Human clinical hemorheological investigations in the field of cardiac rehabilitation, renal transplantation and pharmacological innovation

Ph.D. dissertation

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List of abbreviations

ACC	American College of Cardiology	**hsCRP**	high-sensitivity C-reactive protein
ADP	adenosine diphosphate	**i.e.**	id est
AHA	American Heart Association	**IL6**	interleukin 6
AI	aggregation index	**KDIGO**	Kidney Disease Improving Global Outcome
ASA	acetylsalicylic acid	**LDH**	lactate dehydrogenase
BAO	basic acid output	**LDL**	low-density lipoprotein
BMI	body mass index	**MCHC**	mean corpuscular hemoglobin concentration
BUN	blood urea nitrogen	**MCV**	mean corpuscular volume
CAD	coronary artery disease	**MET**	metabolic equivalent
CGRP	calcitonin gene-related peptide	**NR**	nonresistant
CMV	cytomegalovirus	**NR-PC**	nonresistant, positive control
COX	cyclooxygenase	**NR-RT**	nonresistant renal transplanted
CR	cardiac rehabilitation	**NSAID**	non-steroidal anti-inflammatory drugs
CV	cardiovascular	**PC**	positive control
e.g.	exempli gratia	**PDE**	phosphodiesterase
EBV	Epstein-Barr virus	**PPP**	platelet pure plasma
ECG	electrocardiography	**PRP**	platelet rich plasma
EI	elongation index	**PV**	plasma viscosity
EI_{max}	maximal elongation index	**R**	resistant
ESC	European Society of Cardiology	**RBC**	red blood cell
FDA	Food and Drug Administration	**R-PC**	resistant, positive control
FIS	fatigue impact scale	**R-RT**	resistant, renal transplanted
GGT	gamma-glutamyl transpeptidase	**RT**	renal transplanted
GMBF	gastric mucosal blood flow	**SCORE**	systemic coronary risk evaluation
GOT	glutamic-oxaloacetic transaminase	**$SS_{1/2}$**	shear stress required for half of EI_{max}
GPT	glutamic-pyruvic transaminase	**TNFα**	tumor necrosis factor α
HBsAg	hepatitis B surface antigen	**TRPV**	transient receptor potential vanilloid
Hct	hematocrit	**TXA2**	thromboxane A2
HCV	hepatitis C virus	**UGIC**	upper gastrointestinal complication
HDL	high-density lipoprotein	**UK-HARP-I**	United Kingdom Heart and Renal Protection
HIV	human immunodeficiency virus	**WBV**	whole blood viscosity

Prologue

In the past few decades, mortality of coronary artery disease has decreased substantially in the industrialized countries, but it still remains the leading cause of premature death worldwide. Two main preventive strategies were developed by Geoffrey Rose in 1985. The population strategy aims to reduce the incidence of CAD among a large population with long-time lifestyle and environment changes The high-risk strategy reaches patients with a high cardiovascular risk, and helps to manage the cardiovascular risk factors Although the population strategy is more cost-effective, the effective pharmacological therapy, the successful smoking cessation and cardiac rehabilitation programs resulted in an increased efficiency of the high-risk strategy. Nevertheless, the best prevention could be achieved by the combination of the two approaches.

The appropriate risk management requires precise risk stratification including consideration of all cardiovascular risk factors. To promote the quick but proper risk estimation, it is recommended to use the SCORE system currently. The SCORE system evaluates the 10-year risk of the first fatal atherosclerotic event e.g. myocardial infarction, stroke and peripheral arterial disease using age, gender, smoking, systolic blood pressure and total cholesterol level. Factors for risk assessment are age, gender and genetic background, also known as nonmodifiable parameters of a patient. The traditionally called “major” but modifiable (consequently preventable/treatable) risk factors are hypertension, dyslipidemia, diabetes mellitus, smoking, overweight, lack of regular physical activity. Other modifiable factors are the psychological factors, elevated high-sensitivity C-reactive protein, inflammatory cytokines (e.g. IL-6, TNF-α) and fibrinogen, inflammatory diseases, obstructive sleep apnoea, chronic kidney disease, abnormal hemorheological factors.

After risk stratification an appropriate risk factor management should be started. Correct risk factor management includes lifestyle changes i.e. changes in behavioral patterns, effective communication, stress management, smoking cessation intervention (a cornerstone in successful cardiovascular prevention) with or without pharmacological therapies, healthy diet (mediterranean diet in first line), moderate alcohol consumption (mainly red wine, containing antioxidants – French paradox), reaching BMI <25 kg/m^2 and >20kg/m^2 and regular physical activity (healthy subjects 2.5-5 hours weekly, cardiovascular patients 3X30 minutes weekly). Beneath lifestyle changes several conditions should be medically treated in case of clinical symptoms. Psychosocial problems could be managed by individualized or group psychotherapy and by certain medication. Appropriate treatment should be started for hypertension, diabetes mellitus type 1 and 2, hypercholesterinemia and hypertriglyceridemia and against thromboembolic events.

Clinical role of the hemorheological parameters and methodology

Hemorheology is focusing on the flow properties of the blood. In the last few decades several studies have indicated, that abnormal rheological factors should be handled as risk factors of cardiovascular and cerebrovascular diseases.

Hematocrit:

Hct is the mostly used hemorheological parameter in the clinical routine describing the percentage of the cellular fraction of the whole blood.

Hct was measured by a microhematocrit centrifuge (Haemofuge Heraeus Instr., Germany).

Whole blood and plasma viscosity:
Viscosity is the intrinsic friction of the fluids.
WBV is influenced by the hematocrit, PV, RBC aggregation (at low shear rates) and deformability (at high shear rates).
PV is determined by the quantity of the plasma proteins (fibrinogen and certain globulins) as well as the triglyceride level.
WBV and PV were determined at a shear rate of 90 s^{-1} with a Hevimet 40 capillary viscometer (Hemorex Ltd., Budapest, Hungary). Plasma was prepared by a 10-minute centrifugation of whole blood at 1500 g. Measurements were made at 37 °C.
The ratio of Hct/WBV was utilized to characterize the oxygen transport efficiency of the blood.

Red blood cell aggregation:
RBC aggregation is a reversible process and occurs at low shear conditions or at stasis.
RBC aggregation was measured with both Myrenne (MA-1 Aggregometer, Myrenne GmbH, Roentgen, Germany) and LORCA (Laser-assisted Optical Rotational Cell Analyzer; R&R Mechatronics, Hoorn, The Netherlands) aggregometers using blood samples with native Hct.

Red blood cell deformability:
RBC deformability is a pivotal ability of the cells to pass the capillary system which is required for sufficient tissue oxygenation. Deformability is determined by the internal viscosity of the cell, the membrane viscoelasticity, the surface-volume ratio and the morphology of erythrocytes.
RBC deformability was characterized with a LORCA ektacytometer at 37 °C. The deformability results were analyzed by the Lineweaver-Burke nonlinear equation calculating the maximal EI at infinite shear, and the shear stress value required for half of this maximal elongation. For deformability measurements, blood samples were suspended in a highly viscous (32.6 mPas) polyvinylpyrrolidone solution.

Platelet aggregation:
Platelet aggregation has a key role in the arterial thrombus formation. The activation of this process is mostly evoked by endothelial injury where free collagen releases ADP, epinephrine, serotonin and TXA2 from platelets.
Platelet aggregation measurements were performed by using a Carat TX4 optical aggregometer. Blood samples were centrifuged first at 150 g for 10 minutes to obtain PRP then the centrifugation was repeated at 2500 g to separate PPP. Following the separation procedure platelet aggregation was determined with Carat TX4 optical aggregometer (Carat Diagnostics Ltd, Budapest, Hungary) at 37 °C. Rate of the platelet aggregation was determined as the maximum point on the aggregation index curve and considered clinically significant and effectively inhibited below 40% in case of efficient Aspirin therapy.

Effects of moderate aerobic exercise training on hemorheological and laboratory parameters, and on psychological functioning in ischemic heart disease patients

Introduction

In the past few decades, mortality due to CAD has decreased in the industrialized countries thanks to the optimal pharmacological therapies, modern revascularization procedures and preventive strategies, but it is still the leading cause of death worldwide.

The recent guidelines of the ESC and the AHA/ACC indicate that physical activity has a pivotal role in the primary prevention in healthy subjects, and moreover it reduces the all-cause and CV mortality too.

Impaired hemorheological parameters may have a deleterious effect on the vascular system leading to the development of various CV, cerebrovascular and peripheral arterial diseases. Publications from the last 25 years have clearly revealed a relationship between hemorheological factors and physical training. The possible connections between hemorheology and long-term, moderate aerobic physical activity have not been investigated in a relatively large population with ischemic heart disease.

Our study had the aim of determining the beneficial effects of aerobic physical training on ischemic heart disease patients participating in a long-term (24 weeks) ambulatory CR program.

Methods

79 non-smoker patients with stable ischemic heart disease (39 males and 40 females, mean age: 65.3 ± 5.68 years) were selected for the study. The patients received their medication in accordance with current guidelines for the secondary prevention of CAD and with their co-morbidities. Patients with an ejection fraction < 40%, and MET < 5 or a significant ST depression during a treadmill were excluded from the study. The investigation was approved by the Regional Ethics Committee (licence number: 4378) of the University of Pecs and written informed consent was signed by all subjects.

Study design:

At baseline psychological tests, resting ECG, resting echocardiography, treadmill-based exercise tolerance testing using the Bruce protocol, clinical chemistry, cytokines (TNF-α and IL-6) and hemorheological measurements were performed.

The patients participated in a 24-weeks physical training program lasting for 1 hour 3 times weekly. After 12 weeks, the hemorheological measurements, clinical chemistry (except cytokines) and psychological tests were repeated. At the end of the 24 weeks, the resting ECG measurements, the treadmill tests with the Bruce protocol, the clinical chemistry, the hemorheological measurements and the psychological tests were repeated.

Hemorheological measurements:

Hemorheological measurements were performed according to the above mentioned methodological description (see page 3-4).

Cytokine measurements:

Cytokines were determined with an automated chemiluminescence immunoassay system (Immulite 1000, Siemens).

Psychological surveys:
In order to examine the effects of the 24-week physical training on the patients' subjective experience with fatigue, we applied the FIS. The FIS consists of 40 items which evaluate the impact of fatigue on three aspects of daily life: physical (10 items), cognitive (10 items) and psychosocial (20 items) functions.

Statistics:
Data are shown as means ± S.E.M. Differences were evaluated by a one-way repeated ANOVA statistical test (Tamhane post-hoc test) after using the Kolmogorov–Smirnov test to check on the normality of the data distribution. Multivariate linear regression and stepwise analyses of the data were performed with regard to differences between the baseline and the 24-week MET values.
The psychological data revealed a significant deviation from the normal distribution, and; the nonparametric Friedman test was therefore applied to analyze potential changes in psychological functioning. The analysis of psychological data was restricted to those patients who gave no indication of moderate or severe depression and had no missing surveys (68 patients).
Significance level was defined as $p < 0.05$. SPSS statistical software, version 11.0.1. was used to conduct descriptive analyses and to describe the sample.

Results
Hct displayed a decreasing tendency, while the WBV exhibited a significant reduction ($p<0.05$), resulting in a significantly increased Hct/WBV ratio ($p<0.05$). The PV was significantly decreased after 12 weeks and remained significantly lower at the end of the program ($p<0.001$) (Table 1).

hemorheological parameters	week	mean ± SEM	p value	significance (p<0.05)
whole blood viscosity (mPas)	0	4.35 ± 0.06	0.03	S
	12	4.26 ± 0.07		
	24	4.08 ± 0.08		
plasma viscosity (mPas)	0	1.27 ± 0.02	0.001	S
	12	1.18 ± 0.02		
	24	1.14 ± 0.02		
Hct / WBV (1/Pas)	0	10.2 ± 0.07	0.04	S
	12	10.25 ± 0.13		
	24	10.33 ± 0.19		

Table 1.

The RBC aggregation parameters of the Myrenne and LORCA aggregometers likewise demonstrated significant reductions ($p<0.001$) (Table 2).
The LORCA EIs of erythrocyte deformability increased significantly at 12 weeks compared to the baseline. The increase relative to the baseline was still significant after 24 weeks ($p<0.001$), but the difference between the 12 week and 24-week data was not significant.

The results are also supported by the Lineweaver-Burke nonlinear equation analyses showing a significantly higher EI_{max} and a significantly lower $SS_{1/2}$ ($p<0.05$).

hemorheological para0meters		week	mean ± SEM	p value	significance (p<0.05)
Myrenne	M	0	6.98 ± 0.22	<0,001	S
		12	5.92 ± 0.13		
		24	4.02 ± 0.12		
	M1	0	13.18 ± 0.37	<0,001	S
		12	12.59 ± 0.24		
		24	10.84 ± 0.21		
LORCA	AI	0	66.67 ± 0.67	0.04	S
		12	61.63 ± 1.90		
		24	62.25 ± 1.73		

Table 2.

The clinical chemistry parameters relating to uric acid, triglycerides, hsCRP and fibrinogen decreased significantly during the training period (Table 3).

clinical chemistry	week	mean ± SEM	p value	significance (p<0.05)
uric acid (umol/l)	0	336.39 ± 17.5	0.006	S
	12	327.17 ± 9.3		
	24	312.88 ± 10.7		
triglyceride (mmol/l)	0	1.70 ± 0.09	0.017	S
	12	1.56 ± 0.08		
	24	1.46 ± 0.09		
hsCRP (mg/l)	0	6.02 ± 1.04	<0,001	S
	12	2.98 ± 0.26		
	24	3.04 ± 0.27		
fibrinogen (g/l)	0	3.28 ± 0.14	0.03	S
	12	3.03 ± 0.06		
	24	3.15 ± 0.09		
IL 6 (pg/ml)	0	3.95 ± 0.71	0.06	NS
	24	1.88 ± 0.88		
TNF α (pg/ml)	0	11.53 ± 0.89	0.11	NS
	24	10.21 ± 0.43		

Table 3.

The cytokine measurements did not indicate a significant decrease ($p<0.05$), but only a falling trend as compared with the baseline values (Table 3).
The functional capacity described by the MET significantly improved ($p<0.001$), and the treadmill time increased significantly, by 17.4% ($p<0.001$). Patients lost weight, with the BMI undergoing a significant decrease during the trial ($p<0.001$) (Table 4).

treadmill	week	mean ± SEM	p value	significance (p<0.05)
MET	0	7.93 ± 0.27	4.87E-23	S
	24	9.87 ± 1.09		
treadmill time (min)	0	7.68 ± 2.17	0.001	S
	24	9.02 ± 2.18		
BMI (kg/m^2)	0	28.16 ± 0.62	8.06E-79	S
	24	25.45 ± 1.14		

Table 4.

predictor	standardized β	p value (p<0.05)
Δ whole blood viscosity	0.221	0.07
Δ plasma viscosity	-0.017	0.891
Δ LORCA aggregation index	-0.112	0.314
Δ BMI	-0.201	0,06
Δ elongation index (30Pa)	-0.290	0.018

Table 5.

For the Δ values between the baseline and 24-week measurements were calculated from every parameter showing a significant difference to the baseline. The Δ parameters, which were in positive or negative correlation to the MET were used for the multivariate linear regression analyses: five independent variables were investigated in association with the difference in MET (ΔMET was regarded as the dependent variable). Regression analysis showed that the predictive model provided a good fit to the data with a significant F value ($F(5)$, $p<0.000$), and the five predictors explained 76% of the difference in MET values ($R2 = 0.76$).
The results in Table 5 indicated that the RBC deformability value was a significant independent variable of the regression model and the most strongly related to the variation of the MET values (standardized $\beta = 0.29$). Furthermore, the analyses revealed a strong independent predictive association between the Δdeformability and the dependent variables.
In the course of the psychological study, there was no drop out, and no noteworthy CV event or unplanned hospitalization occurred. Analysis of the FIS data revealed a significant decline in the symptoms of fatigue in the physical ($chi2(2) = 6.12$, $p < 0.05$), the psycho-social ($chi2(2) = 7.09$, $p < 0.05$) and in the cognitive domain ($chi2(2) = 8.85$, $p < 0.05$). More specifically, patients' perception of their physical, cognitive, and social functional limitations caused by fatigue declined significantly over the course of the physical training period.

Discussion

Possible connections between hemorheology and long-term aerobic physical training in a relatively large ischemic heart disease population have not been investigated previously.

Our results pointed to a slight decrease in Hct, while significant decreases were observed in WBV and PV, resulting in a significantly increased Hct/WBV ratio. The RBC aggregation indices and the measured deformability parameters were also significantly enhanced at the end of the training program. The observed beneficial changes in the macrorheological parameters (Hct, viscosity) presumably reduce the CV risk of ischemic heart disease patients. Furthermore, the diameter of the narrowest capillaries in the body (3-5 μm), found in the myocardium, is appreciably less than the resting diameter of a normal RBC (7-8 μm), which highlights the importance of the microrheological parameters (RBC aggregation and deformability). Improvement in RBC aggregation and deformability support capillary flow, while the Hct/WBV ratio indicates a better oxygen-carrying capacity leads to a better oxygen supply of the cardiomyocytes. Our results may suggest that cardiac patients could achieve "hemorheological fitness" by participating in a physical training program for 24 weeks.

The found hemorheological alterations may also contribute to the better exercise tolerability proved by the treadmill test parameter MET and the treadmill time. Moreover, the multivariate linear regression analyses showed that the improvement in RBC deformability is an independent predictor of the positive changes in MET.

Of the clinical chemistry indicators, hsCRP and fibrinogen underwent significant decreases during our study, suggesting that regular long-term physical activity might exert a favorable effect on the inflammation status of patients with CAD. Overproduction of proinflammatory cytokines such as IL-6 or TNF-α could be a marker of chronic inflammation leading to provoked and accelerated atherosclerosis, with a higher risk of CV events and mortality. Our data demonstrated an almost significant decrease in IL-6 and TNF-α levels, suggesting that a longer training program might be required to achieve significant reductions in these parameters.

Hypertriglyceridemia is an independent CV risk factor and elevated serum uric acid level should be considered a risk factor for CV mortality. The significant reductions in our study may reflect a better metabolic state caused by the regular physical activity.

Overweight and obesity are associated with an elevated risk of death in CAD and of all-cause mortality. The BMI was significantly decreased by the end of the 24-week exercise training in our study. The results of the multivariate linear regression model indicated that the better physical exercise tolerance can not be explained merely by the decreases in BMI and obesity.

CAD is often accompanied by an increased subjective feeling of fatigue. Our psychological results showed a significant amelioration as concerns the FIS physical, psycho-social, and cognitive aspects, indicating an improvement in the quality of life among our patients.

Conclusions

Besides the anticipated improvement in functional capacity and the reduction in BMI, the regular, moderate-intensity, long-term physical activity led to favorable hemorheological changes, decreased level of inflammation and improvements in certain metabolic parameters, suggesting that these parameters may play important part in the positive effects of regular physical activity in patients with CAD.

Acknowledgement

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Aspirin resistance as cardiovascular risk factor after kidney transplantation

Introduction

The controlled legal background of the transplantation procedure, the development of modern surgical techniques and immunosuppressive drugs resulted in a better overall survival and life quality after kidney transplantation. Based on the high CV mortality of kidney transplanted patients, to follow the CV prevention guidelines of ESC and AHA/ACC could be highly recommended regarding lifestyle changes and medications used.

While ASA is a widely used antiplatelet agent in the primary and secondary prevention of cardio- or cerebrovascular diseases, ASA resistance could develop in certain circumstances.

For examining the origin of high CV mortality after kidney transplantation this recent study was aimed to investigate the rate of ASA resistance in a RT population and to compare their morbidity and mortality data to a PC population with high cardio- and cerebrovascular risk.

Methods

255 patients were selected into our study between 03/01/2009 and 31/10/2009 to determine the rate of ASA resistance after kidney transplantation. Results were compared to a PC group containing 346 age-matched cardio- and cerebrovascular disease patients recruited from the same time interval. The retrospective analysis of the follow-up period was continued for 3 years until 12/31/2012. Morbidity and mortality data of the participants were collected with retrospective data mining using the e-MedSolution program. Differences were evaluated by using chi-square test analyses. SPSS statistical software, version 11.0.1. was used to describe the sample.

Platelet aggregation measurements were performed 2 hours after blood sampling. The measurements were performed according to the above mentioned methodological description (see page 4).

Results

The demographical analyses demonstrated no differences concerning age and gender between the RT group and the PC group. Our data showed significantly higher rate of ASA resistance in the RT group (36.1%) compared to the PC group (26.9%) ($p<0.001$).

The analyses demonstrated a significantly higher incidence of myocardial infarction, hypertension and diabetes mellitus ($p<0.05$) and increasing incidence in tumor appearance and all-cause mortality of the RT group compared to the PC patients at the end of the three year follow-up period (Table 6).

	RT		PC		p	level of significance
	%	cases	%	cases		
all-cause mortality	2.74	7 / 255	1.44	5 / 346	0.26	NS
myocardial infarction	4.31	11 / 255	1.16	4 / 346	0.01	S
stroke	2.74	7 / 255	2.02	7 / 346	0.56	NS
hypertension	80.39	205 / 255	69.36	240 / 346	0.02	S
diabetes mellitus	28.63	73 / 255	20.81	72 / 346	0.02	S
tumor	8.63	22 / 255	5.78	20 / 346	0.17	NS

Table 6.

The subgroup analyses at finishing of the three-year long follow-up revealed significantly higher incidence of myocardial infarction and stroke in the R-RT group compared to the NR-RT patients ($p<0.05$) (Table 7), increasing occurrence was found in connection with myocardial infarction and stroke as well as in diabetes mellitus in the R-PC group compared to the NR-PC patients (Table 8).

	R-RT		NR-RT		p	level of significance
	%	cases	%	cases		
all-cause mortality	3.26	3 / 92	2.45	4 / 163	0.70	NS
myocardial infarction	5.43	5 / 92	3.68	6 / 163	0.03	S
stroke	4.34	4 / 92	1.84	3 / 163	0.02	S
hypertension	77.17	71 / 92	82.21	134 / 163	0.33	NS
diabetes mellitus	33.70	31 / 92	25.77	42 / 163	0.17	NS
tumor	8.70	8 / 92	8.59	14 / 163	0.90	NS

Table 7.

	R-PC		NR-PC		p	level of significance
	%	cases	%	cases		
all-cause mortality	2.20	2 / 91	1.18	3 / 255	0.48	NS
myocardial infarction	2.20	2 / 91	0.78	2 / 255	0.27	NS
stroke	3.30	3 / 91	1.56	4 / 255	0.31	NS
hypertension	70.33	64 / 91	69.02	176 / 255	0.81	NS
diabetes mellitus	14.29	13 / 91	23.14	59 / 255	0.17	NS
tumor	5.49	5 / 91	5.88	15 / 255	0.89	NS

Table 8.

Comparison of the R-RT and R-PC subgroups at the completion of the three years follow-up showed significantly higher incidence of diabetes mellitus ($p<0.05$), besides an increasing incidence regarding myocardial infarction, hypertension and tumor occurrence (Table 9).

	R-RT		R-PC		p	level of significance
	%	cases	%	cases		
all-cause mortality	3.26	3 / 92	2.20	2 / 91	0.65	NS
myocardial infarction	5.43	5 / 92	2.20	2 / 91	0.25	NS
stroke	4.34	4 / 92	3.30	3 / 91	0.71	NS
hypertension	77.17	71 / 92	70.33	64 / 91	0.29	NS
diabetes mellitus	33.70	31 / 92	14.29	13 / 91	0.01	S
tumor	8.70	8 / 92	5.49	5 / 91	0.39	NS

Table 9.

At the end of the follow-up period the incidence of myocardial infarction and hypertension was significantly higher in the NR-RT than in the NR-PC patients ($p<0.05$) (Table 10).

	NR-RT		NR-PC		p	level of significance
	%	cases	%	cases		
all-cause mortality	2.45	4 / 163	1.18	3 / 255	0.32	NS
myocardial infarction	3.68	6 / 163	0.78	2 / 255	0.03	S
stroke	1.84	3 / 163	1.56	4 / 255	0.83	NS
hypertension	82.21	134 / 163	69.02	176 / 255	0.00	S
diabetes mellitus	25.77	42 / 163	23.14	59 / 255	0.54	NS
tumor	8.59	14 / 163	5.88	15 / 255	0.28	NS

Table 10.

Discussion

The main cause of mortality (35-40%) after kidney transplantation has CV origin generated by the presence of the conventional CV risk factors as well as the non-traditional risk factors like microalbuminuria, uremia, hyperuricemia, calcium and phosphor imbalance and the used immunosuppressive medications. The UK-HARP-I study examined 187 patients after kidney transplantation in connection with the effect of low-dose ASA treatment. This investigation has demonstrated that ASA therapy elevated the risk for minor bleeding complications 3 times higher. Another retrospective study has described that low-dose ASA therapy is associated with better graft survival in renal transplanted patients. The KDIGO guideline has indicated the low-dose ASA therapy after kidney transplantation and the efficiency control of the medication has also been suggested.

Our study revealed a significantly higher incidence of myocardial infarction in the RT group compared to the group of PC patients. This elevated level of CV events may partly be explained by the significantly higher rate of ASA resistance observed among the RT patients compared to the PC group.

Our results also demonstrated that the incidences of myocardial infarction and stroke were significantly higher in the RT group compared to the NR-RT patients. On the other hand these differences between the ASA resistant and the non-resistant subgroups of the control patients were not significant. Prior to this study no evidence was found regarding the CV risk reduction effect of low-dose ASA treatment after kidney transplantation which seems to be more effective than in the population of cardio- and cerebrovascular patients.

The analysis revealed higher incidence of diabetes and hypertension among RT patients showing the important role of these diseases in the development of chronic kidney failure. Higher incidence of diabetes was observed in the R-RT group compared to the R-PC population suggesting a predictive role of diabetes in ASA resistance after kidney transplantation.

Although ASA resistance presumably contributes to the elevated incidence of cardio- and cerebrovascular events after kidney transplantation, higher occurrence of myocardial infarction was found in the NR-RT group than in the NR-PC population. These results suggest that efficient ASA therapy could reduce the CV risk after kidney transplantation but the residual risk remains higher than in the cardio- and cerebrovascular patients emphasizing the role of other factors in the development of the high CV risk.

Conclusion

Our recent study revealed new data about the CV risk of RT patients using low-dose ASA treatment. Authors assume that the control measurements of antiplatelet therapy after kidney transplantation would be clinically useful.

Obviously, further randomized, controlled studies with more patients and longer follow-up period are needed to describe the magnitude of the ASA resistance-induced CV risk increase, as well as the effectiveness of the available therapeutical possibilities (higher dose of ASA, conversion to clopidogrel or prasugrel in case of ASA resistance).

Orally given gastroprotective capsaicin does not modify Aspirin-induced platelet aggregation in healthy male volunteers; human phase I. clinical study

Introduction

CV prevention applies three different platelet aggregation inhibitor agents: a) selective COX inhibitors; b) P2Y12 ADP receptor antagonists; c) in Asian countries, PDE-3 inhibitor. ASA is an important antiplatelet agent in the primary and secondary preventive care of cardio- or cerebrovascular events. But ASA has Janus-faced characteristics; on one hand it prevents cardio,- and cerebrovascular diseases, on the other hand causes gastrointestinal mucosal damages (bleedings, ulcers).

The effect of capsaicin has been widely studied in the gastrointestinal tract. Szolcsányi and Barthó (1981) clearly described the beneficial and harmful effects of capsaicin in peptic ulcer disease of rats depending on the applied doses of capsaicin. The pathomechanism has not yet been described exactly, but several investigations have suggested that capsaicin activates TRPV1, which releases CGRP and substance P in the gastric mucosa.

Aims of the recent study were: a) to investigate the tolerability of capsaicin alone and in co-administration with 500 mg ASA; b) to measure the pharmacokinetic parameters of capsaicin alone and in combination with ASA (the measured kinetic parameters were capsaicin, ASA and salicylic acid, as a metabolite of ASA); c) to study the ASA-induced platelet aggregation in view of ASA absorption and its metabolic rate; d) to evaluate the capsaicin action on the platelet aggregation and on the ASA-induced platelet aggregation inhibition.

The study protocol was approved by the Hungarian Institute of Pharmacy (Budapest, Hungary) and by the National Clinical Pharmacological and Ethical Committee (Budapest, Hungary) (Protocol number: 1.4.1; EudraCT: 20008-0070048-32). Before the enrollment each volunteers gave their signed informed consent.

Methods

The administered capsaicin (Asian Herbex Ltd, Andhra Pradesh, India) was approved for oral drug administration by the Food and Drug Administration (FDA: “17856 A II 26.10.2004 Asian Herbex Ltd: Capsicum USP as manufactured in Andhra Pradesh, India). ASA (DC -90 Aspirin) was synthesized in Shandong Xinhua Pharmaceutical Co. Ltd., China. Preparation of the administered drug and the capsaicin-ASA combination was performed by PannonPharma Co. Ltd. Pécsvárad, Hungary. Pharmacokinetic measurements were carried by using High Pressure Liquid Chromatographic methods.

The trial was conducted in a randomized single-blind manner. According to the findings of a screening process 15 healthy male volunteers were selected for the trial. After 10 hours of fasting, blood was drawn for pre-dose sample and pharmacokinetic measurements, then subjects received one of the five different therapies. Following the drug administration, 8.1 ml of blood was collected for platelet aggregation measurements in the 1st, 2nd, 6th and 24th hour.

Platelet aggregation measurements were performed 2 hours after blood sampling. The measurements were performed according to the above mentioned methodological description (see page 4).

Statistics:

Mean values of the ASA monotherapy in the 1st, 2nd, 6th and 24th hour samples were evaluated by paired Student’s t-test after using the Kolmogorov–Smirnov test to check the

normality of the data distribution. Differences of the mean values in every therapy were calculated by one-way ANOVA test, using Dunnett's post-hoc test. Test results were considered as significant at $p<0.05$. Data are shown as means±SD. SPSS statistical software, version 11.0.1. was used to conduct descriptive analyses and to describe the sample.

Results

Based on the results of the one-way ANOVA analysis, 400 and 800 µg of capsaicin monotherapy caused no significant differences on platelet aggregation (Fig. 1).

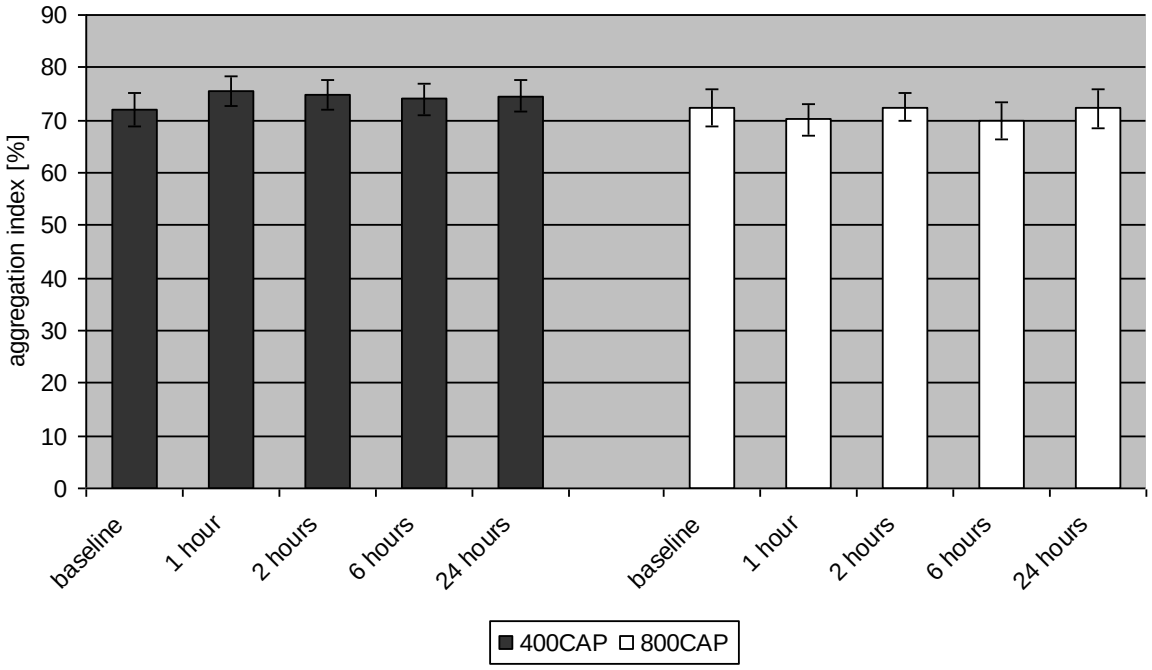

Figure 1.

ASA treatment resulted in a significant and clinically effective platelet aggregation inhibition ($p\leq0.001$), and the two different doses of capsaicin in combination with ASA reached an ASA monotherapy-equivalent level on platelet aggregation (Fig. 2).

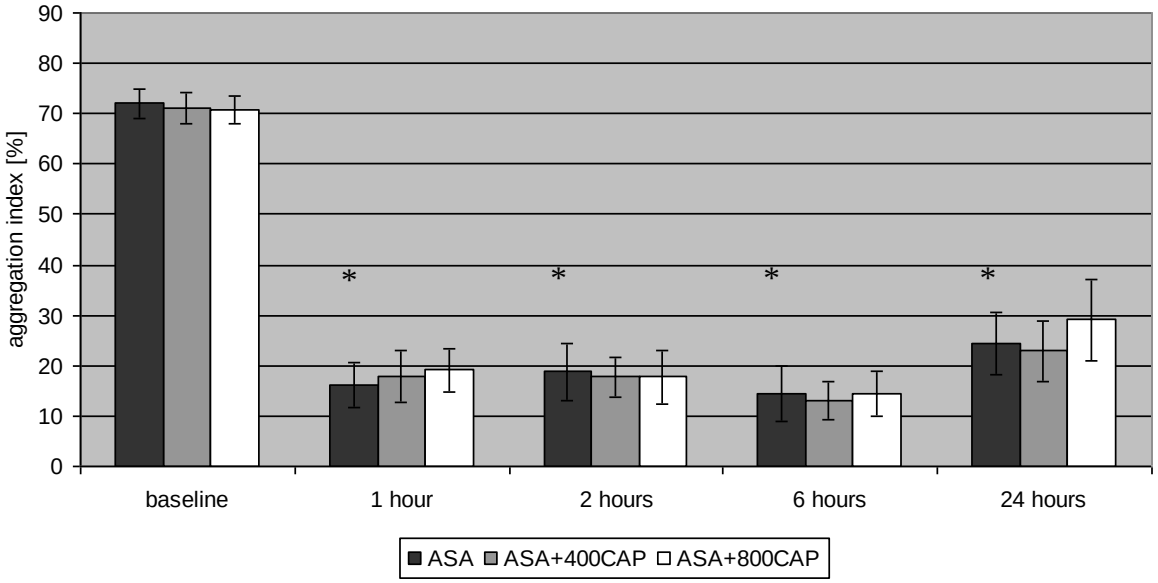

Figure 2.

Neither capsaicin nor dihydrocapsaicin were detected in the sera at any time after administration of capsaicin alone or in combination with ASA. When ASA therapy was administered alone, T1/2 and Tmax values (hr) were 0.559 ± 0.280 and 0.644 ± 0.294 for ASA and 2.545 ± 0.600 and 1.811 ± 0.963 for salicylic acid (mean±SD), respectively. These values did not change significantly after the ASA-capsaicin combination treatment. Cmax value (ng/mL) was 5219.20 ± 1723 for ASA and 21974 ± 3309 for salicylic acid (mean±SD) after ASA administration alone, and these values did not show significant alteration after co-administration of ASA and 400 and 800 μg capsaicin. No significant changes were observed in other pharmacokinetic parameters when ASA was given alone or in co-administration with capsaicin.

Discussion

ASA belongs to the NSAIDs and has various side effects including intracranial bleeding, other non-GI bleeding, tinnitus, dizziness, headache, impaired hearing, hypersensitivity reactions and UGIC, which can later become the origin of a GI bleeding or perforation. High percentages (10-20%) of the ASA users observe dyspepsia, initiating several UGIC (i.e. peptic ulcer, bleeding and perforation). UGIC can be observed among ASA-user patients as 2-3 cases per 1000 patients per year, thus the relative risk for UGIC is 3 to 5 times increased as in the ASA non-user population. For the prevention of UGIC several gastroprotective agents were developed and used in clinical care: a) misoprostol; b) proton pump inhibitors; c) histamine-2 receptor antagonists. Szabo et al. in one trial with more than 100 healthy volunteers showed the BAO decreasing effect of small doses, orally administered capsaicin (200 – 400 – 800 μg). Regarding the still limited numbers of human studies with orally given capsaicin the aim of this study was to investigate whether the pharmacokinetic and antiplatelet effect of ASA could be influenced by two, low, doses of capsaicin.

Our study showed several important results: a) low doses of capsaicin, 400 and 800 μg had no effect on platelet aggregation; b) the combined therapies did not result in any unfavorable influences on the ASA-induced inhibition in platelet aggregation; c) the pharmacokinetic properties of ASA and ASA metabolites were not influenced by the administration of capsaicin; d) concentrations of capsaicin and the metabolites were under the detection limit in sera. In our ex vivo study, the pharmacokinetic results suggest that capsaicin could not be detected in the systemic circulation.

Based on these results, we suppose that low doses of capsaicin do not have any systemic effect and do not influence the antiplatelet effect of ASA. According to the pharmacokinetic findings, capsaicin does not influence the absorption or any other pharmacokinetic characteristics of ASA as well. Furthermore, capsaicin could have only a local influence on the gastric mucosa and therefore could prevent the ASA induced mucosal lesions directly.

Conclusion

Since we found no significant interaction and side effects, our trial could be the first step in a new pharmaceutical development process to study whether capsaicin could be used with ASA in a fix combination to provide an antiplatelet drug with less GI side effects.

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Number of the accepted protocol is 1.4.1, EudraCT: 2008-007048-32.

Summary of the new scientific results

1) Effects of moderate aerobic exercise training on hemorheological and laboratory parameters, and on psychological functioning in ischemic heart disease patients:

1. Long-term (24 week) moderate exercise training improves hemorheological factors in patients with ischemic heart disease. Our results may suggest that cardiac patients could achieve a condition of "hemorheological fitness" by participating in a physical training program for 24 weeks.

2. The multivariate linear regression analyses showed that the improvement in RBC deformability is an independent predictor of the positive changes in MET.

2) Aspirin resistance as cardiovascular risk factor after kidney transplantation:

1. The analysis revealed a significantly higher incidence of myocardial infarction in the RT group compared to the group of PC patients. This elevated level of cardiovascular events may partly be explained by the significantly higher rate of ASA resistance observed among the RT patients compared to the PC group.

2. Our results showed that the incidences of myocardial infarction and stroke were significantly higher in the RT group compared to the NR-RT patients. These differences between R and NR subgroups of the PC patients were not significant.

3. Higher incidence of diabetes was observed in the R-RT group compared to the R-PC population suggesting a predictive role of diabetes in ASA resistance after kidney transplantation.

4. Our results suggest that efficient ASA therapy could reduce the CV risk after renal transplantation but the residual risk remains higher than in the cardio- and cerebrovascular patients.

3) Orally given gastroprotective capsaicin does not modify Aspirin-induced platelet aggregation in healthy male volunteers (human phase I. examination):

1. Low, gastroprotective doses of capsaicin monotherapy caused no significant differences on platelet aggregation in healthy male volunteers. Prior to our study no results were published about the antiplatelet effect of gastroprotective doses of capsaicin in humans.

2. Low, gastroprotective doses of capsaicin in combination with 500 mg ASA reached an ASA monotherapy-equivalent level on platelet aggregation. To confirm these findings, the pharmacokinetic tests showed that the pharmacokinetic properties of ASA and ASA metabolites were not influenced by the administration of capsaicin.

3. The pharmacokinetic investigations revealed that the concentrations of capsaicin and the metabolites were under the detection limit in sera. Accordingly we supposed that low, gastroprotective doses of capsaicin do not have any systemic effect and so could not influence the antiplatelet effect of ASA, the absorption or any other pharmacokinetic characteristics of ASA as well. Furthermore, capsaicin could have only a local influence on the gastric mucosa and therefore could prevent the ASA induced mucosal lesions directly.

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Publications supporting the dissertation

Papers

1. *B. SANDOR*, A. VARGA, M. RABAI, A. TOTH, J. PAPP, K. TOTH, P. SZAKALY Aspirin resistance as cardiovascular risk after kidney transplantation. KARJ. DOI: 10.1007/s13367-014-0027-z (2014)
Impact factor: 0.632

2. *B. SANDOR*, J. PAPP, GY. MOZSIK, J. SZOLCSANYI, I. JURICSKAY, K. TOTH, T. HABON Capsaicin does not modify Aspirin-induced platelet aggregation (human phase I. examination). Acta Physiol Hung. Accepted for publication (2014)
Impact factor: 0.747

3. GY. MOZSIK, T. PAST, T. HABON, ZS. KESZTHELYI, I. SZABO, *B. SANDOR*, J. SZOLCSANYI, M. SZALAI Orally given capsaicinoids do not modify the absorption, metabolism, and excretion of aspirin and its platelet aggregation in human male healthy subjects (human clinical pharmacological phase. i. examinations). JACP. 1, 31-54. (2014)

4. *B. SANDOR*, A. NAGY, A. TOTH, M. RABAI, B. MEZEY, I. CZURIGA, K. TOTH, E. SZABADOS Effects of moderate aerobic exercise training on hemorheological and laboratory parameters in ischemic heart disease patients. PLoS One, Accepted for publication (2014)
Impact factor: 3.534

5. A. NAGY, E. SZABADOS, A. SIMON, B. MEZEY, *B. SÁNDOR*, I. TIRINGER, K. TÓTH, Á. CSATHÓ Association of exercise capacity with different aspects of fatigue in patients with ischemic heart disease. J Cardiopulm Rehabil Prev. Under publication (2014)
Impact factor: 1.679

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3. *B. SANDOR*, J. PAPP, GY. MOZSIK, J. SZOLCSANYI, I. JURICSKAY, T. HABON, K. TOTH Capsaicin does not influence the inhibitory effect of acetylsalicylic acid on platelet aggregation – a human clinical phase I study. 14th International congress of Biorheology and 7th International Conference on Clinical Hemorheology 4-7 July 2012, Isztambul, Biorheol 49, 109-110. (2012)

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