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Induced Hypothermia: From Bench to Bedside in Systemic and Neurological Protection After Resuscitation From Cardiac Arrest

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ABSTRACT

Introduction: Cardiac arrest outside medical center is a major cause of death. The survival rates ranges from 5 percent to 35 percent. In patients who are initially resuscitated, anoxic neurologic injury is the prime cause of morbidity and mortality, besides hazards in multiple organs as the kidneys, for example. Induced hypothermia has proven to improve the prognosis after resuscitation from cardiac arrest. Objective: To demonstrate in experiments where induced mild hypothermia has been able to reduce ischemic and inflammatory damage in animal's brains and consequently the multiplicity of the other organs. Methods: Experiments were carried out with rabbits and rats when mild hypothermia was induced in order to demonstrate its neuroprotective properties. Results: Induced mild hypothermia demonstrated to be able to reduce the deleterious effects caused by brain ischemia and brain inflammation. Conclusions: Hypothermia may be helpful in reducing the ischemic process as well as in reducing the inflammatory cascade caused by ischemia. We believe that induced hypothermia improves prognosis after resuscitation from cardiac arrest reinforcing the application of mild hypothermia in cases of cerebral ischemia mainly after cardiac arrest.

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Abbreviations
PMNL: Polymorphonuclear Leukocytes
TTM: Targeted Temperature Management

Introduction

Population aging has led to the advent of new pathological conditions that require immediate treatment. Patients resuscitated from cardiac arrest outside hospitals, an event which was rarely reported a few years ago, recently has attained a great importance according to the possibility of reversion of this serious condition. However not all patients will have a good recovery mainly because of neurological complications resultant from the ischemic process associated. Therefore, the protection of brain parenchyma must be emphasized at the moment of initial support Therapeutic hypothermia also called Targeted Temperature Management (TTM) refers to deliberate reduction of the core body temperature, typically to a range of about 32°C to 34°C. Neurological protection may be obtained with this drop in body temperature. Even after more than 20 years, TTM is not wildly employed [1-8].

Methods

I. Working in experimental laboratory, we manage to demonstrate that induced mild hypothermia (30°C) could reduce the ischemic area consequent to the coagulation of the middle cerebral artery in rabbits [2]. The coagulation of the middle cerebral artery was carried out in twenty animals. A group of ten animals received the protection provided by mild hypothermia (Figure 1).

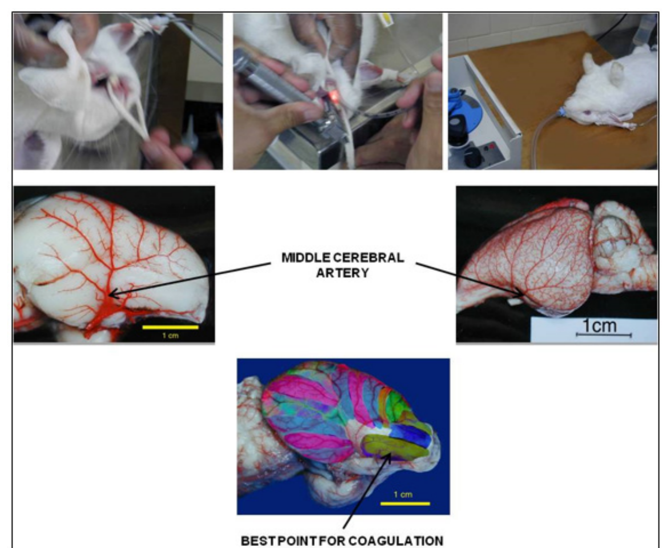

Figure 1: 30°C during 120 minutes

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After 40 hours, the brains were examined and the infarcted area was measured. It could be demonstrated that was a statistically significant difference between both groups. The induced hypothermia demonstrated to be able to provide neuroprotection (Figure 2).

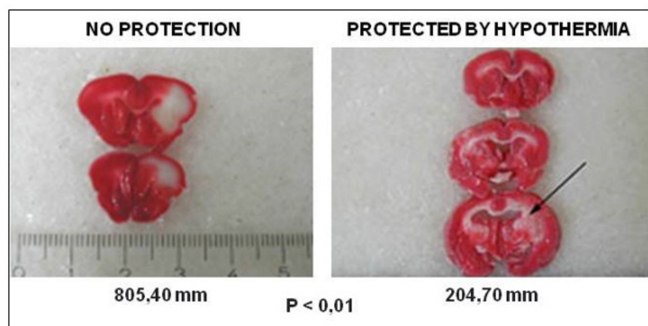


Figure 2: Infarcted area in mm

Mild hypothermia (30°C) locally induced in rabbit's brain has demonstrated to reduce infarcted area of ischemic lesion resultant from the coagulation of the middle cerebral artery [2].

II. In parallel while provoking an intense inflammatory reaction on rat´s brains with a potent inflammatory agent, the reduction of inflammatory reaction could be demonstrated in animals protected by induced hypothermia [3] (30°C)(Figure 3).

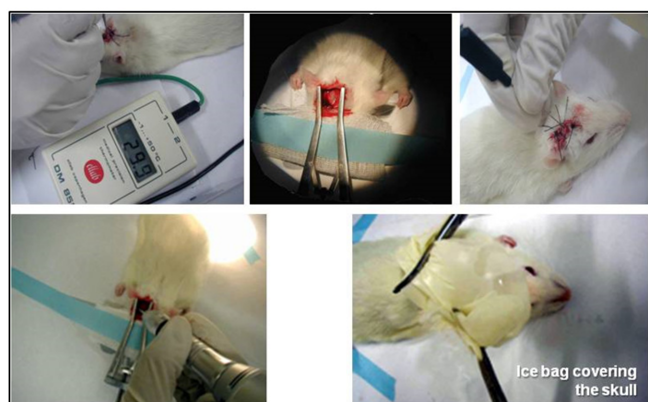


Figure 3: Reduction of induced inflammation by mild hypothermia in brains of rats

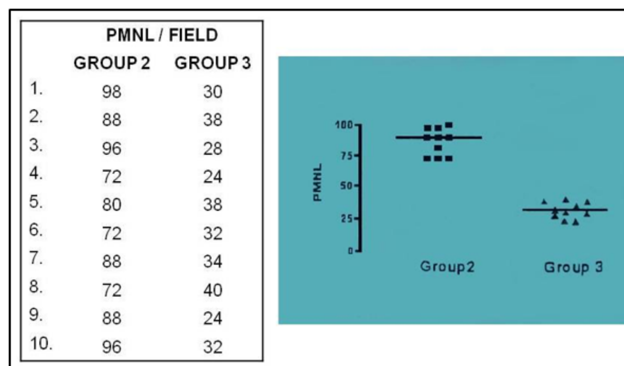
The group 1 was the control, the group 2 and 3 suffered a process of brain inflammation by means of a potent inflammatory substance (carrageenan 5%) topically dropped and the group 3 was neuroprotected by mild hypothermia.

Results

I. The infarcted area observed in group of non protected animals was 805,40 mm while the hypothermic protection was able to reduce de infarcted area to 204,70 mm.

Mild hypothermia (30°c) locally induced in rabbit's brain has demonstrated to reduce infarcted area of ischemic lesion resultant from the coagulation of the middle cerebral artery (Figure 4).

II. On the other hand, the animals that received the cerebral hypothermic protection demonstrated an important reduction in number of polymorphonuclear leukocytes (PMNL) infiltration in induced brain inflammation [3] (Figure 3, Figure 5).



PMNL / FIELD	GROUP 2	GROUP 3
1.	98	30
2.	88	38
3.	96	28
4.	72	24
5.	80	38
6.	72	32
7.	88	34
8.	72	40
9.	88	24
10.	96	32

Figure 4: Groups date and statistical analysis

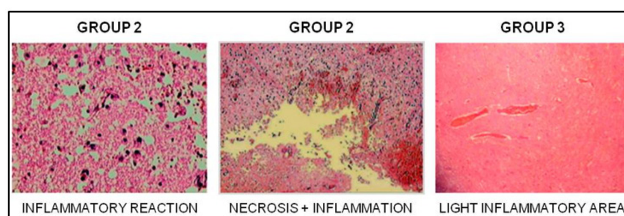


Figure 5: Inflammatory responses

Discussion

Hypothermia has been used for medicinal purposes since ancient times. The therapeutic effect of low temperatures is known for more than 2.500 years, when the Egyptians used cold to treat wounds and inflammations and the Greeks used snow to stop bleeding or reduce the edema in wounded soldiers [1].

Patients resuscitated from cardiac arrest outside hospitals, a quite rare condition a few years ago, recently has turned to be a very important event, due to the possibility of reversion of this serious condition [8-11]. However not all patients will have a good recovery, mainly because of neurological complications resultant from the ischemic process associated. The protection of ischemic brain is mandatory during these events [11-13].

Hypothermia may be helpful in reducing the ischemic process as well in reducing the inflammatory cascade caused by ischemia [3,6,7,8]. Guidelines from the American Heart Association and European Resuscitation Council [13,14,15] have recommended the use of TTM, especially in the United States, application and implementation of these guidelines increased significantly in recent years, with growing consensus on the importance of TTM [9,14,16].

Until nowadays, no drug has proved to be able to have neuroprotective effects during ischemic onset. Therefore, induced hypothermia is the only proven condition capable to improve the prognosis after resuscitation from cardiac arrest [7, 17-20].

Conclusions

Hypothermia may be helpful in reducing the ischemic process as well in reducing the inflammatory cascade caused by ischemia. We believe that induced hypothermia improves prognosis after resuscitation from cardiac arrest reinforcing the application of mild hypothermia in cases of cerebral ischemia mainly after cardiac arrest.

Addendum

Animal protocols were approved by the Federal University of São Paulo animal ethic board. Institutional guidelines were followed in

all protocols. All animal experiments were conducted in accordance with the NIH guide for the care and use of laboratory animals (NIH publication 80-23). All efforts were made to minimize animal suffering, and only the smallest number of animals were used to generate reliable scientific data.

Twenty New Zealand White rabbits weighting from 3100g to 3750 g underwent surgical procedure for coagulation of the left middle cerebral artery. The experiment was divided in two steps. The first step consisted of a group of animals that did not receive the brain protection. In the second group the animals received neuroprotection. The ischemic area of the brain was submitted to mild hypothermia of 29ºC to 30,5ºC over a period of 100 to 120 minutes. The hypothermia was achieved by the placement, over one hemicranium, of a rubber bag containing ice cubes, so that the temperature inside the ice bag was kept constant, near 0ºC (varied from 0,5ºC to 1,5ºC). (*Digital Thermometer* MET 9602 *ELLAB A/S Denmark*). The rectal temperature was measured and did not demonstrate any change during the experiment.

Surgery

The rabbits were anesthetized with intramuscular injection of Ketamine10mg/Kg+ Aceprazolamine1mg/Kg which was supplemented, by intravenous injection of Ketamine 10mg/Kg + Aceprazolamine 1mg/Kg. The animals were intubated and general anesthesia with Halothane was maintained during the procedure. All the procedure was carried out under microscopic magnification. With the animal in the left lateral decubitus position, a scalp incision of approximately 2,5 cm in a C shape, between the left orbital rim and tragus was performed.

The soft tissues were incised and the temporalis muscle was dissected from the cranium and retracted. The exposed skull bone was totally drilled off; the dura was exposed and incised in the region of the inferotemporal fossa. After the dural incision, the brain was gently retracted and the middle cerebral artery was followed down until its origin from the carotid artery. At this point the artery was coagulated. The scalp was closed with sutures. In some cases the coroniod process of the mandible had to be removed in order to permit to visualize the origin of the middle cerebral artery.

Brain protection

In the control group, the animals were maintained under general anesthesia for 100-120 minutes.

For the second group, ice bags were placed on the corresponding hemicranium for brain protection. The hypothermia (29ºC to 30,5ºC) was maintained for 100 to 120 minutes, as described before. After recovery from anesthesia, the endotracheal tube was removed and the animals were taken to specially designed cages where they received food and water. They were observed and all neurological deficits were recorded.

Measurement of infarct volume

Forty to forty five hours after recovery from surgery, the animals were sacrificed. The brains were removed from the cranial vault, placed in ice-cold saline and taken to a refrigerator under a temperature of -14ºC for 10 minutes. The brains were placed in a specially designed brain cutter.

The slices measuring 2 cm were immersed in a solution of 2% 2,3,5-triphenyltetrazolium (TTC) in 0,9% saline, incubated for 30 minutes in the dark, and then they were placed in a 10% formalin solution [1,14,15,16,17].

After one week, with a digital camera, the sections were photographed. The pictures were taken to a computer program (Adobe Photoshop exe 5.0 and Auto Cad R 14) where the infarct areas were measured and the infarct volume was calculated in mm^3.

In the first group, the infarcted area measured, in mm^3 (Figure 6).

1.	114,2
2.	90,60
3.	68,84
4.	34,52
5.	66,40
6.	96,22
7.	47,18
8.	54,80
9.	60,42
10.	72,14

Figure 6: Group I. Infarcted area

In the second group, five animals demonstrated areas of infarct measuring, in mm3 (Figure 7).

11.	32,62
12.	0,00
13.	18,20
14.	0,00
15.	0,00
16.	28,96
17.	0,00
18.	0,00
19.	88,24
20.	38,48

Figure 7: Group II. Infarcted area

The brain slices of five animals did not demonstrated any change of the TTC staining, so we presumed that there was not any macroscopically visible infarcted area. These five brains were taken to the Department of Neuropathology, where the brain slices were embedded in paraffin and stained with hematoxylin and eosin. All the slices were examined by the senior Neuropathologist of the University, who could not find areas of infarct in the five brains examined, confirming our previous results (Figure 8).

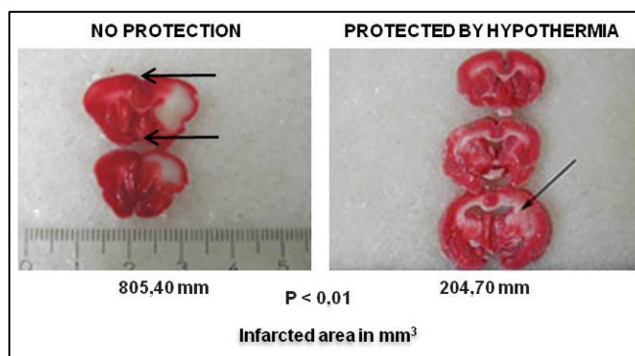


Figure 8: Brain sections of rabbits stained by Triphenyltetrazolium

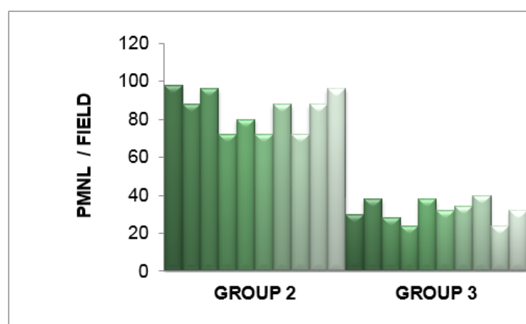


Figure 9: Reduction of induced inflammation by mild hypothermia in brains of rats

**Three groups of ten Wistar E.PM rats weighing between 290g and 330g were studied. Group 1-control group, Group 2-received no protection by hypothermia and Group 3-received protection by hypothermia .General anesthesia was given by means of IM injection of tiletamin chloridrate + zolazepan chloridrate in the proportion of 100mg/kg.

The animals were placed in a specially designed table covered by an homeothermic blanket control unit (Harvard Apparatus Limited Cat 50-7079 Edenbridge Kent). The core temperature was maintained in 37°C and was measured with a flexible fibber with the sensor tip placed into each animal's rectum (Ellab medical precision thermometer DM 852). The scalp incision measuring 18mm was C shaped 4mm from the midline. The muscles and fascia were dissected from the cranium. Under an operating microscope a 10-12 mm burr hole was drilled and the dura was exposed. After the duramater had been incised, 2-3 drops of solution of 5% beta-carrageenin was topically dropped. The scalp was sutured with mononylon 3-0. In the group 3, (the one protected by hypothermia), an ice bag was placed in order to cover the entire head. The ice bag was removed whenever the brain temperature dropped below 29,5°C and replaced when the temperature reached 31° C. Brain temperature was measured with a needle probe placed subcutaneously inserted 5mm into the brain parenchyma (Ellab medical precision thermometer DM 852). The hypothermia was maintained for 120-130 minutes. The control group underwent the same surgical procedure but no inflammatory solution was dropped, nor was hypothermia performed. After 3 days the animals were anesthetized and sacrificed. All bone of the superior part of the skull was withdrawn in order to permit the removal of the whole brain that was immediately fixed in formalin 10,0%. The specimens were allowed to fix for 24 hours, and then embedded in paraffin.

II. Histopathological examination–Brains were sliced into 18-µm-thick coronal sections and stained with hematoxylin and eosin with magnification X40, X100, X200. High microscopy examination aimed to demonstrate the number of PMNL per field. Four fields were examined. Total number of cells was counted using original magnification X 200. Histopathological analysis revealed acute lymphocytic and macrocytic cell predominance; small areas of necrosis were also seen) in some cases without neuroprotection. Inflammatory infiltration was only seen in the specimens where carrageenin was dropped. In brain hemisphere that has not received carrageenin (Group 1) no significant sign of inflammatory reaction was identified. Normal and pathological areas can be seen in. In group 2 and 3, the total number of PLNL is demonstrated in the Figure 4. After 3 days the animals were anesthetized and sacrificed. All bone of the superior part of the skull was withdrawn in order to permit the removal of the whole brain that was immediately fixed in formalin 10,0%. The specimens were allowed to fix for 24 hours, and then embedded in paraffin.

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