Molecular Biology Reports (2020) 47:5025–5032
https://doi.org/10.1007/s11033-020-05568-3

ORIGINAL ARTICLE

Inhibition of suicidal erythrocyte death by pyrogallol

Jibin Liu[1,2] · Abdulla Al Mamun Bhuyan[2] · Ke Ma[2] · Shaqiu Zhang[1] · Anchun Cheng[1] · Florian Lang[2]

Received: 22 April 2020 / Accepted: 5 June 2020 / Published online: 10 June 2020

Abstract

Pyrogallol, a polyphenolic component of *Acacia nilotica* has previously been reported to induce apoptosis of diverse cell types. Pyrogallol is in part effective by influencing gene expression and by interference with mitochondrial function. Despite lack of nuclei and mitochondria, erythrocytes may undergo eryptosis, a suicidal death apparent from phosphatidylserine translocation to the erythrocyte surface and cell shrinkage. Eryptosis is triggered by glucose depletion, by oxidation, by hyperosmotic cell shrinkage and by excessive Ca^{2+} entry. As enhanced eryptosis is a common cause of anemia, uncovering inhibitors and stimulators of eryptosis may, both, be of clinical interest. Here we tested, whether eryptosis of human erythrocytes is modified by pyrogallol. Utilizing flow cytometry, phosphatidylserine abundance at the cell surface was estimated from annexin-V-binding and cell volume from forward scatter. Prior to determinations erythrocytes were incubated with or without glucose, without or with added oxidant *tert*-butyl-hydroperoxide (*t*-BOOH, 0.5 mM), without or with added hyperosmotic sucrose (550 mM) or without or with added Ca^{2+} ionophore ionomycin (1 µM). Treatment of erythrocytes with pyrogallol (2–8 µM) was without significant effect on annexin-V-binding and forward scatter. Glucose deprivation, *t*-BOOH, sucrose and ionomycin, each, triggered annexin-V-binding and decreased forward scatter. Pyrogallol significantly blunted the effects on annexin-V-binding but not on forward scatter. Pyrogallol thus blunts phosphatidylserine translocation in erythrocytes exposed to glucose depletion, oxidative stress, hyperosmotic shock and excessive Ca^{2+} entry.

Keywords Eryptosis · Pyrogallol · Glucose depletion · Oxidative stress · Calcium

Introduction

Pyrogallol, a polyphenolic component of *Acacia nilotica* [1, 2] has been reported to stimulate apoptosis of cancer cells [3–5], fibroblasts [6, 7], endothelial cells [8–10], juxtaglomerular cells [11–13], U937 cells [14], HEK293 cells [15], K562 cells [15] and blood platelets [2]. Pyrogallol-induced apoptosis involves modification of gene expression [16], nuclear DNA fragmentation [1], mitochondria [1], oxidative stress [2, 3, 7–13], inhibition of Bcl-2 proteins [17] and caspase activation [2].

Mature, circulating erythrocytes are lacking nuclei and mitochondria but harbor caspases [18, 19] and may enter eryptosis, a suicidal cell death apparent from phosphatidylserine translocation to the outer cell membrane surface [19–22]. Eryptosis is further typically paralleled by cell shrinkage [23]. Eryptosis may be stimulated by storage [24], energy depletion [19, 25], oxidative stress [19], hyperosmotic shock [19], and excessive Ca^{2+} entry [19]. Excessive eryptosis may result in anemia, because phosphatidylserine exposing erythrocytes are bound to endothelial cells and removed from circulating blood [26, 27]. Eryptosis and subsequent removal of *Plasmodium*-infected erythrocytes may, on the other hand, reduce parasitemia and thus be beneficial in malaria [28]. Accordingly, small molecules stimulating

Jibin Liu and Abdulla Al Mamun Bhuyan have contributed equally to this work.

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s11033-020-05568-3) contains supplementary material, which is available to authorized users.

✉ Anchun Cheng
chenganchun@vip.163.com

✉ Florian Lang
florian.lang@uni-tuebingen.de

[1] Key Laboratory of Animal Disease and Human Health of Sichuan Province, Institute of Preventive Veterinary Medicine, Sichuan Agricultural University, Wenjiang 611130, People's Republic of China

[2] Department of Physiology, Eberhard-Karls-University, Wilhelmstr. 56, 72076 Tuebingen, Germany

[29–34] and small molecules inhibiting [25, 29, 30, 35] eryptosis may be of clinical interest.

The aim of the present analysis was to disclose a potential stimulating or inhibiting effect of pyrogallol on eryptosis. Human erythrocytes isolated from healthy volunteers were incubated in normal Ringer or in absence of glucose (energy depletion), in presence of *tert*-butyl-hydroperoxide (*t*-BOOH, oxidative stress), in hyperosmotic Ringer (sucrose added) or in presence of Ca^{2+} ionophore ionomycin, each without or with addition of pyrogallol. After treatment, flow cytometry was employed to determine abundance of phosphatidylserine at the erythrocyte surface and erythrocyte volume.

Materials and methods

Fresh Li-Heparin-anticoagulated blood drawn from healthy volunteers was 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). The blood was centrifuged at 120 g for 20 min at 21 °C and the platelets and leukocytes-containing supernatant was disposed. Erythrocytes were incubated in vitro at a hematocrit of 0.4% in Ringer solution containing (in mM) 125 NaCl, 5 KCl, 1 $MgSO_4$, 5 glucose, 1 $CaCl_2$, 32 *N*-2-hydroxyethylpiperazine-*N*-2-ethanesulfonic acids (HEPES; pH 7.4) at 37 °C for 48 h. Where indicated, erythrocytes were exposed for 48 h to glucose containing or glucose depleted Ringer solution, for 30 min to the oxidant *t*-BOOH (0.5 mM, Sigma Aldrich, Hamburg, Germany), for 6 h to hypertonic Ringer (addition of 550 mM sucrose, Sigma Aldrich, Hamburg, Germany) or for 60 min to Ca^{2+} ionophore ionomycin (1 µM, Merck Millipore, Darmstadt, Germany), each in the absence and presence of pyrogallol (2–8 µM, Sigma Aldrich, Hamburg, Germany). Exposure time to glucose depletion as well as concentrations of and exposure times to *t*-BOOH, sucrose and ionomycin have been chosen according to previous experiences [36], concentrations of pyrogallol according to studies on the effect of this substance in other cell types [1–15]

After incubation under the respective experimental condition, a 150 Annexin-V-FITC 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. The annexin-V-abundance at the erythrocyte surface was subsequently determined on a FACS Calibur (BD, Heidelberg, Germany). Annexin-V-binding was measured with an excitation wavelength of 488 nm and an emission wavelength of 530 nm. A marker (M1) was placed to set an arbitrary threshold between annexin-V-binding cells and control cells. The same threshold was used for untreated and pyrogallol treated erythrocytes [36].

For each parameter, 50,000 events were counted. The analysis of FACS data was performed using FlowJo software v10.0.7 (FlowJo, Ashland, USA). 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 or *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 [36].

Results

Following incubation for 48 h in standard Ringer solution, the percentage of annexin-V-binding erythrocytes was similarly low without (1.06 ± 0.07%, n = 13) and with (1.19 ± 0.10%, n = 13) presence of pyrogallol (8 µM). The forward scatter was again similar following a 48 h exposure to standard Ringer solution without (480.96 ± 4.73, n = 13) or with (476.86 ± 4.28%, n = 13) pyrogallol (8 µM). Thus, in standard Ringer solution, pyrogallol did not significantly alter phosphatidylserine abundance at the erythrocyte surface or erythrocyte volume.

Energy depletion by a 48 h exposure to Ringer without glucose was followed by a significant increase of the percentage of annexin-V-binding erythrocytes (Fig. 1a). Addition of pyrogallol (2–8 µM) to glucose-free Ringer significantly blunted, but did not abrogate the increase of the percentage of annexin-V-binding erythrocytes (Fig. 1b). Thus, pyrogallol blunted, but did not prevent phosphatidylserine translocation following energy depletion.

Energy depletion by a 48 h exposure to Ringer without glucose was further followed by a significant decrease of forward scatter, which was virtually identical in the absence and presence of pyrogallol (2–8 µM) (Fig. 1c). Thus, pyrogallol did not appreciably alter the shrinking effect of energy depletion (Fig. 1d).

Oxidative stress was induced by treatment with *t*-BOOH. Within 30 min 0.5 mM *t*-BOOH triggered a sharp significant increase of the percentage of annexin-V-binding erythrocytes (Fig. 2a). The effect was slightly, but significantly, blunted in the presence of pyrogallol (2–8 µM). However, in the presence of pyrogallol, *t*-BOOH still significantly increased the percentage of phosphatidylserine exposing erythrocytes (Fig. 2b). Thus, pyrogallol blunted, but did not prevent phosphatidylserine translocation following oxidative stress.

Within 30 min, 0.5 mM *t*-BOOH caused a slight, but significant, decrease of forward scatter (Fig. 2c). The presence of pyrogallol did not significantly modify the decrease of

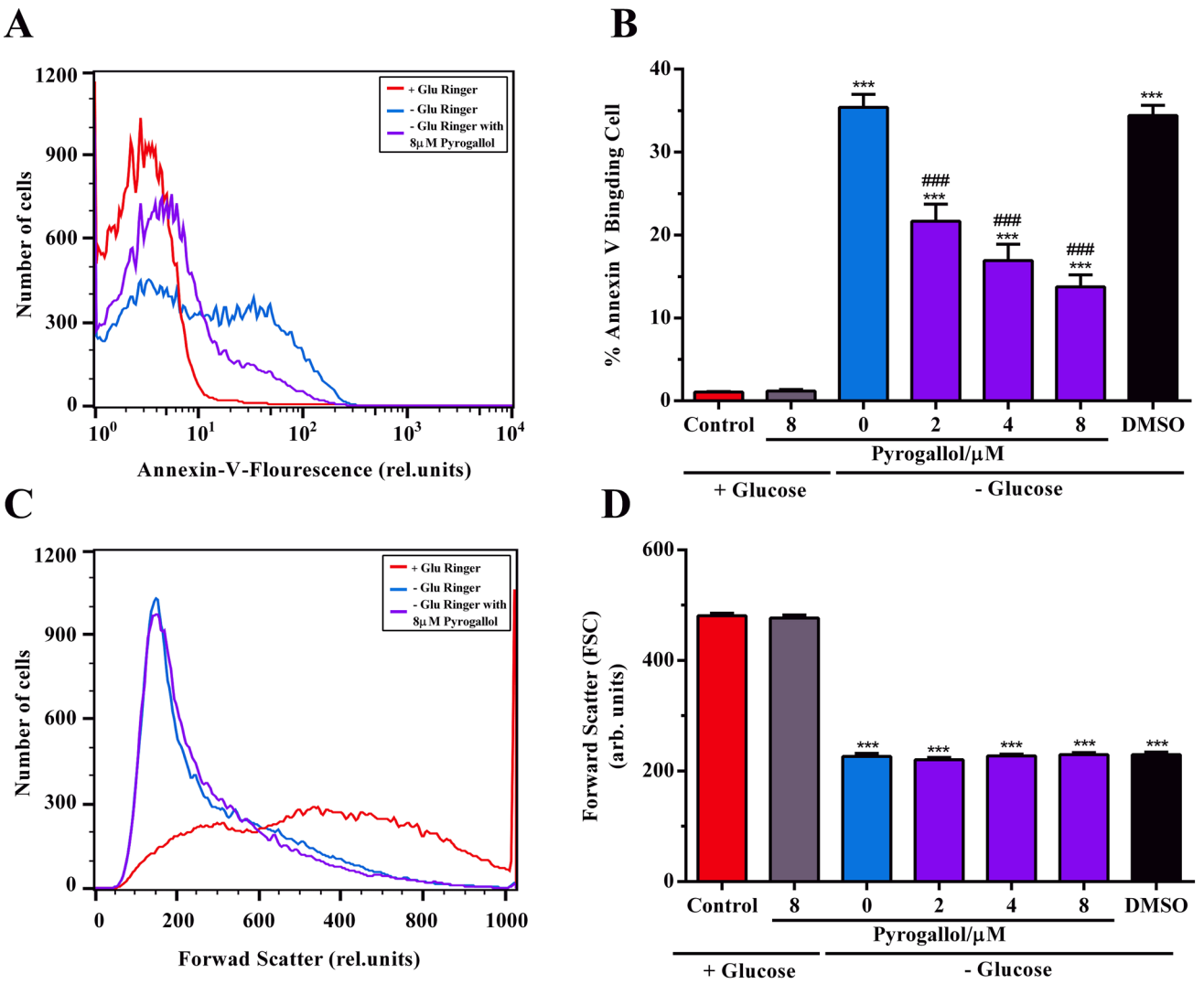

Fig. 1 Pyrogallol sensitivity of phosphatidylserine exposure and cell volume following energy depletion. **a** Original histograms of annexin-V-binding of erythrocytes following exposure for 48 h to glucose containing Ringer solution (red line), Ringer solution without glucose (blue line) and Ringer solution without glucose in the presence of pyrogallol (8 µM) (purple line); **b** Arithmetic means ± SEM (n = 13) of the percentage annexin-V-binding erythrocytes after a 48 h treatment with Ringer solution with (left bar, Control) or without (right bars) glucose in the absence (blue bar) and presence (purple bars) of pyrogallol (2–8 µM). DMSO (black bar) indicates effect of solvent; **c** Original histograms of erythrocyte forward scatter following exposure for 48 h to glucose containing Ringer solution (red line), Ringer solution without glucose (blue line) and Ringer solution without glucose in the presence of pyrogallol (8 µM) (purple line); **d** Arithmetic means ± SEM (n = 13) of the erythrocyte forward scatter after a 48 h treatment with Ringer solution with (left bar, Control) or without (right bars) glucose in the absence (blue bar) and presence (purple bars) of pyrogallol (2–8 µM). DMSO (black bar) indicates the effect of solvent ***($p < 0.001$) indicates significant difference from the presence of glucose, ###($p < 0.001$) indicates significant difference from the absence of pyrogallol (ANOVA). (Color figure online)

forward scatter following *t*-BOOH treatment and in the presence of pyrogallol, *t*-BOOH still significantly decreased the forward scatter of erythrocytes (Fig. 2d). Thus, pyrogallol did not significantly modify the shrinking effect of oxidative stress.

For induction of hyperosmotic shock, Ringer was made hypertonic by the addition of 550 mM sucrose. A 6 h treatment with hypertonic Ringer solution was followed by a significant increase of the percentage of annexin-V-binding erythrocytes (Fig. 3a). The effect was blunted, but not abrogated, in the presence of pyrogallol (2–8 µM) (Fig. 3b). Thus, pyrogallol blunted, but did not prevent phosphatidylserine translocation following hyperosmotic shock.

A 6 h treatment with hypertonic Ringer induced a sharp significant decrease of forward scatter (Fig. 3c). The presence of pyrogallol did not significantly modify the decrease of forward scatter following treatment with hypertonic Ringer and in the presence of 8 µM pyrogallol,

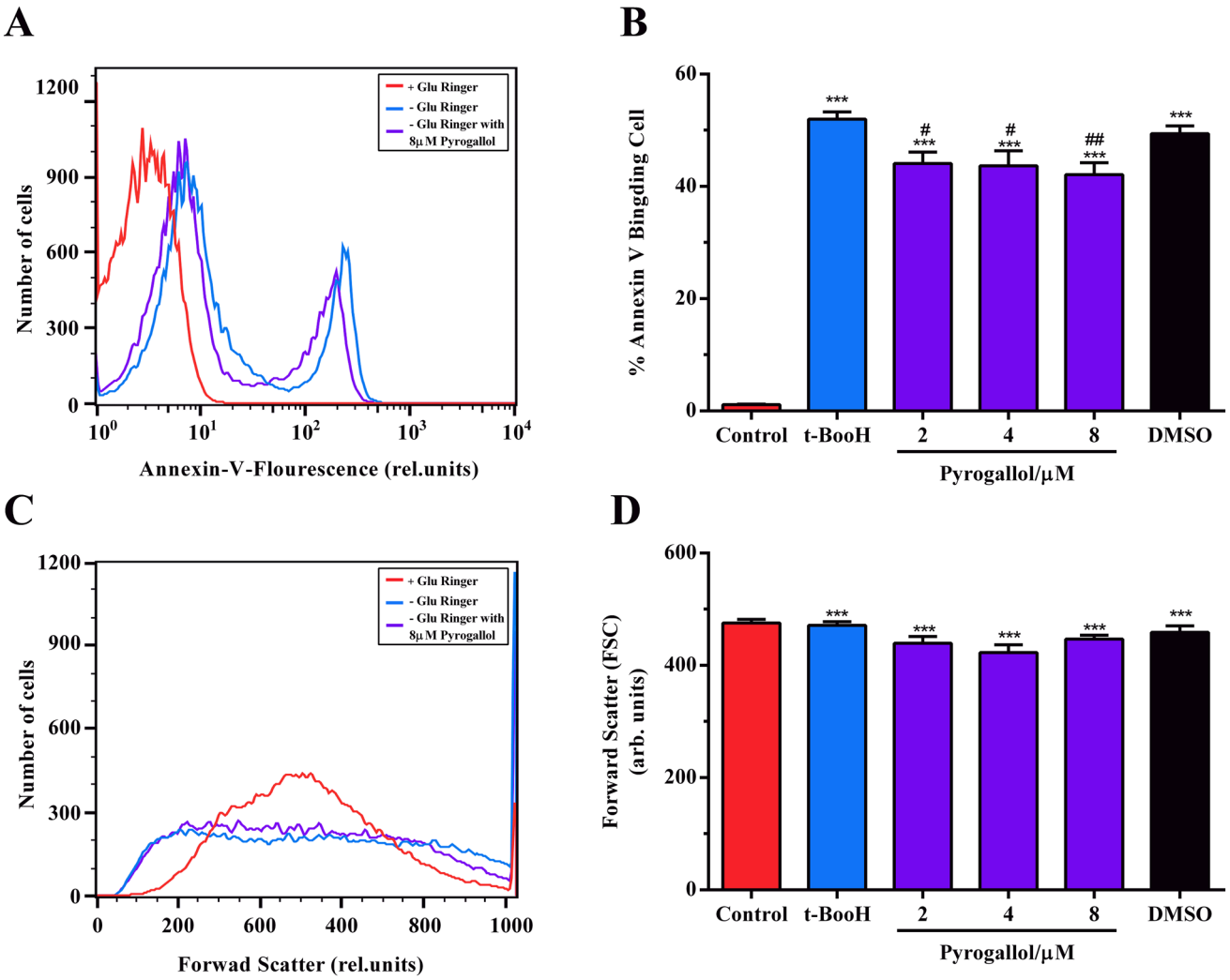

Fig. 2 Pyrogallol sensitivity of phosphatidylserine exposure and cell volume following oxidative stress. **a** Original histograms of annexin-V-binding of erythrocytes following exposure for 30 min to Ringer solution without (red line) or with 0.5 mM *t*-BOOH without (blue line) and with purple line) presence of pyrogallol (8 µM); **b** Arithmetic means ± SEM (n = 8) of the percentage annexin-V-binding erythrocytes after a 30 min treatment with Ringer solution without (left bar, Control) or with 0.5 mM *t*-BOOH (right bars) in the absence (blue bar) and presence (purple bars) of pyrogallol (2–8 µM). DMSO (black bar) indicates effect of solvent. **c** Original histograms of erythrocyte forward scatter following exposure for 30 min to Ringer solution without (red line) or with 0.5 mM *t*-BOOH without (blue line) and with purple line) presence of pyrogallol (8 µM); **d** Arithmetic means ± SEM (n = 8) of the erythrocyte forward scatter after a 48 h treatment with Ringer solution without 0.5 mM *t*-BOOH (left bar, Control) or with 0.5 mM *t*-BOOH (right bars) in the absence (blue bar) and presence (purple bars) of pyrogallol (2–8 µM). DMSO (black bar) indicates effect of solvent. ***($p < 0.001$) indicates significant difference from the absence of *t*-BOOH, $^{\#}(p < 0.05)$, $^{\#\#}(p < 0.01)$ indicates significant difference from the absence of pyrogallol (ANOVA). (Color figure online)

hypertonic Ringer still significantly decreased the forward scatter of the erythrocytes (Fig. 3d). Thus, pyrogallol did not significantly modify the shrinking effect of hyperosmotic shock.

Ca^{2+} overload was accomplished by treatment with Ca^{2+} ionophore ionomycin. Within 60 min 1 µM ionomycin triggered a sharp significant increase of the percentage of annexin-V-binding erythrocytes (Fig. 4a). The effect was significantly blunted, but not abolished in the presence of pyrogallol (2–8 µM) (Fig. 4-B). Thus, pyrogallol blunted, but did not prevent phosphatidylserine translocation following Ca^{2+} overload.

Within 60 min 1 µM ionomycin induced a sharp, significant decrease of forward scatter (Fig. 4c). The presence of pyrogallol did not significantly modify the decrease of forward scatter following ionomycin treatment and in the presence of 8 µM pyrogallol, ionomycin still significantly enhanced the percentage of phosphatidylserine exposing erythrocytes (Fig. 4d). Thus, pyrogallol did not significantly modify the shrinking effect of Ca^{2+} overload.

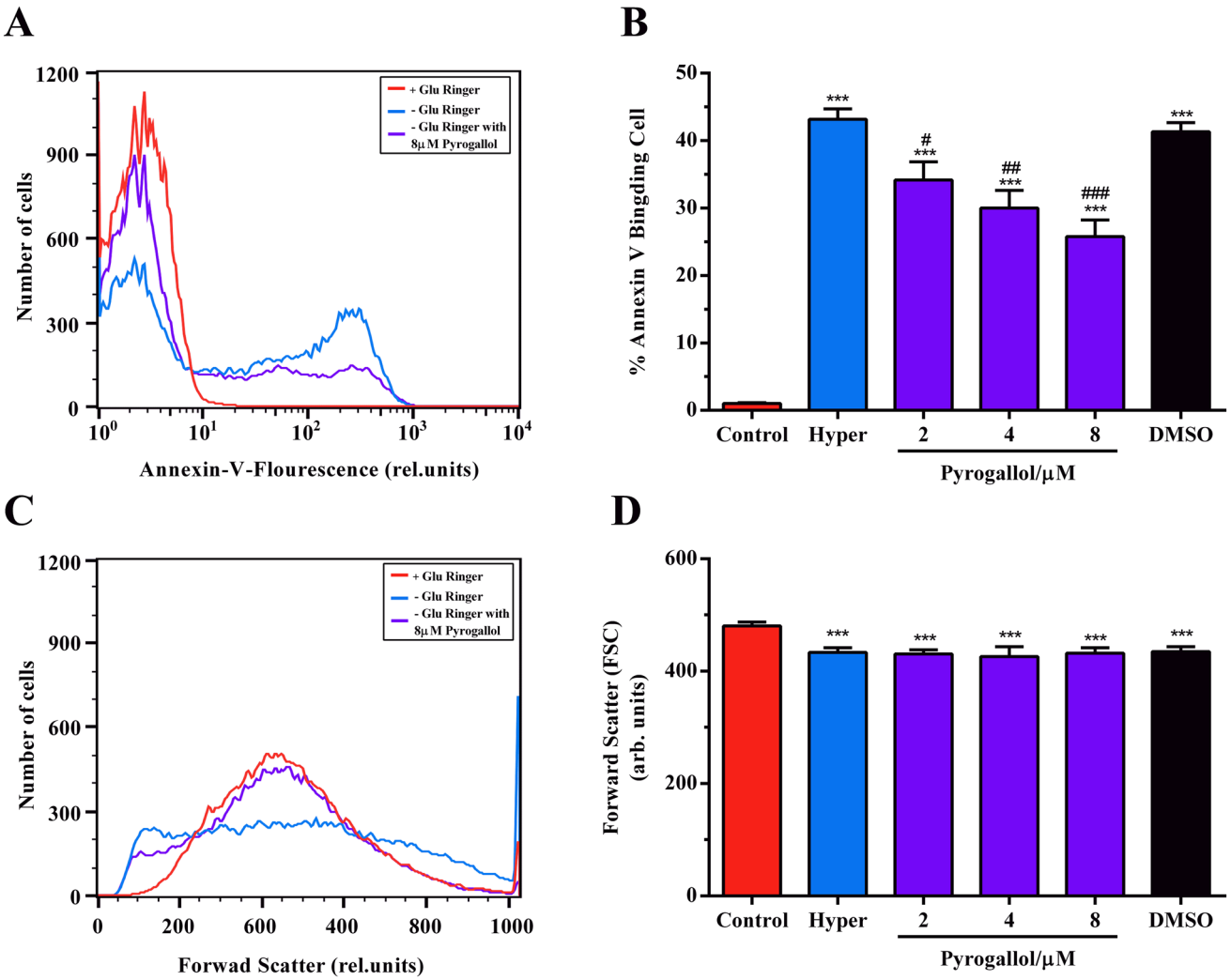

Fig. 3 Pyrogallol sensitivity of phosphatidylserine exposure and cell volume following hyperosmotic shock. **a** Original histograms of annexin-V-binding of erythrocytes following exposure for 6 h to Ringer solution without (red line) or with added 550 mM sucrose without (blue line) and with purple line) presence of pyrogallol (8 µM); **b** Arithmetic means ± SEM (n = 8) of the percentage annexin-V-binding erythrocytes for 6 h treatment with Ringer solution without (left bar, Control) or with added 550 mM sucrose (right bars) in the absence (blue bar) and presence (purple bars) of pyrogallol (2–8 µM). DMSO (black bar) indicates effect of solvent; **c** Original histograms of erythrocyte forward scatter following exposure for 6 h to Ringer solution without (red line) or with added 550 mM sucrose without (blue line) and with purple line) presence of pyrogallol (8 µM); **d** Arithmetic means ± SEM (n = 8) of the erythrocyte forward scatter after a for 6 h treatment with Ringer solution without added 550 mM sucrose (left bar, Control) or with 550 mM sucrose (right bars) in the absence (blue bar) and presence (purple bars) of pyrogallol (2–8 µM). DMSO (black bar) indicates the effect of solvent. ***($p < 0.001$) indicates significant difference from isotonic Ringer, $^{\#}(p < 0.05)$, $^{\#\#}(p < 0.01)$, $^{\#\#\#}(p < 0.001)$ indicates significant difference from the absence of pyrogallol (ANOVA). (Color figure online)

Discussion

The present observations reveal that pyrogallol could inhibit phosphatidylserine translocation to the erythrocyte surface, a hallmark of eryptosis [19]. Without maneuvers stimulating eryptosis pyrogallol did not affect phosphatidylserine translocation. The substance blunted, however, significantly the phosphatidylserine translocation following glucose deprivation, oxidation, hyperosmotic shock, and Ca^{2+} loading. Those maneuvers have been shown in a variety of previous studies to trigger eryptosis [19].

Accelerated eryptosis causes removal of circulating erythrocytes and anemia in diverse clinical conditions including chronic kidney disease [37], HUS (hemolytic uremic syndrome) [38], Autoimmune Haemolytic Anaemia [39], iron deficiency [19], hyperphosphatemia [40], vitamin D excess [41], dehydration [42], diabetes [43], hepatic failure [44], malignancy [45], sepsis [46], sickle cell anemia [19], beta-thalassemia [19], Hb-C and G6PD-deficiency [19], as well as

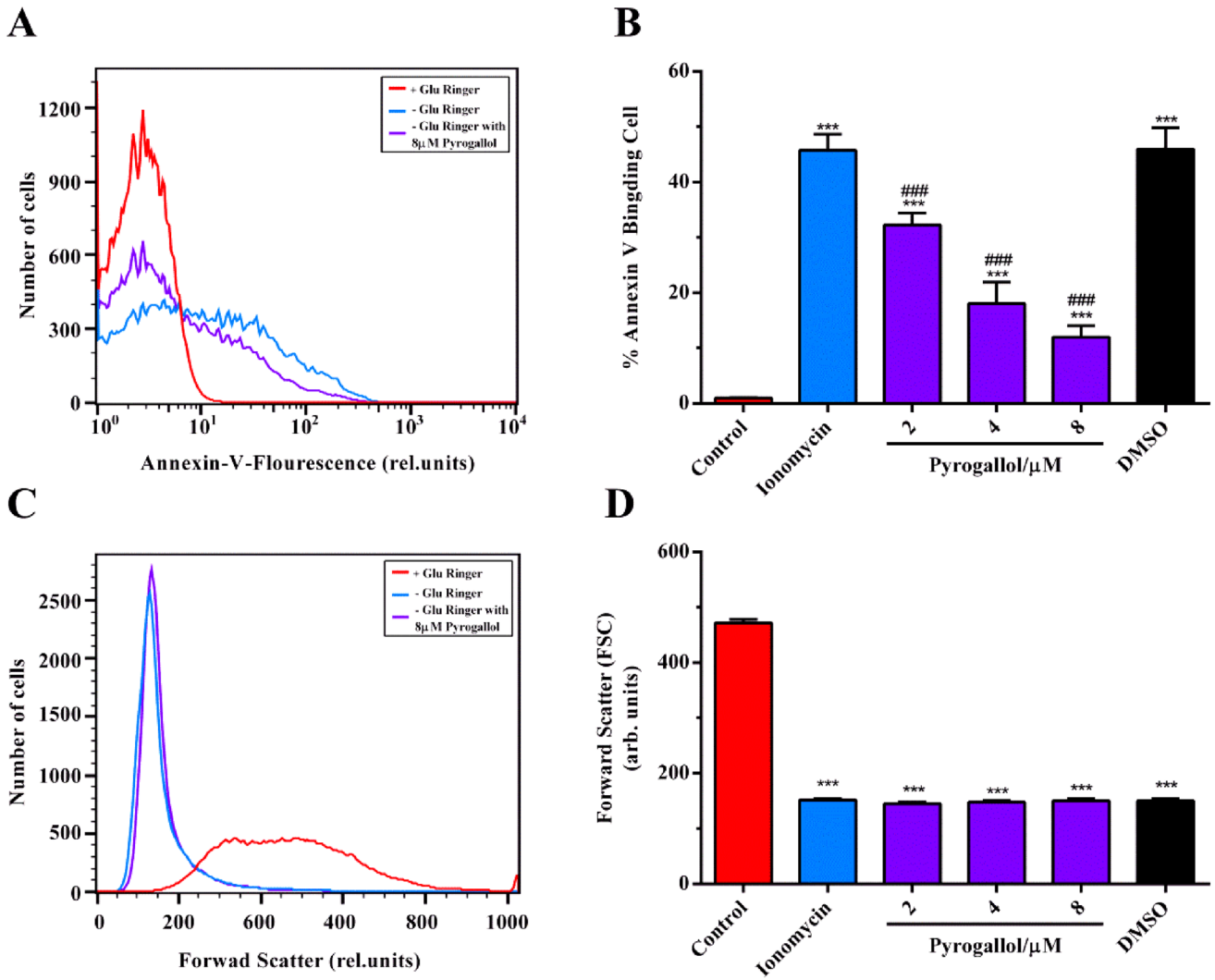

Fig. 4 Pyrogallol sensitivity of phosphatidylserine exposure and cell volume following Ca^{2+} overload. **a** Original histograms of annexin-V-binding of erythrocytes following exposure for 60 min to Ringer solution without (red line) or with 1 µM ionomycin without (blue line) and with purple line) presence of pyrogallol (8 µM); **b** Arithmetic means ± SEM (n = 7) of the percentage annexin-V-binding erythrocytes after a 48 h treatment with Ringer solution without (left bar, control) or with 1 µM ionomycin (right bars) in the absence (blue bar) and presence (purple bars) of pyrogallol (2–8 µM). DMSO (black bar) indicates effect of solvent; **c** Original histograms of erythrocyte forward scatter following exposure for 60 min to Ringer solution without (red line) or with 1 µM ionomycin without (blue line) and with purple line) presence of pyrogallol (8 µM); **d** Arithmetic means ± SEM (n = 7) of the erythrocyte forward scatter after a 48 h treatment with Ringer solution without (left bar, Control) or with 1 µM ionomycin (right bars) in the absence (blue bar) and presence (purple bars) of pyrogallol (2–8 µM). DMSO (black bar) indicates effect of solvent. ***($p < 0.001$) indicates significant difference from the absence of ionomycin, ###($p < 0.001$) indicates significant difference from the absence of pyrogallol (ANOVA). (Color figure online)

Wilson´s disease [46]. The clearance of eryptotic erythrocytes [19] results in anemia as soon as it surpasses the rate of erythropoiesis [19]. Excessive eryptosis is further expected to compromise microcirculation [47], because eryptotic erythrocytes adhere to endothelial cells [48], and favour development of thrombosis [49]. Inhibitors of eryptosis are expected to reverse anemia and improve microcirculation in disorders with accelerated eryptosis. On the other hand, interference with eryptosis may delay removal of defective circulating erythrocytes which may instead enter hemolysis [19]. Hemoglobin thus released may undergo glomerular filtration with subsequent tubular hemoglobin precipitation, nephron occlusion, and thus damage of the kidneys [50].

Conclusions

Pyrogallol blunts the stimulation of eryptotic phosphatidylserine translocation to the erythrocyte surface following energy depletion, oxidative stress, hyperosmotic cell shrinkage, and Ca^{2+} overload.

Acknowledgements The authors acknowledge the meticulous preparation of the manuscript by Lejla Subasic. Jibin Liu is supported by Chinese Scholarship Council.

Author contributions JL, AAMB, AC and FL conceived and designed research. Material preparation, data collection and analysis were performed by JL, AAMB, KM and SZ. The first draft of the manuscript was written by FL and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Funding This study was funded by China Agricultural Research System (CARS-42-17), Integration and Demonstration of Key Technologies for Goose Industrial Chain in Sichuan Province (2018NZ0005), Sichuan Veterinary Medicine and Drug Innovation Group of China Agricultural Research System (CARS-SVDIP).

Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest.

Ethics approval The study is approved by the ethics committee of the University of Tübingen (184/2003V).

References

1. Revathi S, Hakkim FL, Kumar NR, Bakshi HA, Rashan L, Al-Buloshi M, Hasson S, Krishnan M, Javid F, Nagarajan K (2018) Induction of HT-29 colon cancer cells apoptosis by pyrogallol with growth inhibiting efficacy against drug-resistant *Helicobacter pylori*. Anticancer Agents Med Chem 18(13):1875–1884. https://doi.org/10.2174/1871520618666180806104902
2. Bruges G, Venturini W, Crespo G, Lopez Zambrano M (2018) Pyrogallol induces apoptosis in human platelets. Folia Biol (Praha) 64(1):23–30
3. Han YH, Moon HJ, You BR, Park WH (2010) The effects of MAPK inhibitors on pyrogallol-treated Calu-6 lung cancer cells in relation to cell growth, reactive oxygen species and glutathione. Food Chem Toxicol 48(1):271–276. https://doi.org/10.1016/j.fct.2009.10.010
4. Park WH, Park MN, Han YH, Kim SW (2008) Pyrogallol inhibits the growth of gastric cancer SNU-484 cells via induction of apoptosis. Int J Mol Med 22(2):263–268
5. Yang CJ, Wang CS, Hung JY, Huang HW, Chia YC, Wang PH, Weng CF, Huang MS (2009) Pyrogallol induces G2-M arrest in human lung cancer cells and inhibits tumor growth in an animal model. Lung Cancer 66(2):162–168. https://doi.org/10.1016/j.lungcan.2009.01.016
6. Han BR, Park WH (2017) MAPK inhibitors enhance cell death in pyrogallol-treated human pulmonary fibroblast cells via increasing O2(*-) levels. Oncol Lett 14(1):1179–1185. https://doi.org/10.3892/ol.2017.6185
7. Park WH (2016) Pyrogallol induces the death of human pulmonary fibroblast cells through ROS increase and GSH depletion. Int J Oncol 49(2):785–792. https://doi.org/10.3892/ijo.2016.3543
8. Han YH, Moon HJ, You BR, Kim SZ, Kim SH, Park WH (2009) JNK and p38 inhibitors increase and decrease apoptosis, respectively, in pyrogallol-treated calf pulmonary arterial endothelial cells. Int J Mol Med 24(5):717–722. https://doi.org/10.3892/ijmm_00000284
9. Han YH, Moon HJ, You BR, Kim SZ, Kim SH, Park WH (2010) Pyrogallol-induced endothelial cell death is related to GSH depletion rather than ROS level changes. Oncol Rep 23(1):287–292
10. Han YH, Park WH (2010) Pyrogallol-induced calf pulmonary arterial endothelial cell death via caspase-dependent apoptosis and GSH depletion. Food Chem Toxicol 48(2):558–563. https://doi.org/10.1016/j.fct.2009.11.032
11. Han YH, Park WH (2010) Pyrogallol-induced As4.1 juxtaglomerular cell death is attenuated by MAPK inhibitors via preventing GSH depletion. Arch Toxicol 84(8):631–640. https://doi.org/10.1007/s00204-010-0526-8
12. Park WH, Han YH, Kim SH, Kim SZ (2007) Pyrogallol, ROS generator inhibits As4.1 juxtaglomerular cells via cell cycle arrest of G2 phase and apoptosis. Toxicology 235(1–2):130–139. https://doi.org/10.1016/j.tox.2007.03.018
13. Park WH, Han YW, Kim SH, Kim SZ (2007) A superoxide anion generator, pyrogallol induces apoptosis in As4.1 cells through the depletion of intracellular GSH content. Mutat Res 619(1–2):81–92. https://doi.org/10.1016/j.mrfmmm.2007.02.004
14. Saeki K, Hayakawa S, Isemura M, Miyase T (2000) Importance of a pyrogallol-type structure in catechin compounds for apoptosis-inducing activity. Phytochemistry 53(3):391–394. https://doi.org/10.1016/s0031-9422(99)00513-0
15. Mitsuhashi S, Saito A, Nakajima N, Shima H, Ubukata M (2008) Pyrogallol structure in polyphenols is involved in apoptosis-induction on HEK293T and K562 cells. Molecules 13(12):2998–3006. https://doi.org/10.3390/molecules13122998
16. Upadhyay G, Tiwari MN, Prakash O, Jyoti A, Shanker R, Singh MP (2010) Involvement of multiple molecular events in pyrogallol-induced hepatotoxicity and silymarin-mediated protection: evidence from gene expression profiles. Food Chem Toxicol 48(6):1660–1670. https://doi.org/10.1016/j.fct.2010.03.041
17. Tang G, Yang CY, Nikolovska-Coleska Z, Guo J, Qiu S, Wang R, Gao W, Wang G, Stuckey J, Krajewski K, Jiang S, Roller PP, Wang S (2007) Pyrogallol-based molecules as potent inhibitors of the antiapoptotic Bcl-2 proteins. J Med Chem 50(8):1723–1726. https://doi.org/10.1021/jm061400l
18. Maellaro E, Leoncini S, Moretti D, Del Bello B, Tanganelli I, De Felice C, Ciccoli L (2013) Erythrocyte caspase-3 activation and oxidative imbalance in erythrocytes and in plasma of type 2 diabetic patients. Acta Diabetol 50(4):489–495. https://doi.org/10.1007/s00592-011-0274-0
19. Lang E, Lang F (2015) Mechanisms and pathophysiological significance of eryptosis, the suicidal erythrocyte death. Semin Cell Dev Biol 39:35–42. https://doi.org/10.1016/j.semcdb.2015.01.009
20. Cassambai S, Mee CJ, Renshaw D, Hussain A (2019) Tiotropium bromide, a long acting muscarinic receptor antagonist triggers intracellular calcium signalling in the heart. Toxicol Appl Pharmacol 384:114778. https://doi.org/10.1016/j.taap.2019.114778
21. Hernandez G, Villanueva-Ibarra CA, Maldonado-Vega M, Lopez-Vanegas NC, Ruiz-Cascante CE, Calderon-Salinas JV (2019) Participation of phospholipase-A2 and sphingomyelinase in the molecular pathways to eryptosis induced by oxidative stress in lead-exposed workers. Toxicol Appl Pharmacol 371:12–19. https://doi.org/10.1016/j.taap.2019.03.025
22. Alfhili MA, Nkany MB, Weidner DA, Lee MH (2019) Stimulation of eryptosis by broad-spectrum insect repellent N,

N-Diethyl-3-methylbenzamide (DEET). Toxicol Appl Pharmacol 370:36–43. https://doi.org/10.1016/j.taap.2019.03.011
23. Lang PA, Kaiser S, Myssina S, Wieder T, Lang F, Huber SM (2003) Role of Ca^{2+}-activated K^+ channels in human erythrocyte apoptosis. Am J Physiol Cell Physiol 285(6):C1553–C1560
24. McCaig WD, Hodges AL, Deragon MA, Haluska RJ Jr, Bandyopadhyay S, Ratner AJ, Spitalnik SL, Hod EA, LaRocca TJ (2019) Storage primes erythrocytes for necroptosis and clearance. Cell Physiol Biochem 53(3):496–507. https://doi.org/10.33594/000000153
25. Al Mamun Bhuyan A, Lang F (2018) Inhibition of erythrocyte cell membrane scrambling following energy depletion and hyperosmotic shock by Alectinib. Cell Physiol Biochem 51(5):1996–2009. https://doi.org/10.1159/000495777
26. Bissinger R, Bhuyan AAM, Qadri SM, Lang F (2019) Oxidative stress, eryptosis and anemia: a pivotal mechanistic nexus in systemic diseases. FEBS J 286(5):826–854. https://doi.org/10.1111/febs.14606
27. Lang F, Bissinger R, Abed M, Artunc F (2017) Eryptosis: the neglected cause of anemia in end stage renal disease. Kidney Blood Press Res 42(4):749–760. https://doi.org/10.1159/000484215
28. Foller M, Bobbala D, Koka S, Huber SM, Gulbins E, Lang F (2009) Suicide for survival–death of infected erythrocytes as a host mechanism to survive malaria. Cell Physiol Biochem 24(3–4):133–140. https://doi.org/10.1159/000233238
29. Lang E, Lang F (2015) Triggers, inhibitors, mechanisms, and significance of eryptosis: the suicidal erythrocyte death. Biomed Res Int 2015:513518. https://doi.org/10.1155/2015/513518
30. Lang F, Jilani K, Lang E (2015) Therapeutic potential of manipulating suicidal erythrocyte death. Expert Opin Ther Targets 19(9):1219–1227. https://doi.org/10.1517/14728222.2015.1051306
31. Ferdous Z, Beegam S, Tariq S, Ali BH, Nemmar A (2018) The in vitro effect of polyvinylpyrrolidone and citrate coated silver nanoparticles on erythrocytic oxidative damage and eryptosis. Cell Physiol Biochem 49(4):1577–1588. https://doi.org/10.1159/000493460
32. Fink M, Al Mamun Bhuyan A, Zacharopoulou N, Lang F (2018) Taurolidine sensitivity of eryptosis, the suicidal erythrocyte death. Cell Physiol Biochem 51(2):501–512. https://doi.org/10.1159/000495272
33. Fink M, Al Mamun Bhuyan A, Zacharopoulou N, Lang F (2018) Stimulation of eryptosis, the suicidal erythrocyte death, by costunolide. Cell Physiol Biochem 50(6):2283–2295. https://doi.org/10.1159/000495088
34. Yeung KW, Lau PM, Tsang HL, Ho HP, Kwan YW, Kong SK (2019) Extracellular histones induced eryptotic death in human erythrocytes. Cell Physiol Biochem 53(1):229–241. https://doi.org/10.33594/000000132
35. Jemaa M, Fezai M, Lang F (2017) Inhibition of suicidal erythrocyte death by reversine. Cell Physiol Biochem 41(6):2363–2373. https://doi.org/10.1159/000475654
36. Jemaa M, Fezai M, Bissinger R, Lang F (2017) Methods employed in cytofluorometric assessment of eryptosis, the suicidal erythrocyte death. Cell Physiol Biochem 43(2):431–444. https://doi.org/10.1159/000480469
37. Tozoni SS, Dias GF, Bohnen G, Grobe N, Pecoits-Filho R, Kotanko P, Moreno-Amaral AN (2019) Uremia and hypoxia independently induce eryptosis and erythrocyte redox imbalance. Cell Physiol Biochem 53:794–804. https://doi.org/10.33594/000000173
38. 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 (2006) Suicidal death of erythrocytes in recurrent hemolytic uremic syndrome. J Mol Med 84(5):378–388. https://doi.org/10.1007/s00109-006-0058-0
39. Balola AHA, Mayer B, Bartolmas T, Salama A (2019) Sublytic terminal complement components induce eryptosis in autoimmune haemolytic anaemia related to IgM autoantibodies. Cell Physiol Biochem 53(3):453–464. https://doi.org/10.33594/000000150
40. Voelkl J, Alzoubi K, Mamar AK, Ahmed MS, Abed M, Lang F (2013) Stimulation of suicidal erythrocyte death by increased extracellular phosphate concentrations. Kidney Blood Press Res 38(1):42–51. https://doi.org/10.1159/000355752
41. Lang E, Jilani K, Bissinger R, Rexhepaj R, Zelenak C, Lupescu A, Lang F, Qadri SM (2015) Vitamin D-rich diet in mice modulates erythrocyte survival. Kidney Blood Press Res 40(4):403–412. https://doi.org/10.1159/000368517
42. Abed M, Feger M, Alzoubi K, Pakladok T, Frauenfeld L, Geiger C, Towhid ST, Lang F (2013) Sensitization of erythrocytes to suicidal erythrocyte death following water deprivation. Kidney Blood Press Res 37(6):567–578. https://doi.org/10.1159/000355737
43. Nicolay JP, Schneider J, Niemoeller OM, Artunc F, Portero-Otin M, Haik G Jr, Thornalley PJ, Schleicher E, Wieder T, Lang F (2006) Stimulation of suicidal erythrocyte death by methylglyoxal. Cell Physiol Biochem 18(4–5):223–232. https://doi.org/10.1159/000097669
44. Lang E, Pozdeev VI, Gatidis S, Qadri SM, Haussinger D, Kubitz R, Herebian D, Mayatepek E, Lang F, Lang KS, Lang PA (2016) Bile acid-induced suicidal erythrocyte death. Cell Physiol Biochem 38(4):1500–1509. https://doi.org/10.1159/000443091
45. Bissinger R, Schumacher C, Qadri SM, Honisch S, Malik A, Gotz F, Kopp HG, Lang F (2016) Enhanced eryptosis contributes to anemia in lung cancer patients. Oncotarget 7(12):14002–14014. https://doi.org/10.18632/oncotarget.7286
46. 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 (2007) Liver cell death and anemia in Wilson disease involve acid sphingomyelinase and ceramide. Nat Med 13(2):164–170. https://doi.org/10.1038/nm1539
47. Abed M, Towhid ST, Mia S, Pakladok T, Alesutan I, Borst O, Gawaz M, Gulbins E, Lang F (2012) Sphingomyelinase-induced adhesion of eryptotic erythrocytes to endothelial cells. Am J Physiol Cell Physiol 303(9):C991–999. https://doi.org/10.1152/ajpcell.00239.2012
48. Borst O, Abed M, Alesutan I, Towhid ST, Qadri SM, Foller M, Gawaz M, Lang F (2012) Dynamic adhesion of eryptotic erythrocytes to endothelial cells via CXCL16/SR-PSOX. Am J Physiol Cell Physiol 302(4):C644–C651
49. Chung SM, Bae ON, Lim KM, Noh JY, Lee MY, Jung YS, Chung JH (2007) Lysophosphatidic acid induces thrombogenic activity through phosphatidylserine exposure and procoagulant microvesicle generation in human erythrocytes. Arterioscler Thromb Vasc Biol 27(2):414–421
50. Harrison HE, Bunting H, Ordway NK, Albrink WS (1947) The Pathogenesis of the renal injury produced in the dog by hemoglobin or methemoglobin. J Exp Med 86(4):339–356