Bone & Joint Research

Supplementary Material

10.1302/2046-3758.119.BJR-2022-0016.R1

A power calculation was performed assuming a difference of 25% in chondrocyte viability between groups and a standard deviation of 10% within measurements for each group. To achieve a power of 90% with a type 1 error of 0.05 and type 2 error of 0.1, eight animals per group would be required for confocal laser scanning microscope (CLSM) analysis. However, to account for potential loss of samples or a lower measured percentage difference, ten animals/group were used for CLSM at a timepoint of two days after injection.

Five mice per group were used in order to observe for histological evidence of localized joint infection at the time the CLSM analysis was performed (day 2), and to compare the CLSM results with histological evidence of cartilage damage. A further five mice had histological analysis after seven days of infection to ascertain whether any difference in chondrocyte death observed after two days translated into increased histological evidence of cartilage damage at this time. Histological analysis could not be performed on mice used for CLSM, therefore it was necessary to increase the group sizes by ten animals. The experiment was not specifically powered to find a statistical difference in histology scores between groups, as it was felt too many additional animals would be required and contradict the NC3R's principles.

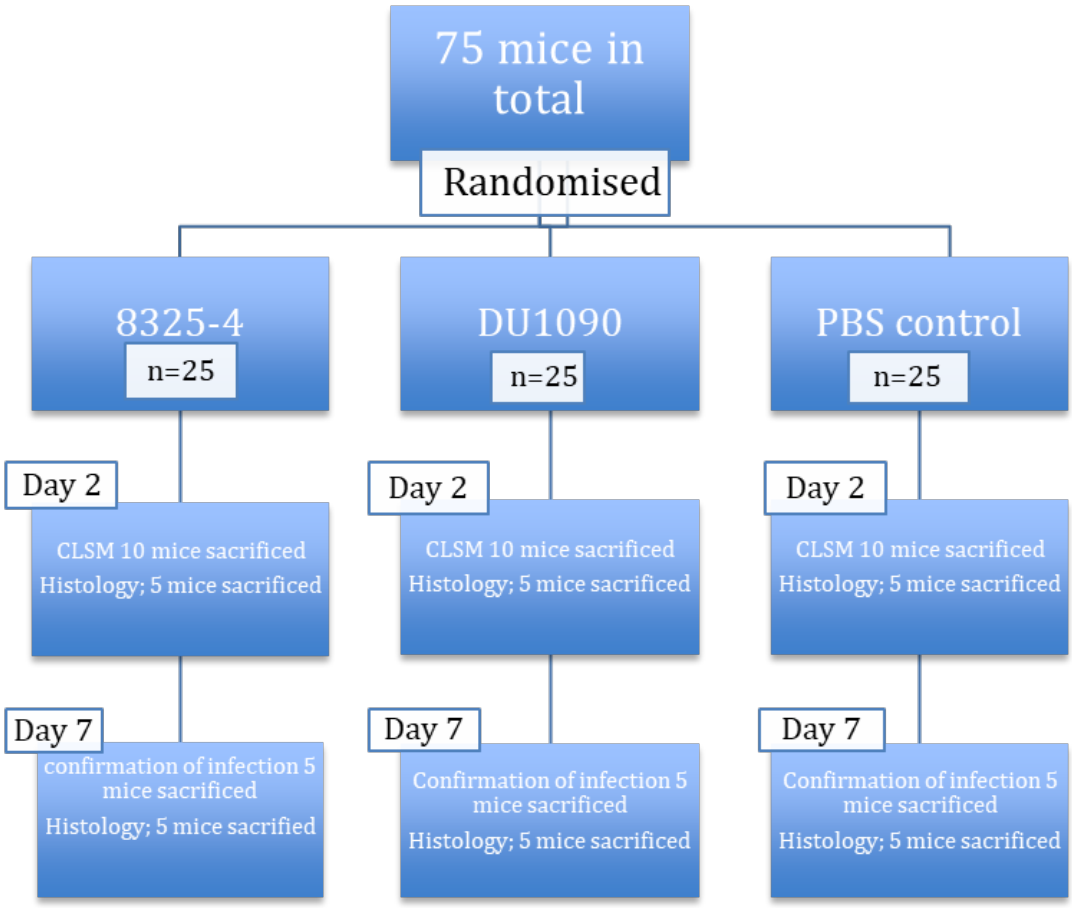

Fig a. A flowchart describing the randomization of mice into the three experimental groups, number of mice sacrificed at each stage and the points at which histology of tissue was performed. CLSM, confocal scanning laser microscopy; PBS, phosphate-buffered saline.

NOTE: Please save this file locally before filling in the table, DO NOT work on the file within your internet browser as changes will not be saved. Adobe Acrobat Reader (available free here) is recommended for completion.

The ARRIVE guidelines 2.0: author checklist

The ARRIVE Essential 10

These items are the basic minimum to include in a manuscript. Without this information, readers and reviewers cannot assess the reliability of the findings.

Item		Recommendation	Section/line number, or reason for not reporting
Study design	1	For each experiment, provide brief details of study design including: a. The groups being compared, including control groups. If no control group has been used, the rationale should be stated. b. The experimental unit (e.g. a single animal, litter, or cage of animals).	Under Materials and methods - Animals, Randomization and Innoculation Under Materials and methods - Animals, Randomization and Innoculation
Sample size	2	a. Specify the exact number of experimental units allocated to each group, and the total number in each experiment. Also indicate the total number of animals used. b. Explain how the sample size was decided. Provide details of any *a priori* sample size calculation, if done.	25 mice to each groups, with a total of 75 mice used
Inclusion and exclusion criteria	3	a. Describe any criteria used for including and excluding animals (or experimental units) during the experiment, and data points during the analysis. Specify if these criteria were established *a priori.* If no criteria were set, state this explicitly. b. For each experimental group, report any animals, experimental units or data points not included in the analysis and explain why. If there were no exclusions, state so. c. For each analysis, report the exact value of *n* in each experimental group.	All animals were included into the study No exclusions were made as there were no significant anomaly in data set Under Materials and methods - Animals, Randomization and Innoculation
Randomisation	4	a. State whether randomisation was used to allocate experimental units to control and treatment groups. If done, provide the method used to generate the randomisation sequence. b. Describe the strategy used to minimise potential confounders such as the order of treatments and measurements, or animal/cage location. If confounders were not controlled, state this explicitly.	Under Materials and methods - Animals, Randomization and Innoculation
Blinding	5	Describe who was aware of the group allocation at the different stages of the experiment (during the allocation, the conduct of the experiment, the outcome assessment, and the data analysis).	Under Materials and methods - Animals, Randomization and Innoculation
Outcome measures	6	a. Clearly define all outcome measures assessed (e.g. cell death, molecular markers, or behavioural changes). b. For hypothesis-testing studies, specify the primary outcome measure, i.e. the outcome measure that was used to determine the sample size.	Under Results - Chondrocyte Viability Under Results - Chondrocyte Viability
Statistical methods	7	a. Provide details of the statistical methods used for each analysis, including software used. b. Describe any methods used to assess whether the data met the assumptions of the statistical approach, and what was done if the assumptions were not met.	Under Materials and Methods - Statistical Analysis Under Materials and Methods - Statistical Analysis
Experimental animals	8	a. Provide species-appropriate details of the animals used, including species, strain and substrain, sex, age or developmental stage, and, if relevant, weight. b. Provide further relevant information on the provenance of animals, health/immune status, genetic modification status, genotype, and any previous procedures.	Seventy-five adult male C57Bl/6 mice aged 12 weeks
Experimental procedures	9	For each experimental group, including controls, describe the procedures in enough detail to allow others to replicate them, including: a. What was done, how it was done and what was used. b. When and how often. c. Where (including detail of any acclimatisation periods). d. Why (provide rationale for procedures).	Under Materials and methods - Animals, Randomization and Innoculation Under Materials and methods - Animals, Randomization and Innoculation Under Materials and methods - Animals, Randomization and Innoculation Under Materials and methods - Animals, Randomization and Innoculation
Results	10	For each experiment conducted, including independent replications, report: a. Summary/descriptive statistics for each experimental group, with a measure of variability where applicable (e.g. mean and SD, or median and range). b. If applicable, the effect size with a confidence interval.	Results - Chondrocyte Viability