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The Effects of Regulating Increased Blood Glucose Levels on Plasma Endothelin-1 Levels After Severe Head Trauma in Rats

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ABSTRACT

AIM: To examine the effects of regulating increased blood glucose levels on plasma ET-1 levels after severe head trauma in rats.

MATERIAL and METHODS: Traumatic diffuse brain injury-induced rats were followed for 7 days and were randomly divided into two groups of 36 rats. Pre- and posttraumatic blood glucose and ET-1 levels were measured in group 1 (control). Posttraumatic blood glucose levels were maintained at normal levels using insulin and both blood glucose and ET-1 levels were measured at 2, 6, 12, 24, and 48 h and 7 days posttrauma in group 2. The study excluded animals that died and had skull fractures.

RESULTS: Posttraumatic plasma ET-1 levels (n=36) were significantly higher than baseline values in group 1 ($p<0.05$). ET-1 levels in group 2 at the 7-day follow-up after trauma were significantly higher than baseline values (n=36) ($p<0.05$). However, the increased ET-1 levels were statistically significantly lower in group 2 than in group 1 ($p<0.05$).

CONCLUSION: The increased ET-1 levels were significantly prevented by keeping blood glucose levels within normal limits with insulin after severe head trauma. Thus, secondary injury to cerebral blood flow can be prevented by reducing the occurrence of vasospasm that starts in the early posttraumatic period or by stimulating the release of nitric oxide. Therefore, further studies on the role of ET-1 and insulin in developing secondary injuries after severe head trauma would be beneficial.

KEYWORDS: Blood glucose levels, Endothelin-1, Hyperglycemia, Traumatic diffuse brain injury

ABBREVIATIONS: CBF: Cerebral blood flow, **CNS:** Central nervous system, **CSF:** Cerebrospinal fluid, **SAH:** Subarachnoid hemorrhage

INTRODUCTION

Traumatic central nervous system (CNS) damage is a leading cause of death and disability. Moderate or major head trauma with high mortality and morbidity constitutes 30%–40% of head traumas (4). Trauma-induced hypoxia and ischemic events cause widespread brain damage by affecting cerebral energy metabolism (22). Hyperglycemia occurs in the early period following severe head trauma, and blood glucose level is negatively correlated with recovery following trauma (10,14).

Blood glucose-level changes, such as hyperglycemia or hypoglycemia, have guided the prognosis of patients with severe head injuries. Hyperglycemia is an undesirable situation in patients with head trauma (36).

Hyperglycemia was found to delay the healing of neuronal damage, impair clinical and neurological response, and increase mortality by altering cerebral metabolism in the presence of hypoxia and ischemia. Periods of cerebral ischemia and hypoxia and increased lactic acid levels cause increased neurologic damage in the presence of hyperglycemia in patients with severe head trauma (33,37). Endothelin-1 (ET-1) leakage from damaged endothelial cells in cerebral microvessels, the presence of hypoxia, and the decrease in perfusion pressure with endothelial cell reduction induce the release of ET-1 (21,40).

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Keeping blood glucose levels within physiological limits is reported to positively affect neurological recovery (19,22,37). Various researchers have suggested the role of endothelial-derived vasoactive peptides in neurological function losses due to brain damage in severe head trauma and ischemic cerebrovascular diseases (28,44). ET-1 is an effective vasoconstrictor agent secreted by vascular endothelial cells that is being studied for its role in neurological damage caused by severe head trauma. ET-1 is thought to increase cerebral ischemia and hypoxia, especially due to its effects on blood vessels, thereby increasing secondary damage in traumatic CNS injuries by changing cerebral energy metabolism (29,38).

Pathogenesis: The mechanical effect of trauma is tissue integrity damage. Primary damage includes neuronal transmission and nerve cell disruption. The primary mechanical damage and the complex following physiopathological events are responsible for the total damage resulting from trauma (7,15,17). Some endogenous factors or substances that occur in response to the primary event cause this delayed damage, resulting in neuronal degeneration (6,7,9,12).

Endothelins: ETs are one of the most effective vasoconstrictor substances known today which were isolated in 1985 by Rubanyi and Vanhoutte (34). ETs were synthesized from vascular endothelial cells. Additionally, the heart, lung, brain, kidney, monocytes, and macrophages synthesize ET (18). Vasoconstriction occurs in the arteries and veins isolated by the strong vasoconstrictor effect of ET (16,27). ETs are released in response to hypoxia and thrombin (41,42). Three ET isoforms, such as ET-1, ET-2, and ET-3, have been identified in addition to two endothelin receptors: ET (A) and ET (B). The ET (A) receptor is highly specific for ET-1, and its stimulation greatly increases the vasoconstrictor response of ET. The ET (B) receptor is different from ET (A). ET (B) responds equally to all ET isoforms and induces vasodilation via nitric oxide and/or prostaglandin release. Endothelin receptors have been demonstrated in the human brain (2,3,23). The vasoconstrictive potency of ET-1 is 10 times greater than that of angiotensin 2, vasopressin, or neuropeptide-Y. Cerebral arteries are hypersensitive to ET-1, which is more potent than the maximal depolarizing effect of potassium. Increasing ischemia due to vasoconstriction is important in pathogenesis (18,20,42).

ET-1 in head trauma: Cerebral blood flow (CBF) changes after head trauma are compatible with brain dysfunction posttrauma (2,6,30). The experimental study conducted by Armstead (2) revealed that ET-1 levels increase in CBF after traumatic brain injury. Additionally, increased ET-1 has increased vasoconstriction in the pial arteries.

This experimental study investigated the changes in plasma blood glucose and ET-1 levels and the effects of regulating increased blood glucose levels on plasma ET-1 levels after severe head trauma in rats.

MATERIAL and METHODS

This experimental study was conducted in the Erciyes University Faculty of Medicine Clinical and Experimental Research Unit. Ethics committee approval was obtained (58681). A traumatic diffuse brain injury model was created in rats using the model presented by Marmarou et al. (26) (Figure 1). Adult rats were injured using a simple weight-drop device consisting of a segmented brass weight free-falling through a Plexiglas guide tube. Skull fracture was prevented by cementing a small stainless-steel disc on the calvaria.

This study used 78 male Wistar albino rats with an average weight of 300–350 g. Rats that died (32) or had skull fracture (10) during the study were excluded from the study. The total number was maintained by replacing excluded rats with new ones. Sedation was provided with intraperitoneal ketamine hydrochloride (60 mg/kg) before the procedure.

Rats with traumatic diffuse brain injury were followed up for 7 days. Blood glucose and ET-1 levels were analyzed in 0.2 ml of blood samples taken from the tail vein pretrauma and 2, 6, 12, 24, and 48 h and 7 days posttrauma.

Experimental groups: This experimental study included 78 rats. They were randomly divided into two groups of 36 rats each. Each group was divided into 6 subgroups with 6 rats each to prevent blood loss. Blood samples were taken from each subgroup once at 2, 6, 12, 24, and 48 h and 7 days

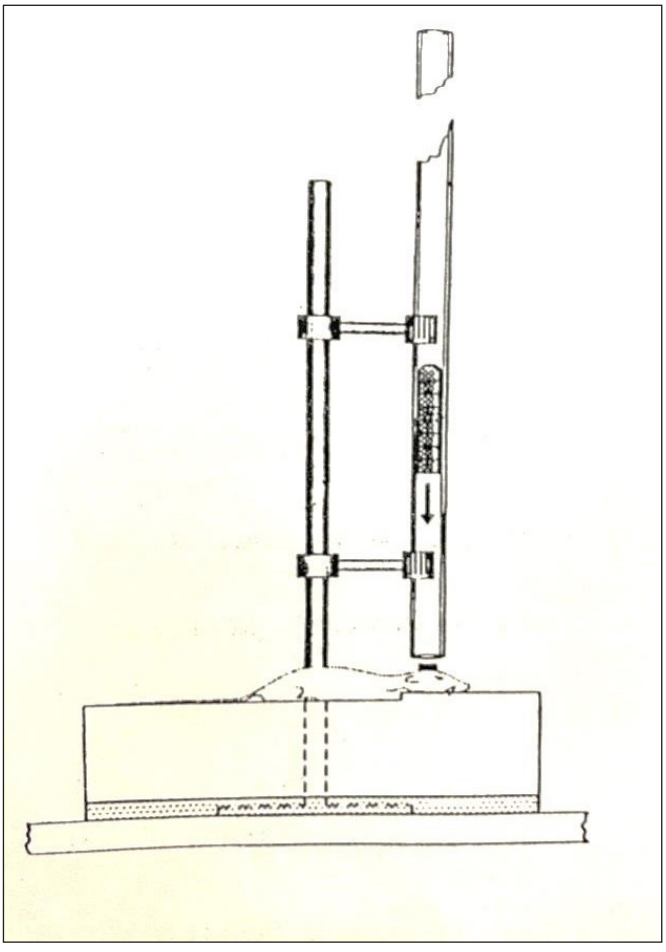

Figure 1: Trauma tool.

Table I: Blood Glucose Values and Mean Values (mmol/l) of rats in Group 1 / Group 2

	Pre-traumatic	2 hours	6 hours	12 hours	24 hours	48 hours	7 days
Group 1	154.2 ± 4.4	198.2 ± 2.5	196.5 ± 2.7	196.8 ± 3.3	192.5 ± 3.1	184.3 ± 5.2	170.0 ± 2.3
Group 2	154.8 ± 4.8	154.2 ± 2.3	150.3 ± 4.8	157.2 ± 4.7	152.7 ± 6.0	156.5 ± 6.6	149.5 ± 4.8

Table II: Endothelin-1 Values and Mean Values (ng/ml) of Rats in Group 1 / Group 2

	Base Values	2 hours	6 hours	12 hours	24 hours	48 hours	7 days
Group 1	63.3 ± 12.5	97.7 ± 7.8	92.7 ± 17.2	96. 6 ± 6.7	92. 7 ± 5.3	88.0 ± 4.4	92.0 ± 14.3
Group 2	63.3 ± 12.5	82.7 ± 5.5	83.3 ± 9.3	80.7 ± 10.3	80.0 ± 12.1	72.7 ± 20.5	8 .0 ± 12.5

Table III: Comparison of Endothelin-1 Values (ng/ml) of Group 1 and Group 2 After Trauma

	Group 1	Group 2	Statistical analysis
Blood glucose	189.7 ± 10.5	151.8 ± 4.6	**p<0.0005**
Endothelin-1	93.1 ± 8.3	81.2 ± 12.5	**p<0.0005**

posttrauma, and blood glucose and ET-1 were studied. Both groups (groups 1 and 2) were subjected to diffuse head trauma in the same way. Six rats were not subjected to head trauma (sham group).

Pre- and posttraumatic blood glucose and ET-1 levels were measured in group 1 (control). Posttraumatic blood glucose levels were maintained at normal levels using insulin in group 2, and both blood glucose and ET-1 levels were measured. In the first 24 h, 2 IU/kg (the first dose was started 2 h after the trauma) of long-acting zinc insulin (Lente) was intraperitoneally administered twice a day. Intraperitoneal glucose injections were administered when necessary to keep blood glucose levels at approximately 5–10 mmol/l (19).

ET-1 plasma samples were measured using an enzyme immunoassay kit (PHOENIX, Pharmaceuticals, Inc. USA).

All results were presented as mean ± standard deviation. The Mann–Whitney U Test was used for intragroup analyses and the Kruskal Wallis Analysis of Variance for between-group analyses.

RESULTS

Blood glucose values were significantly higher in the first 24 h compared to pretraumatic values in group 1 (n=36) (p<0.0001). These values, which were high during the first 24 h, started to decrease at 48 h. It remains slightly higher than normal although it significantly decreased compared with the early posttraumatic period in the 7-day controls. No statistically significant difference was detected although there were minor fluctuations in pre- and posttraumatic blood glucose monitoring in the rats in group 2 (n=36) (p>0.05). Statistical grading for pre- and posttraumatic values are given in Tables I and II.

Endothelin-1

Posttraumatic plasma ET-1 levels of rats with head trauma in group 1 (n=36) were significantly higher than baseline values (p<0.05). Each value measured in the 7-day follow-up posttrauma was statistically significantly higher than the baseline values (p<0.05). High values measured in the first 2 h continued for 7 days.

ET-1 levels in group 2 (n=36) measured in the 7-day posttrauma follow-up were significantly higher than baseline values (p<0.05). However, the increase in ET-1 levels in group 2 was statistically significantly lower than in group 1 (p<0.05). A statistically significant decrease was found in group 2 compared to group 1 at 2, 6, 12, 24, and 48 h post-trauma (p<0.05). This decrease was not statistically significant although a decrease was detected in group 2 in 7 days (p>0.05).

The values of groups 1 and 2 were compared with the baseline values and among themselves, respectively, to examine the difference between the groups. The obtained values are shown in Tables II and III.

DISCUSSION

Studies have focused on some cellular and biochemical factors although the basic pathophysiological mechanism of traumatic secondary brain injuries that occur within minutes or even days following the primary brain injury caused by trauma remained unknown (7). Generally, some endogenous substances that occur as a primary response to trauma initiate a chain of molecular events and cause various vascular and neuronal degenerations (12,15). These peptide substances, which appear as a primary response to trauma, increase cerebral ischemia and hypoxia caused by their strong vasoactive effects, and thus, cerebral energy metabolism changes and lactic acid level increases (10). This vicious circle further advances the secondary damage due to trauma.

Glucose is the most important source of energy for the human brain (11). A direct correlation was found between the severity of head trauma and hyperglycemia (1). Hyperglycemia was

associated with increased mortality (8). Stress hormones increase during severe head trauma. Catecholamine, cortisol, glucagon, and growth hormone increase glycogenolysis and metabolism, and then excessive glucose production occurs. The increase in stress hormones caused hyperglycemia (5,24). Latent diabetes mellitus may occur after severe head trauma or existing diabetes mellitus symptoms may be exacerbated, especially in elderly patients (13).

Hyperglycemia has negative effects on head trauma recovery, and it causes neuronal damage, especially in the presence of ischemia and hypoxia, with a direct relationship between blood glucose levels and head trauma recovery (14,33). De Salles et al. (10) revealed significant hyperglycemia within the first 12 h following head trauma, and mild hyperglycemia continued, although blood glucose values decreased during their 5-day follow-up. Our study detected significant hyperglycemia in all measurements post-trauma in group 1 ($p<0.0001$). Hyperglycemia remained at its highest level during the first 24 h. Blood glucose values were above the normal range at 7 days although with a decrease after 48 h ($p<0.0001$)

Armstead (2) revealed that cortical periarachnoidal cerebrospinal fluid (CSF) ET-1 levels increased in the experimental head trauma model created in newborn pigs. Periarachnoidal CSF ET-1 concentrations were significantly increased following trauma. Again, the study determined that ET-1 reduced the diameter of the pial artery and arterioles. Pial small arterial and arteriolar diameters were also significantly reduced.

A clinical study by Ziv et al. (44) reported a significant increase in ET-1 levels, especially in the first 24 h, in acute ischemic stroke. This increase was proportional to the severity of the neurological status. The increase in plasma ET-1 levels due to stroke remains unclear. The event indicates a nonspecific increase in ET-1 production by the systemic vascular endothelium in response to the general stress associated with acute cerebral infarction (25,35,43). Pavón-Fuentes et al. (31) reported that phycocyanobilin attenuated ET-1-induced focal cerebral ischemia. A clinical study by Suzuki et al. (39) on 27 patients with subarachnoid hemorrhage (SAH) measured the ET-1 levels in the plasma and CSF of patients periodically over 2 weeks and reported that the plasma ET-1 levels increased up to 7–8 times the normal level at the beginning of SAH and the levels remained high throughout the study. A slight increase was detected in patients with vasospasm starting from the third day while CSF ET-1 levels did not show a significant increase in patients without vasospasm.

Our study revealed a significant increase in plasma ET-1 levels 2 h posttrauma in group 1 ($p<0.05$) and continued for 7 days. Increased ET-1 was thought to be caused by posttraumatic astrocyte injury, as Pluta et al. stated (32).

CONCLUSION

Our study revealed that keeping blood glucose levels within normal limits with insulin after severe head trauma significantly prevented the increase in ET-1. Thus, secondary injury to CBF can be prevented by reducing the occurrence of vasospasm that starts in the early posttraumatic period or by stimulating the release of nitric oxide. Therefore, further studies on the role of ET-1 and insulin in the formation of secondary injuries after severe head trauma would be beneficial.

AUTHORSHIP CONTRIBUTION

Study conception and design: MM, RKK
Data collection: MM, AS
Analysis and interpretation of results: MM, RKK
Draft manuscript preparation: MM, RKK
Critical revision of the article: MM, RKK, AS
All authors (MM, RKK, AS) reviewed the results and approved the final version of the manuscript.

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