

Review Article

Accelerated RBC senescence as a novel pathologic mechanism of blood stasis syndrome in traditional East Asian medicine

Sooseong You*, Bongki Park*, Myeong Soo Lee

Medical Research Division, Korea Institute of Oriental Medicine, Daejeon, South Korea. *Equal contributors.

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Abstract: Blood stasis syndrome (BSS) is an important pathologic condition in traditional East Asian medicine, characterized by multiple signs and symptoms, including sublingual varicosity, angiotectasis, slow and choppy pulse, local fixed pain, nyctalga, menstrual cramps, dark-purple tongue and infra-orbital darkness. However, recent studies have been restricted to the circulatory disorder and could not suggest the pathologic core to explain all of the characteristics of BSS. Here, we review the current research on the senescence of red blood cells (RBCs), focusing on the correlation between the pathologic properties of senescent RBCs and BSS-specific manifestations. The accumulation of senescent RBCs and their products induce pathological conditions that affect blood flow resistance and cause thrombosis, vasoconstriction and methemoglobinemia. These pathological alterations are identical to the characteristics of BSS, therefore supporting the hypothesis that accelerated RBC aging could be considered as a novel pathologic mechanism of BSS.

Keywords: Blood stasis syndrome, red blood cell, senescence

Introduction

Blood stasis is a pathological concept in traditional East Asian medicine and refers to stagnant blood that has lost its physiological function within the body [1, 2]. It develops into a blood stasis syndrome (BSS) that is characterized by multiple signs and symptoms, such as sublingual varicosity, angiotectasis, slow and choppy pulse, local fixed pain, nyctalga, menstrual cramps, dark-purple tongue or infra-orbital darkness [1, 3]. In the clinic, these manifestations are frequently observed in patients with ischemic heart disease, cerebral vascular accident, diabetes mellitus, chronic renal failure, severe traumatic injury and dysmenorrhea [2, 4]. Many herbal formulas have been shown to be effective for relieving the BSS-specific manifestations and the severity of these diseases [5-8]. In recent decades, many preclinical and clinical studies have been conducted to reveal the underlying pathogenic correlation between BSS and these diseases. However, almost all of these studies have been restricted to ischemic heart disease and could not sug-

gest the pathologic core to explain all of the characteristics of BSS [9-13].

In this review, we present the current research progress on the senescence of red blood cells (RBCs), focusing on the correlation between the pathologic properties of senescent RBCs and the signs and symptoms of BSS, and we suggest that the accelerated RBC aging process could be considered to be a novel pathologic mechanism of BSS.

Pathologic properties of senescent RBCs

RBCs are naturally exposed to various stressful situations during their lifespan, such as oxidative stress in the lungs, osmotic shock in the kidneys and squeezing through capillary beds [14]. During RBC aging, the accumulation of damage by these stressors induces biochemical and structural alterations that derange the functions of the RBCs.

Decreased deformability

Deformability is the ability of an RBC to change its shape in response to a deforming force with-

out hemolysis, and it is determined by the geometric and viscoelastic properties of the plasma membrane [15, 16]. The lipid bilayer contents of the membrane and the sub-membrane cytoskeletal network of spectrin molecules are primarily responsible for the discocyte morphology of RBCs and provide the membrane with its viscoelastic properties [16, 17]. Oxidative damage to the membrane lipids and the spectrin network occur during RBC aging, which causes morphological changes and decreased membrane viscoelasticity, resulting in impaired deformability [16, 18, 19]. Therefore, older RBCs demonstrate a decreased deformability compared to younger RBCs [20, 21].

Release of microparticles

Microparticles (MPs) are membrane vesicles of less than 1 μm that are released into the blood flow by various types of cells, including platelets, RBCs, white blood cells and endothelial cells [22]. Their cell membrane consists of a phospholipid bilayer, and phosphatidylserine (PS) and phosphatidylethanolamine are specifically enriched in the inner membrane, while phosphatidylcholine and sphingomyelin are enriched in the outer membrane. This asymmetric distribution of phospholipids is actively maintained by three major enzyme systems: a flippase, a floppase and a scramblase [23]. However, various stimuli, such as cell activation, shear stress or apoptosis, induce negatively charged PS externalization on the membrane through the impairment of these enzyme functions and cytoskeletal degradation via Ca^{2+} -dependent proteolysis. This process causes sufficient membrane fluctuation, allowing the formation and release of MPs containing hemoglobin (Hb) and membrane lipids [24, 25]. MPs have also been implicated in RBC senescence [26, 27]. In actuality, RBCs lose approximately 20% of their volume during their lifespan, and this lost volume could be caused by the shedding of MPs from their membrane during RBC aging [28, 29].

Increased methemoglobin

Each human RBC contains approximately 270-million Hb molecules, which are oxygen-transporting metalloproteins that contain four iron atoms. Each ferrous iron (Fe^{2+}) in Hb reversibly binds to one O_2 molecule to provide the oxygen supply for the body. On the converse, methemoglobin (MetHb) is a form of oxidized hemoglobin

in which the ferrous iron is oxidized to ferric iron (Fe^{3+}) [30]. It is normally maintained as a very small proportion of the total hemoglobin (1%), primarily by the reduction of nicotinamide adenine dinucleotide (NADH)-dependent MetHb reductase [31]. Previous studies have reported that senescent RBCs are characterized by an increased proportion of MetHb as a result of reduced NADH-dependent MetHb reductase activity and accumulated oxidative membrane damage [32, 33].

Pathogenetic mechanisms of senescent RBCs and their products

Increased blood flow resistance

The circulatory resistance of the blood has two major components, the rheological properties of the blood and the geometric features of the blood vessels [15, 34]. The rheological properties of the blood are determined by the hematocrit, plasma viscosity, cell aggregation and cell deformability [15]. Especially in the microcirculation, the deformability of an RBC is the principal factor in maintaining normal flow, allowing their transit through capillaries as small as 2-3 μm in diameter because their size, which is approximately 7.5-8.7 μm in diameter and 1.7-2.2 μm in thickness, is larger than the capillary diameter [15, 16]. Therefore, senescent RBCs with impaired deformability increase the local resistance to the blood flow [35, 36].

Thrombosis

RBC-derived MPs in the plasma are involved in several pathological processes, including thrombosis and hemostasis [37, 38]. MPs provide a membrane surface for the initiation of blood coagulation because negatively charged PSs on their outer membrane facilitate the assembly of components of the clotting cascade through an electrostatic interaction with the positively charged γ -carboxyglutamic acid domains in the clotting proteins [37]. In addition, the nitric oxide (NO) scavenging activity of RBC-derived MPs contributes to platelet activation and aggregation through the decreased NO signaling in the platelets, promoting clot formation [39-41].

Vasoconstriction

NO is a critical regulator of basal blood vessel tone. It is synthesized in the endothelial cells and then diffuses to the smooth muscles to

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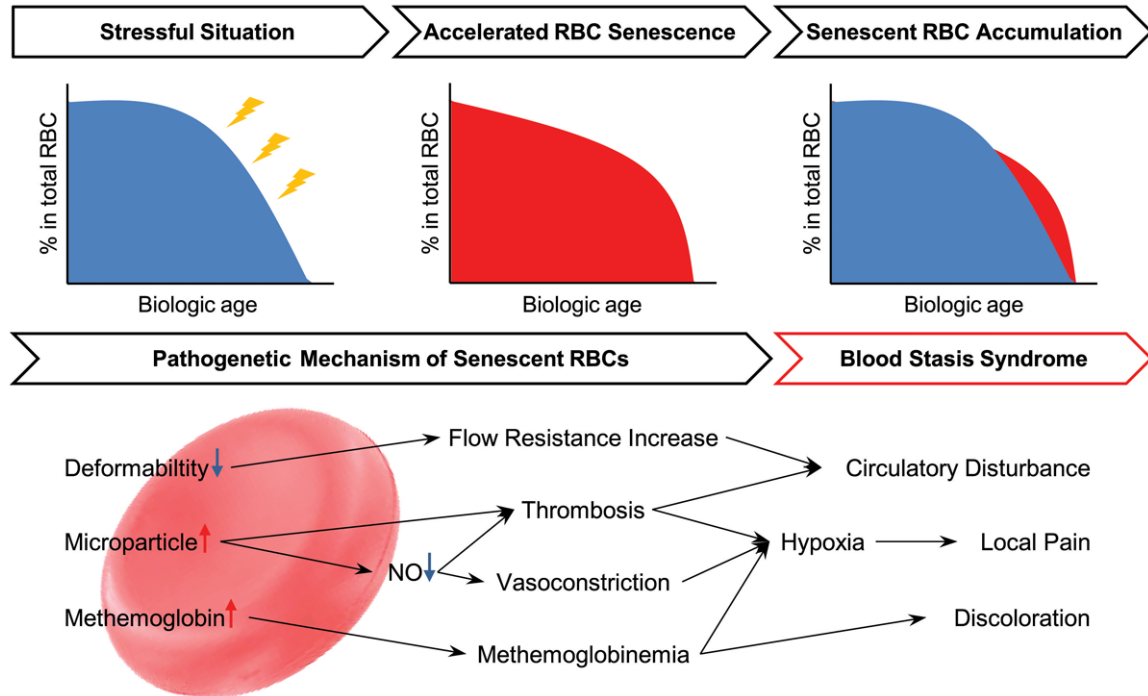


Figure 1. The hypothetical scheme illustrating the pathobiological effects of senescent RBCs on blood stasis syndrome. When the RBC aging process is accelerated during highly stressful situations, the senescent RBCs play pathogenetic roles. The impaired deformability of the RBCs increases the blood flow resistance in the tissues and induces circulatory disturbances. Additionally, increased RBC-derived microparticles activate the body's procoagulant and NO-scavenging activities, therefore facilitating thrombosis and vasoconstriction. This process causes hypoxia in the tissues and ischemic pain, as well as circulatory disturbances. The elevation of methemoglobin leads to a grayish skin color and also contributes to the hypoxia. These pathological alterations caused by senescent RBCs are identical to the diagnostic characteristics of blood stasis syndrome.

activate soluble guanylyl cyclase, ultimately leading to vasodilation and increased blood flow in the tissues [42-45]. As oxygenated Hb reacts with NO to form nitrate and methemoglobin, the NO can be scavenged by Hb in the blood [46, 47]. However, a physical barrier produced by the RBC membrane inhibits the endothelial diffusion of NO to the intracellular Hb [48, 49]. Additionally, the laminar flow within the blood vessel pushes the RBCs inward and away from the endothelial cells, forming a cell-free zone and blocking the access of the RBCs to the endothelium [49-51]. Because of these diffusional barriers, sufficient NO can reach the smooth muscles to sustain vascular homeostasis before being scavenged by Hb. Interestingly, the Hb from RBC-derived MPs are able to more effectively scavenge NO than the Hb from RBCs [52] because the MPs can enter the cell-free zone and easily access the endothelial NO, even in the laminar blood flow [53]. Therefore, the reduction of the endothelial NO concentration by RBC-derived MPs induces vasoconstriction

and decreases the blood flow in the tissues.

Methemoglobinemia

Generally, methemoglobinemia, which is due to abnormally increased MetHb, causes only a grayish pigmentation of the skin and brownish lips and mucous membranes when the MetHb levels are between 3 and 15% of the total hemoglobin. Above 15%, patients develop central cyanosis that is non-responsive to oxygen therapy [30]. The reason is that the ferric iron of MetHb cannot bind to oxygen, which enhances the binding affinity of the remaining ferrous irons to oxygen, shifting the oxygen dissociation curve to the left and decreasing the delivery of oxygen to the tissues [54].

Alterations to the human body during the accelerated RBC aging process

Under normal circumstances, the lifespan of a circulating human RBC is approximately 120

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days [14], indicating that approximately 1% of all circulating RBCs are destroyed and newly generated from hematopoietic stem cells each day [55]. Therefore, the RBC's composition and counts are maintained within the normal range for a particular age. However, under highly stressful situations, such as oxidative stress due to severe trauma [56-59] or exhaustive physical exercise [60-62], shear stress from a cardiovascular device [63, 64], hyperlipidemia [65-67] or hyperglycemia [68, 69], the RBC aging process is accelerated due to the increased damage to the cellular components [63, 70]. The rapid accumulation of senescent RBCs and their products can lead to pathological alterations in the human body.

Circulatory disturbances

The impaired deformability of RBCs and thrombosis increase the blood flow resistance in the tissues, which leads to the development of circulatory disturbances. Impaired deformability is correlated with coronary artery disease, claudication and diabetes mellitus-associated vascular complications [71-75], and the procoagulant activity of RBC-derived MPs has also been observed in the patients with atherosclerosis, ischemic heart disease and nephrotic syndrome [76-78]. These pathologic properties of senescent RBCs can cause BSS-specific signs, such as a slow and choppy pulse, sublingual varicosity and angioectasis, depending on the circulatory disturbance.

Local pain

Thrombosis and vasoconstriction decrease the blood flow in the tissues, and MetHb inhibits the oxygen delivery from the RBCs to the tissues. In this case, hypoxia-induced pain [79, 80] can lead to the development of BSS-specific symptoms, including local fixed pain, nyctalgalgia and menstrual cramps, depending on the site of the lesion.

Discoloration

Skin discoloration, dark-purple tongue and infra-orbital darkness are unique signs of BSS; however, previous studies have been unable to determine the mechanism for the observed discolorations. If the MetHb levels increase to more than 3% of the total Hb in BSS patients, the grayish skin color could be explained by the presence of methemoglobinemia.

Taken together, highly stressful situations should be correlated with the etiology of BSS, and BSS-associated diseases and manifestations are also correlated with accelerated RBC senescence. Furthermore, we explained the characteristics of BSS that arise from the pathologic mechanisms of accelerated RBC senescence (**Figure 1**).

Conclusion

BSS is an important pathologic condition in traditional East Asian medicine, and it is correlated with diseases such as ischemic heart disease, cerebral vascular accident, diabetes mellitus, chronic renal failure, severe traumatic injury and dysmenorrhea, which have been reported in several preclinical and clinical studies [4-8]. However, these studies have been unable to reveal the pathologic mechanism behind the characteristics of BSS. Here, we presented the pathogenetic mechanisms of senescent RBCs, which explain all of the BSS-specific manifestations, such as circulatory disorders and local pain, as well as the grayish skin color.

As RBCs lack a nucleus to synthesize new proteins for repairing stress-induced damage, they lose their deformability and enzyme activity during their aging process, leading to the formation of MPs and methemoglobin. Senescent RBCs in the circulation are selectively removed by macrophages in the liver and spleen, while a proportion of aging RBCs are normally maintained in a healthy individual. However, under highly stressful situations, the RBC aging process is accelerated, and the number of senescent RBCs in the circulatory system increases [63, 70]. The rapid accumulation of senescent RBCs and their products induce pathological conditions that affect the blood flow resistance and lead to thrombosis, vasoconstriction and methemoglobinemia, which lead to pathological alterations, circulatory disturbances, local pain and discoloration of the skin. These findings support the hypothesis that the accelerated RBC aging process may be a novel pathologic mechanism of BSS.

Although intense clinical studies are still necessary to prove the relationship between accelerated RBC senescence and BSS, this new approach could provide an opportunity to develop diagnostic tools using biological mark-

ers and contribute to a more accurate diagnosis and effective treatment of BSS.

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Disclosure of conflict of interest

None.

Address correspondence to: Dr. Myeong Soo Lee, Medical Research Division, Korea Institute of Oriental Medicine, Daejeon, 305-811, South Korea. Tel: 82-42-868-9266; Fax: 82-42-863-9622; E-mail: drmslee@gmail.com; mslee@kiom.re.kr

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