Biometals (2009) 22:453–460
DOI 10.1007/s10534-008-9180-5

Eryptosis triggered by bismuth

Manuel Braun · Michael Föller · Erich Gulbins · Florian Lang

Received: 19 September 2008 / Accepted: 13 November 2008 / Published online: 2 December 2008

Abstract Bismuth is used for multiple industrial purposes and in the treatment of several gastrointestinal diseases. Untoward effects of bismuth include anemia, which could, in theory, result from suicidal erythrocyte death or eryptosis. Hallmarks of eryptosis are cell shrinkage and cell membrane scrambling with phosphatidylserine exposure at the cell surface. Phosphatidylserine-exposing cells are rapidly cleared from circulating blood. Signaling leading to eryptosis includes increase in cytosolic Ca^{2+} activity and formation of ceramide. The present experiments explored whether bismuth elicits eryptosis. To this end, phosphatidylserine exposure was estimated from annexin V-binding, cell shrinkage from decrease of forward scatter in FACS analysis, cytosolic Ca^{2+} activity from Fluo3 fluorescence and ceramide abundance from binding of fluorescent antibodies. A 48 h exposure to bismuth ($\geq$500 μg/l $BiCl_3$) enhanced the percentage of annexin V-binding cells and decreased forward scatter, increased cytosolic Ca^{2+} activity, and stimulated ceramide formation. In conclusion, bismuth stimulates eryptosis, the suicidal death of erythrocytes. The effect may contribute to or even account for the development of anemia during bismuth treatment. Moreover, ceramide formation in intestinal cells may participate in the therapeutic efficacy of bismuth preparations.

Keywords Cell volume · Annexin · Eryptosis · Calcium · Phosphatidylserine

Introduction

Bismuth is used in industry (Larsen et al. 2005; Lee and Gillies 1986; Pamphlett et al. 2000) and as a first-line treatment option in several gastrointestinal diseases (Chey and Wong 2007; Moayyedi et al. 2006; Wagstaff et al. 1988; Zhang et al. 2006). Untoward effects of bismuth include anemia (Moeschlin 1975), which could at least in theory result from stimulation of suicidal erythrocyte death or eryptosis (Lang et al. 2005). Eryptosis is triggered by several anemia-inducing endogeneous substances and xenobiotics (Zappulla 2008), such as cordycepin

This study was supported by the Deutsche Forschungsgemeinschaft SFB 766 and the Carl Zeiss Stiftung. The experimental work and studies on human subjects have been approved by the Ethical commission of the University of Tübingen.

M. Braun · M. Föller · F. Lang
Department of Physiology, University of Tübingen, Tübingen, Germany

E. Gulbins
Department of Molecular Medicine, University of Essen, Essen, Germany

F. Lang (✉)
Physiologisches Institut, der Universität Tübingen, Gmelinstr. 5, 72076 Tübingen, Germany
e-mail: florian.lang@uni-tuebingen.de

(Lui et al. 2007), methylglyoxal (Nicolay et al. 2006), amyloid peptides (Nicolay et al. 2007), paclitaxel (Lang et al. 2006), amantadine (Foller et al. 2008), chlorpromazine (Akel et al. 2006), cyclosporine (Niemoeller et al. 2006a), Bay-5884 (Shumilina et al. 2006), curcumin (Bentzen et al. 2007), valinomycin (Schneider et al. 2007), haemolysin (Lang et al. 2004b), listeriolysin (Foller et al. 2007), aluminum (Niemoeller et al. 2006b), lead (Kempe et al. 2005), mercury (Eisele et al. 2006), copper (Lang et al. 2007), and gold (Sopjani et al. 2008).

Eryptosis is characterized by phosphatidylserine-exposure at the erythrocyte surface (Berg et al. 2001; Brand et al. 2003; Bratosin et al. 2001; Daugas et al. 2001; Lang et al. 2003c). Phosphatidylserine-exposing erythrocytes are rapidly cleared from circulating blood (Kempe et al. 2006). Phosphatidylserine-exposure results from phospholipid scrambling of the cell membrane (Dekkers et al. 2002; Woon et al. 1999).

Stimulators of cell membrane scrambling include increase in the cytosolic Ca^{2+} concentration (Berg et al. 2001; Bratosin et al. 2001; Daugas et al. 2001), which may be secondary to Ca^{2+} entry through Ca^{2+}-permeable cation channels (Duranton et al. 2002, 2003; Huber et al. 2001; Lang et al. 2003a). Besides stimulating cell membrane scrambling, Ca^{2+} activates Ca^{2+}-sensitive K^+ channels (Bookchin et al. 1987; Brugnara et al. 1993) with subsequent exit of KCl and osmotically obliged water leading to cell shrinkage (Lang et al. 2003b). Furthermore, eryptosis was shown to be stimulated by ceramide, which enhances the Ca^{2+} sensitivity of phospholipid scrambling (Lang et al. 2004a) and by oxidative stress (Cimen 2008), which stimulates Ca^{2+} entry and activates caspases (Kriebardis et al. 2007).

The present study tested, whether bismuth stimulates eryptosis. We demonstrate that exposure of human erythrocytes to bismuth chloride triggered eryptosis by increasing intra-erythrocyte Ca^{2+} and by release of ceramide.

Materials and methods

Volunteers

Erythrocytes were drawn from healthy volunteers, who kindly provided informed consent. The study has been approved by the Ethical commission of the University of Tübingen.

Solutions

The 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 $CaCl_2$. Where indicated, $BiCl_3$ (Sigma, Schnelldorf, Germany; dissolved in methanol) was added to the NaCl Ringer at final concentrations ranging from 150 to 2,000 µg/l. The solvent methanol did not elicit eryptosis at the applied concentrations. In Ca^{2+}-free Ringer solution, 1 mM $CaCl_2$ was substituted for 1 mM glycol-bis(2-aminoethylether)-*N,N,N′,N′*-tetra-acetic acid (EGTA).

Measurement of hemolysis

After 48 h of incubation at 37°C, the samples were centrifuged (3 min at 400*g*, RT) 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.

Phosphatidylserine exposure and forward scatter

Erythrocytes were washed once in Ringer solution supplemented with 4 mM $CaCl_2$. The cells were then stained with Annexin V-Fluos (Roche, Mannheim, Germany) at a 1:500 dilution. After 20 min, samples were measured by flow cytometric analysis (FACS-Calibur from Becton Dickinson; Heidelberg, Germany). Cells were analyzed by forward scatter, and annexin V-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 Ca^{2+}

Erythrocytes were washed in Ringer solution and then loaded with Fluo-3/AM (Calbiochem, Bad Soden, Germany) in Ringer solution containing 5 mM $CaCl_2$ and 2 µM Fluo-3/AM. The cells were incubated at 37°C for 20 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 in fluorescence channel FL-1 in FACS analysis.

Determination of ceramide formation

To determine ceramide, a monoclonal antibody-based assay was used. After incubation, cells were stained for 1 h at 37°C with 1 µg/ml anti-ceramide antibody (clone MID 15B4; Alexis, Grünberg, Germany) in PBS containing 0.1% bovine serum albumin (BSA) at a dilution of 1:5. After two washing steps with PBS-BSA, cells were stained for 30 min with polyclonal fluorescein-isothiocyanate (FITC)-conjugated goat anti-mouse IgG and IgM specific antibody (Pharmingen, Hamburg, Germany) diluted 1:50 in PBS-BSA. Unbound secondary antibody was removed by repeated washing with PBS-BSA. Samples were then analyzed by flow cytometric analysis in FL-1.

Statistics

Data are expressed as arithmetic means ± SEM and statistical analysis was made by paired *t*-test or ANOVA, as appropriate, $P < 0.05$ was considered as statistically significant.

Results

A hallmark of eryptosis is scrambling of cell membrane phospholipids with subsequent exposure of phosphatidylserine at the erythrocyte surface (Lang et al. 2005). Annexin V-binding was utilized to detect phosphatidylserine at the erythrocyte surface. As shown in Fig. 1a, b, treatment of erythrocytes with bismuth (≥500 µg/l $BiCl_3$) resulted in a significant increase in annexin V-binding. Despite the cell membrane scrambling, bismuth did not disrupt the integrity of the cell membrane (Fig. 2a) and did not induce marked hemolysis (Fig. 2b).

Another hallmark of eryptosis is cell shrinkage (Lang et al. 2005). Forward scatter was determined as an estimate of cell volume. As shown in Fig. 3, exposure to bismuth (≥500 µg/l $BiCl_3$) significantly decreased the forward scatter, pointing to a decrease of cell volume.

Cell membrane scrambling (Berg et al. 2001; Brand et al. 2003; Bratosin et al. 2001; Daugas et al. 2001; Lang et al. 2003c) and cell shrinkage (Lang et al. 2003b) could be triggered by an increase in the cytosolic Ca^{2+} activity. Fluo3 fluorescence was employed to determine, whether bismuth influences the Ca^{2+} concentration of erythrocytes. As illustrated in Fig. 4, bismuth exposure led to a slight increase in Fluo3 fluorescence, an effect requiring ≥2,000 µg/l $BiCl_3$ for statistical significance. Thus, bismuth leads to a moderate increase in intracellular Ca^{2+} activity.

Eryptosis is further stimulated by the formation of ceramide (Lang et al. 2004a). Fluorescent antibodies were utilized to determine ceramide formation prior to and following bismuth exposure. As illustrated in Fig. 5, exposure to bismuth (2,000 µg/l) resulted in a strong stimulation of ceramide formation.

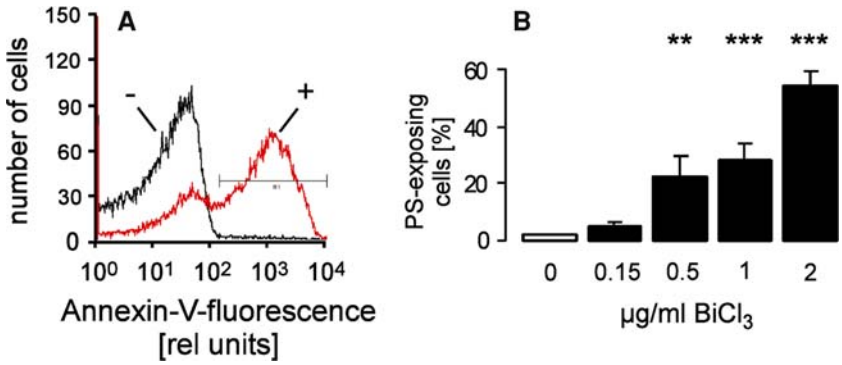

Fig. 1 Stimulation of phosphatidylserine exposure at the erythrocyte surface by bismuth. **a** Histogram of annexin V-binding in a representative experiment of erythrocytes incubated for 48 h in plain Ringer solution (–, *black line*) or in Ringer solution containing 2,000 µg/l $BiCl_3$ (+, *red line*). **b** Arithmetic means ± SEM ($n = 12$–28) of the percentage of annexin V-binding erythrocytes after a 48 h treatment with Ringer solution without (*white bar*) or with (*black bars*) bismuth ($BiCl_3$) at the indicated concentrations. **, *** indicate significant difference ($P < 0.01$, $P < 0.001$) from control (absence of bismuth, ANOVA)

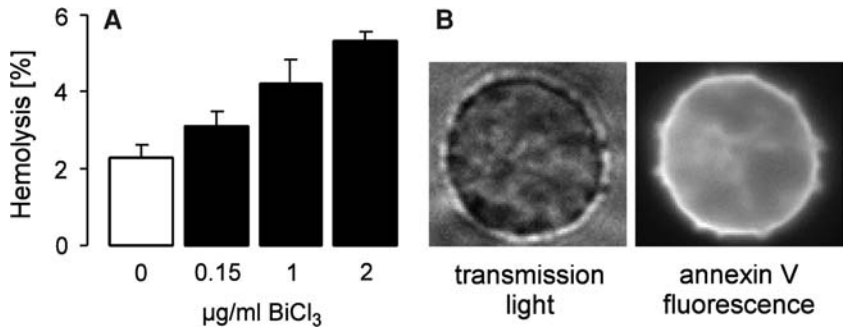

Fig. 2 Integrity of the erythrocyte membrane under the influence of bismuth. **a** Arithmetic means ± SEM ($n = 8$) of the percentage of hemolysed erythrocytes exposed for 48 h to Ringer solution without (*white bar*) or with (*black bars*) bismuth ($BiCl_3$) at the indicated concentrations. **b** Transmission microphotograph (*left panel*) and fluorescence microphotograph (*right panel*) of an erythrocyte stained with fluorescent annexin V. Prior to microscopy, the erythrocyte was exposed for 48 h to bismuth (2,000 µg/l $BiCl_3$) in Ringer solution

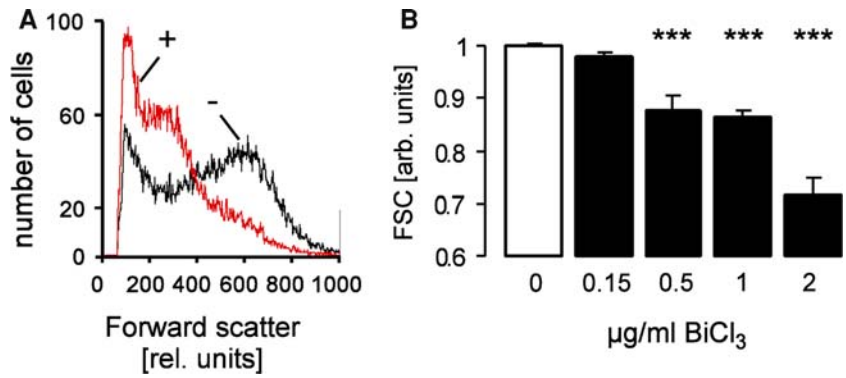

Fig. 3 Erythrocyte forward scatter following exposure to bismuth. **a** Histogram of forward scatter in a representative experiment of erythrocytes incubated for 48 h in plain Ringer solution (–, *black line*) or in Ringer solution containing 2,000 µg/l $BiCl_3$ (+, *red line*). **b** Arithmetic means ± SEM ($n = 12$–20) of the normalized forward scatter of erythrocytes after a 48 h treatment with Ringer solution without (*white bar*) or with (*black bars*) bismuth ($BiCl_3$) at the indicated concentrations. *** indicates significant difference ($P < 0.001$) from control (absence of bismuth, ANOVA)

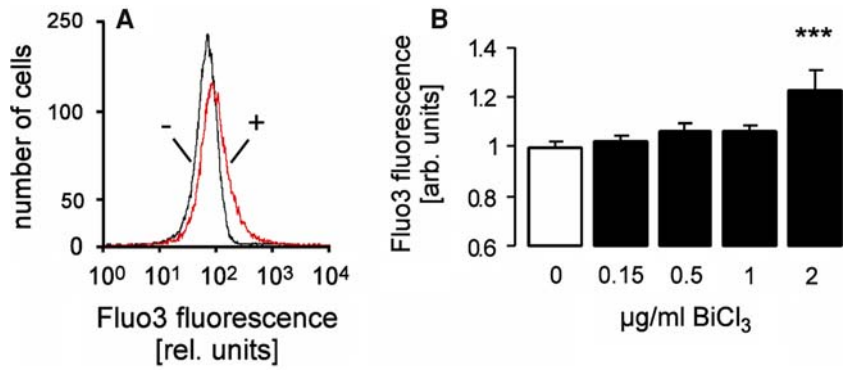

Fig. 4 Increase in the cytosolic Ca^{2+} concentration in erythrocytes following exposure to bismuth. **a** Histogram of Fluo3 fluorescence in a representative experiment of erythrocytes exposed for 48 h to Ringer without (–, *black line*) and with (+, *red line*) 2,000 µg/l $BiCl_3$. **b** Arithmetic means ± SEM ($n = 12$–32) of the normalized Fluo3 fluorescence in erythrocytes exposed for 48 h to Ringer without (*white bar*) or with (*black bars*) bismuth ($BiCl_3$) at the indicated concentrations. *** indicates significant difference from the absence of bismuth (ANOVA, $P < 0.001$)

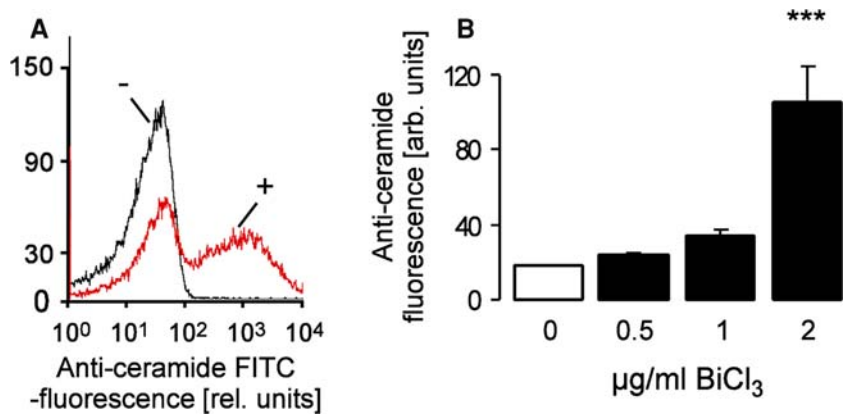

Fig. 5 Influence of bismuth on ceramide formation. **a** Histogram of ceramide abundance in a representative experiment of erythrocytes from healthy volunteers exposed for 48 h to Ringer solution without (–, *black line*) or with (+, *red line*) 2,000 µg/l $BiCl_3$. **b** Arithmetic means ± SEM ($n = 8$) of ceramide abundance in erythrocytes exposed for 48 h to Ringer solution without (*white bar*) or with (*black bars*) bismuth ($BiCl_3$) at the indicated concentrations. *** indicates significant difference from values in control Ringer solution (ANOVA, $P < 0.001$)

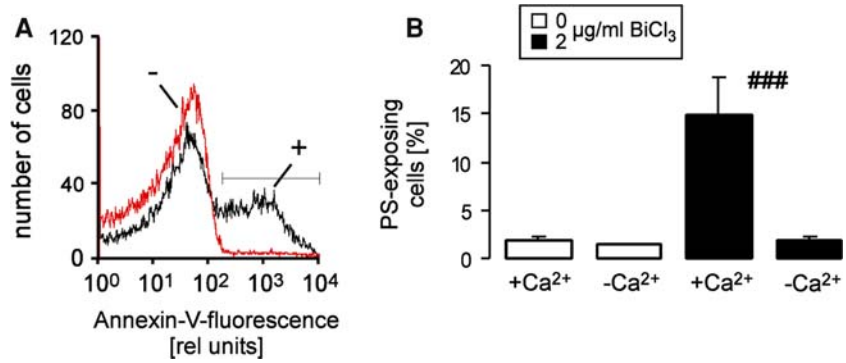

Fig. 6 Stimulation of phosphatidylserine exposure at the erythrocyte surface by bismuth in the presence or absence of extracellular Ca^{2+}. **a** Histogram of annexin V-binding in a representative experiment of erythrocytes incubated for 48 h in Ringer solution containing 2,000 µg/l $BiCl_3$ in the presence (+, *black line*) or absence (–, *red line*) of Ca^{2+}. **b** Arithmetic means ± SEM ($n = 12$) of the percentage of annexin V-binding erythrocytes after a 48 h treatment with Ringer solution without (*white bars*) or with (*black bars*) 2,000 µg/l $BiCl_3$ in the presence (+Ca^{2+}) or absence (–Ca^{2+}) of calcium. ### indicates significant difference ($P < 0.001$, ANOVA) from the respective values in the presence of Ca^{2+}

Further experiments explored whether bismuth-induced cell membrane scrambling was dependent on the presence of Ca^{2+}. As shown in Fig. 6, the effect of bismuth on annexin V-binding was blunted in the nominal absence of Ca^{2+}. Similarly, the effect of bismuth on forward scatter was blunted in the nominal absence of Ca^{2+} (Fig. 7).

Discussion

The present study demonstrates that exposure of human erythrocytes to bismuth triggers eryptosis, characterized by stimulation of cell membrane scrambling and cell shrinkage. The concentrations required to elicit this effect are well in the range of plasma concentrations encountered in vivo (Caravati 2004; Krachler et al. 2000; Wagstaff et al. 1988). Phosphatidylserine-exposing erythrocytes are bound to PS-receptors on macrophages (Fadok et al. 2000), which engulf phosphatidylserine-exposing cells (Boas et al. 1998). Phosphatidylserine-exposing erythrocytes are thus cleared from circulating blood (Kempe et al. 2006). Accordingly, stimulation of eryptosis leads to anemia.

The effect on cell membrane scrambling may in part be due to an increase in the cytosolic Ca^{2+}

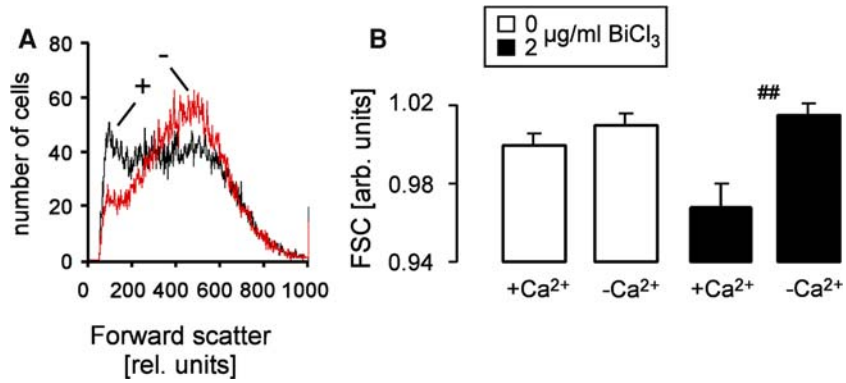

Fig. 7 Decrease of erythrocyte forward scatter by bismuth in the presence or absence of extracellular Ca^{2+}. **a** Histogram of forward scatter in a representative experiment of erythrocytes incubated for 48 h in Ringer solution containing 2,000 µg/l $BiCl_3$ in the presence (+, *black line*) or absence (–, *red line*) of Ca^{2+}. **b** Arithmetic means ± SEM ($n = 12$) of the normalized erythrocyte forward scatter after a 48 h treatment with Ringer solution without (*white bars*) or with (*black bars*) 2,000 µg/l $BiCl_3$ in the presence (+Ca^{2+}) or absence (–Ca^{2+}) of calcium. ## indicates significant difference ($P < 0.01$, ANOVA) from the respective values in the presence of Ca^{2+}

concentration. As shown earlier (Berg et al. 2001; Bratosin et al. 2001; Daugas et al. 2001), an increase in the cytosolic Ca^{2+} activity stimulates phospholipid scrambling of the erythrocyte cell membrane. Ca^{2+} further activates Ca^{2+}-sensitive K^+ channels (Bookchin et al. 1987; Brugnara et al. 1993). Activation of those channels leads to exit of positively charged K^+, hyperpolarisation of the cell membrane and thus increased driving force for Cl^- exit. The cellular loss of K^+ and Cl^- is paralleled by a similar loss of osmotically obliged water. The result is cell shrinkage.

The effect of bismuth may further be due to stimulation of ceramide formation. The strong stimulating effect of ceramide on eryptosis has been observed earlier (Lang et al. 2004a). Ceramide may be formed by activation of a sphingomyelinase (Lang et al. 2007).

The present observations in erythrocytes raise the question, whether similar mechanisms are effective in the favorable influence of bismuth on gastrointestinal disease. Sphingomyelinase has previously been shown to play a critical role in host pathogen interaction (Dreschers et al. 2007; Grassme et al. 1997, 2003, 2005; Gulbins et al. 2004; Kempe et al. 2007), and it is seducing to speculate that the powerful stimulation of ceramide formation by bismuth contributes to or even accounts for the therapeutic efficacy of bismuth. *Helicobacter* has previously been shown to express sphingomyelinase (Lin et al. 1998), but an influence of bismuth on sphingomyelinase activity and its putative role in its therapeutic action has never been explored.

In conclusion, exposure of erythrocytes to bismuth triggers phospholipid scrambling with phosphatidylserine-exposure at the surface of the cell membrane and activation of K^+ channels with subsequent cell shrinkage. The effects are at least in part due to increased cytosolic Ca^{2+} activity and formation of ceramide. Enhanced eryptosis may contribute to the development of anemia following bismuth treatment. On the other hand, the novel cellular mechanisms disclosed here may contribute to the beneficial effects of bismuth in gastrointestinal disease.

Acknowledgments The authors acknowledge the meticulous preparation of the manuscript by Lejla Subasic and Tanja Loch. This study was supported by the Deutsche Forschungsgemeinschaft SFB 766.

References

Akel A, Hermle T, Niemoeller OM, Kempe DS, Lang PA, Attanasio P, Podolski M, Wieder T, Lang F (2006) Stimulation of erythrocyte phosphatidylserine exposure by chlorpromazine. Eur J Pharmacol 532:11–17. doi:10.1016/j.ejphar.2005.12.037

Bentzen PJ, Lang E, Lang F (2007) Curcumin induced suicidal erythrocyte death. Cell Physiol Biochem 19:153–164. doi:10.1159/000099203

Berg CP, Engels IH, Rothbart A, Lauber K, Renz A, Schlosser SF, Schulze-Osthoff K, Wesselborg S (2001) Human

mature red blood cells express caspase-3 and caspase-8, but are devoid of mitochondrial regulators of apoptosis. Cell Death Differ 8:1197–1206. doi:10.1038/sj.cdd.4400905

Boas FE, Forman L, Beutler E (1998) Phosphatidylserine exposure and red cell viability in red cell aging and in hemolytic anemia. Proc Natl Acad Sci USA 95:3077–3081. doi:10.1073/pnas.95.6.3077

Bookchin RM, Ortiz OE, Lew VL (1987) Activation of calcium-dependent potassium channels in deoxygenated sickled red cells. Prog Clin Biol Res 240:193–200

Brand VB, Sandu CD, Duranton C, Tanneur V, Lang KS, Huber SM, Lang F (2003) Dependence of *Plasmodium falciparum* in vitro growth on the cation permeability of the human host erythrocyte. Cell Physiol Biochem 13:347–356. doi:10.1159/000075122

Bratosin D, Estaquier J, Petit F, Arnoult D, Quatannens B, Tissier JP, Slomianny C, Sartiaux C, Alonso C, Huart JJ, Montreuil J, Ameisen JC (2001) Programmed cell death in mature erythrocytes: a model for investigating death effector pathways operating in the absence of mitochondria. Cell Death Differ 8:1143–1156. doi:10.1038/sj.cdd.4400946

Brugnara C, de Franceschi L, Alper SL (1993) Inhibition of Ca(2+)-dependent K+ transport and cell dehydration in sickle erythrocytes by clotrimazole and other imidazole derivatives. J Clin Invest 92:520–526. doi:10.1172/JCI116597

Caravati EM (2004) Bismuth. In: Dart RC (ed) Medical toxicology. Lippincott Williams & Wilkins, Philadelphia, pp 1407–1411

Chey WD, Wong BC (2007) American College of Gastroenterology guideline on the management of *Helicobacter pylori* infection. Am J Gastroenterol 102:1808–1825. doi:10.1111/j.1572-0241.2007.01393.x

Cimen MY (2008) Free radical metabolism in human erythrocytes. Clin Chim Acta 390:1–11. doi:10.1016/j.cca.2007.12.025

Daugas E, Cande C, Kroemer G (2001) Erythrocytes: death of a mummy. Cell Death Differ 8:1131–1133. doi:10.1038/sj.cdd.4400953

Dekkers DW, Comfurius P, Bevers EM, Zwaal RF (2002) Comparison between Ca2+-induced scrambling of various fluorescently labelled lipid analogues in red blood cells. Biochem J 362:741–747. doi:10.1042/0264-6021:3620741

Dreschers S, Franz P, Dumitru C, Wilker B, Jahnke K, Gulbins E (2007) Infections with human rhinovirus induce the formation of distinct functional membrane domains. Cell Physiol Biochem 20:241–254

Duranton C, Huber SM, Lang F (2002) Oxidation induces a Cl(−)-dependent cation conductance in human red blood cells. J Physiol 539:847–855. doi:10.1113/jphysiol.2001.013040

Duranton C, Huber S, Tanneur V, Lang K, Brand V, Sandu C, Lang F (2003) Electrophysiological properties of the *Plasmodium falciparum*-induced cation conductance of human erythrocytes. Cell Physiol Biochem 13:189–198. doi:10.1159/000072421

Eisele K, Lang PA, Kempe DS, Klarl BA, Niemoller O, Wieder T, Huber SM, Duranton C, Lang F (2006) Stimulation of erythrocyte phosphatidylserine exposure by mercury ions. Toxicol Appl Pharmacol 210:116–122. doi:10.1016/j.taap.2005.07.022

Fadok VA, Bratton DL, Rose DM, Pearson A, Ezekewitz RA, Henson PM (2000) A receptor for phosphatidylserine-specific clearance of apoptotic cells. Nature 405:85–90. doi:10.1038/35011084

Foller M, Shumilina E, Lam R, Mohamed W, Kasinathan R, Huber S, Chakraborty T, Lang F (2007) Induction of suicidal erythrocyte death by listeriolysin from Listeria monocytogenes. Cell Physiol Biochem 20:1051–1060. doi:10.1159/000110715

Foller M, Geiger C, Mahmud H, Nicolay J, Lang F (2008) Stimulation of suicidal erythrocyte death by amantadine. Eur J Pharmacol 581:13–18. doi:10.1016/j.ejphar.2007.11.051

Grassme H, Gulbins E, Brenner B, Ferlinz K, Sandhoff K, Harzer K, Lang F, Meyer TF (1997) Acidic sphingomyelinase mediates entry of *N. gonorrhoeae* into nonphagocytic cells. Cell 91:605–615. doi:10.1016/S0092-8674(00)80448-1

Grassme H, Jendrossek V, Riehle A, von Kurthy G, Berger J, Schwarz H, Weller M, Kolesnick R, Gulbins E (2003) Host defense against *Pseudomonas aeruginosa* requires ceramide-rich membrane rafts. Nat Med 9:322–330. doi:10.1038/nm823

Grassme H, Riehle A, Wilker B, Gulbins E (2005) Rhinoviruses infect human epithelial cells via ceramide-enriched membrane platforms. J Biol Chem 280:26256–26262. doi:10.1074/jbc.M500835200

Gulbins E, Dreschers S, Wilker B, Grassme H (2004) Ceramide, membrane rafts and infections. J Mol Med 82:357–363. doi:10.1007/s00109-004-0539-y

Huber SM, Gamper N, Lang F (2001) Chloride conductance and volume-regulatory nonselective cation conductance in human red blood cell ghosts. Pflugers Arch 441:551–558. doi:10.1007/s004240000456

Kempe DS, Lang PA, Eisele K, Klarl BA, Wieder T, Huber SM, Duranton C, Lang F (2005) Stimulation of erythrocyte phosphatidylserine exposure by lead ions. Am J Physiol Cell Physiol 288:C396–C402. doi:10.1152/ajpcell.00115.2004

Kempe DS, Lang PA, Duranton C, Akel A, Lang KS, Huber SM, Wieder T, Lang F (2006) Enhanced programmed cell death of iron-deficient erythrocytes. FASEB J 20:368–370

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 (2007) Suicidal erythrocyte death in sepsis. J Mol Med 85:273–281. doi:10.1007/s00109-006-0123-8

Krachler M, Domej W, Irgolic KJ (2000) Concentrations of trace elements in osteoarthritic knee-joint effusions. Biol Trace Elem Res 75:253–263. doi:10.1385/BTER:75:1-3:253

Kriebardis AG, Antonelou MH, Stamoulis KE, Economou-Petersen E, Margaritis LH, Papassideri IS (2007) Storage-dependent remodeling of the red blood cell membrane is associated with increased immunoglobulin G binding, lipid raft rearrangement, and caspase activation. Transfusion 47:1212–1220. doi:10.1111/j.1537-2995.2007.01254.x

Lang KS, Duranton C, Poehlmann H, Myssina S, Bauer C, Lang F, Wieder T, Huber SM (2003a) Cation channels trigger apoptotic death of erythrocytes. Cell Death Differ 10:249–256. doi:10.1038/sj.cdd.4401144

Lang PA, Kaiser S, Myssina S, Wieder T, Lang F, Huber SM (2003b) Role of Ca2+-activated K+ channels in human erythrocyte apoptosis. Am J Physiol Cell Physiol 285: C1553–C1560

Lang PA, Warskulat U, Heller-Stilb B, Huang DY, Grenz A, Myssina S, Duszenko M, Lang F, Haussinger D, Vallon V, Wieder T (2003c) Blunted apoptosis of erythrocytes from taurine transporter deficient mice. Cell Physiol Biochem 13:337–346. doi:10.1159/000075121

Lang KS, Myssina S, Brand V, Sandu C, Lang PA, Berchtold S, Huber SM, Lang F, Wieder T (2004a) Involvement of ceramide in hyperosmotic shock-induced death of erythrocytes. Cell Death Differ 11:231–243. doi:10.1038/sj.cdd.4401311

Lang PA, Kaiser S, Myssina S, Birka C, Weinstock C, Northoff H, Wieder T, Lang F, Huber SM (2004b) Effect of *Vibrio parahaemolyticus* haemolysin on human erythrocytes. Cell Microbiol 6:391–400. doi:10.1111/j.1462-5822.2004.00369.x

Lang KS, Lang PA, Bauer C, Duranton C, Wieder T, Huber SM, Lang F (2005) Mechanisms of suicidal erythrocyte death. Cell Physiol Biochem 15:195–202. doi:10.1159/000086406

Lang PA, Huober J, Bachmann C, Kempe DS, Sobiesiak M, Akel A, Niemoeller OM, Dreischer P, Eisele K, Klarl BA, Gulbins E, Lang F, Wieder T (2006) Stimulation of erythrocyte phosphatidylserine exposure by paclitaxel. Cell Physiol Biochem 18:151–164. doi:10.1159/000095190

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 13:164–170. doi:10.1038/nm1539

Larsen A, Stoltenberg M, Sondergaard C, Bruhn M, Danscher G (2005) In vivo distribution of bismuth in the mouse brain: influence of long-term survival and intracranial placement on the uptake and transport of bismuth in neuronal tissue. Basic Clin Pharmacol Toxicol 97:188–196. doi:10.1111/j.1742-7843.2005.pto_973132.x

Lee KP, Gillies PJ (1986) Pulmonary response and intrapulmonary lipids in rats exposed to bismuth orthovanadate dust by inhalation. Environ Res 40:115–135. doi:10.1016/S0013-9351(86)80088-3

Lin YL, Liu JS, Chen KT, Chen CT, Chan EC (1998) Identification of neutral and acidic sphingomyelinases in *Helicobacter pylori*. FEBS Lett 423:249–253. doi:10.1016/S0014-5793(98)00087-8

Lui JC, Wong JW, Suen YK, Kwok TT, Fung KP, Kong SK (2007) Cordycepin induced eryptosis in mouse erythrocytes through a Ca2+-dependent pathway without caspase-3 activation. Arch Toxicol 81:859–865. doi:10.1007/s00204-007-0214-5

Moayyedi P, Soo S, Deeks J, Delaney B, Innes M, Forman D (2006) Pharmacological interventions for non-ulcer dyspepsia. Cochrane Database Syst Rev (4):CD001960. doi:10.1002/14651858.CD001960.pub3

Moeschlin S (1975) Clinical-hematological demonstrations: aplastic anemia, acute leukemias, polyneuropathy in Waldenstrom's disease, acute porphyria. Schweiz Med Wochenschr 105:1289–1298

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 18:223–232. doi:10.1159/000097669

Nicolay JP, Gatz S, Liebig G, Gulbins E, Lang F (2007) Amyloid induced suicidal erythrocyte death. Cell Physiol Biochem 19:175–184. doi:10.1159/000099205

Niemoeller OM, Akel A, Lang PA, Attanasio P, Kempe DS, Hermle T, Sobiesiak M, Wieder T, Lang F (2006a) Induction of eryptosis by cyclosporine. Naunyn Schmiedebergs Arch Pharmacol 374:41–49. doi:10.1007/s00210-006-0099-5

Niemoeller OM, Kiedaisch V, Dreischer P, Wieder T, Lang F (2006b) Stimulation of eryptosis by aluminium ions. Toxicol Appl Pharmacol 217:168–175. doi:10.1016/j.taap.2006.09.001

Pamphlett R, Stoltenberg M, Rungby J, Danscher G (2000) Uptake of bismuth in motor neurons of mice after single oral doses of bismuth compounds. Neurotoxicol Teratol 22:559–563. doi:10.1016/S0892-0362(00)00083-0

Schneider J, Nicolay JP, Foller M, Wieder T, Lang F (2007) Suicidal erythrocyte death following cellular K+ loss. Cell Physiol Biochem 20:35–44

Shumilina E, Kiedaisch V, Akkel A, Lang P, Hermle T, Kempe DS, Huber SM, Wieder T, Laufer S, Lang F (2006) Stimulation of suicidal erythrocyte death by lipoxygenase inhibitor Bay-Y5884. Cell Physiol Biochem 18:233–242. doi:10.1159/000097670

Sopjani M, Foller M, Lang F (2008) Gold stimulates Ca2+ entry into and subsequent suicidal death of erythrocytes. Toxicology 244:271–279. doi:10.1016/j.tox.2007.12.001

Wagstaff AJ, Benfield P, Monk JP (1988) Colloidal bismuth subcitrate. A review of its pharmacodynamic and pharmacokinetic properties, and its therapeutic use in peptic ulcer disease. Drugs 36:132–157. doi:10.2165/00003495-198836020-00002

Woon LA, Holland JW, Kable EP, Roufogalis BD (1999) Ca2+ sensitivity of phospholipid scrambling in human red cell ghosts. Cell Calcium 25:313–320. doi:10.1054/ceca.1999.0029

Zappulla D (2008) Environmental stress, erythrocyte dysfunctions, inflammation, and the metabolic syndrome: adaptations to CO2 increases? J Cardiometab Syndr 3:30–34. doi:10.1111/j.1559-4572.2008.07263.x

Zhang L, Mulrooney SB, Leung AF, Zeng Y, Ko BB, Hausinger RP, Sun H (2006) Inhibition of urease by bismuth(III): implications for the mechanism of action of bismuth drugs. Biometals 19:503–511. doi:10.1007/s10534-005-5449-0