Targeting Talk: *Dose Ranging*

by Dr. Douglas Lappi

Q: *We just completed surgeries where we implanted third ventricular cannulas and temporary bilatera cannulas directed into the nucleus tractus solitarius in the brainstem of animals. We injected either the Blank-SAP control toxin or the experimental material Oxytocin-SAP into the bilateral NTS cannulae over a 30-second period. However, within the next week-two weeks post-surgery, we lost 13 of the 19 animals treated; they appeared not to be able to groom properly and lost over 20% of their body weight. This was apparent in both the Blank-SAP and the Oxytocin-SAP groups. We gave a dose of 40 ng/300 nl for each of the reagents. This dose was determined based on a published article using another of ATS's targeted toxins. I'm very surprised by my results. Can you offer any explanation/advice?*

A: This is a particularly disturbing result; it appears that a dose was chosen by comparison to one used with another targeted toxin. Although this can be a good approximating tool to begin a dose-ranging study, it usually doesn't take into account the tissue, system, target molecule — so many parameters that are important to determining the proper dosage.

The literature is quite extensive on targeted toxins, and so there may be a comparable starting dose that has been published. Let's use, for example, 4 µg. Reduce that amount by 20% quantities (4, 3.2, 2.4) and test in a small number of animals to determine a value that is safe and effective. If no trouble is seen at the highest dose, and the effect is minimal, that would indicate a higher dose may be acceptable. You can then test doses in 20% increased increments (4.8, 5.6, 6.4).

The effects you see in your animals should only be reflective of the particular cell type you are eliminating. In the case of control reagents, such as Blank-SAP, no cell type is being targeted, so if you are seeing any kind of result, then you are certainly over-dosing.

Q: *Is there some kind of formula that one can use that will help determine a starting point for establishing a range of doses to test in animals prior to initiating a study? For example, if the targeted toxin is administered intravenously, does it take more or less material than when administered directly into tissue?*

A: Start with a few animals and do dose-ranging as discussed in the previous question. The various modes of application are really too wide to discuss in any detail here, but I, a biochemist by training, always like the approach of thinking about what sort of concentration will be needed to have a cytotoxic effect. Generally, these molecules have an ED_{50} in the nanomolar to picomolar range. Obviously if you inject systemically, the material from the first becomes greatly diluted, relative to an injection directly into tissue, and so you'll need a lot more. If you inject directly into tissue the local concentration can be quite high.

This brilliant artistic expression is a creation of this issue's Nobel Prize winner as featured in the Targeting Teaser on the back cover. The title of this piece is *Hamadryad.*