Comparison of hydrodynamic tail vein injections (HDTVi) with and without anaesthesia: implications for Animal Welfare and scientific outcomes

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Introduction

At Cancer Research UK, Cambridge Institute, we perform hydrodynamic tail vein injections (HDTVi's) to deliver nucleic acids to the liver of live mice to study the effect of gene modulation upon the development of chronic liver disease.

As part of upholding the 3Rs ethos and legal obligations of the project licence we considered use of anaesthesia when performing HDTVi's due to the perceived likelihood of the animal suffering pain or distress when conscious.

Method

To assess the impact on science and an opportunity to refine our processes to elevate Animal Welfare we conducted a pilot study. For the pilot, 40 C57Bl6/J mice which were divided into two cohorts (10 males and 10 females in each cohort), with each group having the procedure performed either with anaesthesia (AB) or without anaesthesia (AA).

The pilot was performed over two days. Day 1 all 20 animals in the group receiving the injection under AB and Day 2 the second group of 20 animals receiving the injection AA.

As a welfare indicator we used full recovery timings following the injection. Full recovery was defined as the animal walking around and behaving normally. To assess the impact on the science we analysed the samples using RAS immunohistochemistry (IHC) staining of the liver.

Results

- The qualitive data from the results showed that the number of stained cells (hepatocytes) in the liver was comparable between mice which received the injection AA or AB therefore indicating that the science is not impacted. However it is important to note that the RAS IHC staining in the AA group was slightly lower than expected, which made quantitative comparisons challenging. This could be attributed to potential batch effects as the AA and AB procedures were performed on different days.
- Recovery after HDTVi AB, overall takes longer.
- The use of a warming cabinet helps recovery for AA but not for AB.

Limitations

One significant limitation of this study was the technicians lack of prior experience in performing HDTVi on anaesthetised mice. This inexperience could have influenced the performance of the procedure and potentially affected the results.

Conclusion

Based on the findings of this pilot study, the use of anaesthesia during hydrodynamic tail vein injections does not appear to affect the scientific outcomes.

While anaesthesia is considered to reduce the risk of an animal suffering pain or distress during the injection and recovery process, the longer recovery times observed in the AB group raise questions about overall Animal Welfare. The extended recovery period could potentially

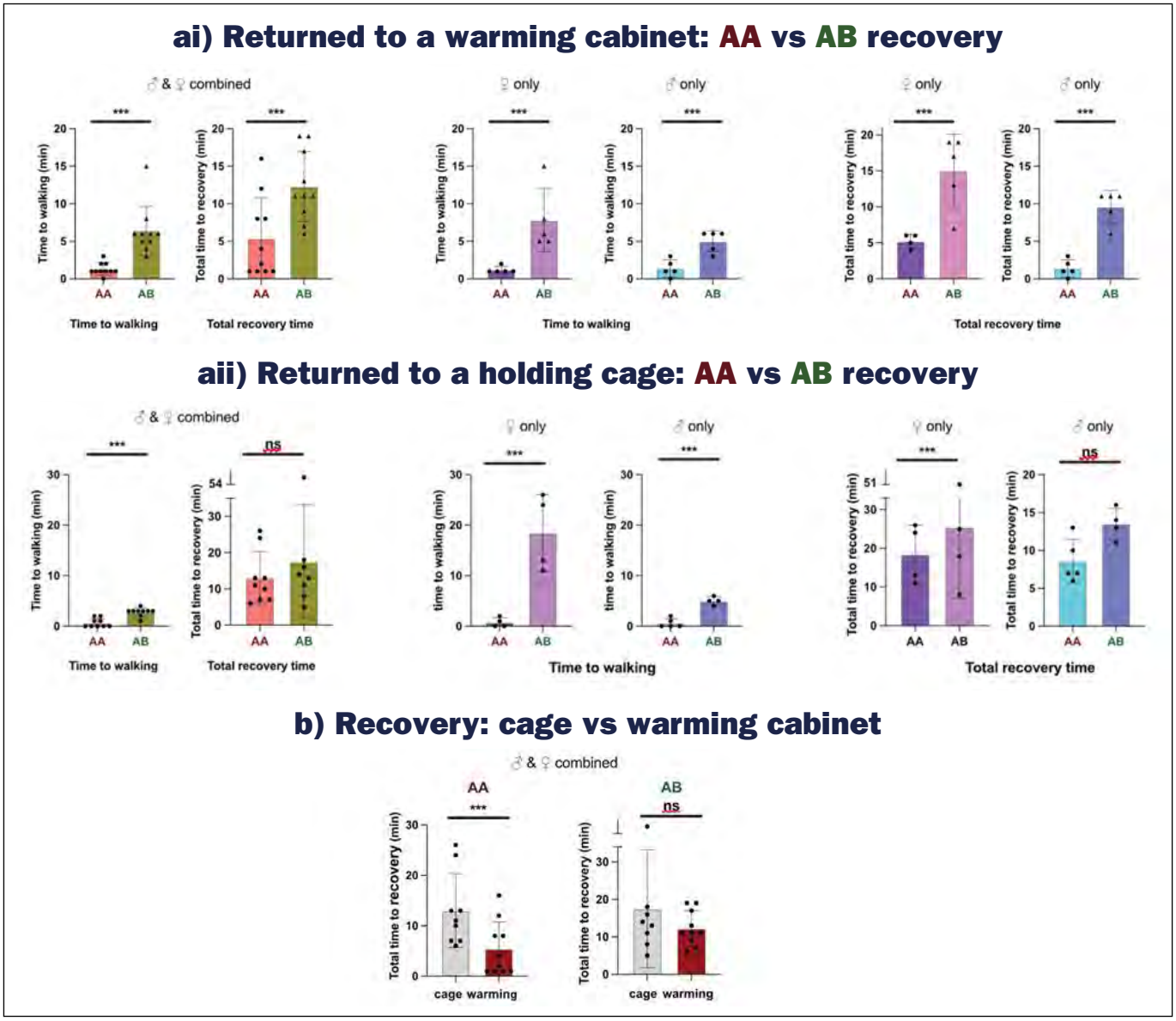

Figure 1: Recovery in mice post-HDTVi, a) Comparison of time to walking and total recovery time for male and female mice returned to i) a warming cabinet, ii) a holding cage, where the procedure was performed without (AA) and under anaesthesia (AB), b) Comparison of total recovery time for both male and female mice returned to both warming cabinets and holding cages, where the procedure was performed without (AA) and under anaesthesia (AB).

introduce additional stress for the animals, which must be weighed against the benefits of reduced acute distress during the initial period following the procedure itself.

Next steps

- To seek clarity on the impact of performing the pilot over two days on the RAS IHC staining we are going to conduct a small-scale pilot study where both AA and AB procedures are performed on the same day allowing for more direct comparisons and minimising potential confounding factors.
- Refine our recovery protocols, particularly investigating the optimal use of warming cabinets for all animals.
- Developing training protocols for personnel performing HDTVi's to ensure proficiency in both AA and AB techniques.
- Explore alternative anaesthesia protocols that might offer the benefits of pain reduction without significantly extending recovery times.

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References

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