HEMORHEOLOGICAL METHODS IN THE INVESTIGATION OF RISK FACTORS OF MYOCARDIAL ISCHEMIA AND OF THE ANTIOXIDANT PROPERTIES OF DIFFERENT CARDIOVASCULAR DRUGS

Ph.D. thesis

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LIST OF ABBREVIATIONS

ADP	adenosine diphosphate
AI	aggregation index
AICS	acute ischemic coronary syndrome
AMI	acute myocardial infarction
ASA	acetylsalicylic acid
CAD	coronary artery disease
IHD	ischemic heart disease
HCT	hematocrit
LORCA	Laser-assisted Optical Rotational Cell Analyzer
PBS	phosphate buffered saline
PMS	phenazine methosulphate
PPP	platelet poor plasma
PRP	platelet rich plasma
PV	plasma viscosity
RBC	red blood cell
RCTT	relative cell transit time
WBV	whole blood viscosity

1. INTRODUCTION

Cardiovascular diseases are the most frequent causes of morbidity and mortality in the developed countries. In spite of the extended research on this field, there still remained obscure parts in the pathomechanism of these diseases. Investigations explored the role of vascular wall, cellular interactions, and many of the underlying biochemical processes. Less attention was paid to the properties of the circulating blood and even less to the relationship of these factors and oxygen free radicals.

Coronary artery disease (CAD), the stenotic lesion of one or more coronary branches is the most frequent type of ischemic heart disease (IHD). In the development of IHD several risk factors may play a role, which can influence each other. While the role of "classic" risk factors (e.g. smoking, hypertension, hypercholesterolemia, diabetes, etc.) has been well known for a long time, the importance of hemorheologic parameters in the development of coronary artery disease has been accepted only for the last two decades.

Coronary vessel system is a special part of the circulation. There is a continuous change in blood flow, perfusion pressure, and shear rate due to the cardiac cycle, moreover, the narrowest capillaries of the body can be found in the myocardium (diameter can be as small as 3-5 μm, several micrometers smaller comparing to that of red blood cells). Therefore the role of rheological alterations can be of higher importance than in other parts of the circulatory system. In several studies hemorheological parameters were proved to be primary cardiovascular risk factors (Framingham Study, Nortwick Park Study, Monica Project, Edinburgh Artery Study).

Acute ischemic coronary syndrome (AICS) is caused by the acute disorder in the blood supply of the myocardium caused by the sudden decline of coronary blood flow or by the sudden raise of myocardial oxygen demand. Mortality and morbidity of patients with this syndrome are substantial, therefore AICS has considerable medical, social and economic importance all over the world. Prevention and elimination of risk factors are the most powerful ways to reduce the mortality and morbidity of this syndrome. As previously mentioned, classical risk factors of

cardiovascular diseases are well known and many of them could be prevented. Previous studies have already confirmed that hemorheological parameters also could have determining role in the development and severity of AICS. Experimental evidences underline that hemorheological alteratlons observed in acute myocardial infarction (AMI) are also involved in the decreased perfusion of the damaged area and the extension of the necrotic regions. Some of the rheological parameters (e.g. plasma fibrinogen level, white blood cell count) known as "non-classic" risk factors have been recently clarified to have determining role in the prognosis of patients after AMI. Although several studies examined a part of rheological parameters in AICS, few of them examined all of these parameters simultaneously. Thus further investigations should reveal which hemorheological factors have significant role in the development of CAD or AICS. The examination of these parameters can help to modify them favorably in the future, which can be important in the prevention and treatment of cardiovascular diseases.

Elevated plasma fibrinogen concentration is associated with increased frequency of coronary heart disease and stroke. It was proven as a primary risk factor for cardiovascular and cerebrovascular disease in healthy individuals and a risk factor for death or recurrence of myocardial ischemia in patients with a previous coronary event. Nevertheless, the numerous studies investigating the role of fibrinogen in red blood cell (RBC) aggregation, the mechanism of its action has not fully been elucidated. Although fibrinogen has a determining role, several other plasma factors also have significant influence on RBC aggregation.

Increased RBC aggregation has a negative effect on the flow dynamics of blood and it is known to be associated with many clinical states (e.g. ischemic heart disease, acute myocardial infarction, cardiogenic pulmonary edema, thromboembolic states, renal failure, diabetes mellitus, venous thrombosis). The generally accepted theory for RBC aggregation is the bridging hypothesis, which supposes that RBC aggregation is due to the bridging between neighboring cells by specific plasma proteins (e.g. fibrinogen, large molecular weight globulins) and can also be induced by large macromolecules such as high molecular weight dextrans and other polymers. On the other hand, a few reports have questioned this hypothesis and proposed a thermodynamic model based on polymer/protein depletion from the intercellular

contact zone. It is important to know that RBC aggregation is a dynamic process and thus represents a balance between forces of aggregation and disaggregation (e.g. mechanical shear, electrostatic repulsion between cells). Thus, in general, the aggregation process depends on the properties of the erythrocytes as well as the mechanical and physicochemical characteristics of their environment.

Because of the several different methods used to determine RBC aggregation in different laboratories, the standardization of aggregation measurements is important for getting comparable results. As these measurements do not completely reflect to the real in vivo situation, comparison and/or parallel measurements by different methods can characterize better the different aspects of this complex process.

Oxygen free radicals are highly reactive chemical species generated in biological systems during numerous physiological and pathophysiological processes. In physiological circumstances they play a role in cellular metabolism and cellular defense systems, on the other hand, large amount of oxygen free radicals is highly toxic for tissues and cells, because they can oxidatively modify and damage a variety of biological systems (e.g. cellular proteins, nucleic acids, carbohydrates, and lipids). Although cells have various defense systems against free radical damages including scavenger enzymes (e.g. superoxide dismutase, catalase, glutathion peroxidase) and nonenzymatic molecules (e.g. glutathion, ubiquinone [coenzyme Q], ascorbate, vitamin E), in case of impaired antioxidant defense or increased production of oxygen free radicals, these reactive agents can take part in formation of serious disorders. They are thought to be involved in a wide range of diseases such as atherosclerosis, ischemia-reperfusion injury, diabetes mellitus, inflammatory diseases and immunological disorders.

Because of the wide range of diseases involving oxygen free radical damages, scavenging these radicals should be considered as a basically important therapeutic approach.

2. AIMS OF THE INVESTIGATIONS

1. Our purpose was to examine the antioxidant properties of a novel, experimental antiarrhythmic-cardioprotective compound, H-2545 and its metabolite H-2954. Besides these new agents we aimed to study the antioxidant capacity of several clinically used cardiovascular drugs.

2. The pyrroline ring in the molecular structure of H-2545 was supposed to be responsible at least partially for the antioxidant capacity. To confirm this theory modification of the mexiletine and trimetazidine molecules with pyrroline ring was planned to be investigated. We wanted to compare the antioxidant properties of the new compounds and the modified drugs to those of the clinically used cardiovascular drugs.

3. Plasma fibrinogen concentration is an important risk factor of cardiovascular diseases. The role of fibrinogen concentration as a risk factor can be due to at least partially to its effect on hemorheological parameters. To challenge this theory the effects of different fibrinogen concentrations on the microrheological parameters, especially on the RBC aggregation were tended to be investigated. We wanted to study the effect of fibrinogen concentration on red blood cell aggregation both in vitro and in vivo. Our purpose was to compare two different RBC aggregation measurement methods to each other and to examine the relationship between RBC aggregation indices measured by these methods and plasma fibrinogen concentration.

4. To reveal the role of the rheological disorders in acute ischemic coronary syndromes we aimed to examine hemorheological parameters and platelet aggregation in patients with AICS. Besides the comparison of these parameters in patients with AICS and healthy subjects we planned the follow-up of their changes after discharge.

5. We wanted to estimate the biological effect of routine antiplatelet therapy in our patients. We intended to use platelet aggregometer in order to determine the level of platelet aggregation induced by different inductor agents.

3. MATERIALS AND METHODS

3.1. Antioxidant properties of different cardiovascular drugs

3.1.1. Sample preparation

Venous blood samples (40 ml) were taken from the antecubital vein of six healthy male volunteers into Vacutainer tubes containing sodium heparin as an anticoagulant. Blood samples were centrifuged at 2500 g for 10 minutes, then plasma and buffy coat were removed. Red cell suspensions were washed twice in phosphate buffered saline (PBS) (pH: 7.4, osmolality: 300 mOsm, glucose: 10 mM). After the last centrifugation supernatant was removed and RBCs were resuspended in PBS. The hematocrit of red blood cell suspension was adjusted to 20 % and suspensions were incubated with 1 mM phenazine methosulphate (PMS) at 37 °C, for 120 minutes. A cardiovascular drug was also added to the incubating medium at two concentrations in "treated" tubes: maximum serum concentration used in vivo, and five times higher concentration (experimental agents were examined also in other concentrations). PMS and drugs were not added to control samples. After incubation blood samples were centrifuged, supernatant was removed and hematocrit was adjusted to 10 % using PBS. Red blood cell filterability of these suspensions and potassium concentration of the supernatant were determined. During sample preparation microhematocrit centrifuge (Hemofuge, Heraeus Instr., Germany) was used to determine the hematocrit of suspensions.

3.1.2. Examined drugs

Carvedilol was provided by Boehringer Mannheim (Mannheim, Germany), the other clinically used drugs (procainamide, mexiletine, propafenon, metoprolol, timolol, sotalol, amiodarone, verapamil, nifedipine, trimetazidine and trolox) were purchased from Aldrich Chemical Co., Inc. (Milwaukee, USA). Experimental drug (H-2545), its metabolite (H-2954), trimetazidine, modified mexiletine (HO-2434), its metabolite (HO-2433) and modified trimetazidine (H-2921R) were synthesized in the Institute of Organic and Medicinal Chemistry of the University of Pécs.

3.1.3. Measurement of RBC filtration

Red blood cell filterability was measured by Carat FT-1 Filtrometer (CARAT Ltd., Hungary) using St. George's technique. In this filtrometer RBC suspension flows through a Nucleopore filter with 5 μm diameter pores. Filtration rate is measured at four pairs of light sources and detectors. The apparatus is interfaced to a computer, which automatically analyses sequential flow rates and thus distinguishes between cell transit time (relative cell transit time - RCTT) and pore clogging rate. Filtration pressure was set to 4 cm of water in our experiments. All measurements were carried out at room temperature (22 ± 1 °C) and were repeated three times at each sample.

Results were expressed in percent of decrease of PMS-induced increase in RCTT:

$$- (RCTT_{PMS} - RCTT_{PMS+cardiovascular\ drug}) / (RCTT_{PMS} - RCTT_{Control}) \times 100.$$

3.1.4. Measurement of potassium concentration

Potassium concentration of the supernatant was measured by OMSZOV OE 851 digital flame photometer (with propane-butane gas burner) (OMSZOV, Hungary) and lithium base solution was used.

Results were expressed in percent of decrease of PMS-induced potassium leaking due to drugs:

$$- (K^+_{PMS} - K^+_{PMS+cardiovascular\ drug}) / (K^+_{PMS} - K^+_{Control}) \times 100.$$

3.2. Relationship between fibrinogen concentration and RBC aggregation measured by different methods

3.2.1. Sample preparation

To determine the effect of fibrinogen concentration on RBC aggregation, measurements were performed in fibrinogen solutions. Blood samples were drawn from the antecubital vein of healthy adult volunteers into EDTA (1.5 mg/ml) and all measurements were performed within three hours after venesection. PBS was prepared as follows: 0.030 M KH_2PO_4 + Na_2HPO_4, 0.122 M NaCl; pH = 7.4,

295 ± 5 mOsmol/kg. Fibrinogen (2 % lyophilized human fibrinogen; American Diagnostica Inc., USA) was dissolved in PBS. Red blood cells were separated from whole blood via 2000 g centrifugation for 10 minutes, then, subsequently, plasma and buffy coat were removed. RBCs were washed three times, twice in PBS and third time in fibrinogen solution; residual portions of the buffy coat were discarded after each centrifugation. The washed RBCs were then resuspended in fibrinogen solutions (0.5, 0.6, 0.75, 0.9 and 1.0 mg/ml concentration, respectively) at a hematocrit of 41.0 ± 0.3 % for aggregation measurements in Myrenne aggregometer. All preparations and measurements were carried out at room temperature (22 ± 1°C).

For the comparative RBC aggregation measurements in whole blood, sixty two blood samples from subjects (including healthy volunteers, patients with ischemic heart disease and diabetes mellitus, mean age: 49.9 ± 16.7 years; 37 males, 25 females) were analyzed in Myrenne MA-1 RBC aggregometer and LORCA (laser-assisted optical rotational cell analyzer) aggregometer. The blood was drawn from the antecubital vein into Vacutainer tube containing lithium heparin (143 IU / 4.5 ml) and all measurements were performed at the native hematocrit of the samples within two hours after venesection. Hematocrit was measured by using microhematocrit centrifuge. Fibrinogen concentration was determined by Clauss's method. Temperature in LORCA was adjusted to 37 °C, all other preparations and measurements were carried out at room temperature (22 ± 1°C).

3.2.2. Aggregation measurements

RBC aggregation was measured in two instruments: Myrenne and LORCA aggregometers (blood samples were taken from the same tube).

1/ Myrenne aggregometer (Model MA-1 Aggregometer, Myrenne GmbH, Germany) employs the light transmission method of Schmid-Schönbein et al. through a transparent cone-plate shearing instrument. The principle of this technique is the increase of light transmission through a red cell suspension, which occurs when individual cells aggregate into rouleaux or rouleaux-rouleaux complexes; gaps in the suspending medium between the aggregates allow more light to pass through the RBC suspension. This aggregometer has two modes of operation: M and M1. For both modes blood sample (30 μl) is first sheared at 600 s^{-1} to disperse all pre-existing

aggregates, then shear rate decreases rapidly to zero (M mode) or low shear (3 s^{-1}: M1 mode). The extent of aggregation is characterized by the aggregation index (AI_M, AI_{M1}), calculated from the surface area below the light intensity curve in a 10 s period of time.

2/ Aggregation measurements by LORCA aggregometer (R&R Mechatronics, Netherlands) are based on the detection of laser back-scattering from the sheared (disaggregated), then unsheared (aggregating) blood, performed in a computer-assisted system. Blood samples (1 ml of oxygenated blood) are injected into the gap between the outer cylinder "cup" and the inner cylinder "bob" of LORCA. During the measurement the cup is driven by a computer controlled stepper motor and temperature is adjusted to 37 °C. Blood sample is sheared at 400 s^{-1}; then shear rate decreases rapidly to zero. Back-scattering data are evaluated by the computer and the aggregation index (AI_L) is calculated from the syllectogram (light scatter vs. time curve during a 120 s period) on the basis that there is less light back-scattered from aggregating red cells.

Data were evaluated as means ± S.E.M. (standard error of mean) by Student's t test and correlation analysis.

3.3. Hemorheological parameters in patients with acute ischemic coronary syndromes

3.3.1. Study population

125 patients with AICS (72 males and 53 females; mean age: 65 ± 12 years) and 68 healthy persons (30 males and 38 females; mean age: 36 ± 6 years) were investigated in a prospective study. All of the patients were admitted to our coronary care unit with acute ischemic heart symptoms and routine examinations (ECG, myocardial enzymes) proved the AICS in each case. 57 patients had significant ST elevation, 55 patients had ST depression or no ST deviation and 13 patients had left bundle branch block or ventricular pacemaker rhythm on admission. 45 patients were on antiplatelet therapy on admission, 34 of them took ASA (average dose: 140 mg/day), 4 of them took 100 mg ASA and 500 mg ticlopidine, 1 patient took 325 mg ASA and 500 mg ticlopidine, 2 patients took 100 mg ASA and 75 mg

clopidogrel, 2 patients took 500 mg ticlopidine, 1 patient took 250 mg ticlopidine and 1 patient took 75 mg clopidogrel daily. After admission thrombolysis was performed in 21 cases and percutaneous coronary intervention in 20 patients. Almost all the patients were on subcutaneous low molecular weight heparins and i.v. nitroglycerin during the acute phase. 29 of them received i.v. platelet IIb/IIIa receptor blocker. 120 patients were on antiplatelet therapy at discharge, 84 of them took ASA (average dose: 190 mg/day), 15 of them took 100 mg ASA and 500 mg ticlopidine, 7 patients took 325 mg ASA and 500 mg ticlopidine, 5 patients took 100 mg ASA and 75 mg clopidogrel, 3 patients took 500 mg ticlopidine and 6 patient took 75 mg clopidogrel daily. There was no relevant change in their medication after discharge.

Blood samples were taken from the cubital vein, and routine blood chemistry and hemorheological parameters - hematocrit, plasma fibrinogen level, plasma and whole blood viscosity, RBC aggregation, RBC deformability and platelet aggregation - were determined.

3.3.2. Hemorheological measurements

Hematocrit

Venous blood collected into lithium-heparin coated Vacutainer tubes was used to determine hematocrit. Hematocrit was measured by centrifuging hematocrit capillaries (80 µl, containing heparin) at 12000 rpm for five minutes in microhematocrit centrifuge (Hemofuge, Heraeus Instr., Germany). Measurements were performed at room temperature (22 ± 1 °C).

Plasma and whole blood viscosity

Venous blood samples were collected into lithium-heparin coated Vacutainer tubes for viscosity measurements. Plasma was prepared by centrifuging one tube of blood at 1500 g for ten minutes. Plasma and whole blood viscosities were determined in Hevimet 40 capillary viscosimeter (Hemorex Ltd., Hungary). 0.5 - 0.5 ml of plasma or whole blood was injected into the capillary tube of the device. In this viscosimeter the flow of the fluid is detected optoelectronically along the capillary

tube and a flow curve is drawn. Shear rate and shear stress are calculated from this curve by a computer program. Viscosity values are determined as a function of these parameters according to Casson's principle.

For the presentation of our results, apparent whole blood viscosity values calculated at 90 s^{-1} shear rate are given. Corrected whole blood viscosity was calculated with a mathematical formula according to Mátrai et al. In this formula apparent whole blood viscosity value at 90 s^{-1} shear rate (WBV_{HCT}) is used and correction is made to 40 % hematocrit:

$$WBV_{40\%} / PV = (WBV_{HCT} / PV)^{40\% / HCT}$$

Measurements were carried out at 37 °C within two hours after venepuncture.

Plasma fibrinogen

4.5 ml blood sample was drawn into a Vacutainer tube containing sodium citrate (0.129 M, 1:10 dilution) and plasma fibrinogen concentration was determined by using Clauss's method.

RBC aggregation

RBC aggregation measurements were carried out from venous blood samples collected into lithium-heparin coated Vacutainer tubes by Myrenne aggregometer (Model MA-1 Aggregometer, Myrenne GmbH, Germany) according to the previously described method. Measurements were performed at room temperature (22 ± 1 °C) and were carried out within two hours.

Measurement of RBC filtration

Red blood cell filterability was measured by Carat FT-1 filtrometer (CARAT Ltd., Hungary) according to the previously mentioned method. Measurements were performed at room temperature (22 ± 1 °C) and were carried out within three hours.

Platelet aggregation

Spontaneous, epinephrine-, ADP- and collagen-induced aggregation of platelets was analyzed. Blood was taken into tubes containing sodium citrate. Samples were centrifuged at 150 g for 10 minutes to produce platelet rich plasma (PRP), which was carefully removed for measurement; and then centrifuged further at 2500 g for 10 minutes to get platelet poor plasma (PPP). 450 µl PRP was measured against 450 µl PPP to determine spontaneous platelet aggregation. 50 µl of ADP (2 µM, 5 µM and 10 µM), epinephrine (5 µM, 10 µM and 15 µM) or collagen (2 µg/ml) was added to PRP so as to measure induced platelet aggregation. Platelet aggregation was measured in Carat TX4 platelet aggregometer (Carat Ltd., Hungary). The principle of its technique is similar to that of used by Myrenne aggregometer: the increase of light transmission through a platelet suspension, which occurs when individual platelets aggregate; gaps in the suspending medium between the aggregates allow more light to pass through the suspension. Activation of platelets, hereby platelet aggregation can be induced by different agents. To eliminate the differences between the absolute light transmission of different samples, PPP is used to determine the base intensity of the plasma and platelet aggregation indices are calculated with the ratio between the light transmission of PPP and PRP.

Antiplatelet medication was considered to be effective if the aggregation indices were lower than the range of untreated persons with 95 % confidence intervals. Measurements were carried out at 37 °C within two hours after venepuncture.

4. RESULTS AND CONCLUSIONS

4.1. Antioxidant properties of different cardiovascular drugs

The filterability of RBCs treated with PMS was reduced significantly compared to control samples, which referred to the increased rigidity of cells. Similarly to changes of RBC filtration, the potassium concentration of the supernatant was significantly higher in PMS treated samples, which again proved the RBC membrane damage.

H-2545 provided highly significant protection against both PMS-induced RCTT increase and potassium leaking. Moreover, H-2545 showed the best scavenger activity at the same concentration, which had the best antiarrhythmic effect without any side effects. The metabolite of the experimental agent, H-2954 also had significant antioxidant activity, however this was better at higher concentration. As this metabolite also has significant antioxidant activity, H-2545 can eliminate free radicals in two steps: in the first step the amino compound is oxidized to the hydroxylamine and in the second step to the nitroxide form, so the scavenger activity of the drug and the metabolite is added up.

All the examined class I antiarrhythmic drugs (procainamide, mexiletine and propafenon) had only a weak, but in some concentrations significant antioxidant effect, which was almost equal in the different subclasses. Although their scavenging activity was statistically significant, it is questionable if this effect can also be clinically significant.

Among the examined β-blockers (metoprolol, carvedilol, timolol) carvedilol had the strongest antioxidant effect, which was significantly higher comparing to those of the other drugs in this group. Metoprolol and timolol also showed a mild, but statistically significant antioxidant property. The significant antioxidant property of β-blockers in our study may imply a potential therapeutic significance in addition to their well-known effects.

In class III, sotalol had similar protective effect to RCTT and potassium leaking as β-blockers, while amiodarone did not show any antioxidant property. Although sotalol belongs to class III, it has strong β-blocker properties, which can explain our results.

Among Ca-channel blockers verapamil provided highly significant protection against PMS-induced damages. The other examined Ca-channel blocker, nifedipine did not have a significant scavenger effect. Ca antagonists are believed to be lipophilic to various degrees and would concentrate presumably in the lipid domains of the phospholipid membranes. Oxygen free radicals generate lipid peroxidation in these membranes, which increases membrane and thus cellular rigidity and disturbs the barrier and transport function of the cell membrane. A Ca-channel blocker with antioxidant property may prevent free radical damages in membranes.

Trimetazidine was able to protect RBCs from the effect of PMS only at higher concentration. Perhaps the lack of lipophilic property accounts for this result, as trimetazidine does not accumulate in plasma membrane, which is one of the main determining factors of RBC deformability.

Comparing to the examined cardiovascular drugs, Trolox (water-soluble form of vitamin E) provided more significant protection against oxidative damage. The scavenger effects of the new experimental agents reached or even exceeded the scavenger capacity of this well-known antioxidant substance. Moreover H-2545 showed significantly higher antioxidant property in therapeutic concentration than any of the examined drugs.

The scavenger effect of the modified drugs (mexiletine and trimetazidine derivatives with pyrrolin ring) was improved significantly after the modification. These agents showed more than three times higher protective effect against PMS-induced damages than the basic molecules. These results suggest that the pyrroline ring is responsible for a significant part of the antioxidant properties. The scavenger properties of the metabolite of the modified mexiletine were also investigated. Similarly to the metabolite of H-2545, HO-2433 also has antioxidant capacity, which suggests that drugs modified with the pyrroline ring can eliminate free radicals in two steps.

4.2 Relationship between fibrinogen concentration and RBC aggregation measured by different methods

Analyzing RBC aggregation indices in fibrinogen solutions, an increase of RBC aggregation measured by Myrenne MA-1 aggregometer could be noted at higher fibrinogen concentrations. Although correlation between fibrinogen concentration and RBC aggregation was significant, above a certain fibrinogen concentration (0.9 g/l) aggregation index did not show a further increase in vitro. Excluding samples with that high fibrinogen concentration the correlation became even stronger and the correlation coefficient was higher. These results may imply that above a certain level no more fibrinogen binding could occur at the surface of the cell.

Analyzing all the human whole blood samples, there was a significant relationship between plasma fibrinogen and AI_L, however, correlation between

fibrinogen and AI_M or AI_{M1} could not be proved. There was no significant correlation between AI_L and AI_M, whereas correlation of AI_L and AI_{M1} was significant. Further analysis based on the scatter plot diagram revealed that in human samples (similarly to the in vitro measurements) there is no further increase in the aggregation index above a certain fibrinogen concentration (4.5 g/l). After excluding samples with the above mentioned high fibrinogen concentration, significant correlation could be calculated between fibrinogen concentration, AI_L and AI_{M1}. Using the data of these samples only, significant correlation could be found not only between AI_L vs. AI_{M1}, but also between AI_L vs. AI_M. The lower in vitro fibrinogen concentration at the plateau phase reflects to that in vivo other factors beside fibrinogen play an important part in the aggregation / disaggregation process.

4.3. Hemorheological parameters in patients with acute ischemic coronary syndromes

Almost all the measured hemorheological parameters (hematocrit, plasma fibrinogen concentration, white blood cell count, plasma and whole blood viscosity, red blood cell aggregation and deformability) were significantly worse in patients with AICS at admission than in control subjects. Although, RBC aggregation index measured in M mode was not statistically different in the two groups that measured in M1 mode was significantly higher in patients than in control subjects.

During the hospital phase hematocrit values of patients with AICS showed a gradual decrease, which became significant after six days and was associated by a slight decrease of whole blood viscosity. On the first two days plasma fibrinogen, C-reactive protein and white blood cell count showed a significant elevation. As plasma fibrinogen is a major determinant of plasma viscosity, its elevation was associated with the elevation of plasma viscosity. During the hospital phase both of the examined microrheological parameters (RBC aggregation and filtration) were found prominently worse at admission and on day two. While some of the other hemorheological parameters decreased considerably by day 6, plasma fibrinogen and plasma viscosity still remained high, moreover, further elevation was noticeable, which calls the attention to the remained hemorheological risk at discharge. The elevation in fibrinogen might increase the risk of repeated acute coronary events,

since fibrinogen plays a central role in platelet and erythrocyte aggregation and is one of the main determinants of plasma and whole blood viscosity.

Hematocrit was elevated at the one-month sampling in correspondence with whole blood viscosity and they showed further elevation at the six-month and one-year examination. CRP values returned to the normal range after discharge, and remained there during the follow-up. White blood cell counts decreased significantly after the acute phase of the disease, but they were slight above than those of control subjects during the whole follow-up. Although plasma fibrinogen concentration decreased significantly after discharge and was moderately lower than the admission value, plasma viscosity did not show the same pattern during the follow-up. Among the microrheological parameters RBC filterability remained in the pathologic range during the entire follow-up period. Though a few hemorheological parameters decreased after the acute phase of the disease, several of them remained significantly higher than those of control subjects and some of them showed even an increase during the follow-up period. These alterations should draw attention to the rheological risk of these patients.

In this study we also examined the efficacy of the commonly used antiplatelet therapy. Our present aim was to estimate the effect of the routine therapy; the results of platelet aggregation measurements were not yet considered in the therapy after discharge. Although almost all the examined patients had some cardiovascular risk factors before the acute coronary event, only one third of them were on antiplatelet drugs regularly and this was measured to be efficient in less than half of the treated subjects. During the hospital phase the efficacy of platelet aggregation inhibitory therapy improved significantly and further enhancement could be seen after discharge. Despite of this improvement, the ratio of inadequately treated patients was still almost 30 % at each follow-up visits. These results show the failure of routine antiplatelet medication and support the importance of a guided therapy.

5. SUMMARY

1. We found that the novel, experimental antiarrhythmic-cardioprotective drug had highly significant antioxidant capacity. This property is presumably due to the molecular segment containing the pyrroline ring, hence the scavenger capacity of a clinically used antiarrhythmic drug was significantly improved by the modification with this segment. We could show that some of the clinically used cardiovascular drugs had significant antioxidant effects that could be useful in the clinical practice.

2. Our study showed that both Myrenne and LORCA red blood cell aggregometers were easily applicable in the clinical practice. Although LORCA aggregometer needs larger amount of blood, it seems to provide more precise information on red blood cell aggregation presumably due to its more standardized conditions. Further investigations in large population are needed to standardize these techniques and clarify the clinical significance of these measurements.

3. In a study we investigated and followed-up the hemorheological parameters in patients with acute ischemic coronary syndrome and estimated the efficacy of routine antiplatelet therapy. Both macrorheological and microrheological parameters were found significantly worse in patients than in healthy subjects. Moreover, some of these parameters showed further impairment after the acute event. Antiplatelet therapy was efficient in less than half of the treated patients at admission; and despite a significant improvement, the ratio of ineffectively treated patients was still considerable during the follow-up. Our results draw attention to the importance of hemorheological parameters as important risk factors of ischemic heart diseases and show the necessity of a more vigorous and guided antiplatelet therapy in the secondary prevention.

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