Ceramide in Suicidal Death of Erythrocytes

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Key Words
Apoptosis • Red blood cells • Anemia • Sepsis • Malaria

Abstract
The suicidal death of erythrocytes or eryptosis is characterized by cell shrinkage, membrane blebbing and cell membrane phospholipid scrambling resulting in phosphatidylserine exposure at the cell surface. Eryptosis is stimulated in a wide variety of diseases including sepsis, haemolytic uremic syndrome, malaria, sickle-cell anemia, beta-thalassemia, glucose-6-phosphate dehydrogenase (G6PD)-deficiency, phosphate depletion, iron deficiency and Wilson’s disease. Moreover, eryptosis is elicited by osmotic shock, oxidative stress, energy depletion as well as a wide variety of endogenous mediators and xenobiotics. Excessive eryptosis is observed in erythrocytes lacking the cGMP-dependent protein kinase type I (cGKI) or the AMP-activated protein kinase AMPK. Inhibitors of eryptosis include erythropoietin, nitric oxide NO, catecholamines and high concentrations of urea. Eryptosis-triggering diseases and chemicals are partially effective by stimulating the formation of ceramide, which in turn fosters cell membrane scrambling. Accordingly, ceramide-induced eryptosis participates in the pathophysiology of several diseases and contributes to the effects of a large number of xenobiotics. The mechanisms underlying ceramide formation in erythrocytes are, however, still ill defined. In case of osmotic cell shrinkage, ceramide formation is apparently due to activation of phospholipase 2, leading to formation of platelet activating factor PAF and PAF-dependent stimulation of ceramide formation, which possibly involves acid sphingomyelinase. Additional experiments are needed to conclusively define the ceramide-generating enzyme and the ceramide-dependent cellular events eventually leading to suicidal erythrocyte death.

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Introduction

The life span of mature, circulating erythrocytes (some 100-120 days) is usually limited by senescence, which eventually results in the clearance of the aged erythrocytes [1-3]. Typical features of erythrocyte senescence include binding of hemichromes to band 3, clustering of band 3, and deposition of complement C3 fragments and anti band 3 immunoglobulins [4].

Prior to senescence, erythrocytes may undergo suicidal death or eryptosis [5-7]. As mature erythrocytes have lost their nuclei and mitochondria, important organelles in apoptosis, erythrocytes lack several classical features of apoptosis, such as mitochondrial depolarization and condensation of nuclei. Nevertheless, eryptosis shares several features of apoptosis, i.e. cell shrinkage, membrane blebbing and phosphatidylserine exposure [8-10]. The present short review compiles the present knowledge on mechanisms regulating, triggering and inhibiting the suicidal erythrocyte death or eryptosis. For a more detailed discussion of mechanisms involved, the reader is referred to earlier, more extensive reviews [5-7, 11-13].

Phosphatidylserine-exposing cells are bound to and subsequently engulfed by macrophages [14, 15] and are thus rapidly cleared from circulating blood [16]. Accordingly, enhanced eryptosis may cause anemia, as long as the accelerated loss of erythrocytes is not fully compensated by enhanced formation of new erythrocytes. Eryptotic erythrocytes may further adhere to the vascular wall [17-23]. Accordingly, excessive eryptosis may interfere with microcirculation. Moreover, the uptake of eryptotic cells by macrophages may trigger the release of pro-inflammatory cytokines which may sustain the hormonal stress response as it occurs in metabolic syndrome, a clinical condition wherein major cardiovascular disease risk factors such as obesity, insulin resistance, and hypertension all share a common abnormal ion profile, related also to a reduced GSH/GSSG ratio, in both nucleated and nonnucleated cells [24].

As listed in table 1, eryptosis has been observed in a wide variety of diseases. The excessive eryptosis may thus contribute to the pathophysiology of those diseases, such as anemia and deranged microcirculation. Excessive eryptosis has further been observed in a variety of gene-targeted mice, which thus disclose molecules involved in the regulation of erythrocyte survival (table 2). Moreover, eryptosis is triggered (table 3) or inhibited (table 4) by a wide variety of endogenous mediators and xenobiotics. The diseases and chemicals are in large part effective through increase of cytosolic Ca\(^{2+}\) activity or through stimulation of ceramide formation. The two mechanisms will thus be discussed in the following.

### Table 1. Diseases associated with enhanced eryptosis

<table>
<thead>
<tr>
<th>Diseases associated with accelerated eryptosis</th>
<th>Effective through</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>iron deficiency</td>
<td>Ca(^{2+})</td>
<td>[16]</td>
</tr>
<tr>
<td>phosphate depletion</td>
<td></td>
<td>[59]</td>
</tr>
<tr>
<td>noxocytosis</td>
<td>Ca(^{2+})</td>
<td>[60]</td>
</tr>
<tr>
<td>sepsis</td>
<td></td>
<td>[44]</td>
</tr>
<tr>
<td>hemolytic anemia</td>
<td></td>
<td>[61]</td>
</tr>
<tr>
<td>hemolytic uremic syndrome</td>
<td></td>
<td>[45]</td>
</tr>
<tr>
<td>renal insufficiency</td>
<td></td>
<td>[62]</td>
</tr>
<tr>
<td>malaria</td>
<td></td>
<td>[63, 64]</td>
</tr>
<tr>
<td>sickle cell disease</td>
<td></td>
<td>[11, 22, 48, 65-68]</td>
</tr>
<tr>
<td>thalassemia</td>
<td></td>
<td>[48, 65, 68-70]</td>
</tr>
<tr>
<td>glucose-6-phosphate dehydrogenase deficiency</td>
<td></td>
<td>[48, 71]</td>
</tr>
<tr>
<td>Wilson’s disease</td>
<td></td>
<td>[13]</td>
</tr>
<tr>
<td>APE1 mutation</td>
<td></td>
<td>[72]</td>
</tr>
<tr>
<td>GLUT1 mutation</td>
<td></td>
<td>[73]</td>
</tr>
</tbody>
</table>

### Table 2. Enhanced or decreased eryptosis in gene-targeted mice

<table>
<thead>
<tr>
<th>Targeted gene</th>
<th>Effective through</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enhanced eryptosis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Defective hemoglobin (sickle cell, thalassemia)</td>
<td>Ca(^{2+})</td>
<td>[67, 68]</td>
</tr>
<tr>
<td>cGMP-dependent protein kinase type I (cGKI) deficiency</td>
<td></td>
<td>[74]</td>
</tr>
<tr>
<td>AMP-activated protein kinase deficiency</td>
<td></td>
<td>[75]</td>
</tr>
<tr>
<td>Klotho deficiency</td>
<td></td>
<td>[76]</td>
</tr>
<tr>
<td>EPO excess</td>
<td></td>
<td>[77]</td>
</tr>
<tr>
<td>APE1 deficiency</td>
<td></td>
<td>[78]</td>
</tr>
<tr>
<td>PAF receptor deficiency</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reduced eryptosis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PDK1 deficiency</td>
<td>Ca(^{2+})</td>
<td>[79]</td>
</tr>
<tr>
<td>TRPC6 deficiency</td>
<td></td>
<td>[39]</td>
</tr>
</tbody>
</table>

Role of Ca\(^{2+}\)

Eryptosis is stimulated by an increase in cytosolic Ca\(^{2+}\) activity [8-10], which triggers cell membrane vesiculation [25], cell membrane scrambling [26-28] and activation of the cysteine endopeptidase calpain, an en-
Table 3. Stimulators of eryptosis (Mechanisms: Ca$^{2+}$ = stimulation of Ca$^{2+}$ entry, Cer. = stimulation of ceramide formation, other = ATP depletion etc.).

<table>
<thead>
<tr>
<th>Stimulators</th>
<th>Concentration</th>
<th>Effective through</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>aluminium</td>
<td>10-30 µM</td>
<td>+</td>
<td>[80]</td>
</tr>
<tr>
<td>amantadine</td>
<td>0.2 µg/ml</td>
<td>+</td>
<td>[81]</td>
</tr>
<tr>
<td>amiodarone</td>
<td>0.1 µM</td>
<td>+</td>
<td>[82]</td>
</tr>
<tr>
<td>amphotericin B</td>
<td>0.5 µg/ml</td>
<td>+</td>
<td>[83]</td>
</tr>
<tr>
<td>amylod</td>
<td>0.5-1 µM</td>
<td>+</td>
<td>[84]</td>
</tr>
<tr>
<td>amiodamide</td>
<td>2.5 µM</td>
<td>+</td>
<td>[85]</td>
</tr>
<tr>
<td>anti-A IgG</td>
<td>0.5 µg/ml</td>
<td>+</td>
<td>[86]</td>
</tr>
<tr>
<td>arsenic</td>
<td>7-10 µM</td>
<td>+</td>
<td>[87, 88]</td>
</tr>
<tr>
<td>arachidonic acid</td>
<td>2 µg/ml</td>
<td>+</td>
<td>[89, 90]</td>
</tr>
<tr>
<td>Bay-Y8844</td>
<td>20 µM</td>
<td>+</td>
<td>[91]</td>
</tr>
<tr>
<td>Bismuth chloride</td>
<td>500 µg/ml</td>
<td>+</td>
<td>[92]</td>
</tr>
<tr>
<td>cadmium</td>
<td>5.5 µM</td>
<td>+</td>
<td>[93]</td>
</tr>
<tr>
<td>chlorpromazine</td>
<td>10 µM</td>
<td>+</td>
<td>[94]</td>
</tr>
<tr>
<td>ciglitazone</td>
<td>5-10 µM</td>
<td>+</td>
<td>[94]</td>
</tr>
<tr>
<td>cisplatin</td>
<td>1 µM</td>
<td>+</td>
<td>[95]</td>
</tr>
<tr>
<td>copper</td>
<td>3 µM</td>
<td>+</td>
<td>[96]</td>
</tr>
<tr>
<td>cordycepin</td>
<td>60 µM</td>
<td>+</td>
<td>[97]</td>
</tr>
<tr>
<td>curcumin</td>
<td>1 µM</td>
<td>+</td>
<td>[98]</td>
</tr>
<tr>
<td>cyclosporine</td>
<td>10 µM</td>
<td>+</td>
<td>[99]</td>
</tr>
<tr>
<td>CD95/Fas ligand</td>
<td>+</td>
<td></td>
<td>[100]</td>
</tr>
<tr>
<td>glycyphorin-C</td>
<td>+</td>
<td></td>
<td>[101]</td>
</tr>
<tr>
<td>gold chloride</td>
<td>0.75 µg/ml</td>
<td>+</td>
<td>[102]</td>
</tr>
<tr>
<td>hemin</td>
<td>1-10 µM</td>
<td>+</td>
<td>[103]</td>
</tr>
<tr>
<td>hemolyzin</td>
<td>1 U/ml</td>
<td>+</td>
<td>[104]</td>
</tr>
<tr>
<td>lead</td>
<td>0.1 µM</td>
<td>+</td>
<td>[105]</td>
</tr>
<tr>
<td>leukotriene C(4)</td>
<td>10 nM</td>
<td>+</td>
<td>[106]</td>
</tr>
<tr>
<td>lipopeptides</td>
<td>1 µM</td>
<td>+</td>
<td>[107]</td>
</tr>
<tr>
<td>lysterolysin</td>
<td>10 µg/ml</td>
<td>+</td>
<td>[108]</td>
</tr>
<tr>
<td>lithium</td>
<td>1 mM</td>
<td>+</td>
<td>[109]</td>
</tr>
<tr>
<td>mercury</td>
<td>1 µM</td>
<td>+</td>
<td>[110]</td>
</tr>
<tr>
<td>methylolpa</td>
<td>6 µg/ml</td>
<td>+</td>
<td>[111]</td>
</tr>
<tr>
<td>methylglucaron</td>
<td>0.3 µM</td>
<td>+</td>
<td>[112]</td>
</tr>
<tr>
<td>pachulin</td>
<td>10 µM</td>
<td>+</td>
<td>[113]</td>
</tr>
<tr>
<td>PAI</td>
<td>0.5-1 µM</td>
<td>+</td>
<td>[114]</td>
</tr>
<tr>
<td>peptidoglycan</td>
<td>50 µg/ml</td>
<td>+</td>
<td>[115]</td>
</tr>
<tr>
<td>prostaglandin E2</td>
<td>100 pM</td>
<td>+</td>
<td>[116]</td>
</tr>
<tr>
<td>radiocontrast agents</td>
<td>5 µM</td>
<td>+</td>
<td>[117]</td>
</tr>
<tr>
<td>retinoic acid</td>
<td>3 µM</td>
<td>+</td>
<td>[118]</td>
</tr>
<tr>
<td>Selenium (sodium selenide)</td>
<td>200 ng/ml</td>
<td>+</td>
<td>[119]</td>
</tr>
<tr>
<td>silver ions</td>
<td>100 nM</td>
<td>+</td>
<td>[120]</td>
</tr>
<tr>
<td>thrombospondin-1-receptor</td>
<td>+</td>
<td></td>
<td>[121]</td>
</tr>
<tr>
<td>CD47</td>
<td>+</td>
<td></td>
<td>[122]</td>
</tr>
<tr>
<td>thymoquinone</td>
<td>3 µM</td>
<td>+</td>
<td>[123]</td>
</tr>
<tr>
<td>tin</td>
<td>30 µM</td>
<td>+</td>
<td>[124]</td>
</tr>
<tr>
<td>valinomycin</td>
<td>1 nM</td>
<td>+</td>
<td>[125]</td>
</tr>
<tr>
<td>Sodium vanadate</td>
<td>10 µg/ml</td>
<td>+</td>
<td>[126]</td>
</tr>
<tr>
<td>vitamin K(3)</td>
<td>1 µM</td>
<td>+</td>
<td>[127]</td>
</tr>
<tr>
<td>zinc</td>
<td>25 µM</td>
<td>+</td>
<td>[128]</td>
</tr>
</tbody>
</table>

Table 4. Inhibitors of eryptosis (Mechanisms: Ca$^{2+}$ = inhibition of Ca$^{2+}$ entry, Cer. = inhibition of ceramide formation, other = inhibition of ATP depletion etc.).

<table>
<thead>
<tr>
<th>Inhibitors</th>
<th>Concentr.</th>
<th>Effective through</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>adenosine</td>
<td>10-30 µM</td>
<td>+</td>
<td>[129]</td>
</tr>
<tr>
<td>amitryptiline</td>
<td>50 µM</td>
<td>+</td>
<td>[130]</td>
</tr>
<tr>
<td>caffeine</td>
<td>50-500 µM</td>
<td>+</td>
<td>[131]</td>
</tr>
<tr>
<td>catecholamines (isoproterenol)</td>
<td>IC50: 1 µM</td>
<td>+</td>
<td>[132]</td>
</tr>
<tr>
<td>ceramide chloride</td>
<td>+</td>
<td></td>
<td>[133]</td>
</tr>
<tr>
<td>EIPA</td>
<td>IC50: 0.2 µM</td>
<td>+</td>
<td>[134]</td>
</tr>
<tr>
<td>EPO</td>
<td>1 U/ml</td>
<td>+</td>
<td>[135]</td>
</tr>
<tr>
<td>fluteceric acid</td>
<td>10 µM</td>
<td>+</td>
<td>[136]</td>
</tr>
<tr>
<td>NBQX/CNOX</td>
<td>10-50 µM</td>
<td>+</td>
<td>[137]</td>
</tr>
<tr>
<td>nilutamic acid</td>
<td>100 µM</td>
<td>+</td>
<td>[138]</td>
</tr>
<tr>
<td>NO (nitroprusside)</td>
<td>1 µM</td>
<td>+</td>
<td>[139]</td>
</tr>
<tr>
<td>NPPB</td>
<td>100 µM</td>
<td>+</td>
<td>[140]</td>
</tr>
<tr>
<td>resveratrol</td>
<td>5 µM</td>
<td>+</td>
<td>[141]</td>
</tr>
<tr>
<td>staurosporine</td>
<td>500 nM</td>
<td>+</td>
<td>[142]</td>
</tr>
<tr>
<td>urea</td>
<td>650 µM</td>
<td>+</td>
<td>[143]</td>
</tr>
<tr>
<td>xanthohumol</td>
<td>1 µM</td>
<td>+</td>
<td>[144]</td>
</tr>
<tr>
<td>zidovudine</td>
<td>2 µg/ml</td>
<td>+</td>
<td>[145]</td>
</tr>
</tbody>
</table>

Role of ceramide

Eryptosis is further stimulated by ceramide [43]. Ceramide enhances the sensitivity of erythrocytes to the eryptotic effect of enhanced Ca$^{2+}$ concentration [43]. The enzyme accounting for the formation of ceramide, has, however, remained elusive. Enhanced eryptosis in sepsis [44] and hemolytic uremic syndrome [45] is secondary to the capability of serum from the respective patients to trigger eryptosis. Possibly, serum contains sphingomyelinase activity in those diseases. Along those lines, evidence for sphingomyelinase activity has been observed in the serum of patients suffering from Wilson’s disease [13]. Mechanisms contributing to the stimulation of ceramide formation include platelet-activating factor PAF [46]. Upon osmotic cell shrinkage, PAF is released from erythrocytes [46]. Erythrocytes express PAF receptors, and PAF stimulates the breakdown of sphingomyelin leading to ceramide formation even under iso-
tonic conditions [46]. Both, ceramide formation and eryptosis following PAF treatment were blunted in gene-targeted mice lacking PAF receptors [46].

**Further mechanisms**

Additional mechanisms underlying stimulation of eryptosis include energy depletion [47], oxidative stress [48-50] or impaired antioxidative defence [51-53]. Oxidative stress activates the Ca\(^{2+}\)-permeable cation channels [42] and erythrocyte Cl channels [54, 55], the latter contributing to eryptotic cell shrinkage [56]. Oxidative stress may further trigger eryptosis by activation of caspases [9, 57, 58].

**Conclusions**

Ceramide formation is one of several mechanisms triggering eryptosis. Additional experiments are needed to conclusively define the ceramide-generating enzyme and the ceramide-dependent cellular events eventually leading to suicidal erythrocyte death.

**Acknowledgements**

The authors acknowledge the meticulous preparation of the manuscript by Tanja Loch and Lejla Subasic. Their research is supported by the Carl-Zeiss-Stiftung and the Deutsche Forschungsgemeinschaft, Nr. La 315/4-3, La 315/6-1, La 315/13-1 and Hu781/4-3.


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Sopjani M, Foller M, Lang F: Gold stimulates Ca2+ entry into and subsequent suicidal death of erythrocytes. Toxicology 2008;244:271-279.


