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Protective effect of TXA on human umbilical vein endothelial cell injury

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Abstract

Objective: The study aims to explore the effects of TXA on vascular endothelial cell injury at different time points, figure out the mechanism and find the best timing of TXA delivery in clinical.

Methods: An in vitro endotheliopathy of trauma model was established on primary human umbilical vein endothelial cells by treating with hydrogen peroxide. TXA was applied to EoT model at different time points to observe the effect on endothelial cell.

Results: Early TXA administration can effectively inhibit the expression of tPA, multi-ligand glycans, hyaluronic acid, TNF-α, ICAM and MMP-9 protein in endothelial cells in vitro, but not in the late administration, which indicated that early TXA administration can inhibit the cell injury induced by H2O2 on endothelial cells, and it has obvious anti-fibrinolytic effect.

Conclusion: Early administration of TXA especially within one hour is recommended to be applied to patients with acute hemorrhagic trauma in clinical.

Keyword: Endotheliopathy of Trauma (EoT); Human umbilical vein endothelial cell; TXA

Introduction

Conventional total knee arthroplasty causes a huge trauma to the body, and its blood loss is about 1~1.5 L. Postoperative patients may need blood transfusion for anemia. However, blood transfusion may induce direct risks including immune response and intravascular hemolysis, thereby seriously endangers the health of patients and increases medical costs [1]. In addition, severe trauma such as total knee arthroplasty can also lead to systemic vascular endothelial cell damage, ultimately leading to Acute Traumatic Coagulopathy (ATC), cellular inflammatory response and endothelial cell barrier dysfunction, which is called "Endotheliopathy Of Trauma (EoT)"[2]. It has been reported that as many as 1/4 to 1/3 of trauma patients have developed coagulopathy before surgery, and if EoT cannot be diagnosed or treated timely in the early phase, it would develop into multiple organ failure, and the mortality rate is extremely high. Therefore, early intervention of EoT is a key factor of improving the prognosis of patients with severe trauma.

As a synthetic anti fibrinolytic drug, TXA is widely used in clinical trauma treatment. Current clinical randomized studies showed that early administration of TXA can play a better hemostatic effect and reduce complications [3]. TXA treatment after 6 hours of trauma is often not ideal. Previous studies have shown that TXA has anti fibrinolytic and anti-inflammatory effects, but the exact mechanism of TXA remains unclear [3]. In recent years, it has been reported that TXA can protect epithelial cells through protecting the epithelial polysaccharide-protein complex

layer in the hemorrhagic shock intracavitary induced intestinal epithelial cell injury model [4]. However, the role of TXA in EoT and the timing of drug administration are still unclear. In this study, EoT model was established by applying H_2O_2 on primary human umbilical vein endothelial cells in vitro culture and explored the effect and mechanism of TXA on EoT at different time points.

Methods

Ethics Statement

The study has gotten approval from Medical Ethics Committee of Shandong Medical College Affiliated Hospital, and all participants provided written informed consent.

Cell Culture

Human Umbilical Vein Endothelial Cells (HUVECs) were provided by Inner Mongolia Medical University, and then cultured in ECM medium (Gibco, USA) containing 10% fetal bovine serum (FBS; Hyclone Laboratories, Logan, USA) and 1% penicillin–streptomycin mixture (Hyclone Laboratories, Logan, USA). Cultures were maintained in an incubator with 5% CO_2 at 37°C.

Grouping Criteria

The cell concentration was adjusted to 1×10^6/L, and 200 μl of the cell suspension was inoculated into 96-well plates and randomly divided into normal control group, H_2O_2 group, H_2O_2 +TXA (0 min) group, H_2O_2+TXA (30 min) group, H_2O_2+TXA (60 min) group and H_2O_2+TXA (90 min) group. All the latter 5 groups were added with 100 μmol/L H_2O_2 for 2 h and added TXA (150 μmol/L) at four time points (0 h, 30 min, 60 min and 90 min) after the H_2O_2 application.

Fluorescence Observation

Briefly, cells were washed with PBS three times and then fixed in 70% ethanol. After that, the fixed cells were washed twice with PBS and stained in Hoechst 33258 (Beijing Biosea Biotechnology, Beijing, P.R. China), incubated for 10 min at room temperature in the dark, and washed with PBS three times. Added 10 μl anti-fluorescence attenuating seal to the sterilized slide and then immediately subjected to the OLYMPUS inverted fluorescence microscope (Olympus, Japan).

Cell Viability Assay

Cell viability was assessed with the cell counting kit-8 (CCK-8, Boster, China) assay according to the manufacturer's guidelines. HUVECs from each group were starved in 2% FBS for 12 h. Cells were then treated with 10 μL CCK-8 solution for 3 h, and the absorbance was read at a 450-nm wavelength.

Western Blotting Assay of ICAM and MMP-9 protein expression in cells

Western blotting assay was established using Bio-Rad Bis-Tris Gel system and performed as previously described [5]. ICAM and MMP-9 protein proteins in the cells were electrophoresed on polyacrylamide gels, and the gels were transferred onto Polyvinylidene Difluoride (PDVF) membranes (Mil lipore) that were incubated with relevant antibodies. Anti-ICEM anti- MMP-9 and β-actin monoclonal antibody were purchased from Epitomics Biotechnology (Epitomics, USA). Subsequently, the membranes were incubated with horseradish peroxidase-conjugated secondary antibodies (Santa Cruz, USA) for 1 hour at room temperature. The protein signals were detected by enhanced chemiluminescence Western blotting substrate (Pierce, Thermo Fisher Scientific, USA).

ELISA Analysis

Supernatants of cell culture were harvested and the presence of tPA, PAI-1, polyligand, hyaluronic acid and TNF-α were measured by commercial ELISA kit (Kai Ji Biotechnology, China) according to the manufacturer's instructions, and then the absorbance value were obtained at 450 nm of the microplate reader (ELx808, BioTek Instruments, USA). Standard curves were prepared and then tPA, PAI-1, Polyligand, hyaluronic acid and TNF-α content values were calculated, respectively.

Statistical Analysis

Statistical analysis was performed using SPSS 16.0 software. All measurement data obtained in this study were described as mean ± standard deviation. The ANOVA variance SNK-q method was used to analyze the difference between the mean of each two groups. $P < 0.05$ was considered significantly different.

Result

Cell Viability

As shown in Figure 1, the nucleus of HUVECs in the normal control group showed uniform low-intensity fluorescence. After H_2O_2 treatment, a large number of cells showed new-moon form at the edge of chromosome, which indicated typical apoptotic cell changes, and the cell viability rate was significantly lower than that of the control group ($P < 0.001$, Figure 2). Compared with H_2O_2 group, cell viability in H_2O_2+TXA (0 min) group and H_2O_2+TXA (30 min) group were significantly higher ($P < 0.01$, Figure 2). The cell viability of H_2O_2+TXA (60 min) group and H_2O_2+TXA (90 min) group was not significantly different from that of H_2O_2 group ($P > 0.05$, Figure 2).

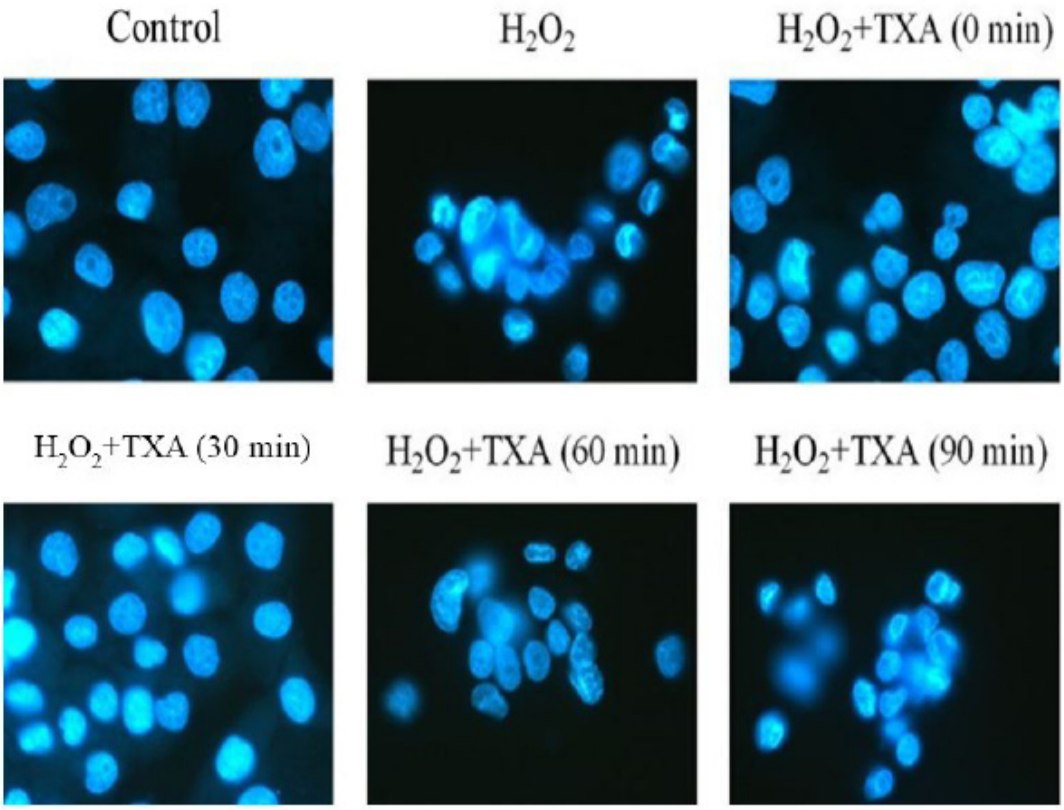

Figure 1: Cell Viability under Fluorescence Observation. HUVECs were stained by Hoechst and observed after a magnification of 400 times by fluorescence microscope. Nucleus was concentrated and dense, and the color was white.

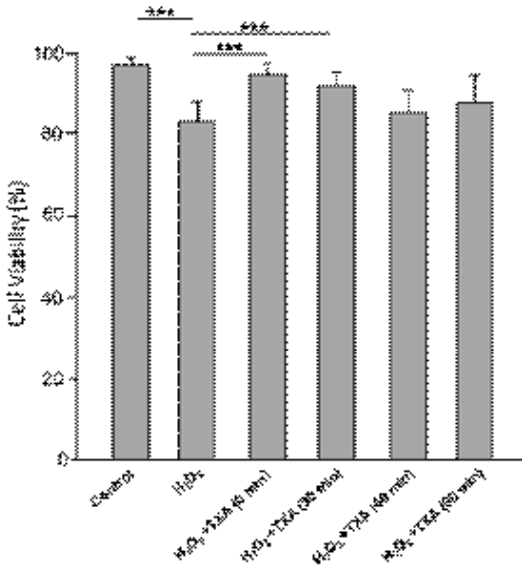

Figure 2: Cell viability test. Cell viability was detected with CCK-8. Values are the mean ± SD; ***P < 0.05.

The effect of TXA on the expression of ICAM and MMP-9 protein in HUVECs

As shown in Figure 3, Table 1 and Table 2, the expression of ICAM and MMP-9 protein were significantly up-regulated after H_2O_2 treatment ($P < 0.001$). Compared with H_2O_2 group, the expression of ICAM and MMP-9 protein in H_2O_2+ TXA (0 min) and the H_2O_2+TXA (30 min) group was significantly decreased ($P < 0.001$). There was no significant difference in the H_2O_2+TXA (60 min) group and H_2O_2+TXA (90 min) group ($P > 0.05$).

Group	ICAM protein expression
Control	1.254 ± 0.326
H_2O_2	5.847 ± 1.247***
H_2O_2+TXA (0 min)	1.365 ± 0.334###
H_2O_2+TXA (30 min)	1.371 ± 0.243###
H_2O_2+TXA (60 min)	6.388 ± 1.231
H_2O_2+TXA (90 min)	5.543 ± 1.024

Compared with control group，***$P < 0.0001$；compared with H_2O_2 group，##$P < 0.001$

Table 1: Effect of TXA on the expression of ICAM protein in HUVECs.

Group	MMP-9 protein expression
Control	0.564 ± 0.102
H_2O_2	2.394 ± 0.481***
H_2O_2+TXA (0 min)	0.787 ± 0.216###
H_2O_2+TXA (30 min)	0.812 ± 0.193###
H_2O_2+TXA (60 min)	2.155 ± 0.462
H_2O_2+TXA (90 min)	2.249 ± 0.512

Compared with control group，***$P < 0.0001$；compared with H_2O_2 group，##$P < 0.001$

Table 2: Effect of TXA on the expression of MMP-9 protein in HUVECs.

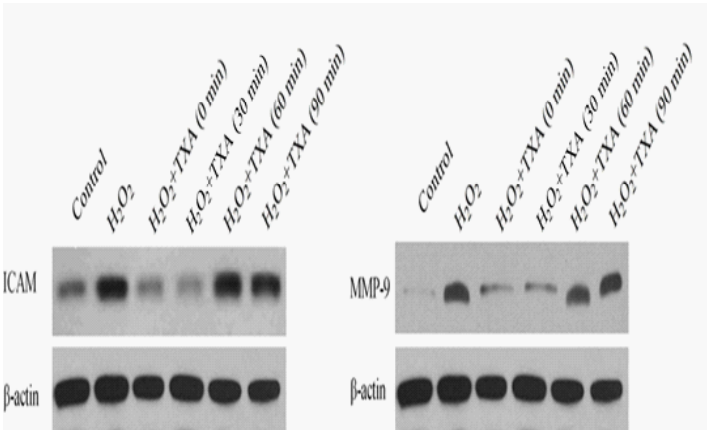

Figure 3: Western blot analysis of the effect of TXA administration on ICAM and MMP-9 protein expression in HUVECs at different time points, with β-actin as an internal reference.

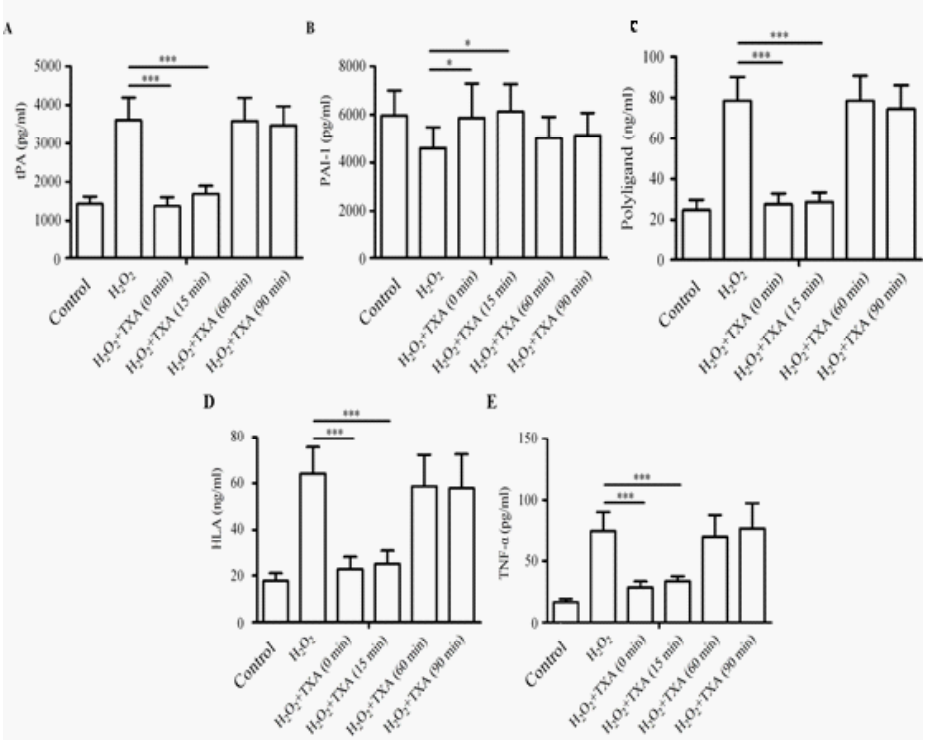

Figure 4: ELISA assay for the expression of tPA, PAI-1, polyligand, hyaluronic acid and TNF-α in HUVECs in vitro, *$P < 0.05$, ***$P < 0.001$.

Effect of TXA on the expression of tPA, PAI-1, polyligand, hyaluronic acid and TNF-α in HUVECs

As shown in Figure 4ACDE, the expression of tPA, polyligand, hyaluronic acid and TNF-α was significantly up-regulated in H_2O_2 group, compared with the control group ($P < 0.001$), while the expression in the H_2O_2+TXA (0 min) group and the H_2O_2+TXA (30 min) group were significantly decreased compared with the H_2O_2 group ($P < 0.001$). Polyligand, tPA, hyaluronic acid and TNF-α in the H_2O_2+TXA (60min) group and the H_2O_2+TXA (90min) group were not expressed differently from that in the H_2O_2 group ($P > 0.05$).

As shown in Figure 4B, H_2O_2 significantly inhibited the expression of PAI-1 in HUVECs compared with the control group. The difference between the two groups was significantly different (5972.4 ± 1029.7 vs. 4613.5 ± 872.1, $P < 0.05$). The expression of PAI-1 in the H_2O_2+TXA (0min) group and the H_2O_2+TXA (30 min) group were (5869.7 ± 1425.2) pg/ml and (6127.5 ± 1147.6) pg/ml, respectively, significantly up-regulated compared with the H_2O_2 group ($P < 0.05$). The expression of PAI-1 in H_2O_2+TXA (60min) group and H_2O_2+TXA (90min) group was not significantly different from H_2O_2 group ($P > 0.05$).

Discussion

As a strong oxidant, H_2O_2 plays an important role in regulating cellular oxidative signaling pathways. However, it has been reported that a certain concentration of H_2O_2 can cause EoT in endothelial cells [6]. H_2O_2 can significantly induce apoptosis in endothelial cells and play a key role in the microcirculation and vascular dysfunction [7]. In this study endothelial cells after treated by 100 μmol/L H_2O_2 were observed the dense staining of many nuclei, which indicated that H_2O_2 could induce damage and death to a large number of endothelial cells.

As a widely used clinical coagulation drug, it has been found that the application of TXA within 3 hours after hemorrhagic trauma can effectively decrease the mortality caused by bleeding. In addition, a prospective clinical trial reported that within 1 hour of acute trauma, the use of TXA has a significant effect on decreasing mortality [3,4]. A recent study of rodent shock models has shown that the mechanism of TXA action is related to the inhibition of the expression of disintegrin, metalloproteinase and tumor necrosis factor by TXA [4,8]. In present study, TXA was applied to the in vitro EoT model at different time points and early TXA administration especially within 1h was found to effectively inhibit cell damage, but not in the late administration,

In this study we found that TXA can inhibit the expression of tPA, multi-ligand glycans, hyaluronic acid, TNF-α, ICAM and MMP-9 but promote PAI-1 expression to reduce endothelial cell injury. As a superfamily of adhesion molecules in endothelial cells, ICAM is up-regulated by inflammatory factors and closely related with mediating cellular inflammatory responses [9,10]. tPA and PAI-1 act as regulators of synthesis and secretion in endothelial cells and play an important role in regulating the balance between the body's fibrinolytic system and the coagulation system. tPA can specifically bind fibrin in thrombus while simultaneously activates lysozyme and fibrinolytic enzyme in the blood and inhibits platelet aggregation. As a rapid inhibitor of tPA, PAI-1 can form a complex binding to tPA, thereby exerting an inhibitory effect on tPA [11,12]. As one of the representative inflammatory cytokines, TNF-α plays an important role in mediating various cell damage and inflammatory reactions in endothelial cells. Down-regulation of TNF-α expression can inhibit adhesion molecules and other cellular inflammation [13]. The polysaccharide-protein complex in endothelial cells acts as a skeleton of the endothelial cell membrane, plays an important barrier function in maintaining vascular permeability, and transmitting extracellular signals into cells [14,15]. Since the polysaccharide-protein complex is difficult to be observed in vivo, the detection of the multi-ligand glycan and hyaluronic acid can indirectly reflect the degradation of the polysaccharide-protein complex. It can also be used as an early marker of endothelial cell damage caused by traumatic shock, as they are often down-regulated in patients with severe trauma [16]. In addition, polysaccharide-protein complexes in vascular endothelial cells can be degraded by extracellular proteases including MMPs after the stimulation of inflammatory factors. Therefore, improving or reversing the degradation process of polysaccharide-protein complex in vascular endothelial cell is expected to reduce mortality and improve prognosis in patients with severe trauma.

Conclusion

The clinical application of TXA requires early administration in patients with acute hemorrhagic trauma and TXA administration pk within one hour can effectively protect the endothelium from inflammatory factors and maintain the normal function of endothelial cells.

References

1. Rodriguez EG, Ostrowski SR, Cardenas JC, Baer LA, Tomasek JS, et al. (2017) Syndecan-1: a quantitative marker for the endotheliopathy of trauma. Journal of the American College of Surgeons 225: 419-427.

2. Flaherty K, Bath PM, Dineen R, Law Z, Scutt P, et al. (2017) Statistical analysis plan for the 'Tranexamic acid for hyperacute primary IntraCerebral Haemorrhage'(TICH-2) trial. Trials 18: 607.

3. Huang Z, Xie X, Li L, Huang Q, Ma J, et al. (2017) Intravenous and topical tranexamic acid alone are superior to tourniquet use for primary total knee arthroplasty: a prospective, randomized controlled trial. JBJS 99: 2053-2061.

4. Yan J, Wang J, Huang H, Huang Y, Mi T, et al. (2017) Fibroblast growth factor 21 delayed endothelial replicative senescence and protected cells from H_2O_2-induced premature senescence through SIRT1. American journal of translational research 9: 4492.

5. Jiang R, Zhang C, Liu G, Gu R, Wu H (2017) MicroRNA□126 Inhibits Proliferation, Migration, Invasion, and EMT in Osteosarcoma by Targeting ZEB1. Journal of cellular biochemistry 118: 3765-3774.

6. Huang AF, Chen MW, Huang SM, Kao CL, Lai H-C (2013) CD164 regulates the tumorigenesis of ovarian surface epithelial cells through the SDF-1α/CXCR4 axis. Molecular cancer 12: 115.

7. Liou C-J, Huang W-C (2017) Casticin inhibits interleukin-1β–induced ICAM-1 and MUC5AC expression by blocking NF-κB, PI3K-Akt, and MAPK signaling in human lung epithelial cells. Oncotarget 8: 101175-101188.

8. Jiang XZ, Gong H, Luo KH, Ventikos Y (2017) Large-scale molecular dynamics simulation of coupled dynamics of flow and glycocalyx: towards understanding atomic events on an endothelial cell surface. Journal of The Royal Society Interface 14: 20170780.

9. Galore-Haskel G, Baruch EN, Berg AL, Barshack I, Zilinsky I, et al. (2017) Histopathological expression analysis of intercellular adhesion molecule 1 (ICAM-1) along development and progression of human melanoma. Oncotarget 8: 99580-99586.

10. Winter M-P, Kleber ME, Koller L, Sulzgruber P, Scharnagl H, et al. (2017) Prognostic significance of tPA/PAI-1 complex in patients with heart failure and preserved ejection fraction. Thrombosis and haemostasis 117: 471-478.

11. Aloni PD, Nayak AR, Chaurasia SR, Deopujari JY, Chourasia C, et al. (2016) Effect of Fagonia arabica on thrombin induced release of t-PA and complex of PAI-1 tPA in cultured HUVE cells. Journal of traditional and complementary medicine 6: 219-223.

12. Altaf M, Stoeckli-Evans H (2017) A copper (II) paddle-wheel structure of tranexamic acid: dichloro-tetrakis [μ-4-(ammoniomethyl) cyclohexane-1-carboxylato-O, O′] dicopper (II) dichloride hexahydrate. Acta Crystallographica Section E: Crystallographic Communications 73: 1421-1425.

13. Targosz-Korecka M, Jaglarz M, Malek-Zietek KE, Gregorius A, Zakrzewska A, et al. (2017) AFM-based detection of glycocalyx degradation and endothelial stiffening in the db/db mouse model of diabetes. Scientific reports 7: 15951.

14. Li T, Liu X, Zhao Z, Ni L, Liu C (2017) Sulodexide recovers endothelial function through reconstructing glycocalyx in the balloon-injury rat carotid artery model. Oncotarget 8: 91350-91361.

15. Kurnik NM, Pflibsen LR, Do A, Bristol R, Singh DJ (2018) Craniosynostosis Surgery and the Impact of Tranexamic Acid Dosing. Journal of Craniofacial Surgery 29: 96-98.

16. Pacheco LD, Hankins GD, Saad AF, Costantine MM, Chiossi G, (2017) Tranexamic acid for the management of obstetric hemorrhage. Obstetrics & Gynecology 130: 765-769.