Özgün Deneysel Araştırma

Alterations in Blood-Brain Barrier After Traumatic Brain Injury in Streptozotocin-Induced Diabetic Rats

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✔ **Aim:** Diabetes mellitus is a chronic metabolic disease that is associated with peripheral microvascular complications and an increased risk of neurological events. Following closed head injury, diabetic rats suffer greater neurological dysfunction, associated with further lipid peroxidation than normal rats. The mechanistic factors in diabetes which cause neurological dysfunction are not clear, but disruption of the blood-brain barrier (BBB) may be one reason. We experimentally investigated alterations in the BBB after traumatic brain injury in streptozotocin-induced diabetic rats.
Methods: Thirty-two adult male Sprague-Dawley rats were divided randomly and evenly into four groups as trauma only, diabetes only, diabetes plus trauma, and sham-operated control. Diabetes was induced by a single injection of streptozotocin. Diabetic rats were exposed to trauma 4 weeks after streptozotocin injection to allow development of chronic diabetes. Permeability to Evans blue dye (EBD) was evaluated to assess the BBB integrity following trauma. Brains perfused with EBD were divided into six anatomically distinct brain regions (cortex, hippocampus, corpus striatum, midbrain, thalamus, and cerebellum), and EBD extravasation was quantified using spectrophotometric methods 60 min after injury.
Results: Spectrophotometric measurements of EBD revealed that BBB permeability was increased significantly in the cerebellum and corpus striatum in the diabetes plus trauma group compared with the trauma only and control groups.
Conclusion: The disruption of the BBB caused by traumatic brain injury is more severe in diabetics than normal subjects, and also region specific with cerebellum and corpus striatum appearing to be most susceptible to trauma-induced microvascular damage in diabetics.

Key words: Blood brain barrier, brain edema, diabetic rats, streptozotocin, traumatic brain injury

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Streptozosin ile diyabetik yapılan ratlarda kafa travması sonrası kan-beyin bariyerindeki değişiklikler

✔ **Amaç:** Diabetus mellitus, periferik mikrovasküler komplikasyonlar ve artmış nörolojik olaylarla beraber olan kronik bir metabolik hastalıktır. Kafa travması sonrası diyabetik ratlarda normal farelere göre daha fazla nörolojik hasar ve artmış lipid peroksidasyonu görülmektedir. Diyabetin nörolojik bozukluk yapma mekanizması bilinmese tam olarak açık değilse de kan-beyin bariyerinin (KBB) bozulması bir neden olabilir. Bu çalışmada streptozosin ile diyabetik yapılan ratlarda kafa travması sonrası KBB'deki değişiklikleri deneysel olarak araştırdık.
Yöntem: Otuziki erişkin erkek Sprague-Dawley rat randomize ve eşit olarak dört gruba ayrıldı: sadece travma, sadece diyabet, diyabet+travma ve sham grubu. Diyabet oluşumu tek doz streptozotosin enjeksiyonu ile sağlandı. Diyabetin kronik değişikliklerinin oturması amacıyla travma, streptozosin enjeksiyonundan dört hafta sonra uygulandı. Travma sonrası KBB devamlılığını değerlendirmek için Evans mavisi boyasına (EMB) geçirgenlik değerlendirildi. EMB ile perfüze edilen beyinler anatomik olarak altı farklı beyin bölgesine ayrıldı (korteks, hipokampus, korpus striatum, midbrain, thalamus ve serebellum) ve EMB ekstravazasyonu hasardan 60 dk. sonra spektrofotometrik olarak ölçüldü.
Bulgular: EMB'nin spektrofotometrik ölçümleri diyabet+travma grubunda diğer gruplara göre özellikle serebellum ve korpus striatum bölgelerinde KBB geçirgenliğinin arttığını göstermiştir.
Sonuç: Travmatik beyin hasarı sonrası KBB'de olan bozulma normallere gore diyabetiklerde daha fazladır ve KBB'de bölgesel olarak bozulma ve travma-kaynaklı mikrovasküler hasar serebellum ve korpus striatum bölgelerinde daha ön plana çıkmaktadır.

Anahtar kelimeler: Kan-beyin bariyeri, beyin ödemi; diyabetik rat; streptozotosin; travmatik beyin hasarı

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Diabetes mellitus (DM) is a chronic progressive disease that often results in vascular complications, including development of microangiopathy [11,29]. This metabolic disorder adversely affects the central nervous system (CNS) [27] and leads to increased incidences of vascular dementia, ventricular hypertrophy, lacunar infarcts, and hemorrhages [3,7,17,28].

Although cerebrovascular accidents are among the primary causes of mortality in patients with DM [33] and diabetes-related cognitive dysfunction has long been recognized [20], only a few studies have investigated the effects of diabetes on the vasculature of the CNS. Microvascular complications of diabetes mellitus include diminished perfusion, abnormal endothelial proliferation, and increased permeability [16,33]. The underlying mechanism is not clearly understood; one of the potential causes may be alteration of the blood-brain barrier (BBB) [22].

The BBB is situated at the level of the endothelial cells and serves to partition the systemic circulation from the brain parenchyma [1]. Its characteristics confer distinct properties that differentiate the BBB from peripheral capillaries [13]. Although diabetes is associated with an increased risk of neurodegeneration [26] and dementia [5], the specific effects of diabetes on the BBB remain controversial. Diabetes has been associated with increased BBB permeability in some studies [12,15,23,30], whereas other studies have indicated that the BBB remained intact in diabetes [8,10,25]. A recent well-designed study demonstrated that streptozotocin-induced diabetes progressively increases BBB permeability in a region-specific manner [14].

Another issue is the effect of head trauma on the BBB in diabetic subjects. Experimental studies have demonstrated that closed head injury is associated with a rapid, transient opening of BBB that begins at the time of the trauma and lasts no more than 30 min in non-diabetic subjects [4]. The morbidity and mortality rates after traumatic brain injury are higher in diabetic patients than in normal subjects. An experimental study demonstrated that diabetic rats experience chronic oxidative stress and suffer greater neurological dysfunction, associated with further lipid peroxidation, following closed head injury [9]. The alterations in the BBB may contribute to the poor outcome scores after closed head trauma in diabetic patients. However, the alteration in the BBB after head trauma has not been evaluated effectively in diabetic subjects. To our best knowledge, no experimental study has examined the changes in the BBB after head trauma in diabetics. Therefore, we experimentally investigated the alterations in the BBB after traumatic brain injury in streptozotocin-induced diabetic rats.

MATERIALS and METHODS

All of the procedures were performed according to the accepted standards of the Guide for the Care and Use of Laboratory Animals. The study was approved by the Animal Research Ethics Committee of our medical faculty. Thirty-two adult male Sprague-Dawley rats weighing between 300 and 350 g were used. The rats were divided randomly and evenly into four groups: trauma only (T; n=8), diabetes only (D; n=8), diabetes plus trauma (DT; n=8), and sham-operated control (C; n=8). Diabetes was induced by a single intraperitoneal injection of strepto-zotocin (Sigma Chemical, St. Louis, MO, USA) at a dose of 50 mg/kg body weight. Rats were classified as diabetic if their fasting blood glucose (FBG) levels exceeded 250 mg/dl, and only animals with FBGs of > 250 mg/dl were included in the diabetic groups (both diabetes only and diabetes plus trauma). The rats were kept alive 4 weeks after streptozotocin injection to allow development of chronic diabetes before they were exposed to trauma.

All of the surgical procedures were performed under general anesthesia induced by intraperitoneal administration of ketamine HCl (50 mg/kg), with maintenance doses as needed. The rats were endotracheally intubated and mechanically ventilated with room air (Harvard rodent ventilator model 683; Harvard Apparatus, South Natick, MA). Trauma was then applied using impact acceleration model of Marmarou et al., the details of which are explained elsewhere [19]. Briefly, after general anesthesia and endotracheal intubation, the scalp of the animal was shaved, a midline incision was made, and the periosteum covering the vertex was reflected. A metallic disk was fixed to the central portion of the skull vault of the rat between the coronal and lambdoid sutures. The animal was then placed in a prone position on a foam bed with a known spring constant, and a 450-g weight from a predetermined height of 2 m was dropped (on the animal). Rebound impact was prevented by sliding the foam bed away from under the tube immediately. The animals were ventilated mechanically during the procedure. After the termination of the procedure, the incision site was sutured. The animals were then extubated, returned to their normal environment, and their access to food and water were allowed. Permeability to Evans blue dye (EBD) was evaluated to assess the BBB integrity using the method described by Chan et al. [6].

Spectrophotometric measurement of EBD

Sixty minutes after the trauma procedure, EBD (4 mL/kg in 2 % saline) was administered via the tail vein and allowed to circulate for 60 mins. To remove the intravascular dye, the animals were perfused with saline through the left ventricle until colorless fluid was obtained from the right atrium. After this washout, the rats were decapitated, their brains were removed, and the distribution of EBD was assessed using the spectrophotometric method described by Chan et al. [6]. The perfused brains were divided into six anatomically distinct regions: cortex, hippocampus, corpus striatum, midbrain, thalamus, and cerebellum. The wet tissue samples taken from each brain region were weighed on preweighed aluminum foil, homog enized with five volumes of 50 % trichloroacetic acid, and centrifuged at 15,000 rpm for 20 minutes. The absorbance was measured at 615 nm. Concentrations of Evans blue dye were calculated in micrograms of dye per gram of wet tissue.

Macroscopic evaluation of alterations in BBB permeability

The remaining brains were placed in 10 % formol. Coronal sections of the brains were made and examined for the extent and intensity of staining due to the extravasation of EBD, which is bound to serum albumin and does not cross the intact BBB. The barrier opening to EBD was graded from 0 (no staining) to +2 (dark blue staining).

Statistical analysis

The group means were compared using one-way analysis of variance (ANOVA). The Tukey honestly significant difference (HSD) post-hoc test was used for comparisons within groups. The results were analyzed using the SPSS for Windows statistical package (SPSS/PC software, Chicago, IL), and $p<0.05$ was accepted as statistically significant.

Results

Spectrophotometric measurement of EBD

One-way ANOVA showed that the spectrophotometry scores of EBD extravasation in various brain regions differed significantly among five groups, (in the cortex, hippocampus, thalamus, cerebellum, and corpus striatum ($p<0.05$, $p<0.05$, $p<0.01$, $p<0.01$, and $p<0.01$, respec-

Table 1: EBD extravasations into brain tissue samples in groups*.

Region	Controls	Diabetes	Trauma	Diabetes+ Trauma
Cortex	0.250±0.11	0.580±0.05	0.328±0.02	0.365±0.14
Hippocampus	0.225±0.04	0.300±0.12	0.503±0.04	0.363±0.35
Thalamus	0.315±0.07	0.268±0.09	0.303±0.11	0.193±0.03
Midbrain	0.305±0.11	0.383±0.04	0.354±0.11	0.389±0.24
Cerebellum	0.220±0.14	0.490±0.25	0.260±0.97	0.530±0.3
Corpus striatum	0.250±0.03	0.460±0.13	0.478±0.20	0.905±0.56

** Data are presented as mean (± SD) µg extravasation per g of brain tissue; EBD: Evans blue dye.*

tively; Table 1).

Cortex

The scores for group D were significantly higher than those for groups DT, T, and C ($p<0.001$; Figure 1a). Moreover, group DT had a significantly higher score than group C ($p<0.02$). Although the scores of group DT were higher than those of group T, the results did not reach statistical significance.

Hippocampus

The trauma group had significantly higher scores than controls ($p<0.03$; Figure 1b). The scores of group DT did not differ from those of groups T and D.

Thalamus

The scores for group DT were significantly lower than those of groups T ($p<0.03$) and C ($p<0.01$; Figure 1c).

Midbrain

The scores of group DT were significantly higher than those of group C ($p=0.05$; Figure 1d).

Cerebellum

The scores of group DT were significantly higher than those for groups T and C ($p<0.05$ and $p<0.02$, respectively). In addition, group D had significantly higher scores than group C ($p<0.05$; Figure 1e).

Corpus striatum

The scores for group DT were significantly higher than those for all other groups (Figure 1f).

Macroscopic evaluation

The macroscopic scores did not differ between the trauma only and diabetes only groups ($p=0.626$). The scores of the trauma only and diabetes plus trauma groups were also similar ($p=0.164$). The macroscopic scores of the diabetes only group were significantly higher than those of the diabetes plus trauma group ($p=0.05$).

DISCUSSION

Our results suggest that diabetes produces a progressive increase in BBB permeability in cases with traumatic brain injury and these changes are region specific mainly affecting cerebellum and corpus striatum, In the trauma and diabetes group BBB permeability. in the cerebellum and corpus striatum increased to a greater extent than trauma alone group. Moreover, in the cortex, diabetes itself caused greater disruption of BBB than did trauma alone.

The intact BBB allows negligible transport of albumin into the brain and serves as a physical barrier to partition the systemic circulation from the brain parenchyma. Consequently, albumin extravasation verified via EBD leakage was used to quantify the impairment in BBB integrity. Although recent reports favor the measurement of inulin or sucrose in addition to albumin [14], the assessment of albumin extravasation is frequently used to measure vascular leakage [31,32].

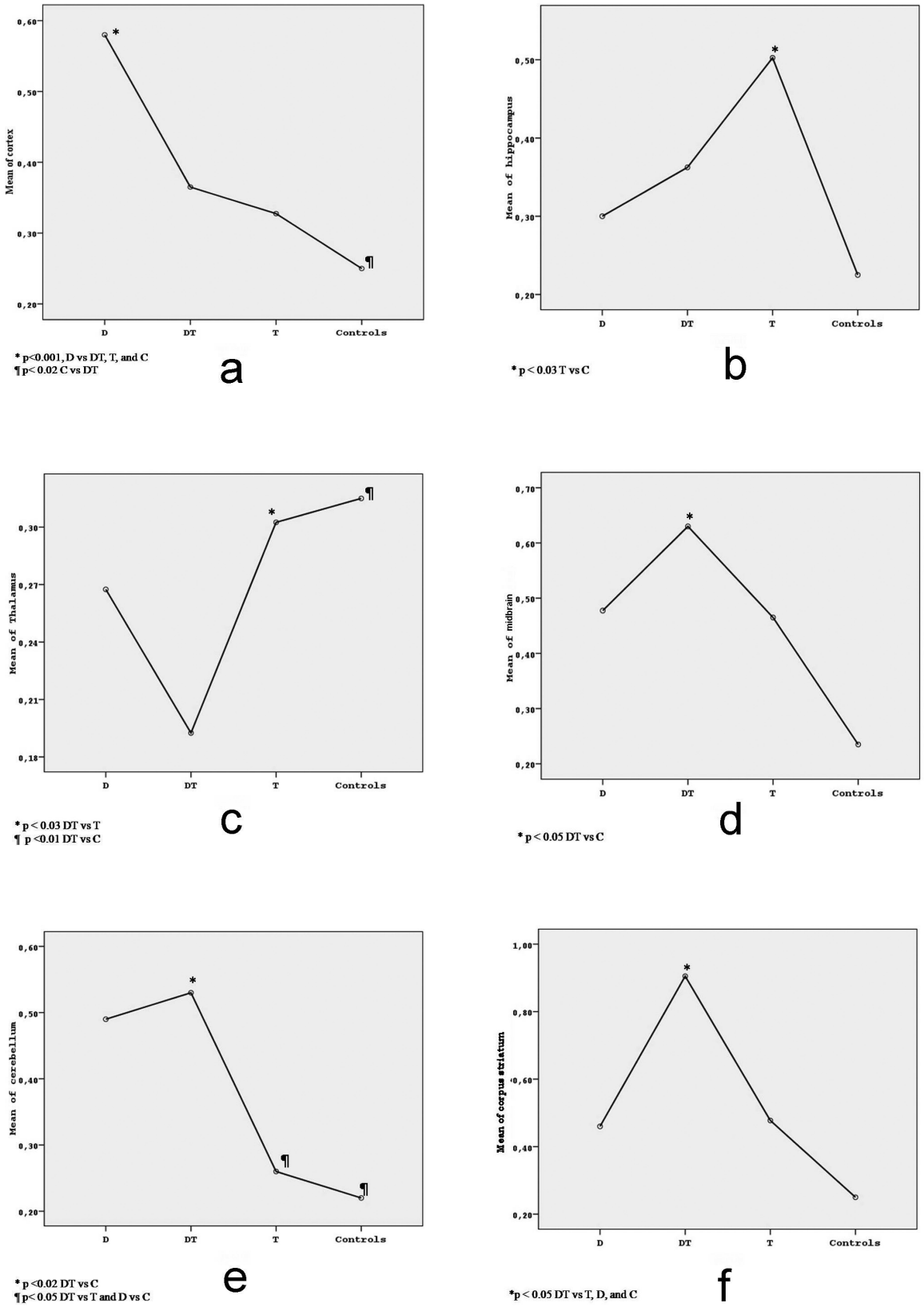

Figure 1: Mean (± SD) scores of groups in (a) cortex, (b) hippocampus, (c) thalamus, (d) midbrain, (e) cerebellum, (f) corpus striatum. (Abbreviations: D:Diabetes; DT:Diabetes+Trauma; T:Trauma; C:Control)

The quantitative measurement of EBD showed that albumin extravasation was significantly higher in the cortex, midbrain, cerebellum, and corpus striatum of injured diabetic rats when compared to controls. This shows that diffuse trauma significantly increases BBB permeability to albumin in these regions in diabetes. When the trauma only group was compared to controls, no difference was found in the BBB permeability in the abovementioned brain regions. Therefore, it is possible that traumatic brain injury causes a significant breakdown of the BBB in the cortex, midbrain, cerebellum, and corpus striatum of diabetic rats, and that these brain regions are significantly more vulnerable to traumatic brain injury in diabetics than in nondiabetics. In the thalamus, the increase in BBB permeability was lower in the diabetes plus trauma group than in the other groups. Conversely, in the corpus striatum, the degree of BBB breakdown was greater in the diabetes plus trauma group than in both diabetes and trauma groups. There is no immediate explanation for these findings.

A variety of pathogenetic mechanisms contribute to CNS dysfunction in diabetic patients. One major contributing factor is the diabetes-related alterations- in the function of the BBB. These alterations can be found in both the barrier and transport components of the BBB functioniality and can be attributed to changes in the physicochemical properties of both endothelial cell membranes and also the tight junctions of the cerebral microvasculature. P-glycoprotein (P-gp) is localized mainly in the apical membrane of cerebral microvascular endothelial cells. It transports substrates toward the blood compartment, limiting the penetration of substances into the brain and modulating their effectiveness and CNS toxicity [24]. However, cyclosporine A (a substrate for P-gp across the BBB) transport to the brain is decreased in streptozotocin-induced diabetic rats. This decreased transport was associated with increased levels of P-gp mRNA and protein in the rat brain [18]. Our observation that BBB permeability in the thalamus was lower in the diabetes plus trauma group than in the other groups may be explained by the regional functional induction of P-gp in the BBB.

Structural changes in cerebral microvessels may also account for some of the observed changes. Some investigators reported increased capillary basement membrane thickening in long-term STZ-induced diabetic animals [2,21]. Other possible mechanisms include changes in biophysical properties and the biochemical composition of endothelial cells, including alterations in lipid fluidity, composition, and neurotransmitter activity in the cerebral microvessels [21]. Iwata et al. [15] reported an unusual homogeneous high-intensity area in the corpus striatum on T1-weighted magnetic resonance imaging of a patient with poorly controlled diabetes. They concluded that this finding was caused by destruction of the BBB resulting from the hyperosmotic state secondary to hyperglycemia. This observation concurs with our finding that the greatest increase in BBB permeability is in the corpus striatum in the diabetes plus trauma group.

Global macroscopic evaluation of the alterations in BBB permeability revealed no significant differences between the groups. This result gives further support to the hypothesis that the impairment in BBB integrity was region specific rather than global. This finding concurs with the results of Huber et al. [14] who stated that there were no differences in BBB permeability between diabetic rats and controls with regard to the entire brain. However, Huber et al. [14] examined diabetic rats and did not include trauma groups. Therefore, our results provide new information about the effect of trauma in diabetic rats.

Whereas total albumin extravasation remained

unchanged, on closer evaluation of brain regions, we noted a significant increase in the concentration of Evans blue stained albumin in the cerebellum and corpus striatum of the diabetes plus trauma group. In a study of diabetic rats only, Huber et al. [14] demonstrated that the microvascular changes were region specific and the major changes occurred in the midbrain. Our results also indicate that changes in the BBB induced by diabetes, trauma, and diabetes plus trauma were region specific.The disruption in the BBB caused by traumatic brain injury is greater in diabetics than normal subjects. This deterioration in BBB functions is region specific, with the cerebellum and corpus striatum appearing to be the most susceptible region to trauma-induced microvascular damage in diabetic rats. In conclusion, the findings in the present study may stimulate new investigations to determine the mechanisms underlying the effect of trauma on BBB functions in diabetics

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