Vascular endothelial growth factor (VEGF) concentration is underestimated by enzyme-linked immunosorbent assay in the presence of anti-VEGF drugs

Running head: VEGF ELISA concentration with inhibitors present

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Supplementary information: List of VEGF enzyme-linked immunosorbent assay (ELISA) kits used in previous papers.

We searched PubMed in May 2015 using the search term “aqueous vegf concentration” and found 17 recent papers that listed the name of the specific ELISA kits used. Twelve of the 17 papers used R&D ELISA kits1-12, 2 used BioVendor kits13,14, and 1 each used GE Healthcare15, MultiSciences16, and Pierce Biotechnology17 kits.

Supplementary Figure 1: Measured concentrations of VEGF by Quantikine

**Human VEGF ELISA kits.**

The concentrations of VEGF measured by Quantikine Human VEGF ELISA kits were lower in the presence of anti-VEGF drugs than in the presence of a control mouse.
IgG. Numbers beside each circle indicate standard deviations. Dotted lines represent calculated free VEGF concentrations. Dashed lines represent minimum detectable dose. Assays were performed in the presence of each of the following. (a) Pegaptanib (52 µg/mL; using the serum half-life in human\textsuperscript{18}: 1625 µg / 4.5 mL \times (0.5)^{28 \text{ days} / 10 \text{ days}}). (b) Aflibercept (0.14 µg/mL; using vitreal half-life in rabbit\textsuperscript{20}: 2000 µg / 4.5 mL \times (0.5)^{56 \text{ days} / 4.8 \text{ days}}. The concentration was calculated for 8 weeks after injection because it was thought that ELISA was strongly blocked by aflibercept due to the low dissociation constant of aflibercept and VEGF). (c) Aflibercept (7.2 µg/mL; concentration calculated for 4 weeks after injection: 2000 µg / 4.5 mL \times (0.5)^{28 \text{ days} / 4.8 \text{ days}}). (d) Aflibercept (440 µg/mL; just after injection, assuming that aflibercept distributes uniformly in the ocular space: 2 mg / 4.5 mL). e) Bevacizumab (4.0 µg/mL; using the concentration–time relation in human vitreous humor\textsuperscript{21}: 1934294 e^{-1.431 \times 28} + 70856 e^{-0.103 \times 28}). (f) Bevacizumab (13 µg/mL; using the vitreal half-life in rabbit\textsuperscript{22}: 1250 µg / 4.5 mL \times (0.5)^{28 \text{ days} / 6.61 \text{ days}}). (g) Ranibizumab (0.11 µg/mL; using aqueous half-life in rabbit\textsuperscript{19}: 500 µg / 4.5 mL \times (0.5)^{28 \text{ days} / 2.84 \text{ days}}. This assay was performed because ELISA was not strongly blocked by aflibercept 4 weeks after injection). (h) Ranibizumab (1.1 µg/mL; mean concentration of 20 retinal vein occlusion patients 1 month after intravitreal injections of 0.5 mg ranibizumab\textsuperscript{23}). (i) Effect of ranibizumab (1.1 µg/mL; equal to (i)) on monocyte chemotactic protein-1 (MCP-1) measurement using a MCP-1 ELISA kit (Quantikine Mouse MCP-1 ELISA kit) is also shown. (j) Mouse IgG (13 µg/mL; equal to bevacizumab (h)). Data labels represent standard deviations (pg/mL). All assays were performed in duplicate according to the manufacturer’s instructions.

References


