Original Paper

Anidulafungin-Induced Suicidal Erythrocyte Death

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Key Words

Phosphatidylserine • Cell volume • Eryptosis • SB203580 • zVAD • Calcium • Hemolysis

Abstract

Background/Aims: The novel antifungal drug Anidulafungin is used for the treatment of diverse fungal infections including candidiasis and aspergillosis. The traditional antifungal drug amphotericin B has previously been shown to trigger eryptosis, the suicidal death of erythrocytes characterized by cell shrinkage and cell membrane scrambling with phosphatidylserine translocation to the erythrocyte surface. Triggers of eryptosis include increase of cytosolic Ca^{2+} activity ($[\text{Ca}^{2+}]_i$), oxidative stress, ceramide, activated protein kinase C (PKC), casein kinase 1α or p38 kinase and activated caspases. Inhibitors of eryptosis include nitric oxide (NO). The present study explored, whether Anidulafungin induces eryptosis. **Methods:** Flow cytometry was employed to estimate phosphatidylserine abundance at the erythrocyte surface from annexin-V-binding, cell volume from forward scatter, $[\text{Ca}^{2+}]_i$ from Fluo3-fluorescence, abundance of reactive oxygen species (ROS) from DCFDA dependent fluorescence, and ceramide abundance at the erythrocyte surface utilizing specific antibodies. Hemolysis was quantified by measuring haemoglobin concentration in the supernatant. **Results:** A 48 hours exposure of human erythrocytes to Anidulafungin (1.5 - 6 $\mu\text{g/ml}$) significantly increased hemolysis and the percentage of annexin-V-binding cells, and significantly decreased forward scatter. Anidulafungin (6 $\mu\text{g/ml}$) slightly, but significantly increased Fluo3-fluorescence and the effect of Anidulafungin on annexin-V-binding was slightly, but significantly blunted by removal of extracellular Ca^{2+} . The effect of Anidulafungin on annexin-V-binding was further significantly blunted by the p38 kinase inhibitor SB203580 (2 μM) and NO donor nitroprusside (1 μM). An increase of extracellular K^+ concentration significantly blunted the effect of Anidulafungin on cell volume but not on annexin-V-binding. Anidulafungin rather decreased DCFDA fluorescence and the effect of Anidulafungin on annexin-V-binding was not significantly blunted by the antioxidant N-acetylcysteine (1 mM). Moreover, the effect of Anidulafungin on annexin-V-binding was not paralleled by significant increase of ceramide abundance and was not significantly blunted by PKC inhibitor staurosporine (1 μM), casein kinase 1α inhibitor D4476 (10 μM) or pancaspase inhibitor zVAD (10 μM). **Conclusions:** Anidulafungin triggers hemolysis and eryptosis with cell shrinkage and phospholipid scrambling of the erythrocyte cell membrane, an effect in part due to Ca^{2+} entry and activation of p38 kinase.

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Introduction

The 1,3-beta-glucan synthesis inhibitor Anidulafungin [1] is a relatively novel antifungal drug effective against a broad range of fungal infections [2-4], such as aspergillosis [5-15] and candidiasis [6-31]. The older commonly used antifungal drug amphotericin B [32] has previously been shown to trigger eryptosis [33, 34], the suicidal death of erythrocytes characterized by cell shrinkage [35] and cell membrane scrambling with phosphatidylserine translocation to the cell surface [36]. Mechanisms engaged in the stimulation of eryptosis include Ca^{2+} entry with increase of cytosolic Ca^{2+} activity ($[\text{Ca}^{2+}]_i$) [36], ceramide [37], oxidative stress [36], energy depletion [36], caspases [36, 38, 39], as well as activation of several kinases including casein kinase 1 α , Janus-activated kinase JAK3, protein kinase C, and p38 kinase [36]. Eryptosis could further be triggered by inhibition of AMP activated kinase AMPK, cGMP-dependent protein kinase, PAK2 kinase and sorafenib/sunitinib sensitive kinases [36]. Cell membrane scrambling is further fostered by cell shrinkage [36].

Eryptosis is stimulated by a wide variety of small molecules [36, 40-70]. Eryptosis could be inhibited by nitric oxide (NO) [36].

The present study tested, whether Anidulafungin triggers eryptosis. To this end, human erythrocytes were exposed to Anidulafungin and phosphatidylserine surface abundance, cell volume, $[\text{Ca}^{2+}]_i$, abundance of reactive oxygen species (ROS) and ceramide determined by flow cytometry.

Materials and Methods

Erythrocytes, solutions and chemicals

Fresh Li-Heparin-anticoagulated blood samples were kindly provided by the blood bank of the University of Tübingen. The study is approved by the ethics committee of the University of Tübingen (184/2003 V). The blood was centrifuged at 120 g for 20 min at 21 °C and the platelets and leukocytes-containing supernatant was disposed. Erythrocytes were incubated *in vitro* at a hematocrit of 0.4% in Ringer solution containing (in mM) 125 NaCl, 5 KCl, 1 MgSO_4 , 32 N-2-hydroxyethylpiperazine-N-2-ethanesulfonic acid (HEPES; pH 7.4), 5 glucose, 1 CaCl_2 , at 37°C for 48 hours. Where indicated, erythrocytes were exposed for 48 hours to Anidulafungin (Selleckchem, Munich, Germany). To test for an involvement of oxidative stress, erythrocytes were exposed for 48 hours to a combination of Anidulafungin and the antioxidant N-acetylcysteine (Sigma Aldrich, Hamburg, Germany). To test for an involvement of protein kinase C or p38 kinase, erythrocytes were exposed for 48 hours to a combination of Anidulafungin and either PKC inhibitor staurosporine (Tocris bioscience, Bristol, UK) or p38 kinase inhibitor SB203580 (Tocris bioscience, Bristol, UK). In order to test, whether the casein kinase 1 α is involved, erythrocytes were exposed for 48 hours to a combination of Anidulafungin and casein kinase 1 α inhibitor D4476 (Sigma Aldrich, Hamburg, Germany). To test for an involvement of caspases, erythrocytes were exposed for 48 hours to a combination of Anidulafungin and pancaspase inhibitor zVAD (Enzo Life Sciences, Lörrach, Germany).

Annexin-V-binding and forward scatter

After incubation under the respective experimental condition, a 150 μl cell suspension of erythrocytes was centrifuged at 1600 rpm for 3 min and, after trashing the supernatant, the erythrocytes were stained with Annexin-V-FITC (1:200 dilution; ImmunoTools, Friesoythe, Germany) in this solution at 37°C for 15 min under protection from light. The annexin-V-abundance at the erythrocyte surface was subsequently determined on a FACS Calibur (BD, Heidelberg, Germany). Annexin-V-binding was measured with an excitation wavelength of 488 nm and an emission wavelength of 530 nm. A marker (M1) was placed to set an arbitrary threshold between annexin-V-binding cells and control cells. The same threshold was used for untreated and Anidulafungin treated erythrocytes. A dot plot of forward scatter (FSC) vs. side scatter (SSC) was set to linear scale for both parameters. The threshold of forward scatter was set at the default value of "52".

Intracellular Ca²⁺

After incubation, erythrocytes were washed in Ringer solution and then loaded with Fluo-3/AM (Biotium, Hayward, USA) in Ringer solution containing 5 mM CaCl₂ and 5 μM Fluo-3/AM. The cells were incubated at 37°C for 30 min and washed once in Ringer solution containing 5 mM CaCl₂. The Fluo-3/AM-loaded erythrocytes were resuspended in 200 μl Ringer solution. Then, Ca²⁺-dependent fluorescence intensity was measured in FL-1 with an excitation wavelength of 488 nm and an emission wavelength of 530 nm on a FACS Calibur. Afterwards, the geomean of the Ca²⁺ dependent fluorescence was determined.

Reactive oxygen species (ROS)

Oxidative stress was determined utilizing 2',7'-dichlorodihydrofluorescein diacetate (DCFDA). After incubation, a 150 μl suspension of erythrocytes was washed in Ringer solution and then stained with DCFDA (Sigma Aldrich, Hamburg, Germany) in Ringer solution containing DCFDA at a final concentration of 10 μM. Erythrocytes were incubated at 37°C for 30 min in the dark and then washed two times in Ringer solution. The DCFDA-loaded erythrocytes were resuspended in 200 μl Ringer solution, and ROS-dependent fluorescence intensity was measured in FL-1 at an excitation wavelength of 488 nm and an emission wavelength of 530 nm on a FACS Calibur (BD). Subsequently, the geomean of the ROS-dependent fluorescence was determined

Ceramide abundance

To determine the ceramide abundance at the erythrocyte surface, a monoclonal antibody was used. After incubation, cells were stained for 1 h at 37°C with 1 μg/ml anti-ceramide antibody (clone MID 15B4; Alexis, Grünberg, Germany) in phosphate-buffered saline (PBS) containing 0.1 % bovine serum albumin (BSA) at a dilution of 1:10. After two washing steps with PBS-BSA, cells were stained for 30 min with polyclonal fluorescein-isothiocyanate (FITC)-conjugated goat anti-mouse IgG and IgM specific antibody (BD Pharmingen, Hamburg, Germany) diluted 1:50 in PBS-BSA. Unbound secondary antibody was removed by repeated washing with PBS-BSA. Samples were then analyzed by flow cytometry in FL-1 at an excitation wavelength of 488 nm and an emission wavelength of 530 nm. Finally, the geomean of the ceramide dependent fluorescence was determined.

Statistics

Data are expressed as arithmetic means ± SEM. As indicated in the figure legends, statistical analysis was made using ANOVA with Tukey's test as post-test and *t* test as appropriate. *n* denotes the number of different erythrocyte specimens studied. Since different erythrocyte specimens used in distinct experiments are differently susceptible to triggers of eryptosis, the same erythrocyte specimens have been used for control and experimental conditions.

Results

The present study explored whether Anidulafungin is able to trigger eryptosis, the suicidal erythrocyte death characterized by cell shrinkage and by phospholipid scrambling of the cell membrane with phosphatidylserine translocation to the cell surface. To this end, erythrocytes drawn from healthy volunteers were incubated for 48 hours in Ringer solution without or with Anidulafungin (1.5 – 6 μg/ml).

Erythrocyte volume was estimated from forward scatter, which was determined utilizing flow cytometry. As illustrated in Fig. 1, Anidulafungin (1.5 – 6 μg/ml) significantly decreased erythrocyte forward scatter. Moreover, Anidulafungin significantly increased the percentage of shrunken erythrocytes (Fig. 1C), an effect reaching statistical significance at 3 μg/ml Anidulafungin concentration. Anidulafungin treatment decreased the percentage of swollen erythrocytes (Fig. 1D), an effect reaching statistical significance at 1.5 μg/ml Anidulafungin concentration. The percentage of hemolytic erythrocytes was estimated from hemoglobin concentration in the supernatant. As illustrated in Fig. 2, a 48 hours exposure to Anidulafungin increased the percentage of hemolytic erythrocytes, an effect reaching statistical significance at 6 μg/ml Anidulafungin concentration.

Fig. 1. Effect of Anidulafungin on erythrocyte forward scatter. (A) Original histogram of forward scatter of erythrocytes following exposure for 48 hours to Ringer solution without (grey area) and with (black line) presence of 6 µg/ml Anidulafungin. (B) Arithmetic means $\pm$ SEM ($n = 12$) of the erythrocyte forward scatter (FSC) following incubation for 48 hours to Ringer solution without (white bar) or with (black bars) Anidulafungin (1.5 - 6 µg/ml). (C) Arithmetic means $\pm$ SEM ($n = 12$) of the percentage of erythrocytes with forward scatter (FSC) <200 following incubation for 48 hours to Ringer solution without (white bar) or with (black bars) Anidulafungin (1.5 - 6 µg/ml). (D) Arithmetic means $\pm$ SEM ($n = 12$) of the percentage of erythrocytes with forward scatter (FSC) >800 following incubation for 48 hours to Ringer solution without (white bar) or with (black bars) Anidulafungin (1.5 - 6 µg/ml). ***($p < 0.001$) indicates significant difference from the absence of Anidulafungin (ANOVA).

Fig. 2. Effect of Anidulafungin on hemolysis. Arithmetic means $\pm$ SEM ($n = 8$) of the percentage of hemolytic erythrocytes following incubation for 48 hours to Ringer solution without (white bar) or with (black bars) Anidulafungin (1.5 - 6 µg/ml). ***($p < 0.001$) indicates significant difference from the absence of Anidulafungin (ANOVA).

Phosphatidylserine exposing erythrocytes were identified utilizing annexin-V-binding, as determined by flow cytometry. As illustrated in Fig. 3, a 48 hours exposure to Anidulafungin increased the percentage of phosphatidylserine exposing erythrocytes, an effect reaching statistical significance at 3 µg/ml Anidulafungin concentration.

Fluo3 fluorescence was taken as a measure of cytosolic Ca^{2+} activity ($[\text{Ca}^{2+}]_i$). As illustrated in Fig. 4, a 48 hours exposure to 6 µg/ml Anidulafungin significantly increased the Fluo3 fluorescence.

Fig. 3. Effect of Anidulafungin on phosphatidylserine exposure. (A) Original histogram of annexin-V-binding of erythrocytes following exposure for 48 hours to Ringer solution without (grey area) and with (black line) presence of 6 µg/ml Anidulafungin. (B) Arithmetic means $\pm$ SEM ($n = 12$) of erythrocyte annexin-V-binding following incubation for 48 hours to Ringer solution without (white bar) or with (black bars) Anidulafungin (1.5 - 6 µg/ml). ***($p < 0.001$) indicates significant difference from the absence of Anidulafungin (ANOVA).

Fig. 4. Effect of Anidulafungin on cytosolic Ca²⁺ activity. (A) Original histogram of Fluo3 fluorescence in erythrocytes following exposure for 48 hours Ringer solution without (grey area) and with (black line) presence of 6 µg/ml Anidulafungin. (B) Arithmetic means $\pm$ SEM ($n = 12$) of erythrocyte Fluo3 fluorescence following incubation for 48 hours to Ringer solution without (white bar) or with (black bars) Anidulafungin (1.5 - 6 µg/ml). *($p < 0.05$) indicates significant difference from the absence of Anidulafungin (ANOVA).

A next series of experiments explored whether the Anidulafungin-induced translocation of phosphatidylserine was sensitive to extracellular Ca²⁺. To this end, erythrocytes were incubated for 48 hours in the absence or presence of 6 µg/ml Anidulafungin in the presence or nominal absence of extracellular Ca²⁺. As shown in Fig. 5, removal of extracellular Ca²⁺ slightly but significantly blunted the effect of Anidulafungin on the percentage of annexin-V-binding erythrocytes. However, even in the absence of extracellular Ca²⁺, Anidulafungin significantly increased the percentage of annexin-V-binding erythrocytes. Thus, Anidulafungin-induced cell shrinkage was in part, but not fully dependent on the presence of extracellular Ca²⁺.

Eryptosis is further stimulated by oxidative stress. Reactive oxygen species (ROS) was thus quantified utilizing 2',7'-dichlorodihydrofluorescein diacetate (DCFDA). As a result, the DCFDA fluorescence was significantly ($p < 0.001$) lower in the presence of 3 µg/ml Anidulafungin (11.02 ± 0.29 a.u., $n = 8$) or 6 µg/ml Anidulafungin (12.11 ± 0.69 a.u., $n = 8$) than in the absence of Anidulafungin (15.79 ± 0.48 a.u., $n = 8$). Thus, Anidulafungin rather decreased oxidative stress.

Fig. 5. Ca²⁺ sensitivity of Anidulafungin-induced phosphatidylserine exposure. A,B. Original histograms of annexin-V-binding of erythrocytes following exposure for 48 hours to Ringer solution without (grey areas) and with (black lines) Anidulafungin (6 μg/ml) in the presence (A) and absence (B) of extracellular Ca²⁺. (C) Arithmetic means ± SEM (n = 11) of annexin-V-binding of erythrocytes after a 48 hours treatment with Ringer solution without (white bars) or with (black bars) Anidulafungin (6 μg/ml) in the presence (left bars, + Calcium) and absence (right bars, - Calcium) of Ca²⁺. ***(*p* < 0.001) indicates significant difference from the absence of Anidulafungin, #(*p* < 0.05) indicates significant difference from the presence of Ca²⁺ (ANOVA).

To explore, whether the effect of Anidulafungin on cell membrane scrambling could be modified by the redox state, the influence of Anidulafungin on annexin-V-binding was tested in the absence or presence of the antioxidant N-acetylcysteine (1 mM). As a result, Anidulafungin (6 μg/ml) increased the percentage of phosphatidylserine exposing erythrocytes to similar values in the absence (from 2.04 ± 0.42 % to 15.66 ± 0.94 %, n = 8) and in the presence (from 1.74 ± 0.22 % to 17.36 ± 0.75 %, n = 8) of N-acetylcysteine (1 mM).

A further stimulator of eryptosis is ceramide. Ceramide abundance at the erythrocyte surface was thus quantified utilizing specific antibodies. As a result, the ceramide abundance was similar following exposure to 6 μg/ml Anidulafungin (15.25 ± 2.17 a.u., n = 5) and in the absence of Anidulafungin (15.65 ± 0.35 a.u., n = 5). Thus, Anidulafungin did not appreciably modify ceramide abundance.

To explore, whether the effects of Anidulafungin involved p38 kinase activity, the influence of Anidulafungin on annexin-V-binding was tested in the absence or presence of p38 kinase inhibitor SB203580 (2 μM). As shown in Fig. 6, addition of SB203580 (2 μM) significantly blunted the effect of Anidulafungin on the percentage of annexin-V-binding erythrocytes. However, even in the presence of SB203580, Anidulafungin significantly increased the percentage of annexin-V-binding erythrocytes. Thus, Anidulafungin-induced cell shrinkage was in part but not fully dependent on p38 kinase activity.

To explore, whether the effects of Anidulafungin involved protein kinase C, the influence of Anidulafungin on annexin-V-binding was tested in the absence or presence of protein kinase C inhibitor staurosporine (1 μM). As a result, Anidulafungin (6 μg/ml) increased the percentage of phosphatidylserine exposing erythrocytes to similar values in the absence (from 3.03 ± 0.79 % to 30.98 ± 1.16 %, n = 8) and in the presence (from 4.78 ± 0.78 % to 30.50 ± 0.93 %, n = 8) of staurosporine (1 μM).

Fig. 6. SB203580 sensitivity of Anidulafungin-induced phosphatidylserine exposure. A,B. Original histograms of annexin-V-binding of erythrocytes following exposure for 48 hours to Ringer solution without (grey areas) and with (black line) Anidulafungin (6 µg/ml) in the absence (A) and presence (B) of SB203580 (2 µM). (C) Arithmetic means $\pm$ SEM (n = 8) of annexin-V-binding of erythrocytes after a 48 hours treatment with Ringer solution without (white bars) or with (black bars) Anidulafungin (6 µg/ml) in the absence (left bars) and presence (right bars) of 2 µM SB203580. *** (p < 0.001) indicates significant difference from the absence of Anidulafungin, ### (p < 0.001) indicates significant difference from the absence of 2 µM SB203580 (ANOVA).

In order to test, whether the effects of Anidulafungin involved casein kinase 1 α , the influence of Anidulafungin on annexin-V-binding was tested in the absence or presence of casein kinase 1 α inhibitor D4476 (10 µM). As a result, Anidulafungin (6 µg/ml) increased the percentage of phosphatidylserine exposing erythrocytes to similar values in the absence (from 1.44 ± 0.22 % to 18.64 ± 1.76 %, n = 8) and in the presence (from 1.58 ± 0.12 % to 20.20 ± 1.88 %, n = 8) of D4476 (10 µM).

A further series of experiments addressed the putative involvement of caspases in Anidulafungin induced eryptosis. To this end, the influence of Anidulafungin on annexin-V-binding was tested in the absence or presence of pancaspase inhibitor zVAD (10 µM). As a result, Anidulafungin (6 µg/ml) increased the percentage of phosphatidylserine exposing erythrocytes to similar values in the absence (from 2.03 ± 0.21 % to 21.50 ± 1.50 %, n = 8) and in the presence (from 2.10 ± 0.24 % to 23.27 ± 1.77 %, n = 8) of zVAD (10 µM).

Additional experiments addressed the sensitivity of Anidulafungin-induced cell membrane scrambling to nitric oxide. As illustrated in Fig. 7, addition of nitroprusside (1 µM) slightly, but significantly blunted the effect of Anidulafungin on the percentage of annexin-V-binding erythrocytes. However, even in the presence of nitroprusside, Anidulafungin significantly increased the percentage of annexin-V-binding erythrocytes.

A final series of experiments explored, whether the Anidulafungin-induced cell membrane scrambling was sensitive to increase of extracellular K⁺ concentration and or cell volume. As shown in Fig. 8, an increase of extracellular K⁺ concentration did not significantly modify the effect of Anidulafungin on the percentage of annexin-V-binding erythrocytes, even though it significantly blunted the effect of Anidulafungin on cell volume.

Fig. 7. Nitroprusside sensitivity of Anidulafungin-induced phosphatidylserine exposure. (A,B) Original histograms of annexin-V-binding of erythrocytes following exposure for 48 hours to Ringer solution without (grey areas) and with (black line) Anidulafungin (6 µg/ml) in the absence (A) and presence (B) of Nitroprusside (1 µM). (C) Arithmetic means $\pm$ SEM (n = 8) of annexin-V-binding of erythrocytes after a 48 hours treatment with Ringer solution without (white bars) or with (black bars) Anidulafungin (6 µg/ml) in the absence (left bars) and presence (right bars) of 1 µM Nitroprusside. *** (p < 0.001) indicates significant difference from the absence of Anidulafungin, # (p < 0.05) indicates significant difference from the absence of 1 µM Nitroprusside (ANOVA).

Fig. 8. Influence of extracellular K⁺ concentration on annexin binding and forward scatter. (A) Arithmetic means $\pm$ SEM (n=20) of annexin-V-binding erythrocytes after a 48 hours treatment with Ringer solution without (white bars) or with (black bars) 6 µg/ml Anidulafungin at 5, 60 or 120 mM K⁺ concentration. (B) Arithmetic means $\pm$ SEM (n=20) of forward scatter of erythrocytes after a 48 hours treatment with Ringer solution without (white bars) or with (black bars) 6 µg/ml Anidulafungin at 5, 60 or 120 mM K⁺ concentration. *** (p < 0.001) indicates significant difference from the absence of Anidulafungin, ### (p < 0.001) indicates significant difference from the respective value at 5 mM K⁺ (ANOVA).

Discussion

The present observations disclose a novel effect of Anidulafungin, i.e. the stimulation of eryptosis, the suicidal erythrocyte death. A 48 hours exposure of erythrocytes from healthy individuals to Anidulafungin resulted in cell shrinkage and cell membrane scrambling with phosphatidylserine translocation to the erythrocyte surface. The concentrations required for this effect were well in the range of peak plasma concentrations determined in Anidulafungin treated patients [71]. The sensitivity to Anidulafungin induced eryptosis is presumably enhanced in clinical conditions with accelerated eryptosis, such as dehydration [72], hyperphosphatemia [73], chronic kidney disease (CKD) [74-77], hemolytic-uremic syndrome [78], diabetes [79], hepatic failure [80], malignancy [36], sepsis [81], sickle-cell disease [36], beta-thalassemia [36], Hb-C and G6PD-deficiency [36], as well as Wilsons disease [82].

The signaling of Anidulafungin on erythrocyte cell membrane scrambling apparently involves to some extent Ca^{2+} entry, as Anidulafungin slightly, but significantly increased Fluo3-fluorescence and the effect of Anidulafungin on annexin-V-binding was slightly, but significantly blunted by removal of extracellular Ca^{2+} . Moreover, the effect of Anidulafungin on annexin-V-binding was significantly blunted by the p38 kinase inhibitor SB203580, pointing to a contribution of p38 kinase to the stimulation of cell membrane scrambling. The effect of Anidulafungin on cell membrane scrambling did not involve oxidative stress, as the drug rather decreased DCFDA fluorescence and the effect of Anidulafungin on annexin-V-binding was not significantly blunted by the antioxidant N-acetylcysteine. Moreover, the effect of Anidulafungin on annexin-V-binding was not paralleled by increase of ceramide abundance and was not significantly blunted by PKC inhibitor staurosporine, casein kinase 1 α inhibitor D4476 or pancaspase inhibitor zVAD. The effect of Anidulafungin on annexin-V-binding was further insensitive to increase of extracellular K^+ concentration, which significantly blunted Anidulafungin-induced cell shrinkage. The effect of Anidulafungin on annexin-V-binding was, however, significantly blunted in the presence of NO donor nitroprusside.

Besides its effect on eryptosis, Anidulafungin triggered hemolysis. Anidulafungin has previously been shown to trigger hemolysis [83]. The main physiological function of eryptosis is the clearance of defective erythrocytes from circulating blood prior to hemolysis [36]. If not preceded by eryptosis, hemolysis leads to release of hemoglobin, which passes the renal glomerular filter, precipitates in the acidic lumen of renal tubules, occludes nephrons and thus triggers renal failure [84]. In malaria, eryptosis accomplishes removal of infected erythrocytes from circulating blood [36]. Accordingly, stimulation of eryptosis by the antifungal drug amphotericin B [33] has previously been shown to counteract the development of parasitemia and thus to favourably influence the clinical course of malaria [34]. It is tempting to speculate that Anidulafungin may have a similar positive effect in malaria.

Due to rapid clearance of eryptotic erythrocytes from circulating blood [36], excessive eryptosis may lead to anemia. However, enhanced eryptosis triggers anemia only, if the accelerated loss of erythrocytes outcasts the formation of new erythrocytes [36]. Phosphatidylserine exposing erythrocytes further adhere to the vascular wall [85], stimulate blood clotting and thus foster thrombosis [86-88]. Enhanced eryptosis may thus impair microcirculation [37, 86, 89-92].

In conclusion, Anidulafungin triggers eryptosis with cell shrinkage and cell membrane scrambling, and triggers hemolysis. The effect on cell membrane scrambling is accomplished in part by Ca^{2+} entry and activation of p38 kinase.

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Disclosure Statement

The authors of this manuscript state that they have no conflicts of interest to declare.

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