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Research Article

Effects of traumatic brain injury on vascular response and fracture healing: an experimental study in a rat model

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ABSTRACT

Objective: This study aimed to investigate the effects of traumatic brain injury (TBI) on vascular response and fracture healing during recovery.

Methods: In this experimental animal study, a total of 63 male Wistar albino rats (200-250 g) were randomly assigned to 3 groups: TBI with tibia fracture (TBI+Fx, n=21), tibia fracture only (Fx only, n=21), and a control group (n=21). Traumatic brain injury was induced in the motor cortex using a controlled impact device, followed by the tibia fracture. The severity of TBI was assessed using rotarod tests. Blood samples were collected on days 1, 7, and 21 post-fracture, while brain and tibia samples were taken on day 21 following decapitation. Levels of antidiuretic hormone (ADH) and angiotensin 1-7 (Ang 1-7) were quantified using Enzyme-linked immunosorbent assays (ELISA). Fracture healing was assessed through micro-CT and histopathological analysis. Aortic segments were evaluated for contractile response and relaxation in isolated organ baths.

Results: Micro-CT analysis revealed significantly greater bone volume (BV) (P=.02) and trabecular number (P=.038) in the TBI+Fx group. Histopathological healing scores were also significantly higher in the TBI+Fx group compared to the Fx only group (P=.019). Potassium chloride (KCl) induced contractile responses were greater in the Fx only group than in the TBI+Fx group (P < .05). Acetylcholine (ACh) induced relaxation was diminished in both Fx and TBI+Fx groups compared to controls (P < .01), whereas sodium nitroprusside (SNP)-induced relaxation was significantly greater in the TBI+Fx group than in the Fx only and control groups (P < .05). On day 21, arginine vasopressin (AVP) levels were significantly higher in the Fx only group compared to the TBI+Fx group (P=.034), with no significant differences observed on days 1 and 7. Plasma Ang 1-7 levels were significantly elevated in the Fx only group on day 21 compared to the TBI+Fx group (P < .05).

Conclusion: Traumatic brain injury was associated with accelerated fracture healing, as evidenced by increased BV, trabecular thickness, and histopathological healing scores. Additionally, TBI appeared to modulate vascular function, possibly via mechanisms involving nitric oxide and calcium signaling. These findings suggest that neuroendocrine changes following TBI may enhance fracture healing, offering potential clinical insights for managing polytrauma patients.

Level of Evidence: N/A

Introduction

Long bone fractures and closed head trauma are increasingly common in individuals who have sustained high-energy trauma.[1] A notable association between traumatic brain injury (TBI) and increased bone formation potential has been identified, as demonstrated by Perkins, who observed increased callus formation and accelerated healing time in patients with concomitant long bone fractures and TBI.[2] However, the complex and varied patterns of injury, coupled with the multiple levels of severity often seen in polytrauma patients, present challenges in understanding TBI's full impact on bone metabolism. In addition, the paucity of clinical trials; the lack of relevant animal models; and the yet-unidentified biochemical, endocrine, and neuronal pathways involved in bone healing in the midst of TBI further complicate the understanding of this phenomenon.[3]

The etiology of hypothalamic-pituitary axis dysfunction after TBI has been attributed to direct mechanical effects, acceleration-deceleration effects typical of motor vehicle accidents, or consequences of brain trauma.[4] Cyran first reported pituitary damage following TBI in 1918.[5] While posterior pituitary damage often results in permanent diabetes insipidus (DI), characterized by the release of pre-synthesized antidiuretic hormone (ADH) into the peripheral circulation, a triphasic response following transection of the pituitary stalk has been described, although rarely observed in clinical practice.[6] Although acute post-traumatic episodes of DI are typically transient, it is recognized that a partial deficiency of arginine vasopressin (AVP) may be overlooked due to the milder nature of the symptoms and the complicating neurological and cognitive disabilities.[7]

Recent research has highlighted the involvement of microRNA, extracellular vesicle discharge, and the

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hippocampus in bone healing after TBI.[8-11] Osteoblasts and osteoclasts express the angiotensin-converting enzyme 2/Mas receptor (ACE2/MasR). Activation of the ACE2/Ang-(1-7)/MasR axis results in the stimulation of osteoblasts and the inhibition of osteoclasts. Activation of the ACE2/Ang-(1-7)/MasR axis reduces dysbiosis-induced bone resorption.[12] However, the role of angiotensin 1-7 in TBI with long bone fractures is still not well understood. The recovery of vascular structures, especially the blood-brain barrier, has been studied before.[13] However, the peripheral vascular response to TBI and trauma has not been the subject of in-depth investigation. It was hypothesized that TBI would alter the recovery course for both the fracture and the vasculature. Therefore, this study's primary aim is to investigate TBI's effects on serum and brain levels of ADH and angiotensin 1-7. In contrast to previous studies that have focused solely on TBI's effect on fracture healing, as a secondary purpose, the research also aims to evaluate TBI's consequences for the vascular response.

Materials and methods

Animals

Male Wistar albino rats weighing 200-250 g were obtained from [Pamukkale University Center for Laboratory Animals] with ethical approval from Pamukkale University Ethical Committee of Animal Research (Approval No: PAUHADYEK 2017/15 Date: 03.08.2017). A total of 20 rats were randomly divided into 3 groups, with 3 rats assigned to the control group, 10 rats to the TBI and tibia fracture (TBI+Fx) group, and 7 rats to the tibia fracture-only (Fx only) group. The asymmetric distribution of rats was used to reduce potential losses in the TBI group during the experiment. Rats were housed under controlled conditions with a 12-hour light/darkess cycle, temperature maintained at 20 ± 2°C, and ad libitum access to food and water. Prior to the experiment, animals underwent a 1-week acclimatization period to minimize stress. All procedures adhered to Institutional Animal Care and Use Committee guidelines and followed Animal Research: Reporting of In Vivo Experiments (ARRIVE) reporting guidelines.[14]

Surgical technique

Surgical anesthesia was induced by ketamine (90 mg/kg body weight) and xylazine (10 mg/kg body weight) administered intraperitoneally (ip). Their right hind legs were shaved and sterilized. A longitudinal midline incision was made by scalpel in the rats' right anterior cruris. The tibia was exposed, and an open fracture model was made at the proximal 2/5 of the tibia; from the fracture line, an intramedullary 0.8 mm k-wire was placed (Figure 1A). Then, the skin was sutured with monofilament suture material.

Traumatic brain injury model

The TBI model was created after a tibia fracture. Using aseptic techniques, after securing the animal to a stereotaxic device, a midline scalp incision was made, and the skin and fascia were reflected to expose the skull. A craniotomy was made in the right hemisphere involving bregma and lambda and between the sagittal suture and the coronal crest using a microdrill and elevation of a 5 × 5 mm bone window. The TBI was produced as previously described in the literature.[15] A standardized TBI was induced using an impacter.[16] Briefly, the impacter shaft was extended, and the impactor tip was centered and lowered over the craniotomy site until it touched the dura mater. Special care was taken to leave the dura intact. The rod was then retracted and the impact tip advanced further to produce a brain injury of moderate severity for the rats (tip diameter, 4 mm; cortical contusion depth, 3 mm; impact velocity, 1.5 m/s) (Figure 1B). Clinically, all brain-injured rats developed apnea with a decrease in respiratory rate immediately after the injury, and then their respiration gradually returned to normal. At the end of the treatment, the scalp was sutured with monofilament suture material, and the rats were placed on a heating pad until recovery from anesthesia.

HIGHLIGHTS

- The courses of vascular response and fracture healing are influenced by traumatic brain injury (TBI) during recovery.
- TBI may enhance fracture healing processes, possibly influenced by neuroendocrine changes.
- The observed vascular responses may be related to changes in nitric oxide synthesis and calcium signaling after TBI.
- Serum levels of angiotensin 1-7 (Ang 1-7) may increase after trauma.

Rotarod test

To determine the severity of head trauma, rotarod tests were performed. The test protocol was adapted from Khan et al[15] (2009) and was used to assess locomotor movement. The apparatus has a drop height of 23 cm and a rotating bar diameter of 5 cm. It has an adjustable speed between 0 and 80 and is equipped with infrared switches that determine the rat's rate of fall. The bar is divided into 4 equal compartments with opaque round dividers at 10 cm intervals (MAY RR 0711, Commat Ltd.). Experiments were started after the rats were positioned on the apparatus with their heads facing forward and away from the experimenter. As the rats moved forward on the rotating bars, the total duration (latency) of the rat falling to the bottom of the apparatus was automatically recorded. The test started at a steady speed of 5 rpm to allow the rats to get used to the apparatus on the first day. Later, the test was performed for 300 seconds at an increasing speed of 0-40 rpm for 3 trials with a 10-minute intertrial interval. All procedures were performed between 11:00 a.m. and 2:00 p.m. Rats were tested for 7 consecutive days. Rats were selected for inclusion in the study protocol on the basis of their average time spent on the rotating bar for at least 60 seconds across all days of testing. The rats were operated on the following day. At 3, 7, and 14 days postoperatively, the test was repeated and all data were recorded. The pre- and postoperative times were compared for each rat. Performance on the postoperative test was compared between groups.

Collection of specimens

Blood was taken from the tail vein on the first and seventh postoperative days using a 15 mL/min catheter (Mediflon, Global Ltd.). On Day 21, blood was collected intracardially with a 5 mL injector (Mediflon, Global Ltd.). The plasma was separated. Brain samples were collected after decapitation. Both samples were stored at −80°C for further analysis. The tibia bones were dissected together with the surrounding tissues after an anterior longitudinal incision, and the intramedullary K-wire was removed and fixed with 10% formalin (070312510; Tekkim Kimya San.Tic.Ltd., Türkiye). Formalin-embedded specimens were transferred to Ege University Central Research Test and Analysis Laboratory (Egematal) for micro-CT analysis.

Micro-CT analysis

The tibia specimens were placed in a standard sample holder of appropriate size and screened with a micro-CT device (Scanco Medical μCT 50, Switzerland). The scanning parameters were determined as 70 kVp energy, 114 μA intensity, 10 μm voxel size,

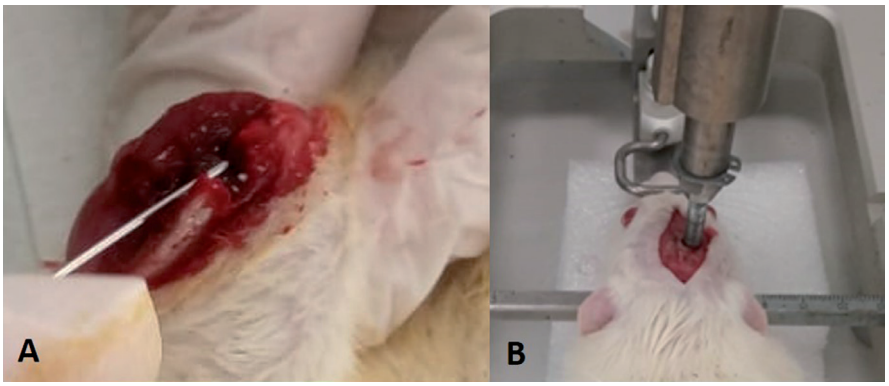

Figure 1. (A) Tibial intramedullary nailing; (B) craniotomy and standardized traumatic brain injury model.

and 600 ms integration time. As the filter, 0.1 mm Al was used. 2D section images were obtained, and 3D analyses were processed with the μCT Evaluation Program (V6.5, Scanco Medical, Switzerland). The micro-CT analysis results were evaluated according to Bouxsein et al.[17] Parameters such as bone volume (BV, mm^3), BV fraction (BV/TV, %), apparent density (mg HA/ccm), trabecular number (Tb. N, mm^{-1}), trabecular thickness (Tb. Th, μm), and trabecular space (Tb. Sp, μm) were evaluated in the impacted and non-impacted groups.

Histopathological assessment

After sacrificing the rats on the 21st day, the tibiae were excised together with the surrounding tissue and fixed with 10% formalin (070312510; Tekkim Kimya San.Tic.Ltd., Türkiye). They were decalcified with a 10% formic acid solution (K43640264227, Merck, Germany) for 10 days. The tibiae were then washed in running water and dehydrated in a series of alcohols. The tissue was cleared in xylene and then embedded in paraffin. The specimens were sectioned transversely and longitudinally. Sections were cut at 5 μm using a Leica RM-2125 rotary microtome (Leica, Wetzlar, Germany) and mounted on lysine-coated glass slides (23573; Marienfeld Laboratory Glassware, Histobond, Marienfeld, Germany). Sections were deparaffinized in xylene, rehydrated through graded ethanols, and stained with hematoxylin and eosin (Merck, Germany). A numerical grading scheme was used for the histological assessment of fracture healing (Table 1).[18]

ELISA

Blood samples collected from the rats 1, 7, and 21 days post-TBI were centrifuged (1000 × g, 20 min), and serum samples were aliquoted and stored at −80°C. Commercially available ELISA kits for antidiuretic hormone (Fine Test, China) and angiotensin 1-7 (Fine Test, China; SunRed, China) were used to analyze plasma and right striatum homogenates according to the manufacturer's protocol. Briefly, standards and biotin-labeled working solution were prepared. Standards and diluted (1:10) samples were added to 100 μL wells and incubated at 37°C for 90 minutes. After 2 washes, biotin-labeled antibody working solution was added to all wells and incubated at 37°C for 60 minutes. After washing the plate 3 times, 100 μL of Strept Avidin-Biotin Complex (SABC) solution was added to each well and incubated at 37°C for 30 minutes. After the addition of Tetramethylbenzidine(TMB) substrate (90 μL) and finally stop solution (50 μL), OD values were recorded at a wavelength of 450 nm using a microplate reader (Biotek-ELX 800). The standard curve was constructed using Curve Expert 1.3 software, and the concentrations in the samples were calculated.

Table 1. Numerical scoring scheme used for the histologic evaluation of fracture healing

Score	Associated finding at fracture site
1	Fibrous tissue
2	Predominantly fibrous tissue with small amount of cartilage
3	Equal mixture of fibrous and cartilaginous tissue
4	Predominantly cartilage with small amount of fibrous tissue
5	Cartilage
6	Predominantly cartilage with small amount of immature bone
7	Equal mixture of cartilage and immature bone
8	Predominantly immature bone with small amount of cartilage
9	Union of fracture by immature bone
10	Union of fracture fragments by mature bone

Aortic segment preparations

On Day 21, under deep anesthesia, the thoracic aorta was dissected free of connective tissue and segmented into transverse rings 4-5 mm in length. Denuded aortic specimens were obtained by gently rubbing the intimal surface with forceps. Under 1.0 g resting tension, the rings were mounted in 20 mL organ chambers filled with Krebs-Henseleit solution of the following composition (mmol/L): NaCl (sodium chloride) 118.30, KCl (potassium chloride) 4.70, magnesium sulfate ($MgSO_4$) 1.20, monopotassium phosphate (KH_2PO4) 1.22, calcium chloride ($CaCl_2$) 2.50, sodium bicarbonate ($NaHCO_3$) 25.00, glucose 11.10, pH 7, at 37°C and bubbled with 95% O_2/5% CO_2. Paired rings with and without endothelium were mounted in parallel in each experiment. Each aortic ring was connected to a force-displacement transducer (FDT 10-A) for isometric tension recordings, and data were recorded using an 8-channel transducer data acquisition system (GTA0303 General Transducer Commat, Ankara, Türkiye) and software (MP 150 version 3.9.1.6 - Biopac Systems Inc.). A single dose of Ach (acetylcholine) (10^{-6} M) was administered to test endothelial denudation in denuded aortic segments, and relaxation of less than 20% indicated adequate endothelial denudation.[19]

Isolated organ bath studies

After 15 minutes of equilibration, each aortic segment was gradually stretched and finally equilibrated at a resting tension of 2.0 g, washed every 20 minutes before drug administration. Contractile responses were studied with KCl (60 mmol/L) or phenylephrine (Phe; 1×10^{-9} - 3×10^{-5} mol/L). The relaxant effects of single concentrations of ACh (1×10^{-5} mol/L) and SNP (sodium nitroprusside) (1×10^{-6} mol/L) were studied in aortic rings precontracted with submaximal phenylephrine.

Data analysis

The response of both intact and denuded aortic segments in the isolated organ bath is expressed as a percentage of the increase in tension from baseline (Phe and KCl), and the relaxation responses to ACh and SNP in Phe submaximally precontracted preparations are expressed as a percentage of the decrease in the increase in contractility. Data were analyzed by *t*-test except for Phe. Phe data were statistically evaluated by one-way analysis of variance with Tukey's post-hoc multiple comparison test using SPSS 10.0 (SPSS Inc.; Chicago, IL, USA). for Windows. *P*-values less than .05 were considered statistically significant.

Results

During the TBI procedure, 1 rat in the TBI+Fx group died. On postoperative Day 2, 1 rat in the TBI+Fx group died, and on Day 3, 1 rat in the Fx-only group died. There was no non-union in either the TBI+Fx or the Fx-only group. Three rats were classified as mild and 5 rats were classified as moderate according to their rotarod results in the TBI group based on the degree of trauma severity (Table 2).

According to micro-CT analysis, the mean BV in the TBI+Fx group was 91.8 mm^3, and in the Fx-only group was 78.8 mm^3. The mean BV/TV ratio was 0.11 in the TBI+Fx group and 0.067 in the Fx-only group, which was a significant difference (P=.02). The mean trabecular number (TbN) volume was 1.34 (1/mm) in the TBI+Fx group and 0.97 (1/mm) in the Fx-only group, another significant difference (P=.038) (Table 3).

According to the standard histopathological evaluation, the mean healing score was 6.125 in the TBI+Fx group and 3.5 in the Fx-only group, which was statistically significant (P=.019). The histopathological healing score was correlated with TV, BV/TV, and TbN (P=.003, P=.004, and P=.002, respectively) using Pearson's correlation analysis test.

Thoracic aortic responses

Contractile

KCl induced contractions in both endothelium-intact and endothelium-denuded preparations. The response obtained in denuded aortas was greater than in intact aortas. In addition, KCl depolarization was greater in the Fx-only group than in the TBI+Fx group (Figure 2).

The phenylephrine (Phe) responses of endothelium-denuded aortas were greater than those of endothelium-intact aortas. The contractile responses to Phe were reduced in the Fx only and TBI+Fx groups, but this was not statistically significant ($P \geq .05$).

Relaxation

ACh relaxation was reduced in both the TBI+Fx and Fx-only groups. While no relaxation was observed in the control group's denuded aortas, ACh-induced relaxations were below 15% in both TBI+Fx and Fx-only groups (Figure 3A).

In intact aortas, SNP relaxation was increased in the TBI+Fx and Fx-only groups. However, the effect in the TBI+Fx group was significantly higher than in the control and Fx-only groups ($P \leq .05$). On the other hand, in the denuded aortas, SNP relaxation was higher in the TBI+Fx and Fx groups than in the control group, but this was not statistically significant ($P \geq .05$) (Figure 3B).

Plasma Ang 1-7 levels

Although plasma angiotensin 1-7 (Ang 1-7) levels were 5000-6000 pg/mL in all 3 groups, on Day 21, the Fx-only group had significantly higher Ang 1-7 levels compared to the TBI+Fx-only group ($P \leq .05$) (Figure 4A).

Right striatum Ang 1-7 levels

Ang 1-7 levels were too low, below the detection limit, and could not be determined.

Plasma arginine vasopressin levels

On Day 21, AVP levels were significantly higher in the Fx-only group compared to the TBI+Fx group (P=.034) (Figure 4B).

Right striatum arginine vasopressin levels

There was no statistically significant difference between groups (P=.092) (Figure 5A and B).

Discussion

In this experimental study, the fracture healing rate was found to be higher in the TBI+Fx group than in the Fx-only group. According to the micro-CT analysis, the mean BV in the TBI+Fx group was significantly higher. The mean TbN volume was 1.34 in the TBI+Fx group and 0.97 in the Fx-only group, which was a significant difference.

According to the standard histopathological evaluation, the mean healing score was 6.125 in the TBI+Fx group and 3.5 in the Fx-only group, which was statistically significant. The histopathological healing score was correlated with TV, BV/TV, and TbN. Tsitsilonis et al[3] found that the TBI group had significantly increased callus volume as early as the second postoperative week based on micro-CT examination of fracture healing. The findings of Yang and colleagues suggest that TBI is associated with faster fracture healing and an increased rate of callus formation.[20] Boes et al[21] performed a biomechanical analysis in rats with TBI. In their study, mechanical tests performed 21 days after TBI showed that callus formation was significantly stiffer in the TBI animals than in the fracture-only animals. Ozan et al[22] evaluated the stiffness of fracture healing in a rat model using a biomechanical test, the 4-point bending

Table 2. Rotarod test results

		Mean of LATENS			
	Rat ID	**Preop Test**	**Postop_3 Test**	**Postop_7 Test**	**Postop_14 Test**
TBI+Fx	1	132.6	56.3	18	41.3
	2	32		died	
	3	98	15.3	7	27.3
	4	68	7.3	18.6	43.6
	5	103	14.6	15	24.6
	6	27	4.3	5.3	5
	7	87.6	8	17.3	17.3
	8	59.6	4.3	14.3	5.6
	9	39	7.3	died	
	10	33.3	6.3	12.6	4.6
Fx Only	Rat ID	Preop Test	Postop_3 Test	Postop_7 Test	Postop_14 Test
	1	178.3	57.6	89.6	132
	2	123.3	49	22.6	40.6
	3	130.6	20.3	24.6	26.3
	4	108.6	11	died	
	5	146.6	33	127	84.6
	6	66.6	69	40.6	41
	7	141.6	37.3	78	108.3

Table 3. Micro-CT results

	Rat ID	TV [mm³]	BV [mm³]	BV/TV	TbN [1/mm]	TbTh [µm]	TbSp [µm]
TBI+Fx	1	480.3	85.5	0.178	2.188	0.3651	0.52
	3	791.6	93.6	0.12	1.69	0.1974	0.69
	4	941.6	132.9	0.1411	2.102	0.2476	0.70
	5	1397.4	84.4	0.0604	0.920	0.2968	1.37
	6	1446.6	91.7	0.0634	0.2346	0.2827	4.78
	7	815.7	108.7	0.133	1.289	0.2428	1.03
	8	709.1	80.7	0.1139	1.318	0.262	0.86
	10	851.5	56.8	0.066	0.995	0.266	1.16
Fx only	1	1123.06	83.2	0.0741	0.3421	0.3124	3.32
	2	1144.2	84.7	0.0741	0.855	0.3379	1.39
	3	1267.1	69.3	0.0547	0.5177	0.2774	2.15
	5	1100.0	70.9	0.0645	1.282	0.2045	0.92
	6	868.5	77.5	0.0893	1.663	0.3759	0.74
	7	1849.0	87.6	0.0474	1.210	0.3372	0.84

BV, bone volume; BV/TV, bone volume fraction; TbN, trabecular number; TbSp, trabecular spaceTbTh, trabecular thickness; TV, tissue volume.

test, and a finite element model. In Ozan et al's[22] study, the biomechanical test results of mean maximum fracture load were significantly higher in the head trauma group (12 rats) compared to the control group (10 rats) (< 0.05). Most studies, including that of Ozan et al,[22] used a TBI model developed by Marmarou et al,[23] whereas in this study, a standardized TBI head trauma model was used.

Potassium chloride depolarization was greater in the Fx-only group than in the TBI+Fx group. Potassium chloride–induced depolarization causes Ca^{2+} influx into the cell and subsequent activation of calmodulin. Ca2+/calmodulin signaling regulates osteoblast differentiation.[24] The reason for the high KCl depolarization in the Fx-only group seems to be related to the entry of more calcium into the cell. The decreased Phe contractile response in the TBI+Fx and Fx-only groups compared to the control group could be interpreted as a decreased intracellular calcium concentration.

The Phe contractile response in the TBI+Fx group was not significantly higher than in the Fx-only group. Endoplasmic reticulum-mediated calcium efflux was observed to be greater in the TBI+Fx group. A decrease in the G-protein-coupled alpha-receptor-mediated contractile response to Phe in the Fx-only group and a significant increase in the KCL response compared to the other groups may indicate Ca^{2}+ desensitization. In the TBI+Fx group, the increased contractile response due to stimulation of alpha receptors with Phe and the decreased response to KCl may be due to Ca++ sensitization.[25]

In this study, ACh-induced relaxations were below 15% in both TBI+Fx and Fx-only groups. The autonomic nervous system plays a role in regulating bone blood flow. Acetylcholine acts through nicotinic and muscarinic receptors. The relaxing effect of ACh is endothelium-dependent and NO-mediated. Bone biomechanics, synthesis, and trabecular formation are increased, and bone resorption is decreased by stimulation of the muscarinic mAChR subtype M3.[26] Although endothelium-dependent ACh responses were reduced in both groups in comparison to the control group, these responses were significantly preserved in the TBI+Fx group in comparison to the Fx-only group. Liu et al[27] reported that bone marrow sympathetic tone was increased after TBI. They also found accelerated fracture healing rates after TBI.

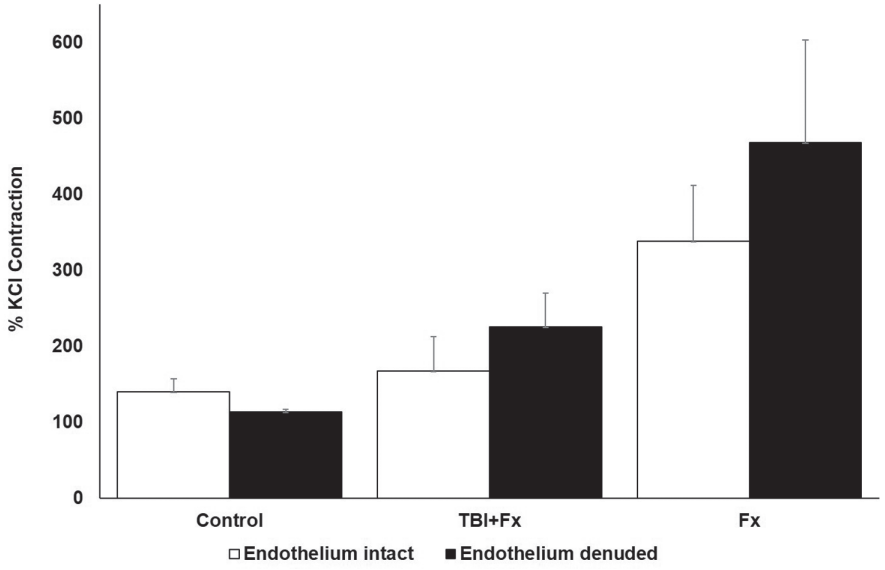

Figure 2. % KCl contraction. **P* < .01 Fx vs. Control, **P* < .05 Fx vs. TBI+Fx in endothelium-intact aorta. □*P* < .05 TBI+Fx vs. Control and ŀ*P* < .01 Fx vs. Control, ŀ*P* < .05 Fx vs. TBI+Fx in endothelium-denuded aorta.

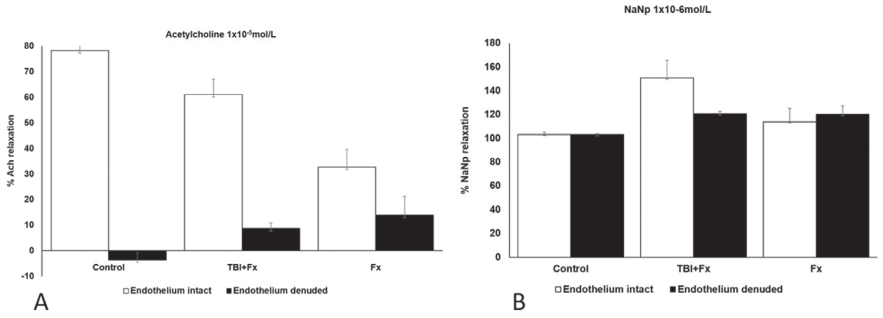

Figure 3. (A) % ACh relaxation. **P* < .001 Fx vs. Control, **P* < .01 Fx vs. TBI+Fx, □*P* < .05 TBI+Fx vs. Control in endothelium-intact aorta. □*P* < .002 TBI+Fx vs. Control and Ⱨ*P* < .01 Fx vs. Control in endothelium-denuded aorta. (B) % NaNp relaxation. **P* < .05 TBI+Fx vs. Fx, □*P* < .005 TBI+Fx vs. Control in endothelium-intact aorta. □*P* < .001 TBI+Fx vs. Control and Ⱨ*P* < .01 Fx vs. Control in endothelium-denuded aorta.

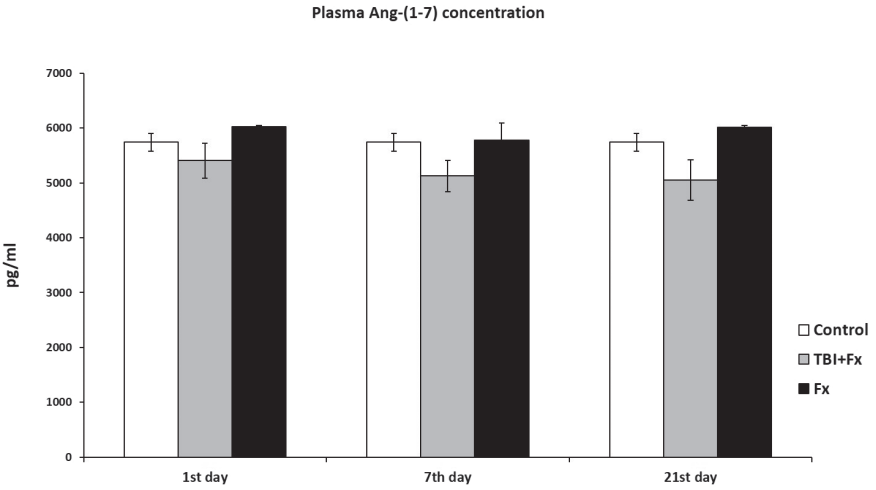

Figure 4. Plasma Ang 1-7 concentration. **P* < .05 TBI+Fx vs. Fx.

In intact aortas, SNP relaxation was found to increase in the TBI+Fx and Fx-only groups. However, the effect in the TBI+Fx group was significantly higher than in the control and Fx-only groups. Sodium nitroprusside releases nitric oxide (NO) by binding to oxyhemoglobin. Nitric oxide activates vascular smooth muscle guanylate cyclase and increases intracellular cGMP production. The effect of SNP is independent of the endothelium. In TBI with long bone fracture, the effect of SNP was significantly higher than in the control group in both intact and endothelium-denuded aortas. The NO pathway has effects at both the vascular and cellular levels.[28] Nitric oxide is an important mediator in the regulation of cerebral blood flow. It has been reported that endothelial NO synthase (eNOS)-derived NO is neuroprotective after acquired brain injury. In addition, eNOS appears to play a key role in the regulation of osteoblast activity and bone formation, promoting bone formation and suppressing bone resorption.[29]

As micro-CT analysis revealed, the mean BV was significantly higher in the TBI+Fx group than in the Fx-only group. Based on the increased SNP relaxation in the TBI+Fx group, the balance between eNOS and iNOS may play a role in bone formation in neuroprotection. This balance could explain the increased bone formation in the TBI+Fx group. The renin-angiotensin system also has anti-inflammatory properties, in addition to its classical properties. ACE2/MasR is expressed in osteoblasts and osteoclasts. It has been shown to stimulate osteoblasts and inhibit osteoclasts. The ACE2/Ang 1-7/Mas receptor (ACE2/Ang 1-7/MasR) axis has recently been shown to have a role in bone remodeling.[12] In this study, although the rate of healing scores in the TBI+Fx group was higher than in the Fx-only group, the plasma levels of Ang 1-7 were lower than in the Fx-only group. In this case, it is plausible that other factors besides plasma Ang 1-7 are effectively involved in bone healing. Zhang et al[30] reported that TBI patients with clavicle fractures had higher serum NGF levels

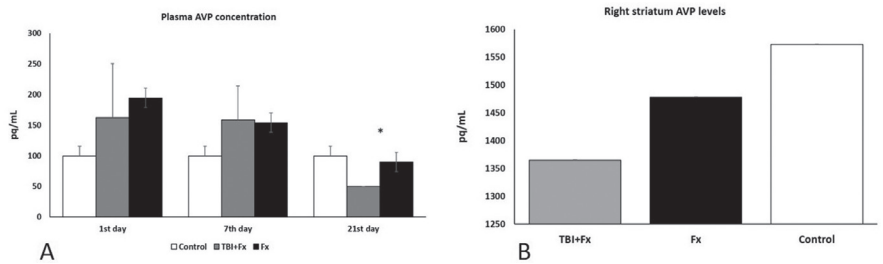

Figure 5. (A) Plasma arginine vasopressin concentration. **P* < .05 TBI+Fx vs. control and Fx. (B) Right striatum arginine vasopressin levels.

and NGF expression in callus tissue. In this study, the expressions of CD31, NGF, and VEGF were correlated with shorter fracture healing time.

On Day 21, AVP levels were significantly higher in the Fx-only group compared to the TBI+Fx group (P=.034). Kumar et al[31] demonstrated hypothalamic-pituitary dysfunction in TBI patients. A review of pituitary dysfunction has been published by Sav et al.[32] Vasopressin, also known as AVP, has a role in skeletal homeostasis. AVP receptors, Avpr1α and Avpr2, are coupled to Erk activation. They are expressed in osteoblasts and osteoclasts.[33] AVP increases the formation of bone-resorbing osteoclasts but decreases the formation of bone-forming osteoblasts. The delayed bone healing in the Fx group may be explained by the high AVP levels.

Mollahosseini et al[34] investigated cytokine levels after TBI. In their article, the elevated serum levels of BMP-2, PDGF, FGF-2, and IL-1β may be associated with accelerated healing in TBI patients with fractures. Luntzer et al[35] also investigated pro-inflammatory cytokines and found that the pro-inflammatory cytokines KC and MCP1 were elevated in TBI patients.

This study has several limitations. Firstly, the small sample size, particularly in the control group, may limit the findings' generalizability. In addition, the study was conducted in a rat model, which, although informative, may not fully replicate the complexity of human trauma and fracture healing processes. The asymmetric distribution of animals between groups was necessary to account for the expected mortality in the TBI+Fx group; however, this may have introduced bias into the interpretation of the results. This study's scope was also limited by the inability to measure certain molecular markers, such as right striatal Ang 1-7 levels, due to detection limits. Intracellular or blood levels of Na, K, and Cl were not measured.

TBI is associated with accelerated fracture healing, as evidenced by increased BV, trabecular thickness, and histopathological healing. Consistent with previous research, neuroendocrine changes associated with TBI appear to facilitate enhanced bone regeneration. In addition, TBI seems to modulate vascular function and reactivity, possibly through mechanisms involving nitric oxide and calcium signaling. The findings provide valuable insights into the potential mechanisms underlying enhanced bone healing in polytrauma patients by elucidating the complex interplay between neuroendocrine factors, vascular responses, and bone metabolism following TBI.

Data Availability Statement: The data that support the findings of this study are available upon request from the corresponding author.

Ethics Committee Approval: This study was approved by the Ethics Committee of Pamukkale University Ethical Committee of Animal Research (Approval No: PAU-HADYEK 2017/15 Date: 03.08.2017).

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