SYSTEMATIC REVIEW PROTOCOL FOR ANIMAL INTERVENTION STUDIES

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Item #	Section/Subsection/Item	Description	Check for approval
	A. General		
1.	Title of the review	Stem cells therapy for chronic temporal lobe epilepsy: a systematic review and meta-analysis of animal studies	
2.	Authors (names, affiliations, contributions)	- [1,6] Osama Abunar - [2,6] Muhammed Sinokrot - [3,6] Ahmed Magdy Soliman - [4,6] Ahmed Said Ali - [1,6] Mohammed Yasser Elsherbeny - [4,6] Abdelrahman abuzied eltonoby - [5] Adham Elkeryouni - [4,6] Ahmed Elgebaly - [2] Rana Mohamed Ali Zaki [1] Faculty of Medicine, Mansoura University, Mansoura - Egypt [2] Faculty of Medicine, Cairo University, Cairo - Egypt [3] Faculty of Medicine, Al Azhar University, Damietta - Egypt [4] Faculty of Medicine, Al Azhar University, Cairo - Egypt [5] Genetic Engineering and Biotechnology Research Institute, University of Sadat City, Menoufia – Egypt [6] Medical Research Group of Egypt	
3.	Other contributors (names, affiliations, contributions)	-	
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5.	Funding sources/sponsors	-	
6.	Conflicts of interest	-	
7.	Date and location of protocol registration	-	
8.	Registration number (if applicable)	-	
9.	Stage of review at time of registration	Title and abstract screening	
	B. Objectives		
	Background		
10.	What is already known about this disease/model/intervention? Why is it important to do this review?	Temporal lobe epilepsy (TLE) is a common type of epilepsy, which is characterized by neural cells loss in the hippocampus. Medical therapy is not effective in >30% of patients and cannot alleviate memory and mood disorders that are common in TLE patients. Other therapies including surgical resection, vagus nerve stimulation and ketogenic diet are either inadequate or have serious side effects. As TLE is very difficult to treat so many animal models were performed to mimic the histopathology and	

		clinical course in human in order to test new therapies before clinical application. Some animal studies showed that stem cell transplantation can suppress epileptogenesis and spontaneous recurrent seizures and also prevent memory and mood dysfunction in TLE models. Recently, many animal studies were performed to test stem cell efficacy in TLE models but they were heterogeneous in modeling of animals, type of cells and assessment of outcomes so it is important to gather all available evidence in one paper to compare methodology and outcomes of each one qualitatively by systematic review and quantitatively by meta-analysis. This review will be very important for pre-clinical researchers to design their future trials and for clinical researchers to determine if the available evidence is enough to begin human trials.	
	Research question		
11.	Specify the disease/health problem of interest	Chronic temporal lobe epilepsy	
12.	Specify the population/species studied	All animal species	
13.	Specify the intervention/exposure	Stem cells	
14.	Specify the control population	Epileptic animal without intervention or with any sham intervention other than stem cells	
15.	Specify the outcome measures	- Functional outcomes as: 1. Seizures frequency, duration and amplitude 2. Memory and learning outcomes - Structural outcomes as: 1. Cells migration to the site of the injury. 2. Cells Differentiation to functional cells. 3. Cells integration with the surrounding cells	
16.	State your research question (based on items 11-15)	What is the effect of stem cell therapy on functional and structural outcomes in animal models for chronic temporal lobe epilepsy?	
	C. Methods		
	Search and study identification		
17.	Identify literature databases to search (*e.g.* Pubmed, Embase, Web of science)	X PubMed X Web of Science X SCOPUS ☐EMBASE ☐Other, namely: ☐Specific journal(s), namely:	
18.	Define electronic search strategies (*e.g.* use the step by step search guide[15] and animal search filters[20,]	We will use the following keywords (("Stem Cells"[Mesh]) AND ("Epilepsy"[Mesh]) OR "Epilepsy, Temporal Lobe"[Mesh]).	

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)	- Title and abstract screening - Full text screening	
22.	Specify (a) the number of reviewers per screening phase and (b) how discrepancies will be resolved	a. Each article will be screened be 2 independent observers. b. Discrepancies will be resolved by discussion but if consensus cannot be reached a 3rd person will take the final decision.	
	Define all inclusion and exclusion criteria based on:		
23.	Type of study (design)	Inclusion criteria: Primary animal studies Exclusion criteria: Reviews, Conference and meeting abstracts etc.	
24.	Type of animals/population (*e.g.* age, gender, disease model)	Inclusion criteria: - All animal species - Chronic temporal lobe epilepsy models Exclusion criteria: - Studies using humans or in vitro studies - Generalized epileptic models - Global brain ischemia/hypoxia models - Acute seizure models	
25.	Type of intervention (*e.g.* dosage, timing, frequency)	Inclusion criteria: Any stem cell intervention	
26.	Outcome measures	Inclusion criteria: 1. Seizures frequency, duration and amplitude 2. Memory and learning outcomes 3. Cells migration to the site of the injury. 4. Cells Differentiation to functional cells. 5. Cells integration with the surrounding cells Exclusion criteria:	
27.	Language restrictions	Inclusion criteria: English articles Exclusion criteria:	
28.	Publication date restrictions	Inclusion criteria: Any time Exclusion criteria:	
29.	Other	Inclusion criteria: Exclusion criteria:	
30.	Sort and prioritize your exclusion criteria per selection phase	Title and abstract screening: 1. Human trials 2. Reviews 3. Non therapeutic trials 4. Conference and meeting abstracts	

		5. In vitro trials 6. Irrelevant models Additional criteria in Full text screening: 1. Non-English articles 2. Cell free extracts (extracts that do not contain stem cells)	
	Study characteristics to be extracted (for assessment of external validity, reporting quality)		
31.	Study ID (*e.g.* authors, year)	First author and year	
32.	Study design characteristics (*e.g.* experimental groups, number of animals)	Number of animals, experimental groups (including type of control group)	
33.	Animal model characteristics (*e.g.* species, gender, disease induction)	- Type of animals (species, strain, sex, age) - Animal model (induction method epilepsy) -	
34.	Intervention characteristics (*e.g.* intervention, timing, duration)	- Intervention (type of stem cells, donor species) - Route - Dose - Frequency - Timing relative to induction of epilepsy	
35.	Outcome measures	- Functional outcomes	
36.	Other (*e.g.* drop-outs)	- Euthanasia	
	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	a. Two reviewers for each study b. differences will be resolved by discussion	
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 ☐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: X Other criteria, namely: National Research Council Institute for Laboratory Animal Research 2011. We picked the relevant criteria from previously published tool: 1. Random allocation of treatment 2. Blinding 3. Animal details 4. Attrition of animals 5. Use of a control group 6. Description of food and feeding methods 7. Description of water source, deliver methods, treatment 8. Reporting of housing/husbandry conditions 9. Description of environmental parameters 10. Description of anesthetics, analgesics, and other substances	

		11. Description of methods of sampling 12. Description of method of euthanasia References : 1. https://www.nap.edu/catalog/13241/guidance-for-the-description-of-animal-research-in-scientific-publications 2. https://www.ncbi.nlm.nih.gov/pubmed/23771496	
	Collection of outcome data		
39.	For each outcome measure, define the type of data to be extracted (*e.g.* continuous/dichotomous, unit of measurement)	- Continuous, dichotomous, and time-to-event data will be extracted depending on the extracted outcomes.	
40.	Methods for data extraction/retrieval (*e.g.* first extraction from graphs using a digital screen ruler, then contacting authors)	- Data will be extracted from tables or paragraphs if mentioned explicitly. Data in graph will be extracted using Plot digitizer. In case of missing data, we will contact the corresponding authors of relevant papers.	
41.	Specify (a) the number of reviewers extracting data and (b) how discrepancies will be resolved	a. Two reviewers for each study b. differences will be resolved by discussion	
	Data analysis/synthesis		
42.	Specify (per outcome measure) how you are planning to combine/compare the data (*e.g.* descriptive summary, meta-analysis)	- Continuous and dichotomous data will be compared by using pair-wise meta-analysis method using Review Manager 5.3 for Windows.	
43.	Specify (per outcome measure) how it will be decided whether a meta-analysis will be performed	- We will perform meta-analysis in each outcome that was reported in at least two trials and measured in similar method.	
	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)	- Continuous data will be pooled as mean difference (MD) or standardized mean difference (SMD) in a meta-analysis model while dichotomous data will be pooled as relative risk (RR) in a random-effect model using the Mantel–Haenszel (M–H) method. We will use Review Manager 5.3 for Windows.	
45.	The statistical model of analysis (*e.g.* random or fixed effects model)	- The random effect model will be used	
46.	The statistical methods to assess heterogeneity (*e.g.* I^2, Q)	- Heterogeneity will be assessed by visual inspection of the forest plots and measured by I-square and Chi-square tests. The Chi-square test measures the existence of a significant heterogeneity while the I-square quantifies the magnitude of heterogeneity in the effect size.	
47.	Which study characteristics will be examined as potential source of heterogeneity (subgroup analysis)	- Suggestions: type of stem cells, animal species, epilepsy induction method	
48.	Any sensitivity analyses you propose to perform	- Not planned	
49.	Other details meta-analysis (*e.g.* correction for multiple testing,	-	

	correction for multiple use of control group)		
50.	The method for assessment of publication bias	- We will use funnel plot tests for detecting publication bias	
Final approval by (names, affiliations):		- Osama Abunar - Muhammed Sinokrot - Ahmed Magdy Soliman - Ahmed Said Ali - Mohammed Yasser Elsherbeny - Abdelrahman abuzied eltonoby - Adham Elkeryouni - Ahmed Elgebaly - Rana Mohamed Ali Zaki	Date: 15/5/2017