SSC Subcommittee Project/Collaborative Project

NAME OF PROJECT: Comparative Evaluation of Murine Bleeding Models
Subcommittee: Animal, Cellular and Molecular Models

- Person responsible (Chair / Principal Investigator):
  - Chair: Tom Knudsen
  - Principal Investigator: Brian C. Cooley

- Design:

  Compare the performance, pros, and cons of three widely applied standard bleeding models in mice under different conditions of bleeding tendencies, e.g. haemophilia and anticoagulant therapy.

- Aim/Objective/Rationale (Needs assessment / Reason):

  Several bleeding models are currently applied in mice. Significant variability exists with regard to methodologies, outcomes, and data reporting. Notably, while direct inter-model comparison is always difficult, even intra-model comparison, e.g. between labs, is presently associated with considerable variability for any specific model. Accordingly, comparative studies, ultimately leading to increased standardization and the application of common guidelines are needed to facilitate cross-laboratory comparison of data.

  The overall objective of the proposed SSC effort is therefore to compare these models directly under standardized conditions and to communicate the findings in order to guide the society towards a higher degree of standardization.

  The specific objective of this study is to provide a methodical comparison among three commonly used bleeding models, under controlled conditions of a spectrum of bleeding phenotypes, to provide data that can be used by future groups to compare select optimal bleeding models for studies. Furthermore, the data will serve as a reference standard for these models under specific, reproducible conditions.

- Methodology (Data expected to collect, sample size and statistical analysis):

  Three murine bleeding models will be employed, all in anaesthetized mice:

  1. Standard tail tip transection\textsuperscript{1,2} amputating the entire tail tip at a defined distance from the tail tip. Bleeding time is monitored in 37°C saline, blood is collected and quantified, to determine time to hemostasis and total amount of blood loss, respectively.
2. **Tail vein transection**\(^3\) transecting the left tail vein at a uniform proximal location in the tail using a specially designed template to achieve consistent depth of incision. Bleeding is monitored as in the tail tip transection model above.

3. **Saphenous vein bleeding**\(^4-5\) surgically exposing the saphenous vein followed by partial transection and longitudinal opening of the vein. Bleeding is monitored by measurement of the time until bleeding cessation, with subsequent repeated disruptions (mechanical clot removal) and re-measurement of time to bleeding cessation over the course of 30 minutes. Primary endpoint is number of hemostatic events over the entire period.

Three bleeding states will be investigated, one in which the animal has a genetic predisposition to bleeding (F8-KO; Haemophilia A), one where wild-type mice are dosed with an anticoagulant (FXa inhibitor; Rivaroxaban), and one where wild-type mice are administered a platelet inhibitor (Clopidogrel). This approach should cover a broad range of bleeding situations for which the models can be used.

**Study groups:**

<table>
<thead>
<tr>
<th>Bleeding Model</th>
<th>Control</th>
<th>Clopidogrel: 2 doses</th>
<th>Rivaroxaban: 2 doses</th>
<th>F8-KO Mice</th>
<th>F8-KO Mice + Recombinant F8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standard tail tip transection</td>
<td>12</td>
<td>12+12</td>
<td>12+12</td>
<td>12</td>
<td>12</td>
</tr>
<tr>
<td>Tail vein transection</td>
<td>12</td>
<td>12+12</td>
<td>12+12</td>
<td>12</td>
<td>12</td>
</tr>
<tr>
<td>Saphenous vein bleeding</td>
<td>12</td>
<td>12+12</td>
<td>12+12</td>
<td>12</td>
<td>12</td>
</tr>
</tbody>
</table>

**Sample size / study population**

Power analyses of existing data sets were done to determine appropriate animal numbers. See details below. In short, it is anticipated that up to 252 mice will be used, 12 mice per group in 21 groups, to evaluate the 3 models under the various experimental conditions.

**Justification of sample size**

Data for F8-KO mice using the tail vein transection model either without therapy or low dose rFVIII therapy (5 U/kg 5 min before injury) yielded a blood loss of 7626 ± 1545 or 747 ± 478 nmol haemoglobin, respectively (means ± SD). Using α=0.05, and a power of 0.9, with a one-
tailed design, a power analysis shows that it will be possible not to overlook a decrease of effect of 590 nmol Hgb in relation to the treatment group with a group size of n=12 (proc power twosamplemeans of two independent treatment groups), which is well within the pharmacological window of the model (747 – 7626 nmol Hgb).

Also, based on previous series done in Dr. Cooley’s lab using the saphenous vein bleeding model either without therapy or with low-dose recombinant Factor IX therapy (100 U/kg given 7 days before model application) yielded 7.50 ± 5.88 or 1.52 ± 1.14 haemostatic events, respectively (means ± SD). Using the same criteria as mentioned above for the TVT model, a power analysis shows that it will be possible not to overlook a decrease of effect of 1.41 haemostatic events in relation to the treatment group also with a group size of n=12 (proc power twosamplemeans of two independent treatment groups), which again is well within the pharmacological window of this model (1.52 – 7.50 haemostatic events).

The above suggested power analyses are for the studies in FVIII deficient mice. For the studies in wild type mice where the mice will be treated with anticoagulants we have no a priori perception of the variation, but it is assumed that the variation will be similar to the bleeding studies in FVIII deficient mice why the group size is also chosen to be 12 mice per treatment group in these series.

Statistical analyses

Data will be analyzed via ANOVA and posthoc Tukey’s HSD tests, using a p<0.05 level to assign statistical significance.

- Study population (Inclusion, exclusion, eligibility) (patient population; recruitment of participating institutions/physicians and subjects; minimum number needed; expected number):

  The experimental animal populations are described in the section above. This proposal does not involve human studies.

- Expected timeline:
  
  o Project stage/set up: 2 months -- order mice and supplies.
  o Launch: 1 month – evaluate and validate tail transection models (control experimental conditions; the saphenous vein bleeding model is standardly done in the PI’s Core Lab).
  o Duration: Experimental phase: 6 months – complete all experiments.
  o Finalization/analysis: 2 months – complete all analyses.
  o Reporting: 1 month -- write final report and develop presentation materials (oral presentation and manuscript for publication).

- Expected outcomes (ie. publications):
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Upon completion of the study, a manuscript for presentation of the data will be developed and submitted. The study will also be submitted for presentation at a forthcoming SSC/ISTH meeting.

- Description of project set/up and management, needed infrastructure and resources (summary):

  The project will be done and managed by the Rodent Advanced Surgical Models Core Lab of the University of North Carolina, McAllister Heart Institute. The PI is Core Director and will plan and implement the experiments and data analysis. All equipment and instrumentation, including computers for analysis, are available through the PI’s Core Lab. Only mice and the technical conduction of the experiments are needed.

  In addition to the above experiments, data generated will be compared to those already on file with several international groups, namely:

  1) INSERM 770, Paris, France, EU.  
     PI: Dr. Cecile Denis

  2) CHOP, Philadelphia, PA, USA.  
     PI: Lacramioara Ivanciu / Denise Sabatino

  3) Novo Nordisk A/S, Denmark, EU.  
     PI: Dr. Peter Bygballe Johansen

* Data on file will not necessarily cover all combinations of genotypes and interventions as investigated in the proposed project, but selections will be made, e.g. comparing the effects of FVIII in F8-KO mice across laboratories.
References:


