Review
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THE AGE-DEPENDENCE OF THE RED BLOOD CELL WATER PERMEABILITY AND ITS PHYSIOLOGICAL RELEVANCE

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Abstract

Developments in the understanding of the molecular basis of water permeability of the red blood cell (RBC) have taken place rapidly since the discovery in 1985 in Cluj-Napoca, Romania, by the group of Benga of a water channel protein (WCP), later called aquaporin 1 (AQP1), in the RBC membrane. However, the physiological role of AQP1 is not yet fully understood. Investigations of RBCs from human subjects of various ages could help shed light on this important issue. We present a short review of our studies on this topic that were published in less "visible" journals and books.

The diffusional water permeability (P_d) of the RBC membrane has the lowest values in the newborns. Then P_d values are increasing in children, reaching at about 7 years a value that remains rather constant in young and mature subjects. The high permeability to water of the RBC membrane can be correlated at these ages with the ability to undertake a high level of physical activity. In elderly individuals (over 65 years) a further small, but statistically significant, increase in the diffusional water permeability of RBC was observed. In this case the higher RBC water permeability can be correlated with a requirement of the RBC membrane to favour the membrane undulations and the rapid entry or exit of solutes of molecular size greater than water, in conditions when the organism is less physically active, probably has lower metabolic rates and lower mean rates of blood circulation.

Key words: water channel proteins, aquaporins, red blood cell membrane, nuclear magnetic resonance, newborn, children, elderly people.

INTRODUCTION

In the last decades rapid advances in the understanding of the mechanisms of water permeability

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of biological membranes have taken place, since the discovery of water channel proteins (WCPs) (reviewed in refs. 1, 2). These are transmembrane proteins that have a specific three-dimensional structure with a pore that can be permeated by water molecules (2). The first WCP was discovered in the red blood cell (RBC) membrane by Benga and coworkers in 1985 in Cluj-Napoca, Romania, reported in publications in 1986 (3, 4) and reviewed in subsequent years (5-13). This protein was later called aquaporin 1 (AQP1). This discovery was the starting point of a new field of biomedical and natural sciences dedicated to the integrated approach of WCPs; the field, which is also a chapter of Cellular and Molecular Biology has been called aquaporinology (14).

Despite the important advances in understanding the structural determinants of water permeation through WCPs, particularly through AQP1, (reviewed in refs. 2, 13) their physiological role in the RBC membrane is not fully understood (discussed in refs. 15-17). Investigations of the water permeability of RBCs from organisms of various species or of RBCs from human subjects of different ages could help shed light on the physiological significance of this transport process. It was concluded that RBC water permeability is a species characteristic (reviewed in refs. 18-20). On the other hand it was found that RBC water permeability is also age-dependent (21-26); the aim of this article is to present here a short review of our studies on this latter topic, studies that were published in less "visible" journals and books.

SUBJECTS AND METHODS

In our studies (21-26) the characteristics of the RBC water permeability were determined in human subjects of all ages, from newborns to children, young, adult subjects and elderly people.

The RBC membrane permeability for water diffusion (P_d) was measured by a methodology involving morphological measurements of the cell volume and cell surface area, and of the water diffusion exchange time by the Mn^{2+}-doping ^1H NMR method (27), as previously evaluated (28). The calculation of the activation energy ($E_{a,d}$) of water diffusion and other details of measurements of water permeability were previously described (29-35).

Data were expressed as mean ± standard deviation (SD). Differences between groups were analysed by Student's t test, using the Microsoft Office Excel software (Microsoft Corporation, Redmond, USA); p values ≤ 0.05 were considered statistically significant.

RESULTS

Figure 1 presents the variation of P_d values for the RBCs from subjects of various ages. It is obvious that in the newborns the RBC water permeability has the lowest values. Then P_d values are increasing in children, reaching at about 7 years a value that remains rather constant in young and mature subjects. In elderly individuals (over 65 years) a further small, but statistically significant, increase in the diffusional

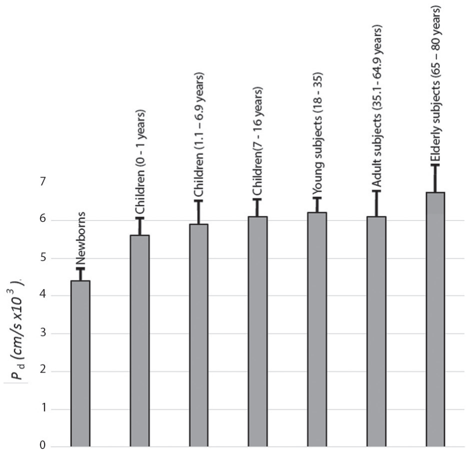

Figure 1. The variation of P_d values for the RBCs from human subjects of various ages. The methodology for measuring the P_d is presented in refs. 28-35. The values (at 37 degrees C) are taken or calculated from our previous publications (refs. 21-26).

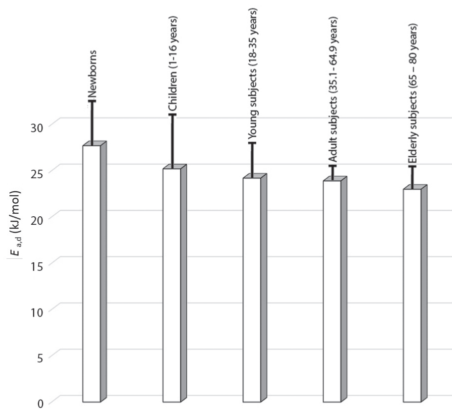

Figure 2. The variation of $E_{a,d}$ values for the RBCs from human subjects of various ages. The methodology for measuring the $E_{a,d}$ is presented in refs. 28-35. The values are calculated from our previous publications (refs. 21-26).

water permeability of RBC was observed. Although the differences in P_d values between the two groups are rather small, these differences are statistically significant: p<0.01 at 15 °C; p<0.04 at 20 °C and 25 °C; p<0.004 at 37 °C and p<0.02 at 42 °C, by the Student's t test. This means that RBCs from elderly people have a higher water diffusional permeability.

The $E_{a,d}$ values of water diffusion in the RBCs from individuals of various ages are presented in Fig. 2. It appears that $E_{a,d}$ values are decreasing from the newborns to children, young and mature subjects. A further decrease occurs in elderly people, however, this is not statistically significant (p>0.05).

DISCUSSION

This is the first analysis of all our comparative studies of water permeability of RBCs from human subjects of various ages, from the newborns to elderly people. It is obvious that RBCs have the lowest water permeability in the newborns, then it is increasing in children, reaching at about 7 years a value that remains rather constant in young and mature subjects. RBCs from elderly people have then a slightly higher water diffusional permeability, as documented by the statistically significant higher values of P_d.

Other parameter of interest is the value of $E_{a,d}$. Schafer and Andreoli (36) described two components of the activation energy for water diffusion across a membrane: one is the energy required for a water molecule to break the hydrogen bonds formed with neighboring molecules, while the other is the activation energy for water diffusion across the membrane. A low value of $E_{a,d}$ for a molecule suggests that the diffusion of the molecule is specialized with respect to the hydrophobic lipid bilayer, i.e. water channel proteins are involved. A higher water permeability of the RBC membrane could be due to a greater number of channels; hence in parallel with a higher P_d value one would expect a lower value of $E_{a,d}$. This means that in the RBC membrane of the newborns are a low number of WCPs; their number increases with age, first rapidly in childhood, reaching at about 7 years a value that remains rather constant in young and mature subjects. A further small increase is probably occurring in elderly people, or, alternatively, the water passage through the channel of WCPs might be regulated differently in the RBC membrane of elderly people.

Benga and Kuchel have previously (15-17) provided possible explanations for the fast exchange of water across the RBC membrane (compared to other cellular membranes), and, consequently, for the physiological role of WCPs in the RBC. The first explanation applies to the whole-body system: the organisms that are more physically active have higher metabolic rates or higher mean rates of circulation of their blood and also have higher RBC water permeability (18-20). It is known that physical activity is related to the development of the whole human body and particularly the muscle system. This is achieved when a human subject is developing from a small child to an adult. This might explain the increase of the RBC water permeability during these age periods of human life.

Other two explanations for the high water permeability of the RBCs are related to molecular- and cellular-scale events. One of this, denoted as the "oscillating sieve" hypothesis states that known membrane undulations of the RBC membrane (37) are energetically favoured by the high water permeability of the membrane. The other explanation, denoted as the "displacement" hypothesis, is based on the known rapid exchange across the RBC membrane of ions (such as Cl^- and $HCO3^-$) and solutes (such as glucose), all of whose molecular volumes are significantly greater than that of water. The RBC membrane has been "naturally selected" to be very permeable to water so that the rapid entry and exit of solutes of molecular size greater than water allows concomitant displacement of water thus obviating a change in cell volume which would affect the concentration of reactants and hence the rates of enzymic and binding reactions, and cell flexibility (18-20).

In conclusion, the diffusional water permeability (P_d) of the RBC membrane has the lowest values in the newborns. Then P_d values are increasing in children, reaching at about 7 years a value that remains rather constant in young and mature subjects. The high permeability to water of the RBC membrane can be correlated at these ages with the ability to undertake a high level of physical activity. In elderly individuals (over 65 years) a further small, but statistically significant, increase in the diffusional water permeability of RBC was observed. In this case the higher RBC water permeability can be correlated with a requirement of the RBC membrane to favour the membrane undulations and the rapid entry or exit of solutes of molecular size greater than water, in conditions when the organism is less physically active, probably has lower metabolic rates and lower mean rates of blood circulation. Further detailed studies could be very useful to elucidate these aspects.

Conflict of interest

We declare that there is no conflict of interest.

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References

1. Benga Gh. Birth of water channel proteins – the aquaporins. Cell Biol Int 2003; 27(9):701-709.
2. Benga Gh. Water channel proteins (later called aquaporins) and relatives: past, present and future. IUBMB Life 2009; 61(2): 112-133.
3. Benga Gh, Popescu O, Pop VI, Holmes RP. p-Chloromercuribenzene sulfonate binding by membranes proteins and the inhibition of water transport in human erythrocytes. Biochemistry 1986; 25(7): 1535-1538.
4. Benga Gh, Popescu O, Pop VI, Mureşan A, Mocsy I, Brain A, Wrigglesworth J. Water permeability of human erythrocytes. Identification of membrane proteins involved in water transport. Eur J Cell Biol 1986; 41: 252-262.
5. Benga Gh. Water transport in human red blood cells. Prog Biophys Mol Biol 1988; 51(3): 193-245.
6. Benga Gh. Permeability through pores and holes. Curr Opinion Cell Biol 1989; 1: 771-774.
7. Benga Gh. Water exchange through the erythrocyte membrane. Int Rev Cytol 1989; 114: 273-316.
8. Benga Gh. Membrane proteins involved in the water permeability of human erythrocytes: binding of p-chloromercuribenzene sulfonate to membrane proteins correlated with nuclear magnetic resonance measurements. In Benga Gh, ed. Water Transport in Biological Membranes. CRC Press, Boca Raton, 1989: 41-61.
9. Benga Gh. Water channels in membranes. Cell Biol Int 1994; 18(8): 829-833.
10. Benga Gh. The first water channel protein (later called aquaporin 1) was first discovered in Cluj-Napoca, Romania. Romanian J Physiol 2004; 41(1-2): 3-20.
11. Benga Gh. Water channel proteins: from their discovery in 1985 in Cluj-Napoca, Romania, to the 2003 Nobel Prize in Chemistry. Cell Mol Biol 2006; 52(7): 25-31.
12. Benga Gh. Water channel proteins (aquaporins and relatives): twenty years after their discovery in Cluj-Napoca, Romania. Acta Endocrinol (Buc) 2006; 2(3): 323-336.
13. Benga Gh. The first discovered water channel protein, later called aquaporin 1: molecular characteristics, functions and medical implications. Mol Asp Med 2012; 33(5-6): 518-534.
14. Benga Gh. Aquaporinology. Acta Endocrinol (Buc) 2014; 10 (1): 1-8.
15. Benga Gh, Kuchel PW. Physiological significance of water channel proteins in the red blood cell membranes: analysis at the level of the cell and of the whole-body systems. The 9th World Multi-Conference on Systemics, Cybernetics and Informatics, Orlando, Fl. 2005; X: 105-110.
16. Kuchel PW, Benga Gh. Why is the transmembrane exchange of water in the red blood cell so fast? Bull Mol Med 2003; 15-16: 29-34.
17. Kuchel PW, Benga Gh. Why does the mammalian red blood cell have aquaporins? BiosSystems 2005; 82(2): 189-196.
18. Benga Gh, Borza T. Diffusional water permeability of mammalian red blood cells, Comp Biochem Physiol 1995; 112B: 653-659.
19. Benga Gh. Diffusional water permeability of red bloods cells from various vertebrate species. Bull Mol Med 2001; 7-8: 27-42.
20. Benga Gh. Comparative studies of water permeability of red blood cells from humans and over 30 animal species: an overview of 20 years of collaboration with Philip Kuchel. Eur Biophys J 2013; 42(1): 33-46.
21. Benga Gh, Morariu VV. Membrane defect affecting water permeability in human epilepsy. Nature 1977; 265(5595): 636-638.
22. Lupea I, Morariu C, Morariu VV, Ordeanu R. Permeabilitatea la apă a eritrocitelor nou nascutului. Pediatria 1978; 27(3): 261-263.
23. Morariu VV, Benga I, Benga Gh. Studii de

rezonanţă magnetică nucleară (RMN) asupra difuziunii apei prin membranele eritrocitare la copiii epileptici. Bul Acad Şt Med 1980; 2: 32-37.
24. Benga Gh, Frenţescu L, Matei H, Ţigan S. Comparative nuclear magnetic resonance studies of water permeability of red blood cells from maternal venous and newborn umbilical cord blood. Clin Chem Lab Med 2001; 39(7): 606-611
25. Benga Gh, Mironescu E, Bâlici S, Ţehaniuc A, Nicula GZ. Water permeability of red blood cells in elderly people. In: Schneider FA, Podea DM, Nanu PD, eds. Gerontology Today. Bucharest: Editura Viaţa Medicală Românească 2007; 31-37.
26. Ţehaniuc A, Benga Gh. Red blood cell water permeability in elderly people. Acta Endocrinol (Buc) 2011; 7 (3): 299-310.
27. Conlon T, Outhred R. Water diffusion permeability of erythrocytes using an NMR technique. Biochim Biophys Acta 1972; 288(2):354–61.
28. Morariu VV, Benga Gh. Evaluation of a nuclear magnetic resonance technique for the study of water exchange through erythrocyte membranes in normal and pathological subjects. Biochim Biophys Acta 1977; 469(3):301–10.
29. Morariu VV, Pop VI, Popescu O, Benga Gh. Effects of temperature and pH on the water exchange through erythrocyte membranes: Nuclear magnetic resonance studies. J Membrane Biol 1981; 62(1-2): 1-5.
30. Morariu VV, Benga Gh. Water diffusion through erythrocyte membranes in normal and pathological subjects: nuclear magnetic resonance investigations. In: Benga Gh, Baum H, Kummerow FA, eds. Membrane Processes: Molecular Biology and Medical Applications. Springer Verlag, New York, 1984: 121-139.
31. Benga Gh, Popescu O, Pop VI. Water exchange through erythrocyte membranes: p-chloromercuribenzene sulfonate inhibition of water diffusion in ghosts studied by a nuclear magnetic resonance technique. Bioscience Rep 1985; 5(3): 223-228.
32. Benga Gh, Borza V, Popescu O, Pop VI, Mureşan A. Water exchange through erythrocyte membranes: nuclear magnetic resonance studies on resealed ghosts compared to human erythrocytes. J Membrane Biol 1986; 89(2): 127-130.
33. Benga Gh, Pop VI, Popescu O, Hodârnău A, Borza V, Presecan E. Effects of temperature on water diffusion in human erythrocyte and ghosts - nuclear magnetic resonance studies. Biochim Biophys Acta 1987; 905(2): 339-348.
34. Benga Gh, Pop VI, Popescu O, Borza V. On measuring the diffusional water permeability of human red blood cells and ghosts by nuclear magnetic resonance. J Biochem Biophys Methods 1990; 21(2): 87-102.
35. Benga Gh, Pop VI, Popescu O, Borza V. The basal permeability to water of human red blood cells evaluated by a nuclear magnetic resonance technique. Bioscience Reports 1990; 10(1): 31-36.
36. Schafer JA, Andreoli TE. Principles of water and nonelectrolyte transport across membranes. In: Andreoli TE, Hoffman JF, Farnestil DD, eds. Membrane physiology. New York: Plenum, 1980: 177–190.
37. Morariu VV, Chis AM, Znamirovschi V. Fluctuations in red cell membranes. Cytobios 1996; 86: 53-64.