

Retinoic Acid Induced Suicidal Erythrocyte Death

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Key Words

Calcium • Phosphatidylserine • Eryptosis • Red blood cells • Apoptosis • Cell death

Abstract

Vitamin A and retinoic acid have previously been shown to confer some protection against a severe course of malaria by fostering the phagocytosis of parasitized erythrocytes. Phagocytosis of erythrocytes is stimulated by phosphatidylserine exposure at the cell surface. The present study has thus been performed to explore the effect of retinoic acid and the specific retinoic acid receptor (RAR) agonist 4-(E-2-[5,6,7,8-tetrahydro-5,5,8,8-tetramethyl-2-naphthalenyl]-1-propenyl) benzoic acid (TTNPB) on erythrocyte annexin V binding, which reflects phosphatidylserine exposure at the cell surface. A 24 hours exposure to either, retinoic acid (3 μ M) or TTNPB (3 μ M), indeed significantly increased annexin binding, an effect paralleled by decrease of forward scatter reflecting cell shrinkage. According to Fluo3 fluorescence, exposure to either, retinoic acid (10 μ M, 24 hours) or TTNPB (10 μ M, 6 hours), significantly increased cytosolic Ca²⁺-activity, a known trigger of

phosphatidylserine exposure. Infection of erythrocytes with *Plasmodium falciparum* increased phosphatidylserine exposure, an effect increased in the presence of TTNPB. In conclusion, retinoid acid and TTNPB trigger phosphatidylserine exposure and cell shrinkage of erythrocytes, typical features of suicidal erythrocyte death or eryptosis. The eryptosis could participate in the accelerated clearance of parasitized erythrocytes from circulating blood following treatment with retinoids.

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Introduction

Vitamin A supplementation has previously been shown to increase the resistance to a severe course of malaria, an effect attributed to enhanced CD36 mediated phagocytosis of parasitized erythrocytes [1-4]. On the other hand, retinoic acid has been reported to modify erythroid growth and differentiation [5-8] and favour the apoptotic death of human erythroleukemia (HEL) and K562 cells [9].

Similar to nucleated cells, erythrocytes may undergo suicidal cell death or eryptosis [10], which shares characteristics with erythrocyte senescence [10-13] and neocytolysis, the premature death of newly formed erythrocytes [14]. Enhanced eryptosis parallels a variety of diseases including sickle cell disease, thalassemia, and glucose-phosphate dehydrogenase deficiency [15], phosphate depletion [16], iron deficiency [17], haemolytic uremic syndrome [18], sepsis [17], malaria [19], or Wilson's disease [20]. Moreover, eryptosis may be triggered by methylglyoxal [21], amyloid [22], paclitaxel [23], chlorpromazine [24], cyclosporine [25], valinomycin [26], lead [27], mercury [28], or copper [20].

The stimulators of eryptosis are at least partially effective through stimulation of Ca^{2+} -entry [15, 29-35] following activation of Ca^{2+} -permeable cation channels [36-41]. Ca^{2+} elicits scrambling of the cell membrane [42, 43] with subsequent phosphatidylserine exposure at the cell surface. Phosphatidylserine exposing erythrocytes are recognized by phosphatidylserine receptors on macrophages [44-46] and thus engulfed and degraded [47]. In addition, intracellular Ca^{2+} activates Ca^{2+} -sensitive K^{+} -channels [48-50], which hyperpolarize the cell membrane thus driving Cl^{-} exit through Cl^{-} channels [51]. The cellular loss of KCl and osmotically obliged water then leads to cell shrinkage [34].

The erythrocyte cation channels are similarly activated by infection of erythrocytes with the malaria pathogen *Plasmodium falciparum* [37, 52]. The survival of the pathogen requires the entry of both Na^{+} and Ca^{2+} i.e. both, Na^{+} - and Ca^{2+} -depletion disrupt the intracellular survival of the pathogen [19]. By the same token, the entry of Ca^{2+} stimulates phospholipids scrambling of the cell membrane with phosphatidylserine exposure at the erythrocyte surface [19].

The present study has been performed to elucidate whether retinoic acid and the specific retinoic acid receptor (RAR) agonist 4-(E-2-[5,6,7,8-tetrahydro-5,5,8,8-tetramethyl-2-naphthalenyl]-1-propenyl) benzoic acid (TTNPB) do influence Ca^{2+} -entry and eryptosis in noninfected and in *Plasmodium falciparum* infected human erythrocytes.

Materials and Methods

Solutions

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 . Caspase inhibitor zVAD-FMK was purchased from Calbiochem (Bad Soden, Germany).

FACS analysis

FACS analysis was performed as described earlier [34]. After incubation in the presence or absence of retinoic acid or TTNBP, cells were washed in annexin-binding buffer containing (in mM) 125 NaCl, 10 HEPES/NaOH (pH 7.4), 5 CaCl_2 . Erythrocytes were suspended in a solution composed of Annexin-V-Fluos (Böhringer Mannheim, Germany) and annexin buffer (dilution of 1:50). After 10 minutes of incubation, samples were finally diluted 1:5 in annexin-binding buffer and measured by flow cytometric analysis (FACS-Calibur from Becton Dickinson). Cells were analysed by forward scatter and annexin-fluorescence intensity was measured in FL-1 with an excitation wavelength of 488 nm and an emission wavelength of 530 nm.

Measurement of intracellular Ca^{2+}

Intracellular Ca^{2+} -measurements were performed as described [29]. Briefly, erythrocytes were loaded with Fluo-3/AM (Calbiochem; Bad Soden, Germany) by addition of 2 μl of a Fluo-3/AM stock solution (2.0 mM in DMSO) to 1 ml erythrocyte suspension (0.16% packed cell volume in Ringer). The cells were incubated at 37°C for 15 min under protection from light. An additional 2 μl aliquot of Fluo-3/AM was added with incubation carried out for 25 min. Fluo-3-AM-loaded erythrocytes were centrifuged at 400 g for 5 min at 22°C and washed two times with Ringer solution containing 0.5% bovine serum albumin (Sigma) and one time with Ringer. For flow cytometry, Fluo-3/AM-loaded erythrocytes were resuspended in 1 ml Ringer (0.16% packed cell volume) containing retinoic acid (10 μM) or TTNPB (10 μM) or vehicle alone (DMSO) and incubated for different time periods as indicated at 37°C . Then, Ca^{2+} -dependent fluorescence intensity was measured in fluorescence channel FL-1 with an excitation wavelength of 488 nm and an emission wavelength of 530 nm.

Measurement of intracellular GSH

Human erythrocytes (5% hematocrit) were incubated for 24 h at 37°C in Ringer solution in the absence or presence of 10 μM retinoic acid or TTNPB. Then, the cells were again washed twice in PBS. All manipulations were then performed on ice. After lysis of 50 μl of the erythrocyte pellet in 250 μl distilled water and centrifugation at 14,000 rpm, 150 μl of the supernatant were deproteinated by adding 150 μl metaphosphoric acid (10%). Glutathione (GSH) was measured with the Glutathione Assay Kit from Cayman Chemicals according to the manufacturer's protocol. The GSH concentration refers to the cytosol of the erythrocytes.

Determination of caspase activity

Caspase activity was measured using the CaspACE In Situ Marker (FITC-VAD-FMK; Promega, Mannheim, Germany). After incubation in the presence or absence of 10 μM retinoic acid or TTNBP for 24 h at 37°C in Ringer solution, the erythrocytes were washed in PBS. HEK 293 cells treated with 50 μM carboplatin for 4 h at 42.5°C served as a positive control. Then, the cells were stained with the In Situ Marker in 200 μl

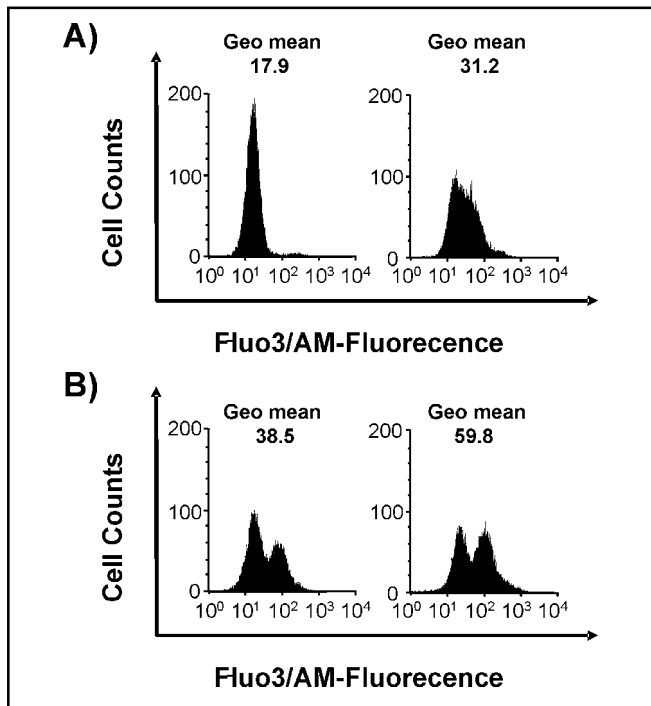


Fig. 1. Effect of TTNPB and retinoic acid on Fluo3-fluorescence in erythrocytes. A. Original histograms of Fluo3-fluorescence of human erythrocytes prior to (left panel) or following (right panel) a 6 hours exposure to 10 μ M of the specific retinoic acid receptor (RAR) agonist 4-(E-2-[5,6,7,8-tetrahydro-5,5,8,8-tetramethyl-2-naphthalenyl]-1-propenyl) benzoic acid (TTNPB). B. Original histograms of Fluo3- fluorescence of human erythrocytes prior to (left panel) or following (right panel) a 24 hours exposure to 10 μ M retinoic acid.

PBS at a 1:500 dilution. After an incubation for 20 min under protection from light, cells were washed in PBS and resuspended. Then, FITC-VAD-FMK-dependent fluorescence was measured in FL-1 on a FACS calibur.

Analysis of calpain activity

Erythrocytes at a hematocrit of 5% were incubated in the absence or presence of 10 μ M retinoic acid for 24 h at 37°C. As a positive control, erythrocytes were exposed to 1 μ M of the calcium ionophore ionomycin for 1 h at 37°C. After 3 washes with PBS 100 μ l of the pellet were hemolysed by hypotonic shock in 20 mM HEPES (pH 7.4) containing a cocktail of protease inhibitors (Roche, Mannheim, Germany). Ghost membranes were pelleted (15,000 g for 20 min at 4°C) and lysed in (mM) 125 NaCl, 25 HEPES/NaOH (pH 7.3), 10 EDTA, 10 N-ethylmaleimide, 10 NaF, 0.1% SDS, 0.5% deoxycholic acid, 1% Triton-X, 10 μ l β -mercaptoethanol. The protein concentration of the samples was determined with the Bradford method (Biorad, Munich, Germany) with bovine serum albumin as standard. Lysates were separated by 10% SDS-PAGE (50 μ g protein per lane) and transferred electrophoretically from the gel to Protan BA83 nitrocellulose membranes (Schleicher and Schuell, Dassel, Germany). Protein transfer was controlled by Ponceau red staining. After blocking the non-specific sites with 5% non-

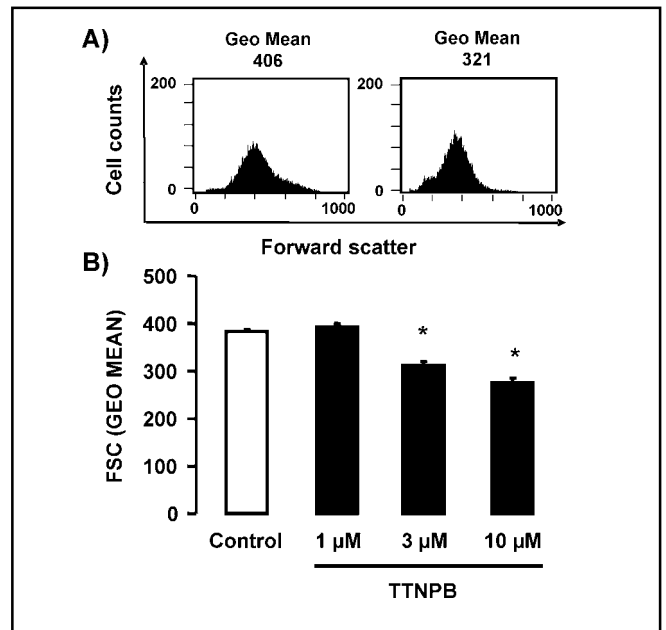


Fig. 2. Effect of TTNPB on erythrocyte forward scatter. A. Original histograms of forward scatter of human erythrocytes prior to (left panel) or following (right panel) a 24 hours exposure to 10 μ M TTNPB. B. Arithmetic means \pm SEM (n = 10) of forward scatter prior to (control, open bar) or following (closed bars) a 24 hours exposure to TTNPB at concentrations ranging from 1 to 10 μ M. * indicates significant (p \leq 0.05) difference from absence of TTNPB (ANOVA).

fat milk, the blots were probed overnight at 4°C with a polyclonal rabbit antihuman μ -calpain (large subunit) antibody (Cell Signaling Technology, Danvers, USA) diluted at 1:500. After washing, the blots were incubated for 1 h at 21°C with a secondary goat anti-rabbit antibody (Cell Signaling Technology, Danvers, MA, USA) conjugated with horseradish peroxidase at a 1:1000 dilution. Antibody binding was detected with the enhanced chemoluminescence (ECL) kit from Amersham.

Infection

For infection of human erythrocytes, the human pathogen *P. falciparum* strains BINH [53] and FCR-3 [54] were grown *in vitro* [55] in banked human RBCs (blood group O+). Parasites were cultured and synchronized by sorbitol treatment as described earlier [37]. Synchronized parasitized erythrocytes were aliquoted in 96-well plates (200 μ l aliquots, 1% hematocrit, 7-10% parasitemia) and grown for 24 h. Parasitemia was defined by the percentage of erythrocytes stained with the DNA/RNA specific fluorescence dye Syto16 (1 μ M, Molecular Probes, Göttingen, Germany). Then, 50 μ l aliquots were washed once in Ringer solution containing 5 mM CaCl₂. The aliquots were stained with 50 μ l Ringer solution containing 5 mM CaCl₂, 1 μ M Syto16, and Annexin V-APC (1:25 dilution, BD, Heidelberg, Germany) and further incubated in the dark for 20 min. For flow

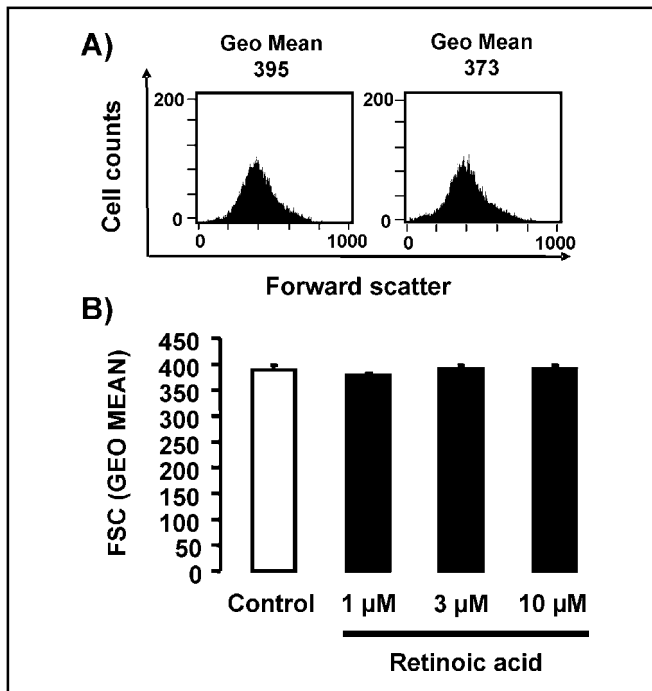


Fig. 3. Effect of retinoic acid on erythrocyte forward scatter. A. Original histograms of forward scatter of human erythrocytes prior to (left panel) or following (right panel) a 24 hours exposure to 10 μ M retinoic acid. B. Arithmetic means \pm SEM (n = 8 - 13) of forward scatter prior to (control, open bar) or following (closed bars) a 24 hours exposure to retinoic acid at concentrations ranging from 1 to 10 μ M.

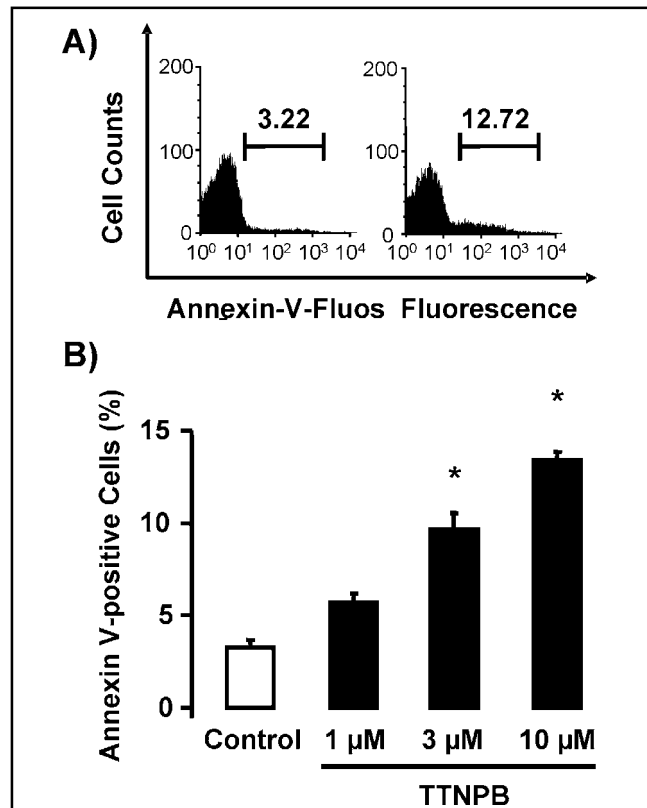


Fig. 4. Effect of TTNPB on erythrocyte annexin binding. A. Original histograms of annexin V binding of human erythrocytes prior to (left panel) or following (right panel) a 24 hours exposure to 10 μ M TTNPB. B. Arithmetic means \pm SEM (n = 10) of annexin V binding prior to (control, open bar) or following (closed bars) a 24 hours exposure to TTNPB at concentrations ranging from 1 to 10 μ M. * indicates significant ($p \leq 0.05$) difference from absence of TTNPB (ANOVA).

cytometry, 150 μ l Ringer solution containing 5 mM CaCl_2 were added and Syto 16 fluorescence was measured in fluorescence channel FL-1 on a FACS-Calibur as well as Annexin-V-APC fluorescence in FL-4 simultaneously.

Statistics

Data are expressed as arithmetic means \pm SEM. Statistical analysis was made by unpaired t-test or ANOVA as appropriate, $p \leq 0.05$ was considered as statistically significant.

Results

As illustrated in Fig. 1A, a 6 hours exposure of human erythrocytes to the specific retinoic acid receptor (RAR) agonist TTNPB (10 μ M) significantly increased Fluo3-fluorescence, an indicator of cytosolic Ca^{2+} -concentration. The average Fluo3 fluorescence increased

from 16.8 ± 0.6 to 28.7 ± 1.5 (n = 8) arbitrary units following a 6 hours incubation with TTNPB (10 μ M). A 6 hours exposure to retinoic acid (10 μ M) had no significant effect on Fluo3-fluorescence (data not shown). Thus, the exposure time has been extended. A 24 hours exposure to retinoic acid (10 μ M) indeed significantly increased Fluo3-fluorescence (Fig. 1B). The average Fluo3 fluorescence increased from 38.9 ± 3.4 to 68.8 ± 5.9 (n = 16) arbitrary units following a 24 hours incubation with retinoic acid (10 μ M).

An increase of intracellular Ca^{2+} -concentration is expected to activate Ca^{2+} -sensitive K^+ channels [48-50] with subsequent hyperpolarization of the cell membrane and thus extrusion of KCl . The loss of KCl and osmotically obliged water should shrink the cells and thus decrease the forward scatter in FACS analysis. Fig. 2 indeed demonstrates that exposure to TTNPB at concentrations

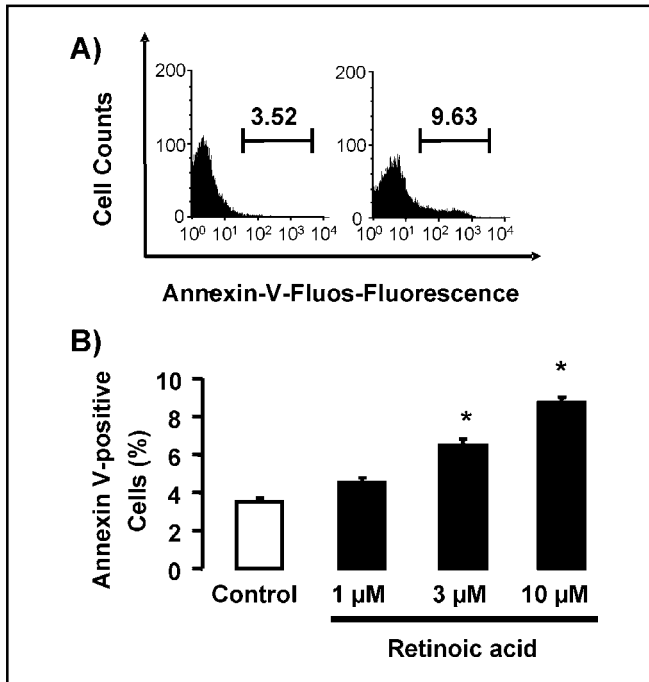


Fig. 5. Effect of retinoic acid on erythrocyte annexin binding. A. Original histograms of annexin V binding of human erythrocytes prior to (left panel) or following (right panel) a 24 hours exposure to 10 μM retinoic acid. B. Arithmetic means \pm SEM ($n = 8 - 13$) of annexin V binding prior to (control, open bar) or following (closed bars) a 24 hours exposure to retinoic acid at concentrations ranging from 1 to 10 μM . * indicates significant ($p \leq 0.05$) difference from absence of retinoic acid (ANOVA).

higher than 3 μM led to a decrease of erythrocyte forward scatter. In contrast to TTNBP, retinoic acid did not significantly shrink erythrocytes after 24 hours of incubation (Fig. 3).

Increased cytosolic Ca^{2+} -concentration is further expected to trigger cell membrane scrambling with subsequent phosphatidylserine exposure at the erythrocytes surface, which should enhance erythrocytic annexin V binding [15, 29-35]. Fig. 4 illustrates that a 24 hours exposure to TTNBP indeed enhanced the percentage of annexin V binding erythrocytes. The concentration needed for a significant increase of the percentage of annexin V binding erythrocytes was 3 μM . The effect of TTNBP was mimicked by retinoic acid (Fig. 5), which similarly increased erythrocyte annexin V binding.

Enhanced cytosolic Ca^{2+} -concentration has further been shown to activate the protease calpain, which degrades cell membrane proteins and leads to cell membrane blebbing [56, 57]. Thus, Western blotting was per-

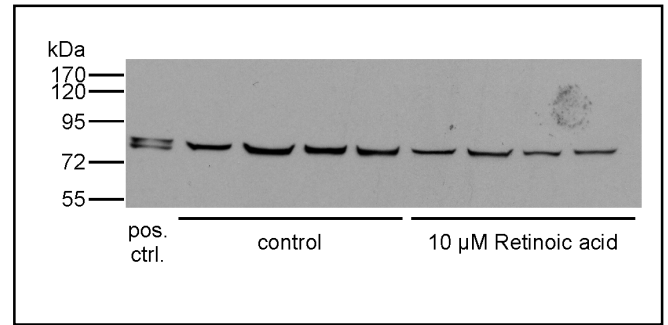


Fig. 6. Effect of retinoic acid on erythrocyte calpain activity. Original western blot showing calpain-specific immunoreactive protein bands of preparations of ionomycin-treated (1 μM for 1 h, left), non-treated (middle) and retinoic acid-treated (10 μM for 24 h, right) erythrocytes. Each lane shows the bands of independent preparations.

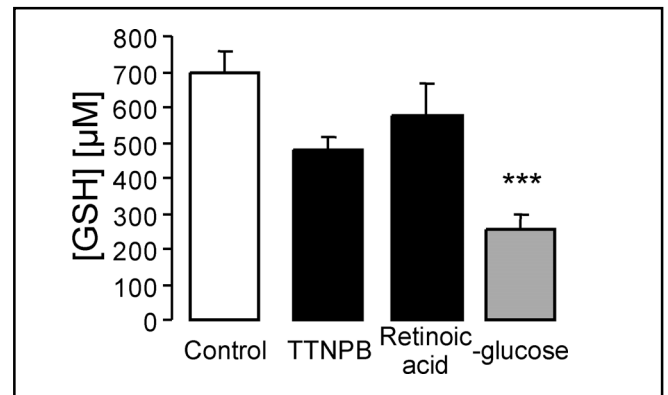


Fig. 7. Effect of retinoic acid and TTNPB on erythrocyte GSH concentration. Arithmetic means \pm SEM ($n = 4 - 5$) of the GSH concentration of erythrocytes following incubation for 24 h in Ringer without (left bar) or with 10 μM retinoic acid or TTNPB (black bars). Exposure to glucose-free Ringer served as a positive control (grey bar). *** indicates significant difference from control (ANOVA; $p \leq 0.001$).

formed to possibly depict calpain activation. As illustrated in Fig. 6, in contrast to the Ca^{2+} -ionophore ionomycin (1 μM , 1 hour), retinoic acid (10 μM , 24 hours) did not lead to appreciable activation of calpain. Thus, at the concentration of retinoic acid employed, the increase of Ca^{2+} -activity was apparently not sufficient for profound calpain activation.

The cation channels are activated by oxidative stress [36]. Thus, additional experiments have been performed to elucidate, whether retinoic acid decreases GSH concentration. As a result, TTNBP and retinoic acid tended to slightly decrease GSH concentration, effects,

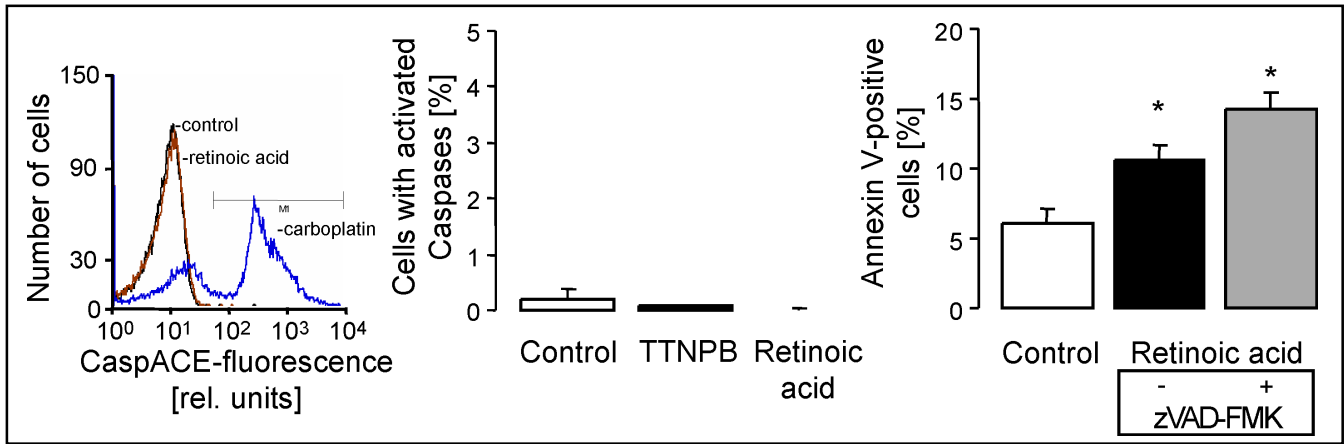
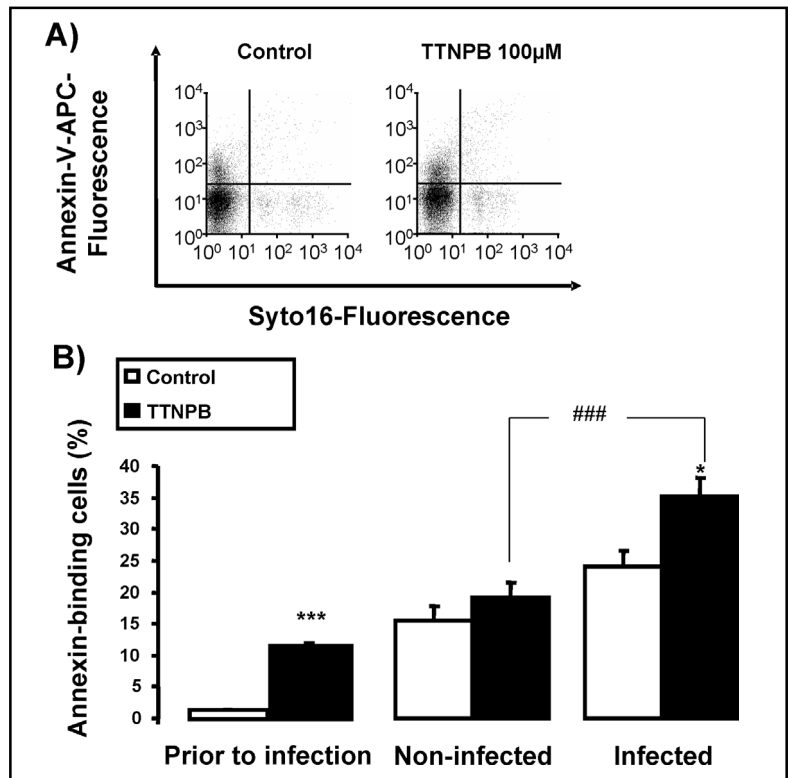


Fig. 8. Effect of retinoic acid and TTNPB on erythrocyte caspase activity. A. Original histogram of CaspACE fluorescence of erythrocytes following a 24 hours exposure to Ringer solution without (black line) or with 10 μ M retinoic acid (red line) or of HEK 293 cells exposed to 50 μ M carboplatin at 42.5°C for 4 h (blue line). B. Arithmetic means \pm SEM (n = 4-5) of CaspACE-positive cells following a 24 hours exposure to Ringer solution without or with 10 μ M retinoic acid or TTNPB. C. Arithmetic means \pm SEM (n = 5) of annexin V of erythrocytes exposed for 24 hours to Ringer solution without (left bar) or with 10 μ M retinoic acid in the absence (middle bar) or presence (right bar) of 10 μ M caspase inhibitor zVAD-FMK. * indicates significant ($p \leq 0.05$) difference from absence of retinoic acid (ANOVA).

Fig. 9. Effect of TTNPB on erythrocyte annexin V binding of *Plasmodium falciparum* infected erythrocytes. A. Original dot blots of annexin V binding (y-axis) and syto 16 fluorescence (x-axis) of infected and non-infected erythrocytes 24 h after infection in the absence (left panel) and presence (right panel) of TTNPB (100 μ M). B. Arithmetic means \pm SEM (n = 12) of annexin V binding of erythrocytes analysed prior to infection (left bars) or 24 hours following infection (middle and right bars). The infected culture contained non-infected (middle bars) and infected (right bars) erythrocytes. Infected erythrocytes were defined as cells stained with the DNA/RNA-specific dye Syto16. *, *** indicate significant ($p \leq 0.05$, $p \leq 0.001$) difference from absence of TTNPB, ### significant ($p \leq 0.001$) difference between non-infected and infected erythrocytes.



however, not reaching statistical significance. For comparison, glucose depletion was followed by the expected statistically significant decrease of GSH concentration (Fig. 7).

Oxidative stress activates caspases in erythrocytes [58]. Thus, additional experiments have been performed

to explore whether retinoic acid stimulates caspase 3. As illustrated in Fig. 8AB, CaspACE fluorescence in erythrocytes was not appreciably modified by a 24 hours exposure to 10 μ M retinoic acid. For comparison, marked increase of CaspACE fluorescence was observed in HEK 293 cells exposed to 50 μ M carboplatin (Fig 8A). Moreover,

the stimulation of annexin V binding was not blunted by the caspase inhibitor zVAD-FMK (Fig. 8C). Thus, caspases are apparently not crucial for the eryptotic effect of retinoic acid.

Infection of erythrocytes with *Plasmodium falciparum* has previously been shown to stimulate phosphatidylserine exposure leading to enhanced annexin V binding [19]. Therefore, experiments were performed to elucidate whether TTNPB preferentially increased annexin V binding of infected erythrocytes. Those experiments were performed in serum-containing medium. The addition of serum significantly decreased the ability of TTNPB to trigger annexin V binding (data not shown), an effect pointing to protein binding of TTNPB. Thus, higher TTNPB concentrations were needed to trigger eryptosis in the presence of serum. As illustrated in Fig. 9B, 100 μ M of TTNPB in medium containing serum stimulated annexin V binding of erythrocytes to a similar extent as 10 μ M of TTNPB in serum-free Ringer (Fig. 4). As illustrated in Fig. 9B, infection enhanced annexin V binding of infected and noninfected cells. The addition of TTNPB (100 μ M) enhanced annexin V binding significantly and preferentially in *Plasmodium falciparum* infected erythrocytes.

Discussion

The present study demonstrates that the specific retinoic acid receptor (RAR) agonist TTNPB as well as retinoic acid itself enhance cytosolic Ca^{2+} -activity in mature human erythrocytes. Retinoic acid has been shown to inhibit the Ca^{2+} -ATPase [59], an effect, which could well contribute to or even account for the increase of intracellular Ca^{2+} -activity.

An increase of intracellular Ca^{2+} -activity is expected to activate Ca^{2+} -sensitive K^+ channels [48-50] with subsequent exit of KCl and osmotically obliged water and thus cell shrinkage. Exposure to TTNPB indeed decreases the forward scatter. No significant change of forward scatter has, however, been observed following exposure to retinoic acid. In theory, retinoic acid could interfere with ion channels participating in KCl exit thus preventing cell shrinkage despite increased cytosolic Ca^{2+} -activity. Alternatively, the increase of cytosolic Ca^{2+} -activity did not reach the levels required for the activation of the Ca^{2+} -sensitive K^+ -channels.

Increased cytosolic Ca^{2+} -activity further stimulates phospholipids scrambling leading to phosphatidylserine exposure at the erythrocyte surface [10]. Indeed both,

TTNPB and retinoic acid, triggered annexin V binding. TTNPB appears to be more potent than retinoic acid.

The effects on forward scatter and phospholipids scrambling are largely, if not exclusively explained by the effect on cytosolic Ca^{2+} -activity. Oxidative stress and caspase activation apparently do not participate in the stimulation of eryptosis by TTNBP or retinoic acid. Retinoic acid and similar compounds, such as acitretin or etretinate, enhance the binding of cAMP to the regulatory unit of protein kinase A (PKA) and thus enhance PKA activity [60]. However, treatment of erythrocytes with forskolin, which should similarly stimulate PKA [61], did not trigger eryptosis (data not shown).

TTNBP potentiates the eryptosis of *Plasmodium falciparum* infected erythrocytes. Stimulation of phosphatidylserine exposure in *Plasmodium falciparum* infected erythrocytes has been observed earlier [19, 62]. Intraerythrocytic *Plasmodium falciparum* imposes oxidative stress on the host cell membrane [37, 55, 63, 64], thus leading to cation channel activation [55, 63, 64] and Ca^{2+} -entry [63]. The oxidative stress and thus stimulation of eryptosis affects not only the infected erythrocytes, but adjacent noninfected cells.

Premature eryptosis is expected to accelerate the clearance of infected erythrocytes from circulating blood and thus to favorably influence the course of malaria [33]. Eryptosis has previously been observed in several anemic conditions, including sickle-cell anemia, beta-thalassemia or glucose-6-phosphate dehydrogenase (G6PD)-deficiency [15] as well as iron deficiency anemia [65]. Sickle-cell anemia [15], beta-thalassemia [15], glucose-6-phosphate dehydrogenase (G6PD)-deficiency [15], and iron deficiency [66] are known to protect against a severe course of malaria. Accordingly, sickle cell trait (HbA/S) erythrocytes infected with *Plasmodium* have been shown to be more rapidly cleared than infected normal erythrocytes (HbA/A) [67]. Moreover, both, iron deficiency [68] and treatment with low concentrations of lead [69] significantly blunted the development of parasitemia in *Plasmodium berghei* infected mice. Most importantly, the decreased parasitemia was paralleled by increased survival of the mice. Thus, it is seducing to speculate that the eryptotic effect of retinoic acid may contribute to the favorable effect of vitamin A on the course of malaria.

Eryptotic erythrocytes may further adhere to the vascular wall [70] and may thus impair microcirculation [71-73]. Enhanced adhesiveness of erythrocytes participates in hemostasis [74-78] and may enhance the risk to

develop thrombosis [62]. Trapping of annexin V binding erythrocytes has previously been observed in the renal medulla following renal ischemia [41]. It is seducing to speculate that eryptosis plays an active role in the breakdown of microcirculation during acute renal failure. Moreover, eryptosis is triggered in haemolytic uremic syndrome [18] and sepsis [17] and may similarly contribute to deranged microcirculation in those disorders. Notably, vitamin A analogues have been shown to enhance concavalin A induced agglutination of human erythrocytes [79].

In conclusion, retinoic acid and the specific retinoic acid receptor agonist TTNPB stimulate eryptosis. The accelerated suicidal death of infected erythrocytes may

well participate in favorable effects of vitamin A on the course of malaria.

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