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Molecular Mechanisms Underlying the Neuroprotective Effect of Hypothermia in Cerebral Ischemia

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1. Introduction

Cerebral ischemia is a serious dynamic event in the brain involving heterogeneous cell types. Neuroprotective agents represent a potential approach for the treatment of acute stroke. Presently, recombinant tissue plasminogen activator (rtPA) is the only drug that is approved for the management of acute ischaemic stroke, except for the antioxidant Edaravone in Japan (Yoshida et al., 2006). Although stroke patients can receive rtPA therapy within the initial 3 h therapeutic window, there is an increased risk of intracranial haemorrhage, disruption of the blood brain barrier, seizures, or the progression of neuronal damage (Laloux, 2001). Thus, there is a continued need to explore novel neuroprotective strategies for the management of ischemic stroke. A large number of therapeutic agents have been tested, including N-methyl-D-aspartate receptor antagonists, calcium channel blockers, and antioxidants, for the management of stroke, but none has provided significant neuroprotection in clinical trials (Green et al., 2003; Kidwell et al., 2001).

Therapeutic hypothermia lowers a patient's body temperature in order to reduce the risk of the ischemic injury to the brain following a period of insufficient blood flow (Lampe and Becker, 2011; Yenari and Hemmen, 2010). The normal human adult body temperature is between 34.4–37.8°C and is maintained at a constant level through homeostasis or thermoregulation. Therapeutic hypothermia is defined as the artificial maintenance of the body temperature at <35°C and is subdivided into 4 different categories: mild (32–35°C), moderate (28–32°C), severe (20–28°C), and profound (<20°C). Nowadays, mild-to-moderate hypothermia (31–33°C) is usually applied for neuroprotection. The practical usage of hypothermia for clinical purposes was begun by the ancient Egyptians, Greeks, and Romans (Polderman, 2004). Ancient people observed the clinical usefulness of hypothermia for accidents and applied it to various diseases/symptoms. Modern clinical interest in hypothermia began in the 1930s with the description of the successful rescue of a drowned person with hypothermia after a prolonged period of asphyxia. After the first scientific report in 1945, which described the clinical application of hypothermia to patients with a severe head injury, hypothermia was subsequently applied to intracerebral aneurysm surgery and cerebral protection during complete circulatory arrest.

In the past few decades, the neuroprotective effects of hypothermia have been well established in experimental animals (Kawai et al., 2000; Miyazawa et al., 2003; Yanamoto et al., 2001) and in patients with cardiac arrest (THCASG, 2002 and Bernard et al., 2002). The initiation of moderate hypothermia within a few hours of severe ischemia can reduce the subsequent neuronal death and profoundly improve behavioural recovery. Although hypothermia is the only clinical intervention that appears to be neuroprotective after the initial injury, its key mechanisms have not been clarified. In other words, the neuroprotective effects of hypothermia provide many insights into the pathology of stroke, and thus may reveal clues for novel drug targets.

2. Neuroprotection against cerebral ischemia by hypothermia

Ischemia and cerebral hemorrhage are the two main causes of strokes. Ischemia accounts for ~85% of all reported incidents of stroke, and occurs when a thrombus or embolus blocks cerebral blood flow, resulting in cerebral ischemia and consequently neuronal damage and cell death. Conversely, hemorrhage occurs following the rupture of any blood vessel in the brain, resulting in rapid cerebral damage, and accounts for the remaining 15% of stroke cases. In each situation, the interruption of blood flow to the brain results in the reduced supply of oxygen and nutrients to the neurons. At the molecular level, the pathophysiology of ischemia is complicated and involves multiple sequential steps: progressive neural injury beginning with the activation of glutamate receptors, followed by the production and release of proinflammatory cytokines, nitric oxide (NO), free oxygen radicals, and proteases. As a result, neurons in an ischemic brain suffer irreversible and fatal damage. From the electrophysiological viewpoint, neurons depolarize massively giving rise to anoxic depolarization (Bureš et al., 1974) or to peri-infarct depolarizations (Gyngell et al., 1995). They are both characterized by swelling of neurons, massive influx of Na^+ and Ca^{2+} into neurons, massive release of K^+ into the interstitial space, release of glutamate, acidification of the tissue (Somjen, 2001; Dreier, 2011; Balestrino, 1995).

The lack of blood supply results in two identifiable areas, namely the core and the penumbra. The core is a neuronal dead area that is not therapeutically accessible, whereas the penumbra is a still salvageable zone (Bandera et al., 2006). As a consequence of the reduced blood supply inside the core, adenosine triphosphate (ATP) levels are reduced, leading to the depression of cellular metabolism. Energy loss results in impaired ion homeostasis, which leads to rapid depolarization and a large influx of calcium and potassium ions. The increased levels of intracellular calcium induces the activation of excitotoxic glutamatergic transmission, NO synthase, caspase, xanthine oxidase, and the release of reactive oxygen species. Glutamate release activates phospholipases, phospholipid hydrolysis, and the release of arachidonic acid. The generation of free radicals and lipid peroxidation and the activation of immediate early genes, such as *c-fos, c-jun*, and the inflammatory cascade, lead to progressive ischemic damage, resulting in necrotic as well as apoptotic cell death. Conversely, the penumbra represents viable tissue surrounding the core and receives a trivial amount of blood from collateral arteries; therefore, the penumbra is the target for drug intervention and has the potential for recovery.

Precisely controlled mild hypothermia has been proven to have neuroprotective properties and reduces the risk of the detrimental effects that often occur during profound hypothermia. Large numbers of the phenomena observed in ischemic brains can be

ameliorated by hypothermia, including the reduction of oxygen radical production, with the subsequent reduction in peroxidase damage to lipids, proteins, and DNA, thereby supporting the behavioral recovery of patients. Hypothermia also decreases microglial activation, ischemic depolarization, cerebral metabolic demand for oxygen, and the release of glycerin and excitatory amino acids. We and others have demonstrated that inflammation potentiates cerebral ischemic injury and that hypothermia can reduce inflammation by suppressing the infiltration of neutrophils into ischemic regions (Ohta et al., 2007; Shintani et al., 2011; Terao et al., 2009; Zheng and Yonari, 2004). Furthermore, the inhibition of reactive oxygen species production by leukocytes and microglia in the ischemia brain (Kil et al., 1996), NF-κB activation (Han et al., 2003), neutrophil infiltration (Wang et al., 2002), and cytochrome c release (Yenari et al., 2002) has been also reported in hypothermia-treated ischemic rat brains.

Recently, Lin et al., (2011) reported that whole-body hypothermia broadens the therapeutic window of intranasally administered recombinant human insulin-like growth factor 1 (IGF-1) in a neonatal rat cerebral hypoxia–ischemia model. They ligated the right common carotid artery of postnatal day 7 rat pups, followed by 8% oxygen inhalation for 2 h. After the hypoxia-ischemia treatment, the pups were divided into 2 groups and maintained under different temperatures, room temperature (24.5 ± 0.2°C) and a cool environment (21.5 ± 0.3°C), for 2 or 4 h before being returned to room temperature. IGF-1 was administered intranasally at 1 h intervals starting at 0, 2, or 4 h after hypothermia. Although the administration of hypothermia or IGF-1 alone at 2 h after hypoxia-ischemia treatment did not provide neuroprotection, the combined treatment of hypothermia and IGF-1 significantly protected the neonatal rat brain from hypoxia-ischemia injury. Hypothermia extended the therapeutic window of IGF-1 to 6 h after hypoxia-ischemia. It was observed that the combination therapy decreased the infiltration of polymorphonuclear leukocytes, the activation of microglia/macrophages, and the attenuation of nuclear factor kappa-B (NF-κB) activation. These findings broaden the potential application of hypothermia, i.e., not only for its neuroprotective effects but also for its synergistic effects by the combined use of hypothermia with already existing therapeutic drugs.

3. Molecular mechanisms of hypothermia-induced neuroprotection

3.1 Proteins influenced by hypothermia

The detailed molecular mechanisms underlying the neuroprotection induced by hypothermia against ischemia have been approached by degrees. In most cases, molecules, whose expression is known to be affected by ischemia, have been identified and the effects of hypothermia on the molecule have been explored retrospectively. Table 1 lists the molecules whose expression is affected by hypothermia during ischemia and/or reperfusion. The majority of these proteins are apoptosis, inflammation-related, and signalling molecules, such as kinases and transcription factors. For example, apoptosis-related molecules, such as B-cell lymphoma 2 (Bcl2), Bcl-associated X protein (Bax), caspases, calpains, cytochrome c, and Fas/Fas ligand (FasL), are up- or down-regulated by hypothermia in accordance with its neuroprotective effect, thereby preventing neuronal cell death. Inflammatory molecules, such as tumor necrosis factor alpha (TNFα), interleukin (IL)-1β, IL-6, monocyte chemotactic protein-1 (MCP-1), macrophage inflammatory protein-3

Molecule	Hypothermia	Expression		Ischemia/observation	Reference
		Ischemia	Hypothermia		
IL-1α	33˚C culture	N.D.	Increase	In vitro cell culture	Yanagawa et al., 2002
IL-1β, TNFα	2 h hypothermia, started 20 min after ET-1 injection	Increase	Decrease	Rat endothelin (ET)-1-induced transient focal cerebral ischemia; hypothermia reduced astrogliosis at 1 and 3 d after stroke onset.	Ceulemans et al., 2011
IL-1β, TNFα	33˚C culture	Increase	Decrease	In vitro cell culture; 30 h oxygen – glucose deprivation (OGD)	Webster et al., 2009
IL-6	33˚C culture	N.D.	Decrease	In vitro cell culture	Yanagawa et al., 2002
IL-18	32˚C for 24 h after hypoxia-ischemia	Increase	Decrease	Hypoxia-ischemia	Fukui et al., 2006
MIP-3α	34˚C after MCAO	Increase	Decrease	2 h MCAO	Terao et al., 2009
IL-1β, IL-6, IL-10, TNFα, ICAM-1	33˚C for 24 h	Increase	Decrease	Pigs were subjected to cardiac arrest following temporary coronary artery occlusion.support.	Meybohm et al., 2010
MCP-1	34 ˚C after MCAO	Increase	Decrease	2 h MCAO	Ohta et al., 2007
Intercellular adhesion molecule-1 (ICAM-1)	33˚C etc.	Increase	Decrease	2 h MCAO; extracellular signal-regulated kinase-1/2 (ERK-1/2) activation, and induction of leukocyte infiltration and inflammatory reaction by ischemia were inhibited by hypothermia.	Choi et al., 2011; Inamasu et al., 2001; Koda et al., 2010
NF-κB	33˚C for 2 h after MCAO	No change	No change	2 h MCAO; NF-kB was translocated from cytoplasm to nucleus by hypothermia.	Han et al., 2003
NF-κB's inhibitory protein (IkB-α)	33˚C for 2 h after MCAO	Increase	Decrease	2 h MCAO	Han et al., 2003
IkB-α, NOS, TNFα	30-34˚C	Increase	Decrease	Phosphorylation of IkB-α was suppressed by hypothermia.	Yenari et al., 2006

Molecule	Hypothermia	Expression		Ischemia/observation	Reference
		Ischemia	Hypothermia		
Bcl-2	30-34°C	Down	Increase	1 h hypoxia-ischemia etc.	Eberspacher et al., 2005; Jieyong et al., 2006; Zhao et al., 2004; Yenari et al., 2002; Zhang et al., 2001
Bcl-2	31-32°C hypothermia for 60 min after MCAO	N.D.	Increase	Rat global cerebral ischemia (20 min); the mortality in rats was evaluated at 72 h and 168 h reperfusion.	Zhang et al., 2010
Bcl2	33°C culture	Increase	Decrease	In vitro cell culture; 5 h OGD at 37°C; human umbilical vein endothelial cells were used in this study.	Yang et al., 2009
Bax	33°C culture	Decrease	Increase	In vitro cell culture; 5 h OGD at 37°C; human umbilical vein endothelial cells were used in this study.	Yang et al., 2009
Bax	34°C during ischemia	Increase	Decrease	Bcl–2, p53, and Mdm–2; no change	Eberspacher et al, 2003, 2005
Fas, FasL	33°C during ischemia etc.	Increase	Decrease	2h MCAO; soluble FasL (sFasL) was decreased by hypothermia, while membrane-bound FasL (mFasL) increased.	Liu et al., 2008; Phanithi et al., 2000
Akt	34°C after ischemia	Increase	Decrease	Hypoxia-ischemia for 1 h	Tomimatsu et al., 2001
Caspase-3	30-34°C	Increase	Decrease	1 h hypoxia-ischemia etc.	Fukuda et al., 2001; Pabello et al., 2005; Phanithi et al., 2000; Tomimatsu et al., 2001
Caspase 8	33°C during ischemia	Increase	Decrease	2h MCAO	Liu et al., 2008
Caspase-3/9	33°C for 10 min before ischemia and maintained 3 h after reperfusion	Increase	Decrease	10 min MCAO and hypotention	Zhao et al., 2004, 2005
Calpain	32°C for 10 min before and during reperfusion	Increase	Decrease	3 h MCAO and 24 h reperfusion	Liebetrau et al., 2004

Molecule	Hypothermia	Expression		Ischemia/observation	Reference
		Ischemia	Hypothermia		
Cytochrome c, AIF	30 or 33°C for 2 h during and/or after MCAO	N.D.	N.D.	2 hr MCAO; cytochrome c release and AIF translocation from mitochondria to nuclei were stimulated by hypothermia.	Zhao et al., 2007
Cytochrome c, AIF	34 or 36°C	Increase	Decrease	50 min MCAO; cytochrome c release and AIF translocation from mitochondria to nuclei were stimulated by hypothermia.	Zhu et al., 2006
Akt	30°C for 10 min before ischemia and maintained for 1 h after ischemia	N.D.	N.D.	1 h MCAO; Akt activity was inhibited by ischemia and stimulated by hypothermia.	Zhao et al., 2005
c-Fos, AP-1	Cold room (1°C) during MCAO	Increase	Decrease	1 h MCAO; c-Jun expression was not affected by hypothermia.	Akaji et al., 2003
AMPK (phospho-rylation)		N.D.	N.D.	Ischemia-induced phosphorylation of AMPK was inhibited by hypothermia.	Li et al., 2011
cold-inducible RNA-binding protein (CIRP)	Moderate (30 ± 2°C) hypothermia for 2 h	Increase	Increase	20 min MCAO	Liu et al., 2010
p53	31-32°C	N.D.	Decrease	Rat global cerebral ischemia (20 min); the mortality in rats was evaluated at 72 h and 168 h reperfusion.	Zhang et al., 2010
p53, PUMA, NOXA	33°C	Increase	Decrease	2 h MCAO; DNA damage-dependent signaling events, including NAD depletion, p53 activation, and mitochondrial translocation of PUMA and NOXA	Ji et al., 2007
hypoxia-inducible factor-1 (HIF-1)	28 – 32°C culture	Increase	Decrease	In vitro cell culture	Tanaka et al., 2010
high-mobility group box 1 (HMGB1)	32°C	N.T.	Decrease		Koda et al., 2010
Hsp70		Increase	Increase	2 h MCAO	Terao et al., 2009

Molecule	Hypothermia	Expression		Ischemia/observation	Reference
		Ischemia	Hypothermia		
Agrin, SPARC (ΒM-40, osteonectin)	32° C	Decrease	Increase	Transient 20 min forebrain ischemia	Baumann et al., 2009
GSK3β	33˚C	N.D.	Decrease	Global cerebral ischemia	Kelly et al., 2005
GSK 3β	Moderate hypothermia (30˚C) blocked degradation of total GSK 3β.	N.D.	N.D.	Dephosphorylated after stroke in normothermia	Zhang et al., 2008
β-catenin		N.D.	N.D.	Phosphorylation of b-catenin was increased and degraded by ischemia. Hypothermia did not inhibit the phosphorylation, but it blocked degradation in the ischemic penumbra.	Zhang et al., 2008
MLK3, MKK4/7, JNK3, c-Jun, FasL	32˚C hypothermia for 10 min before ischemia and maintained for 3 h after ischemia	Increase	Decrease	Alternation of the assembly of the GluR6-PSD95-MLK3 signaling module	Hu et al., 2008
Galanin	33˚C	Decrease	Increase	60 min transient MCAO	Theodorsson et al., 2008
COX-2	33-34˚C	Increase	Decrease	Global ischemic insult	Xiang et al., 2007
MMP-2, MMP-9	33˚C etc.	Increase	Decrease	90 min MCAO	Lee et al., 2005; Nagel et al., 2008
TIMP-2	33˚C during ischemia	N.D.	Increase	2 h MCAO	Lee et al., 2005
BDNF	33-34˚C for 24 h	Decrease	Increase	Permanent MCAO	Xie et al. 2007
α, β, γ-PKCs, CaM kinase II	30 min of ischemia followed by 60 min of reperfusion	N.D.	N.D.	30 min ischemia followed by 60 min reperfusion; hypothermia inhibited translocation of CaM kinase II and α, β, γ-PKC	Harada et al., 2002
PKCε	30°C during MCAO	Decrease	Increase	1 h MCAO	Shimohata et al., 2007
PKCδ	30°C during MCAO	Increase	Decrease	1 h MCAO	Shimohata et al., 2007

Molecule	Hypothermia	Expression		Ischemia/observation	Reference
		Ischemia	Hypothermia		
hypoxanthine phosphoribosyl transferase (HPRT)	33-34°C throughout identical injury and reperfusion periods	No change	No change	8 min hypoxia or ischemia, and 30 min or 4 h of cerebral reperfusion	Cherin et al., 2006
calcium sensing receptor (CaSR)	33°C for 3 h after reperfusion	Increase	Decrease	10 min ischemia followed by 1-3 d reperfusion	Kim et al., 2011
Inhibitory gamma-aminobutyrica cid-B receptor 1 (GABA-B-R1)	33°C for 3 h after reperfusion	Decrease	Increase	10 min ischemia followed by 1-3 d reperfusion	Kim et al., 2011
AMPA receptor subunit GluR2	1 h after reperfusion at 32°C, then warmed to 34°C and 36°C in a step-by-step manner.	Decrease	Increase	1 h MCAO	Colbourne et al., 2003
SOD	33°C during ischemia	Increase	Decrease	2 h MCAO	Maier et al., 2002
iNOS	33°C during or after ischemia	Increase	Decrease	1 h MCAO	Han et al., 2002
GPR78	34±0.5°C	Decrease	Increase	ischemic for 15 min and then reperfused for 3 h under	Aoki et al., 2001
74 proteins including glycolysis, plasticity, and redox-related proteins.	33°C during ischemia	N.D.	Glycolysis and plasticity-related proteins were preserved but redox-related proteins were lowered by hypothermia.	15 min ischemia	Teilum et al., 2007

N.D. = not determined.

Table 1 Proteins of which expression and/or modification are altered by ischemia and hypothermia

alpha (MIP-3α), and NF-κB, are also up-regulated after middle cerebral artery occlusion (MCAO) and down-regulated by hypothermia. These molecules are considered to enhance cell damage through the activation of microglia and/or astrocytes. Recently, more direct and dynamic inflammatory changes have been speculated to be induced by hypothermia

during ischemia. And now it has become clear that the neuroinflammatory response could be detrimental and that even peripheral immune responses can be regulated by the brain (Ceulemans et al., 2011).

Another type of change evoked by hypothermia in ischemic brains is protein modification. For instance, Li et al. (2011) reported that hypothermia reduced the activation of 5' adenosine monophosphate-activated protein kinase (AMPK), a ubiquitously distributed kinase, by the dephosphorylation of its regulatory residues in ischemic mice. They showed that hypothermic neuroprotection was ameliorated by compound C, an AMPK inhibitor, and that genetic deletion of one of the catalytic isoforms of AMPK completely reversed the effects of hypothermia on stroke outcome after acute and chronic survival. Their study provides evidence that hypothermia exerts its protective effects, in part, by inhibiting AMPK activation, at least in experimental focal stroke. AMPK is known to participate in an energy sensing cascade and to serve as a master regulator of metabolism in response to ATP depletion. Recently, additional roles of AMPK in a variety of other cellular processes have been revealed, in the cytoplasm and nucleus, as a controllor of cell polarity and a transcriptional regulator. A more interesting function of AMPK in signaling pathways is its role as a responder to cellular stress and damage, and the relevance of AMPK signaling in various diseases is becoming a hot topic in the investigation of ischemic physiology.

3.2 Inflammatory proteins

3.2.1 Cytokines

Neuroinflammation is involved in the pathogenesis of many central nervous system (CNS) diseases. In stroke, excess inflammatory activation results in brain injury and ultimately causes severe neuronal apoptosis (Zheng and Yonari, 2004). Anti-inflammatory therapies using immunosuppressants (Furuichi et al., 2004) or biogenetics, e.g., an anti-ICAM-1-neutralizing antibody (Matsuo et al., 1994), have been applied in preclinical and clinical trials. In this context, the effect of hypothermia on neuroinflammation has been vigorously explored.

Webster et al. (2009) measured the levels of proinflammatory cytokines, such as TNF-α and IL-β, in microglial culture supernatants after stimulation with lipopolysaccharide (LPS) or 2 h oxygen–glucose deprivation (OGD) exposure followed by 24 h reperfusion. There was a marked increase in the production and release of inflammatory cytokines by microglia following LPS stimulation and OGD, which was attenuated by hypothermia. Glutamate, a major neurotransmitter, was also released from microglia stimulated with LPS at 37°C, but reduced levels were observed when they were stimulated with LPS at 33°C. IL-18 is another proinflammatory cytokine that may contribute to brain injury. Fukui et al. (2006) showed that the effects of hypothermia treatment after hypoxia-ischemia (rectal temperature of 32°C for 24 h) on IL-18 expression. IL-18 expression in the ipsilateral hemispheres of the normothermia group significantly increased at 72 h after hypoxia-ischemia compared with controls; however, IL-18 expression was significantly decreased in the hypothermia group.

NF-kB is a transcription factor that is activated after cerebral ischemia. The activation of NF-kB leads to the expression of many inflammatory genes involved in the pathogenesis of stroke. Yenari and Han (2006) showed that hypothermia decreases the translocation of NF-kB from the cytoplasm to nucleus and its binding activity to NF-kB regulatory proteins. Mild hypothermia appears to suppress the phosphorylation of IkB-α, an NF-kB inhibitory

protein, by decreasing the expression and activity of IkB kinase-γ (IKKγ). As a consequence, hypothermia suppressed the expression of 2 NF-kB target genes, inducible nitric oxide synthase (iNOS) and TNFα.

We previously determined that the therapeutic time window of post-ischemia mild hypothermia was 4 h after reperfusion (Ohta et al., 2007). Thus, we considered that the gene expression changes that occur before the 4 h time point might be important for the neuroprotective effect induced by mild hypothermia, even though the neuroprotection afforded by hypothermia alone during this period was found to be insufficient. After a series of investigations with hypothermia, we hypothesized that the gene expression changes that were observed after the 4 h timepoint, following the discontinuation of hypothermia, are related to the ischemic damage detected at 2 d after MCAO. Genes that were upregulated after the 4 hr timepoint, including *early growth response-2 (Egr-2), neurotransmitter-induced early genes-1* (*Ania-1*), and *macrophage inflammatory protein-3α (MIP-3α)*, were found to be important for the neuroprotection afforded by hypothermia. We selected the following 12 genes that might exert substantial neuroprotective activity: *c-Fos, Egr-1, Egr-4, neuron-derived orphan receptor-1 (Nor1), MAP kinase phosphatase-1* (*MKP-1*), *MKP-CPG21, MIP-3α, monocyte chemotactic protein-1* (*MCP-1*), *brain-derived neurotrophic factor* (*BDNF*), *IL-1β, Ania-1,* and *Ania-7*. Egr-1 is known as a master switch that is activated by ischemia to trigger the expression of pivotal regulators of inflammation, e.g., IL-1α, MCP-1, and MIP-2, in addition to coagulation and vascular hyperpermeability.

3.2.2 Chemokines

Chemokines are also well known to be detrimental factors in the brain. For instance, MCP-1 is considered to be a promising drug target due to its possible role in exacerbating ischemic injury, controlling blood-brain barrier permeability, and driving leukocyte infiltration into the brain parenchyma in stroke (Dimitrijevic et al., 2006, 2007; Schilling et al., 2009). We have observed that *MCP-1* gene expression was upregulated by ischemia and that the expression stimulated by ischemia was supressed by hypothermia (Ohta et al., 2007).

We further conducted comprehensive gene expression analyses of ischemic rat brains with or without hypothermia by using a rat ischemia-reperfusion model in order to elucidate the underlying mechanisms and discover novel target molecules. In this study, we revealed that cerebral *MIP-3a* and *CC-chemokine receptor 6 (CCR6)* genes were significantly induced in the core and penumbra regions of MCAO rat brains, and hypothermia suppressed the expression of both genes (Terao et al., 2009). MIP-3α is expressed in macrophages, dendritic cells, and lymphocytes. Depending on the conditions, MIP-3α can act constitutively or inducibly and serves as a chemoattractant, especially in epithelial immunological systems such as those of the skin and mucosa (Charbonnier et al., 1999; Cook et al., 2000). In the CNS, MIP-3α expression has been reported in autoimmune encephalomyelitis (Ambrosini et al., 2003) and stroke patients (Lu et al., 2004; Utans-Schneitz et al., 1998), but its full role has not yet been determined. CCR6, the sole receptor for MIP-3α, is expressed in multiple leukocyte subsets, and is implicated in diverse inflammatory responses in animal models, such as allergic airway disorders, inflammatory bowel disease, and autoimmune encephalitis (Schutyser et al., 2003). Strikingly, the intracerebral administration of an anti-rat MIP-3α-neutralizing antibody significantly reduced infarct volumes in MCAO rats compared with those of vehicle- and control mouse IgG-treated rats, suggesting that MIP-3α-CCR6 signaling is dominant in the

neuroinflammatory cascades of brain ischemia. Interestingly, the administration of MIP-3α into the striatum induced *CCR6* gene expression in a dose-dependent manner, but not *CCR1* or *CCR2* expression. The intrastriatal injection of IL-1β and TNF-α into control rats upregulated *MIP-3α* and *CCR6* mRNA expression levels in a sequential fashion. Taken together with the robust induction of IL-1β and TNF-α in ischemic brains during an acute phase of MCAO prior to MIP-3α expression, these cytokines may directly evoke MIP-3α production in the CNS. Furthermore, *MIP-3α* mRNA expression was markedly induced by IL-1β and TNF-α in rat astrocytes, but not in microglia or neurons. Astrocytes stimulated by ischemic stress turn into their active form, expressing glial fibrillary acidic protein (GFAP), and appear around the damaged area after ischemic injury (Zoli et al., 1997). Rat primary microglia constitutively express the *CCR6* gene under normal culture conditions, while astrocytes and neurons do not. Interestingly, we found that the expression of *iNOS* and *IL-1β* was induced in MIP-3α-treated microglia. Microglia are activated and accumulate around the injured area following ischemia (Wood, 1995). We observed that MIP-3α was produced by rat primary cultured astrocytes in response to IL-1β and TNF-α treatment, while hypothermia significantly suppressed the expression of both cytokines. Therefore, the activation of astrocytes and microglia may accelerate brain injury-induced neuroinflammation via MIP-3α-CCR6 signaling, whereas hypothermia suppresses this signaling. The physiological roles of MIP-3α-CCR6 signaling in the CNS have yet to be fully determined because various roles for chemokines in the brain have recently been proposed, e.g., neurotransmitters and neuromodulators (de Haas et al., 2007; Rostène et al., 2007). The interactions between MIP-3α-CCR6 signaling and other pathways involved in ischemic pathology, e.g., excitotoxicity, acidotoxicity, oxidative stress, and apoptosis, should also be examined.

3.3 Apoptotic proteins

Apoptosis is another important factor for ischemic damage because of its contribution to the cell death subsequent to ischemia/reperfusion injury (Broughton et al., 2009). To date, mitochondrial dysfunction, oxidative stress, and impaired cerebral energy metabolism have been observed during the neuronal cell death that is responsible for much of the poor neurologic outcome from these events. Recent studies using *in vitro* and *in vivo* neuronal cell death models point toward several molecular mechanisms that are either induced or promoted by the oxidative modification of macromolecules, including the consumption of cytosolic and mitochondrial nicotinamide adenine dinucleotide (NAD+) by poly-ADP ribose polymerase (PARP), opening of the mitochondrial inner membrane permeability transition pore, and the inactivation of key, rate-limiting metabolic enzymes, such as the pyruvate dehydrogenase complex. In addition, the relative abundance of proapoptotic proteins in immature brains and neurons, and particularly within their mitochondria, predisposes these cells to the intrinsic, mitochondrial pathway of apoptosis, which is mediated by the Bax- or Bak-triggered release of proteins into the cytosol through the mitochondrial outer membrane. On the basis of these cell dysfunction and death pathways, several approaches toward neuroprotection are being investigated that show promise for their future clinical application. These strategies include minimizing oxidative stress to avoid unnecessary hypoxia, promoting aerobic energy metabolism by the repletion of NAD+, and providing alternative oxidative fuels, e.g., ketone bodies, directly interfering with apoptotic pathways in mitochondria, and pharmacologically inducing antioxidant and anti-inflammatory gene

expression. Hypothermia is known to reduce oxidative stress, metabolic dysfunction, delayed neuronal death, and short- and long-term neurobehavioral impairment. However, despite successful clinical trials of hypothermia, its neuroprotective mechanisms have not been well investigated.

We investigated the activity of apoptotic proteases, calpains, and caspase-3 in 2 h MCAO rat brains using α-fodrin, a cytoskeletal protein enriched in the synaptosome-rich fraction, as a substrate for each (Fig. 1). α-Fodrin is fragmented by calpains and caspase-3 to 145/150-kDa and 120-kDa cleavage products from its intact 240-kDa protein, respectively (Wang, 2000). Harada et al. (2002) reported that ischemia-reperfusion induced the proteolysis of α-fodrin with the generation of the 150-kDa fragment, while hypothermia inhibited this ischemia-reperfusion-induced proteolysis. As shown in Fig. 1, an anti-α-fodrin antibody detected several bands, including the 240-kDa intact protein and the 145/150-kDa and 120-kDa breakdown products. We also detected the 145/150-kDa bands at 48 h after MCAO in normothermia. Surprisingly, mild hypothermia (34°C) did not change the density of those bands. These finding suggest that the inhibition of protein degradation by hypothermia is not dominant in our model or that α-fodrin is not a suitable substrate to determine apoptotic degradation.
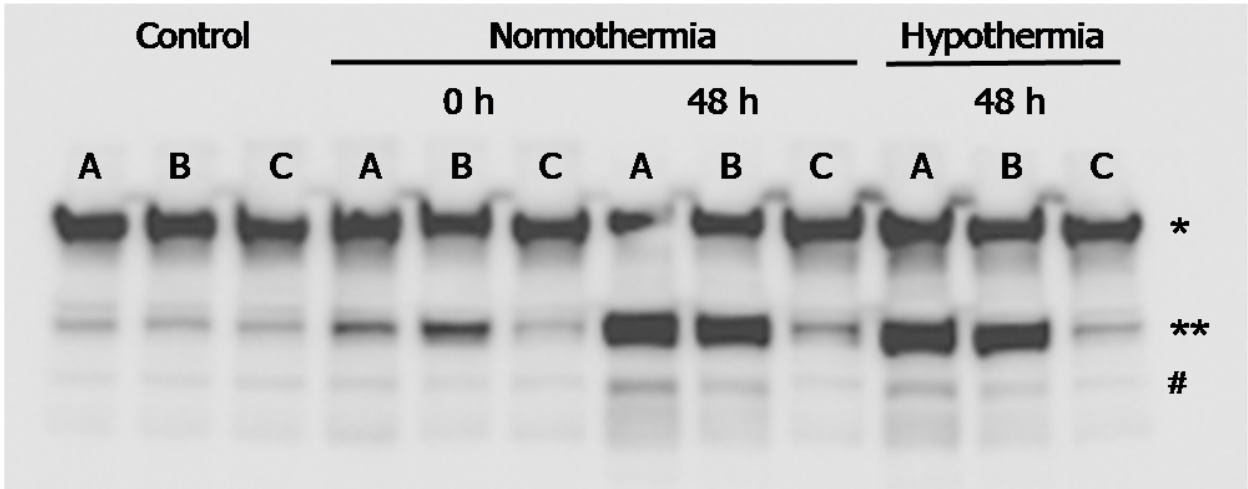

Fig. 1. Lack of effect of hypothermia on α-fodrin degradation in MCAO rat brains

Protease activity in brain tissues of rats maintained at normothermic (37°C) or hypothermic (34°C) conditions at 0 or 48 h after MCAO and in control brain tissues was shown by the degree of α-fodrin degradation. In controls, α- fodrin was detected mainly as a 240-kDa (*) band, although α-fodrin was severely degraded into 145/150- (**) and 120-kDa (#) bands in the core (A) and penumbra (B) regions. Unexpectedly, hypothermia did not affect the degradation of α- fodrin in contralateral (C) regions in MCAO rat brains.

3.4 Cold-inducible RNA-binding proteins

Having some analogy to mild hypothermia, mammalian hibernation serves as a natural model of tolerance to extreme reductions of blood flow, energy consumption, and body temperature, and to the capacity to deliver oxygen to tissues at otherwise lethal levels (Frerichs et al, 1994; Frerichs and Hallenbeck, 1998). As hibernating animals suffer no CNS

damage or cellular loss because of these special adaptive changes (Carey et al, 2003; Storey, 2003), the molecular mechanisms that regulate these adaptations are potential targets for drug discovery. Generally, low temperatures reduce the rate of enzymatic reactions, diffusion, and membrane transport due to the inhibition of chemical reaction rates. Although certain microorganisms are able to adapt to cold environments, apart from the over-expression of a defined set of cold-shock proteins, by the modification of enzyme kinetics, in mammalian cells, the molecular mechanisms that govern adaptation to mild hypothermia are not well known, although they may involve a series of events that modulate transcription, translation, the cytoskeleton, the cell cycle, and metabolic processes. Furthermore, it has been demonstrated that cells exposed to moderate hypothermia, even for short periods of time, have broad changes in their gene expression patterns. Upon exposure to moderate hypothermia, certain specific cold-shock proteins respond immediately to ensure that the cell rapidly adapts to the novel environmental conditions (Fujita, 1999). For decades, it was assumed that the proteins activated upon exposure to mild cold temperatures are somehow responsible for the general metabolic deceleration that occurs when mammalian cells are exposed to mild cold shock. Nonetheless, cold-shock proteins actually "facilitate" the accurate and enhanced translation of specific mRNAs at temperatures below physiological temperatures (Durandy, 2008).

Several hypothermia-induced genes have been identified mainly by using *in vitro* cellular models. For example, Tanaka et al. (2010) showed that the exposure of cultured cells to 32°C for as long as 24 h suppressed the hypoxia-induced activation of hypoxia-inducible factor 1 (HIF-1) and the subsequent upregulation of HIF-1 target genes, e.g., vascular endothelial growth factor (VEGF) or glucose transporter 1 (GLUT-1), although HIF-1 protein stability was not affected by hypothermic treatment. Yang et al. (2009) reported that hypothermia markedly reduced OGD-induced apoptosis in human umbilical endothelial cells (HUVEC), which was induced by changing the media of HUVEC subjected to OGD for 5 h with oxygenated media. The cells were then placed in an incubator for 0–20 h at normothermic (37°C) or hypothermic (33°C) conditions to mimic reperfusion. Hypothermia reduced the expression of cleaved caspase-3 and poly(ADP-ribose) polymerase (PARP), but, in contrast, it reversed the OGD-induced activation of Fas/caspase-8, the increase of Bax and decrease of Bcl-2, and the inhibition of JNK1/2 activation via MKP-1 induction, suggesting that hypothermia represses OGD-induced endothelial cell apoptosis by inhibiting the extrinsic- and intrinsic-dependent apoptotic pathways and the activation of JNK1/2. Lee et al. (2009) showed that moderate hypothermia (30°C) reduces ischemic damage after permanent distal MCAO in rats. They showed that early and delayed hypothermia blocked δ-protein kinase C (PKC) cleavage, suggesting that the degradation of proteins by caspases might be suppressed by hypothermia.

We attempted to identify genes whose expression is specifically induced by mild hypothermia using cDNA subtraction between normothermia- and hypothermia-treated rat cortex-derived primary neurons. The culture temperature of the cells was lowered to 32°C for 14 h and then increased to 37°C. The cells were incubated for 0 (A) or 7 h (B) before their mRNA was collected. Control neurons were maintained at 37°C for 21 h (C). We synthesized cDNA from each mRNA sample as described in our previous report (Terao et al., 2009) and generated a subtraction cDNA library for each condition. To identify the specific genes associated with mild hypothermia, we screened subtracted cDNAs between (A) and (C) or (B) and (C). We identified candidate genes whose expression were specifically induced in

cells incubated at 32˚C compared with controls. All of the genes thus obtained were different gene fragments of the same cold-inducible RNA-binding protein (CIRP) (data not shown). CIRP (also known as A18 hnRNP) is an 18-kD protein that consists of an amino-terminal RNA-binding domain and a carboxyl-terminal glycine-rich domain (Fujita, 1999; Lleonart, 2010). It exhibits structural similarity to a class of stress-induced RNA-binding proteins found in plants. Nishiyama et al. (1997) reported that *cirp* cDNA was induced at a low culture temperature of 32˚C in a mouse-derived cell line. In the last decade, a considerable number of cold-shock proteins have been identified in human cells; however, only 2 of these proteins, CIRP and RNA-binding motif protein 3 (RBM3), have been well characterized since their initial discovery. Although *cirp* expression was clearly identified in cell-based experiments, a comprehensive approach comparing MCAO rat brains with or without hypothermia did not identify the modulation of the *cirp* gene by hypothermia. We speculate that the experimental design of the *in vivo* ischemia-hypothermia model may be not appropriate or that the changes in *cirp* gene expression were too small to be detected on oligonucleotide chips. Since Liu et al. (2010) reported that CIRP expression was upregulated even by ischemia alone, it might be difficult to see the difference in *cirp* gene expression between ischemia alone and ischemia plus hypothermia rat brains. We identified several transcription factors, including AP-1, Pax-4, lmo2, MyoD, ADR1, cdxA, and Pax-8, that have potential binding sites on the human and mouse *cirp* genome promoter regions (data not shown). Importantly, cold-inducible RNA-binding proteins, e.g., CIRP and RBM3, are also able to regulate their expression at the level of translation by binding to different transcripts, thus allowing the cell to respond rapidly to environmental signals. The binding of certain proteins to the 5′-untranslated region (UTR) or 3′-UTR of their mRNAs can affect the rate of translation initiation and the stability of the transcript. Xue et al. (1999) reported that hypothermia induces CIRP expression in the CNS *in vitro* (PC12 neuroblastoma cells) and *in vivo* (rat brains) and that oxidative stress and/or ischemia counteract this phenomenon. In contrast, Liu et al. (2010) reported no correlation between CIRP expression and brain metabolism; therefore, it is suggested that CIRP has unrevealed neuroprotective activity.

More recently, Chip et al. (2011) reported that a low temperature (32˚C) induces the expression of a small subset of proteins, including RBM3. Immunohistochemistry of the developing postnatal murine brain revealed that the spatiotemporal neuronal expression pattern of RBM3 was very similar to that of doublecortin, a marker of neuronal precursor cells. Mild hypothermia profoundly promoted RBM3 expression and rescued neuronal cells from forced apoptosis, as observed in primary neurons, PC12 cells, and cortical organotypic slice cultures. Blocking RBM3 expression in neuronal cells by specific RBM3 small interfering RNAs significantly diminished the neuroprotective effects of hypothermia, while RBM3 overexpression reduced the cleavage of PARP, prevented internucleosomal DNA fragmentation, and reduced lactate dehydrogenase release, suggesting that RBM3 is a novel apoptosis modulator. Taken together, these data indicate that neuronal RBM3 induction in response to hypothermia apparently accounts for a substantial proportion of hypothermia-induced neuroprotection. Therefore, they concluded that RBM3 may be one of the potential neuroprotective factors induced by hypothermia.

3.5 Chaperones

In response to various forms of stress, cells activate a highly conserved heat shock response in accordance with the induction of a set of heat shock proteins (Hsps) that play important

roles in cellular repair and protective mechanisms (Moseley, 2000). Evidence suggests that manipulation of the cellular stress response may offer strategies to protect brain cells from the damage that is encountered following cerebral ischemia or during the progression of neurodegenerative diseases. Hsp70 is a chaperone protein that can fold or refold proteins, coordinate protein trafficking, inhibit protein aggregation or degradation, and exhibits anti-apoptotic and anti-inflammatory activities under physiological conditions. Over-expression of Hsp70 reduced ischemic injury in the mammalian brain (Marber et al., 1995; Zheng et al., 2008). Investigation of the domains within Hsp70 that confer ischemic neuroprotection revealed the importance of the carboxyl-terminal domain (Sun et al., 2006). Arimoclomol, a co-inducer of Hsps, delayed the progression of amyotrophic lateral sclerosis in a mouse model in which motor neurons in the spinal cord and motor cortex degenerate (Kieran et al., 2004). Celastrol, a promising candidate as an agent to counter neurodegenerative diseases, induced the expression of a set of Hsps in differentiated neurons grown in tissue culture (Chow and Brown, 2007). Heat shock "preconditioning" protected the nervous system at the functional level of the synapse, and selective over-expression of Hsp70 enhanced the level of synaptic protection (Ge et al., 2008). Following hyperthermia, constitutively expressed Hsc70 increased in synapse-rich areas of the brain where it associates with Hsp40 to form a complex that can refold denatured proteins. Stress tolerance in neurons is not solely dependent on their own Hsps, but can be supplemented by Hsps from adjacent glial cells; hence, the application of exogenous Hsps at neural injury sites is an effective strategy to maintain neuronal viability.

We identified Hsp70 as a hypothermia-induced protein. Interestingly, we found that Hsp70 was induced by hypothermia after ischemia, although hypothermia by itself did not induce Hsp70 in non-ischemic brains (Terao et al., 2009). Hsp27 was also induced by ischemia, but it was not induced by hypothermia. Other Hsps, including Hsp40, Hsp90, Grp78, Grp94, PDI, and ORP150, were detectable under normal conditions and were not affected by hypothermia or ischemia (Shintani et al., 2011). Recently, Hagiwara et al. (2007) reported that mild hypothermia at 34°C increased the expression level of Hsp70 in LPS-stimulated RAW264.7 cells, although IL-1β, IL-6, and TNF-α expression levels were reduced under the same conditions. In contrast, Tirapelli et al. (2010) recently reported the increase of Hsp70 protein and gene expression in 1 h MCAO rat brains and the role of neuroprotection with hypothermia. However, they showed that the number of Hsp70-positive cells in the ischemic areas was reduced by hypothermia. In our model, we observed the suppression of *Hsp70* mRNA expression by hypothermia, in contrast to the protein level of Hsp70, as observed by Tirapelli et al. (2010). Since we currently do not know the mechanism of induction or the neuroprotective roles of Hsp70 in hypothermia, it is worthy of further investigation.

4. Autophagy

Autophagy is a catabolic process whereby cells respond to energy stress by recycling intracellular components, e.g., proteins, ribosomes, lipids, and even entire organelles (Rabinowitz and White, 2010). In the presence of a sufficient nutrient supply, anabolic reactions predominate within cells, and the autophagy system is maintained at the low levels that are critical for normal cellular homeostasis and survival. Basal levels of autophagic flux are required to degrade long-lived proteins, lipid droplets, and dysfunctional organelles, particularly in post-mitotic cells, e.g., cardiomyocytes and neurons, where the capacity for regeneration is limited. However, autophagy is rapidly activated in response to starvation or is

induced either by an inadequate nutrient supply or by defects in growth factor signaling pathways; when cells are exposed to stress, such as starvation and hypoxia, autophagic mechanisms are triggered to liberate energy substrates and eliminate defective organelles. Apart from conditions of emergent nutrient scarcity, the acceleration of autophagy is often observed in other clinically important circumstances, including neurodegenerative disorders, cancer, misfolded protein accumulation, microbial invasion, and cardiovascular diseases (Beau et al., 2011). There is now increasing evidence that, under certain pathological conditions, autophagy is able to trigger and mediate programmed cell death (type II death) (Galluzzi et al., 2008). Such cell death might be involved in the neuronal death observed after global and focal cerebral ischemia (Balduini et al., 2009 and Sheng et al., 2010). Recently, it was reported that delayed neuronal death occurring in the CA1 pyramidal layer of the gerbil hippocampus after ischemia is apoptotic in nature and autophagosomes/autolysosomes are abundant in these neurons before DNA fragmentation (Xu and Zhang, 2011), i.e., under ischemic conditions, autophagy follows the activation of the mitochondrial pathway of apoptosis; cytochrome c is released from mitochondria, and caspase-9/caspase-3 are activated. Turkmen et al. (2011) recently showed that autophagy and autophagic flux are reduced in cold ischemic kidneys treated with bafilomycin A1. Reduced autophagy and autophagic flux were associated with a significant reduction in apoptotic cell death. These results suggest that hypothermia is able to suppress the autophagic phenomena induced by ischemia; however, since the relationship between autophagy and hypothermia is not well understood, detail analyses should be conducted in the future.

5. Conclusion

Hypothermia is a strong therapeutic methodology for delaying and suppressing ischemic damage in the brain. However, as we do not clearly know the precise mechanisms of hypothermia, the technique is still a kind of art and its usage is very limited. To resolve this situation, we should clarify the detailed molecular mechanisms of hypothermia, identify the dominant effectors for neuroprotection, and substitute the function of hypothermia into a therapeutic drug. In this review, we summarized potential drug targets to be considered for developing "hypothermia-like" drugs. At the present time, as we speculate that these targets are still a piece of the whole picture, we should improve our analytical technology and identify critical factors that change the pathophysiological condition induced by ischemia into a hypothermic one.

6. Acknowledgements

7. References

K. Akaji, S. Suga, T. Fujino, K. Mayanagi, J. Inamasu, T. Horiguchi, S. Sato, T. Kawase (2003) Effect of intra-ischemic hypothermia on the expression of c-Fos and c-Jun, and

DNA binding activity of AP-1 after focal cerebral ischemia in rat brain, *Brain Res.*, 975: 149-157.

E. Ambrosini, S. Columba-Cabezas, B. Serafini, A. Muscella, F. Aloisi (2003) Astrocytes are the major intracerebral source of macrophage inflammatory protein-3alpha/CCL20 in relapsing experimental autoimmune encephalomyelitis and in vitro, *Glia*, 41, 290–300.

M. Aoki, M. Tamatani, M. Yamaguchi, Y. Bando, L. Kasai, Y. Miyoshi, Y. Nakamura, M.P. Vitek, M. Tohyama, H. Tanaka, H. Sugimoto (2001) Hypothermia treatment restores glucose regulated protein 78 (GRP78) expression in ischemic brain, *Brain Res. Mol. Brain Res.*, 95: 117-128.

W. Balduini, S. Carloni, G. Buonocore (2009) Autophagy in hypoxia-ischemia induced brain injury: evidence and speculations, *Autophagy*, 5: 221-223.

M. Balestrino (1995) Pathophysiology of anoxic depolarization: new findings and a working hypothesis, J. Neurosci. Methods, 59: 99-103.

E. Bandera, M. Botteri, C. Sutton, K.R. Abrams, N. Latronico (2006) Cerebral blood flow threshold of ischemic penumbra and infarct core in acute ischemic stroke: a systematic review, *Stroke*, 37: 1334-1339.

E. Baumann, E. Preston, J. Slinn, D. Stanimirovic (2009) Post-ischemic hypothermia attenuates loss of the vascular basement membrane proteins, agrin and SPARC, and the blood-brain barrier disruption after global cerebral ischemia, *Brain Res.*, 1269: 185-197.

I. Beau, M. Mehrpour, P. Codogno (2011) Autophagosome and human diseases, *Int. Biochem. Cell Biol.*, 43: 460-464.

S.A. Bernard, T.W. Gray, M.D. Buist, B.M. Jones, W. Silvester, G. Gutteridge, K. Smith (2002) Treatment of comatose survivors of out-of-hospital cardiac arrest with induced hypothermia, *N. Engl. J. Med.*, 346: 557–563.

B.R. Broughton, D.C. Reutens, C.G. Sobey (2009) Apoptotic mechanisms after cerebral ischemia, *Stroke*, 40: 331-339.

J. Bureš, O. Burešova, J. Krivănek (1974) Anoxic depolarization. In: *The Mechanisms and Applications of Leao's Spreading Depression of Electroencephalographic Activity*, J. Bureš, O. Burešova, J. Krivănek, 72-86, Academia (Publishing House of the Czechoslovak Academy of Sciences) and Academic Press, Prague and New York.

H.V. Carey, M.T. Andrews, S.L. Martin (2003) Mammalian hibernation: cellular and molecular responses to depressed metabolism and low temperature, *Physiol Rev.*, 83: 1153-81.

A-G. Ceulemans, T. Zgavc, R. Kooijman, S. Hachimi-Idrissi, S. Sarre, Y. Michotte (2010) The dual role of the neuroinflammatory response after ischemic stroke: modulatory effects of hypothermia, *J. Neuroinflamm.*, 7: 74-92.

A-G. Ceulemans, T. Zgavc, R. Kooijman, S. Hachimi-idrissi, S. Sarre, Y. Michotte (2011) Mild hypothermia causes differential, time-dependent changes in cytokine expression and gliosis following endothelin-1-induced transient focal cerebral ischemia, *J. Neuroinflamm.*, 31: 60.

A.S. Charbonnier, N. Kohrgruber, E. Kriehuber, G. Stingl, A. Rot, D. Maurer (1999) Macrophage inflammatory protein 3α is involved in the constitutive trafficking of epidermal langerhans cells. *J. Exp. Med.*, 190: 1755–1768.

T. Cherin, M. Catbagan, S. Treiman, R. Mink (2006) The effect of normothemic and hypothermic hypoxia-ischemia on brain hypoxanthine phosphoribosyl transferase activity, *Neurol. Res.*, 28: 831-836.

S. Chip, A. Zelmer, O.O. Ogunshola, U. Felderhoff-Mueser, C. Nitsch, C. Bűhrer, S. Wellmann (2011) The RNA-binding protein RBM3 is involved in hypothermia induced neuroprotection, *Neurobiol. Dis.*, 43: 388-396.

J.S. Choi, J. Park, K. Suk, C. Moon, Y.K. Park, H.S. Han (2011) Mild hypothermia attenulates intercellular adhesion molecule-1 induction via activation of extracellular signal-regulated kinase-1/2 in a focal cerebral ischemia model, *Stroke Res. Treat.*, 2011: 846716. Epub 2011 Mar 16.

A.M. Chow, I.R. Brown, (2007) Induction of heat shock proteins in differentiated human and rodent neurons by celastrol, *Cell Stress Chaperones*, 12: 237-244

F. Colbourne, S.Y. Grooms, R.S. Zukin, A.M. Buchan, M.W. Bennett (2003) Hypothermia rescues hippocampal CA1 neurons ans attenuates down-regulation of the AMPA receptor GluR2 subunit after forebrain ischemia, *Proc. Natl. Acad. Sci. USA*, 100: 2906-2910.

D.N. Cook, D.M. Prosser, R. Forster, J. Zhang, N.A. Kuklin, S.J. Abbondanzo, X.D. Niu, S.C. Chen, D.J. Manfra, M.T. Wiekowski, L.M. Sullivan, S.R. Smith, H.B. Greenberg, S.K.Narula, M. Lipp, S.A. Lira (2000) CCR6 mediates dendritic cell localization, lymphocyte homeostasis, and immune responses in mucosal tissue, *Immunity*, 12: 495–503.

O. B. Dimitrijevic, S. M. Stamatovic, R. F. Keep, and A. V. Andjelkovic (2006) Effects of the chemokine CCL2 on blood-brain barrier permeability during ischemia-reperfusion injury, *J. Cereb. Blood Flow Metab.*, 26: 797–810.

O. B. Dimitrijevic, S. M. Stamatovic, R. F. Keep, A.V. Andjelkovic (2007) Absence of the chemokine receptor CCR2 protects against cerebral ischemia/reperfusion injury in mice, *Stroke*, 38: 1345–1353.

J.P. Dreier (2011) The role of spreading depression, spreading depolarization and spreading ischemia in neurological disease, *Nat. Med.*, 17: 439-447.

Y. Durandy (2008) Pediatric myocardial protection, *Curr. Opin. Cardiol.*, 23: 85-90.

E. Eberspacher, C. Werner, K. Engelhard, M. Pape, L. Laacke, D. Winner, R. Hollweck, P. Hutzler, E. Kochs (2005) Long-term effects of hypothermia on neuronal cell death and the concentration of apoptotic proteins after incomplete cerebral ischemia and reperfusion in rats, *Acta. Anaesthesiol Scand.*, 49: 477-487.

E. Eberspacher, C. Werner, K. Engelhard, M. Pape, L. A. Gelb, P. Hutzler, J. Henke, E. Kochs (2003) The effect of hypothermia on the expression of the apoptosis-regulating protein Bax after incomplete cerebral ischemia and reperfusion in rats, *J. Neurosurg. Anesthesiol.*, 15: 200-208.

K.U. Freichs, C. Kennedy, L. Sokoloff, J.M. Hallenbeck (1994) Local cerebral blood flow during hibernation, a model of natural tolerance to "cerebral ischemia, *J. Cereb. Blood Flow Metab.*, 14: 193-205.

K.U. Freruchs, J.M. Hallenbeck (1998) Hibernation in ground squirrels induces state and species-specific tolerance to hypoxia and aglycemia: an in vitro study in hippocampal slices, *J. Cereb. Blood Flow Metab.*, 18: 168-75.

J. Fujita (1999) Cold shock response in mammalian cells, *J. Mol. Microbiol. Biotechnol.*, 1: 243-255.

H. Fukuda, T. Tomimatsu, N. Watanabe, J.W. Mu, M. Kohzuki, M. Endo, E. Fujii, T. Kanzaki, Y. Murata (2001) Post-ischemic hypothermia blocks caspase-3 activation in the newborn rat brain after hypoxia-ischemia, *Brain Res.*, 910: 187-191.

O. Fukui, Y. Kinugasa, A. Fukuda, H. Fukuda, E. Tskitishvili, S. Hayashi, M. Song, T. Kanagawa, T. Hosono, K. Shimaya,

M.A. Yenari, H.S. Han (2006) Influence of hypothermia on post-ischemic inflammation: role of nuclear factor kappa B (NfkappaB), *Neurochem. Int.*, 49: 164-169.

O. Fukui, Y. Kinugasa, A. Fukuda, H. Fukuda, E. Tskitishvili, S. Hayashi, M. Song, T. Kanagawa, T. Hosono, K. Shimoya, Y. Murata (2006) Post-ischemic hypothermia reduced IL-18 expression and suppressed microglial activation in the immature brain, *Brain Res.*, 1121: 35-45.

Y. Furuichi, T. Noto, J.Y. Li, T. Oku, M. Ishiye, A. Moriguchi, I. Aramori, N. Matsuoka, S. Mutoh, T. Yanagihara (2004) Multiple modes of action of tacrolimus (FK506) for neuroprotective action on ischemic damage after transient focal cerebral ischemia in rats, *Brain Res.*, 1014: 120–130.

L. Galluzzi, E. Morselli, J.M. Vicencio, O. Kepp, N. Joza, N. Tajeddine, G. Kroemer (2008) Life, death and burial: multifaceted impact of autophagy, *Biochem. Soc. Trans.*, 36: 786-790.

P.F. Ge, T.F. Luo, J.Z. Zhang, D.W. Chen, Y.X. Luan, S.L. Fu (2008) Ischemic preconditioning induces chaperone hsp70 expression and inhibits protein aggregation in the CA1 neurons of rats, *Neurosci. Bull.*, 24: 288-296.

A.R. Green, T. Odergren, T. Ashwood (2003) Animal models of stroke: do they have value for discovering neuroprotective agents? *Trend. Pharmacol. Sci.*, 24: 402–408.

M. Gyngell, E. Busch, B. Schmitz, K. Kohno, T. Back, M. Hoehn-Berlage, K-A. Hossmann (1995) Evolution of acute focal cerebral ischemia in rats observed by localized 1H-MRS, diffusion-weighted MRI, and electrophysiological monitoring, *NMR Biomed.*, 8: 206-214.

A.H. de Haas, H.R. van Weering, E.K. de Jong, H.W. Boddeke, K.P. Biber (2007) Neuronal chemokines: versatile messengers in central nervous system cell interaction, *Mol. Neurobiol.*, 36: 137–151.

S. Hagiwara, H. Iwasaka, S. Matsumoto, T. Noguchi (2007) Changes in cell culture temperature alter release of inflammatory mediators in murine macrophagic RAW264.7 cells, *Inflamm. Res.*, 56: 297–303.

H. S. Han, M. Karabiyikoglu, S. Kelly, R. A. Sobel, M. A. Yenari (2003) Mild hypothermia inhibits nuclear factor-κB translocation in experimental stroke, *J. Cereb. Blood Flow Metab.*, 23: 589-598.

H.S. Han, Y. Qiao, M. Karabiyikoglu, R.G. Giffard, M.A. Yenari (2002) Influence of mild hypothermia on inducible nitric oxide synthase expression and reactive nitrogen production in experimental stroke and inflammation, *J. Neurosci.*, 22: 3921-3928.

K. Harada, T. Maekawa, R. Tsuruta, T. Kaneko, D. Sadamitsu, T. Yamashima, K.K. Yoshida (2002) Hypothermia inhibits translocation of CaM kinase II and PKC-α, β, γ isoforms and fodrin proteolysis in rat brain synaptosome during ischemia-reperfusion, *J. Neurosci. Res.*, 67: 664-669.

M. Holzer (2008) Devices for rapid induction of hypothermia, *Eur. J. Anaesthesiol. Suppl.*, 42: 31-38.

The Hypothermia after Cardiac Arrest Study Group (2002) Mild therapeutic hypothermia to improve the neurological outcome after cardiac arrest, *N. Engl. J. Med.*, 346: 549–556.

W.W. Hu, Y. Du, C. Li, Y.J. Song, G.Y. Zhang (2008) Neuroprotection of hypothermia against neuronal death in rat hippocampus through inhibiting the increased assembly of GluR6-PSD95-MLK3 signaling module induced by cerebral ischemia/reperfusion, *Hippocampus*, 18: 386-397.

J. Inamasu, S. Suga, S. Sato, T. Horiguchi, K. Akaji, K. Mayanagi, T. Kawase (2001) Intra-ischemic hypothermia attenuates intercellular adhesion molecule-1 (ICAM-1) and migration of neutrophil, *Neurol. Res.*, 23: 105-111.

X. Ji, Y. Luo, F. Ling, R.A. Stetler, J. Lan, G. Cao, J. Chen (2007) Mild hypothermia diminishes oxidative DNA damage and pro-death signaling events after cerebral ischemia: a mechanism for neuroprotection, 12: 1737-1747.

B. Jieyong, W. Zhong, Z. Shiming, Z. Dai, Y. Kato, T. Kanno, H. Sano (2006) Decompressive craniectomy and mild hypothermia reduces infarction size and counterregulates Bax and Bcl-2 expression after permanent focal ischemia in rats, *Neurosurg. Rev.*, 29: 168-172.

N. Kawai, M. Okauchi, K. Morisaki, S. Nagao (2000) Effects of delayed intraischemic and post-ischemic hypothermia on a focal model of transient cerebral ischemia in rats, *Stroke* 31: 1982–1989.

S. Kelly, D. Cheng, G.K. Steinberg, M.A. Yenari (2005) Mild hypothermia decreases GSK3β expression following global cerebral ischemia, *Neurocrit. Care*, 2: 212-217.

D. Kieran, B. Kalmar, J.R. Dick, J. Riddoch-Contreras, G. Burnstock, L. Greensmith (2004) Treatment with arimoclomol, a coinducer of heat shock proteins, delays disease progression in ALS mice, *Nat. Med.*, 10: 402-405.

H. Y. Kil, J. Zhang, C. A. Piantadosi (1996) Brain temperature alters hydroxyl radical production during cerebral ischemia/reperfusion in rats, *J. Cereb. Blood Flow Metab.*, 16: 100–106.

J.Y. Kim, N. kim, M.A. yenari, W. Chang (2011) Mild hypothermia suppresses calcium-sensing receptor (CaSR) induction following forebrain ischemia while increasing GABA-B receptor 1 (GABA-B-R1) expression, *Transl. Stroke Res.*, 2: 195-201.

Y. Koda, R. Tsuruta, M. Fujita, T. Miyauchi, K. Kaneda, M. Todani, T. Aoki, M. Shitara, T. Izumi, S. Kasaoka, M. Yuasa, T. Maekawa (2010) Moderate hypotheramia suppresses jugular venous superoxide anion radical, oxidative stress, early inflammation, and endothelial injury in forebrain ischemia/reperfusion rats, *Brain Res.*, 1311: 197-205.

P. Laloux (2001) Intravenous rtPA thrombolysis in acute ischemic stroke, *Acta. Neurol. Belg*, 101: 88-95.

C.S. Kidwell, D.S. Kiebeskind, S. Starkman, J.L. Saver (2001) Trends in acute ischemic stroke trials through the 20th century, *Stroke*, 32: 1349–1359.

J.W. Lampe, L.B. Becker (2011) State of art in therapeutic hypothermia, *Annu. Rev. Med.*, 62: 79-93.

S.M. Lee, H. Zhao, C.M. Maier, G.K. Steinberg (2009) The protective effect of early hypothermia on PTEN phosphorylation correlates with free radical inhibition in rat stroke, *J. Cereb. Blood Flow Metab.*, 29: 1589-1600.

J.E. Lee, Y.J. Yoon, M.E. Moseley, M.A. Yenari (2005) Reduction in levels of matrix metalloproteinases and increased expression of tissue inhibitor of metalloproteinase-2 in response to mild hypothermia therapy in experimental stroke, *J. Neurosurg.*, 103: 289-297.

J. Li, S. Benashski, L.D. McCullough (2011) Post-stroke hypothermia provides neuroprotection through inhibition of AMP-activated protein kinase, *J. Neurotrauma.*, 28: 1281-1288.

M. Liebetrau, D. Burggraf, H.K. Martens, M. Pichler, G.F. Hamann (2004) Delayed moderate hypothermia reduces calpain activity and beakdown of its substrate in experimental focal cerebral ischemia in rats, *Neurosci. Lett.*, 357: 17-20.

M.E. Lieonart (2010) A new generation of proto-oncogenes: cold-inducible RNA binding proteins, *Biochim. Biophys. Acta.*, 1805: 43-52.

S. Lin, P.G. Rhodes, Z. Cai (2011) Whole body hypothermia broadens the therapeutic window of intranasally administered IGF-1 in a neonatal rat model of cerebral hypoxia-ischemia, *Brain Res.*, 18: 246-256.

A. Liu, Z. Zhang, A. Li, J. Xue (2010) Effects of hypothermia and cerebral ischemia on cold-inducible RNA-binding protein mRNA expression in rat brain, *Brain Res.*, 1347: 104-110.

L. Liu, J.Y. Kim, M.A. Koike, Y.J. Yoon, X.N. Tang, H. Ma, H. Lee, G.K. Steinberg, J.E. Lee, M.A. Yenari (2008) FasL shedding is reduced by hypothermia in experimental stroke, *J. Neurochem.*, 106: 541-550.

X.C. Lu, A.J. Williams, C. Yao, R. Berti, J.A. Hartings, R. Whipple, M.T. Vahey, R.G. Polavarapu, K.L. Woller, F.C. Tortella, J.R. Dave (2004) Microarray analysis of acute and delayed gene expression profile in rats after focal ischemic brain injury and reperfusion, *J. Neurosci. Res.*, 77: 843–857.

C.M. Maier, G.H. Sun, D. Cheng, M.A. Yenari, P.H. Chan, G.K. Steinberg (2002) Effects of mild hypothermia on superoxide anion production, superoxide dismutase expression, and activity following transient focal cerebral ischemia, *Neurobiol. Dis.*, 11: 28-42.

M.S. Marber, R. Mestril, S.H. Chi, M.R. Sayen, D.M. Yellon, W.H. Dillmann (1995) Overexpression of the rat inducible 70-kD heat stress protein in a transgenic mouse increases the resistance of the heart to ischemic injury, *J. Clin. Invest.*, 95: 1446-1456.

Y. Matsuo, H. Onodera, Y. Shiga, H. Shozuhara, M. Ninomiya, T. Kihara, T. Tamatani, M. Miyasaka, K. Kogure (1994) Role of cell adhesion molecules in brain injury after transient middle cerebral artery occlusion in the rat, *Brain Res.*, 656: 344–352.

P. Meybohm, M. Gruenewald, K.D. Zacharowski, M. Albrecht, R. Lucius, N. Fosel, J. Hensler, K. Zitta, B. Bein (2010) Mild hypothermia alone or in combination with anesthetic post-conditioning reduces expression of inflammatory cytokines in the cerebral cortex of pigs after cardiopulmonary resuscitation, *Crit. Care*, 14: R21.

T. Miyazawa, A. Tamura, S. Fukui, K.A. Hossmann (2003) Effect of mild hypothermia on focal cerebral ischemia. Review of experimental studies, *Neurol. Res.*, 25: 457–464.

P. Moseley (2000) Stress proteins and the immune response, *Immunopharmacology*, 48: 299-302.

Y. Murata (2006) Post-ischemic hypothermia reduced IL-18 expression and suppressed microglial activation in the immature brain, *Brain Res.*, 22: 35-45.

S. Nagel, Y. Su, S. Horstmann, S, Heiland, H. Heiland, H. Gardner, J. Koziol, F.J. Martinez-Torres, S. Wagner (2008) Minocycline and hypothermia for reperfusion injury after focal cerebral ischemia in the rats: effects on BBB breakdown and MMP expression in acute and subacute phase, *Brain Res.*, 1188: 198-206.

H. Nishiyama, H. Higashitsuji, H. Yokoi, K. Itoh, S. Danno, T. Matsuda, J. Fujita (1997) Cloning and characterization of human CIRP (cold-inducible RNA-binding protein) cDNA and chromosomal assignment of the gene, *Gene*, 204: 115-120.

H. Ohta, Y. Terao, Y. Shintani, Y. Kiyota (2007) Therapeutic time window of post-ischemicmild hypothermia and the gene expression associated with the neuroprotection in rat focal cerebral ischemia, *Neuroscience Res.*, 57: 424–433.

N.G. Pabello, S.J. Tracy, A. Snyder-keller, R.W. Keller (2005) Regional expression of constitutive and inducible transcription factors following transient focal ischemia in the neonatal rat: influence of hypothermia, *Brain Res.*, 1038: 11-21.

P.B. Phanithi, Y. Yoshida, A. Santana, M. Su, S. Kawamura, N. Yasui (2000) Mild hypothermia mitigates post-ischemic neuronal death following focal cerebral ischemia in rat brain: immunohistochemical study of Fas, caspase-3 and TUNEL, *Neuropathology*, 20: 105-111.

K. H. Polderman (2004) Application of therapeutic hypothermia in the ICU, *Intensive. Care Med.*, 30: 556-575.

J.D. Rabinowitz, E. White (2010) Autophagy and metabolism, *Science,* 330: 1344-1348.

W. Roste` ne, P. Kitabgi, S.M. Parsadaniantz (2007) Chemokines: a new class of neuromodulator? *Nat. Rev. Neurosci.*, 8: 895–903.

M. Schilling, J-K. Strecker, E.B. Ringelstein, W-R. Schäbitz, R. Kiefer (2009) The role of CC chemokine receptor 2 on microglia activation and blood-borne cell recruitment after transient focal cerebral ischemia in mice, *Brain Res.*, 1289: 79-84.

E. Schutyser, S. Struyf, J. Van Damme (2003) The CC chemokine CCL20 and its receptor CCR6, *Cytokine Growth Factor Rev.*, 14: 409–426.

R. Sheng, L.S. Zhang, R. Han, X.Q. Liu, B. Gao, Z.H. Qin (2010) Autophagy activation is associated with neuroprotection in a rat model of focal cerebral ischemic preconditioning, *Autophagy*, [Epub ahead of print]

T. Shimohata, H. Zhao, J.H. Sung, G. Sun, D. Mochly-Rosen, G.K. Steinberg (2007) Suppression of deltaPKC activation after focal cerebral ischemia contributes to the protective effect of hypothermia, *J. Cereb. Blood Flow Metab.*, 27: 1463-1475.

T. Shimohata, H. Zhao, G.K. Steinberg (2007) Epsilon PKC may contribute to the protective effect of hypothermia in a rat focal cerebral ischemia model, *Stroke*, 38: 375-380.

Y. Shinatni, Y. Terao, H. Ohta (2011) Molecular mechanisms underlying hypothermia-induced neuroprotection, *Stroke Res. Treat.*, 2011: 1-9.

G. G. Somjen (2001) Mechanisms of spreading depression and hypoxic spreading depression-like depolarization, *Physiol. Rev.*, 81: 1065-1096.

K.B. Storey (2003) Mammalian hibernation. Transcriptional and translational controls, *Adv. Exp. Med. Biol.,* 543: 21-38.

Y. Sun, Y.B. Ouyang, L. Xu, A.M. Chow, R. Anderson, J.G. Hecker, R.G. Giffard (2006) The carboxy-terminal domain of inducible Hsp70 protects from ischemic injury in vivo and in vitro, *J. Cereb. Blood Flow Metab.*, 26: 937-950.

T. Tanaka, T. Wakamatsu, H. Daijo, S. Oda, S. Kai, T. Adachi, S. Kizaka-Kondoh, K. Fukuda, K. Hirota (2010) Persisting mild hypothermia suppresses hypoxia-inducible factor-1α protein synthesis and hypoxia-inducible factor-1-mediated gene expression, *Am. J. Physiol. Regul. Integr. Comp. Physiol.*, 298: R661-671.

M. Teilum, M. Krogh, T. Wieloch, G. Mattiasson (2007) Hypothermia affects translocation of numerous cytoplasmic proteins following global cerebral ischemia, *J. proteome Res.*, 6: 2822-2832.

Y. Terao, H. Ohta, A. Oda, Y. Nakagaito, Y. Kiyota, Y. Shintani (2009) Macrophage inflammatory protein-3alpha plays a key role in the inflammatory cascade in rat focal cerebral ischemia, *Neurosci. Res.*, 64: 75-82.

Y. Terao, S. Miyamoto, K. Hirai, H. Kamiguchi, H. Ohta, M. Shimojo, Y. Kiyota, S. Asahi, Y. Sakura, Y. Shintani (2009) Hypothermia enhances heat-shock protein 70 production in ischemic brains, *NeuroReport*, 20: 745–749.

A. Theodorsson, L. Holm, E. Theodorsson (2008) Hypothermia-induced increase in galanin concentrations and ischemic neuroprotection in the rat brain, *Neuropeptides*, 42: 79-87.

D.P. Tirapelli, C.G. Carlotti, J.P. Leite, L.F. Tirapelli, B.O. Colli (2010) Expression of HSP70 in cerebral ischemia and neuroprotective action of hypothermia and ketoprofen, *Arq. Neuropsiquiatr.*, 68: 592-596.

T. Tomimatsu, H. Fukuda, M. Endo, N. Watanabe, J. Mu, M. Kohzuki, E. Fujii, T. Kanzaki, Y. Murata (2001) Effects of hypothermia on neonatal hypoxic-ischemic brain injury in the rat: phosphorylation of Akt, activation of caspase-3-like protease, *Neurosci. Lett.*, 312: 21-24.

K. Turkmen, J. Martin, A. Akcay, Q. Nguyen, K. Ravichandran, S. Faubel, A. Pacic, D. Ljubanović, C.L. Edelstein, A. Jani (2011) Apoptosis and autophagy in cold preservation ischemia, *Transplantation*, 91: 1192-1197.

U. Utans-Schneitz, H. Lorez, W.E. Klinkert, J. Da Silva, W. Lesslauer (1998) A Novel rat CC chemokine, identified by targeted differential display, is upregulated in brain inflammation, *J. Neuroimmunol.*, 92: 179–190.

K.K. Wang (2000) Calpain and caspase: can you tell the difference? *Trends Neurosci.*, 23: 20–26.

G.J. Wang, H.Y. Deng, C.M. Maier, G.H. Sun, M.A. Yenari (2002) Mild hypothermia reduces ICAM-1 expression, neutrophil infiltration and microglia/monocyte accumulation following experimental stroke, *Neuroscience*, 114: 1081–1090.

C.M. Webster, S. Kelly, M.A. Koike, V.Y. Chock, R.G. Giffard, M.A. Yenari (2008) Inflammation and NFκB activation is decreased by hypothermia following global cerebral ischemia, *Neurobiol. Dis.*, 33: 301-312.

P.L. Wood (1995) Microglia as a unique cellular target in the treatment of stroke: potential neurotoxic mediators produced by activated microglia, *Neurol. Res.*, 17: 242–248.

Z. Xiang, S. Thomas, G. Pasinetti (2007) Increased neuronal injury in transgenic mice with neuronal overexpression of human cyclooxygenase-2 is reversed by hypothermia and rofecoxib treatment, *Curr. Neurovasc. Res.*, 4: 274-279.

Y.C. Xie, C.Y. Li, T. Li, D.Y. Nie, F. Ye (2007) Effect of mild hypothermia on angiogenesis in rats with focal cerebral ischemia, *Neurosci. Lett.*, 422: 87-90.

M. Xu, H.L. Zhang (2011) Death and survival of neuronal and astrocytic cells in ischemic brain injury: a role of autophagy, *Acta. Pharmacol.*, doi: 10. 1038/aps.2011.50.

J.H. Xue, K. Nonoguchi, M. Fukumoto, T. Sato, H. Nishiyama, H. Higashitsuji, K. Itoh, J. Fujita (1999) Effects of ischemia and H2O2 on the cold stress protein CIRP expression in rat neuronal cells, *Free Radic. Biol. Med.*, 27: 1238-1244.

Y. Yanagawa, M. Kawakami, Y. Okada (2002) Moderate hypothermia alters interleukin-6 and interleukin-1α reactions in ischemic brain in mice, *Resuscitation*, 53: 93-99.

H. Yanamoto, I. Nagata, Y. Niitsu, Z. Zhang, J.H. Xue, N. Sakai, H. Kikuchi (2001) Prolonged mild hypothermia therapy protects the brain against permanent focal ischemia, *Stroke,* 32: 232–239.

D. Yang, S. Guo, T. Zhang, H. Li (2009) Hypothermia attenuates ischemia/reperfusion-induced endothelial cell apoptosis via alterations in apoptotic pathways and JNK signaling, *FEBS Lett.*, 583: 2500-2506.

M.A. Yenari, T.M. Hemmen (2010) Therapeutic hypothermia for brain ischemia: where have we come and where do we go?, *Stroke*, 41: S72-74.

M.A. Yenari, S. Iwayama, D. Cheng, G.H. Sun, M. Fujimura, Y. Morita-Fujimura, P.H. Chan, G.K. Steinberg, (2002) Mild hypothermia attenuates cytochrome c release but does not alter Bcl-2 expression or caspase activation after experimental stroke, *J. Cereb. Blood Flow Metab.*, 22: 29-38.

M.A. Yenari, H.S. Han (2006) Influence of hypothermia on post-ischemic inflammation: role of NFκB, *Neurochem. Int.*, 49: 164-169.

H. Yoshida, H. Yanai, Y. Namiki, K. Fukatsu-Sasaki, N. Furutani, N. Tada (2006) Neuroprotective effects of edaravone: a novel free radical scavenger in cerebrovascular injury, *CNS Drug Reviews*, 12: 9–20.

Z. Zhang, R.A. Sobel, D. Cheng, G.K. Steinberg, M.A. Yenari (2001) Mild hypothermia increases Bcl-2 protein expression following global cerebral ischemia, *Brain Res. Mol. Brain Res.*, 95: 75-85.

H. Zhang, C. Ren, X. Gao, T. Takahashi, R.M. Sapolsky, G.K. Steinberg, H. Zhao (2008) Hypothermia blocks β-catenin degradation after focal ischemia in rats, *Brain Res.*, 1198: 182-187.

H. Zhang, G. Xu, J. Zhang, S. Murong, Y. Mei, E. Tong (2010) Mild hypothermia reduces ischemic neuron death via altering the expression of p53 and bcl-2, *Neurol. Res.*, 32: 384-389.

H. Zhao, T. Shimohata, J.Q. Wang, G. Sun, D.W. Schaal, R.M. Sapolsky, G.K. Steinberg (2005) Akt contributes to neuroprotection by hypothermia against cerebral ischemia in rats, *J. Neurosci.*, 25: 9794-9806.

H. Zhao, M.A. Yenari, D. Cheng, R.M. Sapolsky, G.K. Steinberg (2005) Moderate hypothermia (30 degree C) for surgery of acute type A aortic dissection, *J. Cereb. Blood Flow Metab.*, 25: 1119-1129.

H. Zhao, M.A. Yenari, D. Cheng, R.M. Sapolsky, G.K. Steinberg (2004) Mild postischemic hypothermia prolongs the time window for gene therapy by inhibiting cytochrome C release, *Stroke*, 35: 572-577.

H. Zhao, J.Q. Wang, T. Shimohata, G. Sun, M.A. Yenari, R.M. Sapolsky, G.K. Steinberg (2007) Conditions of protection by hypothermia and effects on apoptotic pathways in a rat model of permanent middle cerebral artery occlusion, *J. Neurosurg.*, 107: 636-641.

Z. Zheng, J.Y. Kim, H. Ma, J.E. Lee, M.A. Yenari (2008) Anti-inflammatory effects of the 70 kDa heat shock protein in experimental stroke, *J. Cereb. Blood Flow Metab.*, 28: 53-63.

Z. Zheng, M. A. Yenari (2004) Post-ischemic inflammation: molecular mechanisms and therapeutic implications, *Neurological Res.*, 26: 884–892.

C. Zhu, X. Wang, F. Xu, L. Qiu, X. Cheng, G. Simbruner, K. Blomgren (2006) Intraischemic mild hypothermia prevents neuronal cell death and tissue loss after neonatal cerebral hypoxia-ischemia, *Eur. J. Neurosci.*, 23: 387-393.

M. Zoli, R. Grimaldi, R. Ferrari, I. Zini, L.F. Agnati (1997) Short- and long-term changes in striatal neurons and astroglia after transient forebrain ischemia in rats, *Stroke*, 28: 1049–1059.
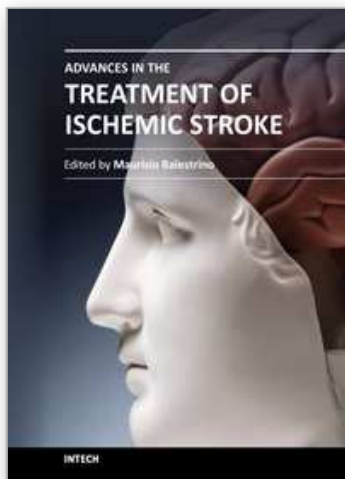
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In recent years research on ischemic stroke has developed powerful therapeutic tools. The novel frontiers of stem cells therapy and of hypothermia have been explored, and novel brain repair mechanisms have been discovered. Limits to intravenous thrombolysis have been advanced and powerful endovascular tools have been put at the clinicians' disposal. Surgical decompression in malignant stroke has significantly improved the prognosis of this often fatal condition. This book includes contributions from scientists active in this innovative research. Stroke physicians, students, nurses and technicians will hopefully use it as a tool of continuing medical education to update their knowledge in this rapidly changing field.

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