Translational realistic expectations of chronic cerebral hypoxemia in rat model after bilateral commom carotid artery ligation. Neurocognitive aspects[1]

Maria Cecília Santos Cavalcanti Melo[I], Diego Gadelha[II], Guilherme Veras Mascena[III], Thárcia Kiara Beserra Oliveira[IV], Carlos Teixeira Brandt[V]

[I]Fellow PhD degree, Postgraduate Program in Surgery, Federal University of Pernambuco (UFPE). Assistant Professor, Department of Ophthalmology, Campina Grande Faculty of Medicine (FCM), Campina Grande-PB, Brazil. Acquisition and interpretation of data, manuscript writing.
[II]Fellow PhD degree, Postgraduate Program in Surgery, Health Sciences Center, UFPE.Associate Professor, Department of Ophthalmology, FCM, Campina Grande-PB, Brazil. Manuscript writing, critical revision.
[III]Assistant Professor, Department of Cardiology, FCM, Campina Grande-PB, Brazil. Interpretation of data, manuscript writing.
[IV]Veterinarian, Fellow Master degree in Agricultural Engineering - UFCG - Paraíba , Federal University of Campina Grande (UFCG), Campina Grande-PB, Brazil. Acquisition and interpretation of data
[V]PhD, Head Professor, Scientific Methodology and Pediatric Surgery, UFPE, Recife-PE, Brazil. Coordinator of Campina Grande Faculty of Medicine (FCM), Campina Grande-PB, Brazil. Scientific and intellectual content of the study, interpretation of data and final critical revision.

ABSTRACT

PURPOSE: To evaluate the effects of chronic cerebral hypoxia on memory of rats submitted to bilateral common carotid artery ligation (BCCL).

METHODS: Every each week, for 16 weeks, 31 rats were tested for memory using a water and land mazes and compared with 30 normal rats (control group A). The variables were expressed by their mean and standard error of the mean (SEM). $p<0.05$ was used for rejecting the null hypothesis. The study was approved by the Ethics Committee for animal investigation.

RESULTS: There was a significant increase in the latency time, in the survival water and land mazes, after four weeks (study group B) follow-up. However, without any medication or therapeutically induced measures, after 16 weeks (study group C) follow-up the latency mean times tend to be similar to control group (A) in the neurocognitive tests.

CONCLUSIONS: Neurocognitive deficits after 16 weeks post-operative follow-up of rats that underwent bilateral common carotid artery ligation is a natural adaptive phenomenon. Thus, is not realistic to allow translational information from this animal model for therapeutically approaches aiming at to prevent, or to improve brain damage in human beings suffering from chronic deprivation of adequate blood supply.

Key words: Hypoxia-Ischemia, Brain. Carotid Arteries. Memory Disorders. Rats.

Introduction

Since early 1960s, although the mortality rate in Sprague- Dawley rats was very high when both carotid arteries were permanent occluded[1], it was realized that in Wistar rats, consistent ischemic damage of the brain was very difficult to maintain consistentely[2].

Permanent occlusion of both common carotid arteries in the rat or bilateral common carotid artery ligation (BCCL) has been used as an experimental model for chronic cerebral hypoperfusion[3]. After BCCL, cerebral blood flow (CBF) immediately declines to 30–60% of its control value, but recovers to approximately ~63% and ~90% of control at four- and eight-weeks, respectively[4,5].

Acute BCCL in rats leads to increase in intracellular calcium[6] to activate ARA-selective cPLA2 and release ARA and increase ARA recycling in phospholipid[7]. Activation of sPLA2 by calcium[8] also may release ARA and ultimately to produce PGE2[9] to mediate compensatory vasodilatation. Nitric oxide, free radicals and neurogenic factors during ischemia also can produce vasodilatation[10,11]. It is likely that some recovery occurred in the present study even at 24 h, associated with the sPLA2 activation, helping to normalize the significant disturbances in uFA and ARA-CoA concentrations evident at 6 h. However, auto regulation remains abnormal and the brain is more vulnerable to additional insults such as hypotension, hypoxia and further ischemia following BCCL[12].

Several therapeutically approaches have been followed to prevent or to improve the neurocognitive deficit produced by BCCL[13-24]. Among them: nitric oxide carrier such as S-nitrosoglutathione (GSNO)[13]; several natural substances like ligustilide (LIG), a main lipophilic component of Danggui (Chinese Angelica root, Radix Angelica sinensis)[14]; Bushen Jiannao Recipe (BJR)[15];effective component group of Chinese herbal medicine[16]; or biochemical substances like DL-3-n-butylphthalide (DL-NBP)[17], ferulic acid[18], 2D-DIGE combined with MALDI-TOF MS[19], Insulin-like growth factor-1 (IGF-1)[20]; or yet cognitive and physical stimulation, named Environmental Enrichment, consisted of one-hour sessions run 3 times per week during 12weeks[21]; electro acupuncture combined with compound Salviae Miltiorrhizae tablet[22]; a mixed compound of ginkgo biloba extract and cilostazol[23], or administration of Pistacia lentiscus L. essential oil[24], just to list the investigations produced in the year 2012. However, none of these studies or the previous ones follow the clinical course of this condition long enough to evaluate the natural history of the so called neurocognitive chronic effect of BCCL without doing any kind of intervention.

The purpose of this investigation was to weekly evaluate the neurocognitive status of the rats that underwent BCCL, without any intervention on the natural history with the minimum follow-up of 16 weeks.

Methods

The research project was approved by the Research Ethics Committee of FCM-Campina Grande-PB, Brazil.

Adult Wistar rats (*Rattus norvegicus*) from the animal colony were used for this study. They were housed in polypropylene cages, four animals per cage, under standard light/dark conditions (lights on 7:00, off 19:00) with food pellets and water *ad libitum*.

Chronic cerebral hypo perfusion was induced by BCCL[1-22], in 31 rats. The animals were anesthetized with ketamine hydrochloride (50 mg/kg - ip) and xilasine (10 mg/kg -ip). The common carotid arteries were exposed via a ventral midline incision, carefully separated from their sheaths and vagus nerves, and permanently doubly tied with 5/0 silk suture approximately 8 to 10mm below the origin of the external carotid artery.

Every each week, for at least sixteen weeks, the rats were tested for memory and learning, using a survival water maze task (SWMT). The apparatus consisted of a rectangular water tank measuring 120 cm in length by 80 cm wide and 60 cm in height. To make the water opaque, 1 kg of powdered milk was added, and water temperature was kept at 23 ± 1°C. A translucent acrylic platform (10 cm in diameter) was located in the one end (north) of the maze during training. The top of the platform was approximately 1.5 cm below the surface of water (Figure 1).

FIGURE 1 - Rectangular survival water maze. Rat starting position for the survival learning memory. Submerse circular platform close to the south end of the maze in opposition of rat initial water mark.

Spatial training of the platform in the water maze was performed for five consecutive days. Each rat received two trials per day for five days with the inter trial interval of twelve hours.

The starting position (south end) for each trial was randomly chosen and counterbalanced across all experimental groups.

The rats were gently placed into the water, facing the middle position of the maze south side wall. Swimming paths of the rats were monitored by a video camera. For each training trial, the latency to escape onto the platform and the path length were recorded. All data were fed into a computer program for posterior independent analysis.

The rats were given a maximum of 60s to find the platform. If they failed to find it within 60s, the training was terminated and a maximum score of 60s was assigned. The rats were then guided to the platform by hand, and it was allowed to stay on it for 10s before removed from the water.

The physical findings, the way of swimming, balance instability and weakness of the limb muscles were also recorded and analyzed. Both the training process and the sixth day evaluations were performed in a randomized fashion in order to eliminate any bias in the analysis.

Similarly, the rats were tested for visual and olfactory functions in a land maze (Figure 2).

FIGURE 2 - Land maze for testing visual and olfactory functions.

The animals were maintained in starving condition on individual cages for 24 hours.

Each rat was placed in the entrance of the land maze and the time was recorded until the rat found the exit of the maze where its original cage with food and water were placed. The environment was in the shadows just with a weak monochromatic blue light. For each week the latency times were recorded and analyzed after four weeks (study group B) and 16 weeks (study group C). In this meantime the animals were housed in polypropylene cages, four animals per cage, under standard light/dark conditions (lights on 7:00, off 19:00) with food pellets and water *ad libitum*.

Thirty normal rats without any disease (control group A) were caged in the same environment and way of life; including food and water,

The quantitative variables were expressed by their mean and standard error of the mean (SEM). $p<0.05$ was used for rejecting the null hypothesis.

Results

The mortality rate for BCCL was 38.7%

There was a significant increase in the latency time, in the survival water maze, after four weeks (study group B) follow-up. However, without any medication or therapeutically induced measures, after 16 weeks (study group C) follow-up the latency mean time tends to be similar to control group (A) (Figures 3 and 4).

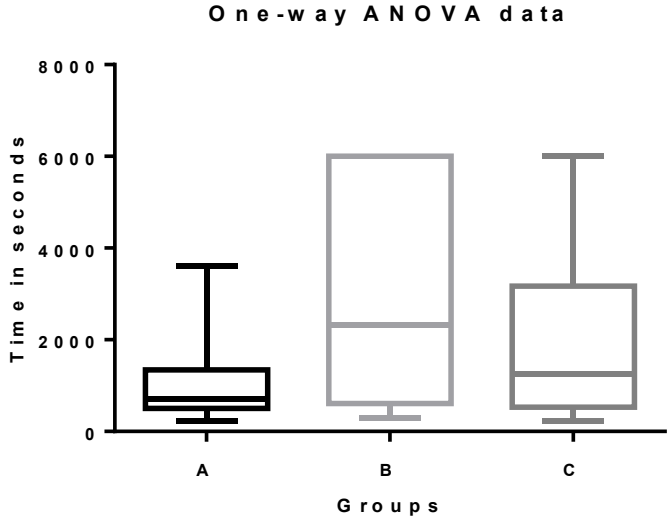

FIGURE 3 - Box plot of the medians of survival water maze times from the control group (**A**) and studied groups at four weeks follow-up (study group **B**) and at more than 16 weeks follow-up (study group **C**) – p = 0.0009.

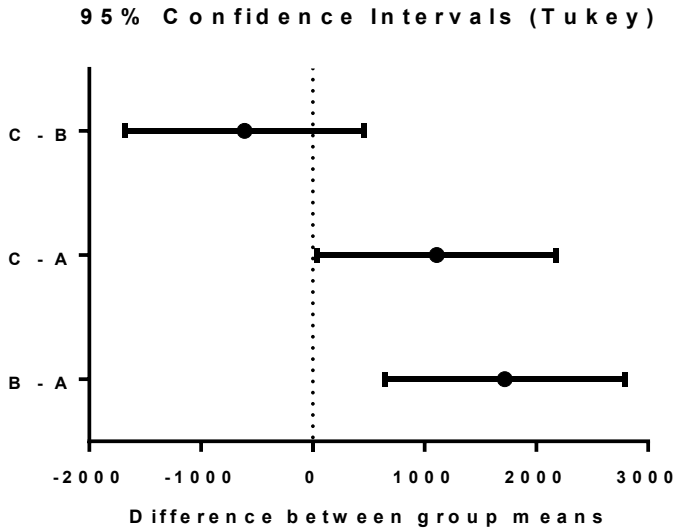

FIGURE 4 - Tukey post-test of survival water maze for comparing the control group (**A**) and studied groups at four weeks follow-up (study group **B**) and at more than 16 weeks follow-up (study group C). No difference comparing latency times from C and B - p=0.3680; small, but significant difference, comparing the mean latency time from groups C and A - p=0.0403; and significant difference comparing study group B and control group A - p=0.0007.

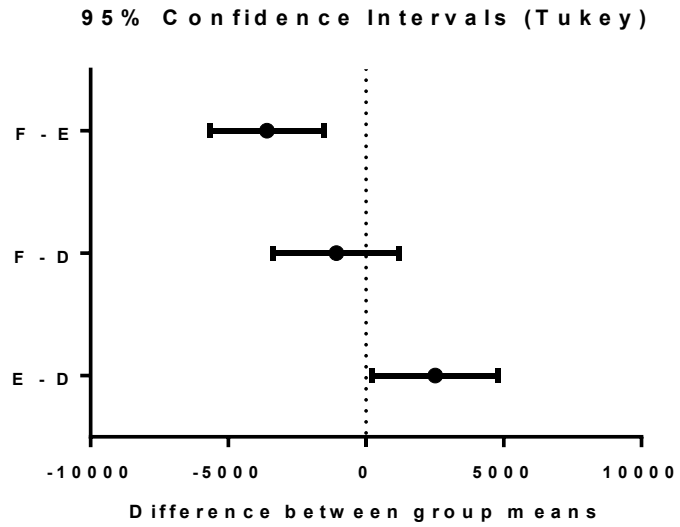

FIGURE 6 - Tukey post-test of survival land maze for comparing the control group (**A**) and studied groups at four weeks follow-up (study group **B**) and at more than 16 weeks follow-up (study group **C**). Significant increase in the mean land maze latency time comparing study group B (**E**) and control group A (**D**) - p=0.0277. No difference between the land maze latency times of control group A (**D**) and study group C (**F**) - p=0.5027. Very significant decrease of the mean land maze time between study group B (**E**) and study group C (**F**) - p=0.0002.

There was a significant increase in the latency time, in the survival land maze, after four weeks (study group B) follow-up. However, without any medication or therapeutically induced measures, after 16 weeks (study group C) follow-up the latency mean time was even significant smaller than the control group (A) (Figures 5 and 6).

As a whole, the latency mean times of water survival maze increased at four weeks follow-up; but, they tend to be similar to normal (control) rats after 16 weeks follow-up, without any treatment measure. Similarly, as regard to land maze times, they increase at four weeks follow-up; but, they were similar to normal (control) rats after 16 weeks follow-up.

At the end of the investigation period the animal of the study group underwent euthanasia, and the anterior portion of the neck were surgically open for the assessment of the ligated carotids. In both sides fibrous cord were seen in these arterial vessels. Additionally, thin arterial branches could be seen running in the carotid fibrous cord and in thigh attachment to the trachea (Figures 7 and 8).

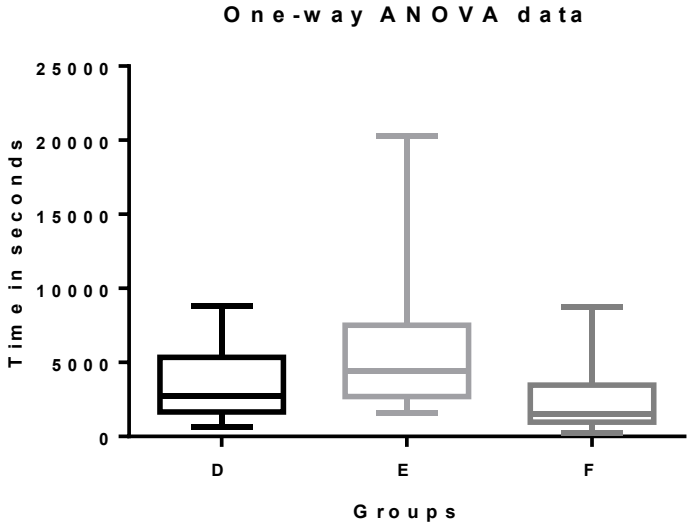

FIGURE 5 - Box plot of the medians of survival land maze times from the control group (**A** – represented by **D**) and studied groups at four weeks follow-up (study group **B** – represented by **E**) and at more than 16 weeks follow-up (study group **C** – represented by **F**) – p = 0.0003.

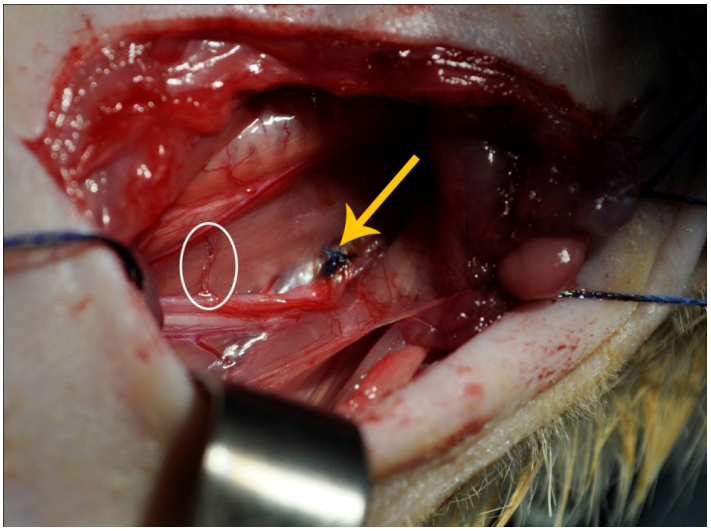

FIGURE 7 - Adaptive arteriogenesis after BCCA. Yellow arrow indicating the ligature of the left carotid artery. White circle and blue arrow indicating one of the thin colateral artery close by to trachea.

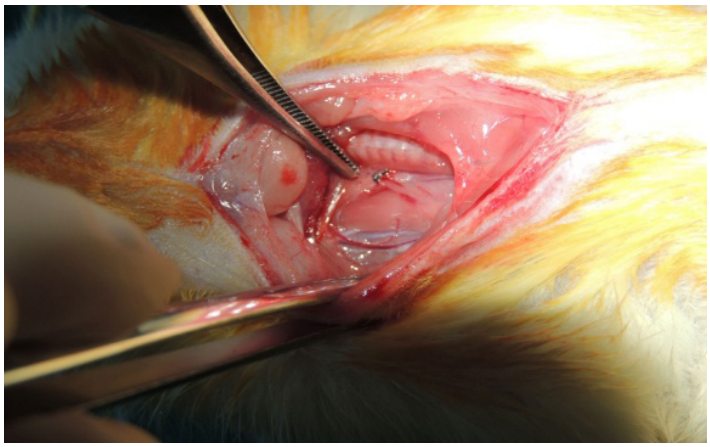

FIGURE 8 - Fibrous cord resulting from left BCCA. Thin arteries (arrows) running attached to the fibrous cord and posterior aspect of this cord.

Discussion

The majority of papers dealing with chronic encephalic hypoxemia due to bilateral common carotid ligation (BCCL) indicate that it is associated with neuron apoptosis, mainly in the hippocampus, cerebral cortex, and caudate affecting the microenvironment vital for the central nervous system functions[3,5,13-24], producing cognitive changes measured by radial maze tests. These changes are supposedly reverted using several therapeutically interventions[13-24].

A chronic, sustained reduction of cerebral blood flow can cause neuropath logical changes that may lead to various neurological deficits such as chronic ischemic infarction or vascular dementia (VD) via the secondary impairment of cerebral glucose metabolism and chronic energy production[25]. However, one has to realize that the vascular impairment of the arterial supply for the brain is different when comparing human being and rats. As the BCCA is performed in rats there is an almost immediately compensation from the collaterals arterial branches from the vertebral arteries.

In murine animals, apart from neuron plasticity, there is a proved adaptive mechanism of encephalic improvement after global ischemic damage (neurogenesis) particularly in the hippocampus area[26,27].

Effects on adaptive cerebral arteriogenesis has been demonstrate in other model of cerebral hypo perfusion, so called three vessel occlusion (bilateral vertebral plus unilateral common carotid artery) occlusion (3-VO)[28,29]. It is likely that the same phenomenon occurs after BCCL in rats.

The findings in the present investigation lend support to the hypothesis that, in rats, there is a natural adaptive mechanisms (hemodynamic, neuronal plasticity, neurogenesis and arteriogenesis), which prevent these animals from dying, and as the post-operative time goes by there is a non-interventional restoration of the rat brain blood perfusion neuron encephalic population, and consequently the natural improvement of the neurocognitive functions. The evidence from this study is that the conclusions from previous investigations[13-24] performed in 2012; as well as in the years before may be not useful for translational human being purpose. As a consequence we suggest that there is a need for searching an alternative animal model, rather than BCCL in rats, which can mimic chronic cerebral hypo perfusion and could allow for therapeutically approaches aiming at to prevent or to improve the neurocognitive deficit produced after chronic decreasing the arterial supply to the encephalon.

Conclusions

From the data one can assume that the improvement of the neurocognitive deficits after 16 weeks post-operative follow-up of rats that underwent BCCL is a natural adaptive phenomenon. Furthermore, the interpretation of these findings is that is not realistic to allow translational information from this animal model for therapeutically approaches aiming at to prevent, or to improve brain damage in human beings suffering from chronic deprivation of adequate blood supply.

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Correspondence:
Carlos Teixeira Brandt
Avenida Boa Viagem, 5030/1302
51011-000 Recife – PE Brasil
Tel.: (55 81)3342-0830
carlosbrandt@bol.com.br

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