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REVIEW ARTICLE

Hypothermia in animal models of acute ischaemic stroke: a systematic review and meta-analysis

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Induced hypothermia is proposed as a treatment for acute ischaemic stroke, but there have been too few clinical trials involving too few patients to draw any conclusions about the therapeutic benefit of cooling. Animal studies of induced hypothermia in focal cerebral ischaemia have tested cooling throughout a wide range of target temperatures, durations and intervals between stroke onset and the initiation of hypothermia. These studies, therefore, provide an opportunity to evaluate the effectiveness of different treatment strategies in animal models to inform the design of future clinical trials. We performed a systematic review and meta-analysis of the evidence for efficacy of hypothermia in animal models of ischaemic stroke, and identified 101 publications reporting the effect of hypothermia on infarct size or functional outcome, including data from a total of 3353 animals. Overall, hypothermia reduced infarct size by 44% [95% confidence interval (CI), 40–47%]. Efficacy was highest with cooling to lower temperatures (≤31°C), where treatment was started before or at the onset of ischaemia and in temporary rather than permanent ischaemia models. However, a substantial reduction in infarct volume was also observed with cooling to 35°C (30%; 95% CI, 21–39%), with initiation of treatment between 90 and 180 min (37%; 95% CI, 28–46%) and in permanent ischaemia models (37%; 95% CI, 30–43%). The effects of hypothermia on functional outcome were broadly similar. We conclude that in animal models of focal cerebral ischaemia, hypothermia improves outcome by about one-third under conditions that may be achievable for large numbers of patients with ischaemic stroke. Large randomized clinical trials testing the effect of hypothermia in patients with acute ischaemic stroke are warranted.

Keywords: ischaemic stroke; animal model; hypothermia; systematic review; meta-analysis

Abbreviations: NOCSS = Nordic Cooling Stroke Study; PAIS = Paracetamol (Acetaminophen) In Stroke

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There is evidence for efficacy of over 500 treatment strategies in animal models of focal cerebral ischaemia (O'Collins *et al.*, 2006), but only recombinant-tissue plasminogen activator (rt-PA), aspirin and stroke unit care have convincingly demonstrated efficacy in clinical trials of acute ischaemic stroke [The National Institute of Neurological Disorders and Stroke rt-PA Stroke Study Group, 1995; CAST (Chinese Acute Stroke Trial) Collaborative Group, 1997; International Stroke Trial Collaborative Group, 1997; Stroke Unit Trialists' Collaboration, 1997]. While differences in design and methodological quality between animal studies and clinical trials may be responsible for some of this discrepancy [Stroke Therapy Academic Industry Roundtable (STAIR), 1999; Gladstone *et al.*, 2002; Macleod *et al.*, 2004; van der Worp *et al.*, 2005], the failure of allegedly neuroprotective compounds in the clinic may be explained in part by the fact that most neuroprotectants inhibit only a single step in the broad cascade of events that leads to cell death (STAIR, 1999; Gladstone *et al.*, 2002).

In contrast, pre-clinical studies have suggested that hypothermia affects a wide range of cell death mechanisms including energy depletion, disruption of the blood–brain barrier, free radical formation, excitotoxicity

and inflammation (Olsen *et al.*, 2003) The potential of hypothermia has recently been underlined by randomized clinical trials in patients with global cerebral ischaemia after cardiac arrest (Bernard *et al.*, 2002; Hypothermia after Cardiac Arrest Study Group, 2002) and in infants with moderate or severe hypoxic-ischaemic encephalopathy (Shankaran *et al.*, 2005), in which cooling reduced mortality and disability. In addition, several prospective observational studies have demonstrated that higher body temperatures are associated with poor outcome after stroke (Reith *et al.*, 1996; Castillo *et al.*, 1998).

Unfortunately, few clinical trials have tested the benefits of therapeutic cooling in patients with acute ischaemic stroke, and those which have been performed have been too small to allow reliable conclusions to be drawn (Olsen *et al.*, 2003; Lyden *et al.*, 2006). This may be explained in part by uncertainty about the optimal depth and duration of hypothermia. Of even greater importance may be the fact that cooling to temperatures <35°C, as used in most animal studies, generally requires sedation, mechanical ventilation, and therefore admission to an intensive care facility, which limits both the numbers recruited to clinical trials and the capacity to widely implement any therapy shown to be effective.

In animal studies of hypothermia in focal cerebral ischaemia, a wide range of depths, durations and delays to initiation of cooling has been tested. Using systematic review and meta-analysis, we set out to describe (i) the conditions under which maximum efficacy was achieved in animal studies; (ii) the efficacy achieved under conditions which might feasibly be reproduced in clinical practice, to inform the design of future clinical trials; and (iii) factors modifying the response to hypothermia in animal experiments.

Material and methods

Search strategy

Studies of hypothermia in animal models of acute ischaemic stroke were identified from (i) PubMed, EMBASE and BIOSIS up till December 31, 2004 with the search strategy [[[<cerebral> OR <brain> OR <neuron> OR <neuronal> OR <nervous>] AND [<ischemia> OR <ischaemia>]] OR <stroke>] AND [<hypothermia> OR <temperature>]; (ii) hand searching of abstracts of scientific meetings of the International Society of Cerebral Blood Flow and Metabolism, the International Stroke Conference ['Joint (International) Conference on Stroke and Cerebral Circulation' before 2000] and the European Stroke Conference during the same time period; (iii) reference lists of identified publications; and (iv) requests to authors of the identified publications for references to other studies.

Eligibility

Studies were included if they were controlled, tested the effect of hypothermia in an animal model of focal cerebral ischaemia by means of occlusion of a cerebral artery, and if outcome was measured as infarct size or neurobehavioural score. Studies were excluded if (i) hypothermia was accomplished with use of a pharmacological agent that may also have an intrinsic neuroprotective property; (ii) cooling was used to counteract (spontaneous) hyperthermia after MCAo; (iii) brain cooling lasted <10 min, for example to counteract heating in models of photochemically induced cerebral infarction; (iv) data were presented in a way not suitable for use in a meta-analysis (e.g. no information on group size); or if (v) mortality was the only outcome measure.

Data extraction and outcome assessment

From each source we identified individual comparisons where outcome was measured in a group of animals cooled to a specific temperature at a specified time and compared with outcome in a control group. Where the treatment group received more than one intervention this was recorded. For each comparison and for each treatment and control group we extracted data for number per group, mean outcome and its standard deviation. Where an outcome was measured serially, only the final measure was used. Where data were presented only graphically we contacted authors to seek the raw data; where these were not available we estimated values by measurement from the publication. When one group of animals was scored in more than one neurological or functional domain, data were combined using meta-analysis to give an overall estimate of effect size and standard error. The primary outcome measure was the effect of hypothermia on infarct size. The effect of hypothermia on neurological or functional outcome was a secondary outcome measure.

If both total infarct volume and infarct volume adjusted for oedema were presented, the latter was used in the analyses. The start of hypothermia was defined as the start of cooling. If only the time point at which the target temperature was achieved was presented and the relevant information could not be obtained from the authors, the start of cooling was arbitrarily set at 15 min before the target temperature was achieved. If combinations of rectal, temporal muscle, epidural or brain temperatures were provided, we used the temperature closest to the brain. If the temperature actually achieved by cooling was not presented, we used the target temperature in the analyses.

We assessed study quality against a 10 item checklist (Macleod *et al.*, 2004), which comprises (i) publication in a peer-reviewed journal; (ii) statement of control of temperature; (iii) randomization to treatment or control; (iv) blinded induction of ischaemia (i.e. concealment of treatment group allocation at time of induction of ischaemia); (v) blinded assessment of outcome; (vi) avoidance of anaesthetics with marked intrinsic neuroprotective properties; (vii) use of animals with hypertension or diabetes; (viii) sample size calculation; (ix) statement of compliance with regulatory requirements; and (x) statement regarding possible conflicts of interest.

We also collected other relevant data including method of induction of ischaemia, duration of hypothermia and time of outcome measurement, as well as the individual component items of the quality checklist above.

Statistical analysis

For the purpose of meta-analysis, we considered all neurobehavioural scales to behave as continuous scales even if they were

clearly ordinal. Data on infarct size and functional scores in hypothermic animals were normalized to outcomes in the control groups. Where multiple neurobehavioural outcomes were reported, we combined effect sizes using weighted mean difference with a random effects model to give an estimate of the overall neurobehavioural outcome. Where a single control group served multiple treatment groups, the size of the control group entered to the meta-analysis was adjusted by division by the number of treatment groups served. Because of the anticipated biological differences between studies, we used a random effects rather than a fixed effects model, as this takes into account heterogeneity between studies. We have explored in simulation the relative performance of weighted versus standardized mean difference analysis when sample size is small, and found weighted mean difference analysis to perform better under these circumstances (http://www.camarades.info/index_files/CAMARADES%20Monograph%201.pdf).

To assess the effect of different variables on the effect of cooling, we stratified analysis according to: depth and duration of hypothermia; duration of ischaemia; time of start of treatment; start of treatment in relation to the onset of reperfusion; study quality; individual components of the study quality checklist; the presence of co-treatments; method of induction of ischaemia; anaesthetic used; species, strain and gender of animal used; and time interval from onset of ischaemia to final assessment of outcome. These stratifications were pre-specified. The significance of differences between n groups was assessed by partitioning heterogeneity and by using the χ^2 distribution with n-1 degrees of freedom (df). To allow for multiple comparisons we set our significance level at $P<0.001$. Publication bias was assessed with a funnel plot and with the Egger regression method (Sterne *et al*., 2001).

Results

We identified 4203 studies via PubMed, 1579 via EMBASE and 3332 via BIOSIS, with considerable overlap between the searches. From a total of 191 full publications considered as possibly reporting the efficacy of hypothermia in animal models of focal cerebral ischemia, 99 were excluded after reading the complete article because they did not meet the eligibility criteria. We found four duplicate and two triplicate publications, reducing the number of included papers by eight. One additional full publication was found via the hand search, and one was provided by the author. We identified 66 relevant abstracts, of which 43 described work later published in full where the final publication had been identified in the search above; eight abstracts reported duplicate data (of which four were included) and four abstracts provided insufficient data. Therefore, a total of 86 full publications and 15 abstracts were included in this review (details available in Appendix). These 101 publications described 277 comparisons: 222 comparisons in 3256 animals reported data as infarct size and 55 comparisons in 870 animals as a neurobehavioural score. The total number of animals included was 3353.

Study quality and publication bias

The median number of study quality checklist items scored was 5 out of a possible 10 (interquartile range, 4–6). We found one study with a quality score of 8, one with a score of 9 and none with a score of 10, preventing robust conclusions on the highest scores. Study quality had a significant impact on reported outcome, with greatest efficacy reported from studies meeting fewest items on the study quality checklist (Fig. 1). These modest quality scores are consistent with quality seen in reviews of other candidate stroke drugs and represent an important potential source of bias. Efficacy was significantly lower in comparisons with random treatment allocation and blinded assessment of outcome (Fig. 2). The Funnel plot did not suggest a substantial publication bias, and consistent with this a significant small-study effect was not seen at Egger regression (data not shown).

Other study characteristics

The median time to start of treatment was 0 min (range, −60–360 min). In 145 experiments, both the time cooling was started and the time the target temperature was achieved were reported. The median time required to achieve the target temperature was 20 min. The median duration of active cooling for all comparisons was 180 min (range, 5 min to 75.5 h). In 50 comparisons, temperature was measured for 12 h or longer after the onset of ischaemia. Overall, the median time of outcome assessment was 48 h (interquartile range, 24–74 h). In 117 comparisons (42%), outcome was assessed at least 3 days after the onset of ischaemia.

Efficacy

Overall, hypothermia reduced infarct size by 43.5% (95% CI, 40.1–47.0%; 222 comparisons; Fig. 3A) and improved

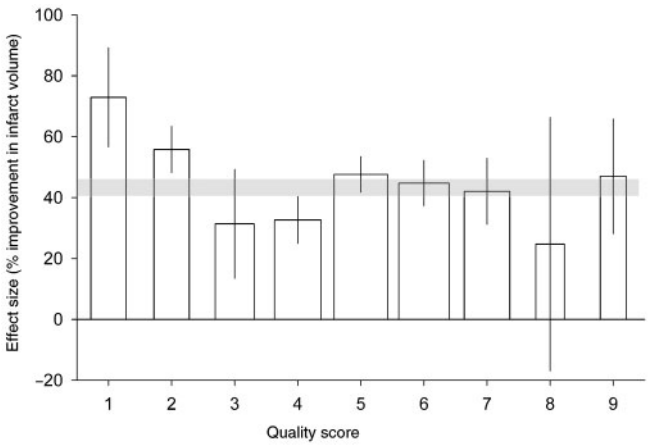

Fig. 1 Point estimates and 95% CIs of effect of cooling on infarct size by reported study quality score. The thickness of each bar reflects the number of comparisons contributing to that data point. The 95% CI for the global estimate is shown as a grey band.

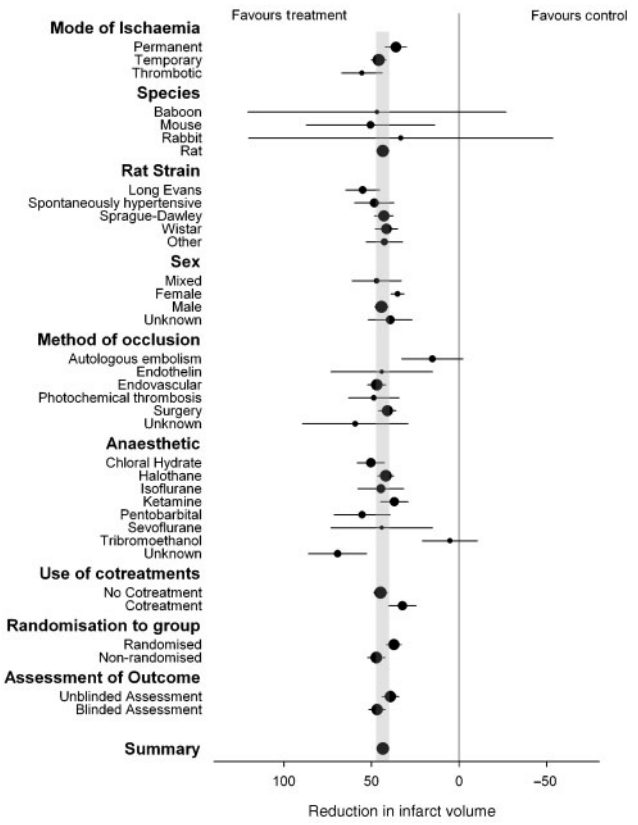

Fig. 2 Point estimate of effect on infarct size and 95% CI by mode of ischaemia, species, rat strain, sex, method of occlusion, anaesthetic, use of co-treatment, randomisation, and assessment of outcome. The grey band indicates the global estimate and its 95% CI.

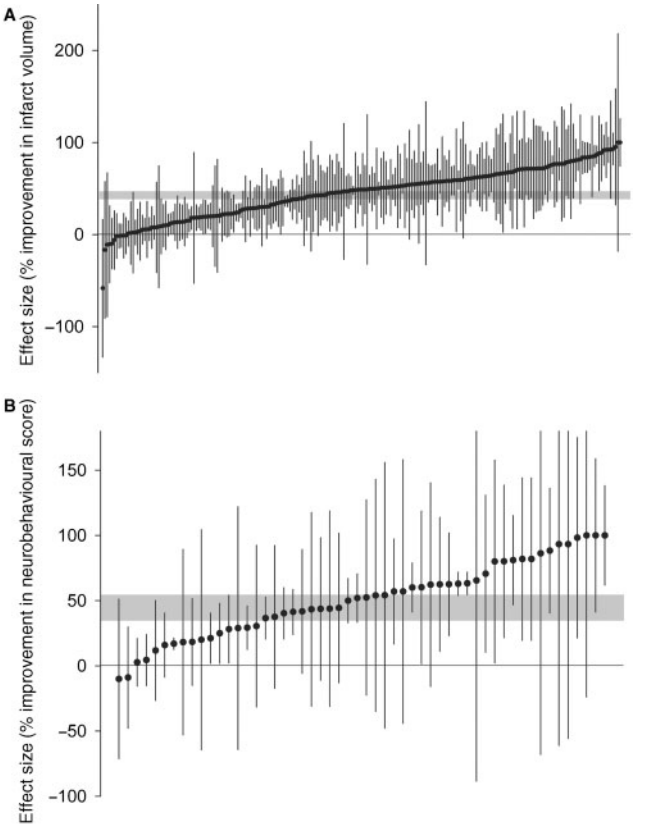

Fig. 3 Point estimates and 95% CIs ranked by effect size for (**A**) 222 comparisons testing change in infarct size and (**B**) 55 comparisons testing change in neurological score. Effect size is the improvement in hypothermic animals expressed as a proportion of the outcome in control animals. The grey band indicates the global estimates and its 95% CI. The solid horizontal line marks where treatment and control are equal.

neurobehavioural scores by 45.7% (95% CI, 36.5–54.5%; 55 comparisons; Fig. 3B). There was substantial between-study heterogeneity for the analysis of infarct volume ($\chi^2 = 1547$, df = 221, $P < 10^{-198}$) and neurobehavioural score ($\chi^2 = 280$, df = 54, $P < 10^{-33}$). The substantial heterogeneity suggests that these results must be interpreted with caution. In 44 of the 55 comparisons testing the effect of hypothermia on neurobehavioural score, infarct size was measured in the same animals. In these comparisons, hypothermia reduced infarct size by 48.1% (95% CI, 41.2–55.6%) and improved neurobehavioural scores by 43.5% (95% CI, 34.2–52.7%).

Factors modifying effect on infarct size

The median target temperature was 33°C (range, 24–35°C). There was a significant depth-response relationship ($P < 0.001$; Fig. 4), but a substantial reduction in infarct volume was still observed with cooling to 35°C (30%; 95% CI, 21–39%). The effect of cooling was most robust when started before or at the start of vessel occlusion, but there was no clear time-dependency thereafter. Both the number of experiments with treatment delays of more than 3 h and the number of animals included in these experiments were too small to draw firm conclusions (Fig. 4). In models of temporary ischaemia, the effect of cooling was largest if started before the onset of ischaemia and continued during both ischaemia and reperfusion, and smallest if animals were cooled only during either ischaemia or reperfusion (Fig. 4). There was a small inverse relation between the duration of hypothermia and effect size (Fig. 4).

Of the 222 comparisons, 214 (96%) involved rats, making any conclusion on differences between species futile (Fig. 2). When the analysis was restricted to data from rats alone the findings remained essentially unchanged (data not shown). Of note, in the two studies performed in dogs in the 1950's (Rosomoff, 1956 and 1957), infarct size was not quantified sufficiently to be included in this part of the meta-analysis. Similarly, it is not possible to discern with any certainty any effect of gender; 196 comparisons concerned male animals, three concerned female animals, two concerned animals of both sexes, and in 21 the sex was unknown (Fig. 2). Hypothermia was slightly more effective in Long Evans and in spontaneously hypertensive rats than

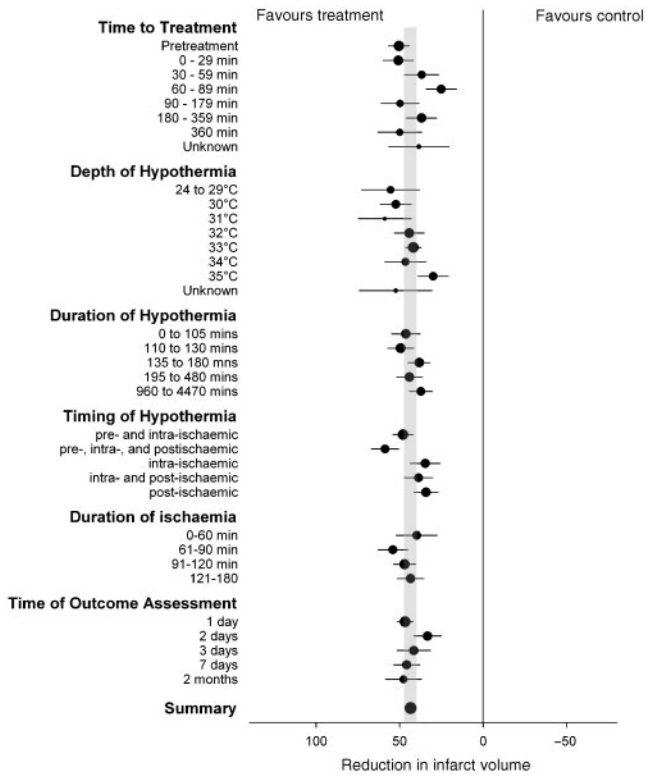

Fig. 4 Point estimate of effect on infarct size and 95% CI by duration of ischaemia in models of reperfusion, time to treatment, depth of hypothermia, duration of hypothermia, timing of hypothermia and time of outcome assessment. The grey band indicates the global estimate and its 95% CI.

in other normotensive rats (Fig. 2). Only one study tested the effect in diabetic rats. Efficacy was higher in temporary than in permanent occlusion models, but the effect in the latter was still substantial (37%; 95% CI, 30–43%; Fig. 2). In models of temporary ischaemia, there was no clear relation between the duration of ischaemia and effect size (Fig. 4). The choice of anaesthetic used had a significant impact on outcome, with higher efficacy reported in studies using chloral hydrate or pentobarbital (Fig. 2). The time of outcome assessment (Fig. 4) and the mode of induction of ischaemia did not affect the benefit of hypothermia, except for a lower efficacy in models of autologous clot embolism (Fig. 2).

Complications

In 19 of the 101 publications, complications were reported in a total of 79 normothermic and 39 hypothermic animals. All but one of these complications concerned premature death, for which in 74 cases (63%) no cause was reported and in 36 cases (31%) the cause was presumed to be large or space-occupying infarction. These animals were excluded from the analyses of infarct size in 68 (86%) and 35 (90%) of the normothermic and hypothermic cases, respectively. In addition, significant decreases in heart rate and blood pressure during hypothermia were reported in three studies with cooling to 24°C. In one study, a decreased respiratory rate and cardiac arrhythmias were reported in rats cooled to 30°C.

Discussion

This systematic review and meta-analysis strongly underscores the efficacy of hypothermia in improving both histological and functional outcome in animal models of acute ischaemic stroke. In accordance with expectations, efficacy was highest with lower temperatures, where treatment was started before or at the onset of ischaemia, and in temporary rather than permanent ischaemia models. However, infarct volume was still reduced by about one-third with cooling to 35°C, with initiation of treatment between 90 and 180 min, and in permanent ischaemia models. In addition, hypothermia was more effective in animals with hypertension compared with normotensive animals, and was effective across the range of scores for methodological quality of the study. These findings suggest that hypothermia has considerable potential as a neuroprotective strategy in patients with acute ischaemic stroke.

Methodological considerations

A notable feature of the present review is the marked heterogeneity between studies, implying that the overall estimate of efficacy should be interpreted with some caution. Conventional ('narrative') reviews selectively citing reports at either end of the spectrum of efficacy might therefore lead to different conclusions about the effect of hypothermia especially within subgroups. Although previous non-systematic reviews have also reported an overall benefit of hypothermia (Miyazawa *et al.*, 2003; Krieger and Yenari, 2004), the substantial heterogeneity between studies (and the importance therefore of identifying all relevant data) provides a substantial impetus for the use of a systematic approach such as ours to reduce selection bias and to increase the precision of estimates given.

Although our analyses of factors influencing the reported efficacy of hypothermia was pre-specified and a stringent significance level was chosen to allow for multiple testing, some of the apparent effects may be due to the play of chance. Our conclusions should therefore be viewed as hypothesis-generating rather than definitive evidence. This meta-analysis has other weaknesses. First, while we consider that our search strategy is likely to have ascertained the majority of relevant publications, it has yet to be validated. Furthermore, we have only been able to include data which have been published in some form: hence, our analysis takes no account of unpublished data. However, both Funnel Plot and Egger Regression suggested that there was not a substantial publication bias in those studies identified.

Secondly, because studies may differ across a number of characteristics in addition to that being studied, it may be that the differences attributed to one characteristic when studies are analysed using this univariate method in fact reflect the influence of a second, alternative study characteristic not included in the analysis. With sufficient data from a range of candidate drugs it should be possible to use multivariate analyses to adjust for confounders, and this would allow more reliable assessment of those variables which significantly modify treatment effects.

Although infarct volume was reduced by about one-third in studies reporting cooling to 35°C, in studies with initiation of treatment between 90 and 180 min after the onset of ischaemia, and in permanent ischaemia models, no single study reported testing outcome in this combination of conditions. Before starting clinical trials testing delayed and mild cooling in patients with acute ischaemic stroke, additional animal studies testing this regimen are required.

This is the first systematic review and meta-analysis of the efficacy of a physiological intervention in animal models of ischaemic stroke. As such, it shows that this technique may usefully be applied not only to pre-clinical drug testing but also to experiments reporting outcome in for instance experiments using transgenic animals or those exploring more fundamental pathophysiological processes.

Implications for the design of clinical trials

For patient comfort, monitoring and the prevention of shivering, hypothermia to levels of 32–34°C generally requires sedation, mechanical ventilation, and therefore admission to an intensive care unit (Feigin *et al.*, 2003; Olsen *et al.*, 2003). Due to the limited availability of intensive care beds in most countries, treatment of even a minority of acute stroke patients is therefore precluded by substantial practical and logistical problems. The fact that intensive care treatment has long been common clinical practice for patients with severe traumatic brain injury or after cardiac arrest may explain why large and definitive randomized clinical trials of moderate hypothermia have been accomplished in these patient populations, whereas such trials have not even been started in patients with acute ischaemic stroke. The only temperature-driven phase III randomized acute stroke trial currently ongoing is the Paracetamol (Acetaminophen) In Stroke (PAIS) trial, which is directed at normalization of temperature without inducing considerable hypothermia (van Breda *et al.*, 2005).

The present observation that cooling to only 35°C consistently reduces infarct volume in animal experiments may help in the design of future randomised clinical trials. A reduction in temperature to 35.5°C in awake patients with acute ischaemic stroke is feasible on a stroke unit using surface cooling in combination with pethidine or other antishivering agents (Kammersgaard *et al.*, 2000). The Nordic Cooling Stroke Study (NOCSS) was a randomized trial which tested the effect of temperature reduction to 35°C in the acute phase of stroke. Unfortunately, the trial was recently terminated because of slow recruitment after the inclusion of 44 patients against a target recruitment of 1000 patients (U.J. Weber, personal communication).

The time dependence of efficacy of hypothermia in clinical use is likely to be limited both by a time window for effectiveness (median time to treatment in animals of 0 min, with efficacy seen up to 3 h) and by differences in the time taken to achieve target temperatures, which are likely to be substantially longer than the 20 min observed in animal experiments. Further animal experiments to determine the effectiveness of hypothermia at later time points (beyond 3 h) would help the design of future clinical trials.

Cooling acute stroke patients to temperatures between 32 and 35.5°C using conventional methods (cooling blankets, ice water and alcohol rubs) is reported to take around 4 h (Olsen *et al.*, 2003). With use of an endovascular cooling device inserted into the inferior vena cava, achievement of a target body temperature of 33°C has been reported in a mean of 77 min (De Georgia *et al.*, 2004). Although external cooling has been shown to improve outcome after cardiac arrest despite a delay of 8 hours between start of treatment and attaining the target temperature (Hypothermia after Cardiac Arrest Study Group, 2002), methods to improve the speed of cooling deserve further study.

Implications for future animal experiments

Small and uncontrolled or non-randomized studies in patients with acute ischaemic stroke have suggested that cooling to 32–33°C carries a high risk of adverse events, including arterial hypotension, cardiac arrhythmias, pneumonia and thrombocytopenia (Olsen *et al.*, 2003). In the only randomized clinical trial of moderate hypothermia published to date, cooling was tolerated well (De Georgia *et al.*, 2004). However, this trial included only 40 patients and was too small for definitive conclusions about safety and efficacy.

Remarkably, complications in either hypothermic or control animals were reported in only one-fifth of the publications in the present review. Most of these complications concerned premature death, for which in about two-thirds of the cases no cause was reported. In most other cases the cause was presumed to be large or space-occupying infarction. Pneumonia or septicaemia were not reported in a single study. The results of this review therefore suggest that experimental procedures in animal models of acute ischaemic stroke, including the induction of infarction and hypothermia, are safe and do not give rise to infections.

By contrast, others have found septicaemia and pneumonia in all animals 3 days after experimental MCA occlusion (Prass *et al.*, 2003). The apparent absence of

infections in the studies included in the present review may be in part explained by the relatively short median time to outcome assessment of 2 days. However, outcome was assessed at least 3 days after onset of ischaemia in 42% of the experiments. Infections may therefore have been either truly absent or have been under-reported, for example because animals have not been scrutinized for signs of pneumonia and septicaemia. In view of the suggested increase in pneumonia after induction of hypothermia, animal studies focusing on hypothermia-induced complications are warranted.

The finding of an inverse relation between the duration of active cooling and efficacy, if true, is remarkable. In animal models of focal cerebral ischaemia, the diverse pathophysiological processes which are invoked exert their deleterious effects over different timecourses extending from the first hours to several days after vessel occlusion (Dirnagl *et al.*, 1999). Such observations would suggest that hypothermia should in fact be more efficacious if prolonged. In clinical trials of cardiac arrest, hypothermia has been proven efficacious if maintained for 12 or 24 h (Bernard *et al.*, 2002; Hypothermia after Cardiac Arrest Study Group, 2002). For these reasons and for the general caveats given before, we believe that this finding should be interpreted with caution; the relationship between duration of hypothermia and outcome should be examined in further animal experiments designed specifically to test the hypothesis that longer durations of hypothermia adversely affect outcome.

In studies that assessed both rectal and brain temperatures, the relation between both was not unequivocal. Some authors reported brain temperature about 1°C higher than that measured rectally (Maier *et al.*, 1998; Yenari *et al.*, 2000), whereas others found an opposite relation (Xue *et al.*, 1992; Barone *et al.*, 1997). Temperature in the temporal muscle is considered to reflect brain temperature well (Huh *et al.*, 2000). For this reason, we did not perform separate analyses for temperatures measured in different parts of the body but combined these data instead.

In the vast majority of experiments, measurement of temperature was discontinued soon after the end of the active cooling period. Where temperature was measured for longer periods (usually in studies reporting prolonged active cooling), a spontaneous increase in temperature was observed in control animals in some (Yanamoto *et al.*, 1996, 1999, 2001), but not all (Corbett *et al.*, 2000) studies after the first six hours. Hyperthermia has consistently been shown to increase infarct volume in animal models of focal cerebral ischaemia (Miyazawa *et al.*, 2003) and may affect the results of studies testing neuroprotective treatment strategies (Noor *et al.*, 2005). It is unknown whether spontaneous hyperthermia also occurred in those experiments in which temperature was not assessed for more than a few hours, nor whether this occurred to the same extent in controls and in animals that had been treated with hypothermia. The effect of spontaneous hyperthermia on the results of studies included in this review therefore remains unknown. Future studies of hypothermia in animal models of stroke should assess body temperature for at least 24 h, and studies of other neuroprotective drugs might also benefit from prolonged monitoring of temperature.

Conclusion

In animal models of focal cerebral ischaemia, hypothermia improves outcome by about one-third under conditions that may be feasible in the clinic, with even modest cooling resulting in a substantial improvement in outcome. Cooling is effective in animals with co-morbidity and with delays to treatment of 3 h. Large randomized clinical trials testing the efficacy of moderate hypothermia in patients with acute ischaemic stroke are warranted.

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