LIMITED PROTECTIVE EFFECTS OF HYPERGLYCEMIA ON CEREBRAL ISCHEMIC DAMAGE IN RABBITS

TAVŞANLARDA SEREBRAL İSKEMİK YIKIM ÜZERİNE HAFİF HİPERGLİSEMİNİN SINIRLI KORUYUCU ETKİSİ

Hakan Hadi KADIOĞLU, Hüsnü KÜRŞAD, Cemal GÜNDOĞDU, Mahmut ARIK, Tülay ONAY

Departments of Neurosurgery **(HHK, MA)**, Anesthesiology and Reanimation **(HK, TO)** and Pathology **(CG)**, Medical School of Atatürk University,Erzurum-Turkey

Özet

Bu çalışmada hipergliseminin tavşanlarda a. Carotis Communis'in tek taraflı ligasyonu ile oluşturulan hemisferal serebral iskemi üzerine olan etkisi incelenmiştir. Çalışmada, 20 tanesi kontrol, 20 tanesi de çalışma grubu olmak üzere toplam 40 adet tavşan kullanıldı. Her iki grupta da halothan inhalasyonu ile anesteziyi takiben sağ a. carotis Communis ligatüre edildi. Çalışma grubuna ligasyon öncesi 5 mg/kg 'dan % 30'luk dekstroz intravenöz olarak verilirken kontrol grubuna aynı dozda % 0.45'lik NaCl verildi. Bağlamadan 6 saat sonra her iki gruptaki denekler intrakardiak KCl enjeksiyonu ile sakrifiye edilerek beyinleri çıkarıldı. Çıkarılan beyinler % 10 formaldehit içerisinde tesbit edildikten sonra coronal kesitler alındı. Her beyin için alınan kesitler ışık mikroskopu ile incelenerek iskeminin en yoğun olduğu alanlar saptandı ve her iki gruptaki iskemik nöronal değişiklikler değerlendirildi.

Sonuçta, hiperglisemi ile sağaltılan grupta gözlenen iskemik nöronal değişikliklerin kontrol grubuna göre oransal olarak daha az olduğu görülerek hiperglisemin iskemik nöronal yaralanmada sınırlı koruyucu bir etkisi olabileceği kanısına varıldı.

Anahtar kelimeler: *Hiperglisemi, Serebral iskemi, Karotid ligasyonu, Tavşan*

Summary

In this study, we attempted to research experimentally the effect of hyperglycemia on the cerebral ischemia induced by unilateral carotid ligation. In our experiments 40 rabbits were used, 20 of which were control group and the others 20 were study group. Right a. Carotis Communis was ligated in both groups following anaesthesia with halothan inhalation. In the study group, 30 % dextrose was infused with a dose 2.5 gr/kg before carotid ligation, while the same dose of 0.45 % NaCl was being infused in control group. At the end of 6 hours after ligation, all the rabbits in both groups were sacrified, and the brains were removed. Coronal sections were made after the brains had been fixated in buffered 10 % formol solution. Each slice of brain was examined carefully under light microscope to establish the most intensive ischemic areas. Then, ischemic neuronal alterations in both group were evaluated by light microscope.

We observed that neuronal ischemic changes were less in the study group than the control one. We therefore, concluded that hyperglycemia may have a limited protective effect in ischemic neuronal injury.

Key words: *Hyperglycemia, Cerebral ischemia, Carotid ligation, Rabbit*

Table 1. *Physiologic Values Observed in Rabbits*

	control group			hyperglycemia group		
	pre-ischemia	40th min of ischemia	6th hour min of ischemia	pre-ischemia	40th min of ischemia	6th hour min of ischemia
temperature (°C)	36,3 ± 0,7	36,5 ± 0,5	36,5 ± 0,6	37,0 ± 0,9	36,3 ±1,2	36,9 ± 0,7
pulse rate (pulse/min)	152 ± 26	153 ± 23	152 ± 23	170 ± 20	174 ±16	177 ± 33
MAP (mmHg)	126 ± 30	119 ± 24*	108 ± 5	140 ± 22	146 ± 14*	125 ± 24
glucose (mg/dl)	156,6 ± 19,5	155,3 ± 14,9**	168,3 ± 16,1**	156,8 ± 23,4	471,6 ± 2,9**	562,5 ± 21,7**
P_{O2} (mmHg)	108,6 ± 4,6*	108,2 ± 4,4	107,6 ± 4,2*	127,0 ± 20,6*	125,3 ± 21,1	128,5 ± 19,3*
P_{CO2} (mmHg)	43,2 ± 4,3	43,6 ± 4,1	43,8 ± 3,6	43,3 ± 2,4	44,7 ± 1,5	45,8 ± 1,7
pH	7,4 ± 0,1	7,2 ± 0,1	7,4 ± 0,1	7,4 ± 0,3	7,4 ± 0,2	7,4 ± 0,1

** p<0,05 as to t-test, ** p<0,0001 as to t-test; there is a very significant relationship between PO2, PCO2 and pH values in control group, PO2 values in hyperglycemia group as to the correlation regression analysis*

Introduction

Cerebral ischemia can appear as a result of pathologic cases, which are different in type and results, such as cardiac arrest, stroke, asphyxi, trauma, neoplasms and infection (1-4). Recently some theories about blood circulation of brain, metobolism and pathogenesis of ischemic-anoxic damage have been proposed (2-7). Some researchers claim that prevention of brain from ischemic damage is a very important problem. It is known that stress response in acute ischemic stroke is hyperglycemia (8). Generally the effect of high glucose level, which is considered to be dangerous in global ischemia does not agree with papers related to focal cerebral ischemia. Although results of some laboratory researches show that there is a negative relation between hyperglycemia and result in focal cerebral ischemia (5,9,10), others show that there is beneficial effects of preischemic hyperglycemia in formation of focal cerebral infarcts (6,11-13). In addition there are few studies about early recognition of preischemic lesions and risk factors and results of the effect of hyperglycemia on ischemic cerebral changes are contradictory (3,14-16). We designed the present research for the purpose of determining the histological changes and degree of ischemic damage resulting in acute ischemic injury treated by moderate hyperglycemia.

Materials and Methods

In the study, male hybrid rabbits with a weight of 2-2.5 kg were used. They were divided into two groups of 20 rabbits. No food was given to rabbits before 24 hours of the study. The anesthesia was provided with inhalation of halothan (2 Br, Cl, 1,1,1-trifluor ethan). The anesthetized rabbit was fixed on the operation table in supine position. Rectal probe was inserted aiming at determining of body temperature. Right femoral artery and right jugulary vein were exposed and catheterized to take blood samples and to monitorize the tension arterial, and to give glucose solutions. Then, right a. carotis communis was exposed and released from the surrounding tissue. Before ligation, arterial blood samples were taken and arterial blood pressure, pulse rate and body temperature were measured. In order to keep body temperature at same level (~ 37°C) electrical fan was used. pH, PO_2, PCO_2 levels in blood samples were measured. After these measurements through jugulary vein, with 2.5 gr/kg doses 30% dextrose solution was infused to study group and 0.45 % saline was infused to control group. Half an hour after infusion right a. carotis communis was fixed with 6.0 silk suture. 40 minutes after ligation blood samples were taken, arterial blood pressure, pulse rate and body temperature were measured. From rabbits which were observed for 6 hours, bloods samples were taken for the third time. After measuring arterial blood pressure, pulse rate, body temperature, rabbits were

sacrified by intracardiac KCl injection. Brains obtained by decapitization were taken to fixation in 10 % formalin for 72 hours. 2 mm coronal sections from brain samples were blocked with paraffin. Obtained slices were stained with hematoxylen & eosin, crystale violet, toluidine blue and examined microscopically. By magnifying by 100 and 400 times neuronal changes were evaluated according to 10 pointed ischemia scale defined defined by LeBlanc et al (17). This classification was done according to no changes in neurons (10 points), a few hypoxic neurons existences (9 points), sparsely hypoxic neurons (8 points), number of hypoxic neurons < 33 % (7 points), amount of hypoxic neurons < 33-66 % (6 points), amouny of hypoxic neurons 66-100 % (5 points), rare necrotic changes in neurons (4 points), proportion of necrotic changes in neurons < 33 % (3 points), proportion of necrotic changes in neurons 33-66 % (2 points), proportion of necrotic changes in neurons 66-100 % (1 points).

The student's t-test for physiological parameters and non-parametric Mann-Whitney U for histopathological data were used in statisticaal analysis in 3.0 statistics programme of GB-STAT version of IBM adapted computer.

Findings

Findings about physiological values of rabbits before and after ligation are summarized in Table 1. No difference in vital findings of control and hyperglycemia groups is observed. Determination of very important increase in blood glucose level in rabbits of hyperglycemia groups statistically shows that applied glucose level is appropriate to the purpose. It is also observed that there is a very important increase glucose level in control group ($p< 0.05$). In addition, according to physiological parameters, there was no statistically difference between two groups and also between the members of each group ($p> 0.05$)

In 31 of brain samples of rabbits in two groups ischemic alterations appeared to be discoloured area macroscopically. Because the base of this study consists of examination of histopathological changes, a volumetric evaluation is not carried out.

Microscopic examination was done according to the scale defined by LeBlanc et al (17). In hyperglycemia group average of ischemic neuronal changes is 7.35 ± 2.4, while in control group it is 5.5 ± 3. It was determined that there was no ischemic alterations in 6 subjects of study group, and 3 subjects of control group (Table 2). In result of the examination, no meaningful difference was found out between data of groups according to nonparametric Mann-Whitney U testing ($Z=1.9882$, $p<0.05$)

Table 2. *Distribution of the Subjects in Both Groups as to Ischemic Neuronal Changes*

grade	control	hyperglycemia
no changes	3	6
I	4	6
II	6	5
III	7	3
total number of subjects with ischemic changes	17	14

Statistically insignificant difference between two groups based on p= 0,91 as to x^2-test

Discussion

In occlusive strokes, one of the very important reason of mortality and morbidity is cerebral ischemic damage (1,3,4,8,11,13,18). As a result of many studies, it is thought that hyperglycemia may supply protection to brain tissue which is exposed to ischemia, however, the mechanism of protective effect of hyperglycemia is not known properly and the effect in focal cerebral ischemia is doubtful (10-14). In global cerebral ischemia, beneficial effect of hyperglycemia could not be seen (2,9,10). On the contrary, it causes worse neuronal damage. It has hardly been to explain how the hyperglycemia affects adversly on global cerebral ischemia while it exerts protective effects on focal cerebral ischemia. In global cerebral ischemia high

glucose concentration causes an increas in the amount of lactic acide in anaeorobic conditions. It is thought that hydrogen ions in high amount, which become free with decomposition of lactic acide, have toxic affect on brain tissue (1-3,5,9,10,18,19). The excessive increase of hydrogen ions in amount cause an increase the entrance of calcium and sodium ions in neuronal cells, and it may also lead to lipolysis, proteolysis, disintegrated of cells membranes and damaging changes such as increased cellulary cathabolism (1,5,9,10,13]. In studies, where permanent focal vasculary occlusion models were used, beneficial effects of preischemic hyperglycemia on formation of focal cerebral infarction are seen (6,11-13). There is a detailed difference between permanent and temporary focal ischemia. Temporary ischemia is not only more susceptible to damage with high glucose level but permanent ischemia is more resisting to dangerous effect of hyperglycemia but also profits from it (20). Because anaeorobic metabolism does not take place in high amount in focal cerebral ischemia, concentration of hydrogen ions will not reach an excessive. Furthermore, giving additional glucose may prevent increase of damage in surrounding parts of ischemic regions. There are evidences that glucose accelerate improvement of tissue damage in medium without lactic acide (11-13). Conversely Hoffman et al (21), in a recent study, reported that in temporary ischemia hyperglisemic conditions should be avoided because of the well-known bad effects of increasing acidosis and the lack of positive effects for a long period in the maintenance of energy condition.

In our study physiological parameters do not show any difference between control and study groups. In control group statiscally a very important increase in glucose level is observed ($p<0.05$). In subjects of hyperglycemia group, a statistical determination of a very important increase in blood glucose level shows that applying 30 % dextrose 2.5 gr/kg dosage is appropriate. With ligation of unilaterally a. carotis communis for 6 hours, hemispheral cerebral ischemia is induced. It is known that this time is adequate for ischemic cellulary changes (11,13,14).

In histological examinations done after ischemia period, it was determined that no cerebral ischemia takes place in 6 subjects of hyperglycemia group and 3 subjects of control group. The result was statistically insignificant. This may be due to developing of remaining perfusion with collateral vessels and local perfusion pressure (18). However, the total ischemia can rarely exists in a cerebral arterial occlusion or acute ischemic attacks.

LeBlanc et al (17) classifies neuronal changes in cerebral ischemia histologically in 10 groups. In this classification grading of ischemic damage was arranged in accordance with existences and proportion of hypoxic or necrotic changes in neurons.

The result obtained from microcopic examination is statistically meaningful, and it shows that hyperglycemia may have a limited protective effect in hemispheral cerebral ischemia. Although this result forms a contrast with findings reported by Chopp et al (5), Kraig and Chesler (9), and Siesjö et al (10), it agrees with results obtained by Ginsberg et al (6), Kraft et al (11), Nedergard et al (12) and Zasslow et al (13).

In conclusion, in focal ischemia due to permanent vasculary occlusion, beneficial effects of hyperglycemia may be related with pathophysiological alterations in borders of infarct areas. We think that reperfusion of this region or sufficiently collateral blood flow may change the protective effect and explain why hyperglycemia brings different results in different models. For this reason, we think that relations between intra- and extracelluar metabolic alterations and different blood glucose concentrations in occlusion-reperfusion models with different periods should be investigated in order to be more evident for this matter.

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Correspondence and Reprints:

Hakan Hadi KADIOĞLU, M.D., Ph. D.

Atatürk University Medical School,
Department of Neurological Surgery,
25240, Erzurum-Turkey