Bone & Joint Research

Supplementary Material

10.1302/2046-3758.108.BJR-2020-0041.R3

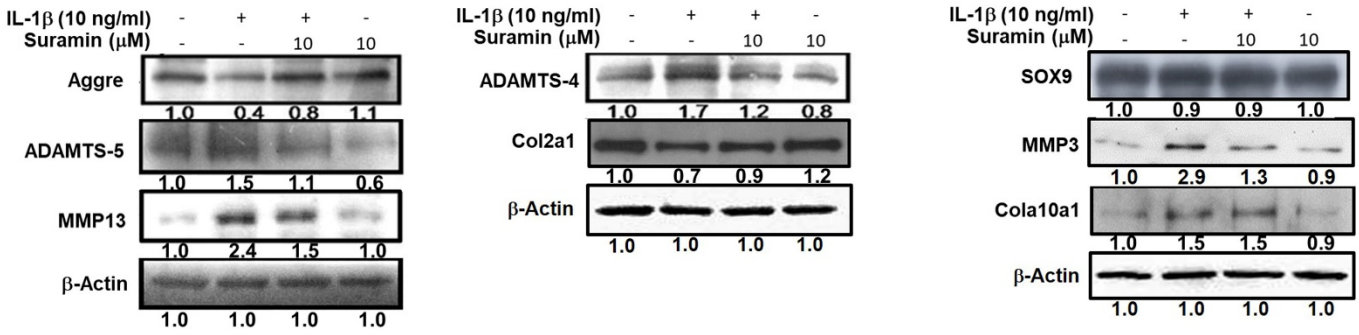

Fig a. Western blotting of protein levels of aggrecan, matrix metalloproteinase (MMP)-3, MMP-13, a disintegrin and metalloproteinase with thrombospondin motifs 4 (ADAMTS-4), and ADAMTS-5 in nucleus pulposus (NP) cells treated with suramin combined with or without interleukin beta (IL-1β) (10 ng/ml) for 24 hours (n = 3 to 5). Col2a1, collagen type II alpha 1; SOX9, SRY-Box Transcription Factor 9.

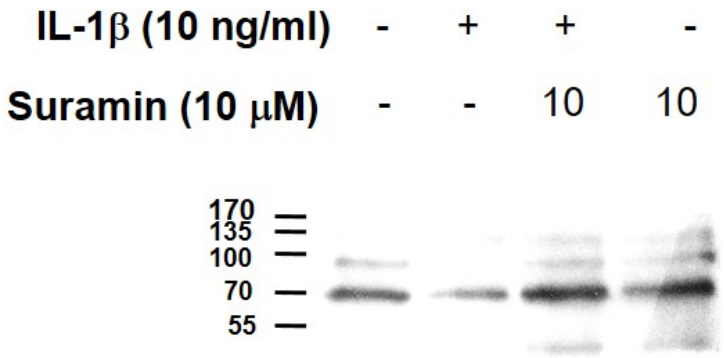

Fig b. Western blotting of protein levels of aggrecan in nucleus pulposus cells treated with suramin combined with or without interleukin 1 beta (IL-1β) (10 ng/ml) for 24 hours (n = 3 to 5).

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).	4 groups: a. control, il-1, il-1+suramin, suramin b. cage of animal
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.	a. total 10 sd rat, 2 sd in each group b. according previous study
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.	The same age and gender no exclusion N = 5
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.	the experiment isnot performed random Lab animals have independent cages for preventing confound
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).	Allocation: research assistant, members of animal center the conduct of the experiment: Dr. post Dr the outcome assessment : post dr. and principal investigation data analysis: principal investigator
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.	cell death: flow cytometry, Western blot molecular marker: col2, aggrecan Primary outcome measure: Safranin O, alcian blue staining, immunohistological staining (Col2, Col10, aggrecan)
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.	statistical methods: t-testsoftware: Image J not appliable
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.	Ten male of 8 weeks old Sprague-Dawley rats Livestock Research Institute, Council of Agriculture, GPI-CRC-PGD genotype GPI (Glucose phosphate isomerase) CRC（

			Calcium Release Channel) PGD (6-Phosphogluconate dehydrogenase)
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).	Ten male of 8 weeks old Sprague-Dawley rats (n = 10 spines, 20 discs) were euthanized and the total of 20 entire disc Briefly, the lumbar spine was approached through an abdominal midline incision, and further dissection was performed through the posterior abdominal wall was performed to directly visualize the L3 -L6 IVDs. The isolated disc show no signs of degeneration (grade 0). lumber spine ex vivo culture
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.	Il-1: Con: 1 +0.2 IL-1:1.5+0.2 Il-1+suramin: 1.1+0.1 Suramin: 1.15+0.2 IL-8: Con: 1 +0.1 IL-1:2.25+0.2 Il-1+suramin: 1.6+0.2 Suramin: 1.+0.1 TNFa: Con: 1 +0.1 IL-1:2.8+0.3 Il-1+suramin: 1.1+0.2 Suramin: 0.75+0.25 ADAMTS-4 Con: 1 +0.15 IL-1:1.7+0.35 Il-1+suramin: 0.9+0.2 Suramin: 1.1+0.2 CI: 78.08 to 85.92