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Role of histone deacetylases and sirtuins in the ischaemic stroke: a protocol for a systematic review and meta-analysis of animal studies

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ABSTRACT

Background Stroke is a major cause of global mortality and disability. Currently, the treatment of acute ischaemic stroke through reperfusion has posed several challenges, raising the need for complementary options to protect the ischaemic penumbra. Recent investigations have indicated that certain epigenetic factors, specifically, histone deacetylases (HDACs) and sirtuins, can be promising for ischaemic stroke therapy, with recent studies suggesting that inhibitors of HDACs or sirtuins may provide neuronal protection after ischaemic stroke. However, the impact of specific HDAC/sirtuin isoforms on the survival of neuronal cells following stroke is still uncertain. This study aims to provide a comprehensive overview of the function of HDACs and their modulators in the treatment of acute ischaemic stroke.

Methods This systematic review and meta-analysis will encompass animal intervention studies that explore the efficacy of modulation of HDACs and sirtuins in the acute phase of ischaemic stroke. The review will be reported according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses guidelines. Electronic searches will be conducted in PubMed, Web of Science and Scopus, with subsequent screening by independent reviewers based on the established eligibility criteria. Methodological quality will be evaluated using the SYRCLE risk of bias tool. The primary outcomes will be infarct volume and functional response, with the secondary outcomes established a priori. Data pertaining to infarct volume will be used for random-effects meta-analysis. Additionally, a descriptive summary will be conducted for the functional response and secondary outcomes.

Discussion No systematic review and meta-analysis on the treatment of ischaemic stroke through HDAC modulation has been conducted to date. A comprehensive analysis of the available literature on the relevant preclinical investigations can yield invaluable insights in discerning the most effective trials and in further standardisation of preclinical studies.

Systematic review registration This systematic review has been recorded in the International Prospective Register of Systematic Reviews (PROSPERO), with the assigned reference number: CRD42023381420

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ Application of thrombolysis as the main therapeutic strategy for acute ischaemic stroke faces several challenges, rendering ischaemic stroke as one of the leading causes of global mortality and long-term disability.

WHAT THIS STUDY ADDS

⇒ Providing novel complementary neuroprotective treatments targeting histone deacetylases (HDACs) and sirtuins can save the penumbra tissue and reduce the disease load.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ This protocol aims to design a systematic review and meta-analysis that compares the efficacy of the interventions targeting each subtype of the HDAC family in the treatment of the preclinical acute ischaemic stroke models.

BACKGROUND

Stroke represents the second leading cause of global mortality and the predominant cause of long-term disability.[1] The ischaemic stroke is the major subtype of stroke[2] caused by an abrupt decrease in brain tissue perfusion due to cerebral artery occlusion which causes multiple pathological events leading to tissue damage and death.[3]

Current therapeutic strategies for ischaemic stroke include thrombolytic therapy administrated within the critical 4.5 hours time window following the onset of ischaemia.[4] Nonetheless, only a limited number of individuals suffering from acute ischaemic stroke receive treatment with tissue plasminogen activators, due to the limited time window. Consequently, there is an increasing need to provide alternative or complementary options for the management of these patients.[5] Within this crucial time window, it is possible to provide neuroprotection

and alleviate neurotoxicity, specifically in the tissue surrounding the infarction core, known as the ischaemic penumbra.[6] Given the multifactorial nature of ischaemic brain tissue pathology,[7] previous attempts at clinical trials have been largely unsuccessful due to the interference of these treatments with vital physiological processes, as well as targeting pathological events.[8 9] Therefore, recognising these specific pathological and neuroprotective elements of the neurovascular unit can provide novel therapeutic approaches for ischaemic stroke.[10]

Epigenetic alterations pertain to inheritable modifications in the expression patterns of genes, devoid of altering the fundamental DNA sequences.[11] Recent studies have shown that epigenetic elements can influence multiple pathological events in ischaemic stroke simultaneously, making them a promising candidate for acute ischaemic stroke therapy.[12] Histone deacetylases (HDACs) pertain to a group of epigenetic molecules which regulate the acetylation of nuclear histones, and thereby chromatin remodelling and gene expression as a result of the tissue environmental changes. HDACs can be categorised into four distinct classes, namely class I, II and IV, which are dependent on Zn^{2+} ions, whereas class III, more commonly known as sirtuins, rely on NAD^{+} ions.[13]

Recent studies have shown that HDAC or sirtuin inhibitors can provide neuroprotection following ischaemic stroke, but the precise involvement of particular HDAC/sirtuin isoforms in the viability or demise of cerebral tissue subsequent to a stroke remains insufficiently understood.[14 15] The current clinical trials of ischaemic stroke treatment based on HDAC modification are predominantly carried out with non-selective HDAC inhibitors.[16] Moreover, several studies indicate that different HDAC isoforms may play distinct roles in cell survival in response to ischaemic injury.[17 18] On the contrary, non-specific HDAC inhibitors may affect the different cell types and hence, increase the risk of unexpected toxicity and side effects.[19] Consequently, investigating more specific HDAC modulators might be helpful in diminishing unwanted side effects and also increasing the likelihood of success in clinical trials in the future.[9 20]

To date, no systematic review and meta-analysis on the treatment of ischaemic stroke with HDAC modulators has been conducted. This study aims to compare the efficacy of modulation of each HDAC family subtype with one another and with the non-specific inhibitors in the treatment of ischaemic stroke. Through this assessment, we might be able to distinguish the more effective HDAC modulation trials in preclinical studies, so that future trials might be one step closer to achieving the desired clinical outcomes. In addition, the evaluation of the methodological quality of the preclinical studies will be conducted through quality appraisal of the published papers, accompanied by subgroup analyses aimed at identifying potential sources of heterogeneity among studies. These measures will be implemented with the purpose of enhancing the standardisation and methodological quality of preclinical trials focused on ischaemic stroke.

Research questions

1. Can administration of HDAC or sirtuin inhibitors or agonists during the acute phase following ischaemic stroke in rodents improve functional outcome, reduce infarct volume, alleviate neurotoxicity or provide neuroprotection in the brain tissue?
2. How does the modification of specific HDAC or sirtuin isoforms affect survival or death of brain neurovascular tissue following acute ischaemic stroke in rodents?

Objectives

The primary aim of this study is to evaluate whether the administration of HDAC or sirtuin agonists or inhibitors during the acute phase—the first 2 weeks—following an ischaemic stroke in rodents can improve the functional outcome, reduce infarct volume, alleviate neurotoxicity or provide neuroprotection in the brain tissue. By conducting a systematic review on preclinical studies, we aim to investigate the potential of HDAC/sirtuin modulators in enhancing stroke outcomes. Additionally, the effectiveness of each HDAC/sirtuin subtype modulation on acute ischaemic stroke treatment will be evaluated and compared with each other.

METHODS

Protocol and registration

A systematic review and meta-analysis on 'Role of HDACs and sirtuins in the ischaemic stroke' will be conducted. A group of preclinical research scientists, specialists in the specific field and data management experts have formulated the systematic review protocol. It was designed in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses Protocols (PRISMA-P)[21] (see online supplemental material). This systematic review has been officially recorded in the International Prospective Register of Systematic Reviews (PROSPERO), with the assigned reference number: CRD42023381420.

Types of studies

This review will encompass animal intervention studies that assess the role of HDAC/sirtuin activators or inhibitors within the acute phase following ischaemic stroke in the brain. All studies regarding the aforementioned subject matter will be included. However, human and in vitro studies, as well as review studies, retracted articles, commentaries, editorials, book chapters and meeting abstracts will be excluded. We will exclude articles with no access to their full-text versions.

Types of animal populations

Eligible animal models for our study include mature, young and healthy rodents (rats/mice) with experimental models of acute brain ischaemic stroke (Middle Cerebral Artery Occlusion (MCAO), Photothrombotic Stroke (PTS), endothelin-1, etc). Hence, chronic models of cerebral ischaemia will not be included. Besides, studies that have not included any animal in their population (eg, only in vitro models, only human population,

etc) will be excluded. Other exclusion criteria will be as follows:

Animals with ageing, prior disease or preconditioning will not be included. This decision is based on the understanding that the aforementioned factors prior to the induction of ischaemia may lead to alterations in the pathophysiology of the neurovascular unit,[22] thereby introducing unwanted confounding variables and amplifying the heterogeneity of the study.

Other exclusion criteria pertaining to the study population involve exclusion of other-than-ischaemic models of stroke (haemorrhagic, etc), other-than-brain ischaemic injury models (spinal cord ischaemic injury, vascular, etc) and global models of cerebral ischaemia (eg, the asphyxial cardiac arrest model). This determination is also guided by the objective of ensuring the included studies are as consistent as possible, thereby facilitating more precise comparisons of the impact of various interventions on ischaemic stroke outcomes.

There will be no limitations regarding sex, strain, or species of rodents. However, sex, strain or species-dependent response to HDAC modulation will be summarised during the data extraction phase.

Types of interventions

The existing literature about HDACs and ischaemic stroke contains cellular pathways that include at least one member of the HDAC family (including sirtuins). Our included studies will contain interventions targeting (activating or inhibiting) at least one component of these pathways, applied in the acute phase (the first 2 weeks) after the ischaemic onset in vivo, in two groups as follows:

1. The intervention targets other-than-HDAC components of these pathways: In this occasion, there should be a negative control group (with a knockout of HDAC enzyme) to ensure that the neuroprotective effect of these interventions is dependent on the associated HDAC.
2. The intervention targets a member of the HDAC family directly: These types of interventions will be included, whether or not they have a negative control group.

The interventions mentioned, which target HDACs directly, can be further categorised into two groups. One group consists of direct but non-specific interventions, while the other comprises direct and specific modulators, the latter of which is currently being investigated further in preclinical stroke trials. It is important to highlight that both of these groups of direct HDAC modulators will be examined, and their impact on ischaemic stroke outcomes will be assessed, in order to determine whether a more focused approach to HDAC modulation can be beneficial, while also identifying the most effective trials.

Additional eligibility criteria regarding the intervention include:

1. The intervention should be a chemical (eg, a pharmacological agent or chemicals extracted from stem cells). Other types of interventions (microRNA, lncRNA, viral vectors, etc) will be excluded. This determination is also motivated by the objective of reducing heterogeneity and facilitating a more homogeneous comparison of the impact of interventions on outcomes related to ischaemic stroke.
2. Studies that provide neuroprotection through interventions that are too general—such as preconditioning, postconditioning, stem cell transplantation, enriched environment, exercise, dietary modifications and neurosurgical interventions—will be excluded; since they can provide neuroprotection independent of HDAC/sirtuin and hence can be a source of multiple possible confounding factors.
3. Studies that have no direct manipulation of HDAC/sirtuins in any of their intervention or control groups will be excluded.
4. Interventions that are initiated before the onset of ischaemia, whether continued after the stroke (peritreatment schema) or not (pretreatment schema), will be excluded.
5. Interventions that are initiated after the acute phase (the first 2 weeks) of ischaemic onset will be excluded. Nonetheless, inclusions will be made for interventions that have been implemented within the acute phase and continued thereafter.
6. There are no limitations on dosage and frequency of interventions. Nevertheless, a more in-depth examination into the impact of dosage and frequency of interventions on the outcomes of ischaemic stroke will be conducted in the data extraction phase.

Types of control comparisons

The populations treated with HDAC/sirtuin activator or inhibitor will be compared with groups receiving no treatment, vehicle-treated or sham groups. We have applied no specific exclusion criteria for control groups.

Types of outcome measures

Primary outcomes

The primary outcomes of this study include infarct volume and functional response after stroke, since the aforementioned are the key outcomes of preclinical ischaemic stroke trials according to the Stroke Therapy Academic Industry Roundtable (STAIR) recommendations.[23] Furthermore, according to the updated STAIR recommendations, in order to demonstrate a sustained therapeutic benefit following drug administration, we will extract outcomes data regarding the histological and behavioural endpoints measured at least 2–3 weeks or longer after ischaemia onset, as well as outcomes measured within the acute phase.[24]

Included studies should contain at least one of the stated primary outcomes (infarct volume, functional response). The study reports that fail to include results for neither infarct volume nor functional outcomes will be excluded.

Secondary outcomes

Defined a priori secondary outcomes of this study include brain water content, alterations in the cell cycle,

differentiation and apoptosis, changes in blood–brain barrier integrity, angiogenesis, pathological responses of glial cells, changes in neuroinflammation, energy balance, etc. Additional secondary outcomes will be specified and reported in the main project during the data extraction phase.

Electronic search strategies for identification of studies

The researchers collaborated with a medical information specialist to develop electronic search strategies. To conduct the search, databases such as PubMed, Web of Science and Scopus will be used, employing Medical Subject Headings, as well as relevant text words, from inception until 31 January 2023. Furthermore, the references of the included full-text studies will be examined. Additionally, results from Google Scholar search will also be screened to ensure sufficient coverage of the literature. Finally, prior to data extraction, we will provide an updated search in the databases.

The search queries were generated for these databases, as exemplified in online supplemental material. The structure and topic descriptors were modified based on the specific database being explored. No limitations pertaining to the date of publication or type of publication will be imposed; nevertheless, English will serve as a language filter.

Data collection and analysis

The importation of citations from literature searches and the elimination of duplicates will be accomplished using EndNote (V.X8.1). Then, the data will be exported to Rayyan AI for first-phase (title and abstract) screening. The second-phase screening of the full-text articles, along with data extraction will be conducted manually. The extracted data will be imported into Microsoft Excel. Afterwards, the comprehensive meta-analysis (CMA) software V.3.7 will be employed for all statistical analyses.

Study selection and data extraction

Three independent reviews (AMa, EA and MD) will screen titles and abstracts according to the eligibility criteria stated above. Disagreements will be resolved through discussion and employing the liberal acceleration policy: The agreement of a single reviewer is sufficient to advance a publication to the next phase of screening, while the unanimous agreement of all reviewers is required to reject a publication. Finally, in the event that a consensus cannot be attained, a fourth reviewer (HM) will be consulted.

Then three independent review authors (AMa, SY and AMo) will screen the full-text of articles, blinded to each other's decisions, according to the predefined criteria. Disagreements among the review authors will be settled by means of discourse, and in the event that a unanimous agreement cannot be reached by the reviewers, a fourth reviewer (HM) will be sought after for consultation. Additionally, articles with no access to their full text will be excluded. Moreover, to enhance elucidation, the PRISMA 2020 flow diagram depicting the systematic study selection

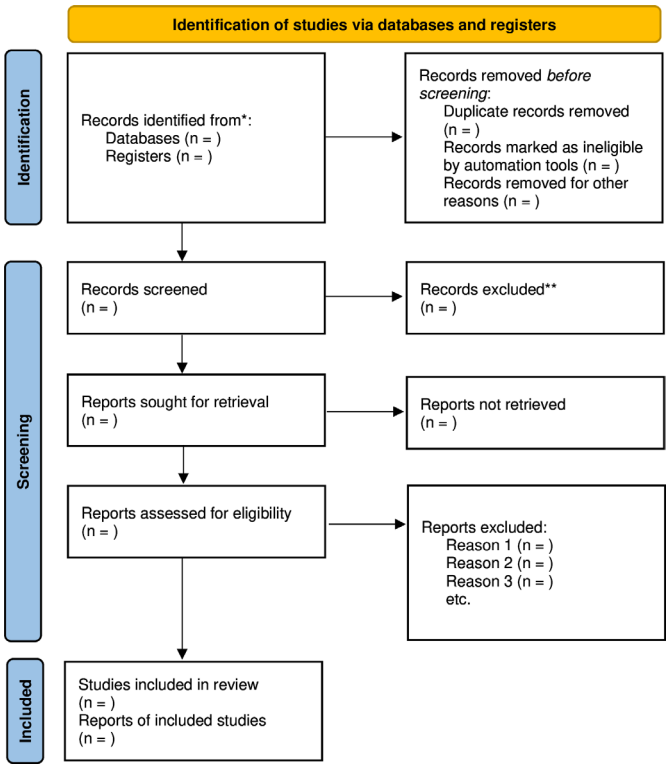

Figure 1 - PRISMA 2020 flow diagram for new systematic reviews study selection process

process will be incorporated into the manuscript of the main project, with its initial rendition available for reference in figure 1.[25]

In the data extraction phase, the review authors will be divided into two groups, as follows:

1. AMa, AGT and EA will independently export infarct volume data from the studies that contain interventions targeting HDAC/sirtuin directly. These data include mean, SD of infarct volume and the number of participants in each of the treatment and control groups. Data will be obtained from the text, tables and figures. The extraction of data from the graphs will be performed by using the digital measuring instrument WebPlotdigitizer. The extracted data will be reported in Microsoft Excel. Any disagreements between the authors will be solved through discussion. Afterwards, to execute a meta-analysis, the extracted data will be transferred to CMA software V.3.7. The results from the meta-analysis will be peer-reviewed and consulted with KM. Studies containing interventions that targeted other-than-HDAC components (see the Type of interventions section) will not be used for meta-analysis.
2. AMo, SY and MD will independently export data regarding other outcomes (functional response and secondary outcomes) of all included studies. Information will be collected from the text, tables and figures. Any disagreements will be resolved through consensus or with the assistance of a fourth or fifth member of the review team (HM or AMa).

Forms for extracting data will be prepared in advance, as follows:

1. Study characteristics (author, year, research setting and funding of studies)
2. Study population (animal strain, age, weight, sex and model of ischaemic stroke)
3. Study intervention (name, type and source of treatment; dose, time and route of administration and effect of the intervention on HDAC/sirtuin)
4. Study outcomes (functional outcome: time, unit and format of measurement, and changes detected; and the secondary outcomes with the changes detected). In addition, infarct volume (time, unit and format of measurement, and changes detected) of the studies that targeted other-than-HDAC components will be extracted.

These extracted data will be reported in Microsoft Excel. In the event of data that are not present, the authors will be contacted via e-mail twice; if there is no response following the second e-mail, the authors will be considered unattainable.

Assessment of risk of bias in included studies

In order to evaluate the methodological quality of the included studies, we will use the SYRCLE (Systematic Review Center for Laboratory Animal Experimentation) risk of bias tool.[26] Three independent review authors (AMo, SY and MD) will evaluate the risk of bias in each study, categorising it as low, unclear or high based on the factors including selection bias, performance bias, detection bias, attrition bias, reporting bias, other sources of bias (in this domain, we will include more reporting items related to our studies).

Any disagreements between individual judgements will be resolved through discussion and consultation with HM and AMa.

Furthermore, to examine the impact of risk of bias on data extraction, we will perform sensitivity analysis by removing high-risk of bias studies in the descriptive summary of other outcomes and the meta-analysis. However, if removing high-risk studies causes the loss of a large amount of data, we will include all studies and state this as a limitation of our study in the main project.

Data analysis

We will conduct a random-effects meta-analysis to assess the effectiveness of HDAC/sirtuin moderators on standardised mean differences of infarct volume with 95% CIs. Furthermore, if feasible, subgroup analyses will be performed on the studies to compare the efficacy of trials on each subtype of the HDAC family. In addition, other possible heterogeneity factors, such as the type of rodents (mice/rat), the model of ischaemic stroke, the route of drug administration, the measure of infarction and the method of infarction measurement will be also analysed as subgroups. Moreover, if possible, we will conduct a meta-regression analysis on the time of drug onset and frequency of drug administration to see whether there's an optimal timepoint and frequency of administration for each enzyme subtype moderator. For the purpose of this assessment, it is necessary to have a minimum of two investigations for each category.

If there are multiple timepoints of infarct volume measurement, all will be included. Additionally, when we encounter multi-arm trials in a study, we will include all the arms of trials. On this occasion, to prevent unit-of-analysis error, we will split the control arms of shared groups, as explained by Rücker *et al*.[27]

The study's heterogeneity in results will be assessed through the Cochrane Q-test and quantified using I^2 values. Furthermore, we will evaluate publication bias in the included studies by examining funnel plots and conducting Egger's regression test.

A descriptive summary will be performed for all other outcomes, including functional response and secondary outcomes, as outlined in the Study selection and data extraction section.

DISCUSSION

Ischaemic stroke stands as a leading cause of global mortality and disability.[1] Currently, thrombolytic therapy constitutes the primary approved treatment for ischaemic stroke patients. Nevertheless, this strategy is encumbered by several limitations, including a limited time window of only 4.5 hours after the ischaemic onset for its application, perceived inefficacy in certain high-risk patients and some major complications such as intracranial bleeding leading to haemorrhagic stroke.[28] Therefore, it seems crucial to develop alternative approaches to thrombolysis, provide neuroprotection and reduce disease mortality and morbidity. In this study, the effect of HDAC modulators on stroke outcomes will be examined.

As mentioned earlier, the ongoing clinical trials for treating ischaemic stroke by modifying HDAC primarily involve the use of non-selective HDAC inhibitors.[16] Furthermore, the therapeutic effects are frequently assessed in relation to the alterations in the total level of histone deacetylation, rather than examining the activity of each specific HDAC subtype individually.[14 15] Therefore, it is essential to explore the precise functions of each HDAC isoform in the context of ischaemic stroke.[9] As a result, recent studies have shifted focus on evaluating the effectiveness of specific HDAC inhibitors in the management of acute ischaemic stroke on preclinical animal models.[29]

Strengths and limitations

Similar to other reviews, our study might encompass various strengths and shortcomings. In accordance with the updated STAIR recommendations,[24] we have established our eligibility criteria and predefined target outcomes to ensure robust scientific inquiry. For instance, our criteria for timing of intervention—the inclusion of interventions administered within the acute phase following ischaemic stroke—can be substantiated from a translational standpoint. This is due to the fact

that the therapeutic trials are predominantly oriented towards the acute phase, rather than before the beginning of ischaemic stroke. Hence, our approach aligns with the clinical authenticity of managing patients who had a stroke.

On the other hand, to improve the accuracy of our comparisons and reduce the impact of confounding variables, we have selectively restricted our inclusion criteria regarding the study population and certain interventions, which may limit the scope of our analysis and pose challenges for translational research.[24 30] Consequently, future studies should consider alternative interventions and their impacts on diverse stroke models to yield a more comprehensive understanding of their therapeutic effectiveness.

An important impediment that our review encounters pertains to the meta-analysis of data regarding the neurological response. Initially, our aim was to perform a meta-analysis on both the primary outcomes; however, following preliminary searches, we noted a multitude of methodological and reporting variations, particularly in the context of diverse scales and measures employed to assess the neurological response. This diversity poses a significant challenge in combining the results across the studies and conducting a comprehensive meta-analysis. Therefore, we have decided to present the relevant data concerning the neurological response in the form of descriptive summary tables.

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Contributors Conceptualisation: AMa, AMo and HM. Search strategy and design: AMa and HM. Title and abstract screening: AMa, MD and EA. Full-text screening: AMa, SY and AMo. Quality appraisal: SY, AMo and MD. Descriptive data extraction: SY, AMo and MD. Data curation: AMa. Meta-analysis: AMa, AGT, EA and KM. Supervision: AMa, HM and KM. Validation: HM and KM. Visualisation: AMa. Writing—original draft: AMa and AGT. Writing—review and editing: AMa, AGT and HM. The authors have read and approved the final manuscript.

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