ORIGINAL PAPERS

Adv Clin Exp Med 2007, **16**, 1, 21–27
ISSN 1230-025X

Marlena Broncel[1], Piotr Duchnowicz[2], Maria Koter-Michalak[2],
Eliza Lamer-Zarawska[3], Julita Chojnowska-Jezierska[1]

In Vitro Influence of Baicalin on the Erythrocyte Membrane in Patients with Mixed Hyperlipidemia

Wpływ bajkaliny *in vitro* na błonę erytrocytarną u pacjentów z mieszaną hiperlipidemią

[1] Department of Internal Diseases with Clinical Pharmacology and Therapy Monitoring Unit, Medical University of Lodz, Poland
[2] Department of Environmental Pollution Biophysics, University of Lodz, Poland
[3] Department of Biology and Botany Silesian Piasts University of Medicine in Wrocław, Poland

Abstract

Background. *Scutellaria baicalensis Georgi* is a popular herb used in China and Japan. It is suspected that baicalin, a potent antioxidative and anti-inflammatory agent, may prevent or slow down the development of atherosclerosis.
Objective. The aim of the study was to evaluate the *in vitro* effect of baicalin on lipid peroxidation (thiobarbituric acid reactive substances, TBARS), cholesterol content, and ATPase activity of erythrocytes from patients with untreated mixed hyperlipidemia.
Material and Methods. The study involved 48 patients with total cholesterol (TC) > 200 mg/dl, LDL cholesterol (LDL-C) > 160 mg/dl, and triglycerides (TG) > 150 mg/dl and 15 healthy persons as the control group. The TBARS concentrations were determined by the Stocks and Dormandy method. The cholesterol concentration was determined using Liberman-Burchard reagent. The activity of Na^+K^+-ATPase was measured by a modified method of Bartosz. The studied parameters were assessed after 24-hour incubation of either whole blood or 2% suspensions of erythrocytes from hyperlipidemia patients and healthy controls with or without a 10 μM baicalin solution.
Results. After incubation, the 2% suspensions of erythrocyte from patients with mixed hyperlipidemia with baicalin showed a significant decrease in TBARS (0.293 ± 0.071 vs. 0.202 ± 0.07 μmol/mg hemoglobin, $p < 0.01$), cholesterol content (4.19 ± 0.72 vs. 2.09 ± 0.61 mg cholesterol/packed cells, $p < 0.01$), and Na^+K^+-ATPase activity (121.94 ± 53.8 vs. 61.24 ± 32.8 nmol Pi/mg proteins x h, $p < 0.01$) compared with the values obtained after incubation without baicalin. Similar changes were noted after incubation of the whole blood of patients with baicalin. In the control group, incubation of both whole blood and the 2% erythrocyte suspensions with baicalin did not show any significant changes in these parameters.
Conclusion. Baicalin shows *in vitro* antioxidant activity, decreases the concentration of cholesterol, and inhibits the activity of Na^+K^+-ATPase in whole blood and 2% erythrocyte suspensions of patients with mixed hyperlipidemia (**Adv Clin Exp Med 2007, 16, 1, 21–27**).

Key words: baicalin, ATPase activity, cholesterol, peroxidation, hyperlipidaemia.

Streszczenie

Wprowadzenie. *Scutellaria baicalensis Georgi* jest popularnym ziołem stosowanym w Chinach i Japonii. Przypuszcza się, iż przez działanie antyoksydacyjne, przeciwzapalne, tarczyca bajkalska może być w przyszłości stosowana jako lek zapobiegający lub zwalniający rozwój zmian miażdżycowych.
Cel pracy. Ocena wpływu bajkaliny w warunkach *in vitro* na peroksydację lipidów (TBARS – poziom substancji reagujących z kwasem tiobarbiturowym), zawartość cholesterolu oraz aktywność ATP-azy w erytrocytach pacjentów z mieszaną hiperlipidemią.
Materiał i metody. Badaniem objęto 48 pacjentów z wyjściowym stężeniem cholesterolu całkowitego (TC) > 200mg/dl, cholesterolu LDL (LDL-C) > 160 mg/dl, triglicerydów TG >150 m/dl oraz 15 osób zdrowych stanowią-

cych grupę kontrolną. Stężenie TBARS oznaczono metodą według Stocksa i Dormandy'ego [9]. Stężenie cholesterolu z zastosowaniem odczynnika Libermana-Burcharda [11], aktywność ATPazy Na^+K^+ według zmodyfikowanej metody Bartosza [13]. Badane wskaźniki oceniano po 24 h inkubacji (pełnej krwi oraz 2% zawiesiny erytrocytów pobieranych od pacjentów z hiperlipidemią i od osób zdrowych) z 10 μM roztworem bajkaliny i bez bajkaliny.
Wyniki. Po inkubacji z bajkaliną 2% zawiesiny erytrocytów pobieranych od pacjentów z mieszaną hiperlipidemią obserwowano istotne zmniejszenie stężenia TBARS (0.293 ± 0.071 *vs.* 0.202 ± 0.07 μmol/mg hemoglobiny, $p < 0.01$), cholesterolu (4.19 ± 0.72 *vs.* 2.09 ± 0.61 mg cholesterol/liczbę upakowanych komórek, $p < 0.01$) oraz aktywności ATPazy Na^+K^+ (121.94 ± 53.8 *vs.* 61.24 ± 32.8 nmol fosfolipidów/mg białka x h, $p < 0.01$) w porównaniu do wartości uzyskanych po inkubacji zawiesiny bez bajkaliny. Podobne zmiany obserwowano po inkubacji z bajkaliną pełnej krwi pacjentów z hiperlipidemią. W grupie kontrolnej nie stwierdzono istotnych zmian badanych wskaźników zarówno po inkubacji pełnej krwi, jak i 2% zawiesiny erytrocytów z bajkaliną.
Wniosek. W warunkach *in vitro* bajkalina wykazuje działanie antyoksydacyjne, zmniejsza stężenie cholesterolu i hamuje aktywność ATPazy Na^+K^+ w pełnej krwi i 2% zawiesinie erytrocytów pobieranych od pacjentów z mieszaną hiperlipidemią (**Adv Clin Exp Med 2007, 16, 1, 21–27**).

Słowa kluczowe: bajkalina, aktywność ATPazy, cholesterol, peroksydacja, hiperlipidemia.

Scutellaria baicalensis Georgi is a popular herb used in China and Japan. The fragmented root of a 2- to 3-year-old plant is used for medical purposes. It contains over 40 various flavonoids, the most important being the four lipophylic ones: the most abundant, baicalin, its aglicon baicalein, and vogonosid and its aglicon ogonin. Many beneficial features of baicalin have been demonstrated in studies, among others its strong anti-oxidative [1–3], anti-inflammatory [4], anti-cancer [5], and hepatoprotective [6] properties. Because of its wide spectrum of pharmacological activity, numerous attempts have been made to apply baicalin as a co-therapeutic agent in the treatment of numerous diseases. It is suspected that baicalin, as a potent anti-oxidative and anti-inflammatory agent, may prevent or slow down the development of atherosclerosis.

Hyperlipidemia is a major risk factor for the development and progression of atherosclerosis and coronary heart disease and it is associated with various alterations in cell reactivity and membrane properties. In erythrocytes, hyperlipidemia is accompanied by increased cell membrane cholesterol content and peroxidation of fatty acids and by decreased membrane fluidity and ATPase activity [7]. This, in turn, causes changes in cell properties: erythrocytes become less flexible, they lose the ability to adjust their shape to the vessel diameter, and aggregate, accelerating the development of atherosclerosis [8]. Because of the versatility of baicalin's properties on the one hand and the lack of data in the literature about its effect on cell membrane on the other, this study was carried out to evaluate the in vitro effects of baicalin on the cholesterol content, lipid peroxidation, and the ATPase activity in whole blood and erythrocyte suspensions of patients with untreated mixed hyperlipidemia.

Materials and Methods

Patients

Forty-eight patients (29 men and 19 women) with mixed hyperlipidemia, aged 40 to 65 years (mean age: 56.73 ± 7.58), participated in the study. These patients were selected on the basis of their plasma total cholesterol (TC) > 200 mg/dl, LDL cholesterol (LDL-C) > 160 mg/dl, and triglycerides (TG) > 150 mg/dl. The exclusion criteria were hypertension, diabetes, renal, hepatic or metabolic disease, coronary heart disease, secondary hyperlipidemia, alcohol abuse, smoking, and obesity (BMI > 35 kg/m^2). Patients receiving treatment with drugs capable of modifying lipid metabolism were also excluded. The control group consisted of 15 healthy individuals (10 men, 5 women, mean age: 56.9 ± 6.35).

These experiments were in accordance with the ethical standards as formulated in the Helsinki Declaration of 1975 (revised 1983) (Consent Number RNN/58/03/KB of the Commission of Medical Research Ethics of the Medical University of Lodz, Poland).

Lipid Analysis in the Plasma

The serum levels of TC, LDL-C, HDL cholesterol (HDL-C), and TG were determined colorimetrically using commercial kits (bioMerieux, France) 12 h after the last meal. The results were expressed in mg/dl of plasma.

Erythrocytes

Blood samples were obtained between 9 and 10 a.m. after a 12-hour overnight fast from patients with mixed hyperlipidemia. Blood collected into ACD (solution consisting of 23 mM of citric acid, 45.1 mM of sodium citrate, and 45 mM

of glucose) was centrifuged at 3000 rpm for 10 min at 4°C to separate plasma and red blood cells. Erythrocytes were washed three times with buffered 0.9% NaCl and suspended in the incubation solution (140 mM NaCl, 10 mM KCl, 1.5 mM $MgCl_2$, 10 mM glucose, 10 mM HEPES, 100 μg/ml streptomycin, and 0.05 mM TRIS-HCl, pH 7.4) at a hematocrit of 2%. Both the whole blood and the 2% suspension of erythrocytes were incubated for 24 hours at 37°C with or without a 10 μM of baicalin (isolated from the root of *Scutellaria baicalensis Georgi*) under non-hermetic conditions. After the incubation with and without baicalin, TBARS level, cholesterol content, and Na^+K^+-ATPase activity in the group of patients with mixed hyperlipidemia and in the control group were assessed.

Peroxidation of Lipids

Lipid peroxidation was determined as the compounds reacting with thiobarbituric acid (TBA) according to the method of Stocks and Dormandy [9].

Extraction and Measurement of Lipids

Extraction of lipids from erythrocytes was carried out using the method of Rodriguez-Vico et al. [10]. The concentration of cholesterol was determined using Liberman-Burchard reagent [11].

Concentration of Hemoglobin

The concentration of hemoglobin was determined using the method of Drabkin [12] and spectrophotometric absorption was measured at 540 nm.

Measurement of Na^+K^+-ATPase Activity

Na^+K^+-ATPase activity was measured by a modified method of Bartosz et al. [13]. This method is based on the assessment of the orthophosphate released from ATP during incubation of erythrocytes with a medium containing 1 mM ATP, 10 mM $MgCl_2$, 100 mM Tris-HCl buffer, pH 7.4, and 0.1 mM ouabain, which was added to the medium to block the Na^+K^+-ATPase. The samples were incubated for 1 h at 37°C and then at 0°C, after which 0.6 M TCA (trichloroacetic acid) was added and then they were incubated for a further 3 min at room temperature. Subsequently, the entire sample was centrifuged at 15,000 rpm for 3 min. The concentration of orthophosphate was determined in the supernatant by the method of van Veldhoven and Mannaerst [14]. Absorbance was read at 610 nm. The concentration of orthophosphate in the sample was read from a calibration curve in the range of 2–20 μM of KH_2PO_4 standard.

Concentration of Protein

The protein concentration was estimated by use of the Lowry et al. method [15].

Statistical Analysis

Results are presented as the mean ± standard deviation. Comparisons between the groups were performed using 1-way ANOVA followed by the post-hoc Tukey test [16]. Student's paired *t*-test was applied to compare data after incubation with and without baicalin within the same treatment group. Values were considered statistically significant at $p < 0.05$. Statistical analysis was performed using STATISTICA© 6.1 software (StatSoft Inc., Tulsa, USA).

Results

The biological characteristics of the patients and the controls are given in Table 1. The concentration of TBARS after incubation of whole blood (0.315 ± 0.068 μmol/mg hemoglobin) and the 2% erythrocyte suspensions (0.293 ± 0.071 μmol/mg hemoglobin) without baicalin was significantly higher in the group of patients with mixed hyperlipidemia than in the control group (0.223 ± 0.064 and 0.213 ± 0.07 μmol/mg hemoglobin, respectively) (Table 2). After incubation of either whole blood or the 2% erythrocytes suspensions with baicalin, a significant decrease in TBARS was observed in the erythrocytes of the patients with mixed hyperlipidemia in comparison to the values obtained after incubation without baicalin (Table 2). In the control group the incubation of both whole blood or the 2% erythrocyte suspensions with baicalin did not show any significant changes in this parameter (Table 2).

The content of cholesterol after incubation without baicalin of whole blood (4.23 ± 0.79 mg cholesterol/packed cells) and the 2% erythrocyte suspensions (4.19 ± 0.72 mg cholesterol/packed cells) was significantly higher in the group of patients with hyperlipidemia than in the control group (2.4 ± 0.25 and 2.33 ± 0.38 mg cholesterol/packed cells, respectively) (Table 3). After incubation of the 2% erythrocyte suspensions with baicalin, the cholesterol content decreased significantly to values close to those of the control group

Table 1. Characteristics of the patients with mixed hyperlipidemia and the control group

Tabela 1. Charakterystyka pacjentów z mieszaną hiperlipidemią i grupy kontrolnej

Groups (Grupy)	Patients with mixed hyperlipidemia (Pacjenci z mieszaną hiperlipidemią) n = 48	Control group (Grupa kontrolna) n = 15	Statistical comparison (Porównanie statystyczne)
Men (Mężczyźni)	29	10	
Women (Kobiety)	19	5	
Mean age – years (Średni wiek – lata)	56.73 ± 7.58	56.9 ± 6.35	ns
Mean BMI kg/m^2 (Średni BMI)	25.8 ± 1.4	24.8 ± 2.2	ns
TC mg/dl	270.73 ± 32.70	179.6 ± 15.8	$p < 0.001$
LDL-C mg/dl	176.8 ± 15.8	100.7 ± 25.9	$p < 0.001$
HDL-C mg/dl	48.45 ± 13.8	57.9 ± 9.6	$p < 0.05$
TG mg/dl	226.77 ± 60.2	115.2 ± 29.5	$p < 0.001$

Table 2. The mean values (mean ± *SEM*) of TBARS concentration after incubation of whole blood and 2% erythrocyte suspension with and without baicalin of patients with mixed hyperlipidemia and healthy controls

Tabela 2. Średnie wartości stężeń TBARS po inkubacji z i bez bajkaliny pełnej krwi oraz 2% zawiesiny erytrocytów pacjentów z mieszaną hiperlipidemią oraz osób zdrowych

	TBARS (µmol/mg hemoglobin) mean ± *SEM* TBARS (µmol/mg hemoglobiny)			
	Control group (Grupa kontrolna)	Patients with mixed hyperlipidemia (Pacjenci z mieszaną hiperlipidemią)	Control group (Grupa kontrolna)	Patients with mixed hyperlipidemia (Pacjenci z mieszaną hiperlipidemią)
	whole blood		2% suspensions of erythrocytes	
Incubation without baicalin (Inkubacja bez bajkaliny)	0.223 ± 0.064	0.315 ± 0.068**	0.213 ± 0.07	0.293 ± 0.071**
Incubation with baicalin (Inkubacja z bajkaliną)	0.195 ± 0.078	0.207 ± 0.056††	0.205 ± 0.06	0.202 ± 0.07††

** $p < 0.01$ vs. control group,
†† $p < 0.01$ vs. values after the incubation without baicalin.

** $p < 0,01$ vs grupa kontrolna,
†† $p < 0,01$, vs wartości po inkubacji bez bajkaliny.

(Table 3). Assuming that cholesterol may diffuse from the erythrocyte membranes to the incubation fluid, its membrane content was assessed after the incubation of the whole blood with baicalin. Also in this case a significant decrease in cholesterol content was noted in the hyperlipidemic group (Table 3). In the group of healthy individuals, no significant changes in this parameter due to baicalin were observed, both after incubation of whole blood or the 2% erythrocyte suspensions (Table 3).

In the erythrocytes of patients with hyperlipidemia, the activity of the Na^+K^+-ATPase (nmol Pi/mg proteins·x h) was significantly lower than in the control group (121.94 ± 53.8 vs. 165.13 ± 59.5 nmol Pi/mg proteins x h, $p < 0.05$). The incubation of the 2% erythrocyte suspensions of hyperlipidemic patients with baicalin produced a significant decrease in ion pump activity in comparison with the value obtained after incubation without baicalin (121.94 ± 53.8 vs. 61.24 ± 32.8 nmol Pi/mg proteins x h, $p < 0.01$) (Table 4).

Table 3. The mean values (mean ± *SD*) of cholesterol concentration after incubation of whole blood and 2% erythrocyte suspension with and without baicalin of patients with mixed hyperlipidemia and healthy controls

Tabela 3. Średnie wartości stężeń cholesterolu po inkubacji z i bez bajkaliny pełnej krwi i 2% zawiesiny erytrocytów pacjentów z mieszaną hiperlipidemią oraz osób zdrowych

	Cholesterol (mg cholesterol/packed cells) mean ± *SEM* Cholesterol (mg cholesterol/liczbę upakowanych komórek)			
	Control group (Grupa kontrolna)	Patients with mixed hyperlipidemia (Pacjenci z mieszaną hiperlipidemią)	Control group (Grupa kontrolna)	Patients with mixed hyperlipidemia (Pacjenci z mieszaną hiperlipidemią)
	whole blood		2% suspensions of erythrocytes	
Incubation without baicalin (Inkubacja bez bajkaliny)	2.4 ± 0.25	4.23 ± 0.79*	2.33 ± 0.38	4.19 ± 0.72*
Incubation with baicalin (Inkubacja z bajkaliną)	2.27 ± 0.35	2.04 ± 0.055††	2.21 ± 0.39	2.09 ± 0.61††

* $p < 0.05$ vs. control group,
†† $p < 0.01$ vs. values after the incubation without baicalin.

* $p < 0,05$ vs grupa kontrolna,
†† $p < 0,01$ vs wartości po inkubacji bez bajkaliny.

Table 4. The mean values of Na^+K^+-ATPase activity in 2% erythrocyte suspension of patients with mixed hyperlipidemia and healthy controls after incubation with and without baicalin

Tabela 4. Średnie wartości aktywności ATPazy Na^+K^+ po inkubacji z i bez bajkaliny 2% zawiesiny erytrocytów pacjentów z mieszaną hiperlipidemią oraz osób zdrowych

	Na^+K^+-ATPase activity (nmol Pi/mg proteins × h) mean ± *SD* Aktywność Na^+K^+-ATPazy (nmol fosolipidów/mg białka × h)	
	Control group (Grupa kontrolna)	Patients with mixed hyperlipidemia (Pacjenci z mieszaną hiperlipidemią)
	2% suspensions of erythrocytes	
Incubation without baicalin (Inkubacja bez bajkaliny)	165.13 ± 59.5	121.94 ± 53.8*
Incubation with baicalin		
(Inkubacja z bajkaliną)	149.40 ± 47.0	61.24 ± 32.8††

* $p < 0.05$ vs. control group,
†† $p < 0.01$ vs. values after the incubation without baicalin.

* $p < 0,05$ vs grupa kontrolna,
†† $p < 0.01$ vs wartości po inkubacji bez bajkaliny.

Discussion

Baicalin is one of the major flavonoids of *Scutellaria baicalensis*. Among its biological activities, it has been reported to exhibit antioxidant effects. In this study the addition of baicalin to the incubated red blood cells and whole blood of patients with mixed hyperlipidemia produced a significant decrease in TBARS. Cellular antioxidants can act by inhibiting the formation of free radicals either by directly scavenging the radicals or by enhancing cellular antioxidant mechanisms. It is supposed that baicalin may protect the cell by one or both of these mechanisms due to the C_2–C_3 double bound of the C ring and the hydroxyl groups at positions 5 and 7 on the A ring [17]. The antioxidant effectiveness of phenolic flavonoids may be related to their ability to enter cells and to localize to biomembranes. Flavonoids anchor to the polar heads of the membrane phospholipids, forming reversible physicochemical complexes [18]. Baicalin and baicalein, being more lipid-soluble, may be able to penetrate membranes with greater ease than other flavonoids, for example rutin. Kimura et al. reported that flavonoides such as wogonin, baicalein, and baicalin inhibited lipid peroxidation induced by ADP-NADP and Fe^{+2} ascorbate in rat liver homogenates [19]. Other authors reported that baicalin could scavenge reactive oxygen species (ROS), including superoxide, H_2O_2, and hydroxyl radical generated from the Fenton reaction and from the reaction system containing xanthine or xanthine oxidase [2]. Shieh et al. compared the effects of the four main flavono-

ids of *Scutellaria* on xanthine oxidase and cytochrome C activity [3]. Of all the studied flavonoids, baicalein appeared to be the most potent inhibitor of xantin oxidase and baicalin of cytochrome C. In another study, using electron spin resonance (ESR), both baicalein and baicalin demonstrated a strong activity on eliminating superoxide radical (O_2) [20]. Bochorokova et al. [21] observed that baicalin and baicalein displayed a significant scavenging effect, while the production of OH radicals generated by the UV photolysis of H_2O_2 was considerably decreased in the presence of baicalin and wogonine glucuronide. Baicalin, beside its beneficial influence on TBARS level, also produced a significant decrease in cholesterol content in red blood cells of patients with hyperlipidemia, regardless of the incubation environment. In healthy individuals, the incubation of both the erythrocyte suspensions and whole blood with baicalin did not produce any significant change in cholesterol content.

Na^+/K^+-ATPase is one of the most important integral proteins of the cytoplasmatic membrane and its activity is modified under the conditions of hyperlipidemia. Most researchers reported a decrease in its activity which depended most strongly on the lipid composition of the cell membranes [22]. An increased level of membrane cholesterol and increased lipid peroxidation were related to lower activity of the protein systems of cation transport. Lower activity of Na^+K^+-ATPase, Na^+/Li^+ exchange, and Na^+K^+ co-transport were reported [23]. Other authors [24] showed an increase in erythrocyte membrane sodium pump activity in rabbits on a cholesterol-rich diet. They showed a twofold greater Na^+K^+-ATPase activity in animals with high serum cholesterol compared with a control group. They hypothesized that the modulation of the sodium pump does not depend on the lipid composition of the erythrocyte cell membranes, but may be modulated by the expression of certain genes. Unlike Makarov's study [24], significantly lower activity of Na^+K^+-ATPase in the erythrocyte suspensions of patients with mixed hyperlipidemia compared with the healthy control group were found in the present study. Based on the literature, the present authors suppose that the decreased Na^+K^+-ATPase activity is related to changes in the lipid composition of the erythrocyte membrane. Yeagle's et al. [25] *in vitro* study showed that a high content of membrane cholesterol in erythrocytes inhibited the activity of Na^+K^+-ATPase and lower cholesterol content was related to higher activity of the enzyme. The authors suggested that the possible mechanism of the Na^+K^+-ATPase inhibition is a direct cholesterol-protein interaction, which leads to the formation of a less active protein conformation. In the present study, significantly lower Na^+K^+-ATPase activity in the erythrocytes of patients with mixed hyperlipidemia was observed, which decreased even more after incubation with baicalin, despite the fact that the content of membrane cholesterol also significantly dropped. The results of the present study suggest that baicalin's inhibiting action on Na^+K^+-ATPase activity could come from its direct influence on the enzyme itself and not on the membrane cholesterol. These results may be the starting point of clinical studies on the antiatherogenic activity of baicalin in patients with hyperlipidemia.

Acknowledgments

Financial resources granted by the Medical University of Lodz scheme No. 503-5006-3 and by the University of Lodz scheme No. 505/371.

References

[1] Shao ZH, Vanden Hoek TL, Quin Y, Becker LB, Schumacker PT, Li CQ: Baicalein attenuates oxidant stress in cardiomyocytes. Am J Physiol Heart Circ Physiol 2002, 282, H999–H1006.

[2] Shi H, Zhao B, Xin W: Scavenging effects of baicalin on free radicals and its protection on erythrocyte membrane from free radical injury. Biochem Mol Biol Int 1995, 35, 981–994.

[3] Shieh DE, Liu LT, Lin CC: Antioxidant and free radical scavenging effects of baicalein, baicalin and wogonin. Anticancer Res 2000, 20, 2861–2865.

[4] Kimura Y, Matsushita N, Yokoi-Hayashi K, Okuda H: Effects of baicalein isolated from *Scutellaria baicalensis* radix on adhesion molecule expression induced by thrombin and thrombin receptor agonist peptide in cultured human umbilical vein endothelial cells. Planta Med 2001, 67, 331–334.

[5] Shimizu I: Sho-saiko-to: Japanese herbal medicine for protection against hepatic fibrosis and carcinoma. J Gastroenterol Hepatol 2000, suppl 15, D84–D90.

[6] Geerts A, Rogiers V: Sho-saiko-to: The right blend of traditional oriental medicine and liver cell biology. Hepatology 1999, 29, 282–284.

[7] Koter M, Franiak I, Strycharska K, Broncel M, Chojnowska-Jezierska J: Damage to the structure of erythrocyte plasma membranes in patients with type-2 hypercholesterolemia. Int J Biochem Cell Biol 2004, 36, 205–215.

[8] Martinez M, Vaya A, Gil L, Marti R, Dalmau J, Aznar J: The cholesterol/phospholipid ratio of the erythrocyte membrane in children with familial hypercholesterolemia. Its relationship with plasma lipids and red blood cell aggregability. Clin Hemorheol Microcirc 1998, 18, 259–263.
[9] Stocks J, Dormandy TL: The autoxidation of human red cell lipids induced by hydrogen peroxide. Br J Haematol 1971, 20, 95–111.
[10] Rodriguez-Vico F, Martinez-Cayuela M, Zafra MF, Garcia-Peregrin E, Ramirez H: A procedure for the simultaneous determination of lipid and protein in biomembranes and other biological samples. Lipids 1991, 26, 2677–2680.
[11] Klyszejko-Stefanowicz L: Cytobiochemia. Wydawnictwo Naukowe PWN 1995.
[12] Drabkin DL: The crystallographic and optical properties of the haemoglobin of man in comparison with dose of other species. J Biol Chem 1946, 12, 703–723.
[13] Bartosz G, Bartosz M, Sokal A, Gębicki JM: Stimulation of erythrocyte membrane Mg^{+2}ATPase activity by dinitrophenol and other membrane-disturbing agents. Biochem Mol Biol Int 1994, 34, 521–529.
[14] van Veldhoven PP, Mannaeters GP: Inorganic and organic phosphate measurements in the nanomolar range. Anal Biochem 1987, 161, 45–48.
[15] Lowry OH, Rosebrough A, Ferr L, Randall RJ: Protein measurement with Folin Phenol reagent. J Biol Chem 1951, 193, 265–277.
[16] Motulsky H: Intuitive biostatistics. Oxford University Press 1995, Oxford.
[17] Soobrattee MA, Neergheen VS, Luximon-Ramma A, Aruoma OI, Bahorun T: Phenolics as potential antioxidant therapeutic agents: Mechanism and action. Mutation Res 2005, 579, 200–213.
[18] Saija A, Scales M, Lanza M, Marzullo D, Bonina F, Castelli F: Flavonoids as antioxidant agents: importance of their interactions with biomembranes. Free Radic Biol Med 1995, 19, 481–486.
[19] Kimura Y, Okuda H, Taira Z, Shoji M, Takemoto T, Arichi S: Studies on Scutellariae radix. IX New component inhibiting lipid peroxidation in rat liver. Planta Med 1984, 50, 290–295.
[20] Gao D, Sakurai K, Chen J, Ogsio T: Protection by baicalein against ascorbic acid- induced lipid peroxidation of rat liver microsomes. Research Commun Mol Pathol Pharmacol 1995, 90, 103–114.
[21] Bochorakova H, Paulova H, Slanina J, Musil P, Taborska E: Main flavonoids in the root of Scutellaria baicalensis cultivated in Europe and their comparative antiradical properties. Phytother. Res 2003, 17, 640–644.
[22] Lu G, Ouyang S, Pei Z: Changes of erythrocyte membrane ATPase activities and plasma lipids in patients with coronary heart disease. Human Yi Ke Da Xue Xue Bao 1999, 24, 68–70.
[23] Lijnen, P, Petrov V: Cholesterol modulation of transmembrane cation transport systems in human erythrocytes. Biochem Mol Med 1995, 56, 52–62.
[24] Makarov VL, Kuznetsov SR: Increased Na^+K^+-pump activity in erythrocytes of rabbits fed cholesterol. Int J Exp Pathol 1995, 76, 93–96.
[25] Yaegle P: Cholesterol modulation of (Na^+K^+)ATPase ATP hydrolyzing activity in human erythrocyte. Biochem Biophys Acta 1983, 727, 39–44.

Address for correspondence:

Marlena Broncel
Department of Internal Diseases with Pharmacology and Therapy Monitoring Unit
Medical University of Lodz
ul. Kniaziewicza 1/5
91-347 Lodz
Poland
tel.: +48 42 6511059
Fax: +48 42 6511059
mobile phone: +48 607698372
e-mail: ttm@ttm.org.pl

Conflict of interest: None declared

Received: 1.08.2006
Revised: 3.01.2007
Accepted: 12.01.2007

Praca wpłynęła do Redakcji: 1.08.2006 r.
Po recenzji: 3.01.2007 r.
Zaakceptowano do druku: 12.01.2007 r.