საქართველოს მეცნიერებათა ეროვნული აკადემიის მოამბე, ტ. 8, №2, 2014
BULLETIN OF THE GEORGIAN NATIONAL ACADEMY OF SCIENCES, vol. 8, no. 2, 2014

Medical Sciences

New Diagnostic Criterion of Graft Rejection in Liver Transplantation

Mogely Sh. Khubutiya*, Mark M. Goldin**

** Member of the Russian Academy of Sciences, N.V. Sklifosovsky Research Institute for Emergency Medicine, Moscow, Russia*
***N.V. Sklifosovsky Research Institute for Emergency Medicine, Moscow, Russia*

ABSTRACT. Redox potential (RP) of blood serum monitoring in patients with liver grafts was performed in the course of postoperative treatment. It was found that abrupt RP shifts of over 25 mV indicated to the onset of a rejection crisis or a graft dysfunction in the patient. A statistically significant population of 31 patients with liver grafts was examined, false positive and false negative results were not observed.

Key words: *electrochemical monitoring, redox potential, transplantation, graft.*

It is well known that the redox potential (RP) of a biological medium is an integral characteristic reflecting the level of pro- and antioxidants in the organism [1-4]. For apparently healthy subjects, the normal range of RP is between –50 mV and –23 mV [5]. Addition of antioxidants to blood serum leads to negative RP shifts, whereas addition of oxidants, to positive RP shifts [6]. It is also known that certain pathological states are characterized by different ranges of blood serum redox potentials. For example, serum RP in patients with acute cerebral pathologies is generally more positive than serum RP in apparently healthy subjects [7].

It would appear that the ratio of activities of pro- and antioxidants should be the dominant response in the assessment of a patient's condition by RP measurement. However, the contribution of adsorption of blood serum components (proteins, lipids, enzymes, etc.) and medications on the surface of the working electrode (usually platinum) should not be overlooked. Indeed, it is known that the potential of platinum electrode shifts in the presence of albumin [8,9]. Therefore, it is important to take into account the effect of adsorption on the measured RP values in order to use RP as a diagnostic criterion. Understanding the effects of ongoing medicinal treatment on the RP values is also crucial in assessing its effectiveness and suggesting corrections to the course of treatment.

The main goal of the present work was to develop a diagnostic criterion for assessment of postoperative condition of patients with liver transplants by monitoring their blood serum RP.

Methods

Postoperative monitoring of blood serum RP was performed in 18 patients with liver transplants in the course of their hospitalization, with a total of 284

examinations performed. The RP measurement was performed in blood serum on a platinum working electrode with a silver/silver chloride reference electrode. The RP values were recorded as the open circuit potential (OCP) values after 30 min of measurements according to a previously described method [10]. For each measurement, 8 mL of patient blood was centrifuged at 1500 g for 15min on a CR 3 12 centrifuge (Jouan) to obtain the blood serum.

The following reagents and medicinal substances were utilized: physiologic saline (0.9% aqueous sodium chloride), 20% human serum albumin solution (NOVA Biologics, Inc.), L-ascorbic acid (Alfa Aesar), Cyclosporine (Novartis), and Mycophenolatemofetil (Roshe) – the latter two as typical immunosuppressants used for treatment of liver transplant patients.

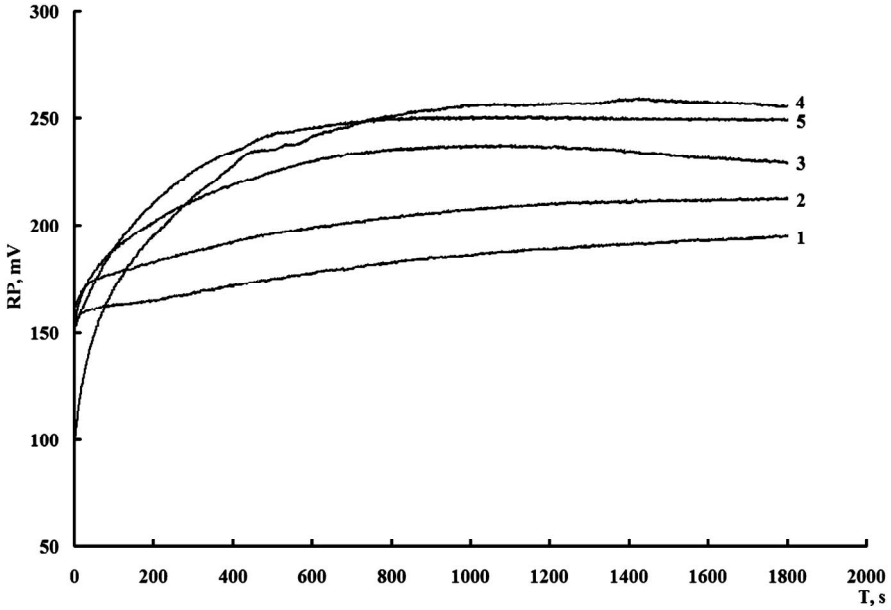

Fig. 1. The effect of albumin concentration on the time dependence of RP. Albumin concentration in physiologic saline: 1 – 20%, 2 – 10%, 3 – 1%, 4 – 0.1%, 5 – 0%.

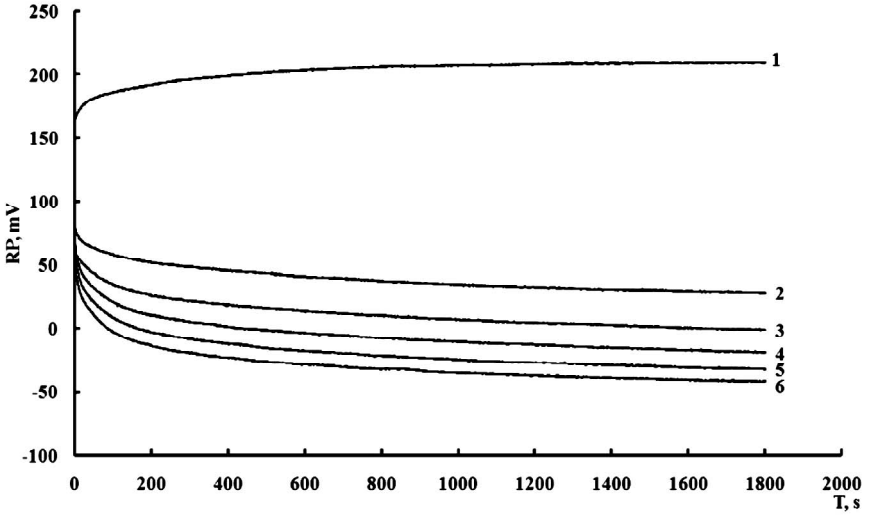

Fig. 2. The effect of ascorbic acid on the RP of 10% albumin. Ascorbic acid concentrations: 1 – 0.00 mM; 2 – 0.57 mM; 3 – 1.14 mM; 4 – 1.70 mM; 5 – 2.27 mM; 6 – 2.84 mM.

Results and Discussion

The effect of certain oxidants and antioxidants on the RP in aqueous solutions was studied previously [6]. In the present work, the same line of investigation was continued with solutions containing albumin. Positive RP shifts were observed with increasing dilution of albumin with physiologic saline, with the RP for the most dilute albumin solution (0.1%) closely approximating the RP of the pure saline solution (Fig. 1). The observed effect is likely due to the adsorption of albumin on the surface of platinum.

with immunosuppressants, it was also important to investigate their effect on the RP of blood serum. In the experiments where the maximum daily dose of Cyclosporine (0.40 g) and Mycophenolatemofetil (2 g) was added to physiologic saline, negative RP shifts were observed (Fig. 3).

On the contrary the addition of the maximum daily dose of Cyclosporine and Mycophenolatemofetil to blood serum led to positive RP shifts (Fig. 4). Though the reason for such difference is unclear at this time, it is possible to introduce a correction for measured

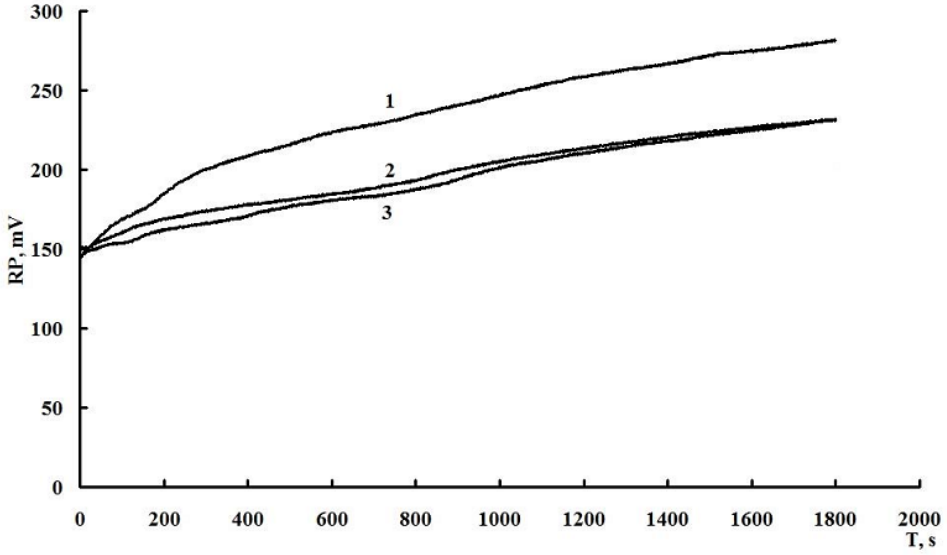

Fig. 3. The effect of immunosupressant addition on the RP of physiologic saline. 1 – initial saline, 2 – saline + 0.5 mg/ml Mycophenolatemofetil, 3 – saline + 0.1 mg/ml Cyclosporine.

The effect of antioxidants on the RP value was also studied. A 10% solution of albumin in physiologic saline was used to model the biological medium, with ascorbic acid used as a typical antioxidant. These data (Fig. 2) show negative RP shifts with increasing ascorbic acid concentration. It is important to note that the RP of solutions containing 2.00 – 3.00 mM ascorbic acid correspond to the normal range of RP for apparently healthy subjects [5]. Moreover, according to [7], the shape of curves in Fig. 2 bears close resemblance to the curves obtained in actual biological media (blood serum).

Since liver transplant patients are usually treated

RP values using data shown in Fig. 4 when RP measurements are performed for patients undergoing immunosuppressant therapy.

RP monitoring was performed in 16 patients with liver transplants. During monitoring, special attention was given to the dynamics of RP changes, as the shape of the RP time dependence may serve as an indicator of various processes occurring in the organism. It was shown that the days with abrupt changes in the RP values preceded or coincided with the onset of a rejection crisis or transplant dysfunction in the patients. The RP monitoring data for patients with favorable (Fig. 5) and

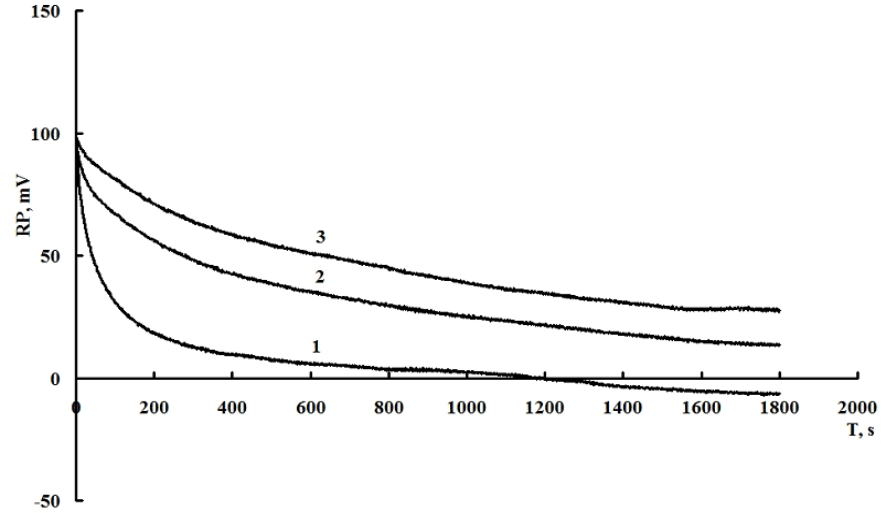

Fig. 4. The effect of immunosupressant addition on the RP of blood plasma. 1 – blood plasma + saline (5:1), 2 – blood plasma + 0.5 mg/ml Mycophenolatemofetil (5:1), 3 – blood plasma + 0.1 mg/ml Cyclosporine (5:1).

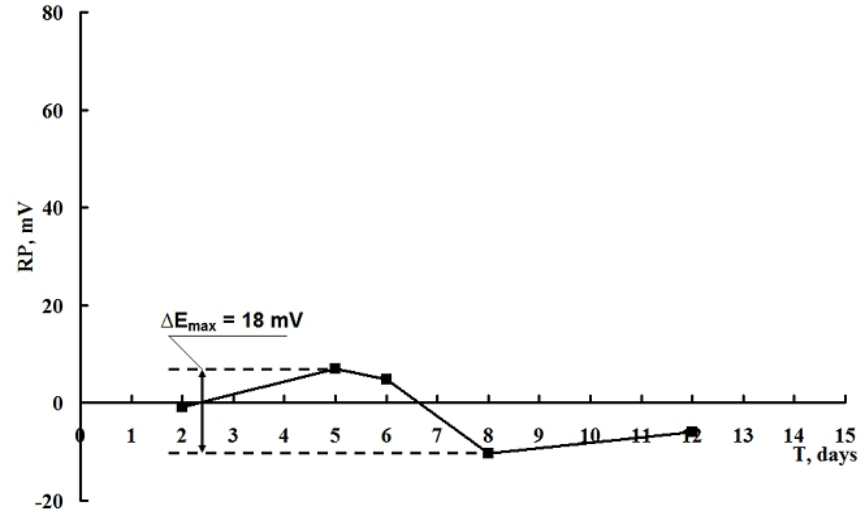

Fig. 5. Redox potential monitoring for Patient K. after liver transplantation (favorable outcome).

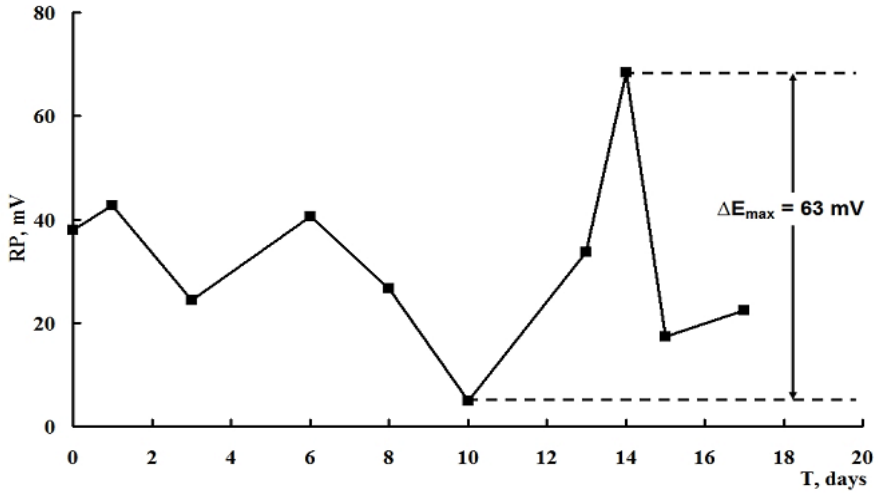

Fig. 6. Redox potential monitoring for Patient S. after liver transplantation (unfavorable outcome).

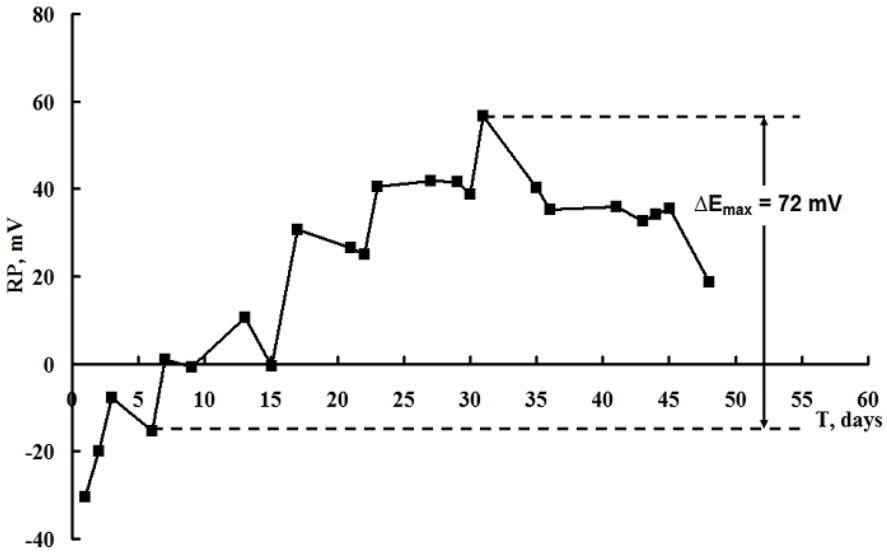

Fig. 7. Redox potential monitoring for Patient Sh. after liver transplantation (unfavorable outcome).

unfavorable (Fig. 6-7) treatment outcomes demonstrate that the maximum range of measured RP values (ΔE_{max}) is much smaller when the treatment outcome is favorable (Patient K. in Fig. 5) than the ΔE_{max} for patients with unfavorable treatment outcomes (Patient S. in Fig. 6 and Patient Sh. in Fig. 7): ΔE_{max} = 18 mV for Patient K. over against ΔE_{max} = 63 mV for Patient S. and ΔE_{max} = 72 mV for Patient Sh.

Thus, it was shown that OCP shifts equal to or greater than 25 mV can serve as a criterion to predict a liver rejection crisis or liver dysfunction in monitoring patients with liver transplants and can be used as an indication that corrections in the course of treatment are necessary.

სამედიცინო მეცნიერებანი

ტრანსპლანტატის შეუთავსებლობის დიაგნოსტიკის ახალი კრიტერიუმი ღვიძლის გადანერგვისას

მ. შ. ხუბუტია*, მ. მ. გოლდინი**

** რუსეთის მეცნიერებათა აკადემიის წევრი, მოსკოვი, რუსეთი*
***ნ.ვ. სკლიფოსოვსკის სახ. სასწრაფო სამედიცინო დახმარების კვლევითი ინსტიტუტი, მოსკოვი, რუსეთი*

ღვიძლგადანერგილი პაციენტების ოპერაციის შემდგომი მკურნალობის პერიოდში მიმდინარეობდა სისხლის შრატში ჟანგვა-აღდგენითი რეაქციის პოტენციალის მონიტორინგი. დადგინდა, რომ ჟანგვა-აღდგენითი რეაქციის მკვეთრი ცვლილება 25 მვ-ზე ზევით შეუთავსებლობის კრიზისზე ან ტრანსპლანტატის დისფუნქციაზე მიუთითებს. ჩატარდა ღვიძლგადანერგილი მოსახლეობის სტატისტიკურად მნიშვნელოვანი ნაწილის - 31 პაციენტის გამოკვლევა. არცერთი ცრუ დადებითი ან ცრუ უარყოფითი შედეგი არ დაფიქსირებულა.

REFERENCES

1. *E. Ziegler* (1965), The Redox Potential of the Blood in Vivo and in Vitro. Charles C. Thomas Publisher.
2. *C. Marmasse, H.J. Grosz* (1964), Nature, **202,** 4927: 94.
3. *H.J. Grosz, B.B. Farmer* (1967), Nature, **213**, 5077: 717-718.
4. *M.M. Goldin, G.J. Blanchard, A.G. Volkov et al.* (2008), ECS Transactions, **11,** 20: 19-28.
5. *M.M. Goldin, A.G.Volkov, M.Sh. Khubutiya et al.* (2008), ECS Transactions, **1,** 1: 39-49.
6. *M.Sh. Khubutiya, M.M. Goldin, M.V. Romasenko et al.* (2010), ECS Transactions, **25**, 19: 63-71.
7. *M.M. Goldin, M.V. Romasenko, A.K. Evseev et al.* (2010), Russ. J. Neurosurg. **4**: 33-39 (in Russian).
8. *J. Anzai, B. Guo, T. Osa et al.*(1994), Chem. Pharm. Bull. (Tokyo) **42,** 11: 2391-2393.
9. *A. Dolatshahi-Pirouz, K. Rechendorff, M.B. Hovgaard et al.* (2008), Colloids and Surfaces B: Biointerfaces, **66,** 1: 53-59.
10. *M.Sh. Khubutiya, A.K.Evseev, V.A. Kolesnikov et al.* (2010), Russ. J. Electrochem., **46,** 5: 537-541.

Received June, 2014