Methods Developed for Pediatric Studies

April 2018 Newsletter

Introduction

“Primum non nocere”, “First, do no harm” is every physician’s guiding principle, but how can these health care providers really abide by this principle and apply it when urged to prescribe medications based on imperfect or absent safety and efficacy data for pediatric populations. Prescriptions that can result in unpredictable and tragic effects. For example, such as what happened with the thalidomide disaster where the drug was given to pregnant women for nausea and morning sickness but resulted in kids born with short or absent limbs (phocomelia).

For far too long, children were considered therapeutic orphans, as they were either exposed to or denied the use of medications that have been approved for adults, but lack safety, dosing and efficacy testing in pediatric populations. Children, however, are not little adults and one size does not fit all. There are metabolic and physiologic differences between these age groups. A child’s whole body is growing. This means that many different kinds of healthy, normal cells are dividing faster than they would be in an adult, which implies that children would be more vulnerable to different chemicals. For example, chemo drugs that are used for cancer treatment and that target rapidly dividing cells could result in detrimental late effects in children due to damage to these cells but may have mild to no effects in adults. In fact, children sometimes suffer from so -called “Late Effects”, which are side effects that will happen in later stages of the child’s life with some of them resulting in detrimental consequences such as learning or cognitive impairments, abnormal growth or infertility. Therefore, drugs administered to children need to have undergone pediatric therapeutic trials. The absence of these population specific studies results in off-label drugs that lack adequate pediatric info in labeling.

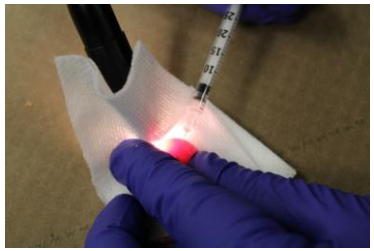
However, since the passing of the BPCA (Best Pharmaceuticals for Children Act) and PREA (Pediatric Resource Equity Act) legislations, both of which incentivize and require pediatric studies, there has been a dramatic increase in the number, timeliness and successful completion of pediatric drug development studies. As a result of these legislative initiatives, as of September 2016, more than 650 products acquired new pediatric information added to the labeling.

Due to this growing requirement for the pharmaceutical industry to specifically test newly discovered drugs in the pediatric populations, we, at ITR, took the leadership and developed a neonatal test system that can be used to evaluate the efficacy and safety of the drugs targeting pediatric patients. We established new techniques that can be specifically used on this population. These include novel intravenous and oral dosing methods as well as a dot-tattoo digital identification technique.

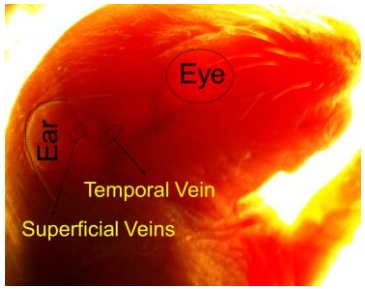
I. Intravenous Dosing of Neonates

Administration of potential therapeutics or test items to neonatal rodents is technically challenging. However, given the importance of such a procedure for toxicology and safety evaluation of drugs targeting pediatric patients, we developed a neonatal test system that serves this purpose. We established a multiple intravenous injection dosing system for neonatal rats using the temporal (or facial) vein, which was located using trans-illuminating light. The injection procedure was performed under a magnifying or dissecting microscope using an insulin syringe with a 31G needle. Successful dosing was evidenced by blanching of the vascular network following injection. Furthermore, infusion of 1% Evans Blue confirmed successful dosing as it was evidenced by immediate blue discoloration of the injected pup. Dosing was performed once every 24 hours and pups were monitored daily for any abnormality. Our results showed that the neonatal rats could be successfully dosed daily until at least post-natal Day 4 and depending on the body size up to day 5. Therefore, our neonatal rat test system can be used for efficacy studies or safety evaluation of pediatric drugs as single or up to five daily doses.

Set up for the procedure



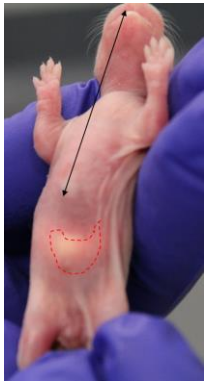
Injection procedure



Anatomical features of rat neonate head under trans-luminating light

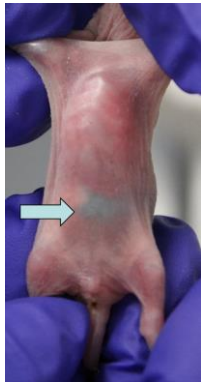
II. Oral Gavage Dosing of Neonates

Although oral gavage is considered a common and well-established experimental procedure in adult rats, its application for evaluation of the drugs designed to be used for children is questionable. Therefore, we also developed an oral gavage procedure for neonatal rats using conventional plastic mouse gavage cannula. Following manual restraint of the pups and pre-measurement of the cannula length on the abdominal surface of the pups from the oral cavity to the xiphoid process and using milk -filled stomach as land mark, oral gavage was performed using an inert dye (tattoo ink). The neonates, especially in earlier post-natal age, accepted the procedure with minimal resistance. Successful dosing was confirmed by discoloration of the white milk-spot post dosing. Necropsy of the neonate 1 hour following dosing showed expected flow of ink in the stomach and intestine. Consequently, the proposed oral gavage procedure in neonatal rats was considered ideal for the safety evaluation of drugs intended for pediatric indication.



Measuring the distance from the oral cavity to the end of the xiphoid process

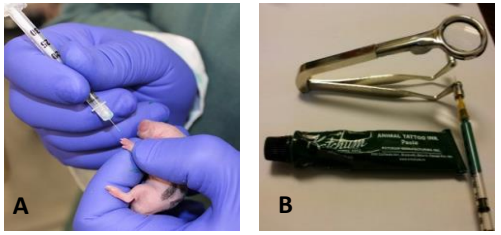
Loaded needle gently advanced to the pre-marked level



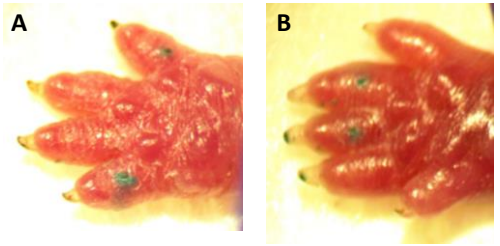
Successful delivery confirmed by discoloration of stomach area

III. Dot Tattoo Digital of Neonates

Effective identification of neonatal animals, especially small rodents is a challenge due to their small body size making it difficult to use commonly used conventional identification methods such as ear tags or tail tattoo. Using microchip or numbering by ink markers have limitations due to the cost or stability of these identifiers. Other methods such as a paw tattoo system often used on reproductive toxicology studies have limitation of the maximum usable number. Traditionally, digit amputation or toe clipping has been used for this purpose, however, the animal welfare aspect of this method remains a significant concern. Therefore, we established a simple and time efficient permanent identification method consisting of dot-tattoo marking of the digits using small gauge needles. This technique allows the separate identification of up to 9999 neonates and was not associated with any significant effects on the animals' overall growth and well-being. It was associated with minimal bleeding and no significant effect on neonatal growth rate and health. Consequently, the proposed animal identification method is considered ideal for use on studies employing neonatal rats for the evaluation of the safety and/or efficacy of drugs intended for pediatric indication.



Tattooing System: A) Insulin Syringe with a 31G Needle B) Aramis Micro-tattoo System



Samples of Dot-Tattoo: A) Front Paw and B) Hind Paw

Conclusion

So far, ITR has shown leadership in pediatric safety and efficacy testing through developing neonatal specific intravenous and oral gavage dosing techniques as well as a novel identification system. We are also working on establishing pediatric inhalation safety pharmacology assays so that we ensure a healthier and safer future for our children.