Ischemic stroke is one of the most common causes of mortality and a leading cause of disability worldwide [15]. Tissue plasminogen activator (tPA) is the only biological intervention used in routine clinical practice in the treatment of acute ischemic stroke, albeit in a select cohort of patients. Other drugs tested in animal models which have been brought forward to clinical trails, such as Tirilazad, have subsequently failed [1]. Despite the fact that more than 37 potential neuroprotective agents have been studied in more than 114 clinical trials, none are clinically efficacious and in use in the western world [6].

Poor methodological quality and publication bias are two factors hypothesised to exacerbate translational failure from animal studies to clinical trials. The Collaborative Approach to Meta-Analysis and Review of Animal Data in Experimental Stroke (CAMARADES) study quality check list has been used to help to address the lack of translation between animal studies and human clinical trials [12]. Previously in studies of animal models of stroke, low methodological quality has been shown to lead to the overestimation of effect size [11;13;21]. Publication bias is thought to exist because studies which do not reach statistical significance, alongside negative studies, will often remain unpublished leading to an overstatement of effect size when published studies are aggregated [22].

The Rho family of GTPases (including RhoA, Rac1 and Cdc42) play major roles in the regulation of many cell behaviours including cell shape, motility, proliferation and apoptosis and are therefore involved in many biological processes [18]. RhoA is known to function as a molecular switch, switching between an active GTP-bound form and an inactive GDP-bound form. ROCK, a downstream effector of RhoA, is activated when bound to the active GTP-bound form of RhoA [3] and is present in 2 isoforms (ROCK1 and ROCK2) [16]. The first identified role of ROCK was in the reorganisation of the cytoskeleton, in particular in the formation of stress fibers and focal adhesion complexes [8]. ROCK can also regulate the production endothelial nitric oxide synthase (eNOS), whereby when ROCK is activated NO expression is decreased. Nitric oxide (NO), normally produced by eNOS, regulates cerebral blood flow (CBF) and vascular tone and protects against ischemic stroke by increasing collateral flow to the ischemic area [19]. ROCK inhibitors have been shown to increase eNOS expression and NO production in vitro and in vivo [19]. There is a major interest in the role of NO as a therapeutic target in ischemic stroke [2;23].

Animal studies have shown that ROCK inhibitors have an neuroprotective effect in models of stroke [19;20]. Administration of selective ROCK inhibitors such as Fasudil, Y-27632 and Hydroxyfasudil increased cerebral blood flow, significantly reduced infarct volume and improved neurological scores [19;20]. ROCK inhibitors may exert these effects by increasing NO production, which results in increased cerebral blood flow to ischemic regions and
decreased infarct size after cerebral ischemia [19] Fasudil and Y-27632 inhibit ROCK by actively competing with ATP for binding on ROCK [4;9;14;19].

The figure below indicates where inhibitory molecules are known to exert their effects in the Rho pathway.

Figure 1. RhoA-activation is inhibited by C3 transferase and by the NSAID ibuprofen. ROCK is inhibited by the Y2763 and fasudil.

Systematic review and meta-analysis identifies all the relevant publications available, gives an unbiased assessment of the data, provides a more precise effect size for the drug than individual publications results and allows more confidence in the drugs when taking them forward to clinical trials [10;12]. This systematic review aims to provide a summary of the effectiveness of Rho kinase inhibitors in reducing lesion size and neurobehavioural score in animal models of focal cerebral ischemia. In this systematic review we will focus on drugs known to inhibit RhoA or ROCK. Meta-analysis will also be performed to investigate the impact of methodological quality and publication bias on the reported effect size of drugs in these studies.

Search Strategy

Three online databases (Pubmed, ISI and EMBASE) will be searched, using the following search terms: 

((C3) OR (C3-transferase) OR (Y27632) OR (Y-27632) OR (Nonsteroidal Anti-Inflammatory) OR (NSAID) OR (Ibuprofen) OR (Rho Kinase) OR (Rho Kinase) OR (Rho) OR (ROCK) OR (RhoA) OR (Fasudil) OR (HA-1077) OR (HA 1077) OR (HA1077) OR (Cethrin) OR (BA-210)) AND ((stroke) OR (ischemia) or (ischaemia) OR (middle cerebral artery) OR (cerebrovascular) OR (MCA) OR (ACA) OR (anterior cerebral artery) OR (MCAO))
NOT (coronary) OR (myocardia*) Limit to ‘other animals’
PubMed Results= 1201

((C3) OR (C3-transferase) OR (Y27632) OR (Y-27632) OR (Nonsteroidal Anti-Inflammatory) OR (NSAID) OR (Ibuprofen) OR (Rho Kinase) OR (Rho-Kinase) OR (Rho) OR (ROCK) OR (RhoA) OR (Fasudil) OR (HA-1077) OR (HA 1077) OR (HA1077) OR (Cethrin) OR (BA-210)) AND ((stroke) OR (ischemia) or (ischaemia) OR (middle cerebral artery) OR (cerebrovascular) OR (MCA) OR (ACA) OR (anterior cerebral artery) OR (MCAO))

NOT (coronary) OR (myocardia*) Limit to ‘animals’
Embase results= 729

((C3) OR (C3-transferase) OR (Y27632) OR (Y-27632) OR (Nonsteroidal Anti-Inflammatory) OR (NSAID) OR (Ibuprofen) OR (Rho Kinase) OR (Rho-Kinase) OR (Rho) OR (ROCK) OR (RhoA) OR (Fasudil) OR (HA-1077) OR (HA 1077) OR (HA1077) OR (Cethrin) OR (BA-210)) AND ((stroke) OR (ischemia) or (ischaemia) OR (middle cerebral artery) OR (cerebrovascular) OR (MCA) OR (ACA) OR (anterior cerebral artery) OR (MCAO))

NOT (coronary) OR (myocardia*) AND animals
Refined by Excluding ‘review, book, letter, clinical trial, case report, patent, editorial’
Web of Knowledge results= 1356

Abstracts will be independently screened by two reviewers to identify those meeting our inclusion criteria (see below), with differences resolved by discussion with a third reviewer.

Inclusion criteria and data extraction
Studies will be included if they reported the effects of a ROCK inhibitor in an in vivo animal model of focal cerebral Ischemia and reported the number of animals per group, a lesion size outcome (infarct volume or infarct area; primary outcome) or a neurobehavioral score (secondary outcome), and the mean and its variance (standard error of the mean or standard deviation). Data will be extracted to the CAMARADES database.

Quality assessment
Studies will be assessed against the CAMARADES published ten item quality check list [12] and given one point for each item reported.
1) A peer reviewed publication, 2) control of temperature stated, 3) were the animal models randomly allocated to groups, 4) was there a blinded induction of ischemia, 5) a blinded assessment of outcome, 6) the anaesthetic used didn’t have intrinsic neuroprotective activity, 7) the use of co morbid animals, 8) sample size calculation, 9) compliance with animal welfare regulations, 10) any statements of potential conflicts of interest.

Collection of data
From each paper the individual comparisons (infarct volume and/or neurobehavioral scores) will be extracted and defined as assessment of outcome in a controlled study where drug was delivered at a given time. The number of animals per group, the mean outcome and the standard error or standard deviation for both the control and treatment group will be extracted for every comparison. When a single control group is used for multiple treatment groups this will be re-calculated in the meta-analysis making the control group divisible by the number of treatment groups it served. When data is only given in graphical form a computerised ruler program (Universal Desktop Ruler) will be used to measure the graphs.

In addition, the anaesthetic used, induction of injury, method of qualification of injury, type of drug, blinded induction of ischemia and assessment of outcome, the type of ischemia, type and sex of animal single/multiple administration of drug and the route of drug delivery will be recorded.

Analysis
Infarct volume/area and neurobehavioral score will be analysed using a weighted mean difference meta-analysis. Studies will be stratified according to drug dose, time of administration, blinded assessment of outcome, random allocation to group, study quality, components of study quality check list, method of induction of ischemia, outcome measurement used, sex and species of animal used, anaesthetic used and use of mechanical intervention.

Significance of the differences will be determined by partitioning heterogeneity of each strata and by using the chi squared distribution with n-1 degrees of freedom.

Publication bias will be assessed using a funnel plot, [7] Egger regression [17] and trim and fill [5]. Finally, trim and fill was used to estimate the number of theoretical missing studies and the effect size in the absence of publication bias. The number of missing studies will be identified using metatrim in STATA and replaced in order to adjust the effect size.

Reference List


[22] Sena ES, van der Worp HB, Bath PM, Howells DW, Macleod MR. Publication bias in reports of animal stroke studies leads to major overstatement of efficacy. PLoS Biol 2010;8:e1000344.