SYSTEMATIC REVIEW PROTOCOL FOR ANIMAL INTERVENTION STUDIES

FORMAT BY SYRCLE (WWW.SYRCLE.NL)
VERSION 2.0 (DECEMBER 2014)

Item #	Section/Subsection/Item	Description	Check for approval
	A. General		
1.	Title of the review	Non-Steroidal Anti-inflammatory Drugs and bone healing in animal Models - Systematic Review and Meta-Analysis	
2.	Authors (names, affiliations, contributions)	Haider Al-Waeli: PhD Candidate, Faculty of Dentistry, McGill University, Canada. (writing manuscripts, reviewer of the first and full text screening, data extraction and meta-analysis) Ana Paula Reboucas – MS Department of Pediatric Dentistry and Orthodontics, Faculty of Dentistry, Federal University of Minas Gerais, Belo Horizonte, Brazil. (reviewer of the first and full text screening, data extraction). Martin Morris- Liaison Librarian, Schulich Library of Physical Sciences, McGill University, Canada. (search strategy, writing the methods and Prisma generation and optimizing search strategy) Belinda Nicolau – Associate Professor at Faculty of Dentistry, McGill University, Canada. (Supervisor professor for the project, editing manuscripts)	
3.	Other contributors (names, affiliations, contributions)	Alaa Mansour- PhD candidate, McGill University (second reviewer for the study quality extraction)	
4.	Contact person + e-mail address	Haider Al-Waeli+haider.al-waeli@mail.mcgill.ca	
5.	Funding sources/sponsors	None	
6.	Conflicts of interest	None	
7.	Date and location of protocol registration	N	
8.	Registration number (if applicable)	N.A.	
9.	Stage of review at time of registration	Full text screening	
	B. Objectives		
	Background		
10.	What is already known about this disease/model/intervention? Why is it important to do this review?	Nonsteroidal anti-inflammatory is a widely prescribed drug for pain relief and inflammation in bone healing cases. Although some studies had associate its use to inhibition of fracture healing and to delay union bone. The effect of anti-inflammatory drugs administration in animal studies for bone healing is controversial, as some	

		researches showed no effects using the drug. The aim of this systematic review and meta -analysis is to assess the outcomes correlated to nonsteroidal anti-inflammatory therapy and bone healing in animal studies.	
	Research question		
11.	Specify the disease/health problem of interest	Bone healing after bone fracture surgery	
12.	Specify the population/species studied	Animal Models	
13.	Specify the intervention/exposure	Nonsteroidal anti-inflammatory agents	
14.	Specify the control population	Use of placebo solution	
15.	Specify the outcome measures	Bone biomechanical (primary Outcome), Bone volume or area (Histology grade or Micro CT) (second outcome)	
16.	State your research question (based on items 11-15)	Does administration of NSAIDs after fracture bone surgery resulted in lower bone morphometric and/or the biomechanical outcome measurements in comparison to control (placebo) administration in rodents animal model?	
	C. Methods		
	Search and study identification		
17.	Identify literature databases to search (*e.g.* Pubmed, Embase, Web of science)	• PubMed • SCOPUS • EMBASE	
18.	Define electronic search strategies (*e.g.* use the step by step search guide[15] and animal search filters[20, 21])	When available, please add a supplementary file containing your search strategy: [Search Strategy Appendix]	
19.	Identify other sources for study identification	■ Reference lists of included studies ☐ Books ☐ Reference lists of relevant reviews ☐ Conference proceedings, namely: ☐ Contacting authors/ organisations, namely: ☐ Other, namely:	
20.	Define search strategy for these other sources		
	Study selection		
21.	Define screening phases (*e.g.* pre-screening based on title/abstract, full text screening, both)	- Initial pre-screening with selection of the relevant studies based on the key components of the review question on title/abstract - Full text screening of the relevant citations	
22.	Specify (a) the number of reviewers per screening phase and (b) how discrepancies will be resolved	- Pre-screening and full text screening will be performed by two reviewers independently - Discrepancies will be solved either by discussion or by a third reviewer (when no agreement is met by the two reviewers)	
	Define all inclusion and exclusion criteria based on:		
23.	Type of study (design)		

		Inclusion criteria: • Original articles • Experimental animal models • • In vivo (designed for bone healing process) Exclusion criteria: • Not an original study • In vitro studies • Clinical trials • Case reports • Review studies • •	
24.	Type of animals/population (*e.g.* age, gender, disease model)	Inclusion criteria: • Animal models • Bone fracture models after surgery Exclusion criteria: • Clinical trials •	
25.	Type of intervention (*e.g.* dosage, timing, frequency)	Inclusion criteria: • Nonsteroidal anti-inflammatory agents in any dose, duration, frequency and type. Exclusion criteria: Studies taking other drug intervention rather than nonsteroidal anti-inflammatory agents • Steroidal anti-inflammatory agents • Antibiotics • Combination of NSAIDs and other interventions	
26.	Outcome measures	Inclusion criteria: Papers reporting bone fracture healing • Bone biomechanical test/maximum force • Histological analysis/ Hu etal healing grade • Micro CT /bone volume or area Exclusion criteria: Studies assessing healing in other tissues rather than bone tissue	
27.	Language restrictions	•	
28.	Publication date restrictions	Inclusion criteria: • No date restriction Exclusion criteria: • N/A	
29.	Other	Inclusion criteria: • N/A Exclusion criteria: • N/A	
30.	Sort and prioritize your exclusion criteria per selection phase	Selection phase: Screening title/abstract and Full text 1. No bone fracture model 2. No use of nonsteroidal anti-inflammatory agents	

		3. No animal study 4. Assessment of healing in others tissues not bone 5. No primary study or review 6.	
	Study characteristics to be extracted (for assessment of external validity, reporting quality)		
31.	Study ID (*e.g.* authors, year)	- Authors, - Year, - Title, - Journal, - Language	
32.	Study design characteristics (*e.g.* experimental groups, number of animals)	number of groups number of animals per group (total and per test) Non selective COX NSAIDs group/s, Selective COX2 group/s, Control group/s number of excluded, reason for exclusion.	
33.	Animal model characteristics (*e.g.* species, gender, disease induction)	Rodent type, weight, age and sex, type of the bone fracture model.	
34.	Intervention characteristics (*e.g.* intervention, timing, duration)	Time of administration, duration, dose mg/kg/day, route of administration.	
35.	Outcome measures	Maximum force of torque of the mechanical bending (N.mm) Bone area Healing grade of histology examination Other bone healing outcomes (pain assessment, cytokines)	
36.	Other (*e.g.* drop-outs)	Reason for drop out of the study	
	Assessment risk of bias (internal validity) or study quality		
37.	Specify (a) the number of reviewers assessing the risk of bias/study quality in each study and (b) how discrepancies will be resolved	- Two independent reviewers - Discrepancies or disagreements will be resolved after discussion with a third reviewer	
38.	Define criteria to assess (a) the internal validity of included studies (*e.g.* selection, performance, detection and attrition bias) and/or (b) other study quality measures (*e.g.* reporting quality, power)	■ By use of SYRCLE's Risk of Bias tool[4] ☐ By use of SYRCLE's Risk of Bias tool, adapted as follows: ☐ By use of CAMARADES' study quality checklist, e.g [22] ☐ By use of CAMARADES' study quality checklist, adapted as follows: Select items that are related to the study design ☐ Other criteria, namely:	
	Collection of outcome data		
39.	For each outcome measure, define the type of data to be extracted (*e.g.* continuous/dichotomous, unit of measurement)	In general, the data will be continuous (generally measured in force for the maximum force of mechanical bending), Units of measurement tend to be N.mm For the Histological healing data will be score of grade, bone volume or area will be measured by mm^2, or $mm^{3.}$	
40.	Methods for data extraction/retrieval (*e.g.* first extraction from graphs using a digital screen ruler, then contacting authors)	1. Extract data from text/tables 3. Contact authors for missing data	

41.	Specify (a) the number of reviewers extracting data and (b) how discrepancies will be resolved	One reviewer (HA) will extract data. A second reviewer (AM) will check the extraction process.	
	Data analysis/synthesis		
42.	Specify (per outcome measure) how you are planning to combine/compare the data (*e.g.* descriptive summary, meta-analysis)	Maximum force measurements will be recorded from each study, sub group analysis will be done regarding type of NSAIDs, rodent model, and time. For grade of healing or bone volume and area will be recorded for meta analysis if not available then descriptive summary will be mentioned for every outcome.	
43.	Specify (per outcome measure) how it will be decided whether a meta-analysis will be performed	If at least 5 studies are found per outcome, data will be pooled for the meta-analysis, high heterogeneity will be investigated to check the refrain form the meta-analysis.	
	If a meta-analysis seems feasible/sensible, specify (for each outcome measure):		
44.	The effect measure to be used (*e.g.* mean difference, standardized mean difference, risk ratio, odds ratio)	We will use mean differences if studies use the same experimental test with the same scoring scale, but standardized mean difference if combining different scale score for the same measurement.	
45.	The statistical model of analysis (*e.g.* random or fixed effects model)	Random effects model	
46.	The statistical methods to assess heterogeneity (*e.g.* I^2, Q)	I^2	
47.	Which study characteristics will be examined as potential source of heterogeneity (subgroup analysis)	Type of NSAIDs, Timing, animal species, time of observation,	
48.	Any sensitivity analyses you propose to perform	We will perform sensitivity analyses to assess if our underlying assumptions are appropriate and our results are robust.	
49.	Other details meta-analysis (*e.g.* correction for multiple testing, correction for multiple use of control group)	We will perform a Holm-Bonferroni correction to correct for multiple testing. We will adjust the p value according to the number of the subgroup analysis. If within one study several doses of NSAIDs are compared to one control group, we will divide the number of control animals by the total number of comparisons made with this group in order to correct for repeated use of one control group.	
50.	The method for assessment of publication bias	Funnel plot (if at least 10 studies included in metaanalysis)	

Final approval by (names, affiliations): Date: