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Trauma-induced Apoptosis of Endothelial Cells

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ABSTRACT

Trauma is a common cause of death in the developed countries leading trauma related coagulopathy. In recent years, 'new type endothelium development' caused by trauma-induced hemorrhagic shock, has been widely understood and researchers trying to figure out the effect of endothelial apoptosis on this process. New modulation of immune response may result in undesired apoptosis and give rise to programmed cell death. In experimental models, apoptosis has been shown in liver, kidney, heart and brain after the damage of ischemia/reperfusion (I/R) during trauma. Apoptosis may be an important factor for the development of serious hemorrhagic shock during trauma. In this review we aimed to reveal the possible relationship between trauma and apoptosis.The balance between pro-apoptotic and anti-apoptotic pathway seals; the fate of every endothelial cell. Apoptotic cell death is important in traumatic hemorrhage and sepsis. Apoptosis increases depending on the severity of hemorrhagic shock during trauma.

1. Introduction

Every year, many people die due to trauma.[1] New concern is that the traumatic coagulopathy is a major cause for death. Not only loosing blood and coagulation proteases but also programmed cell death may trigger this lethal process .[2] Nowadays, the changes in endothelium caused by traumatic hemorrhagic shock, can not explain the damage of the vascular structure. Besides it is related to the impairment in the integrity of the blood/organ barrier, mainly being in the important part of glycocalyx.

Anti-inflammatory response occurs to balance the excessive pro-inflammatory response during trauma when homeostasis is achieved. However, an overreaction of the anti-inflammatory response may cause a compensatory anti-inflammatory response (CARS), or a mixed antagonist response. Although the mixed response basically develops to counterbalance the pro- and anti-inflammatory responses, it can lead to multiple organ failure (MOF) in advanced stages as in systemic inflammatory response (SIRS) and sepsis.[3] All these immune responses are caused by a change in the endothelium phenotype resulting from a complex response in traumatic patients starting at the endothelium level, and the effect of apoptosis on this process is now on the agenda.

Definitions

Traumatic coagulopathy is a pathological condition, in which a complex hemostatic and immunoinflammatory response to injury leads to abnormal clot formation, and may be characterized with either hypo- or hyper-coagulability depending on the response of the vascular bed. Brohi et al.[2] first defined coagulopathy as the activation of fibrinolytic and anticoagulant pathways rather than a result of traumatic tissue injury and shock-induced loss, dysfunction, and dilution of coagulation proteases. All of these factors lead to vascular leak, tissue edema, microthrombus, impaired organ perfusion and ultimately uncontrollable hemorrhage.Endotheliopathy of trauma (EoT) is caused by impairment of glycocalyx, formation of reactive oxygen species (ROS), uncontrolled production of NO (nitric oxide), protease activation, trans-paracellular permeability, instability of vascular endothelial junction, inflammation and vasoreactivity.[4,5] The endothelium which is the largest organ in the body weighs nearly 1 kg and has a surface area of approximately 5000 m^2. It covers all blood and lymph vessels. The presence of a protein layer on theendotheliumwasoriginallysuggestedby Daniel in 1940 andvisualizedforthefirst time byelectronmicroscopy in 1966 .[6]

Endothelial glycocalyx is a gel-like structure with a thickness of 0.2-0.5 mm. It is a network of glycoproteins, proteoglycans and other soluble components and connects to the endothelium with glycosaminoglycans. The glycocalyx has a negatively charged layer primarily composing of proteoglycans and glycoproteins, such as syndecan-1, hyaluronic acid (HA), heparan sulfate (HS) and chondroitin sulfate (CS).Syndecan and hyaluronic acid are the major components of glycocalyx. It is effective for maintenance of vascular permeability. The impairment of glycocalyx leads to capillary leakage by elevating vascular permeability. Inflammatory response develops and coagulation changes as a result of the exposure of endothelial cell to circulating platelets and leucocytes.

Since glycocalyx is a mechano-sensor, damage to it leads to changes in hemodynamic forces such as shear stress and vessel wall tension. Ultimately, impaired glycocalyx endothelial dysfunction, coagulopathy, edema and organ dysfunction were observed. The proteoglycans and

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glycoproteins, the backbone molecules of the glycocalyx, are bound to endothelium. The glycocalyx on the luminal side is composed of soluble plasma components, namely glycosaminoglycans, proteoglycans and sialoproteins. The balance between blood and soluble proteins determines the thickness of glycocalyx. The net negative charge determines the interaction between platelet, erythrocyte and plasma and inhibits microvascular thrombus. As it is a semi-permanent, it acts like a barrier. However, it is not permeable formacromolecules such as albumin, erythrocytes and large molecules like dextrans. Conformational changes in glycocalyx structure, on the other hand, lead to release of NO, affecting vasomotor tone and tissue perfusion. Also, glycocalyx creates a high intravascular oncotic pressure at the endothelial surface and helps the protection of starling forces by binding plasma proteins.

Besides, its role in the protection of wall integrity involves major enzymatic systems and regulates vascular lipid hemostasis, oxidant status, and anticoagulant and anti-inflammatory responses in vessel. Furthermore, the glycocalyx is a physiologically significant binding site for antithrombin III, tissue factor pathway inhibitors, vascular endothelial growth factor, fibroblast growth factor, and lipoprotein lipase. That is to say thatglycocalyx has a criticalimportance in thefate of traumapatients.[7]

Damaged capillaries, damaged glycocalyx, impaired endothelial barrier and leukocyte diapedesis, plasma leak, interstitial edema, and thrombosis constitute the shock pathophysiology in critically ill patients. Andnowallthesefactorsaredefinedby SHINE .[8]

Traumainducedcoagulopathy:

Traumainducedcoagulopathy is known as “bloodvicioustiriad”.[9] Inthe presence of tissue hypoperfusion and hypoxia, catecholamine discharge occurs, ultimately causing thrombin generation through up-regulation of endothelial cell and glycocalyx shedding. Subsequently, thrombomodulin expression increases and protein C is activated and shows a 1000-fold increase.It is again because of thrombin released with tissue damage and such a complex activity elevates activated protein C. Then, inhibition of plasminogen activator inhibitor-1 (PAI-1) activity results in hyperfibrinolysis. Activated protein C activates factor Va and VIIIa, inducing hypo-coagulability which is strengthened with the release of tPA. Additionally, endogenous heparinization occurs when endothelial glycocalyx impairs.

Increased levels of systemic cytokines and hormones lead to endothelial activation, and the endothelial phenotypeturns to thrombotic from anti-thrombotic. Besides, the activation of endothelium down regulates thrombomodulin formation and fibrinolysis while increasing the amount of fibrinogen by causing acute phase response.Thesystemiceffects of alltheseresponsesareknown as the “endotheliopathy of trauma”syndrome. [10]

Syndecan-1 is a heparan-sulfate proteoglycan expressed in both endothelial and epithelial cells and considered as a marker in trauma patients. Thrombomodulin is an anticoagulant protein and play role in protein C activation.Increase in IL-6and TNF levels after traumadown regulates the formation of thrombomodulin. Additionally, this type of thrombomodulin has a disrupted soluablestructurea and a weak marker of endothelial damage.

Theimpairedintegrity of thedamagedendothelium in hemorrhagicshockleadstoexposuretopro inflammatorymediatorsandshedding of syndecan 1, resulting in increasedpermeability.[11]

Gonzalez Rodriguez et al.[10] indicated that the syndecan 1 level was 4 times higher in patients thought to experience auto-heparinization after trauma (116 ng/ml vs.31 ng/ml). In the same study, while the levels of thrombomodulin, interleukin 6 and endocan were also high, protein C level was found to be low.

Apoptosis and Necrosis:

Apoptosis is the process of programmed cell death. Apoptosis is a diffuse and genetically controlled cellular response to stimulants. It is regulated not only by survival factors such as Bcl-2,but also bydeath factors including TNF, Fas ligand (FasL), and Fas.[12] Apoptosis is basically a measure to limit the number of cells in tissues. Apoptotic and inflammatory pathways are related to each other.While necrosis is always abnormal and harmful, apoptosis may be physiological and beneficial to an organism. Apoptosis is energy-dependent and can affect individual cells. Nuclear fragmentation, chromatin condensation, fragmentation of chromosomal DNA, cytoplasmic blebs and cystoplasmic bodies contribute to apoptosis. Apoptosis is associated with cell shrinkage, whereas cells show swelling in necrosis. The integrity of membrane and cell nucleus is preserved in apoptosis but not in necrosis.

There are two mechanisms of apoptosis:

1. Intrinsic (mitochondrial death)
2. Extrinsic (death receptor pathway)

The extrinsic pathway is a cascade of several molecular events.

Fasand TNF receptors are also known as death ligands. Fas which is a proapoptotic factor and a member of tumor necrosis factor receptor superfamily is found in many cells and tissues.Engagement of Fas by FasL results in apoptosis, mediated by the stimulation of caspases.Fas, an integral membrane protein, interacts with death domain proteins such as FAD-associated death domain protein (FADD) and tumor necrosis factor receptor type 1-associated death domain protein (TRADD) when encountered with death signal on the cell surface and transmits that signal to the cytosol. Subsequently,caspase-8 and caspase-10 are activated, inducing the activation of caspase-3. Caspase-8 stimulates caspase-7. Both caspase-8 and caspase-10 elevateBcl-2. With the activation of caspase-3 and caspase-7, a death-inducing

signaling complex (DISC) occurs, leading to ProgrammedCell Death.[13]

Mitochondrial Changes:

Pro-apoptotic factors are released with the influence of free radicals, oxidative damage, radiation, and viral infections. Increased permeability leads to the development of mitochondrial membrane damage. With the release of cytochrome C into the cytoplasm and apoptotic protease activating factors are activated. Caspase-3 stimulates apoptosis by initiating DNA fragmentation with caspase-activated DNase (CAD) activation; chromatin condensation with the chromatin condensation inducer (ACINUS) in cell nucleus;and DNA degradation via cytosolic helicase with an N-terminal caspase-recruitment domain (HELI-CARD). When released into cytosol, AIF (Apoptosis Inducing Factor) migrates to nucleus and induces chromatin condensation, causing a significant amount of DNA fragmentation.[14]

Nature and Functional Characteristics of Caspases:

Cysteine proteases cleave target proteins from Asp units. They are synthesized as inactive precursors and converted to active forms after proteolysis.

There are 12 types of caspases. Even though they are mostly engaged in apoptosis, they also play a role in inflammation. Particularly, caspase-8 and caspase-9 act as activators. Caspase-3, on the other hand, is responsible for key materialsignalamplification.[15] Table 1 summarizes the mechanisms of action for caspases.

Apoptosis and Trauma:

Inflammation and apoptosis are two major factors in the progression of hemorrhagic shock. Free oxygen radicals, metabolites and cytokines formed during ischemia-reperfusion lead to inflammatory response and ultimately apoptosis. Modulation of immune response results in undesired and inconvenient apoptosis and gives rise to programmed death of cells. This process occurs hours or days after trauma and generally takes nearly 3 weeks. Neutrophils migrate to the damaged area after activation and undertake defensive tasks including degranulation, secretion of ROS, and elimination of pathogens. Neutrophil activation is potentiated by the release of inflammatory mediators such as IL-8 and GM-CSF, which ensure that by inhibiting intracellular survival pathway and apoptosis. All these factors intensify inflammation and cause SIRS. Furthermore, neutrophils directly interact with immune cells such as T and B, and NK and form immunosuppression.Neutrophils accumulate in traumatic tissues and cause production of higher amounts of cytotoxic oxygen radicals and proteases.The inflammatory response after major trauma includes elevated cytokine expression, acute phase proteins and complements which extend the life span through activation and sequestration of neutrophils. Complement activation leads to the exacerbation of inflammation by the release of damage-related molecular patterns, the release of proteolytic enzymes such as neutrophil elastase from active leucocytes, and the destruction of proteoglycans by proteolytic enzymes, and strengthening of oxidative stress through active neutrophils. Therefore, apoptotic cell death is important for termination of inflammatoryresponse.[16]

Myeloid cell leukemia 1 (Mcl-1) is an antiapoptotic factor. It hinders the formation of The BAK/BAX heterodimer formation in the external mitochondrial membrane and barriers the release of Site C; therefore, it is important in the intrinsic pathway. In trauma, increase expression of Mcl-1 is the cause of delayed apoptosis.

Apoptosis occurring in case of trauma and ischemia/reperfusion injury was first reported by Hotchkiss et al.[17] Both lymphocyte apoptosis and intestinal epithelial cell apoptosis develop within the first 2-3 hours after injury.

Increase of ROS production in endothelial cell mitochondria after hemorrhagic shock induce leukocyte adherence, the release of apoptogenic factors such as Site C, and the increase of microvascularpermeability.[18] The most important effect of hemorrhagic shock is the elevation of microvascular permeability due to demolished endothelial barrier, which is also thought to be contributed by endothelial cell apoptosis. The most important characteristic of endothelial cell apoptosis is the detachment of cells.

Protease Activated Receptors:

Protease-activated receptors (PARs) are a subfamily of related G protein-coupled receptors and commonly found in endothelial cells. There are 4 members of the PARs family: PAR1, PAR2, PAR3, and PAR4. PAR1, the major thrombin receptor, is important in apoptotic gene expression and closely related to inflammation and coagulation. PAR1 activates the platelets which have a significant role in thecoagulationcascade.[19]

It has beenindicatedthat, withinthefirsthours, plasma DNA concentrationincreases in traumapatientsin proportiontotheseverity of trauma.[20] Although thousands of molecular signals were identified for endothelial apoptosis, the contribution of transcriptomics, glycoproteins, and proteomic changes has been recently understood. Complement regulatory proteins such as CD55, CD59 and CD35 have been identified in the early periodafterpolytrauma.[14]

Guan et al.[21] divided 60 Sprague-Dawley rats into 6 groups: normal control (A, n=6), sham-operation (N, n=6), single hemorrhagic shock (S, n=6), two-site trauma/shock (B, n= 6), four-site trauma/shock (C, n=6), and six-site trauma/shock (D, n=30). The rats experiencing polytrauma were maintained in shock for 60 minutes and then perfusion was reestablished. It was consequently indicated that apoptosis develops in liver, kidney, heart and brain after the damage of I/R during trauma. It was also reported that apoptosis started increasing at the 1st hour and reached the peak level at the 3rd hour.

Hydrocortisone, hyaluronic acid, nitric oxide, sulodexide, lidoflazine, albumin, hydroxietilstarch, N acetylcysteine and metformin have been reported for have protective effects on glycocalyx during trauma.[22]

There are several proteins that can improve vascular stability in fresh frozen plasma (FFP). In this context, FFP is thought to restore coagulopathy by restoring glycocalyx besides replacing coagulation factors. It is also the case for factor-4 PCC but not for albumin.Because barrier dysfunction may suppress interstitial edema, tissue hypoxia, inflammatory cell infiltration, pericyte loss, extracellular matrix breakdown, and apoptosis. It restores tight junctions and inhibits inflammation and edema. It has been also reported to be beneficial for Syncedan 1 expression.[23,24]

Conclusion

The "endotheliopathy of trauma" is now considered as a syndrome. The balance between pro-apoptotic and anti-apoptotic pathway seals the fate of every endothelial cell. Apoptotic cell death is important in traumatic hemorrhage and sepsis. Apoptosis increases depending on the severity of hemorrhagic shock during trauma.

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Table 1: Effect of caspase mechanism

• ensuring detachment from neighboring cells
•reorganization of cytoskeleton (transglutaminases)
•activation of endonucleases (DNA fragmentation)
•resolution of nuclearlamina (condensation)
•production of specificsignals for fagositosis
• activation of specific target proteins to terminate metabolic functions

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