

Effect and mechanisms of resveratrol in animal models of ischemic stroke: A systematic review and Bayesian meta-analysis

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Abstract

Resveratrol (RSV) holds promise as cerebroprotective treatment in cerebral ischemia. This systematic review aims to assess the effects and mechanisms of RSV in animal models of ischemic stroke. We searched Medline, Embase and Web of Science to identify 75 and 57 eligible rodent studies for qualitative and quantitative syntheses, respectively. Range of evidence met 10 of 13 STAIR criteria. Median (Q1, Q3) quality score was 7 (5, 8) on the CAMARADES 15-item checklist. Bayesian meta-analysis showed SMD estimates (95% CI) favoring RSV: infarct size (−1.72 [−2.03; −1.41]), edema size (−1.61 [−2.24; −0.98]), BBB impairment (−1.85 [−2.54; −1.19]), neurofunctional impairment (−1.60 [−1.92; −1.29]), and motor performance (1.39 [0.64; 2.08]); and less probably neuronal survival (0.63 [−1.40; 2.48]) and apoptosis (−0.96 [−2.87; 1.02]). Species (rat vs mouse) was associated to a larger benefit. Sensitivity analyses confirmed robustness of the estimates. Reduction of oxidative stress, inflammation, and apoptosis underlie these effects. Our results quantitatively state the beneficial effects of RSV on structural and functional outcomes in rodent stroke models, update the evidence on the mechanisms of action, and provide an exhaustive list of targeted signaling pathways. Current evidence highlights the need for conducting further high-quality preclinical research to better inform clinical research.

Keywords

Animal models, Bayesian meta-analysis, cerebroprotection, ischemic stroke, resveratrol

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Introduction

Stroke remains as a leading cause of death, permanent disability and dementia worldwide.¹ Major advances in the treatment of acute ischemic stroke came from vascular approaches to dissolve or remove the occluding clot. However, only a minority of patients benefit from such treatments.² Although reperfusion therapies are therefore restricted, they are more clinically beneficial

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than cerebroprotective strategies, which have failed in the translation from bench to bedside.³ Neuroprotectants should be revisited in the era of thrombectomy.⁴

Resveratrol (3, 5, 4'-trihydroxy-trans-stilbene) occurs naturally in skin and seeds of the fruits of various edible plants, especially in grapes.⁵ A popular epidemiological study suggested that the French population has a relatively low incidence of coronary heart disease, despite having a diet relatively rich in saturated fat. Resveratrol (RSV) has been identified as a potential factor responsible for the French paradox.⁶ Previous studies have revealed the multiple bioactivities of RSV including: anti-oxidation, anti-inflammation, anti-cancer, cardioprotection, regulation of glucose homeostasis and neuroprotection.⁷

This systematic review aims to describe which RSV-based interventions have been tested in ischemic stroke animal models, the range of conditions under which RSV efficacy has been tested, and the mechanisms of action involved. A Bayesian meta-analysis accurately assesses both the overall efficacy of RSV and the impact of factors relating to internal and external validity, thus giving valuable insights for translational success. Our goal is to provide evidence to improve the rigor of the conduct and reporting of preclinical research on RSV treatment for ischemic stroke, in addition to inform randomized controlled trials in clinical stroke research.

Research questions

What is the effect of resveratrol on brain structural and functional outcomes in animal models for ischemic stroke?

Which molecular mechanism/s of action underlie the effect of resveratrol on ischemic stroke brain damage?

Methods

All methods were prespecified in a systematic review protocol for animal intervention studies (CRD42021266048) that was registered and published online (July 23, 2021) on the PROSPERO International prospective register of systematic reviews (National Institute for Health Research). Available from: https://www.crd.york.ac.uk/prospero/display_record.php?ID=CRD42021266048. The methodological approach followed specific guidelines for meta-analysis of data from animal studies,⁸ as well as the Preferred Reporting Items for Systematic Reviews and Meta-Analyses guidelines and recommendations (PRISMA 2020).⁹ Data, analytic code and any other material generated during the current study but not published within the article, including supplemental

material, are available from the corresponding authors upon reasonable request.

Literature search strategy

The search process was carried out according to the guidelines to systematically identify all relevant animal studies.¹⁰ Two reviewers (JBS and MALM) identified studies on RSV in animal models of ischemic stroke from electronic searches of Medline (using PubMed interface), Embase and Web of Science, up to and including June 18, 2021. Search syntax was: resveratrol AND (stroke OR ischem* OR ischaem* OR MCAO OR pMCAO OR tMCAO) AND (rat OR rats OR mouse OR mice OR rodent* OR rabbit* OR dog OR dogs OR canine OR cat OR cats OR feline OR pig OR pigs OR porcine OR monkey*) AND (infarct* OR neurofunct* OR neurologic* OR edema OR oedema OR damage OR injury OR neuroprotect* OR mechanism*) AND (brain OR cerebral) NOT review. Search fields were [Title] in Medline (PubMed), Embase, and Web of Science, for resveratrol; [Publication type] in MEDLINE (PubMed) and EMBASE, and [Document type] in Web of Science, for review; and [Title/Abstract] in Medline (PubMed) and Embase, and [Topic] in Web of Science, for all other terms. There were no language restrictions. References were added to a Mendeley Reference Manager library dedicated to this review's topic, which automatically checked for duplicates. Other sources for study identification were the reference lists of included articles and relevant narrative reviews.^{11–14} The search was not extended to unpublished studies or other sources of grey literature.

Study selection: eligibility criteria and screening process

We included studies published as full-length original research articles in any language, provided that the English abstract was available, and abstracts of conference proceedings in English language. In cases of studies with duplicate or overlapping animal cohorts, only the publication with the most complete dataset was included. Protocol articles, review articles and abstracts later published in full were also excluded.

With regard to study design, primary experimental studies with control group (receiving no treatment, vehicle, or other sham intervention), regardless of randomization, were included. Preclinical studies in rats, mice, rabbits, or other laboratory animal species used in models of ischemic stroke, regardless of age and sex, were included. RSV-based interventions were included: RSV (or analogues) administration, alone or in co-treatment with medications commonly used in stroke

patients (i.e., rtPA, statins, blood pressure-lowering medication, aspirin, etc.), regardless of dose, route, method and treatment schedule. Finally, studies analyzing brain damage (i.e., infarct size, brain edema, blood-brain barrier [BBB] impairment, apoptosis, neuronal injury, and/or neurofunctional outcomes) were included.

Observational studies, experimental studies without control group, and reviews, were excluded. In vitro studies (i.e., cell cultures), ex vivo studies (i.e., brain slices), and studies in animal models of global cerebral ischemia, hemorrhagic stroke, traumatic brain injury, or spinal cord injury were excluded. Administration of complex plant extracts containing RSV, or administration of RSV in combination with other compounds, either natural or synthetic, were excluded. Finally, studies reporting results without individual or aggregate data, or no statement of sample size, were excluded.

Search results were screened independently by title and abstract by two reviewers, using the above predefined inclusion/exclusion criteria (MALM and MCR). Full reports were obtained for all titles that appeared to meet the inclusion criteria or where there was any uncertainty. Two reviewers (MCR and MCB) then screened the full-text reports of potentially eligible studies and decided whether these met the inclusion criteria. Discrepancies were resolved through discussion and consensus with the supervisor (JBS).

Data extraction

Two reviewers (MCR and MCB) independently extracted study ID (first author, year, and journal name), study characteristics for the assessment of external validity, and study quality data for the assessment of internal validity.

Study characteristics included experimental groups and sample sizes, animal model (species, strain, gender, age, co-morbidities, type of ischemic stroke, duration of ischemia, and anesthetic used), intervention (type of intervention, RSV dosage, route, method and treatment schedule, and co-treatment), outcome measures, time-points and methods of assessment (infarct size, brain edema, apoptosis, BBB impairment, neuronal injury, and neurofunctional deficit).

Range of evidence was assessed by using a 13-item checklist,¹⁵ taken from the updated STAIR criteria¹⁶: (1) evidence from two or more laboratories, (2) from two or more species, (3) from animals with co-morbidities, (4) from male and female animals, (5) from both permanent and temporary models of ischemia, (6) testing at least two doses of the drug, (7) with some doses given at least 1 h after vessel occlusion, (8) testing using a feasible route of drug delivery, (9) use of

both histologic and behavioral outcomes, (10) outcome measured at least 4 weeks after vessel occlusion, (11) from species other than rodents, (12) interaction studies with medications commonly used in stroke patients, and (13) use of relevant biomarker endpoints.

Study quality was scored to assess risk of bias by using both the former 10-item CAMARADES' checklist,¹⁷ and an adapted checklist¹⁵ that included up to 15 relevant items from the updated STAIR criteria¹⁶: (1) peer reviewed publication, (2) control of temperature, (3) randomization of group allocation, (4) blinded induction of ischemia, (5) blinded assessment of outcome, (6) avoidance of anesthetics with marked intrinsic neuroprotective properties, (7) use of animals with co-morbidities (e.g. hypertension, diabetes), (8) sample size calculation, (9) statement of compliance with animal welfare requirements, (10) statement of potential conflicts of interest, (11) physiological monitoring during stroke induction (in addition to control of temperature, e.g. blood pressure, gases), (12) prespecified inclusion and exclusion criteria, (13) reporting of animals excluded from analysis, (14) reporting of study funding, and (15) injury confirmed via laser Doppler or perfusion imaging.

Two unblinded reviewers (MALM and MCB) independently collected data from outcome measures for all time points, as well as data from molecular targets and suggested mechanisms of action. Discrepancies were resolved through discussion and consensus with the supervisor (JBS). Infarct size (continuous variable, expressed in absolute or percent units) was considered as the primary outcome measure to establish whether RSV might have cerebroprotective effects. Secondary outcome measures were brain edema, BBB impairment, neuronal injury, apoptosis (continuous variables, expressed in absolute or percent units), and neurofunctional outcomes (continuous, expressed in absolute units, or ordinal, in absolute units within the score range). For each outcome measure, the number of animals in which this was assessed, the aggregate value of effect (i.e. mean or median) and a measure of group variance were extracted. Data were extracted from text and tables. When only graphic presentation was available, data were obtained by using WebPlotDigitizer 4.2 (<https://apps.automeris.io/wpd/>) on highly magnified images. Information was also requested directly from authors for checking extracted data or if it was unavailable in the publication. For articles in language other than English, only data from the English abstract were collected.

Analysis

For each outcome measure (infarct, edema, BBB impairment, neuronal injury, apoptosis, and

neurofunctional outcomes), we calculated a standardized mean difference (SMD) as effect size for each comparison. Then, estimates from the different comparisons were pooled using Bayesian hierarchical random effects modeling to account for heterogeneity, with prior distributions set as a normal distribution with mean 0 and SD (standard deviation) 1 for the intercept, and a Cauchy distribution with location 0 and scale 0.5 for the SD. Results were displayed in forest plots. When studies reported multiple comparison experiments, with a control group serving more than one treatment group, this was introduced in the model as a three-level hierarchical structure where comparisons were nested into studies. Heterogeneity was described using τ^2 (estimation of between-study variance). The models were expanded by including quality score and different study characteristics as covariates to identify sources of heterogeneity on the estimated effects of RSV on infarct size and neurofunctional impairment. Two sensitivity analyses were performed. The first one by setting a more stringent prior distribution for the intercept (normal with mean 0 and SD 0.5), and the second one by adjusting a model which included only the studies carried out with blind ischemia induction. Statistical analyses were performed using R (version 4.2) and R packages clickR (version 0.9.27), metafor (version 3.9-11), and brms (version 2.18.0). All estimates included a 95% credible interval (CI).

Results

Literature search

A detailed study selection flow chart is shown in Figure 1. Briefly, a total of 437 records were identified in Medline, Embase and Web of Science databases. Three additional records were identified from other sources. After removing duplicate and not relevant records, 90 full-text articles and one congress abstract were assessed for eligibility. Reasoned exclusions (16 studies)^{18–33} rendered 75 studies included in the qualitative synthesis,^{34–108} and further exclusions (18 studies)^{36,43,44,46,56,61,64,65,69,76,78,83,92,93,98,103,104,107} resulted in 57 studies subjected to meta-analysis of seven stroke outcomes.^{34,35,37–42,45,47–55,57–60,62,63,66–68,70–75,77,79–82,84–91,94–97,99–102,105,106,108}

Study characteristics

A summary of the study characteristics is shown in Table 1. Briefly, studies were carried out mostly (70.67%) in rats (especially Sprague-Dawley), but also (24.00%) in mice (especially C57BL/6). Almost all the studies (88.00%) used adult male animals.

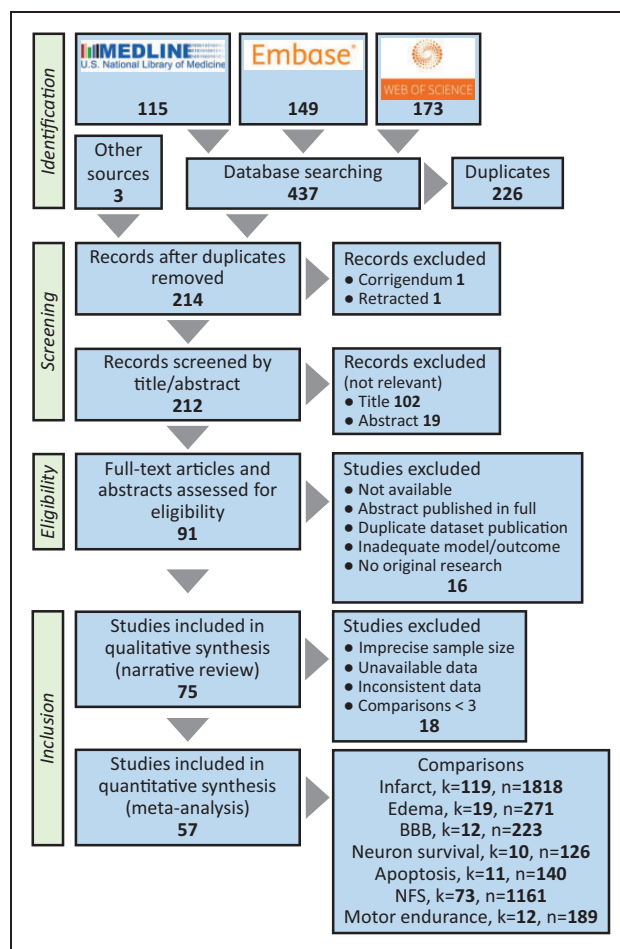


Figure 1. PRISMA flow diagram of study selection. BBB: blood brain barrier; NFS: neurofunctional score.

Only two studies were carried out in adult young and aged female mice, respectively. Intraluminal filament transient middle cerebral artery occlusion (MCAO) of different durations (30–180 min) was the most used (82.67%) ischemia method. Only less than one third of the studies (29.33%) avoided the use of intrinsically neuroprotective anesthetics. Only two studies used the RSV analogs oxyresveratrol and piceatannol, respectively. Median (Q1, Q3) RSV dose was 10 (1, 30) mg/kg. Acute (57.33%) and chronic (46.67%) RSV administrations, before (49.33%) or after (41.33%) the ischemia, were almost equally carried out. Intraperitoneal (57.33%), intravenous (16.00%), and oral gavage (14.67%) were the preferred administration routes. Infarct size (68.00%), and neurofunctional impairment (40.00%) were the main outcome measures, usually assessed up to 24 h after the ischemia (57.33%). Infarct size was usually measured by 2,3,5-triphenyltetrazolium chloride (TTC) staining (76.00%), and also by cresyl violet (CV) staining (5.33%). Other structural outcome measures were edema size (water content),

Table 1. Study characteristics summary.

Variable	n (%)	Variable	n (%)
Species & strain		RSV treatment	
Mouse Balb/c	3 (4.00)	Resveratrol	73 (97.33)
Mouse C57BL/6	13 (17.33)	Resveratrol analog	2 (2.67)
Mouse Kunming	2 (2.67)	RSV regimen	
Rat Long-Evans	2 (2.67)	Acute	43 (57.33)
Rat Sprague-Dawley	39 (52.00)	Chronic	35 (46.67)
Rat Wistar	12 (16.00)	N/A	1 (1.33)
N/A	4 (5.33)	RSV route	
Gender		Intraarterial	1 (1.33)
Male	66 (88.00)	Intracortical	1 (1.33)
Female	2 (2.67)	Intraperitoneal	43 (57.33)
N/A	8 (10.67)	Intravenous	12 (16.00)
Age		Oral gavage	11 (14.67)
Adult	66 (88.00)	N/A	7 (9.33)
Aged	1 (1.33)	RSV timing	
N/A	8 (10.67)	Pre-ischemia	37 (49.33)
Ischemia method		Post-ischemia	31 (41.33)
MCAO intraluminal filament	62 (82.67)	Pre- and post-ischemia	5 (6.67)
MCAO clipping/ligation	7 (9.33)	N/A	7 (9.33)
Cortical photothrombosis	1 (1.33)	Outcome measure	
N/A	5 (6.67)	Infarct	64 (85.33)
Ischemia timing		Edema	20 (26.67)
Transient (30 min)	5 (6.67)	BBB impairment	9 (12.00)
Transient (30 + 30 min)	1 (1.33)	Neuronal injury	11 (14.67)
Transient (45 min)	1 (1.33)	Cell apoptosis	12 (16.00)
Transient (60 min)	13 (17.33)	Neurofunctional impairment	47 (62.67)
Transient (90 min)	11 (14.67)	Motor performance	5 (6.67)
Transient (120 min)	25 (33.33)	Outcome assessment time	
Transient (180 min)	2 (2.67)	≤24 h	43 (57.33)
Transient (N/A)	5 (6.67)	<7 d	13 (17.33)
Permanent	8 (10.67)	≥7 d	14 (18.67)
N/A	4 (5.33)	N/A	5 (6.67)
Anesthetic			
Neutral	22 (29.33)		
Neuroprotective/unknown	53 (70.67)		

BBB: blood brain barrier; MCAO: middle cerebral artery occlusion; N/A: not available; RSV: resveratrol.

BBB impairment (Evans blue extravasation), neuronal injury (hematoxylin and eosin [H&E] staining), and cell apoptosis (terminal deoxynucleotidyl transferase dUTP nick end labeling [TUNEL] assay). Neurofunctional impairment was usually assessed by using different neurofunctional scores (NFS; i.e.: Bederson, Belayev, Clark, Garcia, Longa, Menzies, and modified neurological severity score [mNSS] scales), but also the cylinder, corner, and Morris Water Maze (MWM) tests. Motor performance was specifically measured in some studies by means of the Rotarod test. Key characteristics of each study are detailed in Supplemental Table 1.

Range of evidence

The range of evidence met ten of a possible 13 updated STAIR criteria assessed. Forty-seven studies subjected to

meta-analysis came from many laboratories in 10 countries, mainly China (54.39%) and USA (10.53%). They used two rodent species (mostly different rat strains, but also mice strains). In addition to adult male healthy animals, one study used adult female animals and one study used aged female animals. RSV was mostly tested in temporary models of ischemia, mainly intraluminal filament MCAO, but also in permanent ischemia models. A highly wide range of RSV doses were tested, given up to 5 h after ischemia onset (3 h after reperfusion), and using feasible routes of drug delivery like oral gavage. Seven outcome measures (structural and functional) were assessed, in some cases up to 28 days after the ischemia. Levels of clinically relevant blood biomarkers (i.e., pro- and anti-inflammatory cytokines, lactate dehydrogenase, and NO) were measured.^{41,53,57,90,97,100,102}

Study quality

Table 2 shows methodological quality for each of the 54 studies (with English full-length article available) included in the meta-analysis. Overall, the median (Q1, Q3) quality score (QS) was 5 (4, 6) on the 10-item checklist, and 7 (5, 8) on the extended 15-item checklist.

Effects of resveratrol on cerebral blood flow

Brain perfusion was measured by laser-Doppler flowmetry in 19 studies to confirm blood flow reduction during MCAO and successful subsequent reperfusion. Median (Q1, Q3) occlusion time was 60 (53, 90) min, ranging from 30 to 120 min.^{38,40–42,48,49,53–55,59,67,68,71,79,85,86,88,96,99}

In 10 of these studies, RSV was given as pretreatment, either as acute (single dose) or chronic (daily doses) administrations.^{38,40,49,53–55,59,67,71,79}

Local brain perfusion before, during, and after MCAO was compared between vehicle- and RSV-treated groups in 5 of these studies, with no significant differences.^{38,53,55,71,79} In one of these studies, regional blood flow was also measured by infusing the tracer [¹⁴C]-iodoantipyrine during MCAO. Acute RSV pretreatment did not significantly alter blood flow in the ischemic core or in the penumbra regions, nor did it alter blood flow in contralateral regions.⁷⁹

Effects of resveratrol on ischemic stroke outcomes

Effects of RSV on the seven ischemic stroke outcomes according to the Bayesian model are summarized in Table 3, and depicted in Figure 2. Effects on infarct size were reported in 51 studies describing 119 comparisons. Median (Q1, Q3) sample sizes per comparison were 7 (5, 9) in the control group and 6 (5, 8) in the RSV group. Figure S1 shows individual effect sizes for each of the 119 comparisons. Overall, the estimated probability of RSV reducing infarct was higher than 99.99%, with a pooled SMD estimate of -1.72 (95% CI $[-2.03; -1.41]$), and low between-study heterogeneity in the estimates ($\tau^2 = 1.43$).

Effects on edema size were reported in 14 studies describing 19 comparisons. Sample sizes per comparison were 7 (5, 10) in both the control and the RSV groups. Figure S2 shows individual effect sizes for each of the 19 comparisons. Overall, the estimated probability of RSV reducing edema was higher than 99.99%, with a pooled SMD estimate of -1.61 $[-2.24; -0.98]$, and low between-study heterogeneity in the estimates ($\tau^2 = 1.04$).

Effects on blood brain barrier (BBB) impairment were reported in 8 studies describing 12 comparisons. Sample sizes per comparison were 5.5 (4, 7) in both the control and the RSV groups. Figure S3 shows individual

effect sizes for each of the 12 comparisons. Overall, the estimated probability of RSV reducing BBB impairment was higher than 99.99%, with a pooled SMD estimate of -1.85 $[-2.54; -1.19]$, and low between-study heterogeneity in the estimates ($\tau^2 = 0.66$).

Effects on neuronal survival were reported only in 3 studies describing 10 comparisons. Sample sizes per comparison were 6 (6, 6) in both the control and the RSV groups. Figure S4 shows individual effect sizes for each of the 10 comparisons. Overall, the estimated probability of RSV increasing neuronal survival was 75.93%, with a pooled SMD estimate of 0.63 $[-1.40; 2.48]$, and high between-study heterogeneity in the estimates ($\tau^2 = 29.25$).

Effects on apoptotic cell counts were reported in 8 studies describing 11 comparisons. Sample sizes per comparison were 6 (4, 10) in both the control and the RSV groups. Figure S5 shows individual effect sizes for each of the 11 comparisons. Overall, the estimated probability of RSV reducing apoptosis was 83.38%, with a pooled SMD estimate of -0.96 $[-2.87; 1.02]$, and high between-study heterogeneity in the estimates ($\tau^2 = 68.15$).

Effects on neurofunctional impairment score were reported in 30 studies describing 73 comparisons. Sample sizes per comparison were 7 (5, 8.5) in the control group and 6 (5, 9) in the RSV group. Figure S6 shows individual effect sizes for each of the 73 comparisons. Overall, the estimated probability of RSV reducing neurofunctional impairment was higher than 99.99%, with a pooled SMD estimate of -1.60 $[-1.92; -1.29]$, and low between-study heterogeneity in the estimates ($\tau^2 = 1.00$).

Specific effects on motor performance in the rotarod test were reported in 4 studies describing 12 comparisons. Sample sizes per comparison were 6 (5, 11.5) in the control group and 6 (6, 10.75) in the RSV group. Figure S7 shows individual effect sizes for each of the 12 comparisons. Overall, the estimated probability of RSV improving motor performance was 99.91%, with a pooled SMD estimate of 1.39 $[0.64; 2.08]$, and low between-study heterogeneity in the estimates ($\tau^2 = 0.86$).

Meta-regression to identify sources of heterogeneity and to assess the impact of individual study characteristics and study quality score on the estimated effects of RSV on infarct size and neurofunctional impairment showed that species (rat) was associated to a larger beneficial effect in the case of infarct size (estimate = -1.78 , 95% CI $[-2.79; -0.86]$). The same association was found for neurofunctional impairment. However, in this case the uncertainty regarding the direction of this effect was higher (estimate = -0.74 , $[-2.12; 0.59]$). There was little evidence suggesting study quality affected outcome measures for infarct

Table 2. Study quality report.

Author/s	Year	Journal	QS															QS	
			1	2	3	4	5	6	7	8	9	10	0-10	11	12	13	14	15	0-15
Abdel-Aleem et al.	2016	Arch Physiol Biochem	+	+							+	+	4				+	+	6
Alquisiras-Burgos et al.	2020	Exp Neurol	+		+						+		3		+			+	5
Ashafaq et al.	2021	Int Immunopharmacol	+					+	+		+	+	5					+	6
Clark et al.	2012	PLoS One	+	+	+	+	+	+			+	+	7		+	+	+	+	12
Dong et al.	2008	J Vasc Surg	+	+	+		+					+	5				+	+	7
Dou et al.	2019	Cell Mol Neurobiol	+	+	+			+			+	+	6			+		+	8
Faggi et al.	2018	Int J Mol Sci	+	+	+		+				+	+	6		+			+	9
Fang et al.	2015	Int J Clin Exp Med	+	+	+		+	+			+	+	7						7
Gutiérrez-Aguilar et al.	2020	Brain Sci	+	+							+	+	4		+			+	6
He et al.	2017	Int Immunopharmacol	+	+			+	+			+		5			+		+	8
Hermann et al.	2015	Neurobiol Dis	+	+	+	+	+				+	+	7				+	+	10
Hou et al.	2018	Genes Dis	+		+		+	+				+	5				+		6
Huang et al.	2001	Life Sci	+	+	+	+	+				+		6		+				7
Inoue et al.	2003	Neurosci Lett	+										1				+		2
Jeong et al.	2016	Neurobiol Aging	+	+	+	+	+			+	+	+	8			+	+	+	12
Koronowski et al.	2015	Stroke	+	+	+	+	+				+	+	7		+	+	+	+	11
Koronowski et al.	2017b	Stroke	+	+	+		+			+	+	+	7		+	+	+	+	11
Lei et al.	2019	Exp Ther Med	+	+							+	+	4				+		5
Li et al.	2010	Neurochem Int	+	+	+						+		4				+		5
Li et al.	2012	Brain Res	+	+	+		+	+			+		6				+		7
Li et al.	2015	Mol Med Rep	+		+			+			+	+	5		+		+	+	8
Li et al.	2016	Pharmacol Biochem Behav	+	+			+	+			+		5				+		6
Lin et al.	2013	J Mol Neurosci	+	+	+			+			+	+	6		+	+			8
Liu et al.	2018	J Stroke Cerebrovasc Dis	+	+	+		+	+			+		6		+	+	+		9
Lopez et al.	2016	J Cereb Blood Flow Metab	+		+		+				+	+	5		+		+	+	8
Lu et al.	2020	Nanomedicine	+	+			+				+	+	5		+		+	+	8
Meng et al.	2015	Exp Ther Med	+	+	+						+		4		+		+	+	7
Narayanan et al.	2015	Stroke	+		+	+					+	+	5			+		+	8
Pang et al.	2015	Neuropharmacology	+	+	+						+	+	5				+		6
Park et al.	2019	Lab Anim Res	+								+	+	3				+		4
Pineda-Ramírez et al.	2020	Mol Neurobiol	+	+	+						+		4		+	+	+		7
Ren et al.	2011	Neurochem Res	+	+	+		+	+			+		6		+		+		8
Sakata et al.	2010	Exp Neurol	+	+							+		3				+	+	5
Saleh et al.	2010	Neuroscience	+	+							+		3		+				4
Saleh et al.	2013	Neurosci Lett	+	+							+		3						3
Saleh et al.	2014	PLoS One	+	+							+	+	4		+		+		6
Shi et al.	2016	Biomed Res Int	+					+			+	+	4						4
Shin et al.	2010	J Neuroimmunol	+	+	+		+				+		5			+		+	8
Shin et al.	2012	Neurochem Res	+	+	+						+	+	5			+		+	8
Sinha et al.	2002	Life Sci	+	+				+			+		4		+				5
Su et al.	2016	Neurol Res	+		+	+	+				+	+	6			+	+		9
Teertam et al.	2020	J Clin Neurosci	+		+			+			+	+	5				+		6
Tsai et al.	2007	J Vasc Surg	+	+	+	+	+				+	+	7		+		+		9
Wan et al.	2016	Brain Res Bull	+		+		+				+		4				+		5
Wang et al.	2014	Cell Physiol Biochem	+		+		+	+			+		5						5
Wei et al.	2015	J Mol Neurosci	+	+	+		+				+		5			+		+	8
Wu et al.	2019	J Biomater Tissue Eng	+		+			+					3				+		4
Xu et al.	2018	Front Pharmacol	+	+			+				+	+	5		+	+		+	9
Yang et al.	2016	J Stroke Cerebrovasc Dis	+	+	+		+	+			+		6				+		7
Yao et al.	2021	Front Med	+		+						+	+	4				+		5
Yousuf et al.	2009	Brain Res	+	+				+			+		4						4
Yu et al.	2017	Mol Neurobiol	+	+			+	+			+	+	6			+		+	8
Yu et al.	2021	Life Sci	+				+				+	+	4				+		5
Zou et al.	2017	Int J Clin Exp Med	+	+	+			+			+	+	6				+		7

Study quality (SQ) items are (1) peer-reviewed publication, (2) control of temperature, (3) randomization of group allocation, (4) blinded induction of ischemia, (5) blinded assessment of outcome, (6) avoidance of anesthetics with marked intrinsic neuroprotective properties, (7) use of animals with comorbidities (e.g. hypertension, diabetes), (8) sample size calculation, (9) statement of compliance with animal welfare requirements, (10) statement of potential conflicts of interest, (11) physiological monitoring during stroke induction (in addition to control of temperature, e.g. blood pressure, gases), (12) prespecified inclusion and exclusion criteria, (13) reporting of animals excluded from analysis, (14) reporting of study funding and (15) injury confirmed via laser Doppler or perfusion imaging.

Table 3. Resveratrol effects on ischemic stroke outcomes.

Outcome	Studies (n)	Quality score ^a (median (Q1, Q3))	Comparisons (k)	Heterogeneity (τ^2)	Resveratrol (n)	Control (n)	SMD [95% CI]	Benefit probability (%)
Infarct size	51	7 (5, 8)	119	1.43	836	982	-1.72 [-2.03; -1.41]	>99.99
Edema size	14	8 (5.75, 8)	19	1.04	137	134	-1.61 [-2.24; -0.98]	>99.99
BBB impairment	8	8 (6.5, 9)	12	0.66	112	111	-1.85 [-2.54; -1.19]	>99.99
Neuronal survival	3	7 (6.5, 7)	10	29.25	63	63	0.63 [-1.40; 2.48]	75.93
Cell apoptosis	8	7 (5.75, 8)	11	68.15	70	70	-0.96 [-2.87; 1.02]	83.38
Neurofunctional impairment score	30	7.5 (5.25, 8)	73	1.00	581	580	-1.60 [-1.92; -1.29]	>99.99
Motor performance	4	5.5 (5, 7)	12	0.86	97	92	1.39 [0.64; 2.08]	99.91

^aStudy quality scored using the extended 15-item CAMARADES' checklist.
BBB: blood brain barrier; CI: credible interval; SMD: standardized mean difference.

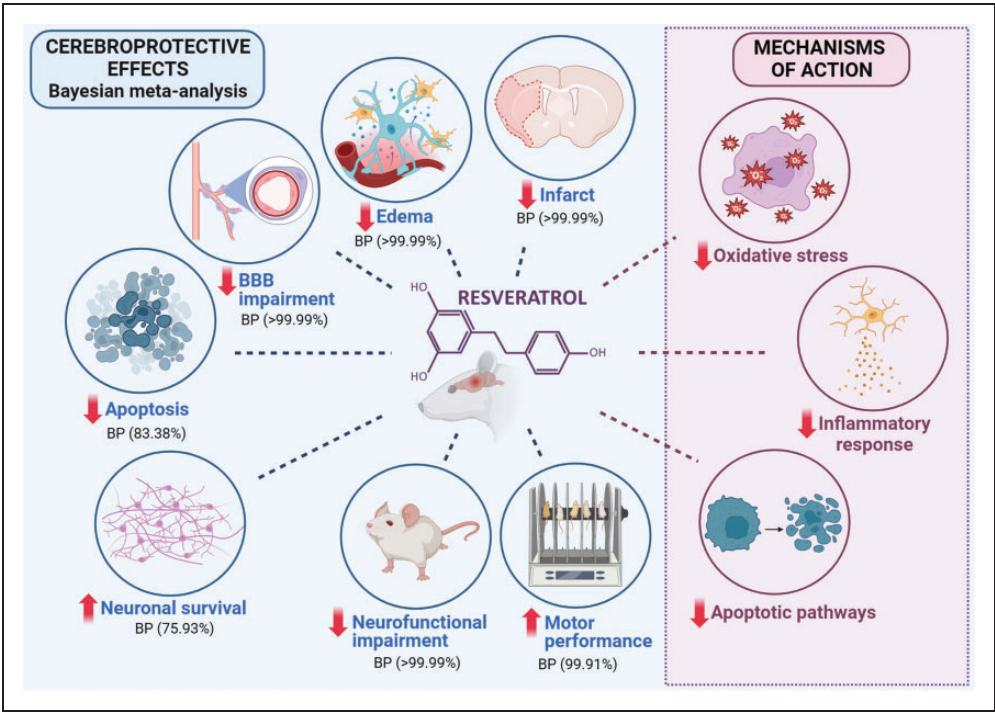


Figure 2. Graphical summary of the cerebroprotective effects of resveratrol in rodent models of ischemic stroke, and the underlying mechanisms of action. BBB: blood brain barrier; BP: benefit probability. Created with BioRender.com.

size or neurofunctional impairment, with estimates in both cases being almost equal to zero and low absolute values for the extremes of the CIs. Highest density intervals (HDI) for all the parameters included in the meta-regression are shown in Figure S8 for infarct and Figure S9 for neurofunctional impairment.

Sensitivity analysis showed that the conclusions for the RSV effects on the different outcomes were robust in relation to the specified priors. All CIs showed an effect for RSV after readjusting the models with the more stringent priors (Table 4). Regarding the analysis for infarct size including only studies carried out with blind ischemia induction, the SMD estimate for the

effect of RSV was smaller but still clearly different from zero (−1.08 [−1.44; −0.74]).

Mechanisms of resveratrol cerebroprotection in ischemic stroke

Fifty-five studies reported the levels of local or systemic molecular markers for targeted signaling pathways and suggested the mechanisms of action underlying the cerebroprotective effects of resveratrol (Supplemental Table 2). As shown in Figure 2, main mechanisms of action were reduction of oxidative stress,^{28,34,37,38,45,49,59,64,68,70,76–78,83,85–88,90,94,99,102} decrease in inflammatory

Table 4. Sensitivity analysis of resveratrol effects on ischemic stroke outcomes.

Outcome	Prior (Intercept)	SMD [95% CI]	Benefit probability (%)
Infarct size	N (0, 1)	−1.72 [−2.03; −1.41]	>99.99
	N (0, 0.5)	−1.59 [−1.88; −1.31]	>99.99
Edema size	N (0, 1)	−1.61 [−2.24; −0.98]	>99.99
	N (0, 0.5)	−1.19 [−1.76; −0.47]	99.93
BBB impairment	N (0, 1)	−1.85 [−2.54; −1.19]	>99.99
	N (0, 0.5)	−1.31 [−1.97; −0.43]	99.85
Neurofunctional impairment score	N (0, 1)	−1.60 [−1.92; −1.29]	>99.99
	N (0, 0.5)	−1.49 [−1.79; −1.20]	>99.99
Motor performance	N (0, 1)	1.39 [0.64; 2.08]	99.91
	N (0, 0.5)	0.98 [0.21; 1.62]	98.88

BBB: blood brain barrier; CI: credible interval; SMD: standardized mean difference.

response,^{34,37,38,41,45,48,53,56,57,59,64–66,69,85,97,100,107,108} and inhibition of apoptotic pathways.^{34,37,45,46,49,50,60,66,68,70,77,86,89,94,96,97,99,102,103,106,107} Resveratrol could also target signaling pathways involved in the reduction of BBB impairment,⁹⁶ edema,^{35,59} excitotoxicity,^{28,58} and lead toxicity,⁷⁸ as well as in the promotion of neurotrophic factors,⁸⁴ neurogenesis,^{49,68,105} neuronal survival,^{62,63,73,103} synaptic plasticity,⁶⁴ microgliosis,⁴⁹ angiogenesis,^{40,49} oligodendrogenesis,¹⁰⁵ autophagy,^{44,48,66,75,94} mitophagy,⁹⁸ ischemic tolerance,^{54,81} and energy metabolism.^{28,83}

Discussion

The results of this systematic review and Bayesian meta-analysis support a beneficial effect of RSV on brain structural and functional outcomes in animal models for ischemic stroke. Convincing evidence of RSV cerebroprotective efficacy is shown in terms of reductions in infarct and edema sizes, and decrease in BBB impairment, as well as by improvements in neurofunctional status and specifically in motor performance. Less evident beneficial effects of RSV on neuronal survival and apoptotic cell death are shown. This systematic review builds on previous narrative reviews claiming potential protective effects of RSV in cerebral ischemia,^{11–14,109} by comprehensively describing which RSV-based interventions have been tested in ischemic stroke animal models, the range of conditions under which RSV efficacy has been tested, and the mechanisms of action involved. This systematic review updates the available evidence on the three main mechanisms of action underlying the cerebroprotective effects of resveratrol (i.e., reduction of oxidative stress, decrease in inflammatory response, and inhibition of apoptotic pathways) and provides an exhaustive list of targeted signaling pathways. Most important, for the first time a Bayesian meta-analysis quantitatively assesses the overall efficacy of RSV on seven relevant stroke outcomes. Two non-prospectively registered

systematic reviews with frequentist meta-analyses of fewer studies and comparisons, carried-out in parallel with the present study, overstated effect sizes of RSV on three stroke outcomes in rats¹¹⁰ and two stroke outcomes in rodents,¹¹¹ maybe because they did not correct for publication bias of the estimates and in some cases used fixed effects models despite high heterogeneity. When compared to Liu's study in rodents,¹¹¹ the present study analyzed more stroke outcomes, including significantly more studies and comparisons, and using a Bayesian modeling approach with regularizing priors that is able to capture uncertainty due to study heterogeneity. This approach provides more accurate and robust pooled estimates of RSV effects, as well as estimated benefit probabilities of RSV for each stroke outcome measure. Moreover, this Bayesian meta-analysis included a meta-regression that identified sources of heterogeneity, sensitivity analyses that confirmed the robustness of the estimates of pooled effect sizes, and an assessment of the risk of bias that could impact on our encouraging results.

Both acute and chronic (from days to months) RSV treatment showed beneficial cerebrovascular effects in rodents and humans. RSV administration rescued neurovascular coupling in aged mice,¹¹² improved cerebral blood flow (CBF) in aged rats,¹¹³ and restored vascular responsiveness of cerebral arterioles in diabetic rats.¹¹⁴ On the other hand, RSV increased CBF during task performance in healthy human adults of both sexes,^{115,116} and enhanced cerebrovascular and cognitive functions in postmenopausal women.^{117–119} Our results show that RSV did not modify CBF in rodents subjected to ischemic stroke. In contrast, both acute and chronic treatment with a mixture of red wine polyphenol compounds (RWPC), containing small amounts of RSV, enhanced CBF in rats subjected to ischemic stroke.^{120,121} Moreover, acute and chronic RSV treatment attenuated the reduction of CBF during global ischemia in rats.^{32,122} Since only 5 studies included in the present review monitored the effects of

RSV on CBF during focal ischemia, this subject deserves further research. Other non-neuronal beneficial effects of RSV were promotion of angiogenesis, oligodendrogenesis and delayed microglial activity, which might help recovery after stroke. These RSV glial effects have been recently reviewed in experimental models of stroke.¹²³

Our data set included results from 51 studies describing 119 comparisons involving 1818 animals for infarct size, the primary outcome measure. Beyond trying to reach as much statistical power as possible, there is no rule of thumb about the number of studies required for a meta-analysis.¹²⁴ The present study, focusing on ischemic stroke, included a number of primary articles higher than that in a recent systematic review on lithium and stroke recovery in rodent models of both ischemic and hemorrhagic strokes,¹²⁵ and a number of animals well above the median (749 [463, 1797]) in thirteen previous experimental stroke meta-analyses.¹²⁶

In addition to the number of studies, there is their methodological quality. Overall, the studies included in this meta-analysis showed medium quality (median QS was 7 on the extended 15-item CAMARADES' checklist),¹⁵ which theoretically might overstate the RSV effect size. However, the meta-regression showed no influence of aggregate QS on the estimates of RSV effects on infarct size or neurofunctional impairment, in agreement with empirical evidence from previous meta-analyses. With regard to individual quality items, just eight studies reported blinded induction of ischemia, the only performance bias with demonstrated influence on effects sizes of the interventions in ischemic stroke animal models.¹²⁶ Of note, in the present study a sensibility analysis excluding unblinded experiments still showed evidence of RSV cerebroprotective efficacy.

With regard to functional outcomes, RSV beneficial effect is based on neurofunctional scores reported in 30 studies describing 73 comparisons involving 1161 animals. However, it should be noted that only easy to perform, but subjective in nature, tests are used to obtain these composite scores, despite the available diversity of motor, sensory and cognitive tests.¹²⁷ In this respect, our meta-analysis also provides evidence for improvement in motor performance by RSV assessed by the rotarod test, a widely used sensitive test.¹²⁸ Other tests to assess grip strength, motor/sensorimotor deficits (cylinder and corner tests), and cognitive impairment (Morris water maze) were seldom reported in the included studies and could not be meta-analyzed.

Meta-regression showed that only rodent species (rat vs mouse) was associated to a larger beneficial effect of RSV in the case of infarct size and, to a

lesser extent, for neurofunctional impairment. RSV preconditioning has been reported to induce an extended window ischemic tolerance with RSV given up to 14 days pre-ischemia.⁵⁴ The highest RSV single dose used in pretreatment for the primary outcome measure (infarct size) was 30 mg/kg.⁶² However, a recent study (published after the literature search or our systematic review was carried out and the meta-analysis finished) showed cerebroprotective effects by 50 mg/kg single dose RSV preconditioning, given 2 days before tMCAO.¹²⁹ Our results showed that RSV timing (pre- vs post-ischemia) did not impact RSV cerebroprotective effects, thus pointing to both prophylactic and curative beneficial effects of RSV against ischemic brain damage. Sensitivity analyses with more stringent intercept priors confirmed the beneficial effects of RSV on infarct and edema sizes, BBB impairment, neurofunctional status, and specifically motor performance. Finally, the meta-analysis showed less probable beneficial effects of RSV in terms of neuronal survival or apoptotic cell death, because of uncertainty due to the limited number of studies reporting on these outcomes and the high between-study heterogeneity. Of note, median quality of these studies was not lower than that of all studies included in the meta-analysis.

Although the range of evidence met ten of a possible 13 updated STAIR criteria assessed, external validity is limited because no study used animals with comorbidities (hypertension, diabetes, etc.), species other than rodents (i.e., large gyrencephalic animal models), and medications commonly used in stroke patients (alteplase, statins, blood pressure-lowering medication, aspirin, etc.). Moreover, there is very limited evidence from female (two studies) or aged (one study) animals. Finally, although clinically relevant blood biomarkers were measured, no study included relevant imaging biomarker endpoints like MRI.

With respect to limitations of the review process, no or incomplete information was extracted from 20 studies that were or appeared to be eligible, because only a conference abstract was available,¹⁰⁷ full-text article could not be retrieved⁶⁴ or was published in Chinese,^{24,39,44,46,61,65,74,76,92,95,98,103,104} and data were inconsistent or unavailable even after requested to authors.^{43,56,69,78,83} Moreover, data from two eligible studies using the RSV analogs oxyresveratrol³⁶ and piceatannol,⁹³ respectively, were not subjected to meta-analysis.

The results of the present meta-analysis showing RSV efficacy and its limitations provide important clues for future animal studies aiming to improve the evidence supporting the suitability of RSV for the treatment of ischemic stroke. The roadmap to fill the gaps in preclinical research should first include improved and properly reported study quality. Some

studies in the present review might have taken measures to reduce bias but not reported them. This highlights the importance of transparent reporting of study methodology in animal research (ARRIVE guidelines 2.0)¹³⁰ to optimize the predictive value of preclinical research findings.¹³¹ Second, studies should focus on unmet or scarcely fulfilled criteria regarding the range of evidence. The addition of an appropriate large gyrencephalic model¹³² would be a critical extra step in the translational pathway toward clinical application. Biologic sex influences many variables that are important to therapeutic response in ischemic stroke.¹³³ The effects of RSV in females with ischemic stroke has been understudied, and given the growing stroke burden in women, this is a direction where more research is needed. The importance of studying aged individuals and comorbidities in stroke has also been underscored, and it is imperative that this applies to both sexes for the future of personalized medicine in stroke treatment.¹³⁴ It is expected that the present results will spur further preclinical research addressing these shortcomings regarding internal and external validity. To this end, in addition to single laboratory initiatives, a multicenter phase III type preclinical study¹³⁵ would increase the translational impact of preclinical research on RSV cerebroprotection in stroke, which should be reassessed in a future meta-analysis update.

Two amendments were made to the registered protocol (PROSPERO CRD42021266048). The first one regarding the adjustment of controls in the case of one control used multiple times. Instead of dividing control sample size by the number of experimental groups, a three-level hierarchical model where comparisons were nested into studies was implemented. The second amendment was related to management of publication bias. Instead of using funnel plots and trim and fill methods, extreme results were controlled by setting regularizing priors for the pooled effects in the Bayesian models.

In conclusion, the current systematic review and Bayesian meta-analysis builds on prior individual publications and narrative reviews claiming a cerebroprotective role for RSV in experimental ischemic stroke. Our results quantitatively state the overall efficacy of RSV in reducing infarct, edema, BBB impairment and apoptotic cell death, as well as in improving neuronal survival, neurofunctional status and specifically motor performance, based on available data from rodent stroke models. Mainly reduction of oxidative stress, decrease in inflammatory response, and inhibition of apoptotic pathways underlie these cerebroprotective effects. However, analysis of the data set also points to improvable study quality and important shortcomings in the range of the evidence. Altogether, current evidence highlights the need for conducting and

reporting further high-quality preclinical research in order to increase both internal and external validity, and thus better inform clinical research. Filling these gaps would increase the chances of translational success determining the suitability of RSV as a treatment for human stroke.

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Supplementary material

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