Cobalt rapidly changes photoreceptor outer segment structure in optical coherence tomography images of the rabbit retina.

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Abstract

Purpose: Cobalt-containing metal hip implants can, in rare, cases cause retinopathy. Previous retinal ganglion cell studies in rabbit showed cobalt (1mM) resulted in a rapid loss of the light response. Here we used optical coherence tomography (OCT) M-scans to study the effect of cobalt on the photoreceptor structure in the ex-vivo rabbit retina.

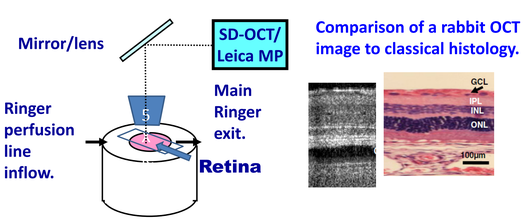
Methods: We studied the effects of cobalt using a retinal eyecup preparation and time lapse OCT, using New Zealand white rabbits were used. The retina was superfused with an oxygenated Ames Ringer on a modified Leica MP multiphoton microscope stage . Time-lapse cross-sectional B-scan images of the rabbit retina were taken at 15 sec intervals. After a control period, cobalt (8-1000μM) was bath applied to the retina for 10min, and then washed out. Select retina were processed for histology. Using OCT M-scans, temporal changes in the dimensions of the retinal structures affected by cobalt were examined and quantified.

Results: We studied how cobalt affected the retinal thickness in OCT M-scans. In the control condition, OCT M-scans showed there was little change in the retinal thickness to sham applications of Ringer's solution (n=5). In contrast, application of 1mM cobalt caused a rapid shrinkage in the retinal thickness averaging -3.3±1.9 μm (n=7) (p<0.01, 1-tailed T-test). In the presence of 1mM cobalt, the width of the retinal pigment epithelium to inner segment (IS) line declined an average of -2.0± 1.2 μm n=7.mean.s.d. (P< 0.01 1-tailed T-test). This suggested a large effect of 1mM cobalt was on the photoreceptor outer segments. In addition, there was reduction in the peak intensity of the IS line of 9± 4.5 AU (SEM) in 1mM cobalt, n=7. No retinal shrinkage was seen in cobalt at doses of 40μM, however there were effects on the photoreceptor IS and outer segment lines in OCT M-scans.

Conclusions: We found cobalt caused rapid changes in the structure of the rabbit retinal tissue. At (0.2-1.0 mM), cobalt caused a retinal shrinkage which was largely due to a reduction in the photoreceptor outer segment width and a disruption of the photoreceptor tips in the OCT image.

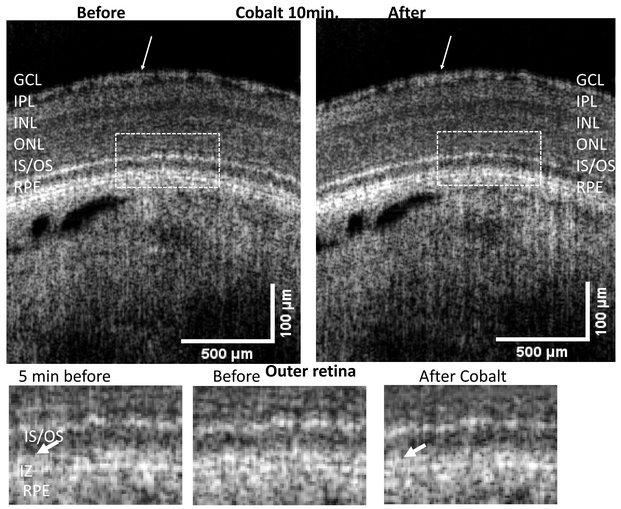
Materials and Methods

We studied the effects of cobalt using a retinal eyecup preparation and time lapse OCT. New Zealand white rabbits were used. Under anesthesia by an approved IACUC protocol, an eye was removed and the back of the eye was mounted in the recording chamber. The retina was superfused with an oxygenated Ames Ringer (~5ml/min) heated to 35C on a Leica MP multiphoton microscope, modified to have an optical coherence tomography channel (840nm SLED). Time lapse cross sectional B-scan images of the rabbit retina (20 frame avg) were taken at 15 sec intervals using a 5X objective lens. After a control period, cobalt (8-1000μM) was bath applied to the retina for 10min, and then washed out In some cases the retina was processed for histology. Using aligned OCT M-scans, temporal changes in the dimensions of the retinal structures affected by cobalt were identified and quantified using the PEAKS BAR plug-in in FIJI (ImageJ).



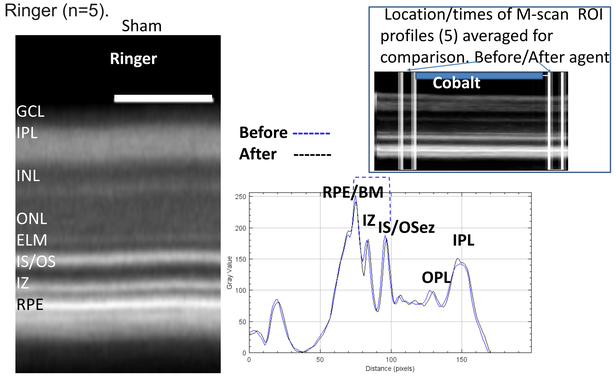
Results

1A. An example of cobalt's effect on the retinal structure can be seen in OCT B-scans of the everted rabbit retinal eyecup. B-scans are shown pre and post agent exposure. Cobalt caused a reduction in the thickness of the retina (arrow). There were also changes in the outer retina (box).



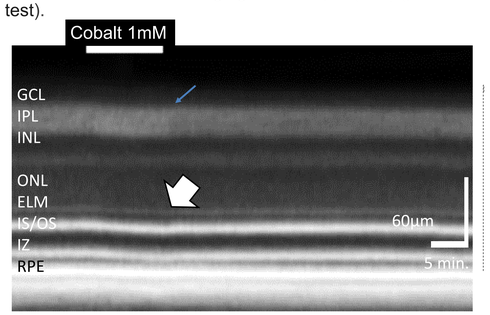
1B. In the outer retina, enlarged B-scans of same region (box) showed subtle losses in the IS/OS line reflectivity and at the interdigitation zone (IZ). (arrow) RPE, retinal pigment epithelium, IZ, interdigitation zone, IS/OSez: Inner/outer segment area (EZ), ELM, external limiting membrane, IPL, Inner plexiform layer, GCL ganglion cell layer. INL, ONL, Inner, outer nuclear layer respectively.

2. We studied how cobalt affected the retinal thickness in OCT M-scans. In the control condition, time lapse OCT (M-scans) showed there was little change in the retinal thickness dimensions to a 10 minute sham application of normal Ringer (n=5).

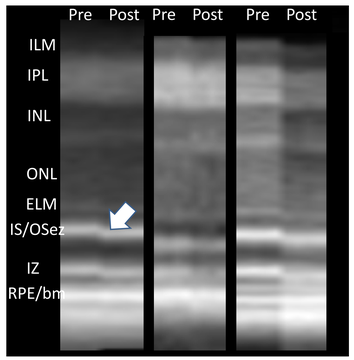


The RPE peak was used to align the OCT M-scans. To prevent bias in OCT layer thickness measurements, all distance peak measurements were referenced to the proximity to the RPE peak line in the ROI zones analyzed. Note in the control and sham Ringer (blue) the M-scan profiles peaks largely overlap each other. AU: Arbitrary Units. Conventions as in Fig. 1.

3.. Application of 1mM cobalt for 10 min. caused a rapid shrinkage in the retinal thickness averaging -3.3±1.9 μm (n=7) (p<0.01, 1-tailed T-test).

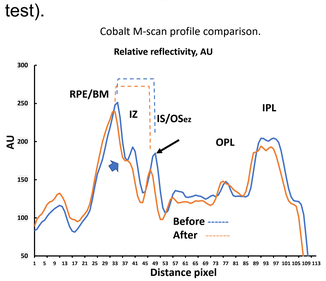


Example of cobalt's effect on the rabbit retinal structure in an OCT M-scan. In the presence of cobalt, there is a slight reduction in GCL layer thickness (blue arrow), and the IPL is shifted slightly. In the outer retina the IS/OS (ez) line (fat arrow) is shifted toward the RPE, and the IZ line is partially disrupted.



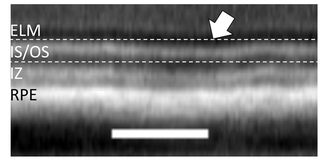
Time lapse B-scan montages of 3 1.25 minute long OCT M-scan image pairs allow comparison of the retinal layers before (Pre) and after (Post) Cobalt application (arrow). Note the IS/OS line is shifted lower after cobalt. Retinal pairs have been stretched slightly to allow registration of layer effects at RPE for cross comparison. Conventions as in Fig. 1.

4A. In OCT M-Scans, a large effect of 1mM cobalt was on the photoreceptor outer segment. In the presence of 1mM cobalt, the proximity of the inner segment (IS/OS) line to the retinal pigment epithelium was shortened an average of -2.0± 1.2 μm n=7.mean±s.d. (P< 0.01, 1-tailed T-test).

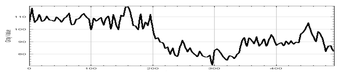


Comparison of the relative reflectivity profile of OCT M-scans of the retina before and after a 10min. bath application of 1mM cobalt. In the presence of cobalt the IS/OS retinal reflectivity profile is shifted slightly to the left. A large decline in the IS/OS reflectivity was observed. In addition, there were also changes in the interdigitation zone (IZ) between the photoreceptor tips and the RPE (fat arrow). AU: Arbitrary Units. Conventions as in Fig. 1.

4B. OCT M-scan of outer segment in cobalt. There was also a reduction in the peak reflectivity of the IS/OS line (dotted zone, arrow). After 1mM cobalt, this line declined an average of 9± 4.5 AU (SEM) , n=7. Bar: 10min cobalt.

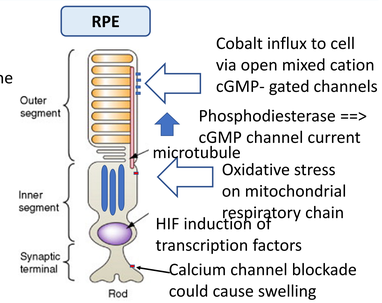


Time lapse M-scan of reflectivity of IS/OS line ROI



Discussion

Possible mechanisms of action of cobalt inducing changes in retinal neurons/photoreceptors. The rapidity of the effect seems unlikely to be due to transcription factors. Conventions as previous.



Adapted: Bibliowiczet al. Progress in Molecular Biology and Translational Science, 2011

Conclusions

1. **We have developed a method to image the effects of metal ions released by implants on the retinal structure in real time. Cobalt causes a rapid thinning of the rabbit retina, that cannot be due to HIF gene activation.**
2. **Cobalt altered the position and reflectivity of the IS/OS (Ez) line, suggesting it had an effect on the photoreceptor outer segments.**
3. **In addition, cobalt 0.2-1mM caused reductions in the photoreceptor IZ thickness, similar to our previous report with cyanide and chloroquine (Majdi et al. IOVS, 2015).**
4. **Cobalt also caused shrinkage in the GCL, an area largely occupied by Müller cell end feet.**
5. **The rabbit eyecup preparation offers a real time assessment of agent effects on the retinal structure, and will be offered as a free FDA regulatory science tool in the near future.**