Eryptosis, a Window to Systemic Disease

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Key Words

Apoptosis • Hemolysis • Red blood cells • Anemia • Sepsis • Malaria

and a rare condition caused by a mutation in GLUT1 turning the carrier into a cation channel.

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Abstract

Similar to apoptosis of nucleated cells, suicidal erythrocyte death or eryptosis is characterized by cell shrinkage, membrane blebbing and membrane phospholipid scrambling with phosphatidylserine exposure at the cell surface. Signaling of eryptosis involves formation of prostaglandin E₂ with subsequent activation of cation channels and Ca²⁺-entry and/or release of platelet activating factor (PAF) with subsequent activation of sphingomyelinase and formation of ceramide. Ca²⁺ and ceramide stimulate cell membrane scrambling. Ca²⁺ further activates Ca²⁺-sensitive K⁺-channels leading to cellular KCl loss and cell shrinkage and stimulates the protease calpain resulting in degradation of the cytoskeleton. Injuries triggering eryptosis may similarly compromise survival of nucleated cells. The case is made that analysis of enhanced eryptosis may direct to the pathophysiology of systemic disease. Examples presented include drug side effects, sepsis, haemolytic uremic syndrome, Wilson's disease, phosphate depletion

Introduction

Circulating erythrocytes are among the most abundant cells. They contribute to almost 10% of cell volume in an adult human organism. In any given patient, they are easily accessible and could be functionally analysed in any detail *ex vivo*. Thus, erythrocytes appear to be ideal model cells for the study of cellular pathophysiology. However, erythrocytes are streamlined for their limited function and thus lack important elements of most cells, such as mitochondria and nucleus. They produce ATP exclusively by degradation of glucose and are considered by most scientists little more than a sac of hemoglobin.

Erythrocytes are well known to experience senescence eventually resulting in the clearance of aged erythrocytes [1-3]. However, most recently, it became obvious that erythrocytes may, similar to nucleated cells, undergo suicidal death, or eryptosis [4-6]. Suicidal death of nucleated cells or apoptosis [7, 8] is characterized by

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loss of cellular K^+ with cell shrinkage, nuclear condensation, DNA fragmentation, mitochondrial depolarization, cell membrane blebbing and breakdown of phosphatidylserine asymmetry of the plasma membrane [9-16]. Eryptosis involves cell shrinkage, membrane blebbing and phosphatidylserine exposure, all typical features of apoptotic nucleated cells [5, 17]. The cell shrinkage is caused by activation of the Ca^{2+} -sensitive K^+ channels [18-20]. The activity of those channels decreases with erythrocyte age [21]. The phosphatidylserine exposure results from phospholipid scrambling of the cell membrane, which in theory may result from activation of a Ca^{2+} -sensitive scramblase [22-24] and/or inhibition of a Ca^{2+} -sensitive and ATP-dependent aminophospholipid translocase [25].

Recently, a wide variety of endogenous mediators, xenobiotics and clinical conditions have been identified, which simultaneously trigger eryptosis and apoptosis. The considerable overlap of mechanisms involved provides the opportunity to understand the mechanisms involved in suicidal death of nucleated cells by analysis of eryptosis. In the following review a brief description of mechanisms involved in eryptosis will be followed by examples highlighting the pathophysiological insight into suicidal cell death.

Mechanisms executing and stimulating eryptosis

A key event of eryptosis is increase in cytosolic Ca^{2+} activity. Ca^{2+} triggers cell membrane vesiculation [26] and stimulates cell membrane scrambling [27-29]. Ca^{2+} further activates Ca^{2+} -sensitive K^+ channels [30-32] with subsequent K^+ efflux, hyperpolarization of the cell membrane, Cl^- exit and loss of cellular water [33]. In addition, Ca^{2+} stimulates the cysteine endopeptidase calpain, which degrades cytoskeletal proteins and thus initiates cell membrane blebbing [34].

Ca^{2+} may enter via nonselective cation channels [35-37], which involve TRPC6 [38] and P2X7-dependent pathways [39-41]. The channels are stimulated by hyperosmotic shock [42, 43], oxidative stress [43, 44] and Cl^- removal [37, 42, 44]. The cation channels are activated by PGE_2 [37].

Phospholipid scrambling is further elicited by ceramide [45] that, for instance, is formed by stimulation with platelet activating factor (PAF) [46]. Eryptosis following energy depletion involves activation of PKC and PKC-dependent phosphorylation of membrane

proteins [47] which allows Ca^{2+} entry [48].

Oxidative stress [49-51] is partially effective through activation of the Ca^{2+} permeable cation channels [44]. Oxidative stress further activates erythrocyte Cl^- channels [52, 53], which are required for erythrocyte shrinkage and thus also participate in the triggering of eryptosis [54]. Oxidative stress further activates aspartyl- and cysteinyl-proteases [55]. Erythrocytes express oxidant-sensitive caspases [5, 56], which are, however, not required for Ca^{2+} -induced cell membrane scrambling [17, 45, 57].

A wide variety of drugs [58], environmental pollutants [58, 59] and endogenous substances [58] could stimulate premature death of erythrocytes. Known stimulators of eryptosis are listed in Table 1. Eryptosis is stimulated by osmotic shock [43], oxidative stress [60-63] and energy depletion [62]. Moreover, a wide variety of substances stimulates eryptosis (see Table 1).

Inhibitors of eryptosis

The Ca^{2+} -permeable cation channels and thus eryptosis are inhibited by erythropoietin [64], which enhances the life span of circulating erythrocytes [65]. Accordingly, the hormone increases the number of circulating erythrocytes not only by inhibiting the apoptosis of erythrocytic progenitor cells [65, 66], but similarly by slowing the clearance of mature erythrocytes. Surprisingly, though, erythrocytes from erythropoietin-overexpressing mice have been observed to die faster *ex vivo* [67]. Possibly, erythropoietin stimulates the expression of genes in progenitor cells, which foster eryptosis and thus accelerate erythrocyte death as soon as the erythropoietin concentrations decline. By this means excessive erythrocytes could be rapidly removed, if they are no more needed. Otherwise, the erythrocytes would remain in circulation for 120 days. For instance, accelerated death of young erythrocytes or neocytolysis, has been observed following a limited exposure to high altitude or space flight [68].

The Ca^{2+} -permeable cation channels [42, 69] and thus eryptosis [69] are further inhibited by amiloride [43] and ethylisopropylamiloride (EIPA) [69] as well as by the catecholamines dopamine, isoproterenol and epinephrine [70]. Moreover, eryptosis is inhibited by flufenamic acid [71], adenosine [72] and nitroxide [73] (Table 2). The effect of nitroxide is partially due to activation of G kinase [73-75], which is important for the maintenance of erythrocyte survival [76]. Accordingly, accelerated eryptosis has been

observed in the G kinase knockout mouse [76].

Erythrocytes are able to take up, store and release nitroxide [77-80]. NO is preferably bound by oxygenated hemoglobin and released by desoxygenated hemoglobin [78, 81-85]. Erythrocytic NO participates in the regulation of vascular tone, and the impaired NO formation in NO-depleted banked erythrocytes may cause vasoconstriction and ischemia following transfusion [82, 84]. Defective erythrocytic NO release may further contribute to pulmonary hypertension [80] and to deranged microcirculation in sickle cell anemia [86], a disease, which is also associated with abnormal Gardos channel activity [87].

Diseases associated with accelerated eryptosis

Accelerated eryptosis contributes to the shortened life span of iron-deficient erythrocytes [88], which display enhanced cation channel activity presumably due to decreased cell volume [44].

Excessive eryptosis has been observed in a wide variety of further diseases or clinical conditions, including sepsis [89], hemolytic uremic syndrome [90], renal insufficiency [64], malaria [91], sickle-cell anemia [92], beta-thalassemia [62], glucose-6-phosphate dehydrogenase (G6PD)-deficiency [62], phosphate depletion [93], and Wilson's disease [94].

The Cu²⁺-accumulation in Wilson's disease, which is caused by inactivating mutations of Cu²⁺-secreting ATP7B [95], leads to mild anemia and eventually to liver cirrhosis [95]. Cu²⁺ triggers ceramide formation in erythrocytes and hepatocytes. Genetic knockout and pharmacological inhibition of acid sphingomyelinase counteracted both, anemia and liver failure in animals with defective ATP7B [94].

Neuroacanthocytosis including the X-linked McLeod syndrome and the autosomal recessive chorea-acanthocytosis are characterized by simultaneous choreatic disorders and acanthocytosis [96]. Chorea-acanthocytosis results from mutations of chorein [97-99], a protein expressed in a wide variety of tissues [100, 101]. The disorder does not only affect the brain and erythrocytes but may involve other tissues such as skeletal muscle [102]. Mutations of chorein may lead to movement disorders by deranged regulation of gephyrin and GABA receptors [103]. The pathophysiological mechanism leading to acanthocytosis remained, however, hitherto elusive.

Stimulators of eryptosis	
Air pollutants [59]	Gold [121]
Aluminium [105]	Hemolysin [<i>Vibrio</i>
Amantadine [106]	parahaemolyticus] [122]
Amiodarone [107]	Lead [123]
Amyloid peptides [108]	Listeriolysin [124]
Anandamide [109]	Mercury [125]
Anti-A-IgG-antibodies [110]	Methyldopa [126]
Arsenic [111]	Methylglyoxal [28]
Szathioprine [112]	Osmotic shock [43]
Bay-Y5884 [113]	Oxidative stress [60-63]
Cadmium [114]	Paclitaxel [127]
CD95/Fas ligand [115]	Platelet activating factor [46]
Ceramide (acylsphingosine) [45]	Peptidoglycans [128]
Chlorpromazine [27]	Prostaglandin E ₂ [37]
Ciglitazone [116]	Retinoic acid [129]
Cisplatin [117]	Selenium [130]
Copper [94]	Tin [131]
Cordycepin [118]	Thrombospondin-I [132]
Curcumin [119]	Valinomycin [133]
Cyclosporine [29]	Vanadate [134]
Energy depletion [62]	Vanadium [134]
Glycophorin-C [120]	Zinc [135]

Table 1. Stimulators of eryptosis

Inhibitors of eryptosis	
Activation of G kinase [76]	Ethylisopropylamiloride (EIPA) [69]
Adenosine [72]	Flufenamic acid [71]
Amiloride [43]	Isoproterenol [70]
Cl ⁻ [136]	Nitroxide [73]
Dopamine [70]	Staurosporine [47]
Epinephrine [70]	Urea [136]
Erythropoietin [64]	Zidovudine [137]

Table 2. Inhibitors of eryptosis

Most recently, a family has been described, which carries a mutation within GLUT1, the primary glucose transporter of erythrocytes and the blood brain barrier, turning the glucose carrier into a an unspecific leaky cation channel [104]. Affected individuals of the family suffered from paroxysmal exertion-induced dyskinesia, epilepsy, mild developmental delay, reduced CSF glucose levels and hemolytic anemia with echinocytosis [104]. Glucose uptake was significantly smaller in *Xenopus* oocytes expressing the mutated GLUT1 than in *Xenopus* oocytes expressing the normal GLUT1. In addition, the cytosolic Na⁺ concentration ([Na⁺]_i) was increased in *Xenopus* oocytes expressing the mutated GLUT1. Accordingly, in patients carrying the mutation, erythrocyte [Na⁺]_i was increased, erythrocyte [K⁺]_i decreased and the cells underwent accelerated eryptosis. Patch clamp and other functional studies in erythrocytes revealed enhanced cation permeability and increased Ca²⁺ entry.

GLUT1 mutations with a reduced glucose uptake were also found in two other families with the same movement disorder but they did not show hemolytic anemia and there was no cation leak induced by these mutations. The cellular mechanism of neuronal disease was thus reflected by reduced glucose transport across the blood-brain-barrier upon physical exertion, whereas the anemia with deformed erythrocytes occurred secondary to a cationic leak with deranged channel activity, cytosolic ion composition and suicidal death of erythrocytes [104].

Conclusions and perspectives

A wide variety of drugs, environmental pollutants and further xenobiotics, endogenous substances, clinical conditions and diseases trigger eryptosis, the suicidal death of erythrocytes. Stimulation of eryptosis involves activation of ion channels and ceramide formation and is governed by a complex signaling network. Additional triggers and signaling pathways are expected to

participate in the machinery leading to suicidal erythrocyte death.

Many of the signaling molecules regulating eryptosis are similarly instrumental in the regulation of apoptosis. Thus, accelerated eryptosis is frequently paralleled by suicidal death of nucleated cells, which may employ similar mechanisms. Thus, the analysis of eryptosis has the potential to unravel the pathogenesis of suicidal death of nucleated cells from less amenable tissues. Accordingly, erythrocytes could be exploited to gain insight into the mechanisms involved in drug side effects, toxicology and pathophysiology of disease.

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