Effect of Anandamide in *Plasmodium Berghei*-infected Mice

Diwakar Bobbala*, Ioana Alesutan*, Michael Föller, Stephan M. Huber¹ and Florian Lang

Department of Physiology and ¹Department of Radiation Oncology, University of Tübingen. *contributed equally and thus share first authorship

Key Words

Malaria • Cell volume • Phosphatidylserine • Red blood cells • Eryptosis • Cell death

Abstract

Eryptosis, the suicidal death of erythrocytes, is characterized by exposure of phosphatidylserine at the erythrocyte surface and cell shrinkage. Triggers of eryptosis include anandamide. Enhanced eryptosis of infected human erythrocytes is expected to delay the development of parasitaemia during infection with *Plasmodium*, the parasite causing malaria. The present experiments aimed to test, whether anandamide influences eryptosis, parasite growth and/or host survival during *in vitro* or *in vivo* infection with *Plasmodia*. Human erythrocytes were infected *in vitro* with *P. falciparum*, and mice *in vivo* with *P. berghei*. Parasitemia was determined with Syto16. Phosphatidylserine-exposing erythrocytes were identified by analysing annexin V-binding in FACS analysis. *In vitro* infection of human erythrocytes was followed by a significant increase in annexin V-binding, an effect slightly enhanced by anandamide ($\geq 50 \mu\text{M}$), which significantly reduced intraerythrocytic DNA/RNA content and *in vitro* parasitaemia.

In vivo administration of anandamide (5 mg/kg b.w. subcutaneously) blunted the parasitaemia (from 36.9% to 24.2% of circulating erythrocytes 21 days after infection) and significantly enhanced the survival of *P. berghei*-infected mice (from 0% to 67% 26 days after infection). The percentage of phosphatidylserine-exposing erythrocytes was significantly increased in anandamide-treated infected mice compared to non-treated infected mice. In conclusion, anandamide stimulated eryptosis of infected erythrocytes thus counteracting parasitaemia and a lethal course of the disease.

Copyright © 2010 S. Karger AG, Basel

Introduction

Suicidal death of erythrocytes or eryptosis is characterized by cell shrinkage and cell membrane scrambling leading to exposure of phosphatidylserine at the cell surface [1-6]. The cell membrane scrambling is stimulated by increased cytosolic Ca^{2+} activity [1, 3, 4, 7] and ceramide [8]. Ca^{2+} enters erythrocytes via Ca^{2+} -perme-

able cation channels, which have been shown to be activated by osmotic shock, oxidative stress and energy depletion [7, 9-12]. In addition, Ca^{2+} activates Ca^{2+} -sensitive K^+ channels [13, 14] leading to KCl exit followed by osmotically obliged water [15]. Thus, Ca^{2+} stimulates suicidal erythrocyte death not only in nucleated cells [16], but as well in human erythrocytes [17].

The phosphatidylserine-exposing erythrocytes are recognized by receptors specific for phosphatidylserine on macrophages [18, 19], which engulf and degrade phosphatidylserine-exposing cells [20, 21]. Thus, eryptotic erythrocytes are rapidly cleared from circulating blood.

Eryptosis is stimulated by infection with *Plasmodium falciparum* [22, 23]. *Plasmodia* induce oxidative stress to host erythrocytes leading to activation of the Ca^{2+} -permeable cation channels resulting in Ca^{2+} entry [24] and subsequent cell membrane scrambling [25].

Rapid clearance of infected erythrocytes during malaria could prevent intraerythrocytic development of trophozoites with the ability to intoxicate macrophages [26, 27]. Thus, at least in theory, accelerated eryptosis could protect against a severe course of malaria [28]. Along those lines, sickle-cell-trait, beta-thalassemia-trait, homozygous Hb-C and G6PD-deficiency lead to premature suicidal erythrocyte death upon infection with *Plasmodium* thus leading to accelerated clearance of ring stage-infected erythrocytes [6, 29-33].

Eryptosis could be stimulated by anandamide [34], a cannabinoid receptor agonist with pleiotropic actions [35-37]. The present study explored whether anandamide augments phosphatidylserine exposure of *Plasmodium falciparum*-infected human erythrocytes and influences parasitaemia and survival following murine infection with *Plasmodium berghei*.

Materials and Methods

Animals, cells and solutions

Animal experiments were performed according to the German animal protection law and approved by the local authorities. Healthy SV129/J wild type mice (aged 4 months, both male and female) were used for the experiments. From the 8th day after infection murine erythrocytes were drawn from animals by incision of the tail vein. Human erythrocytes were drawn from healthy volunteers.

Experiments were performed at 37°C in Ringer solution containing (in mM) 125 NaCl, 5 KCl, 1 MgSO_4 , 32 N-2-hydroxyethylpiperazine-N-2-ethanesulfonic acid (HEPES)/NaOH (pH 7.4), 5 glucose, 1 CaCl_2 . Anandamide was added to the NaCl Ringer at final concentrations varying from 1 μM to 100 μM (Sigma, Schnelldorf, Germany).

Determination of phosphatidylserine exposure

FACS analysis was performed as described [7, 38]. After incubation in the presence or absence of anandamide, suspensions of noninfected erythrocytes were stained with annexin V-FLUOS (Roche, Mannheim, Germany), suspensions of *Plasmodium falciparum*-infected erythrocytes were stained with annexin V-APC (BD Biosciences Pharmingen, Heidelberg, Germany) and/or with the DNA/RNA-specific dye Syto16 (Molecular Probes, Göttingen, Germany) to identify phosphatidylserine-exposing and infected erythrocytes, respectively. For annexin V-binding, erythrocytes were washed, resuspended in annexin V-binding buffer (Ringer solution containing 5 mM CaCl_2 , pH 7.4; the additional Ca^{2+} is required for binding of annexin V), stained with annexin V-APC (dilution 1:20) or annexin V-FLUOS (dilution 1:100), incubated for 20 min at room temperature, and diluted 1:5 with annexin V-binding buffer. Syto16 (final concentration of 20 nM) was added directly to the diluted erythrocyte suspension or co-incubated in the annexin V-binding buffer. Cells were analyzed by flow cytometry (FACS-Calibur, Becton Dickinson, Heidelberg, Germany) in FL-1 for Syto16 or annexin V-FLUOS fluorescence intensity (detected at 530 nm) and in FL-4 for annexin V-APC fluorescence intensity (detected at 660 nm).

In vitro culture of Plasmodium falciparum

For infection of human erythrocytes the human, but not murine pathogenic *Plasmodium falciparum* strain BinH [39] was grown in human erythrocytes *in vitro* [40, 41]. Parasites were cultured as described earlier [42, 43] at a haematocrit of 2% and a parasitaemia of 2-10% in RPMI 1640 medium supplemented with Albumax II (0.5%; Gibco, Karlsruhe, Germany) in an atmosphere of 90% N_2 , 5% CO_2 , 5% O_2 .

In vivo proliferation of Plasmodium berghei

For infection of mice, the murine, but not human pathogenic *Plasmodium berghei* strain ANKA-parasitized murine erythrocytes (1×10^6) were injected intraperitoneally [44] into wildtype mice. Where indicated, anandamide (5 mg/kg b.w.) was administered subcutaneously from the eight day of infection. Blood was collected from the mice from the 8 day after infection by incision of the tail. Parasitaemia was determined by Syto16 staining in FACS analysis.

In vitro growth assays of Plasmodium falciparum-infected human erythrocytes

The *Plasmodium falciparum* BinH strain was cultured and synchronized to the ring stage by sorbitol treatment as described previously [24]. For the *in vitro* growth assay, synchronized parasitized erythrocytes were aliquoted in 96-well plates (200 μl aliquots, 1% haematocrit, 0.5 - 2% parasitaemia) and grown for 48 h in the presence or absence of anandamide (1 μM - 100 μM). The parasitaemia was assessed 0 h and 48 h later by flow cytometry. Parasitaemia was defined by the percentage of erythrocytes stained with the DNA/RNA specific fluorescent dye Syto16.

To estimate DNA/RNA amplification, the culture was ring stage-synchronized, and re-synchronized after 6 h of culture (to narrow the developmental parasite stage), aliquoted (200 μl

Fig. 1. Effects of anandamide on phosphatidylserine exposure of infected and noninfected erythrocytes. A. Arithmetic means $\pm$ SEM ($n = 3$ cultures studied in duplicates) of the percentage of annexin V-binding infected (open bars) and non-infected (closed bars) erythrocytes following infection of human erythrocytes with *Plasmodium falciparum* strain BinH in the presence of the indicated concentrations of anandamide. *** indicates significant difference ($p < 0.001$) from non-infected erythrocytes. B. Time dependence of the arithmetic means $\pm$ SEM ($n = 3$ cultures studied in duplicates) of the percentage of annexin V-binding infected and non-infected erythrocytes following infection of human erythrocytes with *Plasmodium falciparum* in the absence of anandamide. Note: Figures 1A and 1B show the results of independent experiments. The *in vitro* culture was performed by culturing parasitized erythrocytes at a haematocrit of 2% and a parasitaemia of 2-10% in RPMI 1640 medium supplemented with Albumax II in an atmosphere of 90% N_2 , 5% CO_2 , 5% O_2 .

aliquots, 2% haematocrit and 10% parasitaemia) and cultured for further 16 h in the presence or absence of anandamide (1 μ M - 100 μ M). Thereafter, the DNA/RNA amount of the parasitized erythrocytes was determined by Syto16 fluorescence as a measure of intraerythrocytic parasite copies.

Fig. 2. Effects of anandamide on intraerythrocytic DNA amplification and *in vitro* parasitaemia. A. Intraerythrocytic DNA amplification as a function of the anandamide concentration (arithmetic means $\pm$ SEM, $n = 3$ cultures studied in quadruplicates). B. *In vitro* parasitaemia with *Plasmodium falciparum* in human erythrocytes as a function of the anandamide concentration (arithmetic means $\pm$ SEM, $n = 3$ cultures studied in quadruplicates).

Statistics

Data are expressed as arithmetic means $\pm$ SEM, and statistical analysis was made by t-test or ANOVA using Tukey's test as post hoc test, as appropriate. $p < 0.05$ was considered as statistically significant.

Fig. 3. Parasitaemia and survival of *Plasmodium berghei*-infected mice. **A:** Original graphs of parasitaemia-dependent Syto16 fluorescence in untreated animals (upper panels) and animals treated from day 8 until day 21 with 5 mg/kg b.w. of anandamide s.c. (lower panels) 10 (left panels) and 20 (right panels) days after infection with *Plasmodium berghei*. **B:** Arithmetic means $\pm$ SEM of parasitemia in mice without treatment (open circles, $n=10$) or with 5 mg/kg b.w. of anandamide s.c. (closed circles, $n=6$) as a function of days after infection with *Plasmodium berghei*. *, **, *** indicate significant difference ($p<0.05$, $p<0.01$, $p<0.001$) from the untreated animals. The mice were infected by injection of 1×10^6 *Plasmodium berghei* ANKA-parasitized murine erythrocytes intraperitoneally. **C:** Survival of mice without treatment (open circles) or with 5 mg/kg b.w. of anandamide s.c. (closed circles) as a function of days after infection with *Plasmodium berghei*.

Fig. 4. *In vivo* effect of anandamide on phosphatidylserine exposure of infected and noninfected erythrocytes. Arithmetic means $\pm$ SEM ($n = 6-10$) of the percentage of annexin V-binding in infected (open bars) and non-infected (closed bars) erythrocytes retrieved from mice infected with *Plasmodium berghei* on day 10 after infection and untreated (left bars) or treated with (right bars) anandamide. *** indicates significant difference ($p<0.001$) from non-infected erythrocytes, ### indicates significant difference ($p<0.001$) from control.

Results

The percentage of phosphatidylserine-exposing erythrocytes was determined from annexin V-binding in FACS analysis. Infection with *Plasmodium falciparum* significantly enhanced the percentage of annexin V-binding erythrocytes (Fig. 1A). Anandamide slightly augmented the phosphatidylserine exposure of infected erythrocytes (Fig. 1A), an effect, however, not reaching statistical significance. The time course of phosphatidylserine exposure of non-treated erythrocytes upon infection is shown in Fig. 1B.

Intraerythrocytic DNA amplification in erythrocytes was slightly, but significantly decreased by anandamide ($\geq 50 \mu\text{M}$; Fig. 2A). *In vitro* growth of *Plasmodium falciparum* in human erythrocytes was similarly decreased at anandamide concentrations $\geq 50 \mu\text{M}$ (Fig. 2B).

Additional experiments were performed to elucidate the influence of anandamide treatment (5 mg/kg b.w. of anandamide s.c.) on the course of malaria *in vivo*. To this end, mice were infected with *Plasmodium berghei*. Up to day 8 after infection, parasitaemia remained less than 3.8%. Thereafter, the parasitaemia

increased gradually (Fig. 3B). The percentage of parasitized erythrocytes amounted to $36.9 \pm 4.0\%$ in animals without anandamide treatment on day 21 after infection. In anandamide-treated animals the parasitaemia increased only to $24.2 \pm 1.9\%$ 21 days after infection (Fig. 3B).

Anandamide treatment further increased the survival of *Plasmodium berghei*-infected mice. All nontreated animals died within 26 days after the infection, but 67% of the anandamide-treated animals survived the infection for 26 days (Fig. 3C).

Additional experiments were performed to determine whether stimulation of eryptosis accounts for the milder course of malaria in the anandamide-treated mice. As a result, on day 8 after infection, infected erythrocytes exposed significantly more phosphatidylserine than non-infected erythrocytes. More importantly, treatment of the mice with anandamide significantly increased the phosphatidylserine exposure of infected erythrocytes but did not appreciably modify phosphatidylserine exposure of non-infected erythrocytes (Fig. 4).

Discussion

The present observations reveal a novel therapeutic effect of anandamide. During infection with *Plasmodium berghei* anandamide blunted the increase in parasitaemia and, more importantly, substantially enhanced the survival of infected mice. Without treatment, infection of the mice with *Plasmodium berghei* was followed by an invariably lethal course [44]. In contrast, most of the anandamide-treated mice survived the first 26 days of *Plasmodium berghei* infection.

The effect of anandamide could be either due to a direct toxic effect of the drug on the pathogen or due to accelerated host erythrocyte death leading to premature removal of the pathogen within its host cell. A toxic action on the pathogen is reflected by a decrease of the intraerythrocytic DNA/RNA content. The scrambling effect of anandamide may further decrease the life span of infected erythrocytes in circulating blood. Anandamide is known to trigger eryptosis [45]. It stimulates scrambling of the cell membrane and thus fosters the phagocytosis of the affected erythrocyte [20,

21]. Phosphatidylserine exposure is a wellknown hallmark of eryptosis [17].

The effect of anandamide on eryptosis of human erythrocytes was only mild. Possibly the substance was bound to protein and thus, the free concentration lower than in previous experiments, when the erythrocytes were incubated in protein free Ringer solution. Moreover, the oxidative stress induced by *Plasmodium falciparum* may have partially destroyed the substance. The effect on eryptosis was more pronounced in murine erythrocytes. The possibility must be considered that anandamide has distinct actions in between human and mouse erythrocytes. Accordingly, enhanced eryptosis may play a role in the host defence against *Plasmodia* in murine but less so in human erythrocytes.

A variety of substances and conditions has been shown to stimulate phosphatidylserine scrambling of erythrocytes [46-62] including chlorpromazine [63], lead [64] or azathioprine [65]. Moreover, eryptosis contributes to or even accounts for several anemic conditions, such as sickle-cell anemia [66, 67], beta-thalassemia [6], glucose-6-phosphate dehydrogenase (G6PD)-deficiency [6], phosphate depletion [68], iron deficiency [69], Hemolytic Uremic Syndrome [70], sepsis [71], malaria [28, 72-75] and Wilson's disease [51]. Several of those substances or diseases could confer some protection against a severe course of malaria. As a matter of fact, iron deficiency [22] and treatment with chlorpromazine [49], lead [23] or azathioprine [76] have recently been shown to accelerate eryptosis of infected erythrocytes and to be protective against a severe course of malaria.

In conclusion, anandamide accelerates eryptosis of infected erythrocytes. Stimulation of eryptosis of infected erythrocytes blunts the parasitaemia and leads to a favourable course of the disease.

Acknowledgements

This study was supported by the Deutsche Forschungsgemeinschaft (La 315/6-1 and La 315/13-1) and the Carl-Zeiss-Stiftung. The authors acknowledge the meticulous preparation of the manuscript by Lejla Subasic and Tanja Loch.

References

- 1 Berg CP, Engels IH, Rothbart A, Lauber K, Renz A, Schlosser SF, Schulze-Osthoff K, Wesselborg S: Human mature red blood cells express caspase-3 and caspase-8, but are devoid of mitochondrial regulators of apoptosis. *Cell Death Differ* 2001;8:1197-1206.
- 2 Brand VB, Sandu CD, Duranton C, Tanneur V, Lang KS, Huber SM, Lang F: Dependence of *Plasmodium falciparum* in vitro growth on the cation permeability of the human host erythrocyte. *Cell Physiol Biochem* 2003;13:347-356.
- 3 Bratosin D, Estaquier J, Petit F, Arnoult D, Quatannens B, Tissier JP, Slomianny C, Sartiaux C, Alonso C, Huart JJ, Montreuil J, Ameisen JC: Programmed cell death in mature erythrocytes: a model for investigating death effector pathways operating in the absence of mitochondria. *Cell Death Differ* 2001;8:1143-1156.
- 4 Daugas E, Cande C, Kroemer G: Erythrocytes: death of a mummy. *Cell Death Differ* 2001;8:1131-1133.
- 5 Lang F, Gulbins E, Lang PA, Zappulla D, Foller M: Ceramide in suicidal death of erythrocytes. *Cell Physiol Biochem* 2010;26:21-28.
- 6 Lang KS, Roll B, Myssina S, Schittenhelm M, Scheel-Walter HG, Kanz L, Fritz J, Lang F, Huber SM, Wieder T: Enhanced erythrocyte apoptosis in sickle cell anemia, thalassemia and glucose-6-phosphate dehydrogenase deficiency. *Cell Physiol Biochem* 2002;12:365-372.
- 7 Lang KS, Duranton C, Poehlmann H, Myssina S, Bauer C, Lang F, Wieder T, Huber SM: Cation channels trigger apoptotic death of erythrocytes. *Cell Death Differ* 2003;10:249-256.
- 8 Lang KS, Myssina S, Brand V, Sandu C, Lang PA, Berchtold S, Huber SM, Lang F, Wieder T: Involvement of ceramide in hyperosmotic shock-induced death of erythrocytes. *Cell Death Differ* 2004;11:231-243.
- 9 Bernhardt I, Weiss E, Robinson HC, Wilkins R, Bennekou P: Differential effect of HOE642 on two separate monovalent cation transporters in the human red cell membrane. *Cell Physiol Biochem* 2007;20:601-606.
- 10 Duranton C, Huber SM, Lang F: Oxidation induces a Cl(-)-dependent cation conductance in human red blood cells. *J Physiol* 2002;539:847-855.
- 11 Foller M, Kasinathan RS, Koka S, Lang C, Shumilina E, Birnbaumer L, Lang F, Huber SM: TRPC6 contributes to the Ca(2+) leak of human erythrocytes. *Cell Physiol Biochem* 2008;21:183-192.
- 12 Huber SM, Gamper N, Lang F: Chloride conductance and volume-regulatory non-selective cation conductance in human red blood cell ghosts. *Pflugers Arch* 2001;441:551-558.
- 13 Bookchin RM, Ortiz OE, Lew VL: Activation of calcium-dependent potassium channels in deoxygenated sickle red cells. *Prog Clin Biol Res* 1987;240:193-200.
- 14 Brugnara C, de Franceschi L, Alper SL: Inhibition of Ca(2+)-dependent K⁺ transport and cell dehydration in sickle erythrocytes by clotrimazole and other imidazole derivatives. *J Clin Invest* 1993;92:520-526.
- 15 Lang PA, Kaiser S, Myssina S, Wieder T, Lang F, Huber SM: Role of Ca²⁺-activated K⁺ channels in human erythrocyte apoptosis. *Am J Physiol Cell Physiol* 2003;285:C1553-C1560.
- 16 McConkey DJ, Orrenius S: The role of calcium in the regulation of apoptosis. *Biochem Biophys Res Commun* 1997;239:357-366.
- 17 Lang KS, Lang PA, Bauer C, Duranton C, Wieder T, Huber SM, Lang F: Mechanisms of suicidal erythrocyte death. *Cell Physiol Biochem* 2005;15:195-202.
- 18 Fadok VA, Bratton DL, Rose DM, Pearson A, Ezekewitz RA, Henson PM: A receptor for phosphatidylserine-specific clearance of apoptotic cells. *Nature* 2000;405:85-90.
- 19 Henson PM, Bratton DL, Fadok VA: The phosphatidylserine receptor: a crucial molecular switch? *Nat Rev Mol Cell Biol* 2001;2:627-633.
- 20 Boas FE, Forman L, Beutler E: Phosphatidylserine exposure and red cell viability in red cell aging and in hemolytic anemia. *Proc Natl Acad Sci U S A* 1998;95:3077-3081.
- 21 Yamanaka M, Eda S, Beppu M: Carbohydrate chains and phosphatidylserine successively work as signals for apoptotic cell removal. *Biochem Biophys Res Commun* 2005;328:273-280.
- 22 Koka S, Foller M, Lamprecht G, Boini KM, Lang C, Huber SM, Lang F: Iron deficiency influences the course of malaria in *Plasmodium berghei* infected mice. *Biochem Biophys Res Commun* 2007;357:608-614.
- 23 Koka S, Huber SM, Boini KM, Lang C, Foller M, Lang F: Lead decreases parasitemia and enhances survival of *Plasmodium berghei*-infected mice. *Biochem Biophys Res Commun* 2007;363:484-489.
- 24 Duranton C, Huber S, Tanneur V, Lang K, Brand V, Sandu C, Lang F: Electrophysiological properties of the *Plasmodium falciparum*-induced cation conductance of human erythrocytes. *Cell Physiol Biochem* 2003;13:189-198.
- 25 Tyurina YY, Tyurin VA, Zhao Q, Djukic M, Quinn PJ, Pitt BR, Kagan VE: Oxidation of phosphatidylserine: a mechanism for plasma membrane phospholipid scrambling during apoptosis? *Biochem Biophys Res Commun* 2004;324:1059-1064.
- 26 Koka S, Lang C, Niemoeller OM, Boini KM, Nicolay JP, Huber SM, Lang F: Influence of NO synthase inhibitor L-NAME on parasitemia and survival of *Plasmodium berghei* infected mice. *Cell Physiol Biochem* 2008;21:481-488.
- 27 Schwarzer E, Turrini F, Ulliers D, Giribaldi G, Ginsburg H, Arese P: Impairment of macrophage functions after ingestion of *Plasmodium falciparum*-infected erythrocytes or isolated malarial pigment. *J Exp Med* 1992;176:1033-1041.
- 28 Lang F, Lang PA, Lang KS, Brand V, Tanneur V, Duranton C, Wieder T, Huber SM: Channel-induced apoptosis of infected host cells-the case of malaria. *Pflugers Arch* 2004;448:319-324.
- 29 Ayi K, Turrini F, Piga A, Arese P: Enhanced phagocytosis of ring-parasitized mutant erythrocytes: a common mechanism that may explain protection against falciparum malaria in sickle trait and beta-thalassemia trait. *Blood* 2004;104:3364-3371.
- 30 Cappadoro M, Giribaldi G, O'Brien E, Turrini F, Mannu F, Ulliers D, Simula G, Luzzatto L, Arese P: Early phagocytosis of glucose-6-phosphate dehydrogenase (G6PD)-deficient erythrocytes parasitized by *Plasmodium falciparum* may explain malaria protection in G6PD deficiency. *Blood* 1998;92:2527-2534.
- 31 de Jong K, Emerson RK, Butler J, Bastacky J, Mohandas N, Kuypers FA: Short survival of phosphatidylserine-exposing red blood cells in murine sickle cell anemia. *Blood* 2001;98:1577-1584.
- 32 Kean LS, Brown LE, Nichols JW, Mohandas N, Archer DR, Hsu LL: Comparison of mechanisms of anemia in mice with sickle cell disease and beta-thalassemia: peripheral destruction, ineffective erythropoiesis, and phospholipid scramblase-mediated phosphatidylserine exposure. *Exp Hematol* 2002;30:394-402.
- 33 Kuypers FA, Yuan J, Lewis RA, Snyder LM, Kiefer CR, Bunyaratvej A, Fucharoen S, Ma L, Styles L, de Jong K, Schrier SL: Membrane phospholipid asymmetry in human thalassemia. *Blood* 1998;91:3044-3051.
- 34 Bentzen PJ, Lang F: Effect of anandamide on erythrocyte survival. *Cell Physiol Biochem* 2007;20:1033-1042.

- 35 Hwang J, Adamson C, Butler D, Janero DR, Makriyannis A, Bahr BA: Enhancement of endocannabinoid signaling by fatty acid amide hydrolase inhibition: A neuroprotective therapeutic modality. *Life Sci* 2009;83:85-91.
- 36 Lam FF, Luk PW, Ng ES: Pharmacological characterization of receptor types mediating the dilator action of anandamide on blood vessels of the rat knee joint. *Life Sci* 2007;80:1495-1502.
- 37 Reis GM, Pacheco D, Perez AC, Klein A, Ramos MA, Duarte ID: Opioid receptor and NO/cGMP pathway as a mechanism of peripheral antinociceptive action of the cannabinoid receptor agonist anandamide. *Life Sci* 2009;85:351-356.
- 38 Floride E, Foller M, Ritter M, Lang F: Caffeine inhibits suicidal erythrocyte death. *Cell Physiol Biochem* 2008;22:253-260.
- 39 Binh VQ, Luty AJ, Kreamsner PG: Differential effects of human serum and cells on the growth of *Plasmodium falciparum* adapted to serum-free in vitro culture conditions. *Am J Trop Med Hyg* 1997;57:594-600.
- 40 Duranton C, Tanneur V, Lang C, Brand VB, Koka S, Kasinathan RS, Dorsch M, Hedrich HJ, Baumeister S, Lingelbach K, Lang F, Huber SM: A high specificity and affinity interaction with serum albumin stimulates an anion conductance in malaria-infected erythrocytes. *Cell Physiol Biochem* 2008;22:395-404.
- 41 Huber SM, Uhlemann AC, Gamper NL, Duranton C, Kreamsner PG, Lang F: *Plasmodium falciparum* activates endogenous Cl(-) channels of human erythrocytes by membrane oxidation. *EMBO J* 2002;21:22-30.
- 42 Jensen JB, Trager W: *Plasmodium falciparum* in culture: establishment of additional strains. *Am J Trop Med Hyg* 1978;27:743-746.
- 43 Trager W, Jensen JB: Human malaria parasites in continuous culture. *Science* 1976;193:673-675.
- 44 Huber SM, Duranton C, Henke G, Van De SC, Heussler V, Shumilina E, Sandu CD, Tanneur V, Brand V, Kasinathan RS, Lang KS, Kreamsner PG, Hubner CA, Rust MB, Dedek K, Jentsch TJ, Lang F: *Plasmodium* induces swelling-activated ClC-2 anion channels in the host erythrocyte. *J Biol Chem* 2004;279:41444-41452.
- 45 Lang PA, Huober J, Bachmann C, Kempe DS, Sobiesiak M, Akel A, Niemoeller OM, Dreischer P, Eisele K, Klarl BA, Gulbins E, Lang F, Wieder T: Stimulation of erythrocyte phosphatidylserine exposure by paclitaxel. *Cell Physiol Biochem* 2006;18:151-164.
- 46 Attanasio P, Shumilina E, Hermle T, Kiedaisch V, Lang PA, Huber SM, Wieder T, Lang F: Stimulation of eryptosis by anti-A IgG antibodies. *Cell Physiol Biochem* 2007;20:591-600.
- 47 Bentzen PJ, Lang E, Lang F: Curcumin induced suicidal erythrocyte death. *Cell Physiol Biochem* 2007;19:153-164.
- 48 Foller M, Shumilina E, Lam R, Mohamed W, Kasinathan R, Huber S, Chakraborty T, Lang F: Induction of suicidal erythrocyte death by listeriolysin from *Listeria monocytogenes*. *Cell Physiol Biochem* 2007;20:1051-1060.
- 49 Koka S, Lang C, Boini KM, Bobbala D, Huber SM, Lang F: Influence of chlorpromazine on eryptosis, parasitemia and survival of *Plasmodium berghei* infected mice. *Cell Physiol Biochem* 2008;22:261-268.
- 50 Lang F, Gulbins E, Lerche H, Huber SM, Kempe DS, Foller M: Eryptosis, a window to systemic disease. *Cell Physiol Biochem* 2008;22:373-380.
- 51 Lang PA, Schenck M, Nicolay JP, Becker JU, Kempe DS, Lupescu A, Koka S, Eisele K, Klarl BA, Rubben H, Schmid KW, Mann K, Hildenbrand S, Heffer H, Huber SM, Wieder T, Erhardt A, Haussinger D, Gulbins E, Lang F: Liver cell death and anemia in Wilson disease involve acid sphingomyelinase and ceramide. *Nat Med* 2007;13:164-170.
- 52 Mahmud H, Mauro D, Qadri SM, Foller M, Lang F: Triggering of suicidal erythrocyte death by amphotericin B. *Cell Physiol Biochem* 2009;24:263-270.
- 53 Mahmud H, Mauro D, Foller M, Lang F: Inhibitory effect of thymol on suicidal erythrocyte death. *Cell Physiol Biochem* 2009;24:407-414.
- 54 Nicolay JP, Gatz S, Liebig G, Gulbins E, Lang F: Amyloid induced suicidal erythrocyte death. *Cell Physiol Biochem* 2007;19:175-184.
- 55 Nicolay JP, Bentzen PJ, Ghashghaieina M, Wieder T, Lang F: Stimulation of erythrocyte cell membrane scrambling by amiodarone. *Cell Physiol Biochem* 2007;20:1043-1050.
- 56 Niemoeller OM, Kiedaisch V, Dreischer P, Wieder T, Lang F: Stimulation of eryptosis by aluminium ions. *Toxicol Appl Pharmacol* 2006;217:168-175.
- 57 Niemoeller OM, Foller M, Lang C, Huber SM, Lang F: Retinoic acid induced suicidal erythrocyte death. *Cell Physiol Biochem* 2008;21:193-202.
- 58 Niemoeller OM, Mahmud H, Foller M, Wieder T, Lang F: Ciglitazone and 15d-PGJ2 induced suicidal erythrocyte death. *Cell Physiol Biochem* 2008;22:237-244.
- 59 Schneider J, Nicolay JP, Foller M, Wieder T, Lang F: Suicidal erythrocyte death following cellular K⁺ loss. *Cell Physiol Biochem* 2007;20:35-44.
- 60 Sopjani M, Foller M, Gulbins E, Lang F: Suicidal death of erythrocytes due to selenium-compounds. *Cell Physiol Biochem* 2008;22:387-394.
- 61 Wang K, Mahmud H, Foller M, Biswas R, Lang KS, Bohn E, Gotz F, Lang F: Lipopeptides in the triggering of erythrocyte cell membrane scrambling. *Cell Physiol Biochem* 2008;22:381-386.
- 62 Zappulla D: Environmental stress, erythrocyte dysfunctions, inflammation, and the metabolic syndrome: adaptations to CO₂ increases? *J Cardimetab Syndr* 2008;3:30-34.
- 63 Akel A, Hermle T, Niemoeller OM, Kempe DS, Lang PA, Attanasio P, Podolski M, Wieder T, Lang F: Stimulation of erythrocyte phosphatidylserine exposure by chlorpromazine. *Eur J Pharmacol* 2006;532:11-17.
- 64 Kempe DS, Lang PA, Eisele K, Klarl BA, Wieder T, Huber SM, Duranton C, Lang F: Stimulation of erythrocyte phosphatidylserine exposure by lead ions. *Am J Physiol Cell Physiol* 2005;288:C396-C402.
- 65 Geiger C, Foller M, Herrlinger KR, Lang F: Azathioprine-induced suicidal erythrocyte death. *Inflamm Bowel Dis* 2008;14:1027-1032.
- 66 Heibel RP: Beyond hemoglobin polymerization: the red blood cell membrane and sickle disease pathophysiology. *Blood* 1991;77:214-237.
- 67 Wood BL, Gibson DF, Tait JF: Increased erythrocyte phosphatidylserine exposure in sickle cell disease: flow-cytometric measurement and clinical associations. *Blood* 1996;88:1873-1880.
- 68 Birka C, Lang PA, Kempe DS, Hoefling L, Tanneur V, Duranton C, Nammi S, Henke G, Myssina S, Krikov M, Huber SM, Wieder T, Lang F: Enhanced susceptibility to erythrocyte "apoptosis" following phosphate depletion. *Pflugers Arch* 2004;448:471-477.
- 69 Kempe DS, Lang PA, Duranton C, Akel A, Lang KS, Huber SM, Wieder T, Lang F: Enhanced programmed cell death of iron-deficient erythrocytes. *FASEB J* 2006;20:368-370.
- 70 Lang PA, Beringer O, Nicolay JP, Amon O, Kempe DS, Hermle T, Attanasio P, Akel A, Schafer R, Friedrich B, Risler T, Baur M, Olbricht CJ, Zimmerhackl LB, Zipfel PF, Wieder T, Lang F: Suicidal death of erythrocytes in recurrent hemolytic uremic syndrome. *J Mol Med* 2006;84:378-388.
- 71 Kempe DS, Akel A, Lang PA, Hermle T, Biswas R, Muresanu J, Friedrich B, Dreischer P, Wolz C, Schumacher U, Peschel A, Gotz F, Doring G, Wieder T, Gulbins E, Lang F: Suicidal erythrocyte death in sepsis. *J Mol Med* 2007;85:273-281.
- 72 Brand V, Koka S, Lang C, Jendrossek V, Huber SM, Gulbins E, Lang F: Influence of amitriptyline on eryptosis, parasitemia and survival of *Plasmodium berghei*-infected mice. *Cell Physiol Biochem* 2008;22:405-412.

- 73 Foller M, Bobbala D, Koka S, Huber SM, Gulbins E, Lang F: Suicide for survival—death of infected erythrocytes as a host mechanism to survive malaria. *Cell Physiol Biochem* 2009;24:133-140.
- 74 Koka S, Bobbala D, Lang C, Boini KM, Huber SM, Lang F: Influence of paclitaxel on parasitemia and survival of *Plasmodium berghei* infected mice. *Cell Physiol Biochem* 2009;23:191-198.
- 75 Lang PA, Kasinathan RS, Brand VB, Duranton C, Lang C, Koka S, Shumilina E, Kempe DS, Tanneur V, Akel A, Lang KS, Foller M, Kun JF, Kremsner PG, Wesselborg S, Laufer S, Clemen CS, Herr C, Noegel AA, Wieder T, Gulbins E, Lang F, Huber SM: Accelerated clearance of *Plasmodium*-infected erythrocytes in sickle cell trait and annexin-A7 deficiency. *Cell Physiol Biochem* 2009;24:415-428.
- 76 Bobbala D, Koka S, Geiger C, Foller M, Huber SM, Lang F: Azathioprine favourably influences the course of malaria. *Malar J* 2009;8:102.