News Release

Silicone oil, an intraocular surgical adjuvant, induces retinal ferroptosis

Key Points

- Total iron in fluid between SO and the retina was lower in human and rabbit eyes
- The retinae of SO-filled human and rabbit eyes take up Fe
- SO-filled rabbit eyes had increased oxidative stress and decreased GPx4 expression
- The in vitro SO-filled eye model showed ferroptosis in Müller cells
- The ferroptosis in Müller cells can be prevented by ferrostatin-1

Summary

Vitrectomy with silicone oil (SO) endotamponade is an effective treatment for vision-threatening retinal diseases. However, unexplained vision impairment has been reportedly critical side effects. Previously, we reported that the eyes with ocular toxoplasmosis showed retinal ferroptosis with the clinical sign of reduced intravitreal iron (Fe). We also found that total iron levels in sub-silicone oil fluid (SOF) in eyes with SO endotamponade were significantly reduced. We hypothesized that the cause of complications related to SO endotamponade is retinal ferroptosis and that low total iron in SOF is a secondary change that occurs similarly to the changes in ocular toxoplasmosis. In this study, we measured total iron levels in ocular fluid from patients, rabbits with SO endotamponade. Retinal iron taken up from the SOF was evaluated using laser ablation inductively coupled plasma mass spectrometry in human and rabbit eyes. Retinal ferroptosis was confirmed by immunohistochemistry of 4-hydrox-2-nonenal-modified proteins, FeRhoNox-1 staining, western blotting and RT-PCR. We found low total iron levels in the SOF, increased oxidative stress and Fe uptake from the SOF into the retinae of human and rabbit eyes, as well as decreased GPx4 expression, increased FeRhoNox-1 signals and altered Fe-related gene expression in SO-filled rabbit eyes. Of note, the target of ferroptosis was Müller cells. We generated an in vitro silicone oil-filled eye model using MIO-M1 cells (a human Müller cell line). The in vitro SO-filled eye model showed decreased GPx4 expression and increased intracellular catalytic Fe(II), an increase in ferroptosis, prevention of cell death by ferrostatin-1, a ferroptosis inhibitor, and altered Fe-related gene expression. These results indicate that the cause of complications related to SO endotamponade was the induction of retinal (Müller cell) ferroptosis, which can be prevented by ferrostatin-1.

Research Background

Vitrectomy is a type of surgery used to treat problems of the retina and vitreous of the human eyes. It is one of the most difficult eye surgeries and is a very effective treatment for many retinal diseases including retinal detachment, a major vision-threatening retinal disease. However, SO-related vision loss (SORVL), a recent phenomenon that causes severe, unexplained loss of vision, has been critical side effects. Therefore, understanding biological changes in SO-filled eyes is critical for the precise application of SO during surgery.

Recently we reported the low total iron in vitreous fluid (VF) from patients with ocular toxoplasmosis, one of the most globally common intraocular infection, and iron in VF uptake into the retina. Furthermore, retinal ferroptosis was the critical mechanism involved in the induction of retinochoroiditis during ocular toxoplasmosis. Interestingly, reduced intraocular iron that was found in ocular toxoplasmosis was similar to what we previously found in SO-filled eye, in which, we investigated the cause of complications related to SO and found that total iron (Fe) levels in the sub-SO fluid (SOF), fluid accumulating in the space between SO and the sensory retina, were significantly lower than those in control VF.

We hypothesized that the cause of complications related to SO is retinal ferroptosis and that low total iron in the SOF is a secondary change, after adjacent retinal cells absorb iron from the SOF.

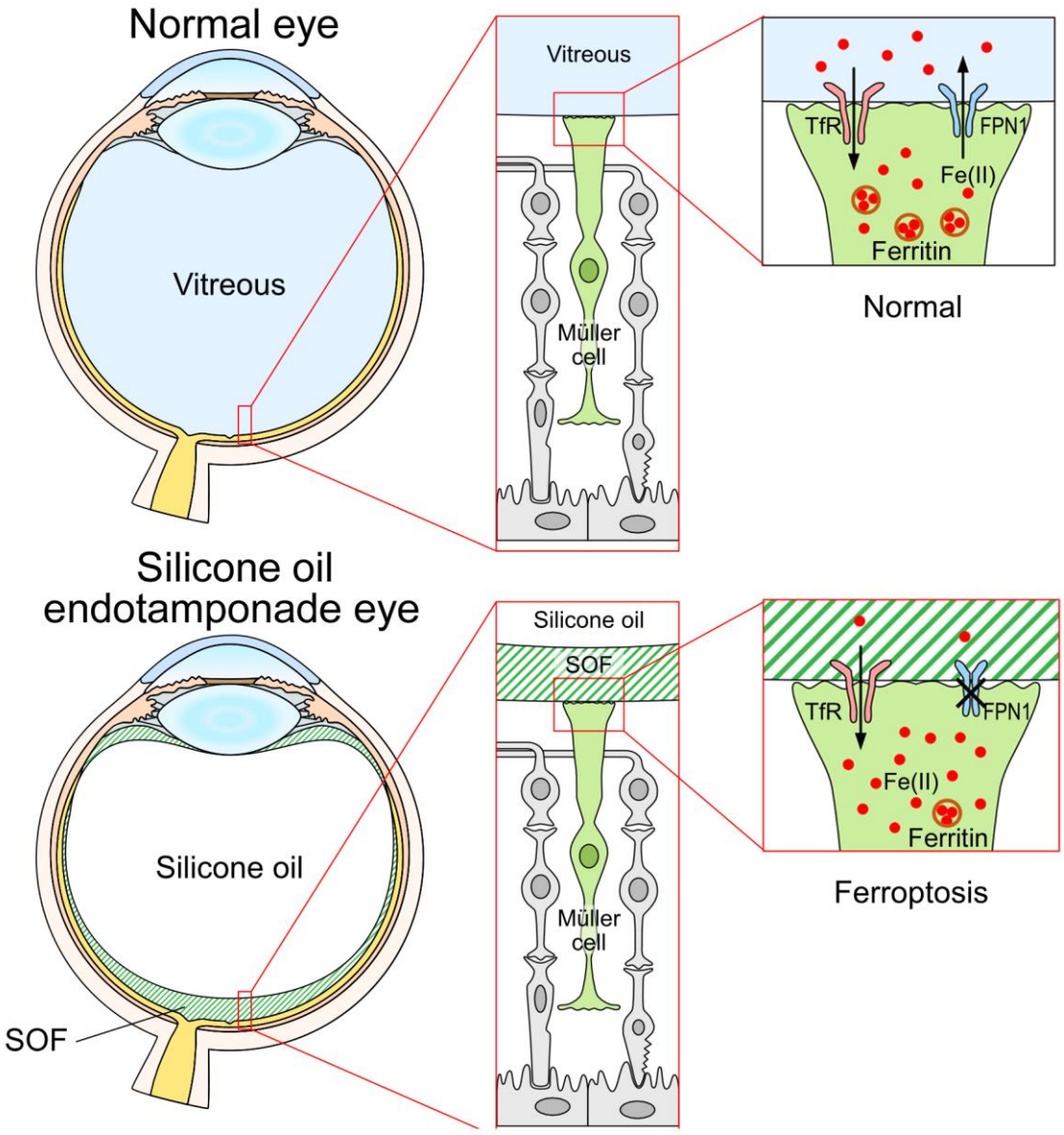
Research Results

We confirmed that the level of total iron in the SOF was lower than that in the VF. Additionally, we showed that LDH activity in the SOF was significantly higher than that in the VF. To demonstrate iron was taken up by the retina in SO-filled eyes, we used LA-ICP-MS to detect ^{56}Fe in a human donor eye with a history of SO endotamponade. The presence of Fe in the neurosensory retina was confirmed. Additionally, we performed vitrectomy, SO endotamponade, and ^{57}Fe IVT in rabbits and found increased iron uptake into the rabbit retinae of SO-filled eyes.

We also confirmed high levels of the oxidation products, 4-HNE and MDA, and FeRhoNox-1 staining in SO-filled rabbit eyes. GPx4 has a critical role in protection against ferroptosis. Therefore, we measured its expression by western blotting and found lower GPx4 expression in SO+ eyes.

We created an in vitro SO-filled eye model. FeRhoNox-1 staining of SO-filled rabbit eyes suggested the involvement of Müller cells; therefore, we developed an in vitro SO-filled eye model containing layers of the MIO-M1 human Müller cell line. We found increased rates of cell death in SO+ eyes with MIO-M1 cells. We also demonstrated that Fer-1 prevented MIO-M1 cell death. These results

indicated that MIO-M1 cells in SO+ eyes accumulated intracellular catalytic Fe(II), an increase in ferroptosis. Therefore, we concluded that the SORVL phenomenon is induced by retinal ferroptosis, and that Müller cells might be strongly associated with SO-induced ferroptosis.
Figure 1.



Research Summary and Future Perspective

This study used human and rabbit SOF and an in vitro SO-filled eye model to show that SO induced retinal ferroptosis, which was found in eyes with ocular toxoplasmosis, and that Müller cells might be closely associated with this phenomenon. SORVL might be related to SO-induced retinal ferroptosis, which can be prevented by ferrostatin-1.

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