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Hyperthermia induced after recirculation triggers chronic neurodegeneration in the penumbra zone of focal ischemia in the rat brain

L.A. Favero-Filho^{1,2}, A.A. Borges^{1,3}, C. Grassl^{1,3}, A.C. Lopes^{1,3},
R. Sinigaglia-Coimbra^{1,4,5} and C.G. Coimbra^{1,2,3}

¹Laboratório de Fisiopatologia Clínica e Experimental, ²Departamento de Neurologia e Neurocirurgia, ³Departamento de Medicina, ⁴Centro de Microscopia Eletrônica, ⁵COLSAN-UNIFESP, Escola Paulista de Medicina, Universidade Federal de São Paulo, São Paulo, SP, Brasil

Correspondence to: C.G. Coimbra, Laboratório de Fisiopatologia Clínica e Experimental, UNIFESP, Rua Pedro de Toledo, 781, 04039-032 São Paulo, SP, Brasil
Fax: +55-11-5539-3123. E-mail: coimbracg.nexp@epm.br

Chronic neurodegenerative processes have been identified in the rat forebrain after prolonged survival following hyperthermia (HT) initiated a few hours after transient global ischemia. Since transient global ischemia and ischemic penumbra share pathophysiological similarities, this study addressed the effects of HT induced after recirculation of focal brain ischemia on infarct size during long survival times. Adult male Wistar rats underwent intra-luminal occlusion of the left middle cerebral artery for 60 min followed by HT (39.0-39.5°C) or normothermia. Control procedures included none and sham surgery with and without HT, and middle cerebral artery occlusion alone. Part I: 6-h HT induced at recirculation. Part II: 2-h HT induced at 2-, 6-, or 24-h recirculation. Part III: 2-h HT initiated at recirculation or 6-h HT initiated at 2-, 6- or 24-h recirculation. Survival periods were 7 days, 2 or 6 months. The effects of post-ischemic HT on cortex and striatum were evaluated histopathologically by measuring the area of remaining tissue in the infarcted hemisphere at -0.30 mm from bregma. Six-hour HT initiated from 6-h recirculation caused a significant decrease in the remaining cortical tissue between 7-day (N = 8) and 2-month (N = 8) survivals (98.46 ± 1.14 to $73.62 \pm 8.99\%$, respectively). When induced from 24-h recirculation, 6-h HT caused a significant reduction of the remaining cortical tissue between 2- (N = 8) and 6-month (N = 9) survivals (94.97 ± 5.02 vs $63.26 \pm 11.97\%$, respectively). These data indicate that post-ischemic HT triggers chronic neurodegenerative processes in ischemic penumbra, suggesting that similar fever-triggered effects may annul the benefit of early recirculation in stroke patients over the long-term.

Key words: Hyperthermia; Ischemic penumbra; Neurodegeneration; Rat focal ischemia

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Introduction

Spontaneous or induced hyperthermia (HT) after transient global ischemia triggers a chronic neurodegenerative process in the rat brain characterized by chronic neuronal death, progressive atrophy of forebrain structures with enlargement of the supratentorial ventricles, chronic inflammation (complement and microglia activa-

tion, tumor necrosis factor- α production, gemistocytic astrocytes, hyperphosphorylated Tau (neurofibrillary tangles) and ubiquitin expression, β -amyloid-containing plaques, and impaired cognition (1-3) over prolonged survival periods.

Transient global ischemia and ischemic penumbra share several pathophysiological features, including delayed neuronal death, apoptosis, mitochondrial dysfunc-

tion, astro- and microglia activation, inflammation and production of oxygen reactive species (4,5). Considering these similarities, we investigated whether HT induced after transient middle cerebral artery (MCA) occlusion triggers chronic degeneration of ischemic penumbra, extending the infarct into the cortex during months of recovery.

Material and Methods

The protocols used were approved by the Ethics Committee for Animal Research of the Federal University of São Paulo. Wistar rats weighing 330-350 g were maintained on a 12-h light/dark cycle, at 22-24°C, with free access to food and water.

One-hour MCA occlusion was induced by the intraluminal suture method (6,7). Briefly, the animals were anesthetized with 3.5% halothane (Halothane Vapor 19.3, Dräger Werk, Germany), intubated orally, and mechanically ventilated under 1.0-1.5% halothane in N₂O/O₂ (100:30) during surgery. A PE-50 polyethylene catheter was inserted into the tail artery for blood pressure recording and sampling. Through a midline cervical incision the branches of the left external carotid artery were isolated and electrocoagulated. After separation of the internal carotid artery from the vagus nerve, the external carotid artery was ligated and a 4.0 monofilament nylon (tip previously rounded by heating) inserted by arteriotomy into the left external carotid artery, and advanced into the internal carotid artery until a mild elastic resistance indicated that the tip was properly lodged, blocking the blood flow to the middle cerebral artery. After

50-min MCA occlusion, anesthesia was discontinued and animals without neurological deficits were excluded. All other rats were re-anesthetized for withdrawal of the occluder after 60-min MCA occlusion. The MP100A data acquisition system (Biopac Systems Inc., USA) enabled arterial pressure recording during experiments. Arterial gases and pH were maintained within the following pre-ischemia ranges: PaO₂ 95-110 mmHg, PaCO₂ 35-40 mmHg, pH 7.35-7.45 (ABL 5, Radiometer, Denmark). Animals with pre-ischemic blood glucose levels higher than 110 mg/dL were excluded (Glucometer 3, Bayer Diagnostics, USA). Colonic temperature (temperature sensor tip positioned into the bowel, 8 cm from the anus) was maintained at 37.0 ± 0.5°C with a heating pad (Homeothermic Blanket Control Unit, USA) until recovery from anesthesia.

Induction of hyperthermia in free-moving rats

Because the rodent brain is dorsally positioned in the head, the use of lamps positioned above may result in more selective heating of the brain. The system used here was devised for homogeneous body heating. Rats were submitted to 2- or 6-h HT within the initial 24-h recirculation using a device designed to deliver a continuous flow of heated room air (2.0 L/min) driven by a vacuum pump into a 50 x 46 x 18 cm acrylic cage in which fully awake animals had free access to fresh water (Figure 1). The device included 2 lateral heating towers, each containing one dimmer-controlled 150-W infrared lamp. Each infrared lamp heated two 1.5 mm-thick horizontal metal plates (45 x 19 cm) arranged 2 cm apart in parallel within each tower. In

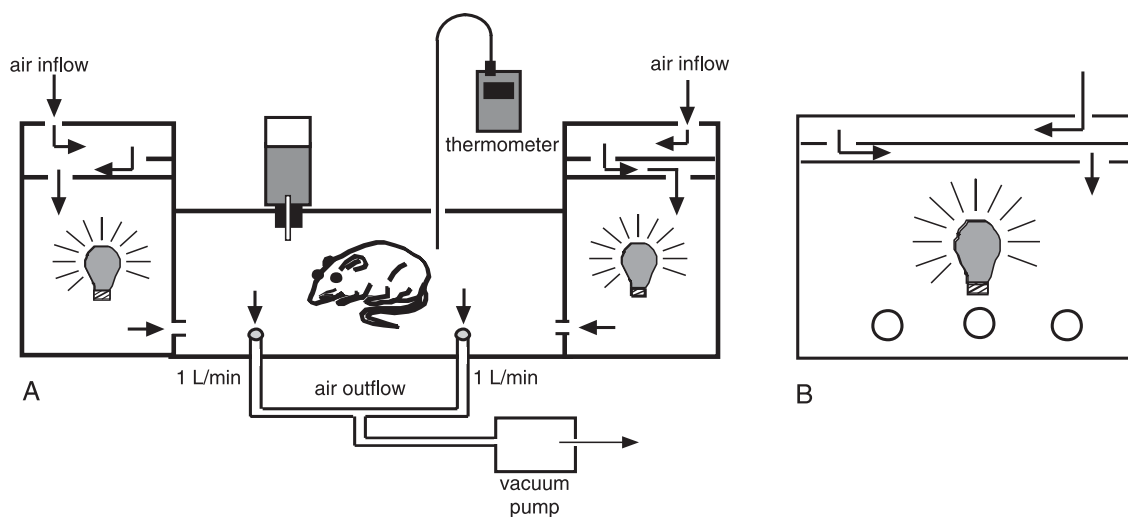


Figure 1. Hyperthermia induced in free-moving rats. *A*, A vacuum-driven flow of room air entered the lateral heating towers and passed between 2 parallel metal plates exposed to a dimmer-controlled infrared lamp, and subsequently into the animal cage through 3 holes in each side. The dimmer switch was set to heat the cage environment as needed (30.0-36.0°C) for the desired colonic temperature (39.0-39.5°C). *B*, Lateral view of the heating tower to show the position of the plate holes to maximize heating of the air flow.

contrast to the heating-lamp system, which induces hyperthermia only in ischemic animals (2,3), the system used here effectively induced HT in sham-operated and normal rats, so that the effect of HT alone could be controlled. After HT, all rats were returned to regular cages for spontaneous resumption of normal colonic temperature.

Experimental groups

The experiments were conducted in 3 consecutive parts, which included 88, 62, and 120 animals, respectively (Table 1). The name of each experimental group provides up to 4 consecutive pieces of information separated

by a slash: initial procedure (ischemia, sham or none), time elapsed from recirculation to HT induction (no HT, 0, 2, 6, or 24 h), duration of HT (no HT, 2-h HT or 6-h HT), and survival period (7 days, 2 and 6 months). Animals submitted to MCA occlusion alone were obtained in all 3 parts to avoid bias related to different animal shipments.

Part I, groups compared for the effects of 6-h HT (39.0-39.5°C induced immediately after 1-h MCA occlusion) on the ischemic damage evaluated at 7 days and 2 months. Part II, groups compared for the effects of 2-h HT induced after 2, 6, or 24 h from recirculation of 1-h MCA occlusion evaluated at 7 days and 2 months. Part III, groups com-

Table 1. Animal groups.

Part	Groups	Description	N
I	isch/no HT/7 days	1-h MCAO → 7-day survival	8
I	isch/no HT/2 months	1-h MCAO → 2-month survival	8
I	isch/0 h/6-h HT/7 days	1-h MCAO → 6-h HT from recirculation → 7-day survival	7
I	isch/0 h/6-h HT/2 months	1-h MCAO → 6-h HT from recirculation → 2-month survival	8
I	sham/no HT/7 days	sham operation → 7-day survival	7
I	sham/no HT/2 months	sham operation → 2-month survival	8
I	sham/0 h/6-h HT/7 days	sham operation → 6-h HT → 7-day survival	7
I	sham/0 h/6-h HT/2 months	sham operation → 6-h HT → 2-month survival	7
I	none/no HT/7 days	normal rats → 7-day survival (aging control)	6
I	none/no HT/2 months	normal rats → 2-month survival (aging control)	6
I	none/0 h/6-h HT/7 days	normal rats → 6-h HT → 7-day survival (HT control)	8
I	none/0 h/6-h HT/2 months	normal rats → 6-h HT → 2-month survival (HT control)	8
II	isch/no HT/7 days	1-h MCAO → 7-day survival	8
II	isch/no HT/2 months	1-h MCAO → 2-month survival	8
II	isch/2 h/2-h HT/7 days	1-h MCAO → 2-h HT from 2-h recirculation → 7-day survival	7
II	isch/2 h/2-h HT/2 months	1-h MCAO → 2-h HT from 2-h recirculation → 2-month survival	7
II	isch/6 h/2-h HT/7 days	1-h MCAO → 2-h HT from 6-h recirculation → 7-day survival	8
II	isch/6 h/2-h HT/2 months	1-h MCAO → 2-h HT from 6-h recirculation → 2-month survival	8
II	isch/24 h/2-h HT/7 days	1-h MCAO → 2-h HT from 24-h recirculation → 7-day survival	8
II	isch/24 h/2-h HT/2 months	1-h MCAO → 2-h HT from 24-h recirculation → 2-month survival	8
III	isch/no HT/7 days	1-h MCAO → 7-day survival	6
III	isch/no HT/2 months	1-h MCAO → 2-month survival	7
III	isch/no HT/6 months	1-h MCAO → 6-month survival	8
III	isch/0 h/2-h HT/7 days	1-h MCAO → 2-h HT from recirculation → 7-day survival	7
III	isch/0 h/2-h HT/2 months	1-h MCAO → 2-h HT from recirculation → 2-month survival	8
III	isch/0 h/2-h HT/6 months	1-h MCAO → 2-h HT from recirculation → 6-month survival	9
III	isch/2 h/6-h HT/7 days	1-h MCAO → 6-h HT from 2-h recirculation → 7-day survival	8
III	isch/2 h/6-h HT/2 months	1-h MCAO → 6-h HT from 2-h recirculation → 2-month survival	9
III	isch/2 h/6-h HT/6 months	1-h MCAO → 6-h HT from 2-h recirculation → 6-month survival	8
III	isch/6 h/6-h HT/7 days	1-h MCAO → 6-h HT from 6-h recirculation → 7-day survival	8
III	isch/6 h/6-h HT/2 months	1-h MCAO → 6-h HT from 6-h recirculation → 2-month survival	8
III	isch/6 h/6-h HT/6 months	1-h MCAO → 6-h HT from 6-h recirculation → 6-month survival	9
III	isch/24 h/6-h HT/7 days	1-h MCAO → 6-h HT from 24-h recirculation → 7-day survival	8
III	isch/24 h/6-h HT/2 months	1-h MCAO → 6-h HT from 24-h recirculation → 2-month survival	8
III	isch/24 h/6-h HT/6 months	1-h MCAO → 6-h HT from 24-h recirculation → 6-month survival	9

Part I, groups compared for the effects of 6-h hyperthermia (HT) [39.0-39.5°C induced immediately after 1-h middle cerebral artery occlusion (MCAO)] on the ischemic (isch) damage evaluated at 7 days and 2 months. Part II, groups compared for the effects of 2-h HT induced from 2, 6, or 24 h recirculation of 1-h MCAO evaluated at 7 days and 2 months. Part III, groups compared for the effects of 2-h HT induced from recirculation or 6-h HT induced from 2, 6, or 24 h recirculation of 1-h MCAO evaluated at 7 days, 2 and 6 months.

pared for the effects of 2-h HT induced from recirculation or 6-h HT induced at 2, 6, or 24 h into recirculation of 1-h MCA occlusion evaluated at 7 days, 2 and 6 months.

Post-ischemic temperature measurements

After ischemia, all rats were maintained on a 12-h light/dark cycle (lights off from 6 pm to 6 am). Colonic temperature was measured with a digital thermometer (DM852, Ellab, Denmark) every 30 min during the period of HT, and at 1:00 pm during the first week of recovery. The probe (PRA-22002-A, Ellab) was lubricated with tap water and the sensor tip placed into the bowel 8 cm from the anus for 20 s, while the animal was minimally restricted, with forepaws left in contact with the cage floor to minimize stress-related temperature shifts.

Histopathology

At 7 days, 2 or 6 months of recovery, the animals were anesthetized and perfusion fixed with 0.9% NaCl followed by phosphate-buffered 4.0% formaldehyde. After fixation *in situ* for 24 h, brains were removed, dehydrated and paraffin embedded. Semi-serial 5 μ m-coronal sections (1-in-10 series) were stained with celestine blue and acid fuchsin for identification of normal and degenerating neurons, respectively (8). The areas of cortex and striatum that remained without signs of infarct (remaining tissue) in the ischemic hemisphere after the end of the survival periods were measured with an image analysis system (Image ProPlus, Media Cybernetics, USA) at a mean level of -0.30 mm (between -0.70 and +0.10 mm) from bregma (9). The area of remaining tissue was reported as percentage of the corresponding area measured in the non-ischemic hemi-

sphere in the same coronal section to control for differences in tissue processing and animal size (10). Remaining tissue was considered 100% when no microscopic signs of tissue damage were found in macroscopically symmetric cerebral hemispheres. The mean value of the percentages obtained in 10-12 coronal sections was used as the (cortical or striatal) area of remaining tissue in the damaged hemisphere of each rat brain (individual values). The cortical and striatal remaining tissue at different survival times were compared using single factor non-parametric ANOVA (Kruskal-Wallis test) followed by the Dunn multiple comparison test and the Mann-Whitney U-test.

Results

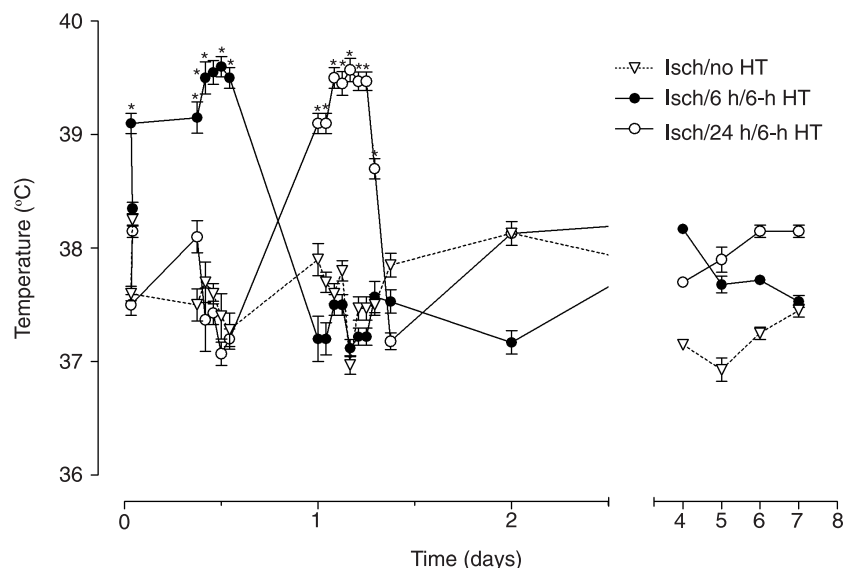
Physiological parameters

The pre-ischemic blood glucose levels, arterial gases and pH did not differ among ischemic and/or sham-operated animals (ANOVA, data not shown). Differences in colonic temperature between MCA occlusion and post-HT MCA occlusion groups during the first week were statistically significant at most of the corresponding measurements (repeated measures ANOVA followed by the Student *t*-test, $P < 0.05$, Figure 2).

Mortality

Evaluation of mortality rate during the initial 24 h from MCA occlusion caused by HT-enhanced acute events such as cerebral edema (11) was confounded by the fact that early deaths are common in this model and usually due to vessel perforation, caused by inappropriate suture insertion depths, resulting in subarachnoid and/or intra-

Figure 2. Mean colonic temperature at 1:00 pm up to 7-day recovery. Data from rats submitted to hyperthermia (HT) initiated at 6-h (ischemia, Isch/6 h/6-h HT) or 24-h (Isch/24 h/6-h HT) recirculation or to middle cerebral artery occlusion alone (Isch/no HT) are shown. Significant differences were observed only at the time points within the periods of induced HT (Isch/no HT vs Isch/24 h/6-h HT, and Isch/no HT vs Isch/6 h/6-h HT groups (* $P < 0.05$, Student *t*-test).



cerebral hemorrhage (12). Premature deaths from hemorrhage were minimized in this study by using rats within a narrow body weight range (330-350 g), so that the appropriate insertion depth became less of a variable from one animal to another. Mortality counted after the end of the most delayed hyperthermic treatment (ischemia/24-h/6-h HT) ceased at 5-day recovery from MCA occlusion in all groups. Analysis of mortality rates (chi-square test) showed a non-significant trend for higher rates in two HT groups ($P = 0.610$, chi-square test), ischemia/24-h/6-h HT (16.6%) and ischemia/0-h/6-h HT (15.4%) compared with MCA occlusion alone (3.5%).

Histopathology

The histopathological results obtained in experimental parts I to III are reported in Table 2 and Figures 3 and 4. In part I, HT did not cause morphological damage to the rat brain in the absence of ischemia (sham-operated and normal rats). No significant differences were found among ischemic groups in parts I and II. In spite of non-significant differences obtained in part I in striatum and cortex ($P = 0.1512$ and 0.1379 , respectively, Kruskal-Wallis test), 6-h HT from recirculation was associated with an early trend for more severe damage to cortex compared with ischemia alone at 7-day recovery ($P = 0.0952$, Mann-Whitney U-test) that was sustained at 2-month survival ($P = 0.0294$, Mann-Whitney U-test). Similarly, early worsening (at 7-day survival) of striatal damage by 6-h HT was also suggested ($P = 0.0556$, Mann-Whitney U-test). Additional enhancement of ischemic damage by 6-h HT was not observed in either the striatum or cortex from 7-day to 2-month survivals.

In part III, significant differences were found in cortex but not in striatum ($P = 0.0354$ and 0.1581 respectively, Kruskal-Wallis test). Following 6-h HT timed from 6-h recirculation, the remaining cortical tissue (mean \pm SEM) significantly decreased from $98.46 \pm 1.14\%$ at 7-day survival to $73.62 \pm 8.99\%$ at 2-month survival ($P < 0.05$, Dunn test; $P < 0.001$, Mann-Whitney U-test) but not from 2- to 6-month survival ($67.89 \pm 11.61\%$). In addition, the remaining cortical tissue decreased from 94.97 ± 5.02 to $63.26 \pm$

Table 2. Amount of tissue remaining in striatum and cortex evaluated at -0.30 mm from bregma in the infarcted hemisphere after ischemia and hyperthermia.

Part	Groups	Striatum (%)	Cortex (%)
I	isch/no HT/7 days	59.00 \pm 8.40	91.60 \pm 8.40
I	isch/no HT/2 months	62.00 \pm 16.95	82.71 \pm 13.58
I	isch/0 h/6-h HT/7 days	14.20 \pm 5.35	51.00 \pm 13.82
I	isch/0 h/6-h HT/2 months	39.00 \pm 14.49	55.00 \pm 15.67
I	sham/no HT/7 days	100	100
I	sham/no HT/2 months	100	100
I	sham/0 h/6-h HT/7 days	100	100
I	sham/0 h/6-h HT/2 months	100	100
I	none/no HT/7 days	100	100
I	none/no HT/2 months	100	100
I	none/0 h/6-h HT/7 days	100	100
I	none/0 h/6-h HT/2 months	100	100
II	isch/no HT/7 days	31.60 \pm 19.35	79.61 \pm 12.90
II	isch/no HT/2 months	49.25 \pm 11.52	81.75 \pm 11.95
II	isch/2 h/2-h HT/7 days	67.43 \pm 13.80	91.20 \pm 7.79
II	isch/2 h/2-h HT/2 months	54.14 \pm 8.47	97.06 \pm 2.25
II	isch/6 h/2-h HT/7 days	51.88 \pm 10.74	82.87 \pm 10.97
II	isch/6 h/2-h HT/2 months	43.00 \pm 13.83	82.33 \pm 10.54
II	isch/24 h/2-h HT/7 days	64.25 \pm 11.84	89.80 \pm 6.81
II	isch/24 h/2-h HT/2 months	50.13 \pm 12.18	76.67 \pm 10.75
III	isch/no HT/7 days	37.66 \pm 13.47	73.25 \pm 11.16
III	isch/no HT/2 months	27.55 \pm 13.01	89.76 \pm 7.73
III	isch/no HT/6 months	39.95 \pm 9.15	90.67 \pm 4.68
III	isch/0 h/2-h HT/7 days	38.75 \pm 16.16	76.94 \pm 12.22
III	isch/0 h/2-h HT/2 months	11.86 \pm 5.50	81.22 \pm 10.51
III	isch/0 h/2-h HT/6 months	19.49 \pm 10.76	62.95 \pm 12.29
III	isch/2 h/6-h HT/7 days	23.89 \pm 11.78	65.41 \pm 10.35
III	isch/2 h/6-h HT/2 months	14.17 \pm 4.84	59.60 \pm 10.09
III	isch/2 h/6-h HT/6 months	28.18 \pm 11.31	72.08 \pm 8.95
III	isch/6 h/6-h HT/7 days	45.19 \pm 13.11	98.46 \pm 1.14
III	isch/6 h/6-h HT/2 months	13.32 \pm 4.63	73.62 \pm 8.99
III	isch/6 h/6-h HT/6 months	27.74 \pm 13.00	67.89 \pm 11.61
III	isch/24 h/6-h HT/7 days	26.83 \pm 1.18	75.91 \pm 9.67
III	isch/24 h/6-h HT/2 months	44.26 \pm 14.80	94.97 \pm 5.02
III	isch/24 h/6-h HT/6 months	24.72 \pm 12.64	63.26 \pm 11.97

See Table 1 for explanation of animal groups in parts I, II and III. No significant differences were found among ischemic (isch) groups in parts I and II. In part III, significant differences were found in cortex but not in striatum ($P = 0.0354$ and 0.1581 , respectively, Kruskal-Wallis test). *Post hoc* analysis detected intergroup differences as shown (* $P < 0.05$, Dunn test).

11.97% from 2- to 6-month survival ($P < 0.05$, Dunn test; $P < 0.05$, Mann-Whitney U-test) in the group treated with HT for 6 h from 24-h recirculation. At 2-month (but not at 7-day or 6-month) survival, the remaining cortical tissue observed in the group treated with 6-h HT initiated from 24-h recirculation was significantly larger than that observed in the group treated with 6-h HT induced from 2-h recirculation (94.97 ± 5.02 vs $59.60 \pm 10.09\%$, respectively ($P < 0.05$, Dunn test; $P < 0.01$, Mann-Whitney U-test). Differences observed in striatum were non-significant ($P = 0.1581$, Kruskal-Wallis test).

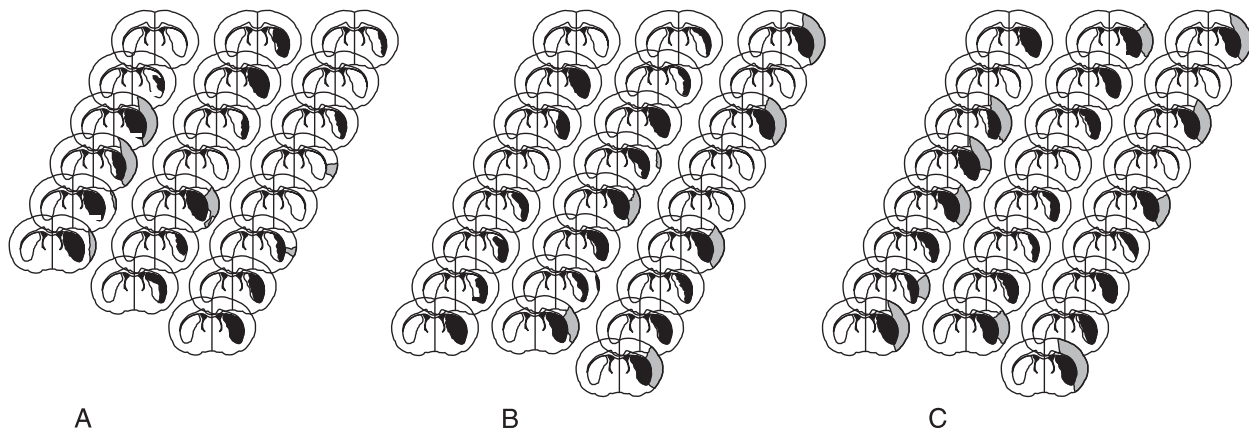


Figure 3. Infarcted areas shown in black (striatum) or gray (cortex) at -0.30 mm from bregma. Coronal section corresponds to individual animal from groups evaluated at 7-day, 2- and 6-month survivals from 1-h middle cerebral artery (MCA) occlusion, respectively (from left to right). A, MCA occlusion alone. B, 6-h HT induced from 6-h recirculation. C, 6-h HT induced from 24-h recirculation.

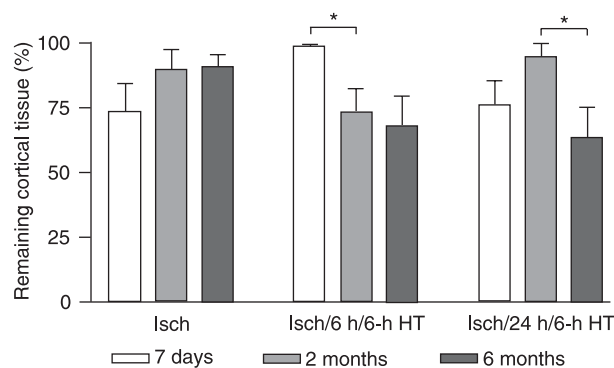


Figure 4. Cortical tissue remaining in the infarcted hemisphere evaluated at -0.30 mm from bregma. Data are reported as mean \pm SEM evaluated at 7-day, 2- and 6-month survivals from 1-h middle cerebral artery occlusion (ischemia, Isch) (* $P < 0.05$, Mann-Whitney U-test).

Discussion

Our results indicate that fever-range HT induced after 6 or 24 h after 1-h MCA occlusion and sustained for 6 h is associated with delayed extension of the infarct in the rat cortex. These delayed changes were not demonstrated in the striatum, but in the cortex - which is mostly composed of salvageable tissue following 1-h MCA occlusion (13) - and became apparent only after long recoveries (2 or 6 months), suggesting that those hyperthermic paradigms trigger chronic neurodegenerative processes in ischemic penumbra. When 6-h HT was delayed for 24 h after recirculation, the expansion of the cortical infarct evolved at a rate even slower than that triggered by 6-h HT delayed for

6 h from recirculation, becoming morphologically apparent after 6-month recovery but not at 2-month recovery. Conversely, 6-h HT induced at recirculation or 2-h HT induced at 24-h recirculation did not produce similar effects up to 2-month recovery, indicating that both timing of induction and length of HT are critical for triggering chronic neurodegeneration. Taken together, these results indicate that the "window of opportunity" for inducing a chronic neurodegenerative process in the penumbra zone (when the required hyperthermia-responsive mechanisms are active) is not opened at recirculation, but is available at 6-h recirculation and about to close at 24 h of recovery from 1-h focal ischemia.

Regarding the acute effects of HT on focal ischemia, 6-h HT may have expanded the cortical infarct within the first week of recovery. This is suggested by the borderline significant trend to larger remaining tissue at 7-day recovery in the group submitted to MCA occlusion alone ($91.6 \pm 8.4\%$) compared with animals submitted to 6-h HT induced at recirculation ($51.00 \pm 13.82\%$). Failure to demonstrate a significant difference in infarct size between HT and normothermic groups at 7-day recovery may result from mortality-related bias. The mortality rates from 1-day to 5-day recovery in rats submitted to MCA occlusion alone and those to 6-h HT at recirculation were 3.5 and 15.4%, respectively. Early HT greatly increases edema (11,14) and, therefore, premature mortality due to intracranial hypertension may have preferentially excluded animals with more severe primary ischemic damage in the HT group. This bias is minimized in studies that evaluate the infarct size within hours after MCA occlusion, when brain edema may not have maximally expanded to cause death second-

ary to intracranial hypertension.

Seven days or even longer recoveries are therefore not appropriate to study the acute effects of HT on the infarct size. However, such acute effects of HT were not the subject of this study, which specifically focused on the longitudinal long-term effects of post-ischemic HT induced at different recirculation time-points within the initial 24-h recirculation.

The early effects of HT on ischemia-reperfusion are well known. HT worsens excitotoxicity and calcium overload, oxygen radical production, lipid peroxidation, proteolysis, blood-brain barrier opening, peri-infarct depolarizations, energy consumption, and DNA damage, ultimately expanding the infarction within hours or days (14). In contrast, the long-term effects of post-ischemic HT on infarct size have not been previously addressed, and the HT-sensitive mechanisms underlying the triggering and persistence of chronic processes in ischemic penumbra are not known.

A suitable model to explain the detrimental effects of HT on the penumbra zone reported in this study must take the following into consideration: 1) the specific "window of opportunity" to trigger chronic degeneration by hyperthermia; 2) the long latency to detect increased infarction size after HT; 3) the known biological responses to HT and ischemia. Some post-ischemic pathophysiological phenomena plausibly integrate all 3 of these items, and may be the object of future research justified with supportive data from animal and human studies, including autoimmunity/inflammation (15-20), Alzheimer-like pathophysiology (2), cumulative mitochondrial DNA mutations (21,22), and apoptosis (23). Our preliminary results indicate that 1-month survival following 6-h hyperthermia initiated from 6-h recirculation, or 3-month survival following 6-h hyperthermia initiated from 24-h recirculation may be appropriate timing for future investigation of pathophysiological

mechanisms. There may be no remaining penumbral tissue undergoing active degeneration to be studied after 4 months of survival.

Using heating lamps to induce hyperthermia causes overheating (by approximately 1.0°C) of the gerbil brain, therefore, reversing the normal difference between body and cerebral temperatures in freely moving rodents (24). This pitfall is avoided by using the system shown in Figure 1, which better reproduces the effects associated with the thermal component of fever in humans.

The impact of HT on the outcome of stroke was first reported in 1976 by Hindfelt (25) and is now widely recognized (14). HT can be detected in about 50% of stroke patients up to 48 h from hospital admission (26). HT occurring during the first few days of recovery correlates with an early increase in infarct volume and edema, poor neurological outcome, prolonged intensive care and increased mortality (14). HT extended for 2 h after tissue plasminogen activator administration increases perfusion deficits and infarct size at 24 h of recovery in a rat model of embolic MCA occlusion (27). Our data suggest that HT occurring several hours after recirculation of ischemic stroke may impair or even annul the early benefit of thrombolytic therapy not only by aggravating early necrotic phenomena, but also by triggering chronically active degenerative processes in the long run. Hence, some of the permanent neurological impairment currently considered to result solely from the primary ischemic insult may rather be brought about by unrecognized late active degeneration.

To the best of our knowledge, this is the first report in the literature on degenerating processes active in the penumbra zone long after reperfusion. Preclinical investigation using post-ischemic hyperthermia-induced penumbral degeneration as an experimental model may allow the development of therapies capable of inactivating these mechanisms.

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