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State of the art

Mechanical clearance of red blood cells by the human spleen: Potential therapeutic applications of a biomimetic RBC filtration method

Clairance mécanique des érythrocytes par la rate humaine : applications thérapeutiques potentielles de la filtration érythrocytaire sur tamis microsphérique

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Abstract

During their lifespan, circulating RBC are frequently checked for their deformability. This mechanical quality control operates essentially in the human spleen. RBC unable to squeeze though narrow splenic slits are retained and cleared from the blood circulation. Under physiological conditions this prevents microvessels from being clogged by senescent, rigid RBC. Retention of poorly deformable RBC is an important determinant of pathogenesis in malaria and may also impact the clinical benefit of transfusion. Modulating the splenic retention of RBC has already been proposed to support therapeutic approaches in these research fields. To this aim, the development of microplates for high throughput filtration of RBC through microsphere layers (microplate-based microsphiltration) has been undertaken. This review focuses on potential therapeutic applications provided by this technology in malaria chemotherapy and transfusion.

Keywords: Erythrocyte; Deformability; Spleen; Filtration; Microspheres; Malaria; Transfusion; Applications

Résumé

La déformabilité des érythrocytes fait l'objet d'un contrôle qualité régulier imposé par la rate humaine. Tout érythrocyte incapable de se déformer suffisamment pour franchir les étroits espaces inter-endothéliaux spléniques est mécaniquement retenu, puis éliminé de la circulation. En conditions physiologiques, cela prévient l'obstruction des microvaisseaux par des érythrocytes sénescents ou dont la biomécanique est altérée. La rétention des érythrocytes peu déformables est un important déterminant de la pathogenèse du paludisme et vraisemblablement du bénéfice clinique de la transfusion. Une modulation de la rétention mécanique splénique des érythrocytes a préalablement été proposée comme alternative thérapeutique dans ces deux champs de recherche. Dans ce but, le développement de microplaques pour la filtration érythrocytaire sur tamis microsphériques (microsphiltration) à haut débit a récemment été entrepris. Dans cette revue, sont présentées les applications thérapeutiques potentielles de cette technologie en chimiothérapie du paludisme et en transfusion.

Mots clés : Érythrocyte ; Déformabilité ; Rate ; Filtration ; Microsphères ; Paludisme ; Transfusion ; Applications thérapeutiques

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1. Pathophysiological framework: major parameters of RBC deformability

Red blood cells (RBC) are 100 to 1000 times more numerous than white cells in the bloodstream and account for almost half of the blood volume. They display elastic properties and their deformability is a key factor of low blood viscosity and resistance to flow. Being deformable enables RBC to navigate along capillaries narrower than their own diameter [1,2]. RBC deformability depends on 3 parameters: internal viscosity, membrane and cytoskeleton biomechanics, and cell geometry [3]. Important morphological features of RBC are size, shape and surface-to-volume ratio. Internal viscosity is correlated essentially to intracellular hemoglobin concentration, which depends on the level of RBC hydration. In sickle cell disease, hemoglobin polymerization becomes the dominant parameter of RBC rigidity during vaso-occlusive crises [4]. Visco-elastic properties of the erythrocyte membrane and cytoskeleton depend more on the biomechanical characteristics of the cytoskeleton than on those of the phospholipid bilayer [5]. The spectrin network, which is the major component of the RBC cytoskeleton, is tightly attached to the phospholipid bilayer and transmembrane proteins by anchoring junction complexes [6]. In hereditary spherocytosis (HS) and other inherited RBC diseases, alterations of cytoskeletal proteins and proteins connecting the spectrin network to the membrane result in a progressive decrease in the RBC surface-to-volume ratio [7] that enhances splenic retention of RBC and leads to anemia. In these conditions, splenic retention can occur in absence of any detectable alteration of the RBC surface and thus independently from conventional ligand–receptor interactions between RBC and macrophages.

2. Quality control of RBC integrity by the spleen

RBC within the splenic circulation follow parallel, slow "open" or fast "closed" microcirculatory pathways [1]. In the fast pathway, RBC flow directly from the peri-follicular spaces to the lumen of sinuses. By contrast, in the slow pathway, RBC circulate in the splenic red pulp upstream from sinuses, without endothelial lining, outside conventional vessels explaining the term "open" microcirculation. To reach the sinus lumen, RBC have to squeeze through narrow apertures between elongated endothelial cells that form the sinus wall [1,8,9]. Crossing these apertures (conventionally named "inter-endothelial slits") is a drastic challenge on RBC deformability [6] and results in the retention of biomechanically altered RBC [1,10,11]. The splenic blood flow is 1 mL/min/G of tissue (i.e., 100–150 mL/min). The slow "open" pathway receives 10% to 20% of the splenic blood flow [1,9], and the biomechanical integrity of RBC is thus checked by the spleen approximately every 2 hours. In humans, clearance of the majority of artificially stiffened RBC (by heating them for 20 minutes) takes a few hours [12]. Other signals for retention and phagocytosis of RBC by the red pulp macrophages of the spleen exist, including conventional ligand–receptor interactions like externalized phosphatidylserine, specific forms of CD47, or opsonized band 3 clusters [13–15]. Such "conventional" ligand–receptor interactions can take place not only in the spleen but also in other macrophage-rich tissues, like the liver or the bone marrow. In comparison with RBC circulation in the fast pathway, RBC flow in the slow pathways of the spleen is approximately 20 times slower, likely facilitating interactions with macrophage receptors [1]. Retention of RBC in the spleen, based either on surface or biomechanical alterations, is expected to result in their removal from the circulation. Macrophages occupy indeed 50% of the red pulp, where retention occurs [1]. The ability of the spleen to scrutinize the surface and biomechanical integrity of RBC is expected to play a central role in many inherited and acquired RBC diseases. This is indeed the case in HS [6], thalassemia [16], sickle cell disease [4], as well as in diabetes [17], malaria [9,18–22], and transfusion of stored RBC [23,24]. This review focuses on the potential medical applications of new biomechanical "spleen-mimetic" approaches in malaria and transfusion.

3. Conventional methods to study RBC deformability–phenotypic relevance of microsphiltration

Since the seminal live microscopic observations of Anton van Leeuwenhoek in 1674, several experimental methods to quantify the deformability of RBC have been developed. Micropipette aspiration (that can simultaneously determine RBC surface and RBC volume), filtration through porous material [25], and more recently ektacytometry [26], optical tweezers [27] and microfluidic chips [28,29]. However, most existing methods to study RBC deformability are not (or not yet) compatible with the stringent requirements for high throughput screening. Micropipette aspiration, optical tweezers and atomic force microscopy are single-cell, quasi-static methods involving localized mechanical cell deformation patterns that reflect only partially the complex process of a RBC crossing a narrow splenic slit [30]. Micropore filtration and ektacytometry allow dynamic study of RBC but cannot quantify the behaviour of a specific RBC subpopulation in a mixture. Using biomimetic microfluidic chips, it is possible to partially reproduce the physiologically relevant, dumbbell-shaped deformation of RBC as they cross splenic slits [2,31,32], but these approaches in their current versions have limited sampling throughput.

In 2011, a tip-based, single-sample filtration device that allows measurement of the capacity of RBC subpopulations to squeeze between calibrated microspheres in a spleen-mimetic way was described and validated [30]. The physiological relevance of "microsphiltration" (i.e., filtration through microsphere layers) was demonstrated by showing similar retention rates of poorly deformable RBC in the microsphiltration device and in human spleens perfused ex vivo. Recently an automated, miniaturized version of the microsphiltration device was developed which permits the robust evaluation of dozens to hundreds of RBC samples in parallel [31].

4. Principle and method of microsphiltration

Microsphiltration is an experimental method developed to analyze the splenic mechanical clearance of RBC subpopulations. The original device was designed as an inverted 1 mL