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MINI REVIEW ON ERYTHROCYTE AGGREGATION

Basic Concepts and Clinical Repercussions

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1. What is the purpose of the present mini review?

The purpose of this mini review is to provide the meaning of erythrocyte aggregation.
Its basic concepts as well as known clinical repercussions and possible therapeutic aids are also briefly described.

2. What do you know about erythrocytes?

Erythrocytes are non-nucleated red cells that behaves like a fluid droplet in flow and not like a solide body: it is fluid, it has the ability to flow.

Biconcave resting shape of erythrocytes with a surface area disproportionately large for its given volume

The red cell membrane rotates around its liquid contents in responses to the shear stress generated in a flowing

medium. By this “tank tread” motion the shear stress acting on the outer cell membrane is transmitted to the liquid citoplasm. As a consequence intra-cellular cytoplasm flow favouring oxygen uptake and release in the pulmonary and peripheral microcirculation respectively.

Erythrocyte submitted to flow undergo rotation

At higher shear stress erythrocytes respond by elongating itself and at cappilaries the red cells adopt a slipper shape.

Erythrocyte ellipsoid shape at high shear stress

The adaptability of the erythrocytes to flow forces in vascular lumen is due to:

a) increase surface to volume ratio
b) fluidity of cell content
c) flexibility of its membrane which is a phospholipid bilayer containing immersed proteins, both in interaction with the cytoskleton components.

Erythrocyte slippe shape at cappilaries bed

3. What means erythrocyte aggregation?

Erythrocyte aggregation (EA) is the erythrocyte tendency to form aggregates whose shape change according to normal or pathological conditions.

Besides the presence of the glycocalyx and its negative charge which repels erythrocytes against each other, the red blood cells aggregates. Why?

The plasma proteins like fibrinogen and α_2-macroglobuline link erythrocyte because of their own length. This is sufficient to overtake the negative electrostatic repulsive strenghts present at erythrocyte surface.

Forms of erythrocytes aggregates

linear (rouleaux) cylindrical of "pieces of money"

Reticular

Pathologic grume of double erythrocytes joint by α_2-macroglo-buline

Grume of lateral network joint by fibrinogen

Pathologic grume

4. What kind of factors influence erythrocyte aggregation?

Plasma ionic strength, pH, temperature, plasma viscosity, superficial ionic charges, age and shape of erythrocytes are influent **biochemical parameters**.
Plasma viscosity, hematocrit (Ht), fibrinogen, α_2-macroglobuline and erythrocyte deformability are influent **haemorheological factors.** For example at constant (Ht), EA increase with the increases of fibrinogen concentration.
Shear stress, shear rate, vessel diameter, blood velocity, vessel wall shear stress, Reynolds number, are influent **haemodynamic factors.**

5. Which is the haemorheological factor dependent from erythrocyte aggregation?

Fåhraeus-Lindqvist effect:

At vessel lumens of 10-20 μm blood viscosity reaches a minimum value, near to that of plasma.

At low shear rates observed at post-capillaries vennules or in venous system an increase in EA induce an increase in blood viscosity and consequently impaired blood velocity. In spite of this, at microcirculatory network the axial alignment of red blood cells decrease blood viscosity at normal physiological conditions.

6. How to quantify erythrocyte aggregation?

The presence of aggregates reduces the number of light-scattering surfaces in a blood sample: light transmission through an aggregate of cell suspensions is therefore

Erythrocyte aggregation could be, for example, quantified in

an aggregometer with a photoelectric device coupled to a cone-plate viscosimeter. The variation of intensity of transmited light obtained by a blood sample when submitted to a fixed shear rate at a fixed time is integrated by a computer and gives the EA tendency.

increased.

7. What are the normal values of erythrocyte aggregation?

The normal values of erythrocyte aggregation are not yet standardized because does not exist the same apparatus in all laboratories.
Each research center have their own values of reference; however, after blood centrifugation the younger erythrocytes have less tendency to aggregate than the older ones.

8. Which are the clinical situations associated with abnormal values of erythrocyte aggregation?

Irreversible erythrocyte aggregation occurs either at absent or lower than 50sec^{-1} shear stress.

For shear rate higher than 50 sec^{-1} the erythrocytes aggregates can be dispersed.
When the shear stress is absent at a distal point of stenosis, for example, the aggregates maintain. In consequence a change in flow behaviour occurs, with stasis, and is simultaneously complicated by local impairment of blood fluidity (inverse of

viscosity).

The disturbance of blood fluidity is especially pronounced in longer capillaries, where flow forces are much smaller than in short capillaries. A negative correlation has been observed between the partial oxygen pressure of tissue supply and erythrocyte aggregation tendency in patients with arteriopathy. Red blood cell aggregation appears to be both an aggravating factor and severity marker of the evolutive stage of low limb insufficiency.

The decreased wall shear stress reported in large artery of hypertensive patients may also be relevant to red blood cell aggregation when vascular changes occur.

Increased values of erythrocyte aggregation were reported in essential hypertension, edema, myocardial ischemia, thrombo-embolic states, retinal venous occlusion, and diabetes.

In chronic peripheral arterial atherosclerosis erythrocyte aggregates are built-up at the post-occlusive areas of the arteries.

In the advanced stages of chronic venous incompetence, erythrocyte aggregates can be found in the enlarged tortuous capillary loops.

9. Which are the therapeutic aids?

The aim of therapy to impair erythrocyte hyperaggregation also decreases blood viscosity.

Looking at **hemodilution** to

In 1163 the council of Tours pronounced that "The Church abhors blood-letting"

apply in the treatment of cerebral ischaemia, there is controversy about the oxygen carying capacity or the viscosity regulates blood flow as the ameliorator factor.

The isovolemic hemodilution decreases erythrocyte aggregation and consequently blood viscosity and increases venous return and local perfusion. Besides this advantages, the isovolemic hemodilution could develop immediate problems such as hypovolemic and hypercoagulability situations.

The **plasmapheresis** is another therapeutic approach to decrease erythrocyte aggregation by decreasing the amount of fibrinogen. The concentration of this protein could also be decreased applying **defibrinogenating enzymes**.

There are some **Ca^{2+} channel blockers** that decrease erythrocyte aggregation by an unknown mechanism.

10. Concluding remarks

Erythrocyte aggregation is a reversible physiological process of cell-cell and protein-cell interactions. Besides some basic concepts that are already known, there are some open questions, namely, the role of erythrocyte subpopulations and their different tendency to aggregate.

In terms of standardised methods it will be needed to develop them for application to therapeutic and clinical

"Red cell aggregation in the form of rouleaux is a generally reversible phenomenon".

"Arterial vessels and capillaries are high shear compartments in which red blood cell are dispersed".

studies. Higher erythrocyte aggregation are associated with vascular disease (arterial or venous).

Measurement of tissue oxygen pressure allows to quantify the effects of various treatments. However, longitudinal multicenter trials must be carried out in order to clarify the diagnostic importance of erythrocyte aggregation measurements.

11. Summary

Blood circulation through the vessels is influenced by constant erythrocyte aggregation-desaggregation process, among other mechanisms affecting blood flow behaviour and vessel wall integrity.

The red blood cell aggregation--desaggregation tendency depends on cell intrinsic properties (erythrocyte charge) and extrinsic factors associated with the suspension medium (pH, temperature and macromolecules).

Beyond this biochemical factors there are hemorheological (plasma, blood viscosity, fibrinogen and hematocrit) and hemodynamic (vessel diameter, shear stress and shear rate) parameters that also have influence on the erythrocyte aggregation-desag-gregation process.

The increase of EA induce increase of blood viscosity at constant hematocrit, fibrinogen concentration and plasma viscosity. Hypertension, diabetes, myocardial ischemic and chronic venous diseases are examples of clinical situations with erythrocyte hyperaggregation values.

Isovolemic hemodilution, plasmapheresis and Ca^{2+} channel blockers are therapeutic aids to decrease the erythrocyte aggregation tendency.

12. Further readings-comments

The following references can be find in the library of the Biochemistry Institute, Faculty of Medicine, Lisbon.

The references could be used to better understanding the underlying mechanisms of the biochemical, hemorheological and haemodynamic factors modulating erythrocyte aggregation.

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13. Lexicon

Reynolds number – Is equal to mean blood velocity divided by blood viscosity, multiplied by vessel diameter.

Rigidity – Stiffening, loss of fluidity. Red blood cells loose their fluidity and become rigid when exposed to acidic metabolic break-down products.

Shear rate – A measure of deformation per time unit or the velocity gradient generated between two fluid layers as they are displaced past each other. Syno-nyms: velocity gradient, shear velocity, shear gradient.

Shear stress – The tangential force which compels fluid layers to glide past each other; and hence to flow. By analogy with shear rate, the literature sometimes uses the term shear force.

Viscosity – Internal friction or "stickiness". A material property of fluids. A measure of the resistance of the fluid elements to gliding motion or flow.