vehicle.

• The intravitreal injections were consistently administered in the inferior-temporal quadrant, either once or up to four times, generally with at least three weeks between injections.
RETINAL EXAMINATION FOR PDN

- 3 sections / globe were examined
- Retina of each globe was roughly divided into three areas:
  - Superior peripheral retina
  - Central retina
  - Inferior peripheral retina
- Peripheral retina
  - (1) portion of the anterior retina that was thinner than the central retina
  - (2) had distinct inner and outer nuclear layers
  - (3) identifiable layer of inner and outer photoreceptor segments
RETINAL EXAMINATION FOR PDN

• PDN were not counted in the disorganized area of peripheral retina and only counted in the peripheral retina that had clearly identifiable retinal layers.

• Only PDN located external to the outer limiting membrane (OLM) and in the layer of inner and outer segments (IS and OS) were counted.

• Because the transition point is arbitrary, the actual number of PDN in the peripheral retina was considered to be a relative number.
RETINAL EXAMINATION FOR PDN

PDN generally had one of two morphologic appearances:

- Approximately the same size as the nuclei in the ONL
- Non-condensed chromatin
- Non-pyknotic PDN \([\text{npPDN}]\)

- Other PDN were much smaller
- Condensed chromatin
- Pyknotic PDN \((pPDN)\)
APPEARANCE

• Types
  - non-pyknotic PDN (npPDN) - most common type
  - pyknotic PDN (pPDN) - scarce
• Mechanism by which PDN occur is unknown, but Lai (1980) described two main stages of movement of these nuclei.
  - first stage – they move through the OLM
    - nuclei have an hourglass shape
  - second stage - nuclei are displaced
• This indicates that
  - nuclei move to displaced location
    - then undergo the process of pyknosis
• Only finding pPDN in the IS & OS layer supports this view
• pPDN are eventually removed by the RPE or subretinal macrophages (Magnum et al, 1980).
• Differentiated from macrophages, detached RPE
RETINAL LOCATION

- PDN occurred throughout all areas of the retina
- It is reported that PDN are more frequently observed in the central retina, near the posterior pole or around the optic disc, and in the peripheral retina
- In this review, more PDN appeared to be located
  - midsagittal and temporal sagittal sections.
  - central or inferior peripheral areas
OCURRENCE

- Displaced photoreceptor nuclei (PDN) have been reported
  - humans
  - monkeys (cynomolgus monkeys)
  - dogs
  - cats
  - pigs
  - rabbits
  - guinea pigs
  - rats
  - mice
  - chickens

- Non-injected globes indicating **spontaneous** cause.
- **Injected globes**, so possibly induced cause.
Table 1. Total and average number of non-pyknotic (npPDN) and pyknotic (pPDN) photoreceptor displaced nuclei in the vertical midsagittal sections of non-injected (NIG) and injected (IG) globes.

<table>
<thead>
<tr>
<th>Study Number</th>
<th>Number of Animals (Total = 32)</th>
<th>Globe</th>
<th>Total (Average number of npPDN)</th>
<th>Total (Average number of pPDN)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-N-09-159</td>
<td>4</td>
<td>Left (NIG)</td>
<td>2 (&lt;1)</td>
<td>0 (0)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Right (IG)</td>
<td>2 (&lt;1)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>2- N-10-031</td>
<td>4</td>
<td>Left (NIG)</td>
<td>13 (3)</td>
<td>0 (0)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Right (IG)</td>
<td>40 (10)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>3- N-10-121</td>
<td>6</td>
<td>Left (NIG)</td>
<td>10 (2)</td>
<td>0 (0)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Right (IG)</td>
<td>12 (2)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>4- N-10-104</td>
<td>6</td>
<td>Left (NIG)</td>
<td>17 (3)</td>
<td>0 (0)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Right (IG)</td>
<td>21 (4)</td>
<td>2 (&lt;1)</td>
</tr>
<tr>
<td>5- N-09-104</td>
<td>12</td>
<td>Left (IG)</td>
<td>61 (5)</td>
<td>0 (0)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Right (IG)</td>
<td>50 (4)</td>
<td>1 (&lt;1)</td>
</tr>
</tbody>
</table>
Table 2. Total and average number of non-pyknotic (npPDN) and pyknotic (pPDN) photoreceptor displaced nuclei in the vertical nasal sagittal (N), midsagittal (M) and temporal sagittal (T) sections of non-injected (NIG) and injected (IG) globes.

<table>
<thead>
<tr>
<th>Study Number</th>
<th>Number of Animals (Total = 33)</th>
<th>Globe</th>
<th>Average of npPDN (Total number of pPDN) N Section</th>
<th>Average of npPDN (Total number of pPDN) M Section</th>
<th>Average of npPDN (Total number of pPDN) T Section</th>
</tr>
</thead>
<tbody>
<tr>
<td>6 –E-10-029</td>
<td>2</td>
<td>Left (NIG)</td>
<td>0 (0)</td>
<td>1 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Right (IG)</td>
<td>0 (0)</td>
<td>1 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>7 –E-10-043</td>
<td>15</td>
<td>Left (NIG)</td>
<td>4 (2)</td>
<td>4 (3)</td>
<td>6 (1)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Right (IG)</td>
<td>2 (4)</td>
<td>3 (2)</td>
<td>2 (9)</td>
</tr>
<tr>
<td>8 –N-08-180</td>
<td>10</td>
<td>Left (NIG)</td>
<td>1 (0)</td>
<td>5 (0)</td>
<td>3 (1)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Right (IG)</td>
<td>0 (0)</td>
<td>2 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>9- N-10-167</td>
<td>6</td>
<td>Left (NIG)</td>
<td>1 (0)</td>
<td>7 (0)</td>
<td>1 (2)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Right (IG)</td>
<td>0 (0)</td>
<td>2 (0)</td>
<td>0 (0)</td>
</tr>
</tbody>
</table>
Table 3. Total and average whole number of non-pyknotic (npPDN) photoreceptor displaced nuclei in superior (S), central (C), or inferior (I) areas of the vertical nasal sagittal (N), midsagittal (M) and temporal sagittal (T) sections of 33 non-injected (NIG) and 33 injected (IG) globes from four studies.

<table>
<thead>
<tr>
<th>Globe</th>
<th>Retinal Area</th>
<th>Total (Average) number of npPDN</th>
<th>Total (Average) number of npPDN</th>
<th>Total (Average) number of npPDN</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>N Section</td>
<td>M Section</td>
<td>T Section</td>
</tr>
<tr>
<td>Left (NIG)</td>
<td>Superior</td>
<td>9 (&lt;1)</td>
<td>30 (&lt;1)</td>
<td>27 (&lt;1)</td>
</tr>
<tr>
<td></td>
<td>Central</td>
<td>44 (1)</td>
<td>89 (2)</td>
<td>26 (&lt;1)</td>
</tr>
<tr>
<td></td>
<td>Inferior</td>
<td>26 (&lt;1)</td>
<td>34 (1)</td>
<td>90 (2)</td>
</tr>
<tr>
<td>Right (IG)</td>
<td>Superior</td>
<td>3 (&lt;1)</td>
<td>16 (&lt;1)</td>
<td>10 (&lt;1)</td>
</tr>
<tr>
<td></td>
<td>Central</td>
<td>5 (&lt;1)</td>
<td>19 (&lt;1)</td>
<td>18 (&lt;1)</td>
</tr>
<tr>
<td></td>
<td>Inferior</td>
<td>21 (&lt;1)</td>
<td>29 (&lt;1)</td>
<td>22 (&lt;1)</td>
</tr>
</tbody>
</table>
Conclusions

- When npPDN or pPDN are noted in a retina which is otherwise normal, the PDN should be diagnosed as PDN.
- Since the term, retinal degeneration, is nonspecific, it should not be used for PDN with no other retinal findings.
- The number of PDN may be quite variable, so factors, such as a procedure (e.g., intravitreal injection), may or may not have a possible influence.
- In this review, more PDN appeared to be located in the midsagittal and temporal sagittal sections and central or inferior peripheral areas.
- pPDN or npPDN may not have diagnostic importance unless accompanied by additional diagnostic retinal changes.
- When PDN are observed in the presence of features of retinal degeneration (e.g., thinning of photoreceptor layers and disorganization and a decrease in photoreceptor cellularity), the PDN may be considered a feature of retinal degeneration.
REFERENCES