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Preclinical Use of Group I Metabotropic Glutamate Receptors (Group I mGluRs) for Ischemic Stroke: Systematic Review and Meta-Analysis

Md. Saidur Rahman[1*], Md. Khalid Hasan[1], Md. Sajal Khan[1]

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ABSTRACT

In ischemic stroke, metabotropic glutamate receptors (mGluRs) have a complex role. Results from studies using both agonists and antagonists of these receptors have been inconsistent. This systematic review and meta-analysis assesses multiple preclinical investigations on group I mGluR agonists and antagonists. Effect sizes for various outcomes, study quality scores, bias risk, and interactions with clinical factors related to functional and histological outcomes were analyzed based on relevant papers. Twelve papers covering 41 treatment groups of 26 interventions from 1999 to 2023 were included. Twelve studies (25 treatment arms) reported structural outcomes, while eight studies (16 treatment arms) reported functional outcomes, with a median quality score of 4 out of 10. Expected results showed larger effect sizes. The mean effect sizes for neurological score and infarct volume improved by -0.75 SMD and -1.37 SMD, respectively, after subgroup adjustments and sensitivity analysis. Effect sizes for neuroprotection, neuronal loss, brain temperature, and NMDA receptor activity with antagonist treatment were 1.73 SMD, -1.35 SMD, -0.05 SMD, and -0.41 SMD, respectively. While antagonists significantly ($P < 0.05$) improved both structural and functional outcomes, agonists only improved structural results. However, SYRCLE's risk-of-bias tool for animal studies identified potential bias. Additionally, clinical variables such as dosage and administration mode of agonist or antagonist medications influenced the effect magnitude. Despite the promising results in preclinical studies, several drugs have failed to prevent ischemic stroke in human clinical trials. Future research using animal models of stroke is recommended to improve study quality, validity, and reduce the risk of bias.

INTRODUCTION

Stroke is one of the leading causes of death and disability in developed nations (Macarena *et al.,* 2017). A damaged or clogged blood vessel stops the flow of blood to a portion of the brain, causing dysfunction in the affected area. There isn't a treatment that works well enough to enhance clinical recovery following a stroke at the moment. Although it may raise the risk of cerebral hemorrhage, tissue plasminogen activator (tPA) is important in the early stages of ischemia (Ning *et al.,* 2010). Restorative therapy, such as group I metabotropic glutamate receptors (mGluRs) blockers and/or activators, may help lessen neurological impairment. In pathological situations, group I mGluRs, which comprise two subtypes, mGluR1 and mGluR5, are intriguing targets for treatment in neurodegeneration and acute and chronic traumas. It has been demonstrated that mGluR1 antagonists reduce neuronal mortality following brain trauma in vivo and mechanical injury in vitro. Group I mGluRs antagonists have also been found to have a neuroprotective effect in ischemic stroke (Faden *et al.,* 2001; Kinga *et al.,* 2007). Although some publications noted that both agonists and antagonists of the mGluR5 receptor are neuroprotective, no positive effects of the mGluR5 agonist were observed in a model of endothelin-1-induced localized ischemia in rats (Riek-Burchardt *et al.,* 2007; Bao *et al.,* 2001). In addition to being linked to new neurons, mGluR5 antagonists are also anticipated to alter the milieu of sick tissue (Norio *et al.,* 2012). Because agonists of these receptors have been shown to either increase or decrease neuronal cell death, and antagonists of these receptors are consistently neuroprotective, the function of group I mGluRs in animal models of ischemia is still debatable (Nicoletti *et al.,* 1999). Both endogenous and exogenous sources may offer viable treatments for ischemic stroke. However, it has been shown that endogenous neuro-regeneration is not enough to restore damaged brain tissue (Lee *et al.,* 2011; Minger *et al.,* 2007). Group I mGluRs agonist and/or antagonist exogenous therapy for ischemic stroke has been successfully implemented, according to several studies (Bao *et al.,* 2001; Hailong *et al.,* 2013; Dorota *et al.,* 2006; Elena *et al.,* 2002). The findings showed that group I mGluRs agonists and/or antagonists may considerably aid in the restoration of brain tissue and neuro-functional outcomes. It is anticipated that the agonist and antagonist of group I mGluRs would mediate homeostasis and tissue healing by controlling the release of brain trophic factors or by interacting with immune cells that live in and infiltrate the central nervous system.

A statistical summary of the findings is called a meta-analysis (Nordmann *et al.,* 2012; O'Rourke, 2007). The estimation of the extent of effect is improved and ambiguity is resolved by combining the data of several studies using a statistical method rather than relying

[1] Department of Anatomy and Histology, Faculty of Animal Science and Veterinary Medicine, Patuakhali Science and Technology University, Bangladesh
[*] Corresponding author's e-mail: saidur@pstu.ac.bd

just on individual studies (Walker *et al.,* 2008). A global estimate of the effectiveness of medication treatment in preclinical stroke models is limited, but there are several research and interventional techniques for the use of agonists and antagonists of group I mGluRs in experimental stroke. Thus, the current study's goal was to assess group I mGluRs' potential as a treatment for ischemic stroke in preclinical research.

MATERIALS AND METHODS

Search Strategy

The publications were looked up using the Science Citation Index, ISI Web of Science, and PubMed for animal models of ischemic stroke until December 2023. The literature review was conducted using the PRISMA flow diagram and guidelines (Figure 1). The supporting information (Table 1) describes the search strategy. The featured publications were authored exclusively in English. Studies that used hemorrhagic stroke models instead of ischemic ones, transgenic studies, neonatal hypoxia/ischemia models, and in vitro research were excluded. By looking at the parent institution, sample size, author list, and outcome, reports of duplicate research were eliminated. Lastly, trials in which the mGluR agonist or antagonist interacted with other biological effects, including gene alteration, or in which the therapies were administered with monitored imaging instead of enhancing outcomes, were not included.

Data Extraction

Authors, year of publication, type of intervention (agonist and antagonist), animal species, cerebral ischemia type, intervention dose, duration of administration, delivery route, anesthetic used, outcome assessment (structural and functional), outcome measure used, mean outcome, SD (standard deviation) or SE (standard error), number of animals per group, study quality, and risk of bias assessment were among the data we extracted (Tables 2–5). The outcome in the control (vehicle) group, as opposed to the treated group, is what we referred to as the treatment comparison. We considered multiple interventions to be another intervention if more than one intervention and treatment duration were provided in a single research. Additionally, we regarded the efficacious doses at a specific time of administration as a distinct treatment arm if the same intervention was delivered in numerous doses. Furthermore, we only took into account the infarct volume from the greatest infarct in control slices and the smallest infarct in the experimental group if data from several brain slices were given in structural results. Another intervention was the assessment of infarct volume throughout varying treatment durations. Lastly, only the lowest neurological score for a given treatment period was included if functional outcomes were recorded for more than one time point. Every available source, including text and figures, was used to extract quantitative data for each study. We used quantitative techniques on highly magnified images to measure mean and SD/SEM values from graphs when the data were solely displayed graphically (GetData Graph Digitizer, version 2.26.0.20).

Quality Assessment

The Quality Score evaluation method reviewed the checklist of animal data from experimental studies (Lees *et al.,* 2012; Vu *et al.,* 2014) and established 10 criteria based on STAIR guidelines (Landis *et al.,* 2012; Fisher *et al.,* 2009; Macleod *et al.,* 2004) for each preclinical study included in the meta-analysis. (1) publication in a peer-reviewed journal; (2) statements explaining temperature control; (3) random assignment of animals to treatment group; (4) allocation concealment; (5) blinded outcome assessment; (6) avoidance of anesthetics with known marked intrinsic neuro-protective properties; (7) use of animals with pertinent comorbidities; (8) inclusion of a sample-size calculation; (9) statement of compliance with animal welfare regulations; and (10) inclusion of a statement declaring the presence or absence of any conflicts of interest. Each reported criterion was worth one point. Higher scores indicate better methodological rigor; the potential score goes from 0 to 10 (Chen *et al.,* 2016).

Risk of Bias Assessment

Using SYRCLE's risk of bias tool for animal studies, two reviewers independently evaluated the risk of bias, such as systematic mistakes (Hooijmans *et al.,* 2014). There are ten entries in this animal research tool. The items in the Cochrane Risk of Bias tool agreed with half of the items. The majority of the discrepancies between the two instruments resulted from the design differences between animal research and RCTs.

Data Analysis

The improvement in outcome in treated (intervention) animals compared to untreated ischemia (control) groups was used to calculate the endpoint effect size of group I mGluRs agonist and/or antagonist therapy. Review Manager 5.3 was the program utilized for the meta-analysis of outcome measures. Because there was significant heterogeneity across treatment doses and time points, we employed the random effect model (Borenstein *et al.,* 2010) for the meta-analysis. To report the improvement in treated groups, we used the inverse-variance (IV) technique approach in units of SD to assess significance and the mean effect size using standardized mean difference (SMD) with a 95% confidence interval (CI) for all included outcomes. A meta-analysis was conducted using the effect size of various doses and time points. Results that were expected to decrease were employed at the left graph level as an experiment and the right graph level as a control; however, the opposite was true for results that were expected to increase. We regarded it as an additional treatment group to estimate efficacy if more than one structural or functional result was reported for the same cohort of animals at the same time point or at a different time point. Sensitivity analysis

was then used to identify the treatment arms that were not appropriate and to ascertain the heterogeneities of the remaining studies. This method can determine why other estimates in strata were considerably different and examine if studies were taken from a homogeneous population.

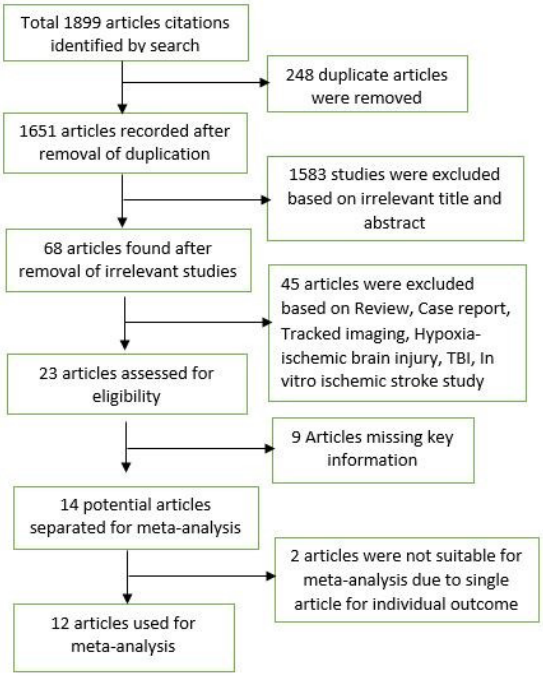

Figure 1: PRISMA Flow Diagram showing summary of study selection procedure.

RESULTS AND DISCUSSION

Study Characteristics

12 out of 1899 papers and 41 treatment groups comprising 26 distinct interventions (5 agonist and 21 antagonist) were selected for the current systematic review and meta-analysis. 10 research used temporary middle cerebral artery occlusion (tMCAO) to induce cerebral ischemia. Two of these investigations used intraluminal blockage of the middle cerebral artery, seven used bilateral carotid artery occlusion (BCAO), and one used endothelin-1 microinjection. Permanent MCAO was found in 2 studies: photothrombosis induced by rose Bengal dye (1 study) and MCAO (1 study). Among 21 antagonist interventions, 12 mGluR5 antagonist (MPEP = 9 and MTEP = 3) and 5 mGluR1 antagonist (2 interventions of LY367385 and 3 interventions of EMQMCM) were recorded. One intervention was treated with combined therapy of MPEP+LY367385, and 3 treatments were carried out with group I (mGluR1 and mGluR5) selective antagonist with MCPG, CBPG, and AIDA, respectively. The other 5 agonist interventions were treated with mGluR5-specific agonist, CHPG (4 interventions) and group I selective agonist, DHPG (1 intervention). Furthermore, 10 out of 26 interventions used Gerbils, 7 interventions used SD Rat, 6 interventions used Wister Rat, and 3 interventions used C57BL/6J mice in the experiments. Regarding the route of administration, 11 interventions of 5 studies were performed via intracerebroventricular (i.c.v.) microinjection (stereotactic), 11 interventions of another 5 studies were delivered by intraperitoneal injection (ip), 3 interventions of one study were applied through the tail vein, and one intervention of one study used the intrathecal route. Anesthetic agents were used in all 26 interventions of 12 studies during stroke induction (Table 2). The present study found 41 treatment groups, of which functional outcome was reported in 16 treatment groups (8 for Neurobehavioral, 4 for NMDA receptor, and 4 for brain temperature) of 8 studies, and structural outcome in 25 treatment groups (12 for infract volume, 8 for neuroprotection and 5 for neuron loss) of all 12 articles (Table 3).

Interpretation of quality score and risk of bias assessment

The interquartile range for the Quality Score checklist items was 3–6, with a median score of 4 out of 10. Peer-reviewed journals have published all of the studies. All studies avoided using anesthetics with known marked intrinsic neuroprotective properties; none used animals with relevant comorbidities (e.g., hypertension); none reported a sample size calculation or allocation concealment during the experiment; 11 studies reported compliance with animal welfare regulations; one study reported possible conflicts of interest; and two of twelve studies reported blinded assessment of the results and randomized allocation to treatment groups (Table 4). The mean effect size in the current meta-analysis remained slightly high (-0.75 SMD with $P > 0.05$ for neurological

score, -1.37 SMD with P < 0.00001 for infract volume, 1.73 SMD with P < 0.00001 for neuroprotection, and -1.35 SMD with P < 0.00001 for neuron loss), even though the overall estimation of the included outcomes represented larger effect sizes even after adjusting these effect sizes by subgroup and sensitivity analysis. Conversely, the effect sizes were less for the NMDA receptor (done by almost identical authors) and brain temperature (performed by nearly identical authors), at -0.41 SMD and -0.05 SMD, respectively; however, both effect sizes were statistically insignificant (P > 0.05). According to this study, the risk of bias was assessed using SYRCLE's risk of bias tool for animal studies (Hooijmans *et al.,* 2014) (Table 5).

Meta-Analysis of Effect Sizes

Neurological score decreased in a meta-analysis of eight treatment arms of four studies with 32 animals in control groups and 60 animals in treatment groups of both agonist and antagonist interventions (Table 1). However, the heterogeneity test among these 4 studies showed that the neurological score significantly favors the treatment (Supplementary Fig. 1). The sensitivity analysis (removal of effect size >3 SMD) improved the reduction of the neurological score effect size (Table 1) and heterogeneity, which significantly favors the treatment again. We did not compare the effectiveness of various training regimens for this outcome because there aren't many studies that evaluate neurobehavioral scores. The effect size increased by -2.07 SMD with 95% CI (-3.35, -0.79) after stratification by agonist or antagonist intervention; heterogeneity: Tau^2 = 1.27; Chi^2 = 12.42, df = 3; I2 = 76%, overall P = 0.002; (Figure 2) in antagonist treatment arms, and it significantly (P < 0.05) affects the treatment. In this case, one intervention's effect size was greater than three SMD.

Table 1: Effect sizes of different outcomes measures

Measures	Before adjustment				After adjustment				ES >3 arms No.
	Mean (ES)	95% CI	No.	P value	Mean (ES)	95% CI	No.	P value	
Neurological score*	-0.23	-0.84, 0.39	4	0.47	-0.00	-0.57, 0.56	3	0.99	-
Infarct volume*	-0.94	-1.45, -0.43	5	0.0003	-0.82	-1.35, -0.28	4	0.003	-
Neurological score#	-2.07	-3.35, -0.79	4	0.002	-1.42	-2.17, -0.67	3	0.0002	1
Infarct volume#	-1.85	-2.52, -1.18	7	<0.00001	-1.63	-2.21, -1.05	6	<0.00001	1
Neuroprotection#	2.48	1.50, 3.47	8	<0.00001	1.73	1.08, 2.38	6	<0.00001	2
Neuron loss#	-1.58	-2.18, -0.99	5	<0.00001	-1.35	-1.85, -0.85	4	<0.00001	1
Brain temperature#	-0.34	-1.43, 0.75	4	0.54	-0.05	-0.62, 0.52	3	0.87	1
NMDA receptor#	-1.11	-2.45, 0.22	4	0.10	-0.41	-1.58, 0.76	2	0.49	2
Neurological score##	-1.09	-1.94, -0.24	8	0.01	-0.75	-1.39, -0.11	7	0.02	1
Infarct volume##	-1.52	-2.00, -1.04	12	<0.00001	-1.37	-1.79, -0.95	11	<0.00001	1

*Note: For each effect size, the 95 % CI does not cross zero, and the p value <0.05 indicates that results favor mGluRs treatments, whereas the P value >0.05 does not favor mGluRs treatments. ES, Effect Size; CI, Confidence Interval; No, Number of intervention. *Agonist interventions, #Antagonist Interventions, ##Agonist and antagonist interventions together.*

Following antagonist treatment, the neurological score significantly decreased (P <0.05) according to sensitivity analysis adjustment of effect sizes (Table 1). Additionally, we assessed the neurobehavioral outcomes that were most commonly observed in the mice, as well as the effect size of group I mGluRs agonist and antagonist therapy, independently. The neurological score outcome for agonist-treated interventions was found to be lower than that of antagonist-treated interventions in terms of both effect magnitude and heterogeneity (Figure 2). The heterogeneity and effect size of the included studies decreased even after sensitivity analysis (removal of height effect size); but, surprisingly, the outcome did not significantly (P > 0.05) favor the therapy before and after correction of interventions.

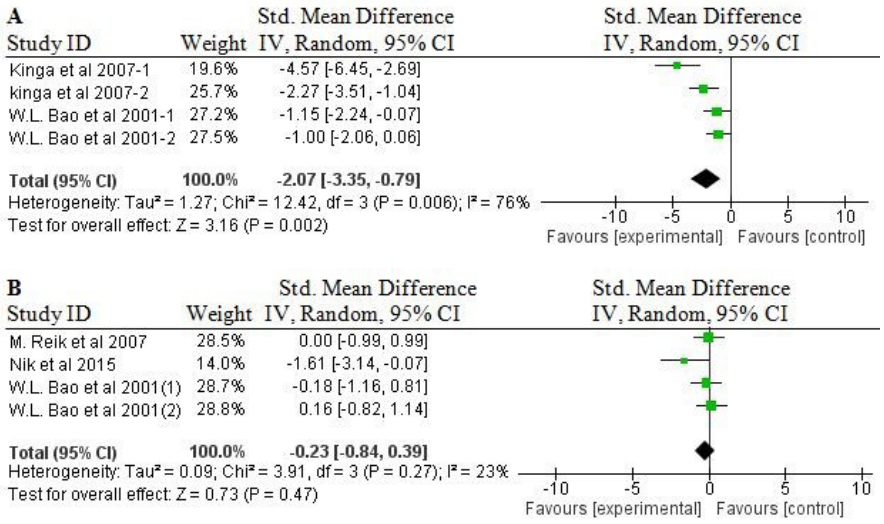

Figure 2: Neurobehavioral Effect size of group I mGluRs across studies. The forest plot showed median effect size and 95% CI for the neurological score of antagonist interventions (A) and agonist interventions (B) compared with the control group. Std, standardized; IV, inverse variance; CI, confidence interval; df, degrees of freedom.

With 105 animals in the experimental groups and 46 in the control groups, the mean infarct size of 5 studies and 12 interventions (both agonist and antagonist) was decreased (Figure 2). Although there were 12 treatment arms where effect size of one treatment was >3.0, and after removal of this inadaptable treatment, the heterogeneity of remaining interventions went down and the effect size was recorded (Table 1). Heterogeneity was $Tau^2 = 0.49$; $Chi^2 = 15.94$, df = 6; I2 = 62%, overall P < 0.00001, and the mean infarct size in 7 antagonist interventions of 3 studies, which included 34 animals in control and 70 animals in experimental groups, was -1.85 SMD with 95% CI (-2.52, -1.18) (Figure 3A). However, there was 1 intervention dominated by effect size 3.78. After removing this inadaptable treatment under sensitivity analysis, the heterogeneity was low and the effect size was -1.63 SMD with 95 % CI (-2.21, -1.05) (Table 1). Contrarily in 5 agonist interventions of 3 studies, the heterogeneity was I2 = 0 %; P = 0.0003 and infract size was -0.94 SMD (95 % CI, -1.45, –0.43) with < 1 SMD effect size in almost all interventions (excluding effect size -2.42 SMD) (Figure 3B). Removal of this biggest inadaptable result in sensitivity analysis showed the effect size, -0.82 SMD with 95 % CI (-1.35, -0.28).

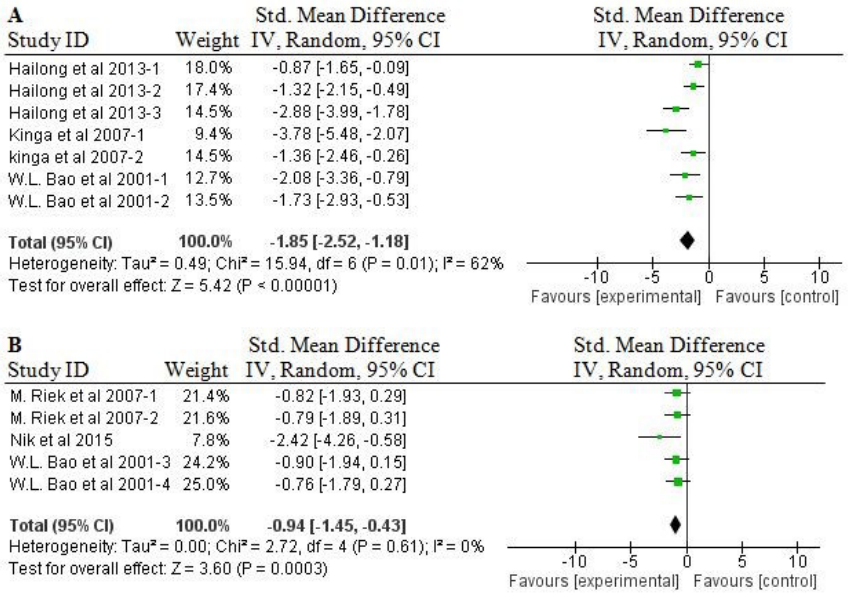

Figure 3: Infarct volume effect size of group I mGluRs across studies. Forest plot showed median effect size and 95% CI for infarct volume of antagonist interventions (A) and agonist interventions (B) compared with the control group. Std, standardized; IV, inverse variance; CI, confidence interval; df, degrees of freedom.

The mean effect size for the neuroprotection or live neuron cells count included 5 studies and 8 antagonist interventions was measured as 2.48 SMD with 95 % CI (1.50, 3.47) where 2 interventions were more than 3 SMD, with the greatest SMD 5.65 in one study. The number of animals (Wister Rat, Gerbils) in both control and experimental groups was 11. Heterogeneity was recorded as $Tau^2 = 1.41$; $Chi^2 = 24.74$, df = 7; I2 = 72 %, overall P < 0.00001 (Figure 4). After removal of inadaptable intervention (effect size > 3 SMD) in sensitivity analysis, the heterogeneity was found lower to $Tau^2 = 0.15$; $Chi^2 = 6.39$, df = 5; I2 = 22 %, overall P < 0.00001 and effect size was 1.73 SMD with 95 % CI (1.08, 2.38) (Table 1). Separately two antagonist interventions in Wister rat showed effect size > 2 SMD but the heterogeneity $Tau^2 = 0.00$; $Chi^2 = 0.02$, df = 1; I2 = 0 %, overall P < 0.0001 (Supplementary Fig. 3A) whereas in 6 interventions of Gerbils showed effect size 2.50 SMD (>2 SMD in 3 interventions with higher SMD 5.65) and heterogeneity was $Tau^2 = 1.95$; $Chi^2 = 24.29$, df = 5; I2 = 79 %, overall P = 0.0001 (Supplementary Fig. 3B). After sensitivity analysis the effect size was found as 1.97 SMD with 95 % CI (0.96, 2.98) (Table 1).

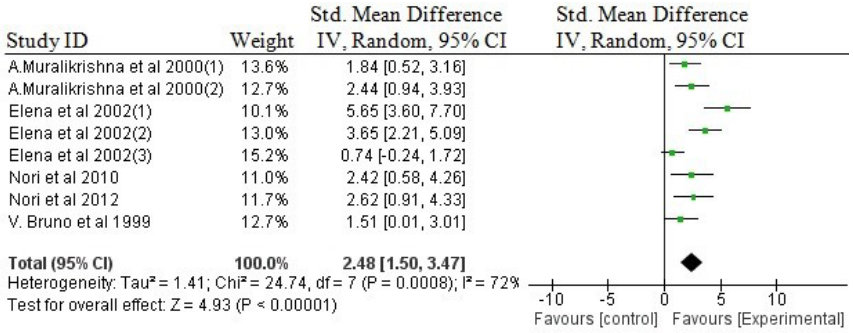

Figure 4: Neuroprotection effect size of group I mGluRs antagonist interventions across studies. The forest plot showed the median effect size and 95% CI for neuroprotection of antagonist interventions compared with the control group. Std, standardized; IV, inverse variance; CI, confidence interval; df, degrees of freedom

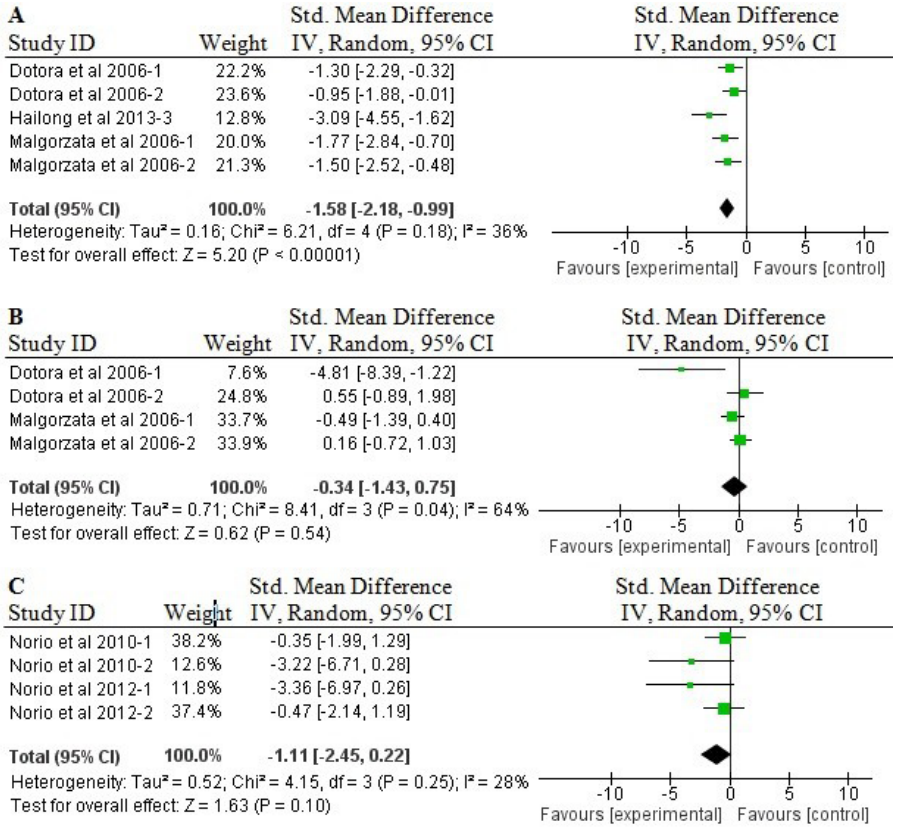

Figure 5: Effect size of group I mGluRs antagonist interventions across studies. Forest plot showed median effect size and 95% CI for neuronal loss (A), brain temperature (B), and effect on NMDA receptor (C) compared with the control group. Std, standardized; IV, inverse variance; CI, confidence interval; df, degrees of freedom.

The size of the effect of antagonist treated neuron loss (5 interventions of 3 studies included 29 animal in control and 49 in experimental groups) and brain temperature (4 interventions of 2 studies included 14 animal in control and 28 in experimental groups) outcomes were reduced by -1.58 SMD with 95 % CI (-2.18, -0.99) and -0.34 SMD with 95 % CI (-1.43, 0.75) respectively. The heterogeneity was measured in neuron loss outcome where in 4 interventions SMD were >1 with greater SMD 3.09 (Figure 5A). The heterogeneity with overall P = 0.54 was found in brain temperature outcome where 3 interventions SMD were less than 1 and in one study SMD was -4.81 (Figure 5B). After sensitivity analysis the heterogeneity was same in both studies but overall P < 0.00001 in neuronal loss and overall P = 0.87 in brain temperature outcomes and effect sizes were -1.35 SMD with 95 % CI (-1.85, -0.85) in neuronal loss and -0.05 SMD with 95 % CI (-0.62, 0.52) in brain temperature outcomes (Table 1). The effect size for NMDA receptor of 4 antagonist interventions of 2 studies was found to be -1.11 SMD with 95 % CI (-2.45, 0.22). Here we found the effect size < 0.5 SMD in 2 interventions and > 3 SMD in another 2 interventions and heterogeneity with overall P = 0.10 (Figure 5C). After adjustment of effect size by sensitivity analysis (removal of effect size > 3 SMD) the heterogeneity I2 = 0 %, and effect size was -0.41 SMD with 95 % CI (-1.58, 0.76) but it was statistically insignificant (P > 0.05) (Table 1).

Discussion

One of the world's major causes of death and disability, ischemic stroke has been the focus of intense research in recent years (Ström *et al.,* 2013). Enhancing this endogenous process could be a treatment approach to improve functional outcome because adult neurogenesis is a restricted process in humans (Greenberg *et al.,* 2007). In this regard, mGluR agonists and/or antagonists may be a good option to provide their protective function in the treatment of stroke. Consequently, several writers have presented conflicting findings regarding the assessment of deficits following experimental ischemia, depending on the species, strain, age, and experimental model employed, as well as the timing of the test (Rosell *et al.,* 2013). Several outcomes, including infarct volume of agonist and antagonist interventions and neuroprotection, neuronal loss, and neurological score of antagonist intervention, significantly favor the treatments, according to this meta-analysis, which primarily looked at preclinical studies of group I mGluRs agonist and antagonist in the treatment of animal ischemic stroke. Nevertheless, no correlation was found between the agonist or antagonist dosage and the delivery method, possibly because the majority of studies used intraperitoneal or intracerebroventricular stereotactic injections to deliver the drug to the lesion. However, our findings point to the need for additional molecular research on group I mGluRs agonists and antagonists for the treatment of ischemic stroke in the future. The current study's median quality score, using the same items from the Quality Score checklist, was 4 (interquartile range: 3–6), which is lower than the preclinical studies on NSCs for ischemic stroke (Chen *et al.,* 2016) and preclinical mesenchymal stromal cells for ischemic stroke (Vu *et al.,* 2014). It is also consistent with the study on preclinical stem cell therapy (Lees *et al.,* 2012). Lower quality studies typically exaggerate intervention or experimental effects (Schulz *et al.,* 1995), while higher quality studies typically provide more effective evaluation of preclinical research impacts (O'Collins *et al.,* 2006).

In this current meta-analysis, moderate effect sizes were observed. For group I mGluRs agonists, the effect sizes for two outcomes, neurological score and infarct volume, were identified. The heterogeneity in neurological scores was lower but not statistically significant (P > 0.05). This meta-analysis reveals that neurological scores significantly favor treatment with group I mGluRs antagonists and disfavor agonist treatment, aligning with previous studies (Chen *et al.* 2016). Differences in drug dose, administration route, type of ischemic stroke, and animal models may contribute to the variations in effect sizes within the agonist or antagonist groups, or between them. The intra-cerebroventricular (icv) route was used by 11 interventions across 5 studies (Riek-Burchardt *et al.,* 2007; Bao *et al.,* 2001; Norio *et al.,* 2012; Norio *et al.,* 2010; Bruno *et al.,* 1999), and the intraperitoneal (ip) route was used by 11 interventions across 5 studies (Kinga *et al.,* 2007; Dorota *et al.,* 2006; Elena *et al.,* 2002; Muralikrishna *et al.,* 2000; Malgorzata *et al.,* 2006). Overall, the assessment indicates that the icv route of administration is more effective than ip injection. Due to these factors, significant heterogeneity existed in behavioral and anatomical outcomes, which we addressed through subgroup and sensitivity analyses, as well as bias risk assessments.

Generally, an effect size >0.8 is considered as a large effect (Schulz *et al.* 1995). In this meta-analysis considerable lower effect sizes were found in the neurological score against agonist interventions and brain temperature outcome against antagonist interventions, but neither of the outcomes were not statistically significant (P >0.05). After removing the inadaptable treatment with effect sizes >3 from a particular treatment group, the rest effect sizes were improved significantly in some cases, but it remained high effect sizes in infract volume, neurological score, neuroprotection, neuron loss, and effects on NMDA receptors in antagonist interventions. For neurological score and infarct volume, the biggest effect sizes were -4.57 SMD and -3.78 SMD, respectively (Kinga *et al.* 2007), but these effect sizes were lower than the study on transplantation of NSCs modified by glial cell line-derived neurotrophic factor (GDNF) gene to native NSCs transplantation after ischemic stroke (Chen *et al.* 2009).

The primary weakness of this investigation was that it was observational rather than experimental. Only the association of studies was provided; all other findings are hypotheses. Even if our search was thorough, there's a chance that some published research was overlooked. The current analysis may overestimate the effects of group I mGluRs agonist and antagonist treatments in ischemic stroke due to the potential use of low-quality research.

Conversely, there were some signs of publication bias influencing the meta-analysis's findings (Feng *et al.,* 2016), a typical occurrence that often affects animal study meta-analyses (Briel *et al.,* 2013; Korevaar *et al.,* 2011). We were unable to compare our data with any meta-analyses on ischemic stroke and mGluRs. The use of mGluRs in experimental ischemic stroke, however, may have been reviewed by our work, which is likely to have documented the primary trend in the field of mGluRs treatment.

CONCLUSION

In preclinical ischemic animal models, group I mGluRs antagonists were primarily linked to significantly better functional and structural outcomes, according to the current systematic review and meta-analysis. It also offered some helpful tools for future therapeutic research on mGluRs in clinical ischemic stroke. Long-term consequences, risk assessment, and improvements in neural structural and functional regeneration and repair should all be taken into account in future research, including mGluRs treatment.

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