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Therapeutic Modulation of Brain Temperature: Relevance of Hypothermia to Inflammatory Changes following Transient Cerebral Ischemia in Rats

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Abstract:

Background : *In stroke, the ischemic crisis activates a series of events, including the inflammatory reactions that are potentiated by reperfusion, eventually leading to neuronal damage. Mild hypothermia has been considered to have a protective effect during ischemic neuronal cell death. The chief aim in this study was to investigate whether changing temperature during and after ischemia could minimize this damage by reducing the inflammatory injury.* ***Material and Methods:*** *The effect of moderate whole body hypothermia (30^0 C) on transient focal cerebral ischemia induced inflammatory injury was investigated. Experimental stroke (transient focal cerebral ischemia) was induced by a 2-hour middle cerebral artery occlusion (MCAO) with the use of a suture inserting into the lumen of the internal carotid artery (ICA) in male Wistar rats. Histopathological evaluation was performed 96 h after reperfusion.* ***Results:*** *MCAO induced inflammatory injury, involving the ipsilateral cortex and basal ganglia with massive infiltration of neutrophils, macrophages and microvascular proliferation, was exhibited in all normothermic rats. However, hypothermic MCAO rats showed minimal inflammatory response.* ***Conclusion:*** *The present study provides experimental evidence for the beneficial role of mild hypothermia using reversible MCAO in rats. Our results indicate that moderate hypothermia has a significant protective effect on the inflammatory injury induced by transient focal cerebral ischemia. Perhaps, the therapeutic effect was related to a reduction in releasing of cytotoxic products and improvement of the cerebral microcirculation.*

Key words: *Focal cerebral ischemia; Transient middle cerebral artery occlusion; Hypothermia; Inflammatory reactions; Rats*

Abbreviation*: MCAO (middle cerebral artery occlusion), MCA(middle cerebral artery), ICA(internal carotid artery), CCA(common carotid artery), ECA(external carotid artery),*

Introduction:

Cerebral ischemia and reperfusion occur frequently either after brain surgery and open heart surgery or after spontaneous thrombolysis and breakup of cerebral emboli in common clinical events. Recirculation affects cerebral ischemia and modifies postischemic events in various ways. There is abundant evidence that an acute inflammatory reaction associated with ischemia and reperfusion contributes to the development of neuronal damage in stroke[1-8]. It has been believed that cytokine production and molecular adhesive events that occur early in ischemia and the subsequent extensive recruitment of leukocytes to the ischemic zone during reperfusion lead to inflammatory injury[9-11]. Initially, ischemia triggers the expression of a number of cytokines, which attract leukocytes into ischemic sites and stimulate the synthesis of adhesion molecules, such as ICAM-1 on migrated leukocytes, endothelial cells, and other types of cells. The upregulation of these inflammatory mediators occurring during ischemia promotes blood-borne inflammatory cell adherence and infiltration during reperfusion. Consequently,

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postischemic leukocytes exacerbate brain injury by physically obstructing capillaries to reduce blood flow during reperfusion and/or releasing cytotoxic products once migrated into the brain parenchyma[12-13].

Ischemic brain temperature is an important determinant of the structural and functional outcome of neuronal and cerebrovascular injury in animal models of experimental stroke[14, 15]. However, the effects of temperature on cerebral ischemia are uncertain. While moderate reductions in ischemic brain temperature provide histopathological protection[16, 17], elevation in ischemic brain temperature has shown to aggravate outcome[18, 19]. Again, it has been suggested a detrimental effect of severe hypothermia (25.9^{0}C) or prolonged hypothermia (48 h) on focal brain ischemia. Brain tissue undergoes cell death, including neurons and glial cells, in conjunction with infiltration of neutrophils, macrophages and microvascular proliferation[1-8].

To our knowledge, there are a few studies that examine the protective effects of moderate hypothermia induced during and immediately after transient focal cerebral ischemia, though, those are not conclusive. In the present study, we used the intraluminal model of transient middle cerebral artery occlusion (MCAO) in the rat to investigate the effect of moderate hypothermia on the inflammatory injury in the ischemic area. Our result shows that moderate hypothermia significantly reduces the inflammatory changes induced by transient focal ischemia.

Materials and Methods

Animals

Male Wistar rats (weighing 270 – 300 g; n=24) were housed in the same animal care facility with food and water available during a 12-hour light/dark cycle throughout the protocol. MCAO was done by advancing a 4-0 surgical nylon suture into the internal carotid artery (ICA) to block the origin of the MCA[20-22]. Rats were fasted overnight before surgery but allowed to free access to water. Animals were anesthetized and maintained with 1.0 – 2.0% halothane in 70% N_2O and 30% O_2 using a face mask. The right femoral artery was cannulated to measure blood gas and blood glucose level before ischemia. Arterial pressure was monitored prior to MCAO and throughout the period of ischemia, and continuously for 20 min after the onset of reperfusion in all animals. Rectal temperature was controlled with a electrical heating pad to maintain the body temperature. All rats were randomly divided into two groups: Group I (Normothermic): MCAO was done at 37^{0}C body temperature (n-10); Group II (Hypothermic): MCAO was induced at 30^{0C} body temperature and hypothermia was maintained throughout the 2 h of ischemia and for an additional 1 h of reperfusion (n=14). The 30^{0}C whole body temperature was instituted 30 min prior to the surgery by spraying alcohol on the skin and fanning room air (20-20^{0}C) toward the animal's body. Then, animals were rewarmed to 37^{0}C using the heating pad

Ischemia (Surgical Procedures)

A 2 cm incision was made at the center of the neck, and the right common carotid artery (CCA), the external carotid artery (ECA), and ICA were exposed through a careful dissection under an operating microscope (Carl Zeiss, Inc., Thornwood, NY, USA). Further dissection was done to identify the pterygopalatine branch. The CCA and ICA were temporarily clamped using microsurgical clips (Codman & Shurtleff, Inc., Randolf, MA, USA). A 5-0 silk suture was tied loosely at the origin of the ECA and ligated at the distal end of the ECA. Then, a 4-0 surgical nylon suture, with its tip rounded by heating near a flame, was introduced into the ECA lumen through a small puncture. The silk suture around the ECA origin was tightened around the intraluminal nylon suture to prevent bleeding, and the microsurgical clips were removed. A length of 18.5 – 19.5 mm of nylon suture, determined according to the animal's weight, was gently advanced from the ECA into the lumen of the ICA until the suture blocked the origin of the MCA. The incision was temporarily closed using skin clips. In both normothermic (Group I) and hypothermic (Group II) animals, halothane anesthesia was maintained throughout the 2 hr of ischemic period and 1 h recirculation to allow accurate temperature control. After 2 h of ischemia, reperfusion was done by withdrawal of the suture until the tip cleared the ICA lumen and reached the origin of ECA.

Additional rats were used to measure body and regional brain temperature during ischemia a recirculation in normothermic (n=3) and hypothermic rats (n=3), without histopathology endpoint. Thirty minutes prior to MCAO, microthermocouples (100 mm) placed into a 27 gauge needle were inserted into the right (lesion side) and left (control side) cortex, caudate putamen, and preoptic areas through 1 mm burr holes in the skull. Brain and rectal temperature were recorded every 5 minutes throughout the experiment using a digital thermometer (Physitemp, Clifton, NJ, USA).

Tissue Preparation

Three days after MCAO, rats were anesthetized with intramuscular ketamine (44 mg/kg) and xylazine (13 mg/kg), and transcardially perfused with heparinized saline and 10% neutral buffered formalin. The head was further fixed in formalin solution for 1 h and then the brain was removed. The brain was cut into 2 mm thick coronal blocks. The brain tissue was processed, embedded, and 6 mm thick paraffin sections from each block were cut and stained with hematoxylin-eosin. Inflammatory injury was evaluated using light microscopy. The right hemisphere was divided into four anatomically distinct regions for detailed histological analysis . Inflammatory response, summation of infiltration by neutrophils, macrophages, and increased numbers of microvessels were evaluated in each distinct region by means of grading scaled presented in Table I. The severity of inflammation was factored into numerical grading. Multiple histological changes within a region were averaged.

Table-I

Brain ischemic damage grading scales

	Grade
Neutrophils	0: not detectable
	1: polymorphonucleus detected
Macrophages	0: not detected
	1: peripheral nucleus with cytoplasmic granules detected
Vasculature	0: normal
	1: increased number of microvessels

Statistics

Wilcoxon two-sample tests were performed to compare the response of inflammation between the normothermic and hypothermic MCAO rats. All data are presented as mean ±SD.

Results:

Blood gas values and serum arterial glucose levels before MCAO were within normal ranges (Table II). The blood pressure fluctuated within 5-10 mmHg during surgery. There were no detectable differences in arterial blood pressure values prior to vessel occlusion and 1 h after the surgery in both normothermic and hypothermic rats. Two animals died 24 h after surgery. The rectal temperatures were maintained almost constant at 37^0C in normothermic and 30^0C in hypothermic rats (Fig. 1). Prior to ischemia, brain temperature was elevated above the rectal temperature by 0.5^0C in normothermic animals and 1.5^0C in hypothermic rats. After the onset of MCAO, brain temperature declined approximately 0.5^0C and fluctuated at 37.2 ±0.7 and 31.2 ±0.6^0C in normothermic and hypothermic animals, respectively.

Table-II

Serum arterial blood gas, glucose, and blood pressure (BP) values

	pH	PCO_2	PO_2	Glucose (mg/dl)	BP (mmHg) Before	BP (mmHg) 1 h After
Gr I	7.41 ±0.07	35.8 ±1.9	139 ±19.9	131 ±29.2	97 ±6.5	95 ±3.2
Gr II	7.33 ±0.04	41.0 ±4.9	191 ±21.7	117 ±14.1	110 ±7.8	101 ±1.9

There were massive infiltration by neutrophils and macrophages as well as increased numbers of microvessels, compared to the contralateral side, were detected in the lesioned region. Figure 1 summarize the inflammatory responses in each of the four regions in both the normothermic and hypothermic ischemic rats. Significant differences were detected between the normothermic and hypothermic animals for inflammatory changes in all the regions ($p<0.01$).

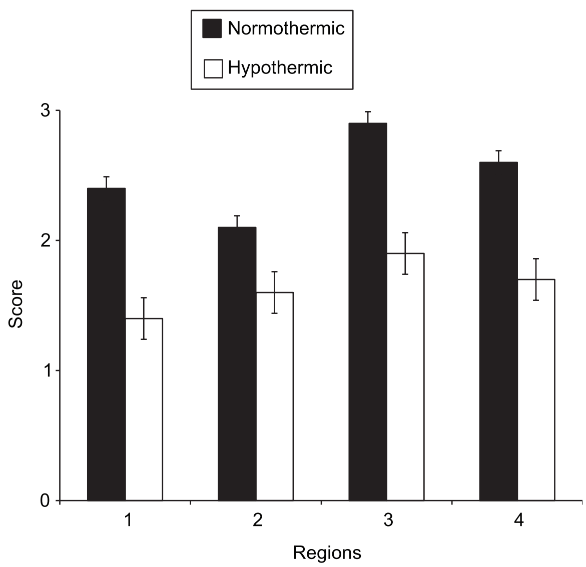

Fig. -1: *Bar chart presents the mean ±SE of inflammatory responses in normothermic MCAO and hypothermic MCAO in the four different brain regions. Region 1: Parietal cortex, 2: Piriform cortex, 3: Caudate putamen and 4: Preoptic area*

Discussion:

In the present study, the therapeutic value of temperature on the transient focal ischemia induced inflammatory injury in the ischemic territory was tested. Our data demonstrated that moderate whole-body hypothermia significantly reduces the degree of inflammatory changes after transient focal cerebral ischemia in the rat.

An extracranial approach to occlude the MCA, by introducing a suture into the ICA, has been recently developed [20-22]. One of the major advantages of this model is focal cerebral ischemia can successfully be induced without opening the cranium. Again, reperfusion can be easily induced by simply withdrawing the suture. The degree of tissue damage and mortality rate is a function of duration ischemia and reperfusion time. We used 2-h duration of ischemia to avoid mortality and 72 h reperfusion time to allow for maturation and clear demarcation of the injury. Our normothermic animals showed a sharply demarcated, reproducible ischemic lesion localized in the frontopareital cortex and basal ganglia (data not presented in this paper). This model of ischemia in our hands is reproducible and allows for detailed histopathological evaluation of ischemic cell damage and therapeutic intervention. Moreover, this model mimics closely the clinical situation because the MCA is the most frequently embolized artery, and recirculation occurs as recanalization is induced surgically or pharmacologically or as a result of spontaneous recanalization.

Because of the minimal lesion, and consequently the absence of a clearly defined infarct in the hypothermic animals, we adapted a scoring system for differential cellular evaluation in four anatomically distinct brain sub regions. Measuring only the area or volume of the lesion fails to demonstrate the anatomical sensitivity and distribution of an ischemic lesion. The present scoring system provides a detailed evaluation of the anatomical distribution of the cellular response and confirms the presence of a reproducible lesion localized at the neocortex and basal ganglia after MCAO.

Hypothermia reduces the cerebral ischemia induced inflammatory responses in the ischemic territories in the present study; though the exact mechanism of hypothermic protection in cerebral ischemia remains unknown. Ischemia induced neurotransmitter release, cerebrovascular permeability, as well as hemodynamic and metabolic abnormalities have been shown to be both temperature-sensitive and associated with ischemic cell death. It has been believed that normothermic MCAO induces a regional reduction in cerebral blood flow and development of a localized cerebral infarction[23]. Brain tissue undergoes cell death, including neurons and glial cells, in addition to infiltration of neutrophils and macrophages and microvascular proliferation[24]. The impact of pro-apoptotic transmembrane protein that can transduce cell death signal is not overruled during cerebral ischemia[25]. Phanithi et al[26] has suggested that mild hypothermia provides protection by reducing the expression of such fatal protein, thereby mitigating the apoptotic neural death. Additionally, it has been suggested that mild hypothermia can significantly reduce neuronal damage by promoting survival, after reversible MCA occlusion.

Furthermore, the protective effect of moderate hypothermia has been attributed, to a greater extent, to a decreased metabolic rate[27], decrease in adenosine triphosphate (ATP) depletion[28], protein synthesis inhibition[29], reduced post-ischemic free radical production[30], increase cerebral blood flow, and reduced neurotransmitter release[31,32]. Whatever the mechanism in the protective action of moderate hypothermia, the present study was limited to inflammatory responses and it stresses the need for further investigation to explore the mechanism.

In conclusion, our data demonstrate that transient ischemia induced by using the intra-arterial suture method to occlude the MCA results in a reproducible brain injury and that moderate hypothermia has a profound protective effect on the inflammatory brain injury after transient MCAO.

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