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DEVELOPMENT OF A POTENTIOMETRIC METHOD FOR THE EVALUATION OF REDOX STATUS IN HUMAN SERUM

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The aim of our study was to evaluate in human subjects, some serum redox parameters such as Apparent Redox Potential (ARP) and Redox Stability Index (RSI), and to correlate them with the subject's global metabolic profile as well as with biochemical markers of oxidative stress, such as low density lipoproteins and erythrocytes susceptibility to lipid peroxidation – LDLox and ESP, respectively. Baseline ARP (ARP_0) was evaluated using a 727 Tistand Potentiometer (Metrohm AG, Switzerland), with a combined Pt electrode with internal reference. The *in vitro* response of the serum to redox stress was studied in dynamics, by incubating the serum samples with a prooxidant system – quinhydrone. We pointed out significant relationships between the redox parameters with the serum levels of molecules with known redox stress implications: glucose and uric acid.

INTRODUCTION

In living organisms oxidation-reduction systems play such an essential part that life itself might be defined as a continuous redox reaction. Basic mechanisms underlying transformation of organic constituents include metabolic sequences in which electron transfer occurs. Reactive oxygen species (ROS) are continuously generated in normal metabolic pathways. Redox homeostasis describes the normal physiologic process of reduction and oxidation in order to repair the unstable, damaging, reduced, ROS. This homeostatic balance between ROS and antioxidant capacity is in contrast to redox stress (redox imbalance) which implies a loss of this unique homeostasis resulting in an excess production of ROS either through the process of reduction or oxidation.[1]

Oxidative stress implies a loss of redox homeostasis with an excess of ROS by the singular process of oxidation. Both redox and oxidative stress may be associated with an impairment of antioxidant defensive capacity as well as an overproduction of ROS. Redox homeostasis is the normal physiological oxido-reduction process which consists in counteracting oxygen free radical reactions using a complex endogenous system, enzymatic and non-enzymatic, of antioxidants.[2] The balance between the oxidative action of free radicals and the level of antioxidants in a body is essential for life and characterizes a living organism's capacity of resistance to stress.

The aforementioned redox stress is the factor which leads to lipid peroxidation, protein oxidation and DNA damage, thus generating the bio-degrading of the cell; these functional cell changes cause at the general level onset of auto-immune phenomena, cardiovascular distress, neurological degeneration syndrome, mutations and tumorigenic processes as well as ageing process generally.[3,4]

Oxidopathies are recently defined as states involving accelerated oxidative molecular injury extended to all tissues, red blood cells and all plasma components, as a result of an imbalance between the endogenous antioxidant systems and the pro-oxidant species generated in the human body.[5] The exact mechanisms underlying oxidopathies remain to be unravelled. On the other hand, the influence of different pharmacologically active molecules on the human redox endogenous systems in the daily clinical practice remains a permanent challenge for the physician.

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Previous studies demonstrated the usefulness of potentiometric methods for the evaluation of redox status of the human body and so, for a redox approach to health and disease.[6-9]

In this paper we investigated the usefulness of state potentials' evaluation of human serum in correlation with the classical biochemical parameters characterizing normal/ abnormal metabolism.

EXPERIMENTAL

Potentiometric measurement

To determine the serum redox potential we proposed an original *in vitro* method enabling a differentiated evaluation for the biological samples' antioxidant capacity as resulting exclusively from redox hydrophilic biomolecules. The method is based on potentiometric evaluation of the status of oxidant and reducing species in samples of human serum. We used a micro Pt/AgCl combination redox electrode, with an internal reference, and a Tistand 727 Potentiometer (Metrohm AG, Switzerland). Measurement of redox potential was performed in a standardized volume of 1mL serum.

The baseline apparent redox potential of the human serum (ARP_0) was assayed; than a mild prooxidant chemical system (quinhydrone, 0.18%) was added to the biological samples. After incubating at 25˚C for 1h respectively 3 hours, a final apparent redox potential were recorded (ARP_f).

Quinhydrone mimics the prooxidant conditions developing *in vivo* and consumes the reducing species leading to an increase of the apparent redox potential in time. This dynamic recording of the data allowed the calculation of a difference between the final value of the ARP (ARP_f) and the initial one (ARP_0), thus defining the redox stability index (RSI). This parameter illustrates the serum sample capacity to counteract the prooxidant agent. The lower the RSI, the higher the activity of the hydrosoluble antioxidant protective systems in the serum.

Study design

The study included 72 randomly selected patients, 35-55 years old, hospitalized at the "Ana Aslan" National Institute of Geriatrics and Gerontology, Bucharest. Selected patients were non-smokers and did not have any kidney, liver or haematological disease. Patients were not given any antioxidant therapy, as well as hypoglycemic or nitrovasodilating treatment for at least 12 hours before blood sampling. Venous blood samples were obtained from the subjects after an overnight fast.

Biochemical methods

For the evaluation of general metabolic parameters we used enzymatic commercial kits (Merck). The The NO stable end products, NO_x (NO_2^- + NO_3^-) were assayed using the Griess reagent with a method previously described.[10] Lipid peroxidation markers ESP and LDLox were assayed using methods described elsewhere.[11]

Statistical analysis

Results are expressed as means ± standard deviation (SD). Differences between means were evaluated using Student's paired t-test. The strength of association between pairs of variables was assessed by Pearson correlation coefficient. Multiple regression analysis was performed to evaluate the independent relation between biochemical parameters by using the SPSS software 9.0 and EXCEL 7.5 programs of Microsoft Software. The level of significance was set at $p < 0.05$.

RESULTS

We correlated the analytical, potentiometric parameters with the serum biochemical parameters illustrating the general metabolic profile: glucose (G), total cholesterol (TC), triglycerides (TG), uric acid (UA). The subjects under study were heterogeneously distributed from the biochemical point of view. Because the purpose of the study was to evaluate the influence of hyperglycemia and/or hyperuricemia on the redox stress parameters, we divided these patients according to their glucose and uric acid serum levels, knowing that these two molecules are responsible for a great part of the redox reactions taking place in serum.

We also evaluated the endogenous formation of plasma nitric oxide – NO, as marker for endothelial nitric oxide synthase activity – eNOS, and a marker of cardio-vascular risk, and two lipid peroxidation markers: susceptibility of erythrocytes and low density lipoproteins to lipid peroxidation - ESP and LDLox, respectively.

In the first phase, the studied patients were divided according to their serum glucose levels into two groups: one group of 53 normoglycemic patients (G ≤110mg/dL) and another of 19 hyperglycemic ones (G >110mg/dL). The values for the biochemical parameters in the two groups are presented in Table 1.

From the statistical analysis of the data we concluded that hyperglycemia was associated with a significant increase of the ESP (p=0,044) and with a very significant increase of the NO plasma level (p=0,0055). The LDL susceptibility to induced lipid peroxidation was increased, even though non-significantly, in subjects with disturbances in glucose metabolism.

For the normoglycemic patients results revealed significant positive correlations between the LDLox and the redox stability index either evaluated after 1 hour or 3 hours of incubation with the prooxidant agent (Fig. 1). No correlations were found between the evaluated parameters in the hyperglycemic group of subjects.

Table 1

The serum biochemical parameters evaluated for the normoglycemic and for the hyperglycemic group of patients

Serum biochemical parameter	Normoglycemic patients (n=53)	Hyperglycemic patients (n=19)
Glucose (mg/dL)	96.43±7.81	168.05±55.76
Total cholesterol (mg/dL)	231.96±51.70	219.10±39.29
Triglycerides (mg/dl)	121.02±45.55	153.42±60.45
Uric acid (mg/dL)	4.74±1.60	4.78±1.74
ESP (nmoles MDA/g haemoglobin)	768.34±150.76	861.68±188.34
LDLox (nmoles MDA/dL serum)	6.15±4.65	6.24±4.98
NO_x (NO_2^- + NO_3^-) (µmoles/L plasma)	4.54±3.91	7.18±3.23
ARP_0 (mV)	-60.50±18.12	-52.94±11.67
RSI 1h (mV)	15.62±9.83	13.94±8.22
RSI 3h (mV)	32.79±13.60	30.78±8.83

ESP – erythrocyte susceptibility to lipid peroxidation; MDA – malondialdehyde; LDLox – low density lipoprotein susceptibility to lipid peroxidation; NOx – nitric oxide metabolic pathway products (nitrites+nitrates); ARP_0 – baseline apparent redox potential of the human serum; RSI – redox stability index.

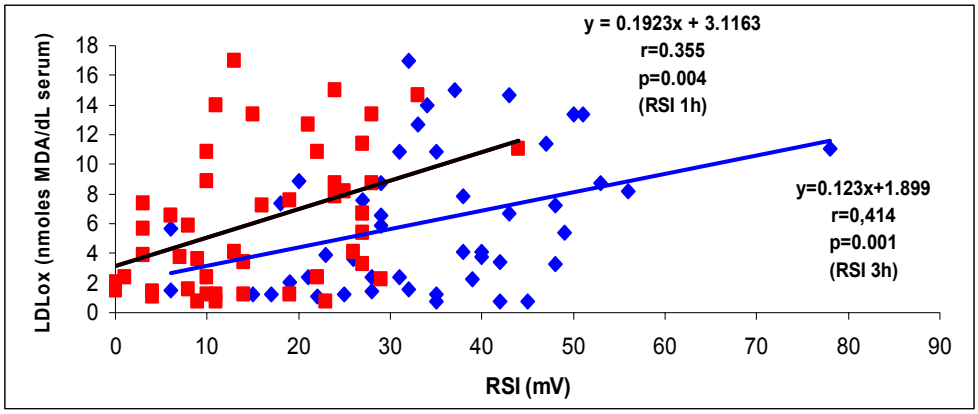

Fig. 1 – Correlation between low density susceptibility to lipid peroxidation (LDLox) and the redox stability index (RSI), determined after the incubation for 1hour and 3hours with quinhydrone, in normoglycemic patients (n=53).

Table 2

The serum biochemical parameters evaluated for the normouricemic and for the hyperuricemic group of patients

Serum biochemical parameter	Normouricemic patients (n=37)	Hyperuricemic patients (n=35)
Glucose (mg/dL)	113.02±44.49	117.77±41.74
Total cholesterol (mg/dL)	224.08±55.28	233.31±41.14
Triglycerides (mg/dl)	116.32±54.35	143.26±45.13
Uric acid (mg/dL)	3.64±0.75	6.22±1.02
ESP (nmoles MDA/g haemoglobin)	795.03±171.85	792.33±162.03
LDLox (nmoles MDA/dL serum)	5.57±4.30	7.69±4.86
NO_x (NO_2^- + NO_3^-) (µmoles/L plasma)	4.12±2.90	6.76±4.40
ARP_0 (mV)	-59.59±17.96	-57.37±15.94
RSI 1h (mV)	15.43±10.63	14.91±8.06
RSI 3h (mV)	31.57±13.66	33.54±11.50

ESP – erythrocyte susceptibility to lipid peroxidation; MDA – malondialdehyde; LDLox – low density lipoprotein susceptibility to lipid peroxidation; NOx – nitric oxide metabolic pathway products (nitrites+nitrates); ARP_0 – baseline apparent redox potential of the human serum; RSI – redox stability index.

In the second phase of our study, in order to evaluate the influence of uric acid on redox serum parameters, the subjects were grouped according to their serum uric acid level, in 37 normouricemic (UA≤5.5 mg/dL) and 35 hyperuricemic ones (UA>5.5 mg/dL). The values for the biochemical parameters in the two groups are presented in Table 2.

Analysing the data we concluded that hyperuricemia was associated with significantly higher values of the LDLox ($p=0.04$) and of the NOx levels ($p=0.004$). The baselines ARP_0 and RSI for the two groups of patients were fairly even.

DISCUSSION

In order to evaluate the biological significance of this potentiometric assay, in the present study we analysed comparatively parameters of the hydrophilic and lypophilic oxidative status for the selected patients, in order to demonstrate that redox stress parameters ARP and RSI evaluation might constitute competent criteria for metabolic characterization in health and disease.

Even if the RSI was not significantly different between the two groups, the ARP value was significantly higher for the hyperglycemic patients, suggesting a decrease of the hydrosoluble reducing molecule concentration. This means that in normoglycemic patients, the blood has powerful antioxidant systems able to protect lipoproteins and erythrocytes from oxidation, and to defend the body from redox stress.

Hyperglycemia induces a large number of alterations at the cellular level of vascular tissue that potentially increase oxidative stress through several pathways. A major mechanism appears to be a hyperglycemia-induced intracellular ROS production, due to an alteration of mitochondria electron transport chain. Another mechanism consists in autooxidation of glucose when superoxide anion and hydrogen peroxide are yielded.[12] This increase in ROS production impairs the function of antioxidant systems, and that explains the lack of relationships between the redox stress parameters in the hyperglycemic group.

The increase of the uric acid level induces redox imbalance, impairing the antioxidant systems and increasing the susceptibility of LDL to lipid peroxidation. Also, the increase of uric acid level is correlated with an increase of the endogenous vasodilatator NO level, indicating the cardiovascular risk at hyperuricemic patients.[13]

The clinical study presented here represents a preliminary study meant to evaluate the clinical relevance of redox parameters ARP and RSI in the global metabolic evaluation of patients. Our results show that the proposed redox parameters change in correlation with the global metabolic characterization of the patients and thus might be useful for the physiopathological evaluation of biological samples.

The oxidative stress induced by hyperglycemia leads to the imbalance of the hydrophilic homeostasis (illustrated by ARP) and also of the lypophilic antioxidant mechanisms (LDLox) while hyperuricemia is correlated with an increase of the LDLox. So ARP and RSI represent useful parameters for the evaluation of the cardiovascular risk.

It becomes increasingly clear that redox potential is one of the most complex indicators of the physiological and physiopathological processes.

The appropriate choice of oxidative or antioxidative therapies in daily clinical practice is a permanent challenge, so an accurate biochemical and redox investigation of the internal environment of the body before therapy and also after the administration of some drugs might prove extremely useful.

CONCLUSIONS

Our results suggest that the assessment of the apparent redox potential and its variation under the influence of a prooxidant system might be useful in the complex evaluation of the global systems involved in maintaining the redox homeostasis. Results show the importance of evaluating the redox stress parameters in the investigation and diagnosis of oxidopathies, and also as possible applications regarding the efficiency of antioxidant therapies.

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