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Hemorheological changes in ischemia-reperfusion: an overview on our experimental surgical data

Norbert Nemeth*, Istvan Furka and Iren Miko

Department of Operative Techniques and Surgical Research, Institute of Surgery, Medical and Health Science Center, University of Debrecen, Hungary

*** Corresponding author:**

Norbert Nemeth, M.D., Ph.D., Department of Operative Techniques and Surgical Research, Medical and Health Science Centre, University of Debrecen, H-4032 Debrecen, Nagyerdei krt. 98., Hungary, Phone/Fax: +36-52-416-915, E-mail: nemeth@med.unideb.hu

Abstract

Blood vessel occlusions of various origin, depending on the duration and extension, result in tissue damage, causing ischemic or ischemia-reperfusion injuries. Necessary surgical clamping of vessels in vascular-, gastrointestinal or parenchymal organ surgery, flap preparation-transplantation in reconstructive surgery, as well as traumatological vascular occlusions, all present special aspects. Ischemia and reperfusion have effects on hemorheological state by numerous ways: besides the local metabolic and micro-environmental changes, by hemodynamic alterations, free-radical and inflammatory pathways, acute phase reactions and coagulation changes. These processes may be harmful for red blood cells, impairing their deformability and influencing their aggregation behavior. However, there are still many unsolved or non-completely answered questions on relation of hemorheology and ischemia-reperfusion. How do various organ (liver, kidney, small intestine) or limb ischemic-reperfusionic processes of different duration and temperature affect the hemorheological factors? What is the expected magnitude and dynamics of these alterations? Where is the border of irreversibility? How can hemorheological investigations be applied to experimental models using laboratory animals in respect of inter-species differences? This paper gives a summary on some of our research data on organ/tissue ischemia-reperfusion, hemorheology and microcirculation, related to surgical research and experimental microsurgery.

Keywords: hemorheology, microcirculation, ischemia-reperfusion, experimental models

1. Introduction and background

"Surgical research probably offers better opportunities for success that it ever has before because of the advances in other sciences and in technology." – wrote Jonathan Rhoads in the Foreword of the textbook Surgical Research [72]. It is also true for the multidisciplinary science of hemorheology.

"Hemorheology is concerned with the deformation and flow properties of cellular and plasmatic components of blood in macroscopic, microscopic and submicroscopic dimensions, and with the rheological properties of the vessel structure with which blood comes into direct contact" - so defined this science Alfred L. Copley in 1951 [17]. Although its roots can be traced back into the distant past [17], the science of hemorheology is still a developing field of medicine. With the modern hemorheological instruments and standards [5, 29] new opportunities have been provided for the experimental surgical research work, also for investigating the pathophysiology of circulation and microcirculation in ischemia-reperfusion injury.

Since the pioneer work and early results of Bywaters and Beall in 1941 and Harman in 1948, the significance of reperfusion had called the attention for the additional damage of ischemia-insulted or even remote tissues and organs [15, 30]. In 1960 Haimovici had clearly reported the connection between the appearing acute renal failure and the revascularization of the previously ischemic limb, calling this phenomenon as reperfusion syndrome [28]. Ischemia-reperfusion still means a serious clinical problem, concerning its pathophysiology, as well as prevention and therapeutical approaches [13, 20, 21, 22, 25, 27, 32, 36, 39, 44]. Processes that involve free radical reactions and composite inflammatory pathways, as well as related endothelial dysfunction, had been widely investigated [2, 16, 24, 26, 27, 33, 52, 82, 83]. Hemorheological variables, such as whole blood viscosity, plasma viscosity, red blood cell deformability and aggregation, together with their determining and influencing factors, all can be affected during ischemia and ischemia-reperfusion in various manners [3, 4, 26, 34, 40].

The magnitude of these changes, the border of reversibility and irreversibility, local versus systemic rheological/micro-rheological alterations in respect of metabolic and hemodynamic changes, the events of the early postoperative days, the benefit of cooling and various preventive-therapeutic ways are principal questions for us. Like sailing between *Scylla and Charybdis*, *ischemia and reperfusion* mean two dangers: where is the acceptable distance from those dangers, where is the minimal damage in case of ischemia and reperfusion on various tissues?

In surgical research hemorheological methods can be involved depending on the experimental design. In our hemorheological research laboratory we could provide a complex panel for investigating whole blood and plasma viscosity (Hevimet-40 capillar viscometer), red blood cell deformability tested by bulk filtrometry (Carat FT-1 bulk filtrometer) and by ektacytometry (Rheoscan D-200 slit flow ektacytometer and LoRRca MaxSis Oscmoscan rotational ektacytometer), red blood cell aggregation by light-transmission aggregometry (Myrenne MA-1 erythrocyte aggregometer) and laser back-scattering method (LoRRca), fibrinogen concentration (Sysmex CA-550 automated coagulometer), hematological parameters (Sysmex F-800 microcell counter), as well as blood pH, lactate concentration and blood gases (ABL555 Radiometer Copenhagen). Using this laboratory 'armamentarium', following the methodological and standardization recommendations and considerations [5, 29], we could analyze colorful hemorheological alterations in experimental surgical and microsurgical research models, focusing on ischemia-reperfusion of various tissues and organs.

However, the hemorheological changes during and after tissue/organ ischemia-reperfusion is quite complex, and often being very difficult to evaluate properly. The reasons include the composite pathophysiological processes and the still partly explored ischemic-reperfusionic micro-rheological changes. In addition, it is still not clarified completely, how the measurable hemorheological parameters in *ex vivo* samples are related to the circulatory-microcirculatory alterations *in vivo* [3, 6].

2. Ischemia-reperfusion and hemorheological alterations – an overview

The main findings from some of our experimental surgical and microsurgical ischemia-reperfusion models are summarized in Table 1.

Alterations in hemorheological parameters during ischemia and the following reperfusion period can be originated from numerous mechanisms that include local metabolic changes, free radical reactions, acute phase reactions, causing complex changes in the systemically detectable hemorheological profile, as well as in the microcirculation [4, 14, 34, 40, 52, 59, 65].

2.1. Local metabolic and micro-environmental alterations

Mammalian and human erythrocytes may show definitive variety of cell shapes along the stomatocyte-discocyte-echinocyte sequence, depending on the micro-environmental conditions. Normally they are biconcave discocytes. Anionic amphipaths, alkalic pH or ATP depletion induce echinocytes, initially reversibly, but later the sphero-echinocytes mean irreversible forms. Cationic amphipaths or acidic pH induce concave stomatocytes, being irreversible when becomes sphero-stomatocyte [41, 48, 59, 69, 70, 71].

Ischemia leads to local metabolic changes (decrease in pH, increase of H^+ and lactate) altering the mechanical properties of blood cells. The pH of stagnant blood decreases in the area excluded from the circulation during the ischemic process, which turns the red bloods cells' discocyte shape into a stomacyte or sphero-stomacyte form. When the ATP depletion and as well calcium accumulation are the dominant, then the echinocyte, sphero-echinocyte forms may appear. Both morphological transformations accompany worsening micro-rheological characteristics: primarily in the deterioration of red blood cells' deformability and disturbed aggregation [14, 48, 59, 62, 63, 70] (Figure 1).

It is known since decades, that the magnitude of tissue damage is depending on the duration of the ischemia [74]. It seems that it is also true for hemorheological variables. During

ischemia the rheological properties of blood are significantly worsened in the excluded region, which deterioration can be detected even after 15 minutes of limb strangulation, as demonstrated by Kayar et al. [38]. It can be impaired further with the prolongation of the ischemic time period [23, 61]. After releasing the vessel (when removing vascular clamps or completing revascularization), the metabolites entering into the systemic circulation together with the damaged red blood cells cause further changes in the microcirculation and even in remote organs and tissues. During stasis hematocrit increases and the altered fluid distribution results in elevated protein concentration (or plasma loss) and increased plasma viscosity. The additional changes in volume, deformability and aggregation of erythrocytes may also increase the whole blood viscosity [4, 22, 52, 61, 85].

Szokoly et al. demonstrated in a rat model of 2-hour hind limb ischemia that arterio-venous values of blood pH, pO_2, pCO_2 and hematocrit show significant differences in the first hour of the reperfusion, expressing a decreased venous blood pH in the ischemically insulted limb versus arterial values [77].

In a canine hind limb ischemia-reperfusion model it was demonstrated that hemorheological parameters significantly impairs in the excluded region during ischemia (3 h): increased whole blood and plasma viscosity, increased blood cell volume and local hematocrit with worsened cell deformability [61].

Concerning the local versus systemic metabolic changes, a cerebral hypoperfusion porcine model clearly showed that lactate accumulation in the superior sagittal sinus blood causes definitive erythrocyte deformability impairment [60]. The investigation of local and systemic rheological differences and changes provided important information in canine liver ischemia-reperfusion [23] and rat intestinal ischemia-reperfusion [12], and as well as after the ischemia of latissimus dorsi muscle flap in a canine model [78] (Table 1).

Furthermore, from the pathophysiological concerns the mechanical trauma to red blood cells could not be neglected, when considering the micro-rheological changes [37].

2.2. Effects of free-radical reactions

It is known from McCord's and his co-workers researches that the production of toxic oxygen metabolites occurs during the reperfusion of previously ischemic tissues, by a xanthine-oxidase (XO)-dependent process [47]. Ischemia leads to the degradation of ATP to hypoxanthine, which provides a substrate for XO. Normally, more than 90% of the XO in tissues exists in xanthine-dehydrogenase form (XD), which cannot transfer electrons to molecular oxygen to form superoxide. During ischemia, by a calcium- and protease-dependent process, XD is converted to XO, which uses oxygen as an electron acceptor and generates superoxide. The released oxygen-centered free radicals initiate chain reactions: damages the cell membrane (lipid peroxidation), the transmembrane proteins (receptors, ion pumps) with the formation sulfhydryl cross-links, the hemoglobin molecules (methemoglobin, Heinz-body formation), as well as the structural proteins [4, 7, 19, 63]. Red blood cells are rich in iron, which catalyzes the free radical reactions through the Fenton-reaction; making these cells highly sensitive against ischemia-reperfusion damage [7, 47, 52]. Furthermore, Vega et al. demonstrated that XO released from the ischemic muscle is taken up by the liver where it mediates Kupffer-cells and polymorphonuclear neutrophil activation [81].

In a rat model of 1-hour ischemia the ischemic insult and the following reperfusion significantly affected erythrocyte deformability by the 1st and 2nd postoperative days. Pretreatment with the XO-inhibitor allopurinol (50 mg/kg) could prevent the deterioration of red blood cell deformability [58]. Renal ischemia of a canine model using 45-minute ischemic time also resulted in significantly impaired red blood cell deformability on the 1st and 2nd postoperative days. The changes also could be prevented by allopurinol (100 mg/kg) [64].

In addition to the role of XO, there are different potential sources of the superoxide radical during ischemia-reperfusion, including activated neutrophils and disturbed mitochondrial electron transport chain [2, 10, 36, 52, 67] (Figure 1). The deranged nitric-oxide (NO) synthase

pathways also play important role in the pathophysiology of ischemia-reperfusion [2, 26, 52, 82, 83]. Although the NO itself may have improving effect on red blood cell deformability [4, 8, 9], the nascent peroxynitrite (NO plus superoxide anion) is a very aggressive free radical [52].

In the last decade increasing number of papers presented information about the complex role and effects of biological gases [e.g. 75]. Nitric oxide (NO) is the most extensively investigated biological gas. It has been reported that red blood cells also act as an enzymatic source of NO [8, 9]. During the complex hemodynamical changes under ischemia-reperfusion NO play an important role in the local flow regulation [9, 52]. It has definitive therapeutical relations, too [18, 45].

The critical importance of the early postoperative days had been enforced by other hemorheological data, too. A 3-hour limb ischemia in a canine model showed that impairment of red blood cell deformability has a peak on the 2^{nd} and 3^{rd} postoperative days [61]. The rheological changes were associated with increased white blood cell and platelet count together with alterations in blood coagulation parameters [76]. It was interesting to see that the local cooling during ischemia did not reduce, rather increased the hemorheological disturbance [61]. Intestinal ischemia of 30 minutes in canine model also resulted in worsened erythrocyte deformability dominantly on the 3^{rd} postoperative day [11].

2.3. Acute phase reactions

In systemic circulation there are various hemorheological changes accompanying acute phase reactions after ischemia-reperfusion. Most of these alterations are non-specific: increase of fibrinogen concentration and α_2-macroglobulin, increase in immunoglobulin levels, decrease in albumin level, rise in leukocyte count, increase or decrease of platelet count, as well as hemoconcentration and erythrocytes' micro-rheological changes [4, 40, 85].

Relative hemoconcentration by the first postoperative day was frequently seen in the follow-up experimental surgical models. However, if the whole blood viscosity (WBV) values

are normalized for a constant hematocrit (Hct) (e.g. for 40%) also counting with plasma viscosity (PV), the increasing blood viscosity could be observable till the 3rd or 5th postoperative days after the ischemic insult [61]. For this analysis the Matrai formula can be used according to the followings: $WBV_{40\%} / PV = (WBV_{Hct} / PV)^{40\% / Hct}$ [46].

Also, an increase of fibrinogen concentration was demonstrated after the 3-hour hind limb ischemia over the 1st to 5th postoperative days in a canine model. The rise in fibrinogen was accompanied by continuous elevation of plasma viscosity values [61]. The increased fibrinogen concentration contributes to the elevation of plasma viscosity and the increase in red blood cell aggregation [4, 40, 62, 73].

Furthermore, the ischemia-reperfusion-caused tissue damage and the induced inflammation together with the alterations in the endothelial function may contribute to the magnitude of the systemic changes [4, 25, 26, 27, 50, 51, 67, 80].

2.4. Microcirculatory changes

The formation of "no-reflow" phenomenon is characteristic for tissue ischemia-reperfusion, which despite of the restarting circulation in large caliber vessels during reperfusion causes slowing or total arrest in the circulation [1, 49]. The phenomenon is caused by microvascular spasm, swelling of endothelial cells, increase of capillary permeability, interstitial edema, micro-thrombi, neutrophils adhesion, and local acidosis. In addition, the presence of deteriorated deformability and/or enhanced aggregation capability of red blood cells contribute to the microcirculatory disturbance [1, 49, 68, 82].

Since hemorheological parameters play important role determining tissue microcirculation [34, 35, 43, 65, 66], increase of plasma viscosity, impairment of red blood cell deformability, enhanced erythrocyte aggregation, as well as local accumulation of erythrocytes cause deterioration in the tissue microcirculation in various manner and dynamics [4, 31, 34, 68, 79]. Wolf et al. also found an early increase of red blood cell accumulation after warm

pulmonary ischemia (20 minutes) in the rat. Already 3 minutes after the staring of reperfusion significant erythrocyte accumulation was observed in the lung capillaries, that was normalized over 30-60 minutes during the reperfusion [85]. Similarly, in liver intermittent ischemia-reperfusion model (during Pringle/Baron maneuver) we also observed hematocrit elevation after 15 minutes of clamping of the hepatoduodenal ligament in portal venous blood, accompanied with increased aggregation index. The magnitude of the changes was depending on the repeating number of this maneuver [23]. However, in latissimus dorsi flap ischemia-reperfusion canine model we could see continuous elevation of the local hematocrit in thoracodorsal vein during the first hour of the reperfusion, while the increase of aggregation index values existed only in the first 10-15 minutes of the reperfusion [78].

Devices for monitoring microcirculation are very useful in ischemia-reperfusion studies [35, 42, 49, 57], however, the best would be to have such a method in the future that may measure the hemorheological parameters *in vivo*, and in parallel with the circulation/microcirculation.

3. Experimental considerations

Since experimental surgical and microsurgical research models are mostly performed using laboratory animals, several questions and concerns are raised because of the inter-species differences of physiological and morphological parameters as well as of pathophysiological pathways.

Over the past decades, numerous studies have been devoted to the investigation of hemorheological differences among the various animal species using different measuring techniques [5, 29]. Although the development of measuring techniques and the appearance of new measuring devices help us with finding adequate answers to a lot of question in this field, there are still plenty of unsolved problems concerning species-dependent hemorheological characteristics [84].

When planning and conducting animal experiments several considerations should be evaluated [86]. Studies are needed for analyzing the inter-species hemorheological differences of laboratory animals, of which magnitude or sensitivity is strongly depending on the measurement technique, often being laboratory-specific [53, 84]. Besides inter-species variations gender differences are also important [54], which may also influence the magnitude of changes. If needed, proper methodological (sampling, handling), measurement adaptation have to be done with standardization of measurement techniques [5].

However, in experiments much more influencing factors should be taken into considerations.

- Concerns *a priori*: planning of experiment and techniques, counting on inter-species and gender differences (also the estrus cycle of animals), carefully planning of blood sampling and handling (site of sampling, required blood sample volume versus available sample volume, anticoagulant, storage), standardized measurement conditions, depending on the method/device.
- Concerns *a posteriori*: extrapolation, reliability.

All these issues have importance for correct evaluation, and so the data obtained could be comparable and may provide useful result for the clinical medicine.

4. Summary and open questions

During our researches we used several experimental models and demonstrated significant deterioration of red blood cells' micro-rheological properties following ischemia. The main conclusions were the followings: (1) The erythrocyte deformability significantly deteriorate during limb I/R and on the following 1^{st}-3^{rd} postoperative days; (2) the majority of these harmful effects can be preventable by antioxidant drugs. (3) Ischemic time and temperature are determinant factors in the extent of changes. (4) The real extent of local micro-rheological changes is still unclear, and mainly in the context of microcirculatory disturbances further investigations are required.

Open questions, research concerns and problems to be solved still include: the exploration of the magnitude of the micro-rheological changes, finding boundaries of reversibility, comparability question of *in vivo* changes versus *ex vivo* measurements (significance of micro-environmental conditions), relations of hemodynamic, microcirculatory and micro-rheological alterations (significance of parallel investigations), local versus remote effects, as well as investigation and searching for targeted micro-rheological therapeutic tools. All being in the frame of the laboratory animal science concerns and the measuremental-technical possibilities.

For a more accurate and better comprehension of local and systemic hemorheological changes, induced by ischemia-reperfusion and hypo- or hyperperfusion, further complex investigations are needed. These investigations would be more informative if the hemodynamic, microcu8ruliatory and hemorheological (from local samples) measurements could be performed in parallel, together with the micro-environmental conditions.

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7. Tables

Table 1. Selections of our main hemorheological results from various ischemia-reperfusion-related experimental surgical models.

Organ/region	Species	Duration of ischemia	Main changes found	Reference
Hind limb	rat	1 h	Significantly worsened red blood cell deformability on the 1st-2nd postoperative days, being preventable by giving allopurinol.	[58]
	rat	1 h	The magnitude of the red blood cell deformability impairment may show gender differences that can be deteriorated after gonadectomy, causing more expressed post-ischemic alterations.	[55]
	rat	1 h	Post-ischemic impairment of skeletal muscle tissue microcirculation could be investigated: remarkable deterioration in blood flux.	[57]
	rat	2 h	Decreasing pH, increasing local hematocrit during the first hour of the reperfusion, with widening arterio-venous differences.	[77]
	canine	3 h	Significantly worsened red blood cell deformability on the 2nd-3rd postoperative days. Hemoconcentration on the 1st day, elevating fibrinogen concentration and increasing plasma viscosity over the 1st to 5th postoperative days. Local cooling caused more expressed impairment.	[61]
Kidney	canine	45 min	Worsening red blood cell deformability in the first 30 minutes of the reperfusion and on the 1st-2nd postoperative days, that could be prevented by allopurinol.	[64]
Liver	canine	3x15 min	Using Pringle (Baron) maneuver, local hematocrit, red blood cell aggregation and leukocyte count markedly increased in portal blood samples after the third 15-min clamping.	[23]
Small intestine	rat	30 min	Portal and caval blood samples showed worsened red blood cell deformability mainly in the first 30 minutes of the reperfusion. Erythrocyte aggregation enhanced in portal venous blood samples.	[12]
	canine	30 min	Impaired red blood cell deformability was found on the 3rd postoperative day.	[11]
M. latissimus dorsi flap	canine	1 h	Compared to the control side, local hematocrit in thoracodorsal vein increased over the first 60 minutes of the reperfusion. Red blood cell aggregation increased dominantly in the first 15 minutes of the reperfusion.	[78]
Testis	rat	30 min	Red blood cell deformability moderately decreased, while erythrocyte aggregation increased in a large magnitude by the 1st postoperative day.	[56]

8. Figure legends

Figure 1

Schematic graph of the events and effects, influencing red blood cell deformability during ischemia-reperfusion.

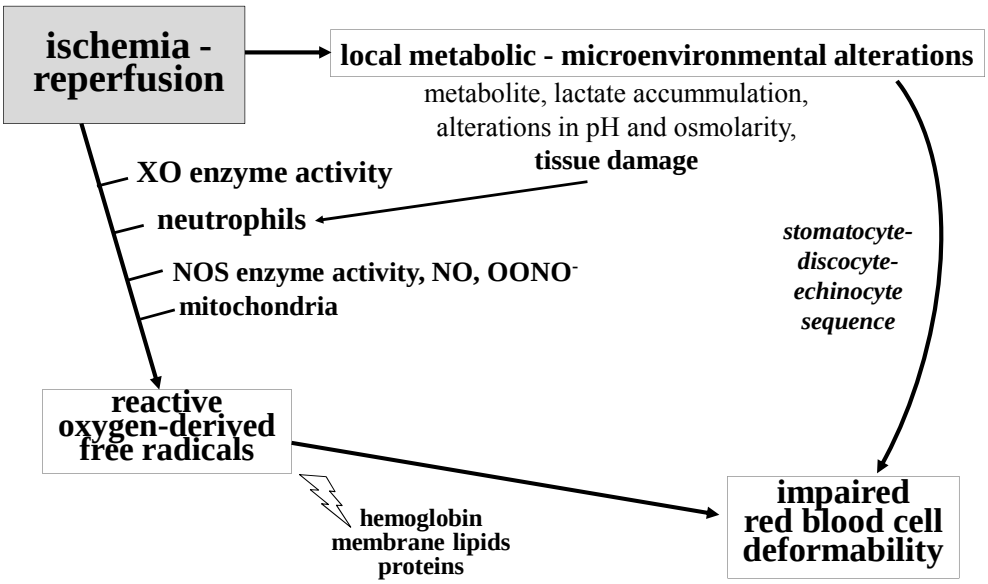

Figure 1.