Intravenous Dosing of Neonatal rats as a Test System for toxicology and safety evaluation of drugs targeting pediatric patients

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Abstract

Administration of potential therapeutics or test items to neonatal rodents is technically challenging. However, considering the metabolic and physiologic difference between adult and neonates, in order to evaluate the efficacy or safety of the drugs targeting pediatric patients, establishing a neonatal test system is essential. We have developed a multiple intravenous injection dosing system for neonatal rats using the temporal (or facial) vein. A total of twenty neonates (10 of each sex) from two dams were divided into control and drug groups. In control group, neonatal rats were anesthetized with either halothane or isoflurane inhalation. The temporal vein was located using trans-luminating light and following a gentle manual restrain. Injection procedure was performed by placing a magnifier or dissecting microscope. For injection, using an insulin syringe with a 31G needle, 50 µl of PBS was injected slowly. Successful dosing was evidenced by blanching of the vascular network following the injection. Furthermore, infusion of 1% Evans Blue confirmed successful dosing as it was evidenced by immediate blue discoloration of the injected pup. Animals in the control group were also anesthetized with same method but were not injected. 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 postnatal Day 4 and depending on the body size up to Day 5. Therefore, we suggest that 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.

Introduction

The effect of drugs on the body is affected by many physiological as well as pathological factors. Life stage is one of the major factors affecting pharmacokinetic and pharmacodynamics of drugs. Children and especially neonates have significantly different metabolic and physiologic capacities compared to the adults. This is an important factor to be considered when safety and toxicity evaluation of those drugs are being evaluated. Therefore, using the results of these studies which are performed in adult animals as a basis for evaluating the safety of the drugs targeting neonates or children might result in serious ethical issues. Therefore, regulatory agencies are intensively reviewing the details of this topic.

In general, an ideal model for evaluating drugs for human pediatric use would be pediatric animals. However, administration of potential therapeutics or test items to neonatal rodents is technically challenging and therefore, establishing a neonatal test system model is essential. In this study we discuss a multiple intravenous injection dosing system for neonatal rats using the temporal (or facial) vein.

Materials & Methods

All animal experimental procedures used in this study have been peer-reviewed by ITR Canada’s Animal Care Committee. Two pregnant Sprague Dawley rats (15 days gestational age) were subjected to 1 week acclimation before parturition. After parturition, a total of twenty neonates (10 of each sex) from two dams (produced 16 and 18 pups in total) were divided into TEST and CONTROL groups. In the TEST group, neonatal rats were anesthetized by either halothane (until Day 4 post-natal) or isoflurane inhalation (After Day 5 and until the end of the study). Temporal vein was located using trans-luminating light (Fig.2). Under a gentle manual restrain, the injection procedure was performed using a magnifier or dissecting microscope (Fig.3). For the TEST group, 50 µl of PBS was injected slowly until the insulin syringe with a 31G needle was filled. First injection was done in a window of 24 hours of birth and were designated as day 1 and was continued as once daily till the end of tentative seven days experiment. Successful dosing was evidenced by blanching of the vascular network following the injection. Furthermore, infusion of 1% Evans Blue was used to confirm successful dosing. Animals in CONTROL group were not anesthetized with same method but were not injected. Dosing was performed every 24 hours and pups were monitored daily for any abnormality.

Results and Discussion

The results of this study demonstrated the possibility of daily intravenous injections to neonatal rats, from day 1 up to day 5 post-natal. Successful dosing was evidenced by blanching of the vascular network following the infusion. Furthermore, infusion of 1% Evans Blue confirmed successful dosing as it was evidenced by immediate blue discoloration of the injected pup (Fig.4).

Our results showed that the neonatal rats could be successfully dosed daily until at least, postnatal (PN) Day 5. The success rate at PN Day 4 was 100% in both male and female pups. At postnatal Day 5, the success rate was 80% (4/5) in male versus 100 % (5/5) in females. At Day 6, none of the 5 male pups, were dosed (5%) and the success rate in female was 80% (4/5). Therefore, there was a decrease in success rate after 5 days PN that was more significant in the male pups. However, the decrease in success rate after Day 5 PN correlated to the increasing body size rather than gender of the pups. In fact, increased body size led to less visibility of the vascular system through skin layers, interfering with intravenous dosing. Further, we suggest using dams of different size, but rather than decreasing, the test results, were reviewed in having pups with proper body size. The daily injection did not result in any significant detrimental effect. No mortality nor abnormalities were reported in any of the groups. Anesthesia and recovery following both methods were smooth and without any complication. There was a slight decrease in body weight that was only significant in female test animals compared to control non-injected animals (10.3±0.9 versus 12.5±0.6 grams, respectively). This was attributed to a mild effect of manipulation during the injection of the TEST group, compared to CONTROL that were not manipulated.

We suggest that by including an actual control group that receive vehicles, PBS or normal saline free effect with the result. In conclusion, we suggest that our neonatal rat test system can be used for efficacy studies or safety evaluation of pediatric drugs as single or multiple dose up to at least five daily doses.

References

