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The influence of merocyanine 540 and protoporphyrin on physicochemical properties of the erythrocyte membrane

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Abstract

The interaction of the red cell membrane with merocyanine 540 or protoporphyrin led to four phenomena, most probably interrelated. (i) The morphology changed from the normal discoid to an echinocytic form. This morphological change persisted when followed over a period of 24 h. (ii) Simultaneously, cell deformability was decreased, as revealed by viscosity measurements and a cell-filtration technique. (iii) Both drugs caused swelling of the erythrocytes in isotonic medium, due to a very-short-term increased permeability of the membrane, also for larger molecules such as lactose. The pathway of this temporary leak seems to be unrelated to the Na^+/K^+-ATPase, the K^+/Cl^- and the $Na^+/K^+/Cl^-$ cotransport systems, the Ca^{2+}-activated Gardos pathway, the oxidation/deformation-activated leak pathway and the so-called residual transport route. Despite the morphological changes, K^+-leakage induced by mechanical stress was not increased. (iv) During osmotic swelling, the critical hemolytic volume was found to be increased in the presence of either merocyanine 540 or protoporphyrin. The increased critical volume protected erythrocytes against osmotic hemolysis.

Keywords: Protoporphyrin; Merocyanine 540; Erythrocyte deformability; Osmotic hemolysis; Hemolysis

1. Introduction

Viral infectivity of blood and blood products has been reduced substantially through improved donor counseling and blood screening procedures, but still there is a continued risk of virus transmission by transfusion of blood and blood products. This risk has been reduced considerably for coagulation factor concentrates by the application of virucidal procedures [1,2]. These procedures, however, are not applicable to blood products containing cell components, due to the fragility of these cells. In this context there is a growing interest in the possibility of photodynamic treatment of cellular transfusion products with sensitizer and light doses that will inactivate viruses, without appreciable damage of blood cells. In many preclinical studies, sensitizers were used with a high affinity to viral DNA [3,4]. The advantage of these sensitizers would be the elimination of viral infectivity, with minimal damage of blood cells. The disadvantage is, however, the possible mutagenesis of the virus, with unmasking of oncogenic potential [5,6]. Mutagenic effects are much less probable with sensitizers, which primarily locate in the viral lipoprotein envelope, but such dyes will also accumulate in the membrane of blood cells, which may result in damage during illumination. Evaluation of this damage and search for methods to circumvent these problems is an essential part of research in this field [7–10].

A complicating factor is that compounds (including some sensitizers) which interact with the erythrocyte membrane, may affect the shape of the cells. For instance, neutral and negatively charged amphiphiles tend to change the discoid cells to echinocytes, whereas positively charged amphiphiles generate invaginated stomatocytes [11,12]. Such changes may affect the physicochemical properties of the cells, resulting in decreased deformability [13]. If a sensitizer, used for photodynamic sterilization, were to

Abbreviations: DIDS, 4,4′-diisothiocyano-2,2′-disulfonic acid stilbene; MC540, merocyanine 540; MCV, mean cellular volume; PBS, phosphate-buffered saline; V_0, MCV of control cells in PBS.

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have such an effect on the red blood cells (even without illumination), this might have a disadvantageous effect on the rheological properties and survival of the transfused blood cells. Therefore, the dark effects of two sensitizers known to localize in the red cell membrane, i.e., merocyanine 540 and protoporphyrin, on selected physicochemical properties of red blood cells were investigated.

2. Materials and methods

Merocyanine 540 was purchased from Eastman Kodak, Rochester NY. Stock solutions (1 mg/ml) were prepared in 50% ethanol. Protoporphyrin IX was obtained from Porphyrin Products, Logan, UT. Stock solutions were made in 4 mM NaOH. DIDS was purchased from Pierce, Rockford, IL, glutaraldehyde from Polysciences, Warrington, PA, inulin-[^{14}C]carboxylic acid and [D-*glucose*-1-^{14}C]lactose from Amersham International, Amersham, UK, and [^{3}H]raffinose and $^{86}Rb^{+}$ from DuPont NEN, Dordrecht, The Netherlands. All other chemicals were of analytical grade and used without further purification. Polycarbonate filters (5 μm) were obtained from Poretics, Livermore, CA.

Heparinized human blood was centrifuged shortly after collection. The erythrocytes were washed three times in PBS. Cells were resuspended at a hematocrit of 2% in PBS or in PBS containing either MC540 or protoporphyrin. After incubation during 5 min the cells were spun down and the hematocrit was adjusted to 10 or 70% by removal of the adequate amount of medium. In some experiments chloride in medium and cells was replaced by nitrate, by preincubation of the erythrocytes with $NaNO_3$ as described by Sheerin et al. [14].

Cell morphology was assessed by light microscopy after fixation of the cells in PBS containing 2% glutaraldehyde for 1 h. Cell deformability was measured with a Brookfield LVT cone-plate viscometer, as described previously [15]. Cell filterability was measured by recording pressure–time curves during filtration of a 2% red blood cell suspension at constant rate through 5 μm polycarbonate filters, according to Miller et al. [16].

Osmotic fragility was measured as described by Horowitz et al. [8]. The mean cellular volume was measured by centrifuging 0.3 ml of a 10% erythrocyte suspension during 20 min at 3000 rpm in a Hamburger-type hematocrit tube, correcting the packed cell volume for trapped medium. Medium trapped between packed cells was determined by adding 2% ^{14}C-labeled inulin to the medium, prior to centrifugation. Samples of 50 μl packed cells were resuspended in 2 ml PBS and the radioactivity in the supernatant of this suspension was measured by scintillation counting.

Cellular uptake of lactose, raffinose and Rb^{+} was measured by incubation of the cells in PBS, containing trace amounts of [^{14}C]lactose, [^{3}H]raffinose or $^{86}Rb^{+}$. After incubation, the intracellular tracer concentration was determined by hemolyzing 50 μl packed cells in 2 ml distilled water. After bleaching of hemoglobin with H_2O_2 radioactivity in the hemolysate was measured and corrected for trapped medium.

K^{+} loss induced by mechanical stress was measured by subjecting a 10% erythrocyte suspension to a shear stress of 200 dyne/cm^2 in a Brookfield DV III viscometer during 2 h, as described by Sugihara et al. [17].

Na^{+}, K^{+} and Li^{+} determinations were carried out with a Corning 410C flame photometer. Intracellular Na^{+} and Li^{+} concentrations were determined by hemolyzing 50 μl packed cells in 3 ml distilled water and measuring Na^{+} or Li^{+} in the hemolysate, correcting again for trapped medium.

Membrane phospholipid asymmetry was monitored by the prothrombinase assay as described by Comfurius et al. [18].

The critical hemolytic volume was assayed as described previously [19].

Preincubation of erythrocytes with 10 μM bumetanide was performed at 37°C for 30 min as described by Johnson and Tang [20], with 12 μM DIDS according to Lepke et al. [21], with 2.5 mM furosemide for 10 min at 37°C according to Garay et al. [22] and with 1 mM ouabain for 5 min at 22°C.

All experiments were carried out at least in triplicate, utilizing blood from different donors.

3. Results

3.1. Red cell morphology

Morphological changes of erythrocytes induced by MC540 and protoporphyrin are shown in Fig. 1. With increasing sensitizer concentrations it appeared that all cells became echinocytic at a concentration of 17.5 μM MC540 or 20 μM protoporphyrin. This shape change persisted during prolonged incubation (24 h) in the presence of the sensitizer at room temperature.

3.2. Red cell deformability

After exposure of the cells to either MC540 or protoporphyrin the viscosity of a 70% cell suspension was significantly increased, especially at low shear rates (Fig. 2). At this hematocrit value the viscosity of the cell suspension is a reflection of red cell deformability [16]. The decreased deformability is corroborated by the increased pressure during passage of a 2% erythrocyte suspension through a 5 μm polycarbonate filter (Fig. 3).

3.3. Sensitizer-induced swelling in isotonic medium

MC540 and protoporphyrin induced an increase of the mean cellular volume in isotonic medium to about 1.13 V_0.

This increase in cellular volume occurred immediately after addition of the drug. Lowering the temperature to 0°C during incubation had no effect on this phenomenon. Storage of the swollen cells in the continued presence of the sensitizer during 24 h at room temperature revealed that the volume increase persisted during this time interval.

If Na^+ in the medium was replaced by K^+, Li^+ or choline$^+$, drug-induced isotonic swelling was not affected. Also, substituting $NaNO_3$ for NaCl in cells and medium did not prevent isotonic swelling. Further, pretreatment of the cells with DIDS, EGTA, furosemide, bumetanide or ouabain or substituting sucrose for NaCl in the medium had no effect on isotonic swelling (not shown).

Isotonic swelling of the erythrocytes in PBS was attended with an increase of the intracellular Na^+ concentration from 14.6 ± 1.2 (mean ± S.E., $n = 16$) to 25.2 ± 2.3 ($n = 20$) mequiv/l packed cells with MC540 and to 27.2 ± 2.1 ($n = 18$) mequiv/l with protoporphyrin. This Na^+ uptake was not counterbalanced by an equal K^+ loss; the intracellular K^+ concentration decreased with only 0.7 ±

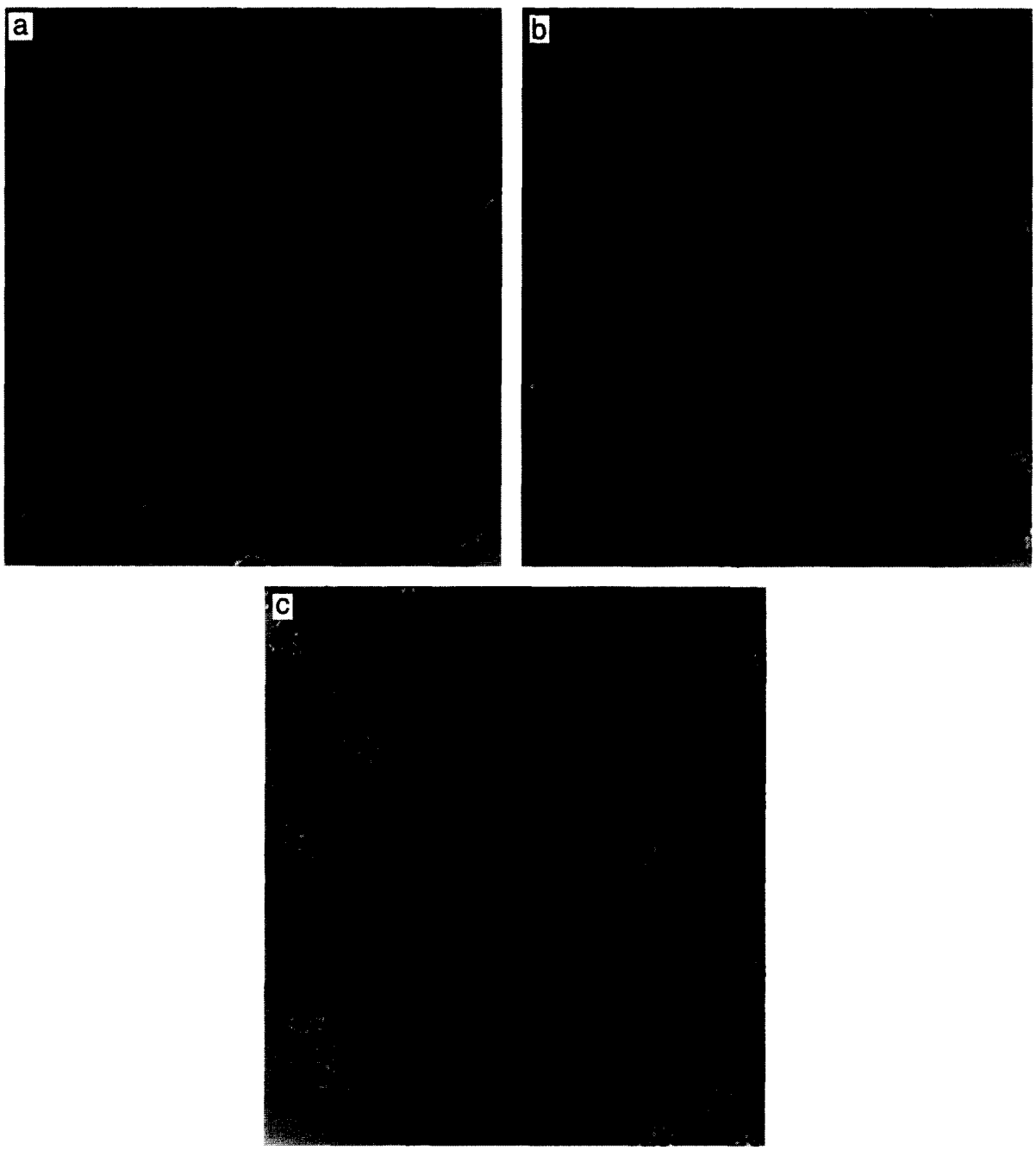

Fig. 1. Light micrographs of normal erythrocytes (a) and erythrocytes exposed to MC540 (b) or protoporphyrin (c). Cell suspensions were incubated with 17.5 μM MC540 or 20 μM protoporphyrin during 5 min at 22°C.

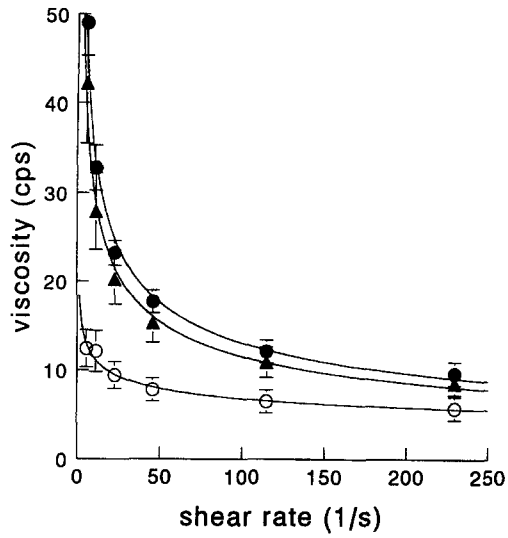

Fig. 2. The viscosity of 70% red cell suspensions at varying shear rates. ○, untreated erythrocytes; ●, erythrocytes incubated with 17.5 μM MC540 at a hematocrit of 2%; ▲, erythrocytes incubated with 20 μM protoporphyrin at a hematocrit of 2%.

0.1 mequiv/l in the presence of MC540 and with 2.7 ± 0.3 mequiv/l with protoporphyrin. When Na^+ in the medium was replaced by Li^+, addition of MC540 or protoporphyrin led to increased Li^+ uptake, with a final intracellular concentration of 11.3 ± 0.8 mequiv/l with MC540 and 12.6 ± 1.1 mequiv/l with protoporphyrin. The intracellular cation concentrations were measured 2 min after addition of MC540 or protoporphyrin. On prolonged incubation in the presence of MC540 no further uptake of Na^+ or Li^+ and no K^+ leakage above the level of control cells took place. Apparently exposure of the cells to the sensitizer resulted in an immediate, but transient increased permeability to Na^+ and Li^+, leading to isotonic cell swelling, after which the passive permeability returned to control levels. This was confirmed by experiments in which ouabaine-treated erythrocytes were incubated with trace amounts of $^{86}Rb^+$. A very slow uptake of Rb^+ occurred, representing the passive permeability of the membrane for this ion. Addition of MC540 caused an instantaneous extra uptake of Rb^+, to a final intracellular concentration of 10–14% of the Rb^+ concentration in the medium. Again, during prolonged incubation Rb^+ uptake had returned to control levels. When Rb^+ was added 15 s after addition of the sensitizer, passive Rb^+ uptake did not deviate from that in control cells. With protoporphyrin the results were identical with one exception: during prolonged incubation both K^+-leakage and passive Rb^+ uptake were slightly, but significantly higher as compared to control cells.

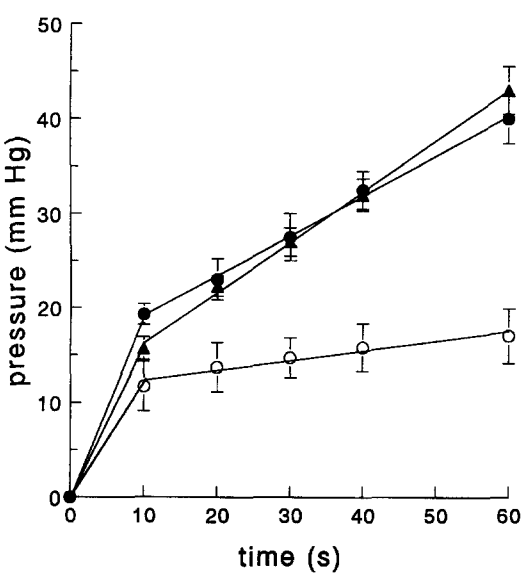

Fig. 3. Pressure-time curves for 2% erythrocyte suspensions filtered through 5 μm polycarbonate filters at a constant flow rate of 5 ml/min. ○, control cells; ●, in the presence of 17.5 μM MC540; ▲, in the presence of 20 μM protoporphyrin.

During the process of isotonic swelling the erythrocyte membrane appeared to be also permeable to somewhat larger molecules, like lactose. When ^{14}C-labeled lactose was present during incubation of the cells with MC540 or protoporphyrin, lactose was taken up by the cells, yielding an intracellular concentration of about 12% of the medium concentration, whereas untreated control cells exhibited no measurable uptake of radioactivity. In similar experiments with [^{3}H]raffinose, no cellular uptake of radioactivity could be measured. When [^{14}C]lactose was added 15 s later than MC540 or protoporphyrin, no uptake of radioactivity occurred, indicating that also the increased permeability for larger molecules was only very temporary.

To investigate whether the increased mean cellular volume and the shape change had an effect on the K^+ leakage induced by mechanical stress, the cells were subjected to a shear stress of 200 dyne/cm^2 for 2 h at 37°C. Pretreatment with MC540 or with protoporphyrin had no effect on the K^+ leakage induced by mechanical stress, which amounted to 0.039 ± 0.004 mequiv K^+/l packed cells per min (corrected for unstressed K^+ loss).

3.4. Membrane phospholipid asymmetry

Normally, the phospholipids in the erythrocyte membrane are distributed asymmetrically with, for instance, most of the phosphatidylserine located in the inner leaflet. Echinocytosis, induced by an increased intracellular concentration of Ca^{2+} is accompanied by a redistribution of phospholipids, as reflected by, for example, the appearance of phosphatidylserine in the outer leaflet of the membrane [23]. Under the experimental conditions described by these authors, we found a similar increase of phosphatidylserine in the outer leaflet from 1% in control cells to 29% after elevation of the intracellular Ca^{2+} concentration. Addition of either MC540 or protoporphyrin to the medium had no effect on these results. On the other hand, exposure of the cells to MC540 or protoporphyrin without elevation of the

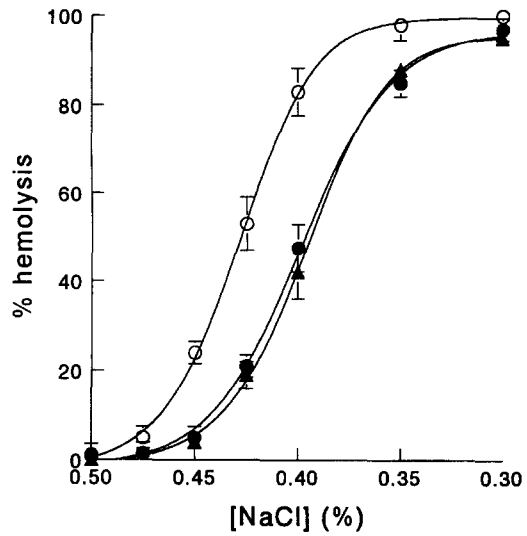

Fig. 4. The effect of MC540 and protoporphyrin on osmotic hemolysis. Red blood cells were added to drug-containing hypotonic NaCl solutions. ○, control, no additions; ●, 17.5 μM MC540 added; ▲, 20 μM protoporphyrin added.

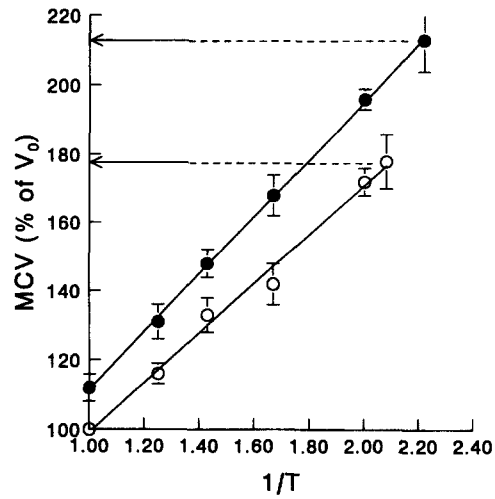

Fig. 6. The relationship between MCV (expressed in % of V_0: the MCV in PBS) and the reciprocal of the tonicity of the medium ($1/T$). Erythrocytes were suspended in the NaCl solution, containing: ○, no additions; ●, 17.5 μM MC540. The arrows indicate the critical hemolytic volume. With protoporphyrin similar results were obtained.

intracellular Ca^{2+} concentration did not result in any increase of phosphatidylserine in the outer leaflet of the membrane.

3.5. Reversibility of the membrane effects

All effects described above appeared to depend on the presence of MC540 or protoporphyrin in the membrane. Upon washing of the erythrocytes three times with PBS, containing 0.5% bovine serum albumin (effectively removing the sensitizers from the membrane) cell morphology, deformability and isotonic volume returned to normal, control values.

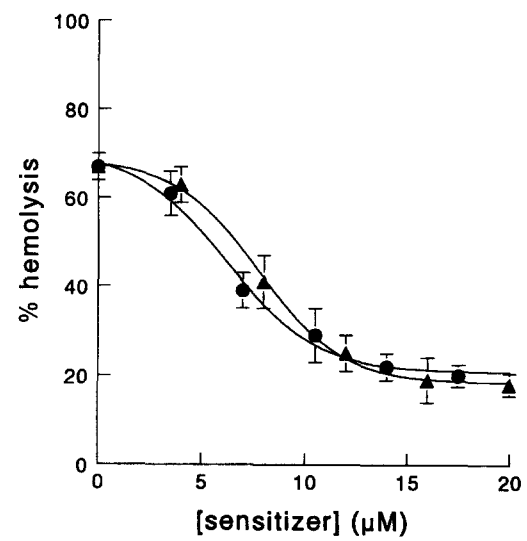

Fig. 5. The effect of MC540 and protoporphyrin on osmotic hemolysis of erythrocytes in 0.425% NaCl solutions, containing the indicated concentration of the sensitizer. ●, MC540; ▲, protoporphyrin.

3.6. Protection against osmotic hemolysis

To further characterize the effects of these drugs on the red cell membrane, the influence of MC540 and protoporphyrin on osmotic hemolysis was determined. When untreated red blood cells were suspended in hypotonic NaCl solutions containing either MC540 or protoporphyrin, both drugs protected the cells against osmotic hemolysis (Fig. 4), in a dose-dependent way (Fig. 5). The protection against osmotic hemolysis could not be attributed to inhibition of osmotically driven water transport: cell swelling was even more pronounced in the presence than in the absence of sensitizer under these experimental conditions (Fig. 6). At low NaCl concentrations osmotic hemolysis occurs when the cells have attained the critical hemolytic volume. In the presence of either MC540 or protoporphyrin osmotic hemolysis started at lower NaCl concentrations than in the control (Fig. 4), with the cells swollen beyond the normal critical volume, which was 1.78 ± 0.08 V_0 for untreated control cells, and 2.10 ± 0.09 V_0 in the presence of MC540 or protoporphyrin.

Under hypotonic conditions, leading to incomplete osmotic hemolysis, the percentage K^+ leakage from normal erythrocytes slightly exceeded the percentage hemoglobin release, a phenomenon known as prelytic K^+ loss. This prelytic K^+ loss was not augmented in the presence of MC540 or protoporphyrin, but rather slightly inhibited (not shown).

4. Discussion

Incubation of erythrocytes with either MC540 or protoporphyrin induced a shape change from the normal discoid

form to that of evaginated echinocytes (Fig. 1). This is in accordance with earlier observations indicating that negatively charged amphiphiles cause discoid cells to become echinocytes, whereas positively charged amphiphiles generate invaginated stomatocytes [11,24]. The different shapes induced by negatively and positively charged amphiphilic agents is presumably related to preferential intercalation of negatively charged molecules in the outer, and positively charged molecules in the inner leaflet of the membrane bilayer [25]. The sensitizer concentrations used in the present experiments are identical or close to the concentrations used in preclinical experiments on photodynamic virus inactivation.

In earlier studies we have shown that the positively charged anesthetic chlorpromazine not only caused a shape change to stomatocytes, but also evoked a pronounced decrease of cell deformability [13]. It seemed conceivable that also a shape change to echinocytes would be associated with an effect on cell deformability. This was corroborated by the results depicted in Figs. 2 and 3: both MC540 and protoporphyrin caused a substantial increase of the viscosity of a 70% cell suspension and an increase of filtration pressure during filtration of a 2% cell suspension through a 5 μm polycarbonate filter. Both phenomena directly reflect decreased deformability of the cells, as pointed out by Miller et al. [16].

As described in the results section both MC540 and protoporphyrin induced a significant increase of the MCV in isotonic medium, indicating uptake of water and solutes from the medium. This was confirmed by the observation that isotonic swelling in PBS was attended with an instantaneous increase of the intracellular Na^+ concentration of about 11 mequiv/l packed cells, whereas in LiCl Li^+ was taken up to an intracellular concentration of about 12 mequiv/l. This cation uptake correlates well with the observed increase of the cellular volume. On continued incubation in the presence of MC540, no further uptake of Na^+ or Li^+ was observed, suggesting that the permeability had returned to control levels. This was confirmed by the Rb^+ and lactose influx experiments: a fast uptake occurred when these solutes were added before MC540, but not when added 15 s later than the sensitizer. In similar experiments with raffinose it appeared that this trisaccharide was not taken up by the cells during exposure to the sensitizer. Thus the isotonic swelling should be ascribed to an immediate but transient increase of the permeability of the membrane for small ions and molecules, subsequent to the addition of the sensitizer. With protoporphyrin identical results were obtained, except for a slightly increased passive permeability for K^+ and Rb^+ during prolonged incubation in the presence of the sensitizer.

In further experiments it was investigated whether the immediate, transient increase of the permeability of the membrane for small ions and molecules occurred via one of the well-characterized transport or leak pathways of the erythrocyte membrane. Several pathways have been described, including the (Na^+/K^+)-ATPase system, sensitive to ouabain, the K^+/Cl^- cotransport pathway, inhibited by furosemide [22] and by substituting nitrate for chloride [26], the $Na^+/K^+/Cl^-$ cotransport system, blocked by bumetanide [27], the EGTA-sensitive Gardos channel [28], the so-called residual pathway, stimulated by replacement of extracellular NaCl by sucrose [29] and the mechanical stress-induced cation flux pathway, inhibited by DIDS [20]. As isotonic swelling was not affected by ouabain, furosemide, bumetanide, EGTA, DIDS or replacement of Na^+ by either nitrate or sucrose, a transient activation of any of these pathways was not involved in MC540- and protoporphyrin-induced water and solute uptake. More likely the temporary increase of membrane permeability to small ions and molecules is caused by membrane destabilization, due to the preferential insertion of molecules in one of the leaflets of the membrane bilayer. Presumably, this intercalation process is followed by some kind of rearrangement to correct the molecular imbalance in the membrane and to restore its barrier function. Apparently, such a rearrangement does not lead to a major loss of the phospholipid asymmetry in the membrane. In echinocytes, induced by elevation of the intracellular Ca^{2+} concentration, we found a substantial increase of phosphatidylserine in the outer leaflet of the membrane, as described before by Lin et al. [23]. In the presence of sensitizer alone, however, no redistribution of phosphatidylserine was observed, indicating that the Ca^{2+}-induced redistribution is not caused by the echinocytosis per se.

When red blood cells are subjected to mechanical stress, for instance by exposing them to a shear stress in a viscometer, K^+ leakage is increased [17,20]. As shown in the Results section, the sensitizer-induced volume increase and shape change was not accompanied by an increased K^+ leakage upon mechanical stress.

Protection of red blood cells against osmotic hemolysis by insertion of various molecules into the lipid bilayer has been described before, but the explanation of this phenomenon is still a matter of dispute with two different opposing ideas: intercalation of amphiphiles in the membrane may either cause an increased membrane permeability for cations [30,31], or may increase the critical hemolytic volume [32]. Increased passive permeability to cations in hypotonic medium would lead to an efflux of ions, decreasing the difference in osmotic pressure between cell interior and buffer, thus reducing the cell volume and protecting against osmotic lysis. Indeed, several membrane-intercalating agents actually cause an increased permeability of the membrane for cations and with these agents this mechanism could explain the observed protection [30,31]. However, several other membrane-intercalating agents, also protecting against osmotic hemolysis, do not cause increased cation permeability [33,34]. This is also the case with MC540 and protoporphyrin: notably in hypotonic media both sensitizers slightly inhibited, rather

than augmented, passive K^+ permeability. In accordance, cell swelling in hypotonic medium was not inhibited but, contrarily, slightly augmented (Fig. 6). In these cases an increase of the critical hemolytic volume could explain the protection against osmotic hemolysis. For MC540 and protoporphyrin this was confirmed by direct measurements of the critical volume, that increased from 1.78 V_0 in untreated control cells to 2.10 V_0 in the presence of sensitizer (Fig. 6). This increase can not be simply explained by the surface area of the intercalated sensitizer molecules. Under the present experimental conditions MC540 binding amounts to $9.3 \cdot 10^6$ molecules per cell, as shown by Allan et al. [12]. These authors further showed that this would cause a maximal membrane surface expansion of 2.7%. This would allow a volume increase of about 5%, much less than the observed increase of the critical hemolytic volume. Therefore, it seems likely that the intercalation of the sensitizer allows a more extensive stretching of the membrane, before it actually ruptures.

It seems likely that also other photosensitizers, potentially useful for photodynamic sterilization of transfusion products, have similar effects on the physicochemical properties of blood cells. Regarding the possible consequences of these effects on the rheology and survival of transfused blood cells, the phenomena described may have practical implications.

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References

[1] Gomperts, E.D. (1986) Am. J. Hematol. 23, 295–305.
[2] Mannucci, P.M. and Colombo, M. (1988) Lancet ii, 782–785.
[3] Melnick, J.L. and Wallis, C. (1982) in The Science of Photomedicine (Regan, J.D. and Parrish, J.A., eds.), pp. 545–559, Plenum Press, New York.
[4] Hanson, C.V. (1992) Blood Cells 18, 7–25.
[5] Rapp, F. and Li, J.L.H. (1982) in The Science of Photomedicine (Regen, J.D. and Parrish, J.A., eds.), pp. 571–594, Plenum Press, New York.
[6] Lytle, C.D., Carney, P.G., Felten, R.P., Bushar, H.F. and Straight, R.C. (1989) Photochem. Photobiol. 50, 367–371.
[7] O'Brien, J.M., Montgomery, R.R., Burns, W.H., Gaffney, D.K. and Sieber, F. (1990) J. Lab. Clin. Med. 116, 439–447.
[8] Horowitz, B., Williams, B., Rywkin, S., Prince, A.M., Pascual, D., Geacintov, N. and Valinsky, J. (1991) Transfusion 31, 102–108.
[9] Ben-Hur, E., Malik, Z., Dubbelman, T.M.A.R., Margaron, P., Ali, H. and Van Lier, J.E. (1993) Photochem. Photobiol. 58, 351–355.
[10] Ben-Hur, E., Rosenthal, I. and Granot, Y. (1993) Photochem. Photobiol. 57, 984–988.
[11] Daleke, D.L. and Huestis, W.H. (1989) J. Cell Biol. 108, 1375–1385.
[12] Allan, D., Hagelberg, C., Kallen, K.J. and Haest, C.W.M. (1989) Biochim. Biophys. Acta 986, 115–122.
[13] VanGastel, L.F.J., VanSteveninck, J. and DeBruijne, A.W. (1973) Biochem. Biophys. Res. Commun. 55, 1240–1245.
[14] Sheerin, H.E., Snyder, L.M. and Fairbanks, G. (1989) Biochim. Biophys. Acta 983, 65–76.
[15] DeBruijne, A.W. and VanSteveninck, J. (1974) Biochem. Pharmacol. 23, 3247–3258.
[16] Miller, L.H., Usami, S. and Chien, S. (1971) J. Clin. Invest. 50, 1451–1455.
[17] Sugihara, T., Rawicz, W., Evans, E.A. and Hebbel, R.P. (1991) Blood 77, 2757–2763.
[18] Comfurius, P., Bevers, E. and Zwaal, R.F.A. (1994) in Methods in Molecular Biology, Vol. 27 (Graham, J.M. and Higgins, J.A., eds.), pp. 131–142, Humana Press, Totowa.
[19] Van Steveninck, J., Gjosund, W.K. and Booij, H.L. (1967) Biochem. Pharmacol. 16, 837–841.
[20] Johnson, R.M. and Tang, K. (1993) Biochim. Biophys. Acta 1148, 7–14.
[21] Lepke, S., Fasold, H., Pring, M. and Passow, H. (1976) J. Membr. Biol. 29, 147–177.
[22] Garay, R.P., Nazaret, C., Hannaert, P.A. and Cragoe, E.J. (1988) Mol. Pharmacol. 33, 696–701.
[23] Lin, S., Yang, E. and Huestis, W.H. (1994) Biochemistry 33, 7337–7344.
[24] Deuticke, B. (1968) Biochim. Biophys. Acta 16, 494–500.
[25] Sheetz, M.P. and Singer, S.J. (1974) Proc. Natl. Acad. Sci. USA 71, 4457–4461.
[26] Olivieri, O., Bonollo, M., Friso, S., Girelli, D., Corrocher, R. and Vettore, L. (1993) Biochim. Biophys. Acta 1176, 37–42.
[27] Chipperfield, A.R. (1986) Clin. Sci. 71, 465–476.
[28] Ihrig, I., Hessel, E., Seidler, G., Erdmann, A. and Bernhardt, I. (1991) Biochim. Biophys. Acta 1069, 171–174.
[29] Bernhardt, I., Hall, A.C. and Ellory, J.C. (1988) Studia Biophys. 126, 5–21.
[30] Isomaa, B., Hägerstrand, H., Paatero, G. and Engblom, A.C. (1986) Biochim. Biophys. Acta 860, 510–524.
[31] Hägerstrand, H. and Isomaa, B. (1991) Chem.-Biol. Interact. 79, 335–347.
[32] Seeman, P. (1972) Pharmacol. Rev. 24, 583–655.
[33] Johnson, R.M. (1994) Biophys. J. 67, 1976–1881.
[34] Jennings, M.L. and Schulz, R.K. (1990) Am. J. Physiol. 259, C960–C967.