RED CELL DEFORMABILITY: PHYSIOLOGICAL, CLINICAL AND PHARMACOLOGICAL ASPECTS

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SYNOPSIS

Twenty five thousand billions of red cells circulate through 300 miles of capillary network. The red cell diameter is 7 to 8 u and the capillary diameter 3 to 5 micron. Red cell deformability is therefore an essential condition for survival. It is impaired in a certain number of clinical conditions. A French task force, covering 40 university hospitals, is presently investigating the clinical implications of red cell filterability, and its possible therapeutical consequences.

INTRODUCTION

Red cell deformability has been described for the first time in the end of the VIIth century by Van Leeuwenhoek (15): "I saw that the blood in the thinnest arteries sometimes stopped; and when in the same artery the blood was pushed on again, I saw that then several corpuscules of blood became twice as long as they were broad, and that the two ends seemed to be pointed." In 1922 Krogh (5) presented a film at Yale University describing the deformation of red blood cells passing through capillaries. Since then these morphological aspects have been illustrated in much detail by the scanning electronic micrographs of Bessis and the films of Branemark.

The fundamental point remains that cells with a 7 to 8 u diameter have to squeeze through 5 or 3 micron wide capillaries so that oxygen can get to the tissues. Gregersen (3) has pointed out that in some animal species deformability can be much lower than in man.

In these species however capillaries are wider. This human peculiarity, which allows us to have a very dense network of capillaries, also makes us dependant upon red cell deformability, which rests on certain biochemical qualities of the red cell.

PHYSIOLOGICAL ASPECTS OF DEFORMABILITY

Deformability can be measured directly by only one method: the micropipette, first used in this aim by Rand and Burton (10). Applying a specific aspiration pressure to a red cell will allow it to be more or less aspired through a micropipette of known diameter. Deformability can thus not only be measured, but also watched through the microscope. Viscosimetric techniques, correlating viscosity of a suspension of red cells with various shear rates lead to very precise measurements of parameters involved in deformability. At low shear stress red cell aggregation interferes with the measures. At high shear

stress deformability is predominant. Filtration through Nuclepore filters has a great advantage to other techniques: it's simplicity. The method can be performed in any non-specialized hematology laboratory, whereas micropipetting and viscosimetry need highly sophisticated environment. Clinical applications of this method have been set up by Reid and Dormandy (11, 12). The biophysics of filtration follow Poiseuille's law: flow through a single pore depends on its length, radius, pressure gradient and on the viscosity of the fluid concerned. This viscosity largely depends on red cell deformability, but also is influenced by hematocrit and fibrinogen. Resuspension of red cells at constant haematocrit in a defined isotonic fluid eliminates these parameters.

The intrinsic deformability of the cell is determined by three major factors according to Chien (2): the viscoelastic properties of the membrane, the viscosity of the intracellular hemoglobin rich fluid (internal fluid viscosity) and the geometry of the cell. As has been shown by Skalak (13), one of the most striking features of red cell deformability is that it takes place at constant volume (around 92 u^3) and constant area (around 140 u^2). The biochemical properties of the membrane are responsible for its mechanical qualities. They have been reviewed recently by Palek (9) and by Lux (7). The red cell membrane is undercoated by a heterogenic protein known as Spectrin. This protein consists of two major polypeptides of molecular weights 240,000 and 215,000, which are very much similar to muscle myosin. The membrane also contains Actin, which has the same molecular weight as muscle Actin. The Spectrin-Actin meshwork plays a fundamental part in red cell deformability. However this part is dependant on red cell concentrations of ATP and Ca++. Weed and La Celle (16) have shown that ATP depletion and calcium accumulation induce a marked decrease in deformability. It has also been shown that phosphorylation by ATP of Spectrin is essential for its ability to react with Actin. This phosphorylation implicates a kinase which seems to be cAMP independant. Another red cell kinase is cAMP dependant, but it's role is not clear yet. The red cell 2-3 DPG is also implicated in two ways. First, in physiological concentrations, it inhibits Spectrin dephosphorylation. Second, at the same concentrations, it has a higher affinity for reduced hemoglobin than ATP, preventing "chelation" by deoxygenated hemoglobin of ATP.

Therefore red cell deformability seems largely dependant on Spectrin-Actin interaction, which in turn is dependant on ATP, Ca++, cAMP and 2-3 DPG. It must be remembered that the red cell is a very poor producer of ATP, having no mitochondria.

CLINICAL ASPECTS OF DEFORMABILITY

Since early 1977 a French task force, now comprising over 40 university groups, has been working on red cell filterability as a method of measure of red cell deformability.

MATERIAL AND METHOD

The filtration method applied has been described by Reid and Barnes (11). All the groups within the task force have been equipped with identical material including tubing and glassware, so that to ensure that results should be as homogeneous as possible. A very large batch of 5 micron pore filters was specially manufactured by Nuclepore for the same reasons. Measures were always performed at least in triplicate, less than two hours after sampling, at room temperature.

Patients and normals were those provided by the usual activities of the task force groups.

RESULTS

Filtration has been studied (6) as a cardiovascular risk factor. We have, in 72 patients, correlated red cell filterability (using two batches of filters) expressed in ul/s, with the number of cardiovascular risk factors present (high blood pressure, overweight, diabetes, hyperuricemia, hyperlipemia, smoking). Statistical analysis has been performed using regression analysis for each batch of filters, and both significance levels have been combined using Fisher's method. Fig. 1 illustrates the relationship between the number of risk factors and filterability. There is a statistical difference between groups as a whole ($p < 0.01$). The difference is highly significant between 0 and 4 risk factors ($p < 0.0005$). Filterability decrease is also directly correlated with the number of cigarettes smoked per day ($p < 0.05$). This is significant in men ($p < 0.05$), but not in women.

In diabetic patients (4) filterability is significantly decreased ($p < 0.001$). The group has shown, using artificial pancreas, that filterability is directly correlated with blood glucose levels in vivo. However, red cells incubated in vitro with various concentrations of glucose

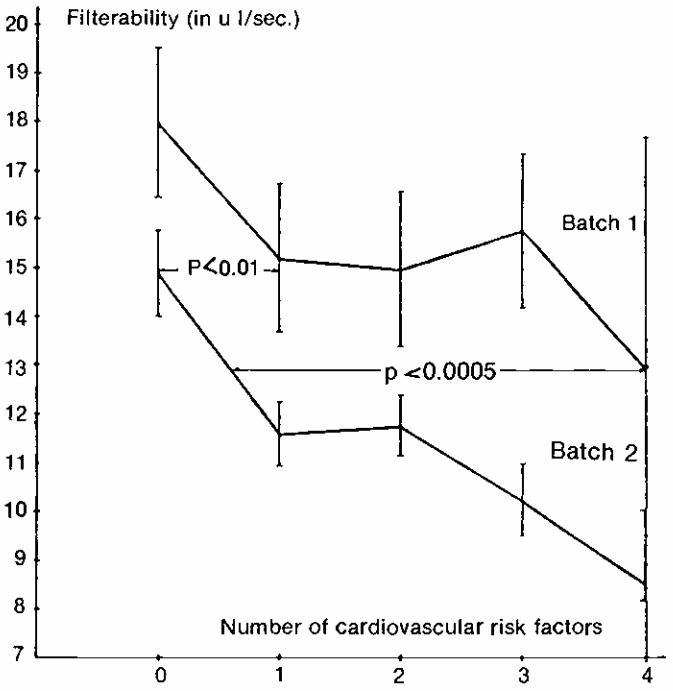

Fig. 1. Relationship between number of cardiovascular risk factors and filterability.

In 72 patients with cardiovascular risk factors (smoking, high blood pressure, overweight, diabetes, hyperuricemia, hyperlipemia), a correlation exists between reduction of blood filterability and the number of risk factors.

do not alter their filterability. Decrease seems therefore linked to diabetes and not to glucose per se. No short term corrlation was found between filterability and haemoglobin A1c, considered as a sign of diabetic equilibrium. Long term studies are under way.

In pregnancy filterability is reduced. This is also found in women taking Oestro-progestative oral contraception.

In cerebro-vascular impairment (1) filterability is reduced. In a series of 200 normal subjects filterability is 33 + 6 sec./ml. whereas in 80 patients (Fig. 2) it is 57 + 28 sec./ml ($p < 0.001$). What is more, filterability seems to have a pronostic value: mortality is much higher in patients with very disturbed values.

Filterability is also disturbed in various other conditions: haemodialysis, severe burns, certain cases of Raynaud phenomenon.

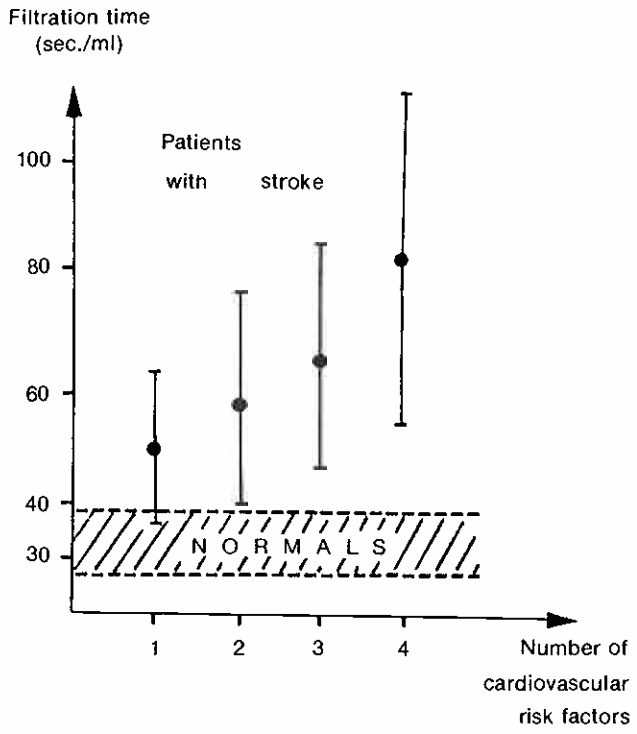

Fig. 2. Filtration time in patients with stroke is higher than in normals. It is also correlated with the number of cardiovascular risk factors.

PHARMACOLOGICAL ASPECTS

Red cell deformability being linked to red cell ATP, cAMP and 2-3 DPG concentrations, it is tempting to try and explain in this way the mode of action of pentoxifyllin, a drug increasing red cell deformability. It has been shown by Stefanovitch (14) that pentoxifyllin increases red cell ATP. It is also known that the drug increases cAMP. Studies are under way concerning it's action on 2-3 DPG.

In patients having suffered from stroke red cell filterability has been measured for three weeks. In this way it has been shown (8) that filterability, highly reduced on day 1, will return rapidly to more normal values in patients receiving Pentoxifyllin. This is not the case in patients receiving vasodilators. The difference is highly significant (Fig. 3).

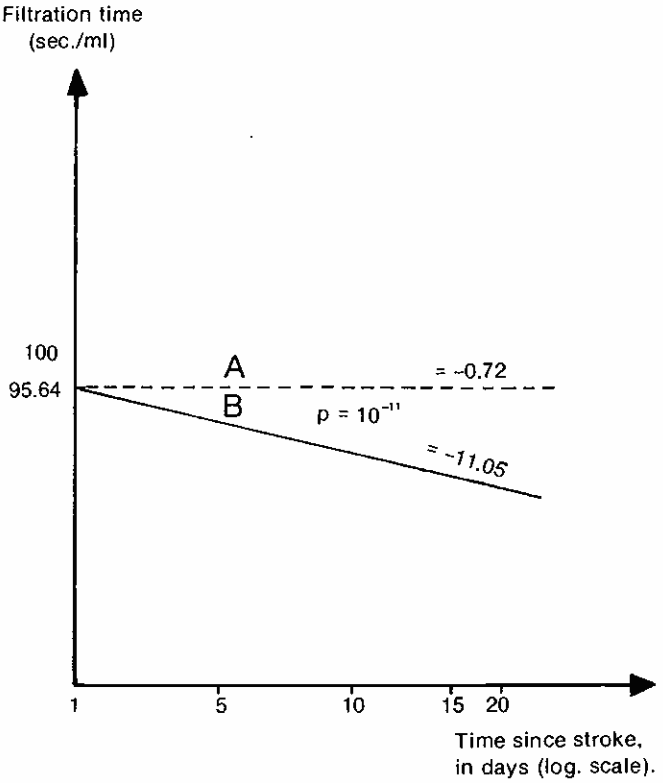

Fig. 3. Filtration time progressively decreases in stroke patients treated with pentoxifyllin (B). This is not the case in patients receiving vasodilator therapy (A). The difference in slope between both groups is highly significant.

CONCLUSION

Red cell deformability could very well be an essential feature of circulation. Pharmacological attempts to enhance circulation by means of vasodilators, anticoagulants, inhibitors of platelet aggregation, have not yet brought all the therapeutical answers. It is hoped that the concept of red cell deformability, with the pharmacological consequences it has, will add one more milestone to the therapeutical possibilities in vascular disease and circulatory impairment.

REFERENCES

1. Boisseau M.R., Lorient, M.F., Bricaud H: Red cell deformability and risk factors in one hundred patients with cerebrovascular thrombosis. VIIth Int. Congr. Thromb. Haemost. London 1979 in Thrombos. Haemost. 42: 107, 1979.
2. Chien S., Principles and techniques for assessing erythrocyte deformability. Blood Cells. 3: 71-99, 1977.
3. Gregersen M.I., Bryant C.A., Chien S., Dellenback R.J., Magazinovic V. Usami S.: Species differences in the flexibility and deformation of erythrocytes. in 5th Europ. Conf. Microcirculation, Gothenburg 1968. Bibl. Anat. N° 10 pages 104-108. Karger, Basel — New-York; 1969.
4. Juhan I: Deformabilite des hematies chez les diabetiques. La Nouvelle Presse Medicale. 7: 759, 1978.
5. Krogh A.: The anatomy and physiology of capillaries. Yale University Press, New Haven, Connecticut. 1929.
6. Lagrue G., Marcel G.A., Faucher G., Grumel J.M., Beiler D. Branellec A. Red cell filterability and cardiovascular risk factors. VIIth Int. Congr. Thromb. Haemost. London 1979 in Thrombos. Haemost. 42: 106, 1979.

7. Lux S.E.: Spectrin — Actin membrane skeleton of normal and abnormal red blood cells. Seminars in Hematology. 16 (1) : 21-51, 1979.
8. Martin P. Vives P. Deformabilite des globules rouges et accidents vasculaires cerebraux. Interet de la Pentoxifylline. Gazette Medicale de France. 86 (23) : 2585-2587, 1979.
9. Palek J., Liu S.C.: Dependance of spectrin organization in red blood cell membranes on cell metabolism: implications for control of red cell shape, deformability and surface area. Seminars in Hematology: 16 (1) : 75-93, 1979.
10. Rand R.P., Burton A.C.: Mechanical properties of the red cell membrane. Membrane stiffness and intracellular pressure. Biophys. Journ. 4: 155, 1964.
11. Reid H.L., Barnes A.J., Lock P.J., Dormandy J.A., Dormandy T.L.: A simple method for measuring erythrocyte deformability. J. Clin. Pharmacol. 29 : 855-858, 1976.
12. Reid H.L., Dormandy J.A., Barnes A.J., Lock P.J. Dormandy T.L.: Impaired red cell deformability in peripheral vascular disease. Lancet. 1: 666-668, 1976.
13. Skalak R. Tozeren A., Zarda R.P. Chien S.: Strain energy function of red blood cell membranes. Biophys J. 1 3: 245-264, 197 3.
14. Stefanovich V: Effect of Pentoxifyllin on erythrocyte adenine nucleotide levels in rats. IRCS Med. Sci. 3 : 91, 1975.
15. Van Leeuwenhoek A: 65th Missive in Mary P. Wiedeman — Microcirculation — Benchmark papers in human physiology, page 35; Dowden, Hutchinson and Ross Inc.; Stroudsburg, Pennsylvania. 1974.
16. Weed R.R., La Celle P.L.: Merrill E.W.: Metabolic dependance of red cell deformability. J. Clin. Invest. 48: 795-809, 1969.