Targeting Talk: Dosing, Volume, and Animal Care

by Dr. Ronald G. Wiley

Q: When performing intraparenchymal injections of immunotoxin, what is the proper volume to use? Is it better to induce two half-portions per hemisphere or is a higher concentration better? At what concentration do you expect necrosis or inflammation?

A: There is no one answer to the question of injection volume. Practically speaking, we have observed that large or extended structures such as the entire cholinergic basal forebrain (CBF) of rats are difficult to ablate with one single injection of 192-Saporin. The best results were obtained with 3-5 separate 0.5-1.0 µl injections. For even larger targets such as the CBF in primates, other strategies may be necessary. Oldfield and co-workers have reported success in delivering cytotoxic chemotherapy to large volumes of brain using long, slow infusions of solutions containing a low concentration of toxin (convective delivery). This procedure delivers toxin by bulk fluid flow rather than diffusion and avoids high local toxin concentrations around the infusion catheter or pipette. High local concentrations of toxin may compromise selectivity and produce non-specific cytotoxicity. This can occur when neurons and glia take up toxic amounts of saporin by bulk fluid phase endocytosis, rather than receptor-mediated endocytosis. With direct intraparenchymal injections, local necrosis can occur with surprisingly small doses of toxin. For example, 60 ng of SP-SAP or dermorphin-SAP into the rat striatum injected in 1 µl typically produces some necrosis in the center of the injection site. With immunotoxins such as 192-Saporin or anti-DBH-SAP, 200 ng in 0.5 µl may barely produce a trace of local damage.

Q: We are interested in the anti-Thy-1 nephritis model in rats. I want to know the titer of OX7-SAP and how much we have to expend for each rat to establish the model?

A: The "titer" of OX7-SAP is rather difficult to define. It is not known precisely how many molecules of this immunotoxin are necessary to kill a thymic-derived (Thy-1-expressing) lymphocyte *in vivo*. Also, since OX7-SAP kills Thy-1-expressing lymphocytes, it may prove difficult to induce Thy-1 nephritis with the immunotoxin. In humans, proteinuria was reported in clinical trials using immunotoxins for treatment of cancer, but we do not know of any comparable data in rats. Probably the only way to determine the appropriate dose would be a dose ranging study.

Selected references on convective delivery of toxin to brain:

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