Therapeutic Hypothermia for Acute Ischemic Stroke
What Do Laboratory Studies Teach Us?

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Background and Purpose—The significance of brain temperature to outcome in cerebral ischemia is recognized. Numerous variations of depth, duration, and delay of cooling have been studied in animal models. It is important to become familiar with these studies to design appropriate clinical trials. With that in mind, a critical review of the pertinent literature is presented, taking into consideration potential limitations in translating such laboratory work to the clinical level.

Methods—Hypothermia is an especially robust neuroprotectant in the laboratory and has been shown to alter many of the damaging effects of cerebral ischemia. Most laboratory research on therapeutic cooling in cerebral ischemia has been conducted in rodent models of temporary and permanent middle cerebral artery occlusion and report the effects of mild or moderate hypothermia arranged during or after ischemia.

Results—Intraischemic cooling vastly reduces infarct size in most occlusion models. Tissue salvage with delayed onset of cooling is less dramatic but is commonly observed when cooling is begun within 60 minutes of stroke onset in permanent and 180 minutes of stroke onset in temporary occlusion models. Prolonged postischemic cooling further enhances efficacy.

Conclusions—Laboratory studies have shown that intraischemic hypothermia is more protective than postischemic hypothermia and more benefit is conferred with temporary occlusion than permanent occlusion models. The efficacy of postischemic hypothermia is critically dependent on the duration and depth of hypothermia and its timing relative to ischemia. **(*Stroke*. 2004;35:1482-1489.)**

Key Words: animal models ■ middle cerebral artery occlusion ■ hypothermia ■ ischemia

Brain injury in cerebral ischemia emerges from the intricate interaction of molecular events set in motion by ischemia and pathological events associated with the restoration of cerebral blood flow. The progression of ischemia and reperfusion are time-sensitive, suggesting that therapeutic interventions are time-critical and phase-specific.[1,2] The time-dependency of thrombolytic therapy is an example of time-critical intervention; thrombolytics applied too late may actually worsen damage. Reperfusion injury is the interaction of ischemic neurovascular tissue with restored blood flow instigating free radical toxicity and inflammatory responses. The ensuing infarct volume is an interaction of the quantity of reduced cerebral blood flow, the class and timing of reperfusion, and the magnitude of reperfusion injury inflicted.

Although several studies have shown that various pharmacological agents improve outcome after experimental stroke, no study has convincingly shown a benefit in humans. An especially robust neuroprotectant in the laboratory is hypothermia, which has been shown to alter a variety of effects of cerebral ischemia, including reduction in metabolic and enzymatic activity, glutamate release and re-uptake, inflammation, reactive oxidant production, and the expression of a host of other genes. Although stroke models vary in methodology, several laboratories have consistently shown that hypothermia reduces the extent of neurologic damage and improves neurologic function. It may be too simplistic to conclude that the beneficial effects of hypothermia are conferred through a specific neuroprotective action; it is more likely that hypothermia influences a variety of cell death mechanisms, analogous to the advocated combinatorial neuroprotective strategy.[3,4] Given its multiple synergistic effects on ischemia and reperfusion, hypothermia has great potential to be successful at the clinical level.

An association between body temperature, initial stroke severity, infarct volume, and clinical outcome has been recognized.[5] Mild to moderate hypothermia has been found to reduce ischemic brain edema in the setting of massive ischemic strokes.[6,7] Several preliminary clinical reports indicate benefits of mild to moderate hypothermia adjunctive to thrombolytic therapy.[8–10] Whether hypothermia can correct the underlying vascular occlusion remains to be seen, but combined use of hypothermia and thrombolytics must con-

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sider the time sensitivity of and possible interactions between the two. Furthermore, both coagulation and thrombolysis may be influenced by temperature.[11] Nevertheless, numerous experimental reports document the neuroprotective effects of hypothermia. Variations in depth, duration, and delay of hypothermia have been studied in animal models of permanent middle cerebral artery occlusion (pMCAo) and transient middle cerebral artery occlusion (tMCAo). Given past failures of pharmacological neuroprotectants, despite promising preclinical data, it is critical that investigators become familiar with interpreting animal studies to design appropriate clinical trials. With that in mind, we present a critical review of the pertinent literature, taking into consideration potential limitations in translating such laboratory work to the clinical level.

Models of Hypothermia in Focal Cerebral Ischemia

Animal stroke models have attempted to mimic certain aspects of focal cerebral ischemia. Generally, this entails occluding the middle cerebral artery (MCA) with electrocautery, an external clip, or an intraluminal occluding device. Occlusive periods of 1.5 to 3 hours followed by reperfusion (transient ischemia) or permanent vessel occlusion result in well-defined infarcts. Other models have taken this 1 step closer to human stroke by injecting preformed emboli into the internal carotid artery. Reperfusion can be accomplished by administering thrombolytic agents. In general, most models create relatively reproducible infarcts within the ipsilateral MCA territory and can be likened to large-vessel strokes that occur in humans. However, infarct volumes vary greatly depending on the model used (eg, pMCAo versus tMCAo), the duration of the experiment (a few hours postischemia versus weeks of follow-up), and study design. For instance, the intraluminal MCA occlusion model produces larger infarcts, encompassing the entire MCA territory, compared with models in which the distal MCA is occluded, which results in injury confined to the cortex. Variation in experimental results can be expected because different laboratories may use different strains from different vendors. A recent study demonstrated significant strain differences in the response to mild hypothermia, even when studies were performed in the same laboratory.[12] Infarct size in some ischemia models may depend on the presence or absence of collaterals. For example, the spontaneously hypertensive rats (SHR) tend to have poor collaterals and thus larger infarcts, whereas Long Evans rats appear to have remarkable protection, especially during reperfusion.[12] It is also important to control physiological variables such as temperature, blood glucose, blood pressure, arterial pH, and pCO_2, which are known to influence infarct size. The intraluminal model may also affect regions supplied by the anterior choroidal artery, leading to hyperthermia from hypothalamic ischemia.[13,14] Some investigators may not continuously monitor temperature throughout the experiment, which creates uncertainty as to whether hyperthermia may exacerbate injury in control normothermic subjects. Most laboratories are not able to conduct stroke experiments in conscious animals, so choice of anesthetic can also influence experimental results. Some investigators also believe that infarct size may vary seasonally; infarcts produced at one time of the year may differ from infarcts produced at different times. Most researchers carefully control these variables within their own laboratories, but one must keep in mind variation across laboratories when critically reviewing the literature. Although this review is an attempt to distill years of animal studies, care must be taken when comparing results from one laboratory to another. Ideally, specific paradigms should be tested by the same laboratory. Nevertheless, such a review may prove instructive in designing appropriate clinical trials.

Mild Hypothermia in Models of pMCAo

Models of permanent occlusion often involve clipping or cauterization of 1 or more of the major cerebral arteries or simply leaving in place an intraluminal embolus (surgical suture or thrombus). This model best represents large-vessel ischemic strokes in which recanalization does not occur, but probably does not model small-vessel lacunar strokes. "Permanent focal ischemia" is not always accompanied by permanent reduction of regional cerebral blood flow (rCBF) to a level critical to neuronal survival. The rCBF levels increase, presumably by collateral flow, to 30% to 50% of baseline values within 3 hours of ischemia onset.[13] The experimental paradigms we reviewed (Tables 1 and 2) varied in occlusion methods, delay, depth, and duration of hypothermia, as well as timing and quantification of outcome. Coagulation or clipping of the proximal MCA was used alone or in combination with ipsilateral or bilateral common carotid occlusion. Hypothermia was initiated at ischemia onset or delayed up to 3 hours, temperatures varied from 24°C to 34.5°C, and duration varied from 1 to 24 hours. Histology was obtained between 6 hours and 21 days postischemia.

The resulting infarct volumes were reduced by hypothermia by as much as 84%, depending on the experimental paradigm. Brief periods of 1 to 6 hours of deep hypothermia (24°C) were protective in a proximal pMCAo model.[15,16] Infarct volume after MCAo in SHRs was reduced by 84% in a study of 6 hours of deep hypothermia.[15] Even a brief duration (1 hour) of deep hypothermia followed by 2 hours of rewarming reduced infarct volume by 27% and 82% in SHR and Wistar rats, respectively.[16,17] In contrast, brief periods (1 to 6 hours) of mild to moderate hypothermia (30°C to 34.5°C) had conflicting effects in Sprague-Dawley and Wistar rats.[18–22] The key differences between these studies were the duration of the experiment and the use of different strains. Although positive results were observed when brains were examined a few hours after stroke onset, such results should be interpreted with caution because it is possible that the infarcts had not been given sufficient time to mature. In fact, 1 study in a global cerebral ischemia model showed that delayed hypothermia improved neuron survival a few days later, but this protection was no longer observed at 1 month,[23] suggesting that this hypothermia paradigm simply prolonged the inevitable. Furthermore, triphenyl tetrazolium chloride (TTC) is frequently used to delineate infarct size, but whether it is reliable at time points earlier than 24 hours or later than 3 days has been controversial,[24] leaving the interpretation of studies using this technique difficult. However, 1 study

TABLE 1. Models of pMCAo and Prompt Onset of Hypothermia

Study	Model	Temperature	Duration	Histology	Difference in Stroke Volume Hypothermia *vs* Controls (%)
Baker[15]	**SHR, CAU, pMCAo**	**24°C**	**6 h**	**6 h**	**−84**
Baker[16]	**Wistar, CAU, pMCAo**	**24°C**	**1 h plus 90 min rewarming**	**24 h**	**−82**
		24°C	**1 h plus 90 min rewarming**	**72 h**	**−58**
Kader[18]	**Wistar, CAU, pMCAo plus ipsilateral ICAo**	**30°C**	**1 h**	**24 h**	**−55**
		33°C	**1 h**	**24 h**	**−60**
		34.5°C	**1 h**	**24 h**	**−60**
Morikawa[20]	Sprague-Dawley, CAU, pMCAo plus 30 min hypotension, pMCAo	30°C	4 h	72 h	None
		30°C	2 h	72 h	None
Moyer[19]	**Wistar, CAU pMCAo plus 60 min bilateral CCAo**	**32°C**	**1 h**	**24 h**	**−77**
Onesti[17]	**SHR, CAU, pMCAo**	**24°C**	**1 h plus 120 min rewarming**	**24 h**	**−27**
Ridenour[21]	SHR, LIG, pMCAo	33°C	2 h	96 h	None
Xue[22]	**Wistar, clips, permanent CCA plus MCA**	**32°C**	**6 h**	**6 h**	**−61**
Yanamoto[13]	**Sprague-Dawley, CAU, pMCAo plus CCA clips, pMCAo plus ipsilateral ICA, ECA, and contralateral ICA**	**30°C**	**24 h**	**48 h**	**−44**
		33°C	**24 h**	**21 d**	**−48**

CAU indicates extraluminal cauterization; CCA, common carotid artery; ECA, external carotid artery; ICA, internal carotid artery; ILS, intraluminal suture; LIG, extraluminal ligature; MCA, middle cerebral artery; p, permanent; o, occlusion.

Bold font denotes model revealed significant benefit, and regular font denotes model did not reveal significant benefit.

revealed that prolonged mild to moderate hypothermia reduced infarct volume by 44% at 2 days after stroke and as much as 48% at 3 weeks after stroke.[13]

Results of studies evaluating delayed hypothermia in pMCAo models are inconsistent. Coagulation or clipping of the proximal MCA was used alone or in combination with ipsilateral or bilateral common carotid occlusion. Hypothermia was initiated 0.5 to 3 hours after insult, temperatures varied from 24°C to 33°C, and duration varied from 1 to 24 hours. In 10 publications on permanent models, 22 experimental paradigms were tested, from which 15 revealed beneficial effects. On the average, hypothermia resulted in a 60% reduction in infarct size (Tables 1 and 2). As little as 1 hour of moderate to deep hypothermia delayed for 1 hour after ischemia onset was beneficial in some models[16,18] but not in others.[19,25] There is no experimental evidence that brief deep hypothermia ameliorates infarct volume if initiated later than 60 minutes after onset of ischemia in permanent occlusion models.[16] Studies in other cerebral ischemia models indicate that hypothermia can protect even if cooling is delayed by several hours, provided the duration of cooling is prolonged. Colbourne and Corbett reported that they could delay cooling by up to 6 hours, provided cooling was maintained for 24 to 48 hours in a global ischemia model.[26]

TABLE 2. Models of pMCAo and Delayed Onset of Hypothermia

Study	Model	Delay	Temperature	Duration	Histology	Difference in Stroke Volume Hypothermia vs Controls (%)
Baker[16]	**Wistar, CAU, pMCAo**	**30 min delayed**	**24°C**	**1 h plus 90 min rewarming**	**24 h**	**−63**
		60 min delayed	**24°C**	**1 h plus 90 min rewarming**	**24 h**	**−63**
	Wistar, CAU, pMCAo	120 min delayed	24°C	1 h plus 90 min rewarming	24 h	+36
		180 min delayed	24°C	1 h plus 90 min rewarming	24 h	+8
Doerfler[25]	**Wistar ILS**	**60 min delayed**	**32°C**	**5 h**	**6 h**	**−65**
	ILS	60 min delayed	32°C	5 h	24 h	+16
Kader[18]	**Wistar, CAU pMCAo plus ipsilateral ICAo**	**60 min delayed**	**33°C**	**1 h**	**24 h**	**−55**
Moyer[19]	Wistar, CAU pMCAo plus 60 min bilateral CCAo	40 min delayed	32°C	1 h	24 h	None

Bold font denotes model revealed significant benefit, regular font denotes model did not reveal significant benefit.

TABLE 3. Models of tMCAo and Prompt Intraischemic Onset of Hypothermia

Study	Model	Temperature	Duration	Histology	Difference in Stroke Volume Hypothermia vs Controls (%)
Chen[37]	**Wistar ILS 120 min**	**30°C**	**3 h**	**96 h**	Qualitative Assessment: **Complete hemispheric infarcts reduced to selective neuronal injury or small foci of infarcts**
Xue[22]	**Wistar, clips tCCA plus MCA**	**32°C**	**3 h**	**24 h**	**−92**
	Permanent contralateral CCA 180 min	**32°C**	**3 h**	**72 h**	**−85**
Karibe[38]	**Sprague-Dawley ILS 120 min**	**32−33°C**	**130 min**	**24 h**	**−87**
Huh[35]	**Sprague-Dawley ILS 120 min**	**32°C**	**3 h + 2 h rewarming**	**72 h**	**−31**
Maier[36]	**Sprague-Dawley ILS 120 min**	**33°C**	**2 h**	**24 h**	**−68**
		30°C	**2 h**	**24 h**	**−51**
		33°C	0.5 h	72 h	None
		33°C	**1 h**	**72 h**	**−84**
		33°C	2 h	72 h	None hemisphere **−72 cortex**
Maier[28]	**Sprague-Dawley ILS 120 min ILS 90 min**	**33°C**	**2 h**	**72 h**	**−54 cortex, −46 striatum**
		33°C	**1.5 h**	**72 h**	**−37**
		33°C	**1.5 h**	**7 d**	**−48**
		33°C	**1.5 h**	**60 d**	**−56**
Morikawa[20]	**Sprague-Dawley, clip, tMCAo 120 min**	**30°C**	**2 h**	**72 h**	**−74 cortex −63 striatum**
Ridenour[21]	**SHR, clip, tMCAo 60 min**	**33°C**	**2 h**	**96 h**	**−48**
Schmidt Elsaesser[32]	**Sprague-Dawley ILS 90 min**	**33°C**	**2 h**	**7 d**	**−63**
Yanamoto[27]	**Sprague-Dawley**	33°C	2 h	48 h	None
	MCAo-clip+ 2CCA-clips 120 min	**33°C**	**5 h**	**48 h**	**−42**
		33°C	**24 h**	**48 h**	**−65**
	MCAo-clip + ipsilatera ICCA-clip 120 min	33°C	5 h	30 d	None
		33°C	**24 h**	**30 d**	**−51**
Zausinger[39]	**Sprague-Dawley ILS 90 min**	**33°C**	**1.5 h**	**72 h**	**−94 cortex −27 striatum**

Bold font denotes model revealed significant benefit, regular font denotes model did not reveal significant benefit.

However, such studies have not been conducted in the focal models. The reasons for these discrepancies are unclear, but they suggest that the protective effects of hypothermia against pMCAo are less consistent than tMCAo followed by reperfusion.

Two studies assessed neurological deficits after pMCAo.[13,21] Neurological scores at 24 and 96 hours in SHR after 2 hours of mild hypothermia revealed no benefit. In contrast, there was a trend toward improved outcomes at 24 hours in SHRs subjected to 2 hours of mild hypothermia in a 1-hour tMCAo model.[21] Yanamoto et al demonstrated long-term favorable outcome in neurological function in their model of intraischemic prolonged hypothermia in pMCAo. Significantly improved outcomes with hypothermia were assessed as early as 48 hours after ligation and sustained for 3 weeks.[13]

Mild Hypothermia in Models of tMCAo

More work has been published in the temporary occlusion models, and relative to the pMCAo model, the results have been more consistent from laboratory to laboratory. After tMCAo, protective effects of mild to moderate hypothermia can be demonstrated more reliably (Tables 3 and 4). Most models use either clips applied to the proximal MCA or filaments inserted into the cervical carotid and advanced into the proximal MCA. Duration of ischemia varied between 90 minutes and 6 hours, with the majority using a 2-hour transient occlusion. In 3 of these investigations, animals were surviving as long as 1 month[27] or 2 months[28,29] after the ischemic challenge. The majority of studies used mild hypothermia.[21,27–34] One study compared deep to moderate hypothermia[35] and another compared moderate to mild hypothermia.[36] Interestingly, both studies indicate that mild to

TABLE 4. Models of tMCAo and Delayed Intraischemic or Postischemic Onset of Hypothermia

Study	Model	Delay	Temperature	Duration	Histology	Difference in Stroke Volume Hypothermia vs Controls (%)
Xue[22]	**Wistar, clips tCCA plus MCA**	**180 min**	**32°C**	**3 h**	**6 h**	**−61**
		90 min	**32°C**	**1.5 h**	**24 h**	**−49**
	Permanent contralateral CCA 180 min	**90 min**	**32°C**	**3 h**	**24 h**	**−73**
Karibe[38]	**Sprague-Dawley ILS 120 min**	**10 min**	**32–33°C**	**110 min**	**24 h**	**−76**
		30 min	**32–33°C**	**90 min**	**24 h**	**−57**
	Sprague-Dawley ILS 120 min	60 min	32–33°C	60 min	24 h	None
Kawai[30]	**Sprague-Dawley ILS 120 min + ECA ligation**	**90 min**	**33°C**	**22 h**	**24 h**	**−59 cortex**
	Sprague-Dawley ILS 120 min + ECA ligation	90 min	33°C	22 h	24 h	None striatum
Kollmar[31]	**Wistar ILS 120 min**	**60 min**	**33°C**	**5 h**	**6 d**	**−30**
Colbourne[34]	**SHR, clip MCAo 90 min**	**60 min**	**33–35°C**	**24 h+24 h**	**5 d**	**−47**
Corbett[29]	**Wistar ILS 30 min plus Hypotension**	**30 min**	**34°C**	**48 h**	**60 d**	**−72 cortex**
		30 min	**34°C**	**48 h**	**60 d**	**−13 striatum**
Maier[28]	**Sprague-Dawley ILS 120 min**	**90 min**	**33°C**	**2 h**	**72 h**	**−66 cortex −61 striatum**
		120 min	**33°C**	**2 h**	**72 h**	**−47 cortex**
	Sprague-Dawley ILS 120 min	180 min	33°C	2 h	72 h	Striatum unchanged Cortex and striatum unchanged
Huh[35]	Sprague-Dawley ILS 120 min	120 min	27°C	3 h + 2 h rewarming	72 h	None
	Sprague-Dawley ILS 120 min	**120 min**	**32°C**		**72 h**	**−46**
Yanamoto[33]	Sprague-Dawley MCAo-clip+CCA-clips 180 min	180 min	33°C	1 hour	24 h	None
		180 min	**33°C**	**21 h**	**24 h**	**−32**
		210 min	33°C	21 h	24 h	None
		180 min	**33°C**	**21 h**	**48 h**	**−31**
Yanamoto[27]	Sprague-Dawley MCAo-clip+CCA-clips 120 min	120 min	33°C	22 h	48 h	None
Zhang[40]	**Wistar ILS 120 min**	**60 min**	**30°C**	**3 h**	**7 d**	**−48**
Zhang[41]	Wistar ILS 120 min	120 min	30°C	1 h	7 d	Qualitative assessment: None cortex None striatum
		120 min	30°C	3 h	7 d	**Reduced cortex** None striatum

Bold font denotes model revealed significant benefit, regular font denotes model did not reveal significant benefit.

moderate hypothermia (32°C to 33°C) produces better results, and argue that difficulties in regulating biological parameters under moderate to deep hypothermia in small animals and protracted recovery from anesthesia may explain the difference.

The timing of hypothermia, whether intraischemic* or postischemic,[22,28,29,33–35,40,41] is another important distinction between the reported studies. Finally, the timing of hypothermia, either at the onset of ischemia[20–22,27,28,32,35–39] or with some delay,[22,27,28,30,31,38,39] is an important consideration when applying the results to human stroke. Studies in which occlusion lasts for 2 to 3 hours and in which hypothermia is delayed as late as 3.5 hours after ischemia onset are of particular clinical interest because these most closely mimic the human stroke scenario in which thrombolysis is applied.† In most investigations, hypothermia was applied for a brief duration, usually between 30 minutes and 5 hours. A few studies used prolonged mild hypothermia ranging between 21 and 48 hours.[27,29,30,33,34] All studies of intraischemic hypothermia revealed benefit or at least a trend toward reduced infarct volume. When follow-up was prolonged[27,28] or onset of hypothermia delayed,[28,30,31,38] animals subjected to prolonged periods of hypothermia exhibited better outcomes,

*References 20–22, 27, 28, 30–32, 35–39.

†References 22, 27, 28, 30, 33, 35, 38, 40, 41.

although only 1 study directly compared different durations of hypothermia. In this study, Sprague-Dawley rats were subjected to 2 hours of bilateral common carotid plus unilateral MCA occlusion. Intraischemic hypothermia was initiated at onset for either 5 or 24 hours. One month later, only animals subjected to prolonged hypothermia revealed significantly smaller infarcts.[27] In general, the superiority of prolonged over brief periods of hypothermia was apparent in models applying hypothermia late, such as after ischemic reperfusion.[22,27–29,33–35,40,41]

Two studies directly comparing pMCAo and tMCAo revealed the important observation that hypothermia was effective only against ischemia with reperfusion.[20,21] In 1 of these studies, Sprague-Dawley rats were subjected to 2 hours of tMCAo at 30°C, resulting in a significant reduction in infarct volume compared with rats maintained at normal temperature. The same level of hypothermia in a permanent model was not protective.[20] This suggests that hypothermia may be optimized at the clinical level by recanalization strategies, either pharmacologic or mechanical.

Studies have evaluated neurological deficits at 24 to 72 hours,[28,35,36] 1 month,[34] or serially.[33] In general, the neuroscore results are less sensitive than infarct volumes, but nevertheless show benefits that persist over time.

How Do Animal Studies on Hypothermia in Focal Cerebral Ischemia Translate Into Human Clinical Trials?

In most experimental models, the time window for hypothermic protection closely resembles that of thrombolysis and prolonged continuation may enhance efficacy. A 30% to 50% reduction of infarct size can be obtained if mild hypothermia is administered within the first hour of reperfusion after 2 hours of tMCAo. No benefit has been observed with most permanent occlusion models or when mild hypothermia is administered later than 3 hours after onset of ischemia. Similarly, in humans, positron emission tomography studies revealed that at 3 hours after stroke onset, irreversible tissue damage makes up two thirds of the final infarct volume.[42] Mild hypothermia may reduce infarct size by one third at this late time point and may be even more effective in patients treated earlier. Although it may be argued that patient enrollment in clinical studies may be facilitated by extending the allowable time window, there is no experimental evidence to suggest that hypothermia can achieve relevant improvement beyond 3 hours. One study in global cerebral ischemia did show that prolonged cooling delayed 6 hours resulted in marked neuroprotection, but this has not been shown in focal ischemia models.[26] Given the different response rates in temporary versus permanent occlusion models, hypothermia is more likely to work in concert with early reperfusion. Timely administration of thrombolytics substantially increases the odds for early reperfusion. Intravenous and intraarterial thrombolysis were compared in 1 rabbit small clot model, which showed that either route of administration led to similar improvement in neurological deficit.[43] Although intravenous thrombolysis is widely used and can be applied expeditiously, intraarterial thrombolysis is largely inaccessible and fairly restrictive.

Neuroprotective effects of hypothermia are demonstrable at brain temperatures <35°C in rodents[11,36] and appear to intensify with lower temperatures. Although maximal tissue preservation may be produced by deep brain hypothermia, currently there is no easy way to induce it in humans. Mild and moderate hypothermia, however, have been suggested for clinical stroke trials. Mild hypothermia has proven superior to moderate hypothermia in the laboratory[36] and can be achieved with relative ease. Moderate hypothermia requires life support that would restrict its use to mainly tertiary referral centers. Although greater depths of hypothermia, despite having more serious side effects, may prove more protective, mild hypothermia appears to be the most appropriate target temperature at this time. Mild hypothermia (33°C) for phase III clinical trials is suggested on the basis of experimental evidence and safety/feasibility evaluations in humans.

Many models of postischemic hypothermia suggest that the duration of cooling should depend on the time elapsed between onset of ischemia and induction of hypothermia, with longer periods of hypothermia suggested in cases in which hypothermia is delayed.[44,45] Few data are available to guide the rewarming process, however. For unknown reasons, patients with massive brain injuries may experience rebound intracranial hypertension when rapidly rewarmed after prolonged periods of mild to moderate hypothermia. Whether this occurs in experimental stroke models has not been widely studied. For practical purposes, a 24-hour cooling period followed by 12-hour slow rewarming, such as 0.25°C/h, may prove feasible and beneficial in the clinical setting.

Several important limitations have precluded the widespread use of mild to moderate (32°C to 33°C) hypothermia in patients with acute ischemic stroke. Previously available cooling techniques were ineffectual and not practical. Surface cooling with external cooling blankets is slow and cumbersome and requires general anesthesia to counteract vasoconstriction and shivering. In recent trials of surface cooling, >3 hours were generally required to cool patients to target temperature. Furthermore, with surface cooling, precise control of the core temperature is difficult. Pharmacologic paralysis to suppress shivering requires intubation and sedation, which affect hemodynamic parameters and preclude clinical assessment in patients with stroke. A novel method of controlling core temperature, which uses an intravenous heat-exchange device, may be advantageous. The major benefit of this approach is that heat can be directly removed from the thermal core circumventing the heat sink and insulating effects of peripheral tissues. In recent studies of intravenous cooling with large pigs, mild hypothermia was achieved in ≈ 1 hour, and core temperature was precisely maintained over prolonged periods.[46,47] However, even with these efficient intravenous cooling devices, the heat generated by shivering is not adequately overcome. Thus, additional antishivering interventions remain necessary. Other mostly proprietary techniques currently being explored include selective head cooling caps and selective intraarterial cooling technologies.

Conclusion

Research on brain cooling in focal ischemia has established hypothermia's robust and permanent functional and histologic protection. The studies have shown that intraischemic hypothermia is more protective than postischemic hypothermia, and more benefit is conferred in temporary than in permanent occlusion models. The efficacy of postischemic hypothermia depends on the time of initiation and duration and depth of hypothermia. To obtain protection, experimental studies indicate that hypothermia should probably be applied within 3 hours of ischemia onset. Although longer time-windows may be acceptable if cooling is prolonged, this has not been adequately studied in focal ischemic models.

Hypothermia has been suggested as the "gold standard" to which other therapies should be compared in the laboratory. Because none of the previous human neuroprotective trials for stroke showed benefit, designing the appropriate clinical trials of cooling for stroke is a difficult proposition. The experimental literature suggests that combining reperfusion with cooling may be most effective. Although hypothermia is remarkably neuroprotective in animal models, it may lack efficacy in human trials because it may be underdosed or overdosed. Adverse systemic effects may outweigh the benefits of brain cooling in a clinical trial. The right balance of time of induction and depth and duration of hypothermia will ultimately determine whether therapeutic hypothermia becomes a friend or a foe of acute stroke intervention.

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References

1. Lee JM, Zipfel GJ, Choi DW. The changing landscape of ischaemic brain injury mechanisms. *Nature.* 1999;399:A7–A14.
2. White BC, Sullivan JM, DeGracia DJ, O'Neil BJ, Neumar RW, Grossman LI, Rafols JA, Krause GS. Brain ischemia and reperfusion: molecular mechanisms of neuronal injury. *J Neurol Sci.* 2000;179:1–33.
3. Grotta JC. Acute stroke therapy at the millennium: consummating the marriage between the laboratory and bedside. The Feinberg lecture. *Stroke.* 1999;30:1722–1728.
4. Hallenbeck JM, Frerichs KU. Stroke therapy. It may be time for an integrated approach. *Arch Neurol.* 1993;50:768–770.
5. Reith J, Jorgensen HS, Pedersen PM, Nakayama H, Raaschou HO, Jeppesen LL, Olsen TS. Body temperature in acute stroke: relation to stroke severity, infarct size, mortality, and outcome. *Lancet.* 1996;347:422–425.
6. Schwab S, Schwarz S, Spranger M, Keller E, Bertram M, Hacke W. Moderate hypothermia in the treatment of patients with severe middle cerebral artery infarction. *Stroke.* 1998;29:2461–2466.
7. Schwab S, Georgiadis D, Berrouschot J, Schellinger PD, Graffagnino C, Mayer SA. Feasibility and safety of moderate hypothermia after massive hemispheric infarction. *Stroke.* 2001;32:2033–2035.
8. Naritomi H, Shimizu T, Oe H, Kinugawa H, Sawada T, Hirata T. Mild hypothermia in acute embolic stroke: a pilot study. *J Stroke Cereb Dis.* 1996;6:193–196.
9. Shimizu T, Naritomi H, Kakud W, Kinugawa H, Yanagimoto S, Teratani T, Sawada T, Hwang S-H, Lee B-C. Mild hypothermia is effective for the treatment of acute embolic stroke if induced within 24 hours after onset but not in the later phase. *J Cereb Blood Flow Metab.* 1997;17:42.
10. Krieger DW, De Georgia MA, Abou-Chebl A, Andrefsky JC, Sila CA, Katzan IL, Mayberg MR, Furlan AJ. Cooling for acute ischemic brain damage (cool aid): an open pilot study of induced hypothermia in acute ischemic stroke. *Stroke.* 2001;32:1847–1854.
11. Yenari MA, Palmer JT, Bracci PM, Steinberg GK. Thrombolysis with tissue plasminogen activator (tPA) is temperature dependent. *Thromb Res.* 1995;77:475–481.
12. Ren Y, Hashimoto M, Pulsinelli WA, Nowak TS Jr. Hypothermic protection in rat focal ischemia models: strain differences and relevance to "reperfusion injury." *J Cereb Blood Flow Metab.* 2004;24:42–53.
13. Yanamoto H, Nagata I, Niitsu Y, Zhang Z, Xue JH, Sakai N, Kikuchi H. Prolonged mild hypothermia therapy protects the brain against permanent focal ischemia. *Stroke.* 2001;32:232–239.
14. He Z, Yamawaki T, Yang S, Day AL, Simpkins JW, Naritomi H. Experimental model of small deep infarcts involving the hypothalamus in rats: changes in body temperature and postural reflex. *Stroke.* 1999;30:2743–2751.
15. Baker CJ, Onesti ST, Barth KN, Prestigiacomo CJ, Solomon RA. Hypothermic protection following middle cerebral artery occlusion in the rat. *Surg Neurol.* 1991;36:175–180.
16. Baker CJ, Onesti ST, Solomon RA. Reduction by delayed hypothermia of cerebral infarction following middle cerebral artery occlusion in the rat: a time-course study. *J Neurosurg.* 1992;77:438–444.
17. Onesti ST, Baker CJ, Sun PP, Solomon RA. Transient hypothermia reduces focal ischemic brain injury in the rat. *Neurosurgery.* 1991;29:369–373.
18. Kader A, Brisman MH, Maraire N, Huh JT, Solomon RA. The effect of mild hypothermia on permanent focal ischemia in the rat. *Neurosurgery.* 1992;31:1056–1061.
19. Moyer DJ, Welsh FA, Zager EL. Spontaneous cerebral hypothermia diminishes focal infarction in rat brain. *Stroke.* 1992;23:1812–1816.
20. Morikawa E, Ginsberg MD, Dietrich WD, Duncan RC, Kraydieh S, Globus MY, Busto R. The significance of brain temperature in focal cerebral ischemia: histopathological consequences of middle cerebral artery occlusion in the rat. *J Cereb Blood Flow Metab.* 1992;12:380–389.
21. Ridenour TR, Warner DS, Todd MM, McAllister AC. Mild hypothermia reduces infarct size resulting from temporary but not permanent focal ischemia in rats. *Stroke.* 1992;23:733–738.
22. Xue D, Huang ZG, Smith KE, Buchan AM. Immediate or delayed mild hypothermia prevents focal cerebral infarction. *Brain Res.* 1992;587:66–72.
23. Dietrich WD, Busto R, Alonso O, Globus MY, Ginsberg MD. Intraischemic but not postischemic brain hypothermia protects chronically following global forebrain ischemia in rats. *J Cereb Blood Flow Metab.* 1993;13:541–549.
24. Liszczak TM, Hedley-Whyte ET, Adams JF, Han DH, Kolluri VS, Vacanti FX, Heros RC, Zervas NT. Limitations of tetrazolium salts in delineating infarcted brain. *Acta Neuropathol (Berl).* 1984;65:150–157.
25. Doerfler A, Schwab S, Hoffmann TT, Engelhorn T, Forsting M. Combination of decompressive craniectomy and mild hypothermia ameliorates infarction volume after permanent focal ischemia in rats. *Stroke.* 2001;32:2675–2681.
26. Colbourne F, Corbett D. Delayed and prolonged postischemic hypothermia is neuroprotective in the gerbil. *Brain Res.* 1994;654:265–272.
27. Yanamoto H, Nagata I, Nakahara I, Tohnai N, Zhang Z, Kikuchi H. Combination of intraischemic and postischemic hypothermia provides potent and persistent neuroprotection against temporary focal ischemia in rats. *Stroke.* 1999;30:2720–2726.
28. Maier CM, Sun GH, Kunis D, Yenari MA, Steinberg GK. Delayed induction and long-term effects of mild hypothermia in a focal model of transient cerebral ischemia: neurological outcome and infarct size. *J Neurosurg.* 2001;94:90–96.
29. Corbett D, Hamilton M, Colbourne F. Persistent neuroprotection with prolonged postischemic hypothermia in adult rats subjected to transient middle cerebral artery occlusion. *Exp Neurol.* 2000;163:200–206.
30. Kawai N, Okauchi M, Morisaki K, Nagao S. Effects of delayed intraischemic and postischemic hypothermia on a focal model of transient cerebral ischemia in rats. *Stroke.* 2000;31:1982–1989.
31. Kollmar R, Schabitz WR, Heiland S, Georgiadis D, Schellinger PD, Bardutzky J, Schwab S. Neuroprotective effect of delayed moderate hypothermia after focal cerebral ischemia: an MRI study. *Stroke.* 2002;33:1899–1904.
32. Schmid-Elsaesser R, Hungerhuber E, Zausinger S, Baethmann A, Reulen HJ. Combination drug therapy and mild hypothermia: a promising treatment strategy for reversible, focal cerebral ischemia. *Stroke.* 1999;30:1891–1899.
33. Yanamoto H, Hong SC, Soleau S, Kassell NF, Lee KS. Mild postischemic hypothermia limits cerebral injury following transient focal ischemia in rat neocortex. *Brain Res.* 1996;718:207–211.

34. Colbourne F, Corbett D, Zhao Z, Yang J, Buchan AM. Prolonged but delayed postischemic hypothermia: a long-term outcome study in the rat middle cerebral artery occlusion model. *J Cereb Blood Flow Metab.* 2000;20:1702–1708.
35. Huh PW, Belayev L, Zhao W, Koch S, Busto R, Ginsberg MD. Comparative neuroprotective efficacy of prolonged moderate intraischemic and postischemic hypothermia in focal cerebral ischemia. *J Neurosurg.* 2000;92:91–99.
36. Maier CM, Ahern K, Cheng ML, Lee JE, Yenari MA, Steinberg GK. Optimal depth and duration of mild hypothermia in a focal model of transient cerebral ischemia: effects on neurologic outcome, infarct size, apoptosis, and inflammation. *Stroke.* 1998;29:2171–2180.
37. Chen H, Chopp M, Zhang ZG, Garcia JH. The effect of hypothermia on transient middle cerebral artery occlusion in the rat. *J Cereb Blood Flow Metab.* 1992;12:621–628.
38. Karibe H, Chen J, Zarow GJ, Graham SH, Weinstein PR. Delayed induction of mild hypothermia to reduce infarct volume after temporary middle cerebral artery occlusion in rats. *J Neurosurg.* 1994;80:112–119.
39. Zausinger S, Hungerhuber E, Baethmann A, Reulen H, Schmid-Elsaesser R. Neurological impairment in rats after transient middle cerebral artery occlusion: a comparative study under various treatment paradigms. *Brain Res.* 2000;863:94–105.
40. Zhang RL, Chopp M, Chen H, Garcia JH, Zhang ZG. Postischemic (1 hour) hypothermia significantly reduces ischemic cell damage in rats subjected to 2 hours of middle cerebral artery occlusion. *Stroke.* 1993; 24:1235–1240.
41. Zhang ZG, Chopp M, Chen H. Duration dependent post-ischemic hypothermia alleviates cortical damage after transient middle cerebral artery occlusion in the rat. *J Neurol Sci.* 1993;117:240–244.
42. Heiss WD, Thiel A, Grond M, Graf R. Which targets are relevant for therapy of acute ischemic stroke? *Stroke.* 1999;30:1486–1489.
43. Clark WM, Russell D, Madden KP, Zivin JA. Intraarterial versus intravenous tissue-type plasminogen activator treatment in experimental stroke. *Stroke.* 1994;25:1699–1700.
44. Colbourne F, Sutherland G, Corbett D. Postischemic hypothermia. A critical appraisal with implications for clinical treatment. *Mol Neurobiol.* 1997;14:171–201.
45. Colbourne F, Grooms SY, Zukin RS, Buchan AM, Bennett MV. Hypothermia rescues hippocampal ca1 neurons and attenuates down-regulation of the ampa receptor glur2 subunit after forebrain ischemia. *Proc Natl Acad Sci U S A.* 2003;100:2906–2910.
46. Inderbitzen B, Yon S, Lasheras J, Dobak J, Perl J, Steinberg GK. Safety and performance of a novel intravascular catheter for induction and reversal of hypothermia in a porcine model. *Neurosurgery.* 2002;50: 364–370.
47. Dae MW, Gao DW, Ursell PC, Stillson CA, Sessler DI. Safety and efficacy of endovascular cooling and rewarming for induction and reversal of hypothermia in human-sized pigs. *Stroke.* 2003;34: 734–738.