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Biochemical Pharmacology 66 (2003) 605–612
Biochemical Pharmacology
www.elsevier.com/locate/biochempharm

The study of the quercetin action on human erythrocyte membranes

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Received 20 November 2002; accepted 8 May 2003

Abstract

Quercetin is a naturally occurring flavonoid that exerts multiple pharmacological effects. In our previous study, we showed that quercetin greatly affects the lipid membrane. In this report, a study of quercetin on human erythrocyte membrane has been performed to determine the influence of this flavonoid on the fluidity and the conformational changes of membrane proteins. An additional aim of the study was to find how quercetin presence affects the resistance of membrane to haemolytic agents. The results showed that incorporation of quercetin into the erythrocyte membranes caused the changes of the partition coefficient of the Tempo spin label between the water and polar head group phases. In the studies, the W/S ratio has been used as a monitor of changes in protein conformation and in the environment within the membrane. It was observed that quercetin caused an increase in protein–protein interactions in human erythrocyte membranes. Haemolytic action of quercetin in the dark was also investigated. This compound showed protective effect against hypotonic haemolysis. However, in the heat-induced haemolysis quercetin caused acceleration of haemolysis. Dark reaction of erythrocyte with quercetin resulted in a shrinkage of the cells and alteration of their shapes. From the results we have concluded that modification of erythrocyte membrane by quercetin proceeds *via* reaction with membrane lipids and proteins.
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Keywords: Quercetin; Human erythrocyte membranes; EPR; Maleimide spin label; Tempo spin label

1. Introduction

Quercetin (3,3′,4′,5,7-pentahydroxyflavone) is one of the most abundant bioflavonoids commonly present in most edible fruits and vegetables [25]. It has been estimated that about 25–50 mg of quercetin are consumed in the daily diet [8]. Flavonoids, among them quercetin, have been used for treatment of inflammation, arteriosclerosis, bleeding, allergy and swellings [8,10]. Quercetin arrests cell cycle [30], induces apoptosis [21,26,30], scavenges free radicals [18], prevents tumour development and carcinogen activation [25] and others. It is also known that quercetin inhibits heat shock protein expression [13].

A lot of reports point out that the effects of quercetin on cells are associated with changes of cell membrane properties. In animal cells flavonoids affect the membrane ion transport by inhibition of Ca^{2+}-Mg^{2+} ATPase, Na^{+}-K^{+} ATPase, mitochondrial ATPase, and cAMP- and cGMP-phosphodiesterase [9,24]. Quercetin inhibits enzymatic activity of phospholipase A_2 and protein kinases [8,16]. The influence of quercetin on cell membrane seems to be related with Ca^{2+} influx and/or Ca^{2+} metabolism [17,22]. Our earlier experiments indicated that in liposomes quercetin caused an increase of membrane fluidity and a decrease of the cooperativity and the temperature of the phase transition [31]. It was found that quercetin can protect against oxidative damage of erythrocyte membrane of mouse [7]. However, little is known about quercetin interaction with human erythrocyte membrane.

Thus, the aim of the study was to estimate the action of quercetin upon the human erythrocyte membrane. Using the spin label method the influence of quercetin on the fluidity and the conformational changes of membrane proteins was examined. We were also interested how quercetin presence affects the resistance of membrane to haemolytic agents.

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doi:10.1016/S0006-2952(03)00344-7

2. Materials and methods

2.1. Chemicals

Quercetin (Sigma) dissolved in DMSO (Merck) was used in the studies. The solution was always kept in the dark. Spin labels: 16-doxylstearic acid (16-SASL), 2,2,6,6-tetramethyl-1-piperidinyloxyl (Tempo) and 4-maleimido-Tempo were purchased from Sigma. Spin labels were dissolved in absolute ethanol and stored at 4°. All other chemicals were of the best quality commercially available.

2.2. Membrane preparation

Erythrocyte membranes were obtained by hypotonic lysis according to the procedure of Dodge *et al*. [6] at 4° and then suspended in PBS, pH 7.4. Protein concentration was estimated by the method of Bradford [4] using BSA as a standard.

2.3. EPR measurements

The erythrocyte ghosts were incubated for 1 hr at 37° with quercetin solutions (0 and 50 μg/mL) in isotonic saline buffer. Concentration of DMSO in the control and sample was 0.5%. In order to incorporate spin label to erythrocytes, the suspension of erythrocyte membranes in a phosphate buffer (5 mM, pH 8) was vigorously vortexed in a glass tube with a film of 16-SASL and Tempo. The concentration of the labels applied was 2.5×10^{-4} mol per 1 mg of total protein in the erythrocyte membranes. Samples to be measured were placed in a 1.3 mm diameter capillary (Hyland Lab. Inc.) and sealed with miniseal wax. EPR spectra were recorded with a SE/X-28 B (UNIPAN) spectrometer working in the X band and equipped with a variable temperature-stabilising unit. EPR spectra were recorded in the following conditions: modulation amplitude 5 G in a case of spectra scanning and 10 G for determining an accurate position of the maxima, time constant 0.3 s, scan time 2 min, scan range 3200–3300 G.

In order to examine conformational changes in the membrane, the membranes were incubated in the absence or with various amounts of quercetin (1, 10, 50 μg/mL) in the dark for 1 hr at 37 and 42°. After incubation, the erythrocyte membranes were washed three times with PBS and labelled with maleimide spin label for 12 hr at 4° in the dark, in a ratio 1 mg of label per 25 mg of membrane protein. The spin-labelled samples were washed several times with PBS, pH 7.4, to remove unbound spin label. EPR spectra were measured at room temperature using a SE/X-28 B (UNIPAN) spectrometer working in the X band and equipped with variable temperature-stabilising unit. Typical instrumental parameters during these measurements were as follows: modulation amplitude 2 G, time constant 1 s, scan time 4 min, scan range 3300–3400 G.

2.4. Haemolysis in isotonic solution

Erythrocytes were obtained from peripheral blood taken by venipuncture. Sodium citrate was used to prevent clotting. Packed red blood cells were washed three times with PBS (154 mM NaCl, 10 mM sodium phosphate, pH 7.4) and resuspended in the same saline solution at 50% v/v. Fifty microlitres of erythrocyte suspension was added to 3 mL quercetin solution (1–25 μg/mL) in PBS in a siliconized glass tube. The control sample contained DMSO at the same concentration as that of quercetin. The highest concentration of DMSO was 0.5%. Each experiment was performed in triplicate. After 60 min of incubation (20 or 37°), the reaction mixtures were centrifuged at 1800 *g* for 5 min and the absorbance of the supernatant was measured at 540 nm. The relative haemolysis was determined by comparison with a sample showing 100% haemolysis.

Six independent experiments were performed with erythrocytes obtained from different blood donor.

2.5. Heat-induced haemolysis

The inhibition of heat-induced haemolysis was carried out as for haemolysis in isotonic solution. The erythrocytes were incubated with quercetin solutions in isotonic saline phosphate buffer at 54° for 30 min in a water bath in the dark.

2.6. Haemolysis in hypotonic solution

The extent of protection of erythrocytes was measured after 30 min incubation of erythrocytes (20°) with quercetin solutions in hypotonic solution (56 mM NaCl in 10 mM sodium phosphate buffer, pH 7.4).

The haemolysis rate was calculated in relation to the haemolysis in the control which was taken as 100%. The actual value of haemolysis in this control was $63.3 \pm 14.9\%$ in hypotonic solution and $59.63 \pm 13.6\%$ in heat-induced haemolysis in comparison with that in water.

2.7. Measurement of erythrocyte diameter

After incubation of erythrocytes with quercetin solutions in isotonic saline buffer (20°, 60 min in the dark), the cells were fixed with 4% glutaraldehyde (30 min) in isotonic saline solution. The diameters of 200 cells were measured in a microscope with a micrometric ocular. Only non-deformed cells were measured.

2.8. Scanning electron microscopy (SEM)

For SEM red blood cells were fixed with 4% glutaraldehyde and 1% osmium tetroxide in isotonic saline buffer. The erythrocytes were dehydrated in a graded acetone series and then coated with gold. The samples were observed with the Tesla BS 300 microscope.

2.9. Quantitative analysis of erythrocytes shapes

For the quantitative analysis of the erythrocytes' shapes, the cells were incubated with different quercetin solutions (10, 25, 50 μg/mL) in isotonic saline buffer for 60 min at 20° in the dark. Then the cells were fixed with 4% glutaraldehyde for 30 min and suspended in isotonic saline buffer. The erythrocytes placed in Bürker's chamber were analysed under light microscope. In each sample 6000 cells were observed.

The results were analysed by Student's t test and χ^2 test.

3. Results

3.1. Haemolysis of red blood cells in solutions with different osmotic pressure at different temperature conditions

To examine the resistance of membrane to haemolytic agents, erythrocytes were suspended in various solutions. Incubation of erythrocytes suspended in isotonic solution containing 1–25 μg/mL quercetin at 20° did not show a haemolytic effect of the compound. Neither did an increase of the incubation temperature to 37° cause a haemolytic action of quercetin. However, the incubation of erythrocytes with quercetin at 54° caused haemolysis in all the samples studied (Table 1). At a concentration of 1 μg/mL, the haemolysis rate was a little higher than in control. In samples with 10 and 25 μg/mL quercetin haemolysis was much more higher than in control and did not show dependence on the compound concentration. The highest value of haemolysis in such conditions was noticed after the incubation of cells with 10 μg/mL of the studied drug. Quercetin concentration-dependently decreased the rate of haemolysis in hypotonic solutions (Table 2). The rate of haemolysis in hypotonic solution at a concentration of 1 μg/mL was lower by 36%, whereas in the samples containing 25 μg/mL quercetin by 77% as compared to the control.

Table 1
The effect of quercetin on heat-induced haemolysis in human red blood cells

Concentration (μg/mL)	Haemolysis rate ± SD (%)
1	64.57 ± 1.83
10	86.89 ± 2.72*
25	83.59 ± 3.13*

1, 10, 25—doses of quercetin used in the experiment. Cells were incubated for 30 min at 54° in isotonic saline phosphate buffer. The haemolysis rate was calculated in relation to the haemolysis in the control which was taken as 100%. The actual value of haemolysis in control was 59.63 ± 13.6%. Six independent experiments were performed. SD—standard deviation.

* $P \leq 0.001$.

Table 2
The effect of quercetin on hypotonic haemolysis in human erythrocytes

Concentration (μg/mL)	Haemolysis rate ± SD (%)
1	64.08 ± 1.58*
10	28.51 ± 6.14*
25	22.93 ± 1.76*

Cells were treated with different concentrations of quercetin (1, 10, 25 μg/mL) in hypotonic phosphate buffer. The extent of protection of erythrocytes was measured after 30 min incubation at 20°. The haemolysis rate was calculated in relation to the haemolysis in the control which was taken as 100%. The actual value of haemolysis in control was 63.3 ± 14.9%. Six separate experiments were carried out. SD—standard deviation.

* $P \leq 0.001$.

3.2. Erythrocytes size and shapes after quercetin treatment

For analysis of the erythrocytes shapes and measurements of erythrocytes diameter, light and scanning electron microscopes were used. Quercetin caused changes in the size and shape of cells. Measurements carried out after 1 hr incubation of erythrocytes with quercetin in isotonic solution (37°) showed a reduced diameter of red blood cells (Table 3). The diameter of erythrocytes incubated with quercetin at a concentration of 10, 20 and 50 μg/mL was smaller by 7.94, 16.83 and 24.41% than that of control cells. Studies by using scanning electron microscope also showed changes in the shape of cells. Among erythrocytes treated with quercetin at a concentration of 10 μg/mL and higher (25, 50 μg/mL), irregular cells with numerous extrusion on their surface or cells with ruffled edges were found (Plate 1). The number of deformed erythrocytes in a suspension containing 10, 25 and 50 μg/mL quercetin was 7.2, 23.3 and 47.7%, respectively, whereas in control 1.05% of cells showed a changed appearance (Table 4).

3.3. The effect of quercetin on structural and dynamic properties of erythrocyte membranes

To examine the influence of quercetin on the fluidity and the conformational changes of membrane proteins the spin

Table 3
The effect of quercetin on the size of the red blood cells

Concentration (μg/mL)	Cell diameter ± SD (μm)
Control	8.44 ± 0.96
10	7.77 ± 0.84*
20	7.02 ± 0.89*
50	6.38 ± 0.93*

Control—cells incubated only in isotonic saline buffer for 60 min at 20°. All the remaining cells were incubated with different quercetin solutions (10, 20, 50 μg/mL) in the same experimental conditions. After fixation with glutaraldehyde the diameters of 200 cells were measured. SD—standard deviation.

* $P \leq 0.001$.

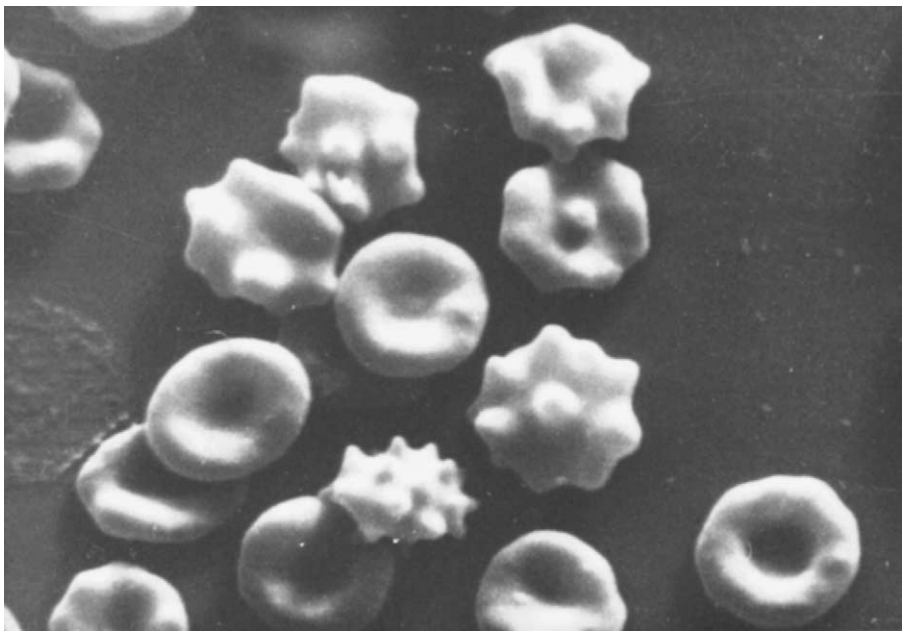

Plate 1. Scanning electron micrograph of erythrocytes treated with quercetin (50 μg/mL).

Table 4
The effect of quercetin on the shape of the erythrocytes

Concentration (μg/mL)	Deformed cells (%)
Control	1.1
10	7.2*
25	23.4*
50	47.7*

Control—cells incubated only in isotonic saline buffer for 60 min at 20° in the dark. All the remaining cells were treated with different quercetin solutions (10, 25, 50 μg/mL) in the same experimental conditions. After fixation with 4% glutaraldehyde erythrocytes were observed under the light microscope. 6000 cells were analysed in each examined sample.

* $P \leq 0.001$.

label method was used. Spin labels of *n*-SASL are commonly used to monitor structural and dynamic properties of lipid bilayer because the shape of their EPR spectra is strongly dependent on the motional freedom of the free radical segment of spin label molecule within the membrane [28]. The lower the maximum splitting ($2T'_{\parallel}$; the parameter closely related with order parameter within the membrane), the higher the fluidity of the membrane in the vicinity of free radicals (Fig. 1). As it may be seen in Fig. 2, incorporation of quercetin into the erythrocyte membrane had no effect on the fluidity of hydrophobic core of the lipid membrane at the depth penetrated by 16-SASL. In the ghost of human erythrocytes incubated for 60 min at 37° the obtained values of $2T'_{\parallel}$; of 16-SASL spin label were similar in control membranes and in membranes with 50 μg/mL quercetin. The influence of quercetin on the membrane in the region of polar head groups with the use

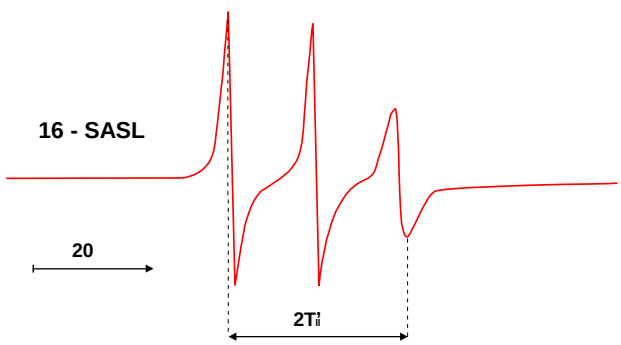

Fig. 1. Typical EPR spectrum of 16-doxylstearic acid spin label incorporated into ghost of human erythrocytes. The measured empirical parameter $2T'_{\parallel}$ is indicated. Spectrum scanned at modulation amplitude 5 G, maxima at 10 G.

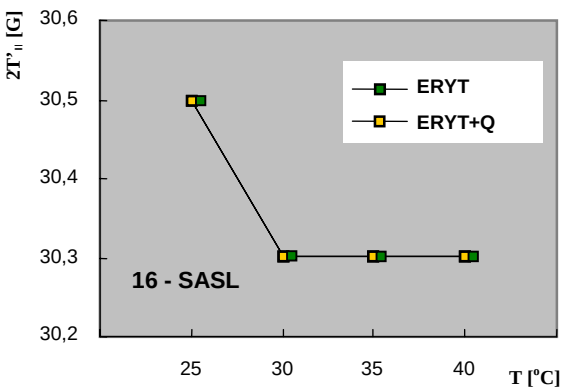

Fig. 2. The effect of quercetin on maximum splitting parameter ($2T'_{\parallel}$) as a function of temperature of 16-doxylstearic acid (16-SASL) spin label doped into ghost of human erythrocytes. ERYT—ghost of human erythrocytes incubated for 60 min at 37° in isotonic saline buffer in absence of quercetin. ERYT + Q—erythrocyte membranes incubated with 50 μg/mL quercetin in the same experimental conditions. The concentration of the label applied was 2.5×10^{-4} mol per 1 mg of total protein in the erythrocyte membranes. Measurements were performed at various temperatures (25–40°).

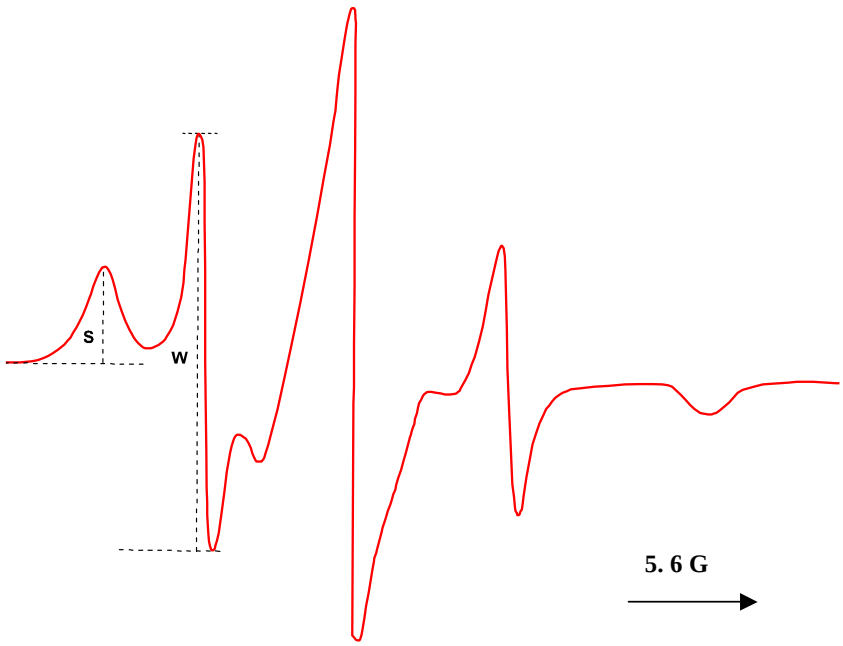

Fig. 3. A typical EPR spectrum of 4-maleimido-Tempo spin label in erythrocyte membrane (W—peak of spin label weakly and S—strongly immobilised). The spectra of two fractions of the spin label are superimposed: weakly and strongly immobilised to the membranes.

of Tempo spin label was examined. High-field EPR spectra of Tempo spin label show two peaks: one correspondent to relatively mobile fraction of spin label in the water phase (A) and the fraction of the spin label immobilised within membrane (B) (Fig. 4). In the spectra, the changes of partition coefficient (B/A) were analysed. As can be seen in Fig. 5, quercetin caused an increased penetration of spin label into the membrane. The highest incorporation of Tempo spin label into the ghosts of human erythrocytes was noted at 25°, whereas the smallest at 35°.

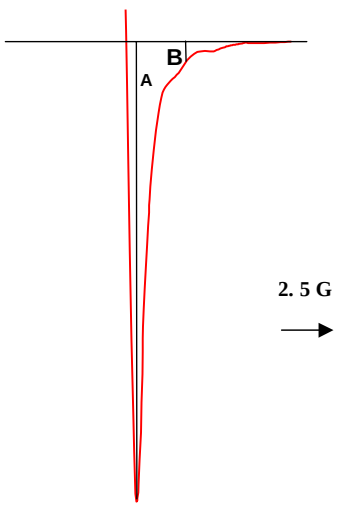

Fig. 4. Fragment of the high-field EPR spectrum of Tempo spin label doped into ghosts of human erythrocytes. Temperature 30°. A expresses the intensity of the minimum corresponding to the relatively mobile fraction of spin label in the water phase and B expresses the shoulder shifted by 2.5 G with respect to the minimum, representing the fraction of the spin label immobilised within the membrane.

3.4. The effect of quercetin on erythrocyte proteins—an EPR spin label study

In order to demonstrate that quercetin also interact with the erythrocyte proteins, the membranes were labelled with maleimide spin label and analysed by EPR. Maleimide spin label binds covalently to membrane proteins giving rise to EPR absorption which represent, respectively, weakly (W) and strongly (S) immobilised state of the labelled sites in the membrane proteins (Fig. 3). In these

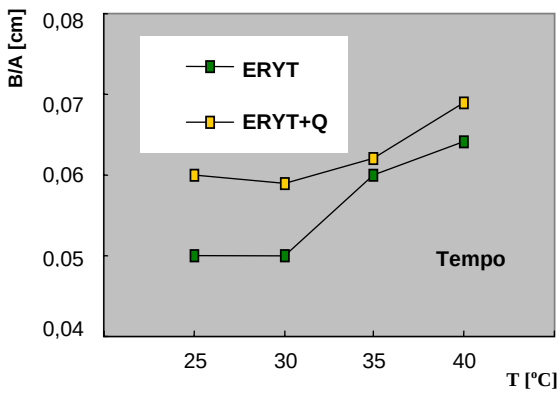

Fig. 5. Temperature dependence of partition coefficient B/A of Tempo spin label between lipid and water phases doped into ghost of human erythrocytes. ERYT—ghost of red blood cells incubated for 60 min at 37° in isotonic saline buffer without quercetin. ERYT + Q—membranes treated with 50 μg/mL quercetin in the same experimental conditions. Tempo spin label was applied at a concentration of 2.5×10^{-4} mol per 1 mg of total protein in the membranes. B/A—partition coefficient where A expresses the relatively mobile fraction of spin label in the water phase and B represents the fraction of the spin label immobilised within the membrane.

Table 5
The effect of quercetin on 4-maleimido-Tempo spin label binding to ghost of human erythrocytes

Incubation temperature (°)	Quercetin concentration (μg/mL)	W/S ratio ± SEM
37	Control	4.59 ± 0.18
	1	4.18 ± 0.09
	10	4.33 ± 0.13
	50	2.36 ± 0.15**
42	Control	5.54 ± 0.92
	1	4.7 ± 0.12*
	10	4.55 ± 0.23*
	50	1.94 ± 0.10**

Control—membranes incubated in the absence of quercetin. Erythrocytes ghosts were incubated for 60 min at 37 and 42° with various amounts of quercetin (1, 10, 50 μg/mL) in the dark. After washing three times with PBS, labelling with maleimide spin label for 12 hr at 4° in the dark in a ratio 1 mg of label per 25 mg of membrane protein was done. The spin-labelled samples were washed several times with PBS, pH 7.4, to remove unbound spin label. EPR spectra were measured at room temperature. Five separate experiments were performed. SEM—standard error of mean.
*$P \leq 0.01$; **$P \leq 0.001$.

studies, the W/S ratio has been used as monitor of the changes in protein conformation and the environment within the membrane [32]. This parameter is particularly sensitive to changes in the membrane structure.

An increase in the W/S ratio indicate conformational changes and imply a decrease in protein–protein interaction. The effect of temperature and quercetin (1, 10, 50 μg/mL) on the W/S ratio are summarised in Table 5. Heat treatment alone (42°, 1 hr) caused a small increase in the W/S ratio. The values of W/S altered from 4.6 ± 0.2 at 37° to 5.5 ± 0.9. Simultaneous exposure of the erythrocyte membranes to quercetin and hyperthermia diminished the W/S ratio with the increasing concentration of drug. The results indicate that quercetin produced a small reduction in the segmental motion of spin-labelled binding sites in the case of 1 and 10 μg/mL at 37° and a significant reduction in the case of 50 μg/mL at the same temperature. At 42° we observed a statistically significant reduction in all three cases leading to an increase in protein–protein interactions in human erythrocyte membranes (Table 5). Small changes in the values of W/S ratio were obtained for erythrocytes' ghosts incubated with quercetin at concentrations of 1 and 10 μg/mL. The highest changes of the W/S ratio were noticed in the case of 50 μg/mL.

4. Discussion

It was shown that quercetin in isotonic solution at 20 or 37° did not induce haemolysis at the concentration used. However, lack of haemolytic effect of quercetin does not mean that this compound has no effect on the cell membranes as it was found effective in conditions for denaturating proteins (54°) or in solutions with a low osmotic pressure. The range of concentration used in the present experiment (1, 10, 25, 50 μg/mL) was similar as compared with studies of other authors that concerned also the examination of red blood cells [2,3,7]. Physiological concentration of quercetin are lower. Hollman *et al*. [11] have found a plasma concentration of about 0.65 μmol/L quercetin after consumption of a meal containing 150 g of fried onions (equivalent to 64 mg of pure quercetin). It is supposed that local concentration of quercetin, e.g. in the intestinal microvilli may be higher. But it seems that to produce the effects observed on many enzymatic systems *in vitro* experiments, the expected concentration of quercetin should be higher. Nevertheless, many *in vivo* experiments indicate that orally introduced quercetin does exhibit certain physiological or pharmacological effect. At the same time, it was implied that repeated dietary intake of quercetin would lead to a build-up of concentration in plasma. Quercetin can thus contribute significantly to the antioxidant defences present in blood plasma [11,12]. The protective effect of quercetin on haemolysis in hypotonic solution increased with the increasing concentration of the compound. Inhibition of phthalocyanine-sensitised photohaemolysis of human erythrocytes by quercetin has also been reported by Ben-Hur *et al*. [2]. Similar protective effect was observed by other researchers. It was shown that quercetin prevented haemolysis of erythrocytes by about 50% at 90 μg/mL. The protection provided by the flavonoid is explained by the authors as its competitive quenching of singlet oxygen. Quercetin has also been found to show protective effects against lipid peroxidation in human erythrocytes. In this manner, it acts as antioxidant and free radical scavenger, thus protecting membrane phospholipids [3]. Another possible explanation of inhibited hypotonic haemolysis is changed permeability of the membrane or increase in the surface area/volume ratio of the cell [1]. The increased proportion of the cell surface to its volume may be caused by intercalation of quercetin into the membrane or by cell shrinking. In our case, simultaneous occurrence of both phenomena is also possible. The observed reduction of the diameter of red blood cells as well as the changes in the configuration of their surface suggest an increased surface area of the erythrocyte with respect to its volume. A comparison of the magnitude of the protective effect with the decrease in the diameter indicates, however, that another mechanism causing the observed changes may occur.

The proteins of the cytoskeleton and integral membrane proteins are responsible for the shape of red blood cells [15,27]. Therefore, the interaction of quercetin with membrane proteins may lead to changes of the shape and size erythrocytes and cause the altered reaction of the cell to haemolytic agents.

The chemical structure of quercetin (Fig. 6) with five polar groups enables prediction of its localisation in the membrane at the polar–unpolar interface. Quercetin indeed is localised at the polar head groups region of phospho-

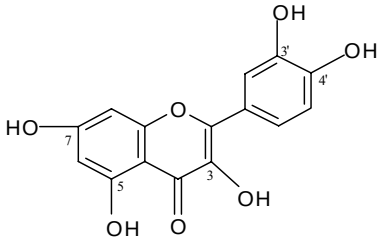

Fig. 6. Chemical structure of quercetin.

lipids as it was indicated by EPR data [20,31]. The effect of quercetin on the erythrocyte membrane may be better understood by analysing the effect of this flavonoid in model phospholipid membranes as studied by calorimetry and ultrasound absorption. According to the differential scanning calorimetry (DSC) and ultrasound absorption data, quercetin decreased cooperativity and temperature of the phase transition of liposomes [31].

Quercetin really affects the lipid membrane but the extent to which the examined flavonoid acts depends on the type of membrane. Considering the influence of quercetin on the fluidity of erythrocyte membrane, one should remember that such a membrane also contains proteins. The fluidity of such complex system will also be dependent on mutual interactions between components. The composition of lipids in such a membrane is also different. Due to variety in lipid composition and presence of proteins, the erythrocyte membranes do not display well-defined sharp phase transition, typical of model membranes formed with synthetic lipids. Nevertheless, the results obtained in this study resemble the results obtained in the examination of DPPC liposomes at low temperatures. They indicate the effect of quercetin on the membrane fluidity in the gel phase of the membrane. They also give evidence that quercetin affects not only lipids but also membrane proteins. Our results are in agreement with the studies reporting that quercetin is associated with erythrocytes to a great extent [7].

Localised near the surface of the membrane [20,23,31] quercetin protects liposomal phospholipids against peroxidation by aqueous oxygen radicals [14,29] and prevents the consumption of lipophilic α-tocopherol [29]. Oxidative damage of erythrocyte membrane of mouse can also be prevented by the examined drug [7]. Therefore, the antioxidant activity of quercetin in its protective effect must be considered.

The data mentioned above indicate that quercetin strongly affects structural and dynamic properties of the membranes. The impact of quercetin on the lipid membrane can lead to changes in permeability of the membrane. It is likely that the protective activity of quercetin on the red cell in low osmotic solution results from an increase in membrane permeability.

The changes in the W/S ratio observed in the quercetin-treated erythrocyte membranes have shown that interaction of this compound occurs at the level of cytoskeletal proteins. This is consistent with the observations in the literature, where the effect of quercetin on alterations of membrane proteins linked with haemolysis was also found [2,7]. The addition of the flavonoid prevented the alteration of membrane proteins, such as spectrin and band 3 proteins. Our studies also indicate that quercetin binding to the cytoskeletal proteins is responsible for increased protein–protein interaction. The preferential binding of maleimide spin label to SH groups of cytoskeletal proteins: spectrin, actin, bands 2.1, 4.1 proteins and to the cytoplasmic region of band 3 proteins has been reported by Butterfield [5], Wyse and Butterfield [32] and Palmieri and Butterfield [19].

The present study shows a relatively strong modifying effect of quercetin on the red blood cell membrane. This impact is associated with changes of the structure and properties of the cell membrane. At the molecular level modes of quercetin action operate by an increase in protein–protein interactions and by fluidisation of the region of polar head groups. The interaction of quercetin with membrane proteins and lipids may cause the altered reaction of the cell to haemolytic agents, as it was shown in the present study. Quercetin had a protective effect against hypotonic haemolysis. Dark reaction of erythrocyte with quercetin resulted in a shrinkage of the cells and alteration of their shapes. Modifying action of quercetin on the cell membrane may cause activation of different mechanisms dependent on the physical state of biomembranes.

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