SELECTIVE HYPOTHERMIA

An experimental study on traumatic brain injury in rats

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Abstract – Objective: To evaluate the efficiency of selective hypothermia in the treatment of the traumatic brain injury in rats. **Method:** After the trauma produced for the model of cortical impact, a small craniectomy in the right frontoparietal region was carried through; after the procedure the animals had been divided in two groups of 15 each. Group A, without treatment with hypothermia (control group) and group B, treated with selective hypothermia for a period to 5 to 6 hours. After this time all the animals were sacrificed, their brains had been removed and histopathological analysis was carried through. **Results:** Comparison between both groups was done using the counting of neurons injured for field. Counting in the control group n=15 had an average of 70.80 neurons injured for field against an average of 21.33 neurons injured for field in group B (submitted to the treatment with hypothermia), with n=15 also. The difference was statiscally significant. **Conclusion:** Based in the quantification of the neurons injured for field, the effectiveness of the treatment with selective hypothermia was demonstrated.

KEY WORDS: selective hypothermia, hypothermia, neuroprotection, traumatic brain injury.

Hipotermia seletiva: estudo experimental de traumatismo crânio-encefálico em ratos

Resumo – Objetivo: Avaliar a eficiência da hipotermia seletiva no tratamento do traumatismo crânio-encefálico (TCE) em ratos. O trauma foi produzido por um modelo de impacto cortical desenvolvido exclusivamente para o estudo. **Método:** Após o TCE produzido pelo modelo de impacto cortical, foi realizada pequena craniectomia na região fronto-parietal direita; após o procedimento os animais foram divididos em dois grupos de 15 cada um. O grupo A, sem tratamento com hipotermia (grupo controle) e grupo B, tratado com hipotermia seletiva por período de 5 a 6 horas. Depois deste tempo todos os animais foram sacrificados, seus encéfalos foram removidos e realizada a análise anatomopatológica. **Resultados:** Na comparação entre o grupo tratado com hipotermia e o grupo controle utilizou-se a contagem de neurônios lesados por campo. Tal contagem no grupo A (controle/sem tratamento) com n=15 teve media de 70,80 neurônios lesados por campo contra a media de 21,33 nerônios lesados por campo no grupo B (submetido ao tratamento com hipotermia), com n=15 também. Diferença estatísticamente significativa pôde ser demonstrada. **Conclusão:** A análise anatomopatológica dos encéfalos dos animais estudados, baseada na quantificação dos neurônios lesados por campo demonstrou efetividade do tratamento com hipotermia seletiva com diferença estatística significativa.

PALAVRAS-CHAVE: hipotermia seletiva, hipotermia, neuroproteção, traumatismo crânio-encefálico.

The use of hypothermia in clinical practice has, since 1940, been supported by numerous reports from the 50s and 60s[1-4]. However, the side effects occurred due to its use such as coagulopathy, cardiac arrhythmias, severe pneumonia and metabolic disturbances, led to a decrease in the number of hypothermia procedures as therapeutic practice and a decline in the conventional therapeutic arsenal[5,6]. In the 1980's and 1990's, several authors published studies on the use of mild and moderate hypothermia offering new guidelines for its use and demonstrating once more its efficacy. In their studies, they claimed that lower degrees in whole body cooling presented fewer complications[7-9].

Inspired by the positive findings of that time, new studies[10-20] investigated the use of head cooling, also known as selective hypothermia. These studies demonstrated encouraging results, and so did the studies published by Prandini et al. on ischemic brain lesions[21] and Prandini et al. on treatment of brain inflammatory lesions[22]. The aforementioned promising results for hypo-

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thermia gave rise to this study. The use of mild and moderate systemic hypothermia and its benefits for brain protection have been extensively reported. On the other hand, fewer publications on selective hypothermia are found. Consequently, a larger number of studies are required to show its effectiveness.

This assertion led to the development of an experimental animal model to demonstrate the hypothermia effectiveness, in a selective way, in traumatic brain injuries[23] as well as to develop a technique which could potentially be adapted for use in humans.

METHOD

For the present study, 30 (thirty) Wistar/EPM male rats were used, their weight ranging between 300 and 400 grams. They were kept in the bioterium of the Federal University of São Paulo under ideal lighting, feeding and hygiene conditions. This study was analyzed and approved by the Ethics Commission of UNIFESP. The procedures were developed in the Neuromicrosurgery Laboratory of UNIFESP.

Controlled cortical impact model

In order to induce the encephalic cranial trauma on the rats, we developed a controlled cortical impact device. The device consists of a pneumatic piston, built with adequate dimensions for the model, supported by two height-regulating metallic rods; metallic platform for the rats' head likely to be adapted and adjusted for either foam or rubber platforms; control panel with a pressure gauge to calibrate and measure the impact intensity; and a trigger switch.

Trauma inducement and surgical procedures

After anesthesia with Zoletil 50® 0.1 mg/Kg (combination of tiletamine/zolazepam - Virbac S/A - France), the rats underwent a 150 psi impact on the right frontal parietal region. After trauma inducement, they were observed and anesthetized again. A small linear incision was performed on the right frontal parietal region, followed by a 3 X 3 mm craniectomy, without injuring the dura mater. The usual surgical care procedures about hemostasis were performed, and a small cotton wound dressing was left on the incision.

Selective hypothermia

After craniectomy, the rats were divided into two randomly selected groups of 15 rats each: Group A, without treatment (control group) and Group B, the group which underwent hypothermic treatment. The rats treated with hypothermia received latex ice bags, specially manufactured for this study, set on the frontal parietal region. As the ice melted, the bags were replaced by new ones. Throughout the analysis period, which lasted from 5 to 6 hours, all rats of both groups were kept anesthetized with Zoletil 50®, lying on a thermic mattress, their body temperature

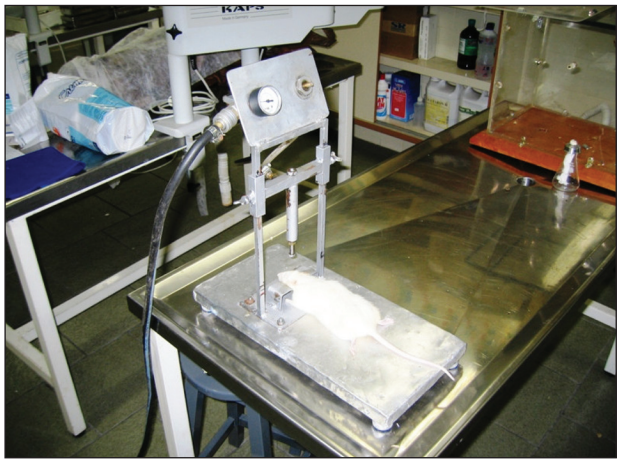

Fig 1. Anesthetized animal on the cortical impact model.

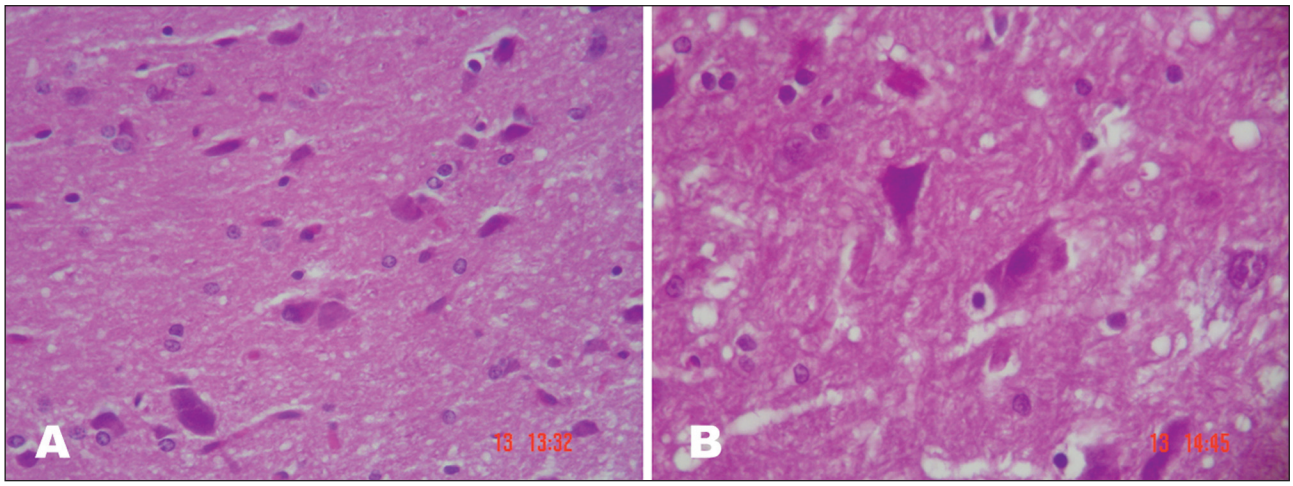

Fig 2. (A) Hystopathologic analysis of the animal treated with hypothermia. Right fronto-parietal region of brain (Hematoxiline-eosine 400 X); (B) Histopathologic analysis of the animal without the treatment with hypothermia, showing a large number of altered neurons and more intense brain edema.Right fronto-parietal region of brain (Hematoxiline-eosine 400 X).

Table 1. Results of control group and treated with selective hipothermia group (number of injured neuron).

Group A = Control group	Group B = Treated with selective hypothermia
Animal 1A = 12	Animal 1B = 2
Animal 2A = 30	Animal 2B = 5
Animal 3A = 50	Animal 3B = 12
Animal 4A = 30	Animal 4B = 20
Animal 5A = 90	Animal 5B = 25
Animal 6A = 90	Animal 6B = 60
Animal 7A = 80	Animal 7B = 2
Animal 8A = 90	Animal 8B = 80
Animal 9A = 80	Animal 9B = 2
Animal 10A = 80	Animal 10B = 10
Animal 11A = 90	Animal 11B = 25
Animal 12A = 90	Animal 12B = 20
Animal 13A = 80	Animal 13B = 12
Animal 14A = 90	Animal 14B = 20
Animal 15A = 80	Animal 15B = 25

ranging from 33 to 36ºC in both groups. Group B (hypothermia) had their brain surface temperature between 29-31ºC measured with the precision thermometer Ellab® DM 852.

After 5 to 6 hours, the rats received an additional anesthesia dosage and were killed by decapitation.

Brain removal and storage

After decapitation, the brains were carefully removed, without incurring lesions, and the whole brains were stored in glass containers with a 20% formol solution. In order to prevent the brains from becoming deformed, a 3-0 cotton thread was inserted through the cerebral trunk and the rats were left hanging without touching the bottom of the container.

Histopathologic analysis

The removed brains of the 30 rats, duly numbered and identified, were submitted to an histopathologic analysis. In order to quantify the generated lesions, the author followed the procedures established by medical practice[18,24], and selected the percentage count of damaged neurons. The brains were cut at coronal sections in right frontoparietal region with 5μ-thick sections, stained with hematoxiline-eosine, and counted by means of an optical microscope, enlarged 400. Other alterations were observed in the analysis including hemorrhagic petechias, brain concussions and edemas; however, they were not included in this study (Fig 2).

RESULTS

Variables and data

The variable of concern in this study was the counting of the injured neurons. The data obtained for each group of rats are in Table 1.

There was significant difference between the number of neurons counted in brain of the animals of both exper-

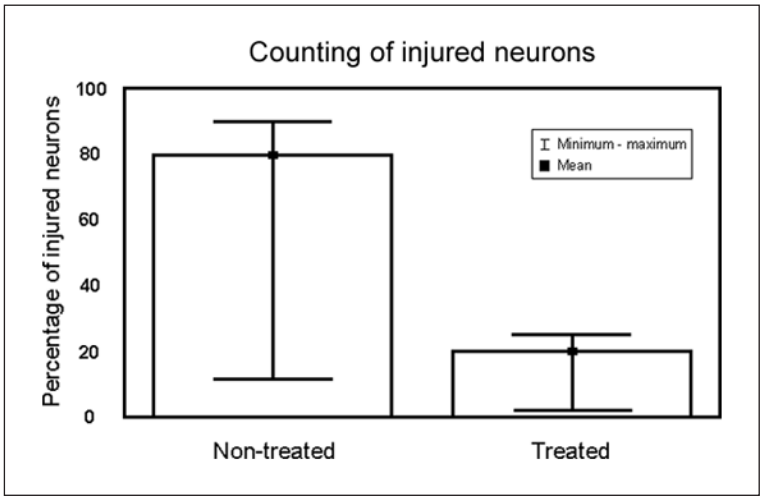

Fig 3. Comparison of results obtained from the treated and non-treated groups (with hypothermia).

Table 2. Comparative results.

Group	N	Average	Mean	Minimum	Maximum	Standard deviation
A (non-treated)	15	70.80	80.00	12.00	90.00	26.53
B (treated)	15	21.33	20.00	2.00	80.00	21.83

imental groups in favor of the group treated with selective hypothermia (p<0.00001, Mann-Whitney). See Table 2 and Figure 3.

DISCUSSION

Hypothermia, regardless of the type used (systemic or selective), aims at preventing secondary brain injuries, and, ultimately, providing brain protection. The brain tissue of the primary brain injury hardly ever survives. On the other hand, the mechanisms that generate secondary brain injury, such as brain edema, ischemia and decrease in cerebral perfusion pressure (CPP) caused by the increase in intracranial pressure (ICP) are considered the possible targets in the acute phase treatment. The factors that promote these physiological changes such as free radicals, blood-brain barrier dysfunction, excitatory amino acid levels and high intracellular calcium concentrations are taken into consideration at the time of the treatment[25].

The primary brain injury involves the destruction of brain tissue, vascular lesion and release of potassium and vasoactive substances such as endogenous opioids, catecolamines, serotonin and excitatory amino acids such as aspartate and glutamate. These changes are developed immediately following brain ischemia, which follows synaptic paralysis and temporary cardiopulmonary dysfunction. In animal studies as the ones published by Baker et al.[26], the initial release of the excitatory amino acids glutamate and aspartate, controlled by the constitutive nitric oxide (NO) radicals, is reported in the rise of intracellular calcium ions in the injured brain tissue. The release of glutamate, high levels of intracellular calcium and the presence of constitutive free radicals cause severe lesion in the intracellular homeostasis and membrane dysfunction as well.

Wang et al.[27] claim that the brain reactions that happen to protect the injured tissue, such as the neutralization of neuronal acidosis, the normalization of the neurotoxic glutamate concentrations, the removal of free radicals, the restoration of neurotrophic factors and the pH control of glial cells, are not sufficient in most of the cases.

The brain tissue rich in protrombine, when severely injured, generates microcirculation disturbances with rapid progression into brain ischemia followed by brain edema and ICP rise. Brain ischemia, brain edema and ICP rise are the most significant pathophysiological alterations at early acute stages of severe brain injuries. Due to the pathophysiological alteration, the ICP rise leads to the decrease in cerebral perfusion pressure (CPP) and brain ischemia with compartmental syndromes[27].

When ICP is above 20-25 mmHg, the cerebral perfusion pressure (CPP) decreases, as these values are higher than the ones for brain capillary pressure. Consequently, in the intensive care unit (ICU) it is essential that the patients with brain trauma have their CPP kept above 80mmHg and the ICP below 20mmHg. Macintosh[28] showed that tissue destruction, vascular ingurgitation, brain edema and inflammatory cytokines with blood-brain barrier dysfunction were considered the major causes of brain edema and ICP rise. Before ICP rise and progression of brain edema, functional changes in the vascular permeability begin between 1 and 15 hours. Before the progression of diffuse edema, selective and specific neuronal lesions in the basal ganglia were reported. The release mechanism of excitatory amino acids (glutamate) has been said to be the major responsible for the neuronal death. Consequently, it is extremely important to prevent neural excitement when handling severe brain traumas.

The brain edema and the intracranial hypertension start to progress after 24 hours, and are followed by reactions involving free radicals. At this point, intravascular release of thromboplastine, activation of intravascular coagulation and consumption of tromboplastine fibrinolysis agents lead to changes in vascular permeability of the microcirculation. Vascular ingurgitation, microcirculation disturbances, ICP rise and reactions involving free radicals generate a malignant ischemic cycle, leading to brain edema and intracranial hypertension. As the lesions take place in the injured tissue, infiltrate of immunoprotective cells is observed, with neutrophils and macrophages producing cytokines, nitric oxide (NO) and superoxide radicals. The release of cytokines increases intravascular coagulation by activating adhesion molecules to the intima of the vascular wall and activates alterations on vascular permeability. The complications due to systemic infections promote these alterations more easily and more severely, and free radicals cause larger brain lesions[25].

Neuroprotection – It is believed that the total recovery of the tissue of the primary brain injury is difficult to attain. Consequently, the objective of the intensive therapy is the care and the protection of secondary injuries caused by brain edema, ICP rise, free radical reactions, changes in intravascular coagulation, blood-brain barrier dysfunction, loss of vascular self regulation and reduction on blood flow caused by the primary injury. The neurons consume more oxygen and metabolic substrates than any other cell of the body and are extremely sensitive to hypoxia and energy crisis. Therefore, the care of severely injured patients focuses more on neuroprotection than on neuronal restoration. The care in intensive therapy concentrates on providing oxygen demand and cerebral blood flow (CBF), control of ICP and/or the decline of brain metabolism using barbiturics or hypothermia. For the restoration therapy to be successful, appropriate neuronal oxygenation,

administration of metabolic substrates and management of the secondary lesion are required[25,28].

The effects of hypothermia described in medical literature include its effects on excitatory amino acids, free radicals, decline in vascular permeability, nitric oxide, cerebral blood flow, (due to the decline in the regional oxygen flow and oxygen consumption), decline of the inflammatory reaction and decrease in the genic expression and apoptosis[21,22,25-28].

The current use of systemic hypothermia and prospective use for selective hypothermia – Mild (34-36ºC) and moderate (28-33.5ºC) systemic hypothermia have been reported to present better results obtained in the treatment of severe brain injury with fewer systemic complications such as arrhythmia and blood dyscrasia. Nevertheless, a study by Harris et al.[29] claims that more controlled, multi-center and random studies are necessary in order to permanently define the indications for use and the procedures of this therapeutic practice.

Recent published studies have proposed the use of systemic hypothermic treatments[25,27] with the brain temperature between 32ºC and 34ºC for severe brain injury, without compromising the systemic circulation and metabolism, and performing neuro-hormonal management and stabilization of the immune function. The initial "targets" of this therapeutic procedure for severe traumatic brain injury with hypothermia are not the decline of the brain metabolism to prevent brain ischemia and the decrease of ICP. Currently, in the attempt to restore the nerve tissue injured due to trauma, researchers advocate the use of hypothermia to attenuate the neuro-hormonal reactions of the hypothalamic-pituitary-adrenal axis, complemented with the adequate administration of oxygen and substrates in order to stabilize the brain metabolism[25].

Other applications of hypothermia as well as its use during complex brain aneurysm surgeries, brain strokes and post cardiac arrest have yielded promising results.

Several studies, which describe the mild and moderate systemic hypothermia side effects, include findings about the decline of the enzymatic activity, changes in pharmacokinetics and in pharmacodynamics, and changes in the neuromuscular response. Complications include postoperative tremors and myocardial ischemia, operative wound infections and coagulopathies concomitant with peroperatory blood loss[20,25,28].

In the last 50 years, several attempts to develop techniques of hypothermia exclusively targeted at the brain have been described, and more common used term in literature was selective hipothermia. These techniques include perfusion, injections of cold substances or direct cooling of the brain by means of several methods and helmet for head cooling. However, since Senning and Olsson's pioneer study[30], few clinical studies on selective hypothermia have been carried out with adequate methodology that would promote the adoption of the therapy on a regular basis. Larger, controlled and random studies are necessary.

The objective to propose a new therapy to treat severe brain trauma with selective hypothermia (cooling of the brain, keeping the corporal temperature constant) was researched and developed following the literature parameters which report the new findings about traumatic brain injury and hypothermia. The original technique discussed in this study proposes the bone removal of a brain region (craniectomy), with the preservation of the dura mater, the animal anesthetized, and the selective hypothermia treatment with an ice bag, gently set on the exposed region. The aim is to propose a viable procedure, easily replicated in future clinical trials.

For the generation of the brain trauma in rats, a controlled cortical impact model was developed, specifically built for the trial, based on available data. The need to show its efficacy also led the author to determine the parameter with which measure the extent of the generated brain lesion. The counting of injured neurons was the criterion selected as it would be difficult to analyze the data obtained by means of determining the extent of the lesion due to brain edema or the hemorrhagic petechias. This option was done because brain edema and hemorrhagic petechias have difficult quantification.

The results obtained have shown that the group treated with selective hypothermia presented a lower percentage of injured neurons when compared to the control group. It was possible to determine the efficacy of the selective hypothermia despite the short treatment time (5 to 6 hours). Other pathological conditions, such as brain edema and hemorrhagic brain patechias were described by the pathologist, who reports a lower incidence of edema in the group treated with hypothermia. However, these data were not used in the analysis of this study.

Moreover, the positive findings stimulate further focused research, initially with an extended period of treatment of 24 hours. Once the efficacy of the treatment is proven in more experimental studies, pertinent clinical studies on humans may be developed. The techniques proposed are easy, fast and cost effective, likely to become an option therapeutic treatment in traumatic brain injury.

In conclusions, the group treated with hypothermia had fewer injured neurons when compared with the group of animals who did not undergo hypothermia treatment.

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