D-Luciferin delivery

D-luciferin is the substrate for firefly luciferase. Traditional dosing for in vivo imaging is recommended at 150mg/kg via intraperitoneal administration. This concentration works well across species (mice, rat, rabbit, monkey, etc). This dose was arbitrarily determined, in most cases a lower dose may work also. The rule of thumb is consistency within an experiment. In certain cases of extreme sensitivity/low signal (e.g. gene delivery) it may be advantageous to double the dose to 300mg/kg. Doubling the dose does not double the signal since one is close to saturation. However, even at 450mg/kg one does not reach saturation in vivo. Also, at 450mg/kg, no toxicity has been observed, but the benefit in signal intensity over 300 mg/kg is minimal. D-luciferin concentration is the limiting factor in signal intensity in in vivo settings. In vitro, ATP is the limiting factor since cells are dead and not producing ATP, therefore one has to supplement. With intraperitoneal delivery, one creates a depot injection. The luciferin is slowly absorbed in the peritoneal vascularization. Since D-luciferin is a small molecule it freely diffuses across membranes into and out of all organs. Luciferin is not metabolized but excreted by the kidneys. Therefore we face a simple pharmacokinetics model of influx and efflux. When influx equals efflux we have a stable concentration of luciferin and therefore stable signal (if luciferase is constitutively expressed). This is the so said plateau phase and for longitudinal studies, it is recommend to repetitively image in the plateau phase at the same time point post injection. It is important to assess an initial timeline during new model validation, since peak signal and plateau phase will vary depending on the model. E.g., it is commonly observed to have a delayed plateau for brain imaging because of the blood brain barrier. Luciferin does pass the blood brain barrier but one observes delayed influx vs other organs and of course also delayed efflux. As a word of caution, the substrate for renilla is coelenterazine. This molecule is a substrate for the pGP pump and the BBB is rich in pGP's does unless there is leakage of the BBB, native coelenterazine will not pass the BBB. Alternative syntetic coelenterazines may exhibit different behavior. In order to increase signal intensity, another approach is to change the route of administration to intravenous delivery as a bolus injection as opposed to an IP depot injection. Either tail vein injections or retro orbital injections are practiced. Obviously, with a bolus injection one instantaneously injects the full dose into the vascular space. Signal will peak two minutes post injection, is short lived and disappears by 30 min post injection. As opposed to the IP injections, in which the signal lasts for at least 2 hours. In certain cases, one needs consistent signal over time for frequent imaging with shorter intervals than the washout kinetics. In this case alternatives such as continuous perfusion with tail vein catheters or implanted osmotic pumps are possibilities. Traditionally, with the continuous perfusion, luciferin concentrations are 100 fold less - decreasing signal intensity two orders of magnitude. Therefore this technique may not apply for weak signals. However there are great publications for eg circadian rhythm studies with luciferase reporters.

上海乐辰生物科技有限公司

上海市宝山区共江路 299 号 207 室　200431

Tel: 021-60444836　400-0021-908　Fax: 021-66209682

Http://www.sinochrome.net　E-mail: tech@sinochrome.net

Sinochrome
Solutions for *in vivo* imaging