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Original Paper

Geldanamycin-Induced Phosphatidylserine Translocation in the Erythrocyte Membrane

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Key Words
Phosphatidylserine • Geldanamycin • Calcium • Ceramide • Cell volume • Eryptosis

Abstract
Background/aims: Geldanamycin, a benzoquinone ansamycin antibiotic, and its analogues induce apoptosis of tumor cells and are thus considered for the treatment of cancer. Similar to apoptosis of nucleated cells, erythrocytes may enter eryptosis, the suicidal erythrocyte death characterized by cell shrinkage and by cell membrane scrambling with phosphatidylserine-exposure at the erythrocyte surface. Triggers of eryptosis include increase of cytosolic Ca^{2+}-concentration ($[Ca^{2+}]_i$) and formation of ceramide. The present study explored, whether geldanamycin modifies $[Ca^{2+}]_i$, ceramide formation, cell volume and phosphatidylserine abundance at the erythrocyte surface. ***Methods:*** Erythrocyte volume was estimated from forward scatter, phosphatidylserine-abundance from annexin V binding, hemolysis from hemoglobin release, ceramide formation from binding of fluorescent antibodies and $[Ca^{2+}]_i$ from Fluo3-fluorescence. ***Results:*** A 48 hours exposure to geldanamycin significantly decreased forward scatter (≥ 5 μM), significantly increased annexin-V-binding (≥ 25 μM), but did not significantly modify Fluo3-fluorescence (up to 50 μM). The annexin-V-binding following geldanamycin treatment was not significantly modified by removal of extracellular Ca^{2+} but was paralleled by significantly increased ceramide formation (50 μM). ***Conclusions:*** Geldanamycin stinulated eryptosis, an effect at least partially due to ceramide formation.

Introduction

The benzoquinone ansamycin antibiotic geldanamycin has been shown to inhibit heat shock protein Hsp90 [1-12], which prevents stress-induced cellular damage [2], stabilizes various oncogenic kinases [1, 7, 9, 10, 12] and influences gene expression e.g. by up-

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regulating NF-κB [13, 14]. As Hsp90 expression is particularly high in cancer cells and is associated with tumor cell progression, invasion and formation of metastases, as well as development of drug resistance [2], geldanamycin and its analogues have been considered for treament of cancer [2, 3, 12, 15-19]. Geldanamycin has been shown to induce apoptosis [1, 5, 6, 8-10, 15, 20-23], an effect paralleled by altered gene expression, downregulation of Akt, p38 MAPK activation, mitochondrial depolarization, reactive oxygen species formation, decline of reduced glutathion, lipid peroxidation and caspase activation [5, 9, 15, 20, 21]. On the other hand, geldanamycin may counteract neuronal injury, an effect attributed to destabilization of RIP1 protein [4, 7, 24].

Similar to apoptosis of nucleated cells, erythrocytes may undergo eryptosis, the suicidal erythrocyte death characterized by cell membrane scrambling and cell shrinkage [25]. Eryptosis may be triggered by increased cytosolic Ca^{2+} concentration ($[Ca^{2+}]_i$) resulting from Ca^{2+} entry through Ca^{2+}-permeable cation channels [26, 27] or from permeabilization of the cell membrane e.g. by hemolysin [28]. Increased $[Ca^{2+}]_i$ leads to cell shrinkage by activation of Ca^{2+}-sensitive K^+ channels [29] with K^+ exit, hyperpolarization, Cl^- exit and thus cellular loss of KCl and osmotically obliged water [30]. Increased $[Ca^{2+}]_i$ further triggers phospholipid scrambling of the cell membrane with translocation of phosphatidylserine to the erythrocyte surface [31]. Ca^{2+} sensitivity of phospholipid scrambling is enhanced by ceramide [32]. Eryptosis may further be stimulated by energy depletion [33] and caspase activation [34-38]. Kinases participating in the regulation of eryptosis include AMP activated kinase AMPK [27], cGMP-dependent protein kinase [39], Janus-activated kinase JAK3 [40], casein kinase 1α [41, 42], p38 kinase [43], PAK2 kinase [44] as well as sorafenib [45] and sunitinib [46] sensitive kinases.

Eryptosis is a physiological mechanism preceding and actually preventing hemolysis of defective erythrocytes [32]. Excessive cell swelling may lead to rupture of the erythrocyte cell membrane, resulting in hemolysis with release of cellular hemoglobin, which is filtered in renal glomerula and subsequently occludes renal tubules [47]. The activation of K^+ channels during eryptosis counteracts cell swelling and thus hemolysis [30].

The present study explored, whether geldanamycin modifies erythrocyte $[Ca^{2+}]_i$, erythrocyte volume and/or phosphatidylserine abundance at the erythrocyte surface.

Materials and Methods

Erythrocytes, solutions and chemicals

Leukocyte-depleted erythrocytes 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 (184/2003V). 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), 5 glucose, 1 $CaCl_2$; pH 7.4 at 37°C for 48 h. Where indicated, erythrocytes were exposed to geldanamycin (Enzo, Lörrach, Germany) at the indicated concentrations. In Ca^{2+}-free Ringer solution, 1 mM $CaCl_2$ was substituted by 1 mM glycol-bis(2-aminoethylether)-N,N,N',N'-tetraacetic acid (EGTA).

FACS analysis of annexin-V-binding and forward scatter

After incubation under the respective experimental condition, 50 µl cell suspension was washed in Ringer solution containing 5 mM $CaCl_2$ and then stained with Annexin-V-FITC (1:200 dilution; ImmunoTools, Friesoythe, Germany) in this solution at 37°C for 20 min under protection from light. In the following, the forward scatter (FSC) of the cells was determined, and annexin-V fluorescence intensity was measured with an excitation wavelength of 488 nm and an emission wavelength of 530 nm on a FACS Calibur (BD, Heidelberg, Germany).

Measurement of intracellular Ca^{2+}

After incubation erythrocytes were washed in Ringer solution and then loaded with Fluo-3/AM (Biotium, Hayward, USA) in Ringer solution containing 5 mM $CaCl_2$ and 2 µM Fluo-3/AM. The cells were

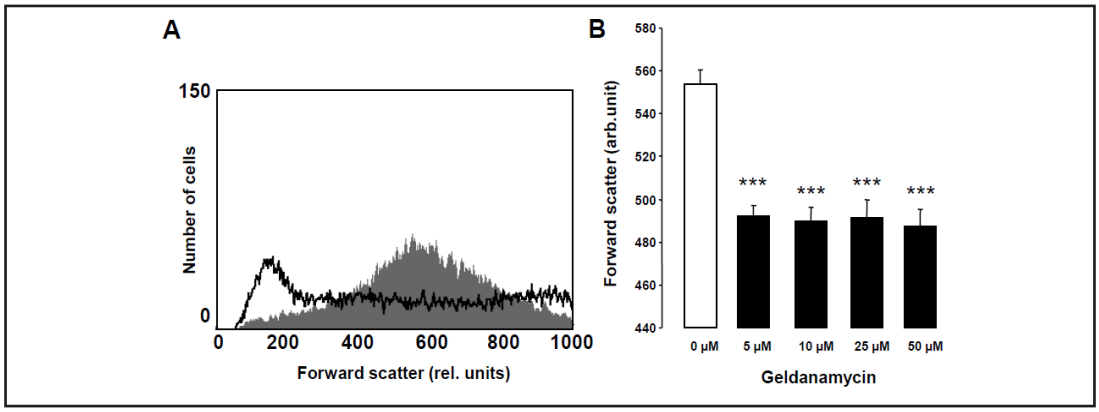

Fig. 1. Effect of geldanamycin on erythrocyte forward scatter. (A) Original histogram of forward scatter of erythrocytes following exposure for 48 hours to Ringer solution without (grey shadow) and with (black line) presence of 50 µM geldanamycin. (B) Arithmetic means ± SEM (n = 12) of the normalized erythrocyte forward scatter (FSC) following incubation for 48 hours to Ringer solution without (white bar) or with (black bars) geldanamycin (5-50 µM). *** ($p<0.001$) indicates significant difference from the absence of geldanamycin (ANOVA).

incubated at 37°C for 30 min and washed twice in Ringer solution containing 5 mM $CaCl_2$. The Fluo-3/AM-loaded erythrocytes were resuspended in 200 µl Ringer. Then, Ca^{2+}-dependent fluorescence intensity was measured with an excitation wavelength of 488 nm and an emission wavelength of 530 nm on a FACS Calibur.

Determination of ceramide formation

For the determination of ceramide, a monoclonal antibody-based assay was used. After incubation, cells were stained for 1 hour at 37°C with 1 µg/ml anti ceramide antibody (clone MID 15B4, Alexis, Grünberg, Germany) in PBS containing 0.1% bovine serum albumin (BSA) at a dilution of 1:5. The samples were washed twice with PBS-BSA. Subsequently, the cells were stained for 30 minutes with polyclonal fluorescein isothiocyanate (FITC) conjugated goat anti-mouse IgG and IgM specific antibody (Pharmingen, Hamburg, Germany) diluted 1:50 in PBS-BSA. Unbound secondary antibody was removed by repeated washing with PBS-BSA. The samples were then analyzed by flow cytometric analysis with an excitation wavelength of 488 nm and an emission wavelength of 530 nm.

Measurement of hemolysis

For the determination of hemolysis the samples were centrifuged (3 min at 400 g, room temperature) after incubation, and the supernatants were harvested. As a measure of hemolysis, the hemoglobin (Hb) concentration of the supernatant was determined photometrically at 405 nm. The absorption of the supernatant of erythrocytes lysed in distilled water was defined as 100% hemolysis.

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 addressed the effect of geldanamycin on eryptosis. Hallmarks of eryptosis include cell shrinkage. Thus, cell volume was estimated utilizing forward scatter. The forward scatter was determined by flow cytometry. As shown in Fig. 1A,B, a 48 hours

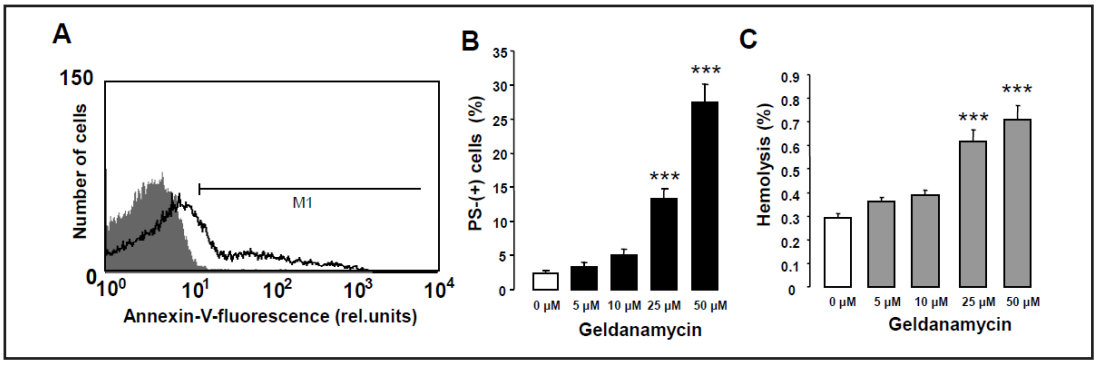

Fig. 2. Effect of geldanamycin on phosphatidylserine exposure and hemolysis. (A) Original histogram of annexin V binding of erythrocytes following exposure for 48 hours to Ringer solution without (grey shadow) and with (black line) presence of 50 µM geldanamycin. (B) Arithmetic means ± SEM (n = 12) of erythrocyte annexin-V-binding (PS-(+) cells) following incubation for 48 hours to Ringer solution without (white bar) or with (black bars) presence of geldanamycin (5-50 µM). (C) Arithmetic means ± SEM (n = 4) of the percentage of hemolysis following incubation for 48 hours to Ringer solution without (white bar) or with (grey bars) presence of geldanamycin (5-50 µM). *** ($p<0.001$) indicate significant differences from the absence of geldanamycin (ANOVA).

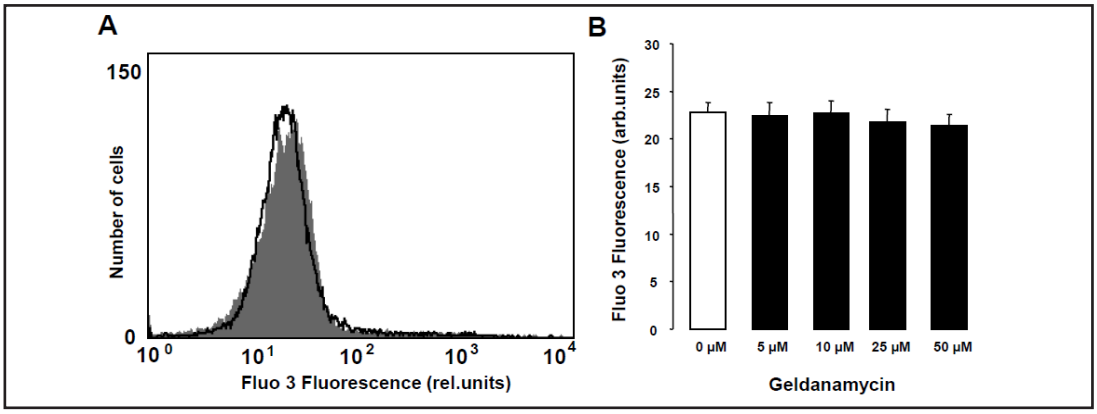

Fig. 3. Effect of geldanamycin on erythrocyte cytosolic Ca^{2+} concentration. (A) Original histogram of Fluo3 fluorescence in erythrocytes following exposure for 48 hours to Ringer solution without (grey shadow) and with (black line) presence of 50 µM geldanamycin. (B) Arithmetic means ± SEM (n = 12) of the Fluo3 fluorescence (arbitrary units) in erythrocytes exposed for 48 hours to Ringer solution without (white bar) or with (black bars) geldanamycin (5-50 µM).

exposure to geldanamycin resulted in a decrease of forward scatter, an effect reaching statistical significance at 5 µM geldanamycin concentration.

The second hallmark of eryptosis is cell membrane scrambling with subsequent increase of phosphatidylserine abundance at the cell surface. Accordingly, phosphatidylserine exposing erythrocytes were identified by annexin-V-binding in flow cytometry. As illustrated in Fig. 2A,B, a 48 hours exposure to geldanamycin increased the percentage of annexin-V-binding erythrocytes, an effect reaching statistical significance at 25 µM geldanamycin concentration.

In order to test, whether geldanamycin exposure leads to hemolysis, the percentage of hemolysed erythrocytes was estimated from hemoglobin concentration in the supernatant. As shown in Fig. 2B, a 48 hours exposure to geldanamycin increased the hemoglobin concentration in the supernatant, an effect reaching statistical significance at 25 µM geldanamycin concentration. Notably, the percentage of hemolytic erythrocytes remained almost one magnitude smaller than the percentage of phosphatidylserine exposing erythrocytes (Fig. 2C).

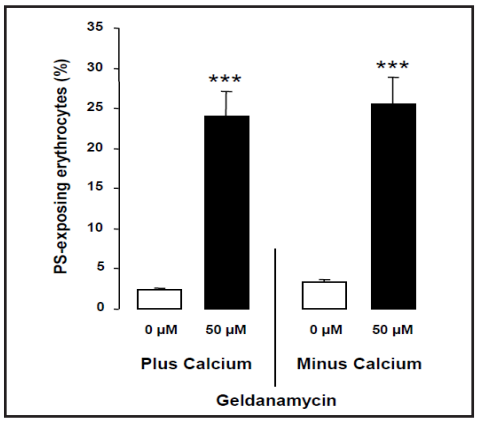

Fig. 4. Effect of Ca^{2+} withdrawal on geldanamycin- induced annexin-V-binding. Arithmetic means ± SEM (n = 6) of the percentage of annexin-V-binding erythrocytes after a 48 hours treatment with Ringer solution without (white bar) or with (black bars) 50 µM geldanamycin in the presence (left bars, Plus Calcium) and absence (right bars, Minus Calcium) of calcium. *** ($p<0.001$) indicates significant difference from the absence of geldanamycin (ANOVA).

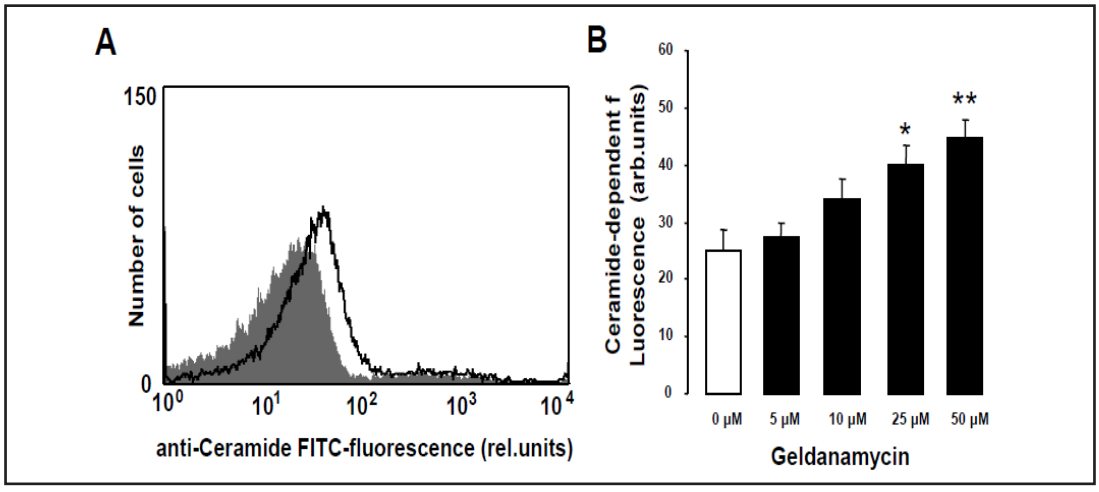

Fig. 5. Effect of geldanamycin on ceramide formation. (A) Original histogram of ceramide surface abundance of erythrocytes following exposure for 48 hours to Ringer solution without (grey shadow) and with (black line) presence of 50 µM geldanamycin. (B) Arithmetic means ± SEM (n = 4) of ceramide abundance after a 48 hours incubation in Ringer solution without (white bar) or with 50 µM geldanamycin (black bar). * ($p<0.05$) indicates significant difference from the absence of geldanamycin (*t* test).

In an attempt to elucidate the mechanisms underlying the triggering of erythrocyte shrinkage and cell membrane scrambling following geldanamycin exposure, cytosolic Ca^{2+} concentration ($[Ca^{2+}]_i$) was determined utilizing Fluo3 fluorescence. To this end, erythrocytes were loaded with Fluo3-AM and Fluo3 fluorescence determined in FACS analysis following incubation in Ringer solution without or with geldanamycin (1-50 µM). As illustrated in Fig. 3, a 48 hours exposure of human erythrocytes to geldanamycin up to 50 µM remained without significant effect on Fluo3 fluorescence.

To further elucidate the role of Ca^{2+}, an additional series of experiments explored whether extracellular Ca^{2+} entry was required for the effect of geldanamycin on cell membrane scrambling. To this end, erythrocytes were exposed to 50 µM geldanamycin for 48 hours either in the presence of 1 mM Ca^{2+} or in the absence of Ca^{2+} and the presence of Ca^{2+} chelator EGTA (1 mM). As illustrated in Fig. 4, the effect of geldanamycin on annexin-V-binding was virtually the same in the presence and nominal absence of Ca^{2+}.

Additional experiments explored, whether geldanamycin stimulates the formation of ceramide, which has previously been shown to trigger eryptosis even at constant $[Ca^{2+}]_i$. Ceramide abundance at the erythrocyte surface was determined utilizing an anti-ceramide antibody. As shown in Fig. 5, a 48 hours exposure to geldanamycin increased the abundance of ceramide at the erythrocyte surface, an effect reaching statistical significance at 25 µM geldanamycin concentration.

Discussion

The present study uncovers a novel effect of geldanamycin, i.e. the stimulation of suicidal erythrocyte death. Exposure of human erythrocytes to geldanamycin is followed by erythrocyte shrinkage and erythrocyte membrane scrambling, both hallmarks of eryptosis. The concentrations required were similar to those previously observed *in vivo* [48].

Similar to geldanamycin, a wide variety of xenobiotics stimulate eryptosis [46, 49-75]. However, most xenobiotics triggering eryptosis do so by increasing cytosolic Ca^{2+} concentration [32]. They are effective by activation of the endogenous Ca^{2+} permeable non-selective cation channels, which involve somehow the transient receptor potential channel TRPC6 [26] and are activated by oxidative stress [75] . Activation of those channels shrinks erythrocytes by stimulating entry of extracellular Ca^{2+} with subsequent increase of cytosolic Ca^{2+} concentration ($[Ca^{2+}]_i$), and activation of Ca^{2+} sensitive K^+ channels [29, 75]. The activation of the Ca^{2+} sensitive K^+ channels leads to K^+ exit, cell membrane hyperpolarisation, Cl^- exit and thus cellular loss of KCl with osmotically obliged water [30]. Even though geldanamycin does not appreciably affect $[Ca^{2+}]_i$, it still leads to a marked and robust decrease of forward scatter. The cell shrinkage is already maximal at geldanamycin concentrations, which have little effect on phosphatidylserine exposure. Possibly, geldanamycin shrinks erythrocytes by Ca^{2+} independent activation of K^+ channels, an effect already maximal at the lowest geldanamycin concentrations used and thus seemingly lacking dose-dependence.

The effect of geldanamycin on cell membrane scrambling is at least partially due to formation of ceramide, which sensitizes the cells to the eryptotic effects of $[Ca^{2+}]_i$ and is thus capable to trigger eryptosis without increase of $[Ca^{2+}]_i$ [32].

Excessive eryptosis contributes to several clinical disorders [25] including diabetes [38, 75, 76], renal insufficiency [75] , hemolytic uremic syndrome [77], sepsis [78], malaria [79, 80], sickle cell disease [81], Wilson's disease [80], iron deficiency [82], malignancy [83], phosphate depletion [84], and metabolic syndrome [71]. Again, those disorders are mostly effective by increasing cytosolic Ca^{2+} concentration [32].

Eryptotic erythrocytes are cleared from circulating blood with subsequent development of anemia, if the accelerated loss of erythrocytes is not compensated by enhanced formation of new erythrocytes [25]. Phosphatidylserine exposing erythrocytes may further adhere to endothelial CXCL16/SR-PSO of the vascular wall [85], which may, at least in theory, interfere with blood flow [85-90]. Phosphatidylserine exposing erythrocytes may further stimulate blood clotting and thus favour the development of thrombosis [86, 91, 92]. In view of the present observations, those potential side effects must be considered during the use of geldanamycin.

Conclusions

Geldanamycin triggers eryptosis, an effect at least partially due to stimulation of ceramide formation. Geldanamycin stimulates cell membrane scrambling and cell shrinkage and thus suicidal death of human erythrocytes by mechanisms not requiring Ca^{2+} entry.

Conflict of Interest

The authors declare no conflict of interest.

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References

1 Farina AR, Tacconelli A, Cappabianca L, Cea G, Chioda A, Romanelli A, Pensato S, Pedone C, Gulino A, Mackay AR: The neuroblastoma tumour-suppressor TrkAI and its oncogenic alternative TrkAIII splice variant exhibit geldanamycin-sensitive interactions with Hsp90 in human neuroblastoma cells. Oncogene 2009;28:4075-4094.
2 Gorska M, Popowska U, Sielicka-Dudzin A, Kuban-Jankowska A, Sawczuk W, Knap N, Cicero G, Wozniak F: Geldanamycin and its derivatives as Hsp90 inhibitors. Front Biosci 2012;17:2269-2277.
3 Kim T, Keum G, Pae AN: Discovery and development of heat shock protein 90 inhibitors as anticancer agents: a review of patented potent geldanamycin derivatives. Expert Opin Ther Pat 2013;23:919-943.
4 Kwon HM, Kim Y, Yang SI, Kim YJ, Lee SH, Yoon BW: Geldanamycin protects rat brain through overexpression of HSP70 and reducing brain edema after cerebral focal ischemia. Neurol Res 2008;30:740-745.
5 Lee CS, Kim YJ, Lee SA, Myung SC, Kim W: Combined effect of Hsp90 inhibitor geldanamycin and parthenolide via reactive oxygen species-mediated apoptotic process on epithelial ovarian cancer cells. Basic Clin Pharmacol Toxicol 2012;111:173-181.
6 Li Q, Zhou Y, Yao C, Ma X, Wang L, Xu W, Wang Z, Qiao Z: Apoptosis caused by Hsp90 inhibitor geldanamycin in Leishmania donovani during promastigote-to-amastigote transformation stage. Parasitol Res 2009;105:1539-1548.
7 Wen XR, Li C, Zong YY, Yu CZ, Xu J, Han D, Zhang GY: Dual inhibitory roles of geldanamycin on the c-Jun NH2-terminal kinase 3 signal pathway through suppressing the expression of mixed-lineage kinase 3 and attenuating the activation of apoptosis signal-regulating kinase 1 via facilitating the activation of Akt in ischemic brain injury. Neuroscience 2008;156:483-497.
8 Wu WC, Wu MH, Chang YC, Hsieh MC, Wu HJ, Cheng KC, Lai YH, Kao YH: Geldanamycin and its analog induce cytotoxicity in cultured human retinal pigment epithelial cells. Exp Eye Res 2010;91:211-219.
9 Schumacher JA, Crockett DK, Elenitoba-Johnson KS, Lim MS: Proteome-wide changes induced by the Hsp90 inhibitor, geldanamycin in anaplastic large cell lymphoma cells. Proteomics 2007;7:2603-2616.
10 Trentin L, Frasson M, Donella-Deana A, Frezzato F, Pagano MA, Tibaldi E, Gattazzo C, Zambello R, Semenzato G, Brunati AM: Geldanamycin-induced Lyn dissociation from aberrant Hsp90-stabilized cytosolic complex is an early event in apoptotic mechanisms in B-chronic lymphocytic leukemia. Blood 2008;112:4665-4674.
11 McCollum AK, Lukasiewicz KB, Teneyck CJ, Lingle WL, Toft DO, Erlichman C: Cisplatin abrogates the geldanamycin-induced heat shock response. Mol Cancer Ther 2008;7:3256-3264.
12 Soga S, Akinaga S, Shiotsu Y: Hsp90 inhibitors as anti-cancer agents, from basic discoveries to clinical development. Curr Pharm Des 2013;19:366-376.
13 Crevecoeur J, Merville MP, Piette J, Gloire G: Geldanamycin inhibits tyrosine phosphorylation-dependent NF-kappaB activation. Biochem Pharmacol 2008;75:2183-2191.
14 Yan P, Qing G, Qu Z, Wu CC, Rabson A, Xiao G: Targeting autophagic regulation of NFkappaB in HTLV-I transformed cells by geldanamycin: implications for therapeutic interventions. Autophagy 2007;3:600-603.
15 Lesko E, Gozdzik J, Kijowski J, Jenner B, Wiecha O, Majka M: HSP90 antagonist, geldanamycin, inhibits proliferation, induces apoptosis and blocks migration of rhabdomyosarcoma cells in vitro and seeding into bone marrow in vivo. Anticancer Drugs 2007;18:1173-1181.
16 Kiang JG, Smith JT, Agravante NG: Geldanamycin analog 17-DMAG inhibits iNOS and caspases in gamma-irradiated human T cells. Radiat Res 2009;172:321-330.
17 Fukumoto R, Kiang JG: Geldanamycin analog 17-DMAG limits apoptosis in human peripheral blood cells by inhibition of p53 activation and its interaction with heat-shock protein 90 kDa after exposure to ionizing radiation. Radiat Res 2011;176:333-345.
18 Pedersen NM, Breen K, Rodland MS, Haslekas C, Stang E, Madshus IH: Expression of epidermal growth factor receptor or ErbB3 facilitates geldanamycin-induced down-regulation of ErbB2. Mol Cancer Res 2009;7:275-284.
19 Pritchard JR, Cosgrove BD, Hemann MT, Griffith LG, Wands JR, Lauffenburger DA: Three-kinase inhibitor combination recreates multipathway effects of a geldanamycin analogue on hepatocellular carcinoma cell death. Mol Cancer Ther 2009;8:2183-2192.
20 Dey A, Cederbaum AI: Geldanamycin, an inhibitor of Hsp90 increases cytochrome P450 2E1 mediated toxicity in HepG2 cells through sustained activation of the p38MAPK pathway. Arch Biochem Biophys 2007;461:275-286.

21 Jeon YK, Park CH, Kim KY, Li YC, Kim J, Kim YA, Paik JH, Park BK, Kim CW, Kim YN: The heat-shock protein 90 inhibitor, geldanamycin, induces apoptotic cell death in Epstein-Barr virus-positive NK/T-cell lymphoma by Akt down-regulation. J Pathol 2007;213:170-179.
22 Sugimoto K, Sasaki M, Isobe Y, Tsutsui M, Suto H, Ando J, Tamayose K, Ando M, Oshimi K: Hsp90-inhibitor geldanamycin abrogates G2 arrest in p53-negative leukemia cell lines through the depletion of Chk1. Oncogene 2008;27:3091-3101.
23 Li S, Ni S, Wu L, Li L, Jiang B, Wang H, Sun G, Gan M, Li J, He W, Lin L, Wang Y, Bai S, Si S: 19-[(1'S,4'R)-4'-Hydroxy-1'-methoxy-2'-oxopentyl]geldanamycin, a Natural Geldanamycin Analogue from Streptomyces hygroscopicus 17997. J Nat Prod 2013;76:969-973.
24 Chen WW, Yu H, Fan HB, Zhang CC, Zhang M, Zhang C, Cheng Y, Kong J, Liu CF, Geng D, Xu X: RIP1 mediates the protection of geldanamycin on neuronal injury induced by oxygen-glucose deprivation combined with zVAD in primary cortical neurons. J Neurochem 2012;120:70-77.
25 Lang F, Gulbins E, Lerche H, Huber SM, Kempe DS, Föller M: Eryptosis, a window to systemic disease. Cell Physiol Biochem 2008;22:373-380.
26 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.
27 Foller M, Sopjani M, Koka S, Gu S, Mahmud H, Wang K, Floride E, Schleicher E, Schulz E, Munzel T, Lang F: Regulation of erythrocyte survival by AMP-activated protein kinase. FASEB J 2009;23:1072-1080.
28 Lang PA, Kaiser S, Myssina S, Birka C, Weinstock C, Northoff H, Wieder T, Lang F, Huber SM: Effect of Vibrio parahaemolyticus haemolysin on human erythrocytes. Cell Microbiol 2004;6:391-400.
29 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.
30 Lang PA, Kaiser S, Myssina S, Wieder T, Lang F, Huber SM: Role of Ca^{2+}-activated K^+ channels in human erythrocyte apoptosis. Am J Physiol Cell Physiol 2003;285:C1553-C1560.
31 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.
32 Lang F, Gulbins E, Lang PA, Zappulla D, Foller M: Ceramide in suicidal death of erythrocytes. Cell Physiol Biochem 2010;26:21-28.
33 Klarl BA, Lang PA, Kempe DS, Niemoeller OM, Akel A, Sobiesiak M, Eisele K, Podolski M, Huber SM, Wieder T, Lang F: Protein kinase C mediates erythrocyte "programmed cell death" following glucose depletion. Am J Physiol Cell Physiol 2006;290:C244-C253.
34 Bhavsar SK, Bobbala D, Xuan NT, Foller M, Lang F: Stimulation of suicidal erythrocyte death by alpha-lipoic acid. Cell Physiol Biochem 2010;26:859-868.
35 Foller M, Huber SM, Lang F: Erythrocyte programmed cell death. IUBMB Life 2008;60:661-668.
36 Foller M, Mahmud H, Gu S, Wang K, Floride E, Kucherenko Y, Luik S, Laufer S, Lang F: Participation of leukotriene C(4) in the regulation of suicidal erythrocyte death. J Physiol Pharmacol 2009;60:135-143.
37 Lau IP, Chen H, Wang J, Ong HC, Leung KC, Ho HP, Kong SK: In vitro effect of CTAB- and PEG-coated gold nanorods on the induction of eryptosis/erythroptosis in human erythrocytes. Nanotoxicology 2012;6:847-856.
38 Maellaro E, Leoncini S, Moretti D, Del Bello B, Tanganelli I, De Felice C, Ciccoli L: Erythrocyte caspase-3 activation and oxidative imbalance in erythrocytes and in plasma of type 2 diabetic patients. Acta Diabetol 2013;50:489-495.
39 Foller M, Feil S, Ghoreschi K, Koka S, Gerling A, Thunemann M, Hofmann F, Schuler B, Vogel J, Pichler B, Kasinathan RS, Nicolay JP, Huber SM, Lang F, Feil R: Anemia and splenomegaly in cGKI-deficient mice. Proc Natl Acad Sci USA 2008;105:6771-6776.
40 Bhavsar SK, Gu S, Bobbala D, Lang F: Janus kinase 3 is expressed in erythrocytes, phosphorylated upon energy depletion and involved in the regulation of suicidal erythrocyte death. Cell Physiol Biochem 2011;27:547-556.
41 Kucherenko Y, Zelenak C, Eberhard M, Qadri SM, Lang F: Effect of casein kinase 1alpha activator pyrvinium pamoate on erythrocyte ion channels. Cell Physiol Biochem 2012;30:407-417.
42 Zelenak C, Eberhard M, Jilani K, Qadri SM, Macek B, Lang F: Protein kinase CK1alpha regulates erythrocyte survival. Cell Physiol Biochem 2012;29:171-180.
43 Gatidis S, Zelenak C, Fajol A, Lang E, Jilani K, Michael D, Qadri SM, Lang F: p38 MAPK activation and function following osmotic shock of erythrocytes. Cell Physiol Biochem 2011;28:1279-1286.
44 Zelenak C, Foller M, Velic A, Krug K, Qadri SM, Viollet B, Lang F, Macek B: Proteome analysis of erythrocytes lacking AMP-activated protein kinase reveals a role of PAK2 kinase in eryptosis. J Proteome Res 2011;10:1690-1697.

45 Lupescu A, Shaik N, Jilani K, Zelenak C, Lang E, Pasham V, Zbidah M, Plate A, Bitzer M, Foller M, Qadri SM, Lang F: Enhanced Erythrocyte Membrane Exposure of Phosphatidylserine Following Sorafenib Treatment: An in vivo and in vitro Study. Cell Physiol Biochem 2012;30:876-888.
46 Shaik N, Lupescu A, Lang F: Sunitinib-sensitive suicidal erythrocyte death. Cell Physiol Biochem 2012;30:512-522.
47 Harrison HE, Bunting H, Ordway NK, Albrink WS: The Pathogenesis of the Renal Injury Produced in the Dog by Hemoglobin or Methemoglobin. J Exp Med 1947;86:339-356.
48 Supko JG, Hickman RL, Grever MR, Malspeis L: Preclinical pharmacologic evaluation of geldanamycin as an antitumor agent. Cancer Chemother Pharmacol 1995;36:305-315.
49 Abed M, Towhid ST, Mia S, Pakladok T, Alesutan I, Borst O, Gawaz M, Gulbins E, Lang F: Sphingomyelinase-induced adhesion of eryptotic erythrocytes to endothelial cells. Am J Physiol Cell Physiol 2012;303:C991-999.
50 Bottger E, Multhoff G, Kun JF, Esen M: Plasmodium falciparum-infected erythrocytes induce granzyme B by NK cells through expression of host-Hsp70. PLoS One 2012;7:e33774.
51 Felder KM, Hoelzle K, Ritzmann M, Kilchling T, Schiele D, Heinritzi K, Groebel K, Hoelzle LE: Hemotrophic mycoplasmas induce programmed cell death in red blood cells. Cell Physiol Biochem 2011;27:557-564.
52 Firat U, Kaya S, Cim A, Buyukbayram H, Gokalp O, Dal MS, Tamer MN: Increased caspase-3 immunoreactivity of erythrocytes in STZ diabetic rats. Exp Diabetes Res 2012;2012:316384.
53 Ganesan S, Chaurasiya ND, Sahu R, Walker LA, Tekwani BL: Understanding the mechanisms for metabolism-linked hemolytic toxicity of primaquine against glucose 6-phosphate dehydrogenase deficient human erythrocytes: evaluation of eryptotic pathway. Toxicology 2012;294:54-60.
54 Ghashghaeinia M, Cluitmans JC, Akel A, Dreischer P, Toulany M, Koberle M, Skabytska Y, Saki M, Biedermann T, Duszenko M, Lang F, Wieder T, Bosman GJ: The impact of erythrocyte age on eryptosis. Br J Haematol 2012;157:606-614.
55 Ghashghaeinia M, Toulany M, Saki M, Bobbala D, Fehrenbacher B, Rupec R, Rodemann HP, Ghoreschi K, Rocken M, Schaller M, Lang F, Wieder T: The NFκB pathway inhibitors Bay 11-7082 and parthenolide induce programmed cell death in anucleated Erythrocytes. Cell Physiol Biochem 2011;27:45-54.
56 Jilani K, Lupescu A, Zbidah M, Abed M, Shaik N, Lang F: Enhanced Apoptotic Death of Erythrocytes Induced by the Mycotoxin Ochratoxin A. Kidney Blood Press Res 2012;36:107-118.
57 Kucherenko YV, Lang F: Inhibitory Effect of Furosemide on Non-Selective Voltage-Independent Cation Channels in Human Erythrocytes. Cell Physiol Biochem 2012;30:863-875.
58 Lang E, Jilani K, Zelenak C, Pasham V, Bobbala D, Qadri SM, Lang F: Stimulation of suicidal erythrocyte death by benzethonium. Cell Physiol Biochem 2011;28:347-354.
59 Lang E, Qadri SM, Jilani K, Zelenak C, Lupescu A, Schleicher E, Lang F: Carbon monoxide-sensitive apoptotic death of erythrocytes. Basic Clin Pharmacol Toxicol 2012;111:348-355.
60 Lupescu A, Jilani K, Zbidah M, Lang E, Lang F: Enhanced Ca^{2+} Entry, Ceramide Formation, and Apoptotic Death of Erythrocytes Triggered by Plumbagin. J Nat Prod 2012;10.1021/np300611r
61 Lupescu A, Jilani K, Zbidah M, Lang F: Induction of apoptotic erythrocyte death by rotenone. Toxicology 2012;300:132-137.
62 Lupescu A, Jilani K, Zelenak C, Zbidah M, Qadri SM, Lang F: Hexavalent chromium-induced erythrocyte membrane phospholipid asymmetry. Biometals 2012;25:309-318.
63 Polak-Jonkisz D, Purzyc L: Ca Influx versus Efflux during Eryptosis in Uremic Erythrocytes. Blood Purif 2012;34:209-210.
64 Qadri SM, Bauer J, Zelenak C, Mahmud H, Kucherenko Y, Lee SH, Ferlinz K, Lang F: Sphingosine but not sphingosine-1-phosphate stimulates suicidal erythrocyte death. Cell Physiol Biochem 2011;28:339-346.
65 Qadri SM, Kucherenko Y, Lang F: Beauvericin induced erythrocyte cell membrane scrambling. Toxicology 2011;283:24-31.
66 Qadri SM, Kucherenko Y, Zelenak C, Jilani K, Lang E, Lang F: Dicoumarol activates Ca^{2+}-permeable cation channels triggering erythrocyte cell membrane scrambling. Cell Physiol Biochem 2011;28:857-864.
67 Qian EW, Ge DT, Kong SK: Salidroside protects human erythrocytes against hydrogen peroxide-induced apoptosis. J Nat Prod 2012;75:531-537.
68 Shaik N, Zbidah M, Lang F: Inhibition of Ca^{2+} entry and suicidal erythrocyte death by naringin. Cell Physiol Biochem 2012;30:678-686.
69 Vota DM, Maltaneri RE, Wenker SD, Nesse AB, Vittori DC: Differential erythropoietin action upon cells induced to eryptosis by different agents. Cell Biochem Biophys 2013;65:145-157.
70 Weiss E, Cytlak UM, Rees DC, Osei A, Gibson JS: Deoxygenation-induced and Ca^{2+} dependent phosphatidylserine externalisation in red blood cells from normal individuals and sickle cell patients. Cell Calcium 2012;51:51-56.

71 Zappulla D: Environmental stress, erythrocyte dysfunctions, inflammation, and the metabolic syndrome: adaptations to CO_2 increases? J Cardiometab Syndr 2008;3:30-34.
72 Zbidah M, Lupescu A, Jilani K, Lang F: Stimulation of suicidal erythrocyte death by fumagillin. Basic Clin Pharmacol Toxicol 2013;112:346-351.
73 Zbidah M, Lupescu A, Shaik N, Lang F: Gossypol-induced suicidal erythrocyte death. Toxicology 2012;302:101-105.
74 Zelenak C, Pasham V, Jilani K, Tripodi PM, Rosaclerio L, Pathare G, Lupescu A, Faggio C, Qadri SM, Lang F: Tanshinone IIA stimulates erythrocyte phosphatidylserine exposure. Cell Physiol Biochem 2012;30:282-294.
75 Lang E, Qadri SM, Lang F: Killing me softly - suicidal erythrocyte death. Int J Biochem Cell Biol 2012;44:1236-1243.
76 Calderon-Salinas JV, Munoz-Reyes EG, Guerrero-Romero JF, Rodriguez-Moran M, Bracho-Riquelme RL, Carrera-Gracia MA, Quintanar-Escorza MA: Eryptosis and oxidative damage in type 2 diabetic mellitus patients with chronic kidney disease. Mol Cell Biochem 2011;357:171-179.
77 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 (Berl) 2006;84:378-388.
78 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;85:269-277.
79 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.
80 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, Hefter 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.
81 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.
82 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.
83 Qadri SM, Mahmud H, Lang E, Gu S, Bobbala D, Zelenak C, Jilani K, Siegfried A, Foller M, Lang F: Enhanced suicidal erythrocyte death in mice carrying a loss-of-function mutation of the adenomatous polyposis coli gene. J Cell Mol Med 2012;16:1085-1093.
84 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.
85 Borst O, Abed M, Alesutan I, Towhid ST, Qadri SM, Foller M, Gawaz M, Lang F: Dynamic adhesion of eryptotic erythrocytes to endothelial cells via CXCL16/SR-PSOX. Am J Physiol Cell Physiol 2012;302:C644-C651.
86 Andrews DA, Low PS: Role of red blood cells in thrombosis. Curr Opin Hematol 1999;6:76-82.
87 Closse C, Dachary-Prigent J, Boisseau MR: Phosphatidylserine-related adhesion of human erythrocytes to vascular endothelium. Br J Haematol 1999;107:300-302.
88 Gallagher PG, Chang SH, Rettig MP, Neely JE, Hillery CA, Smith BD, Low PS: Altered erythrocyte endothelial adherence and membrane phospholipid asymmetry in hereditary hydrocytosis. Blood 2003;101:4625-4627.
89 Pandolfi A, Di Pietro N, Sirolli V, Giardinelli A, Di Silvestre S, Amoroso L, Di Tomo P, Capani F, Consoli A, Bonomini M: Mechanisms of uremic erythrocyte-induced adhesion of human monocytes to cultured endothelial cells. J Cell Physiol 2007;213:699-709.
90 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.
91 Chung SM, Bae ON, Lim KM, Noh JY, Lee MY, Jung YS, Chung JH: Lysophosphatidic acid induces thrombogenic activity through phosphatidylserine exposure and procoagulant microvesicle generation in human erythrocytes. Arterioscler Thromb Vasc Biol 2007;27:414-421.
92 Zwaal RF, Comfurius P, Bevers EM: Surface exposure of phosphatidylserine in pathological cells. Cell Mol Life Sci 2005;62:971-988.