

Ultra-wide field acridine orange digital fluorography revealed that suppression of infiltrated macrophages by tissue plasminogen activator in laser-induced choroidal neovascularization model

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Background:

We have reported that tissue plasminogen activator (tPA) suppressed choroidal neovascularization (CNV) in murine laser-induced CNV model.¹⁾ The purpose of this study was to evaluate the influence of tPA in infiltrated macrophages in laser-induced CNV model using ultra-wide field acridine orange (AO) digital fluorography.

Methods:

CNV was induced by laser injury in C57BL/6J male mice, and the intravitreal injection of tPA (40 IU/ μ l) or PBS was performed right after laser injury. The infiltrated macrophages were evaluated on day 0, 3 hours, 1, 3, 5 and 7 days after laser using ultra-wide field AO digital fluorography (Optos 200Tx; Optos, Dunfermline, Scotland, United Kingdom). The number of AO positive cells/disc area were counted using the open-source image-analysis software FIJI.

Results:

In PBS group, the number of AO positive cells/disc area were significantly upregulated on day 1 and 3 ($p < 0.01$) (Figure 1).

And the time course of the number of AO positive cells were correlated with the previous data which analyzed time course of macrophage fraction after laser injury using flow cytometry (Figure 2).

On day 3, tPA significantly suppressed the number of AO positive cells/disc area compared with PBS-injected eyes (2.0 ± 0.08 vs. 6.0 ± 0.33 , $p < 0.01$) (Figure 3).

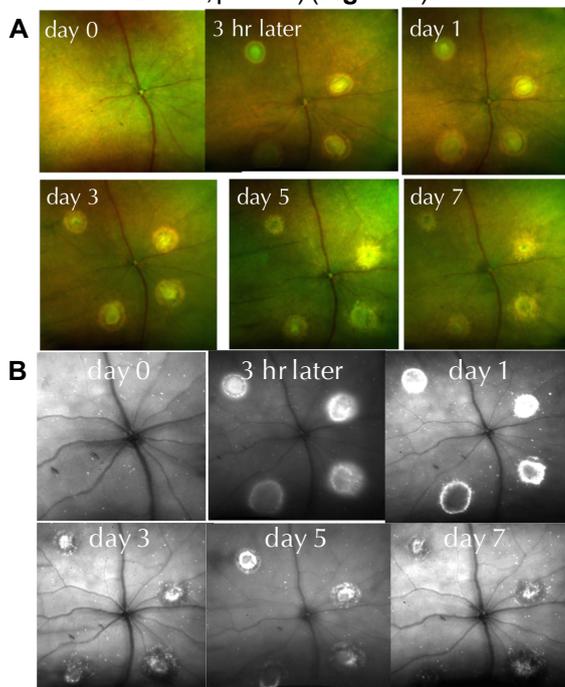
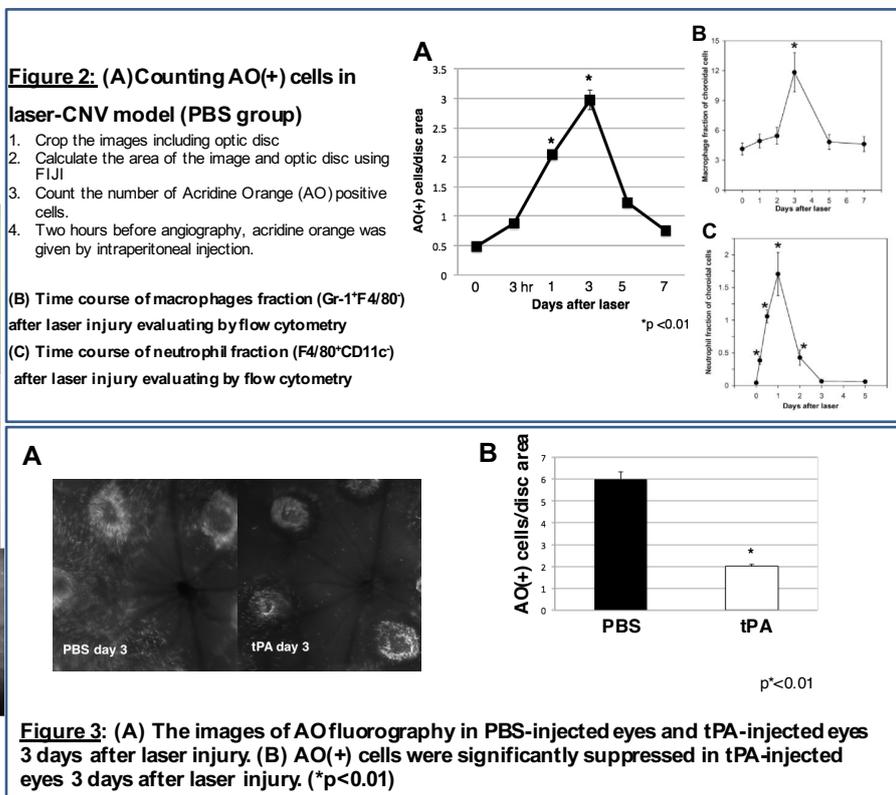


Figure 1: The images of color fundus photo (A) and AO fluorography (B) in PBS-injected eyes.



Discussion:

- tPA suppress fibrin/fibrinogen after laser injury¹⁾, and fibrin depletion decreases inflammation.²⁾
- Macrophages incubating with fibrin express IL-10, which is pro-angiogenic.³⁾

Conclusions:

- Infiltrated macrophages were known as source of pro-angiogenic factor. Our results demonstrated that tPA suppress CNV via inhibit macrophages infiltration in murine laser-induced CNV model.
- Ultra-wide field acridine orange digital fluorography using Optos may serve as a valuable tool to evaluate infiltration of inflammatory cells in vivo.

References:

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