Medicine
Neuroscience

CCR5 antagonists as neuroprotective and stroke recovery enhancing agents: a preclinical systematic review and meta-analysis

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eLife Assessment

This systematic review presents **valuable** insights into CCR5 antagonist drugs for neuroprotection and stroke management. The strength of the evidence is **convincing**, and the review methods and reporting adhere to the expected standards. A sensitivity analysis based on the risk of bias assessment of the included studies would be beneficial, and a more focused/detailed acknowledgment of key limitations of the review would add value to the quality of the reporting and interpretations of the findings.

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Abstract

Background

C-C chemokine receptor type 5 (CCR5) antagonists may improve both acute stroke outcome and long-term recovery. Despite their evaluation in ongoing clinical trials, gaps remain in the evidence supporting their use.

Methods

With a panel of patients with lived experiences of stroke, we performed a systematic review of animal models of stroke that administered a CCR5 antagonist and assessed infarct size or behavioral outcomes. MEDLINE, Web of Science, and Embase were searched. Article screening and data extraction were completed in duplicate. We pooled outcomes using random effects meta-analyses. We assessed risk of bias using the Systematic Review Centre for Laboratory Animal Experimentation (SYRCLE) tool and alignment with the Stroke Treatment Academic Industry Roundtable (STAIR) and Stroke Recovery and Rehabilitation Roundtable (SRRR) recommendations.

Results

Five studies representing 10 experiments were included. CCR5 antagonists reduced infarct volume (standard mean difference −1.02; 95% confidence interval −1.58 to −0.46) when compared to stroke-only controls. Varied timing of CCR5 administration (pre- or post-stroke induction) produced similar benefit. CCR5 antagonists significantly improved 11 of 16 behavioral outcomes reported. High risk of bias was present in all studies and critical knowledge gaps in the preclinical evidence were identified using STAIR/SRRR.

Conclusions

CCR5 antagonists demonstrate promise; however, rigorously designed preclinical studies that better align with STAIR/SRRR recommendations and downstream clinical trials are warranted.

Registration

Prospective Register of Systematic Reviews (PROSPERO CRD42023393438)

Introduction

C-C chemokine receptor type 5 (CCR5) is expressed across a variety of leukocyte subtypes, endothelial cells, and cell types in the brain (e.g., neurons, microglia, astrocytes), and is thought to play crucial roles in post-stroke neuroinflammation, blood-brain barrier repair, and neuronal survival / repair processes.[1] CCR5 antagonists have emerged as potential therapeutic candidates for stroke, demonstrating both neuroprotection and improved neural repair/recovery in preclinical animal models.[2–6] However, no CCR5 antagonist drug has an approved indication in the stroke context, necessitating studies to establish safety and efficacy this population. This has led to an ongoing clinical trial to investigate efficacy of CCR5 antagonists in combination with post-stroke rehabilitation.[7] Assessment of the preclinical evidence supporting CCR5's role in stroke is needed to identify areas of potential benefit, and knowledge gaps, that should be addressed by future preclinical research.[8]

The stroke neuroprotection and recovery communities have advocated for alignment of preclinical and clinical study parameters through publication of consensus recommendations for preclinical research, in an effort to enhance the translation of new stroke therapies.[9–11] Examples include identification of more sensitive and clinically relevant preclinical outcome measures and incorporation of potentially important effect modifiers of treatment efficacy, such as age, sex, and stroke-related comorbidities (hypertension, diabetes, etc.).[9–11] These recommendations aim to improve the translation of novel stroke therapeutics from preclinical to clinical populations, but the degree to which preclinical evidence for CCR5 antagonists satisfy these recommendations is unknown.

We sought to comprehensively evaluate the preclinical evidence for CCR5 antagonist drugs as both neuroprotective and stroke recovery-promoting agents.[9,11] Both perspectives are necessary to fully understand the therapeutic potential of stroke-related treatments, as distinct biological principles, time windows for treatment, and outcomes of interest underpin each of these treatment domains.[12,13] We conducted a systematic review and meta-analysis of the CCR5 literature in conjunction with a panel of individuals with lived experiences of stroke. This review sought to explore how the preclinical evidence for CCR5 antagonist drugs aligned with guidance for preclinical stroke research provided by previous expert committees and the parameters of an ongoing clinical trial (The Canadian Maraviroc Randomized Controlled Trial To Augment Rehabilitation Outcomes After Stroke, CAMAROS, NCT04789616).[9–11]

Materials and methods

We registered the review protocol on the International Prospective Register of Systematic Reviews (CRD42023393438).[14] The findings are reported in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (Table S1)[15] and Guidance for Reporting the Involvement of Patients and the Public (Table S2).[16] A panel of eight patients and caregivers with lived and living experience of stroke informed project development and were actively involved throughout research conduct (see Table S2 for more information).

Eligibility criteria

- *Animals:* Any preclinical *in vivo* animal models of adult stroke were included. Human, invertebrate, *in vitro*, *ex vivo*, and neonatal animal studies were excluded.
- *Model:* Focal ischemic or intracerebral hemorrhagic stroke models were included, while animal models of global ischemia were excluded.
- *Intervention:* Studies administering a CCR5 antagonist drug (e.g., maraviroc, D-Ala-Peptide T-Amide (DAPTA), Takeda 779 (TAK-779)). Study arms in which CCR5 was genetically manipulated (e.g., CCR5 knockout strain) were excluded.
- *Comparator:* Vehicle-treated control groups where stroke was induced. CCR5 antagonist control groups without stroke were excluded.
- *Outcome:* Studies reporting at least one of the following: infarct size, behavioral tests, mortality, adverse events, and spasticity were included.
- *Study design and publication characteristics:* Controlled interventional studies (randomized, pseudo-randomized, or non-randomized) published as full journal articles in any language or year were included. Abstracts, review articles, opinion-based letters/editorials, and unpublished grey literature were excluded.

Information sources and search strategy

An information specialist with experience in systematic searches of the preclinical literature developed a comprehensive search strategy on October 25, 2022, based on a previously published strategy for identifying animal experimentation studies.[17] The search strategy underwent peer-review using the Peer Review of Electronic Search Strategies (PRESS) checklist.[18] We searched MEDLINE (OVID interface, including In-Process and Epub Ahead of Print), Web of Science, and Embase (OVID interface). See the Supplemental Methods for the full search strategy and exact search dates for each database.

Selection process and data collection

We deduplicated citations and uploaded them into DistillerSR® (Evidence Partners, Ottawa, Canada). Two reviewers independently screened citations by title and abstract using an accelerated method (one reviewer required to include, two reviewers required to exclude). We then screened and extracted data from full-text articles in duplicate. Graphical data was extracted using Engauge Digitizer.[19] A third reviewer with content expertise in preclinical stroke studies audited all data extraction. Conflicts between reviewers were resolved by consensus discussion. See Supplemental Methods for the complete list of data extraction elements.

Effect measures and data synthesis

We performed quantitative analyses using the R (version 4.1.2) "metafor" package (version 4.0.0)[20] with inverse variance random effects modelling. We expressed continuous outcome measures as standardized mean differences (SMDs) with 95% confidence intervals (CIs) and assessed statistical heterogeneity of effect sizes using the Cochrane I^2 statistic.[21] This was necessary due to the variety of outcome measures and measurement scales used across studies, which is a common feature of preclinical systematic reviews. Sensitivity analyses were performed using original measurement scales where possible. From patient partner input, subgroups were analyzed based on timing / dose / route of intervention, stroke model, stroke type, species, type of behavioral outcome (i.e., motor, cognitive), and comorbidities.[22] Our planned subgroup analyses on study quality, specific regions/areas of the brain, and post-stroke rehabilitation paradigms were not performed due to insufficient number of studies. We did not assess publication bias using Egger plots due to fewer than 10 studies being included in the analysis, as per Cochrane recommendations.[23]

Risk of bias assessment

Two independent reviewers used the Systematic Review Centre for Laboratory Animal Experimentation (SYRCLE) risk of bias tool to assess each study as having a "Low Risk", "Unclear Risk", "Some Concerns of Risk", or "High Risk" across domains such as randomization, blinding and outcome reporting.[24] "Some Concerns of Risk" indicated reporting of a domain (i.e., randomization), but lacking methodological details. This differed from "Unclear Risk" where there was no mention of the domain in the study.

Comprehensiveness of preclinical evidence and alignment with clinical trials

We assessed comprehensiveness of the *overall body* of preclinical evidence for CCR5 antagonists as a neuroprotective treatment using The Stroke Treatment Academic Industry Roundtable (STAIR) I, VI, and XI Consolidated Recommendations.[11,25,26] These recommendations encompass "candidate treatment qualification" (e.g., dose, timing of dose, outcomes) and "preclinical assessment and validation" (e.g., age, sex, sample size, animal type).[11] We excluded domains that were redundant with risk of bias (e.g., randomization, blinding, etc.) and included additional

items relevant to stroke recovery studies from the Stroke Recovery and Rehabilitation Roundtable (SRRR) Translational Working Group consensus recommendations.[9] Two reviewers extracted data to determine if the overall evidence across studies satisfied each of the STAIR and SRRR recommendations. A third reviewer audited this analysis. We then assessed alignment of existing preclinical evidence with an ongoing clinical trial of CCR5 antagonists for stroke (CAMAROS, NCT04789616).[7]

Protocol deviations

We incorporated an additional assessment using the PRIMED[2] tool for assessing the readiness of stroke neuroprotection therapies to be translated to clinical trials based on the body of preclinical evidence.[27] Additionally, a list of outcomes used to determine CCR5's potential mechanisms of action were extracted (Figure S1, Table S3) based on feedback from individuals that reviewed the initial manuscript drafts.

Results

Study selection

Our search identified 263 citations, which was reduced to 166 unique studies after deduplication. Five studies representing 10 experiments met the eligibility criteria (**Figure 1**).[2–6]

Study and animal model characteristics

Most studies used ischemic stroke (n=4/5). This was induced via intraluminal suture (n=2), cauterization (n=1), or photothrombosis techniques (n=1; **Table 1**). Hemorrhagic stroke was induced in one study via autologous whole blood injection. All studies used mouse (n=4) or rat (n=1) models, comprised of exclusively male animals (n=5). Relevant stroke comorbidities highlighted by patient partners and STAIR/SRRR recommendations (e.g., hypertension, diabetes) were not used in any study.

Intervention characteristics

Maraviroc was used in six of the experiments (n=6/10), TAK-779 in three of the experiments (n=3), and D-Ala-Peptide T-Amide (DAPTA) in one (n=1; **Table 2**). These CCR5 antagonists were delivered intraperitoneally (n=4), intranasally (n=2), intracerebroventricularly (n=2), subcutaneously (n=1), and intravenously (n=1) at a dose range from 0.01 to 100 mg/kg. Most studies delivered a single dose of the drug (n=6); experiments with multiple administrations (n=4) ranged from 3–63 doses. Time of initial treatment administration varied widely. Two studies (3 experiments) administered treatment pre-stroke (10-15 minutes),[2,3] and 4 studies (6 experiments) in the acute, potentially neuroprotective, post-stroke period (50 minutes – 24 hours post-stroke).[3–6] One study (1 experiment) was conducted in the late sub-acute/early chronic period beginning at three to four weeks post stroke, which would be oriented towards recovery, rather than neuroprotective, effects.[6] Patient partners had identified several *a priori* interests (physical therapy alongside CCR5 administration, spasticity), which were also aligned with SRRR recommended considerations for preclinical stroke recovery studies (see Supplemental Methods).[9] These were not reported in any included studies.

Meta-analysis of infarct volume

Infarct volume was reported in six experiments (n=6/10) from four different studies with an overall pooled analysis demonstrating marked neuroprotection with CCR5 antagonists (SMD - 1.02, 95% CI −1.58 to −0.46, $p < 0.0001$, I^2=34%; **Figure 2**). Five of these experiments measured

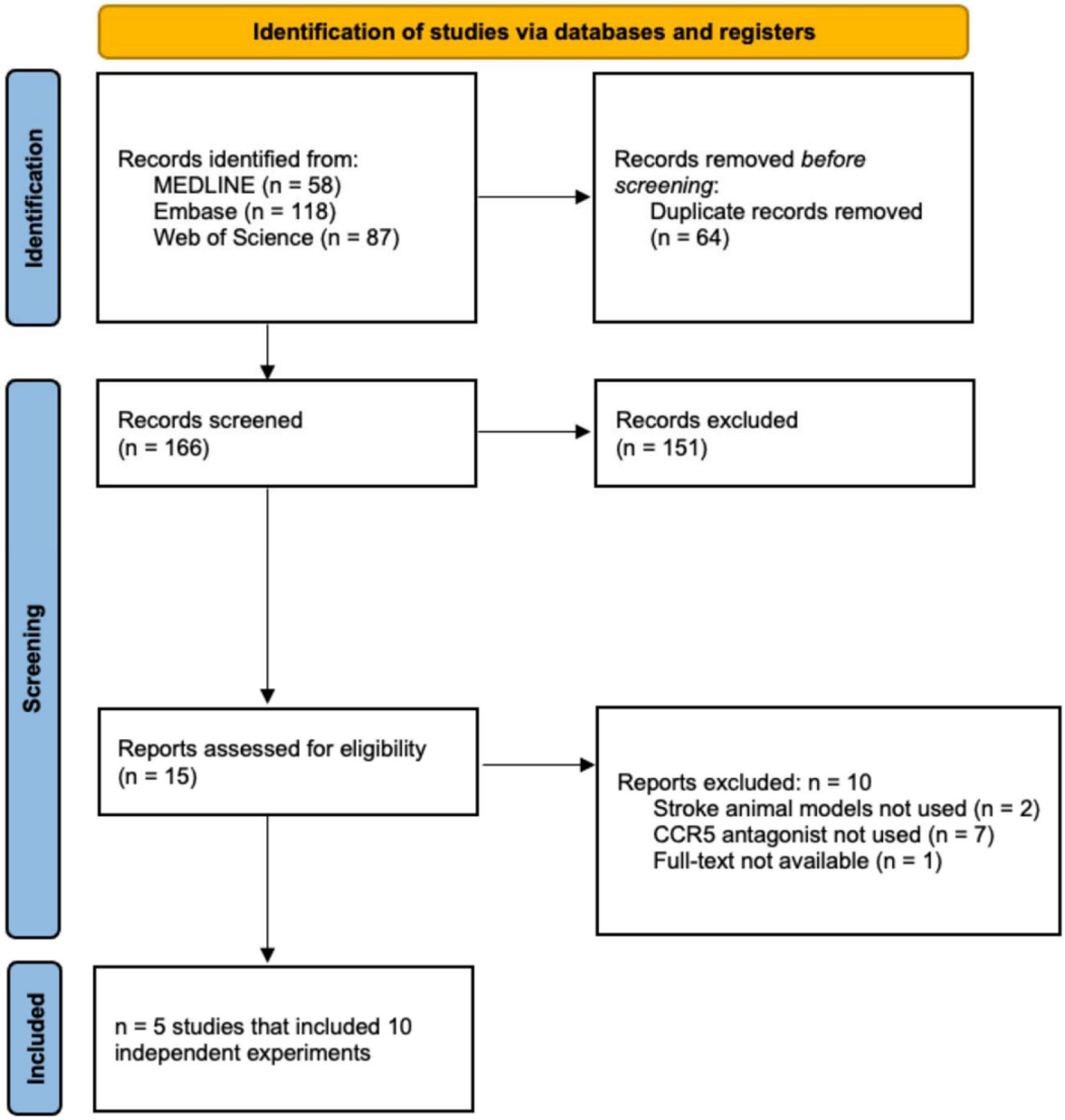


Figure 1.

Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) flow diagram.

Table 1.

Summary of study and animal model characteristics of included articles.

Author	Year	Country	Species	Strain	Stroke Type	Stroke Model	Sex	Weight	Age
Li et al.[2]	2016	China	Rat	Wistar	Ischemic	Intraluminal suture	Male	260–300 g	N/A
Takami et al.[3]	2002	Japan	Mice	ddY	Ischemic	Intraluminal suture	Male	N/A	4 weeks
Chen et al.[4]	2022	China	Mice	C57/BL6	Ischemic	Permanent middle cerebral artery occlusion	Male	25–30 g	8–10 weeks
Yan et al.[5]	2021	China	Mice	CD1	Hemorrhagic	Intracerebral hemorrhagic	Male	30–40 g	N/A
Joy et al.[6]	2019	USA	Mice	C57/BL6	Ischemic	Photothrombotic	Male	25–30 g	8–20 weeks

Table 2.

Summary of intervention characteristics.

Author	Drug	Dose (mg/kg)	Route	Timing	Doses (total #)	Outcomes Measured (Treatment n/Control n)	Outcome Window (post-stroke))
Li et al.[2]	DAPTA (D-Ala-Peptide T-Amide)	0.01	SC	15 min pre-stroke	1	- Infarct volume (5/5) - Neurological deficit score (5/5)	24 h
Takami et al.[3]	TAK-779	5	ICV	10 min pre-stroke	1	- Infarct volume (10/6)	48 h
		50	ICV	10 min pre-stroke		- Infarct volume (13/6)	
		0.25	IV	50 min post-stroke		- Infarct volume (17/18)	
Chen et al.[4]	Maraviroc	20	IP	1.5 h, 24 h, and 48 h post-stroke	3	- Infarct volume (5/5) - Longa score (5/5) - Neurological deficit score (5/5)	72 h
Yan et al.[5]	Maraviroc	0.15	IN	1 h post-stroke	1	- Garcia test (6/6) - Limb placement (6/6) - Corner turn test (6/6)	72 h
						- Foot fault (8/8) - Rotarod (8/8)	3 weeks
						- Probe quadrant duration (8/8)	25 days
				24 h post-stroke		- Garcia test (6/6) - Limb placement (6/6) - Corner turn test (6/6)	72 h
Joy et al.[6]	Maraviroc	100	IP	24 h post-stroke through daily injections for 9 weeks	63	- Infarct volume (5/5)	9 weeks
						- Grid walk (10/10) - Forelimb (10/10)	8 weeks
				24 h post-stroke then daily for 3 weeks	21	- Grid walk (10/10) - Cylinder test (9/8)	9 weeks
				3 to 4 weeks post-stroke then daily for 11 weeks	56	- Grid walk (9/9) - Cylinder test (9/9)	11 weeks

Abbreviations: ICV = intracerebroventricular; IN = intranasal; IP = intraperitoneal; IV = intravenous; SC = subcutaneous

infarct volume at 1-3 days post-stroke, and one experiment measured infarct volume at a delayed time point of 63 days post-stroke. No significant differences between pre- or post-stroke administration were observed (*P*=0.47). Post-hoc sensitivity analysis removing one experiment with extreme values[2] demonstrated that neuroprotection in the remaining two experiments remained statistically significant while reducing heterogeneity (SMD −0.81, 95% CI −1.25 to −0.37, p < 0.001, I^2=0%). A second sensitivity analysis excluded one study that measured infarct volume in mm[3] so that all other studies could be meta-analyzed using mean differences on the percentage scale (Figure S2; MD −9.1%, 95% CI −11.6 to −6.7%, p < 0.001, I^2=0%). This demonstrated a similar neuroprotective effect as the other analyses. Further sub-group analyses by route of administration, time of administration, stroke model, species, CCR5 antagonist, dose, and whether behavior tests were assessed are described in Figure S3-S5.

Synthesis of behavioral outcomes without meta-analysis

Motor behavioral outcomes were reported in six experiments from three studies and represented seven different behavioral tasks. An additional study reported motor behavioral outcomes without standard deviations or standard errors, and thus could not be included (authors did not respond to email requests for data).[4] A cognitive outcome (Morris Water Maze) was measured in one study. Overall, CCR5 inhibition was effective in 11 of 16 behavioral outcomes tested (**Figure 3**). Meta-analysis and planned subgroup analysis were contra-indicated due to an inadequate number of studies for each given outcome measure, which necessitated the synthesis without meta-analysis presented below.[28]

Behavioral outcomes are presented by time of CCR5 antagonist administration, as discussed in the Intervention Characteristics section above, as administration time directly relates to the treatment context and patient population to which the results apply (i.e., acute neuroprotection vs. late sub-acute/early chronic neural repair). Li et al. reported that pre-stroke administration (relevant to surgical contexts with high risk of thrombosis) of DAPTA did not result in significantly greater performance on the neurological deficit score.[2]

Regarding acute post-stroke administration times with the potential for *neuroprotective* effects (up to 24 hours post-stroke based on observed infarct reductions in **Figure 2**), Yan et al. observed that CCR5 antagonist (maraviroc) administration one-hour following stroke resulted in greater motor performance on the corner test, limb placement, modified Garcia, foot fault, and rotarod tasks compared to vehicle-treated controls. Cognitive outcomes in the Morris Water Maze task (proportion of time spent in the probe quadrant) were also improved by this one-hour post-stroke administration. Significantly improved motor performance was not observed if CCR5 antagonist was administered 24 hours post-stroke, using this model of *intracerebral hemorrhage* (**Figure 3**).[5] In contrast, using a *focal ischemia* model, Joy et al. observed that CCR5 antagonist (maraviroc) administration initiated 24 hours post-stroke and continued daily for 3 weeks (which could potentially represent a mix of neuroprotective and recovery-induced effects) resulted in greater performance on the cylinder and grid walk tasks compared to vehicle-treated controls.

Joy et al., also performed one experiment with *late sub-acute/early chronic* administration (a potentially critical time for neural repair) initiated at three to four weeks post-stroke and continued daily for 11 weeks, that demonstrated significantly improved performance for the grid walk, but not cylinder, task.[6] This experiment was initiated outside the plausible range for a neuroprotective effect, implying behavioral improvement involved *recovery-promoting mechanisms*. However, equivalent infarct volumes were not demonstrated between the treated and control groups in this cohort, which could potentially lead to confounding effects.

Figure 2.

CCR5 antagonists reduce infarct volume.

Data is presented as a forest plot with standardized mean differences and 95% confidence intervals. Effect sizes <0 favours drug treatment and >0 favours control. Data is stratified by timing of CCR5 antagonist administration (pre- or post-stroke induction). The 'RE Model for All Studies' represents a pooled estimate of the CCR5 antagonist drug effect on infarct volume from all studies combined. Separate pooled estimates are also reported for post-stroke and pre-stroke CCR5.

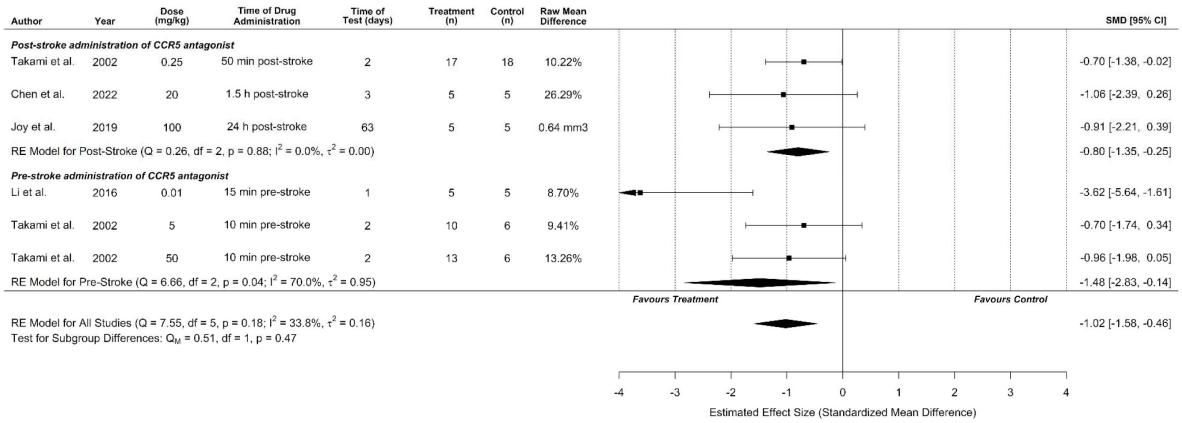
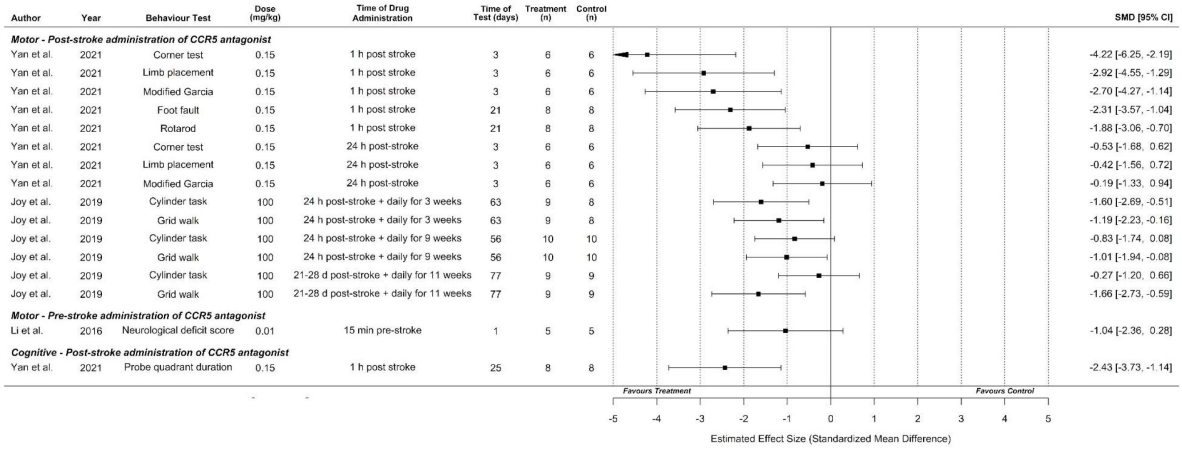


Figure 3.

Meta-analysis for all included preclinical CCR5 antagonist studies that reported motor and/or cognitive behavioral outcomes.

Data is presented as a forest plot with a standardized mean difference and 95% confidence intervals. Effect sizes <0 favours drug treatment and >0 favours control.



Risk of bias

All articles had a 'high' risk of bias in at least one domain of the SYRCLE tool.[24] Most domains within each study demonstrated an 'unclear' risk of bias. All studies reported randomizing animals; however, as commonly observed in the preclinical literature,[29–31] only one of these studies provided sufficient detail to ensure that the randomization method had a low risk of bias. The SYRCLE domains with the highest risk of bias were incomplete outcome data, with 80% of studies (n=4/5) failing to provide complete data for all animals initially included in the study, as well as selective outcome reporting, with 60% (n=3/5; all studies of maraviroc) not providing complete data for all expected outcomes discussed in the methods (Figure S6).

Comprehensiveness of preclinical evidence and alignment with clinical trials

We assessed comprehensiveness of the preclinical evidence using the STAIR and SRRR recommendations[9,11,25,26] as well as alignment with study parameters of the CAMAROS trial.[7] A summary of assessment items from STAIR XI is provided in **Table 3**, with additional items from STAIR I, VI, and SRRR recommendations included in Table S4.

For CCR5 antagonists as a post-stroke neuroprotectant, the overall body of evidence satisfied all five STAIR XI domains assessing 'candidate treatment qualification' (**Table 3**). Overall, a range of doses and clinically relevant administration times for neuroprotection were evaluated across a variety of motor and cognitive behavioral domains. All studies tested both behavioral and histological outcomes and demonstrated neuroprotective effects, but most studies failed to measure and control post-stroke temperature, which could potentially confound the observed neuroprotection (Table S4).[32] Most histological measurements were also assessed at <72 hours, which could confound the observed neuroprotective effects if cell death was merely delayed.[32] For CCR5 antagonists as a post-stroke recovery-inducing treatment, one experiment assessed the effects of initiating CCR5 administration in a similar post-stroke phase as the CAMAROS trial. This experiment (Joy et al.)[6] did not demonstrate that each treatment group had equivalent baseline stroke volumes, which may potentially confound observed behavioral effects. Furthermore, the maximum dose used in mice (100 mg/kg) was not sufficient to attain cerebrospinal fluid levels of maraviroc observed in humans using the CAMAROS dosing regime (mice: 13.8±5.4 ng/mL; humans 33.6–60 ng/mL).[6]

Areas of concern were identified in all STAIR XI domains assessing 'preclinical assessment and validation' (**Table 3**). Although adult animals were used in most of the preclinical studies, the reported weights and ages of these animals corresponded to young adults, rather than the aged adults that better represent the stroke population. All experiments used only male rodents that were free of common stroke comorbidities. It was also unclear if behavioral testing was performed during the inactive circadian phase or active (dark) phase, which could result in confounding if CCR5 antagonists affect arousal of animals during their inactive period.[33] Furthermore, none of the studies had their protocols or results directly replicated by an independent laboratory, or across multiple sites in a multilaboratory study.[9,34] Regarding stroke recovery, no studies assessed behavioral effects on upper extremity skilled reaching / grasping or potential interactions of CCR5 antagonists with rehabilitative therapies (Table S4).[35–37] Both elements are highly relevant to the CAMAROS trial, as one of the primary outcomes of this trial is upper extremity performance on the Fugl-Meyer and maraviroc administration will be paired with an 8-week exercise program. These findings were supported by the PRIMED[2] tool, which resulted in a Readiness for Translation Score of "medium" (on a scale of "low", "medium", "high"). This tool highlighted similar promising elements, as well as weaknesses, as our analysis above (**Table 3**, Table S4), identifying the limited preclinical evidence for the effects of CCR5 antagonists in clinically relevant sexes, ages, species, and disease comorbidities, without sufficient dose-response

Table 3.

Alignment of included preclinical studies with STAIR recommendations and CAMAROS trial parameters.

Recommendation	Overall preclinical evidence	CAMAROS trial alignment	Notes
Candidate treatment qualification			
1) Establish treatment dose-response	✓	X	• **Preclinical** – doses across all studies from 0.01 to 100 mg/kg • 6 doses tested for infarct volume, 3 doses for behavioral effects • Joy et al. demonstrated that 100 mg/kg of maraviroc in mice results in cerebrospinal fluid levels lower than in humans (13.8±5.4 vs. 33.6–60 ng/mL) • **CAMAROS** – participants take 300 mg doses twice daily for 8 weeks • **Interpretation** – maximum preclinical dose may not represent clinical levels
2) Treatment given after clinically relevant delayed times (one to 4.5 hours post-stroke)	✓	✓	• **Preclinical** – 3/5 studies (all maraviroc) administered drug from 1 to 24 hours post-stroke (acute phase) • Joy et al. initiated maraviroc administration at 20 days post-stroke for 11 weeks (subacute phase) • **CAMAROS** – participants recruited between 5 days and 6 weeks post-stroke (acute / early subacute) • **Interpretation** – one experiment in a time window relevant to clinical trial
3) Both histologic and behavioral outcomes at acute (1 to 3 days) and chronic (7 to 30 days) time points	✓	✓	• **Preclinical** – 2/5 studies included both histologic and behavioral outcomes • Mainly tasks of spontaneous movement (e.g., cylinder) or motor coordination (e.g., grid walk) • Tests ranged from 3 days (acute) to 11 weeks (chronic) post-stroke for all included studies, but short term assessment may only delay cell death[32] • **CAMAROS** - motor learning (Fugl-Meyer Upper Extremity Assessment Score and 10-Meter Walk Test Score) measured as the primary outcome at baseline, 4-week (late subacute), 8-week (late subacute), and 6-month (chronic) • **Interpretation** – challenging to directly compare preclinical and clinical motor tasks, but testing range is comparable. Most relevant Joy et al. experiment (subacute administration) did not measure histologic outcomes
4) Treatment reaches presumed target and causes expected physiological effects that can be assessed with a clinically relevant biomarker	✓	NA	• **Preclinical** – 5/5 studies demonstrated trends for CCR5 antagonism to reduce infarct volume. Plausible that CCR5 influences stroke-relevant mechanisms • **CAMAROS** – does not include outcomes to assess treatment mechanisms
5) Treatment able to pass the blood-brain barrier	✓	NA	• **Preclinical** – 1/5 studies used mass spectrometry to demonstrate that maraviroc is present in the brain and cerebrospinal fluid • **CAMAROS** – does not include outcomes to assess presence of drug in the brain
Preclinical assessment / validation			
1) Aging/adult age	X	X	• **Preclinical** – 4/5 studies used rodents with weights/ages consistent with young adulthood; 0/5 studies used rodents ≥10 months old. • **CAMAROS** – Age ≥18 year old adult participants eligible for recruitment • **Interpretation** – Preclinical studies consistent with CAMAROS eligibility criteria; however, no preclinical study examined middle-aged or elderly animals that would be more representative of the trial and overall stroke populations
2) Male and female animals	X	X	• **Preclinical** – 0/5 studies included female animals • **CAMAROS** – both male and female sexes eligible for recruitment • **Interpretation** – effects of CCR5 antagonists for stroke recovery in female animals represents a critical knowledge gap
3) Animals with co-morbidities	X	X	• **Preclinical** – 0/5 studies included animals with common stroke co-morbidities (e.g., diabetes, hypertension, etc.) • **CAMAROS** - participants with stroke co-morbidities eligible for recruitment • **Interpretation** - effects of CCR5 antagonists for stroke recovery in animals with common stroke comorbidities represents a critical knowledge gap
4) Evidence from two or more laboratories	✓	X	• **Preclinical** – 5/5 studies demonstrated stroke-related benefits; however, no study had their results replicated by independent laboratories • **CAMAROS** – trial protocol will be replicated by multiple study sites • **Interpretation** – future preclinical experiments could consider assessing effects using a multilaboratory approach, or independently replicate the effects of existing preclinical studies
5) Gyrencephalic species	X	X	• **Preclinical** – 5/5 studies conducted in lissencephalic (rodent) species. • **CAMAROS** – humans participants • **Interpretation** – effects of CCR5 antagonists for stroke recovery in gyrencephalic species represents a critical knowledge gap
6) Tests during the awake phase of animals	X	X	• **Preclinical** – 0/5 studies reported assessing outcomes at night (i.e. awake phase of rodents) • **CAMAROS** – follow-up occurs during daytime hours (i.e. awake phase of humans) • **Interpretation** – future preclinical studies should conduct behavioral testing during the awake phase to better align with human testing conditions

information to inform trials (Table S5). Overall, our assessments highlight a variety of knowledge gaps and areas of misalignment between the preclinical evidence and clinical trial parameters that could be improved with further preclinical experimentation.

Discussion

The overall body of preclinical evidence for CCR5 antagonists in stroke demonstrates potential acute neuroprotection with corresponding impairment reduction, as well as improved functional recovery in the sub-acute/early chronic phase. Our systematic review also highlights evidence gaps that could impact successful clinical translation of CCR5 antagonists. Our analysis of 10 independent experiments, identified that acute administration of CCR5 antagonists within the first 24 hours post-stroke was associated with a marked reduction in infarct volume. This neuroprotective reduction of infarct volume did not significantly vary based on treatment dose or any other experimental characteristics.[3,4,6] Overall, the majority of behavioral effects appeared to be in a positive direction, but the low number of included studies precluded meta-analysis of these results. Indeed, no individual behavioral experiment included more than ten CCR5-treated animals, and given the wide range of dosages, timings, routes, stroke models, and rodent strains involved, the certainty of these findings is limited and should be interpreted cautiously. Further investigation is warranted to determine the optimal timing of administration and behavioral domains under which CCR5 antagonists exhibit the strongest post-stroke neuroprotective and recovery-inducing effects.

Despite the positive direction of treatment effects across all studies of CCR5 antagonists, we found a substantial risk of bias in the underlying studies.[24] As is commonly observed in the preclinical literature, all studies either did not adequately report their randomization / blinding methods and exhibited evidence of selective / incomplete reporting.[29–31] Such features are associated with biased overestimations of preclinical treatment efficacy, which raises further concerns about the reliability and validity of the present findings.[38–40]

Comprehensiveness of the preclinical evidence for CCR5 antagonists was assessed in relation to STAIR and SRRR consensus recommendations.[9,11,25,26] These recommendations aim to provide investigators and regulators with “assurance that the candidate treatment shows signals of efficacy and safety, before embarking on an expensive clinical development program”.[11] The included studies provide good initial evidence for acute neuroprotection, as well as mechanistic and behavioral evidence for enhanced recovery in the late sub-acute/early chronic post-stroke phase. However, demonstration of efficacy under a wider range of conditions, such as in aged animals, females, animals with stroke-related comorbidities, more relevant timing of dose administrations, or in conjunction with rehabilitative therapies are necessary to provide further confidence in these findings. In addition, all studies used unique doses, timings, and outcomes, so independent replication of the most promising study parameters would further increase certainty in the evidence.[9,11]

In relation to the ongoing CAMAROS trial assessing maraviroc in the subacute post-stroke phase,[7] the most relevant preclinical evidence comes from one experiment within the Joy et al. study[6] where maraviroc was initially administered at three to four weeks post-stroke and continued daily for 11 weeks. This experiment demonstrated that administration of maraviroc in the late sub-acute/early chronic post-stroke phase improved functional recovery on the grid walk, but not cylinder, task. These experimental conditions could potentially align with the putative therapeutic window and outcomes of interest being assessed in CAMAROS (e.g. 10-minute walk test co-primary outcome).[7] However, caution is warranted as this pivotal supporting preclinical evidence is based on a low sample size (n=9). Moreover, potential differences in dosing, severity of infarct, concomitant rehabilitative therapy, and other factors discussed above could influence the

degree to which these results successfully translate to the clinical environment. Nevertheless, this study provides a plausible biological mechanism and "proof of concept" for how CCR5 antagonism might enhance neuroplasticity that improves functional recovery after stroke.

Our present synthesis of the preclinical evidence for CCR5 antagonists used novel approaches to increase its utility for assessing certainty of the findings and identification of knowledge gaps.

First, we engaged patients with lived experiences of stroke throughout the review process to ensure that our research questions, outcomes, and interpretations aligned with the priorities of the ultimate end-user of stroke research. Second, we incorporated consensus recommendations for both preclinical neuroprotection and recovery research as an evidence evaluation tool, which we found often aligned with the priorities of our patient panel.[9,11,25,26] This guided assessment of the alignment of preclinical evidence with parameters of ongoing clinical trials, as well as appraisal of comprehensiveness of the preclinical evidence with a focus on translational validity[41] rather than only internal validity and risk of bias.[24] We also used this method to provide concrete avenues for future preclinical studies to close knowledge gaps and improve certainty in the effects of CCR5 antagonists under clinically relevant experimental conditions. Similar approaches should be considered by future preclinical systematic reviews to improve interpretation of the preclinical evidence from a translational perspective.

In conclusion, CCR5 antagonists show promise in preclinical studies for stroke neuroprotection, corresponding reduction in impairment, as well as improved functional recovery related to neural repair in the late sub-acute/early chronic phase. However, high risk of bias and the limited (or no) evidence in clinically relevant domains underscore the need for more rigorous and transparent preclinical research to further strengthen the overall evidence base. Addressing these concerns will not only enhance the reliability of preclinical evidence but also better inform the design and execution of clinical trials of CCR5 antagonists, such as the ongoing CAMAROS trial.[7] The integration of expert recommendations, such as STAIR and SRRR, should guide future preclinical investigations and synthesis of the body of preclinical evidence in stroke recovery research.[9,11] Our present approach serves as a template by which the preclinical evidence supporting translation to clinical trials can be weighed when justifying early clinical trials of novel interventions for stroke neuroprotection and recovery.

Non-standard Abbreviations and Acronyms

- CAMAROS: Canadian Maraviroc RCT to Augment Rehabilitation Outcomes After Stroke
- CCR5: C-C chemokine receptor type 5
- DAPTA: D-Ala-Peptide T-Amide
- SRRR: Stroke Recovery and Rehabilitation Roundtable
- STAIR: Stroke Treatment Academic Industry Roundtable
- SYRCLE: Systematic Review Centre for Laboratory Animal Experimentation
- TAK-779: Takeda 779

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Disclosures

None.

Supporting information

Supplementary Materials. Supplemental MethodsFigures S1-S6Tables S1-S5

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Reviewer #1 (Public review):

Summary:

The paper is well-organized, with clearly defined sections. The systematic review methodology is thorough, with clear eligibility criteria, search strategy, and data collection methods. The risk of bias assessment is also detailed and useful for evaluating the strength of evidence. The involvement of a patient panel is noticeable and positive, ensuring the research addresses real-world concerns and aligning scientific inquiry with patient perspectives. The statistical approach used for analyzing seems appropriate.

The authors are encouraged to take into account the following points:

As the authors have acknowledged, there is a high risk of bias across all included studies, particularly in randomization, selective outcome reporting, and incomplete data, which could be highlighted more explicitly in the paper's discussion section, particularly the potential implications for the generalizability of the results. The authors can also suggest mitigation strategies for future studies (e.g., better randomization, blinding, reporting standards, etc.). None of the studies include female animals, and the use of young adult animals (instead of aged models) limits the applicability of the findings to the human stroke population, where stroke incidence is higher in older adults and perhaps the gender issue must be included to reflect the translational aspects. The authors can add to the paper's discussion section that perhaps future preclinical studies should include both sexes and aged animals to align better with the clinical population and improve the translation of findings. Another point is the comorbidity. Comorbidities such as diabetes and hypertension are prevalent in stroke patients. How can these be considered in preclinical designs? The authors should emphasize the importance of future research incorporating such comorbid models to enhance clinical relevance.

None of the studies had independent replication of their findings, which is a key limitation, especially for a field with high translational expectations. This should be highlighted as a critical next step for validating the efficacy of CCR5 antagonists.

The studies accessed limited cognitive outcomes (only one reported a cognitive outcome). Given the importance of cognitive recovery post-stroke, this is a gap to highlight in the discussion. Future studies should include more diverse and comprehensive behavioral assessments, including cognitive and emotional domains, to fully evaluate the therapeutic potential.

The timing of CCR5 administration across studies varies widely (from pre-stroke to several days post-stroke) complicating the interpretation and comparison of results. The authors are encouraged to add that future preclinical studies could focus on narrowing the therapeutic window to more clinically relevant time points.
The paper identifies some alignment with clinical trials, but there are several gaps, too, particularly in the types of behavioral tests used in preclinical studies versus those in clinical trials. If this systematic review and meta-analysis aim to formulate a set of recommendations for future studies, it is important that the authors also propose specific preclinical behavioral tasks that could better align with clinical measures used in trials, like functional assessments related to human stroke outcomes.

The discussion needs some revisions. It could benefit from an expanded explanation of CCR5's mechanistic role in neuroplasticity and stroke recovery. For instance, linking CCR5 antagonism more closely with molecular pathways related to synaptic repair and remyelination would enhance the quality of the discussion and understanding of the drugs' potential.

While the tool is used to assess the risk of bias, it might be helpful to integrate a broader framework for evaluating the quality of included studies. This could include sample size justifications, statistical power analysis, or the use of pre-registration in animal studies. These elements can also introduce bias or minimize those if in place.

Please also highlight confounding factors that might have influenced the results in the included studies, such as variation in stroke models, dosing regimens, or behavioral assessment methods.

There is some discussion of the meta-analysis' limitations due to the few studies, but this point could be more thoroughly addressed. Please consider including a more critical discussion of the limitations of pooling data from heterogeneous study designs, stroke models, and outcome measures. What can this lead to? Is it reliable to do so, or does it lack

scientific rigor? The authors are encouraged to formulate a balanced discussion adding, positive and negative aspects.
The conclusion should more explicitly acknowledge that while CCR5 antagonists show potential, the findings are still preliminary due to the limitations in the preclinical studies (high bias risk, lack of diverse animal models). Overall, the conclusion can end with a call for rigorous, well-controlled, and replicated studies with improved alignment to clinical populations and trials to show that the conclusion remains inconclusive, considering what has been analyzed here.

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Reviewer #2 (Public review):

Summary:

This is an interesting, timely, and high-quality study on the potential neuroprotective capabilities of C-C chemokine receptor type 5 (CCR5) antagonists in ischemic stroke. The focus is on preclinical investigations.

Strengths:

The results are timely and interesting. An outstanding feature is that stroke patient representatives have directly participated in the work. Although this is often called for, it is hardly realized in research practice, so the work goes beyond established standards.

The included studies were assessed regarding the therapeutic impact and their adherence to current quality assurance guidelines such as STAIR and SRRR, another important feature of this work. While overall results were promising, there were some shortcomings regarding guideline adherence.

The paper is very well written and concise yet provides much highly useful information. It also has very good illustrations and extremely detailed and transparent supplements.

Weaknesses:

Although the paper is of very high quality, a couple of items that may require the authors' attention to increase the impact of this exciting work further. Specifically:

Major aspects:

(1) I hope I did not miss that (apologies if I did), but when exactly was the search conducted? Is it possible to screen the recent literature (maybe up to 12/2024) to see whether any additional studies were published?

(2) Please clearly define the difference between "study" and "experiment," as this is not entirely clear. Is an "experiment" a distinct investigation within a particular publication (=study) that can describe more than one such "experiment"? Thanks for clarifying.

(3) Is there an opportunity to conduct a correlation analysis between the quality of a study (for instance, after transforming the ROB assessment into a kind of score) and reported effect sizes for particular experiments or studies? This might be highly interesting.

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