Ceramide in Suicidal Death of Erythrocytes

Florian Lang, Erich Gulbins¹, Philipp A. Lang, Donatella Zappulla²
and Michael Föller

Department of Physiology, University of Tübingen, Tübingen, ¹Department of Molecular Biology, University of Duisburg-Essen, ²Institute of Physiology and Department of Internal Medicine, University of Catania Medical School, Catania

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

Diseases associated with accelerated eryptosis	Effective through			References
	Ca ²⁺	Cer.	Other	
iron deficiency	+			[16]
phosphate depletion			+	[59]
neocytolysis			+	[60]
sepsis		+		[44]
hemolytic anemia	+	+	+	[61]
hemolytic uremic syndrome	+	+		[45]
renal insufficiency	+			[62]
malaria	+			[6, 63, 64]
sickle cell disease			+	[11, 22, 48, 65-68]
thalassemia			+	[48, 65, 68-70]
glucose-6-phosphate dehydroge- nase (G6PD) deficiency			+	[48, 71]
Wilson's disease		+		[13]
AE1 mutation	+			[72]
GLUT1 mutation	+			[73]

Table 1. Diseases associated with enhanced eryptosis (Mechanisms: Ca²⁺ = stimulation of Ca²⁺ entry, Cer. = stimulation of ceramide formation, other = inhibition of ATP depletion etc.)

Targeted gene	Effective through			References
	Ca ²⁺	Cer.	Other	
<i>Enhanced eryptosis</i>				
Defective hemoglobin (sickle cell, thalassemia)	+			[67, 68]
cGMP-dependent protein kinase type I (cGKI) deficiency	+			[74]
AMP-activated protein kinase deficiency	+			[75]
Klotho deficiency	+			[76]
EPO excess	+			[77]
AE1 deficiency	+			[78]
PAF receptor deficiency		+		[46]
<i>Reduced eryptosis</i>				
PDK1 deficiency	+			[79]
TRPC6 deficiency	+			[39]

Table 2. Enhanced or decreased eryptosis in gene-targeted mice (Mechanisms: Ca²⁺ = stimulation of Ca²⁺ entry, Cer. = stimulation of ceramide formation, other = inhibition of ATP depletion etc.).

part effective through increase of cytosolic Ca²⁺ activity or through stimulation of ceramide formation. The two mechanisms will thus be discussed in the following.

Role of Ca²⁺

Eryptosis is stimulated by an increase in cytosolic Ca²⁺ activity [8-10], which triggers cell membrane vesiculation [25], cell membrane scrambling [26-28] and activation of the cysteine endopeptidase calpain, an en-

Stimulators	Concentration	Effective through			References
		Ca ²⁺	Cer.	Other	
aluminium	10-30 µM	+			[80]
amantadine	0.2 µg/ml	+			[81]
amiodarone	0.1 µM	+			[82]
amphotericin B	0.5 µg/ml	+		+	[83]
amyloid	0.5-1 µM		+		[84]
anandamide	2.5 µM	+			[85]
anti-A IgG	0.5 µg/ml	+			[86]
arsenic	7-10 µM	+	+	+	[87, 88]
azathioprine	2 µg/ml	+			[89, 90]
Bay-Y5884	20 µM	+			[91]
Bismuth chloride	500 µg/ml	+	+		[92]
cadmium	5.5 µM	+		+	[93]
chlorpromazine	10 µM	+			[26]
ciglitazone	5-10 µM	+			[94]
cisplatin	1 µM	+			[95]
copper	3 µM		+		[13]
cordycepin	60 µM	+			[96]
curcumin	1 µM	+	+		[97]
cyclosporine	10 µM		+	+	[28, 98]
CD95/Fas/ ligand				+	[99]
glycophorin-C				+	[100]
gold chloride	0.75 µg/ml	+			[101]
hemin	1-10 µM	+	+		[102]
hemolysin	1 U/ml	+			[103]
lead	0.1 µM	+			[104]
leukotriene C(4)	10 nM	+			[105]
lipopeptides	1 µM			+	[106]
listeriolysin	10 ng/ml	+		+	[107]
lithium	1 mM	+			[108]
mercury	1 µM		+	+	[109]
methyl dopa	6 µg/ml		+	+	[110]
methylglyoxal	0.3 µM		+		[27]
paclitaxel	10 µM	+	+		[111, 112]
PAF	0.5-1 µM		+		[46]
peptidoglycan	50 µg/ml	+	+		[113]
prostaglandin E ₂	100 pM				[38]
radiocontrast agents	5 mM	+		+	[114]
retinoic acid	3 µM	+			[115]
Selenium (sodium selenite)	200 ng/ml	+	+		[116]
silver ions	100 nM			+	[117]
thrombospondin-1-receptor					
CD47				+	[118]
thymoquinone	3 µM			+	[119]
tin	30 µM	+	+	+	[120]
valinomycin	1 nM			+	[121]
Sodium vanadate	10 µg/ml	+			[12]
vitamin K(3)	1 µM		+		[122]
zinc	25 µM	+	+		[123]

Table 3. Stimulators of eryptosis (Mechanisms: Ca²⁺ = stimulation of Ca²⁺ entry, Cer. = stimulation of ceramide formation, other = ATP depletion etc.).

zyme degrading the cytoskeleton and thus causing cell membrane blebbing [29]. Ca²⁺ further stimulates Ca²⁺-sensitive K⁺ channels [30-32], the subsequent efflux of K⁺ hyperpolarizes the cell membrane, which drives Cl⁻ exit [33]. The cellular loss of KCl with osmotically obliged water leads to cell shrinkage [33].

The increase in cytosolic Ca²⁺ activity may result from entry through non-selective cation channels [34-38]. The entire molecular identity of the cation channels re-

Inhibitors	Concentr.	Effective through			References
		Ca ²⁺	Cer.	Other	
adenosine	10-30 µM	+		+	[124]
amitriptyline	50 µM		+	+	[125]
caffeine	50-500 µM	+		+	[126]
catecholamines	IC50: 1 µM	+			[127]
(isoproterenol)					
chloride		+			[40]
EIPA	IC50: 0.2 µM	+			[128]
EPO	1 U/ml	+			[62]
flufenamic acid	10 µM	+			[129]
NBQX/CNQX	10-50 µM	+			[130]
niflumic acid	100 µM			+	[56]
NO (nitroprusside)	1 µM			+	[131]
NPPB	100 µM			+	[56]
resveratrol	5 µM	+		+	[132]
staurosporine	500 nM			+	[47]
urea	650 µM		+	+	[133]
xanthohumol	1 µM	+		+	[134]
zidovudine	2 µg/ml	+			[135]

Table 4. Inhibitors of eryptosis (Mechanisms: Ca²⁺ = inhibition of Ca²⁺ entry, Cer. = inhibition of ceramide formation, other = inhibition of ATP depletion etc.).

mains incompletely understood but apparently involves TRPC6 [39]. Stimulators of those channels include osmotic shock [40, 41], oxidative stress [41, 42] and Cl⁻ removal [38, 40, 42].

Role of ceramide

Eryptosis is further stimulated by ceramide [43]. Ceramide enhances the sensitivity of erythrocytes to the eryptotic effect of enhanced Ca²⁺ 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 Ca²⁺-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.

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