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Protective effect of quercetin against cigarette tar extract-induced impairment of erythrocyte deformability

Aynun Nahar Begum, Junji Terao*

Department of Nutrition, School of Medicine, The University of Tokushima, Tokushima 770-8503, Japan

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Abstract

Quercetin (3,3′,4′,5,7-pentahydroxyflavone) is one of the most abundant flavonol-type flavonoids rich in diet and suggested to possess a beneficial role in blood circulation. This study was conducted to know the effect of quercetin aglycone and one of its possible metabolite, quercetin-3-*O*-*β*-*D*-glucuronide on cigarette tar extract-induced impairment of erythrocyte deformability. Erythrocyte suspension containing quercetin aglycone, quercetin-3-*O*-*β*-*D*-glucuronide or quercetin-3-*O*-*β*-*D*-glucoside was forced to flow through microchannels with equivalent diameter of 7 μm and its transit time was measured as an index of erythrocyte deformability using microchannel array method. Both quercetin aglycone and quercetin-3-*O*-*β*-*D*-glucuronide, but not quercetin-3-*O*-*β*-*D*-glucoside, substantially increased erythrocyte deformability indicating that the former two compounds affect the physicochemical state of erythrocyte by interacting with its membranes. Aqueous cigarette tar extract caused marked decrease in erythrocyte deformability with concomitant increase of membranous lipid peroxidation. In that case, quercetin aglycone suppressed the impairment of erythrocyte deformability as well as membranous lipid peroxidation. The same effect was found in quercetin-3-*O*-*β*-*D*-glucuronide, eventhough its effect was lower than that of quercetin aglycone. Thus, *not only* quercetin aglycone *but also* its conjugate metabolite protects erythrocyte membrane from the damage of smoking by scavenging reactive oxygen species generated from cigarette tar. Intake of quercetin-rich food may be helpful to protect membranous damage in erythrocytes from smoking.

Keywords: Quercetin; Quercetin-3-*O*-*β*-*D*-glucuronide; Erythrocyte deformability; Cigarette tar; Lipid peroxidation

1. Introduction

Cigarette smoke causes a formation of reactive oxygen species (ROS) and toxic chemical loads and is associated with increases in the incidence of cardiovascular diseases as well as other circulatory diseases [1]. Whole smoke can be divided into two phases, that is, gas phase and tar phase [2]. Aqueous tar extracts from cigarette tar phase contain semiquinone radical and superoxide anion resulting in the production of hydrogen peroxide and hydroxyl radical [3]. On the other hand, erythrocyte has been extensively investigated to sustain peroxidative membrane damages *via* ROS [4–6]. Oxidized erythrocytes would be more prone to form aggregates and increase the viscosity of blood stream and thus, ROS might impair blood flow in the microcirculation [7]. Apparently, several lines of evidence suggest that the peroxidative damage to erythrocyte membranes induces an impairment in its physiological functions including deformability [8–11]. It is now well documented that smoking affects the flow properties of blood and alters the rheological behavior of erythrocytes [12–14]. It is therefore likely that ROS generated from tar extract initiates peroxidative damage in erythrocyte membrane resulting in the impairment of erythrocyte deformability.Quercetin (3,3′,4′,5,7-pentahydroxyflavone) is one of the abundant flavonol-type flavonoids and is found to accumulate in blood plasma as conjugated metabolites by the intake of quercetin-rich food [15]. An epidemiological study has shown that the consumption of certain flavonoids including quercetin is correlated with decreasing risk of coronary artery disease mortality [16]. Antioxidant activity of flavonoids has attarcted much attention in the prevention of atherosclerosis and thrombosis by protecting low density lipoprotein (LDL) against oxidative damage as well as by lowering the cytotoxicity of oxidized LDL and platelet aggregation [17]. Erythrocyte deformability is also likely to play an essential

* Corresponding author. Tel.: +81-88-633-7087; fax: +81-88-633-7089.

E-mail address: terao@nutr.med.tokushima-u.ac.jp (J. Terao).

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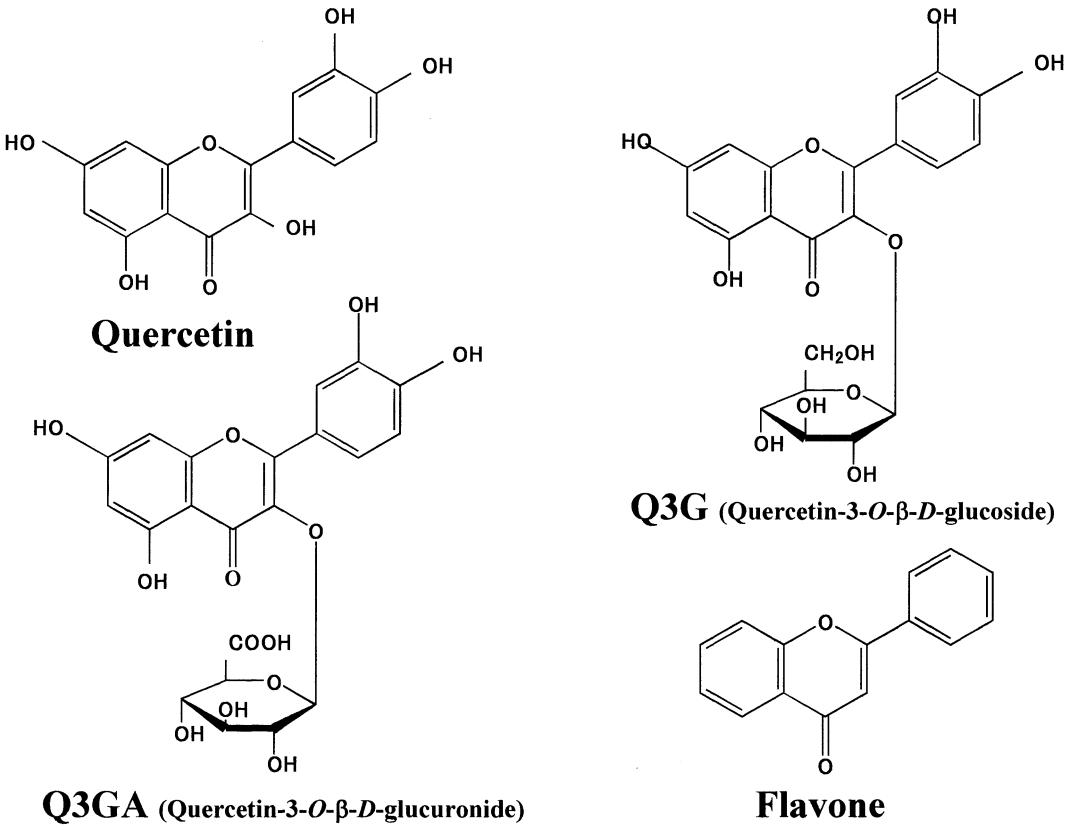

Fig. 1. Structures of quercetin and its related compounds used in the study.

role in the circulatory system from the view point of vascular disease prevention [18]. Nevertheless, the effect of flavonoids on erythrocyte deformability is ambiguous. Moreover, the role of quercetin on the protection against smoking-induced impairment of erythrocyte deformability have been still unresolved.

We recently identified quercetin-3-*O*-*β*-*D*-glucuronide (Q3GA) as an antioxidative metabolite in rat plasma after oral adminstration of quercetin [19]. This paper describes the protective effect of quercetin aglycone and its related compounds including Q3GA (Fig. 1) on cigarette tar extract-induced impairment of erythrocyte deformability. Erythrocyte deformability was estimated by a reproducible microchannel array method developed by Kikuchi et al. [20].

2. Materials and methods

2.1. Materials

Quercetin was obtained from Sigma Chemical Co (St. Louis, MO). Quercetin-3-*O*-*β*-*D*-glucoside (Q3G) was from Extrasynthase (Genay, France). Flavone was purchased from Nacalai Tesque, Inc. (Kyoto, Japan). Quercetin-3-*O*-*β*-*D*-glucuronide (Q3GA) was chemically synthesized and isolated as described elsewhere [19].

2.2. Blood collection and erythrocyte preparation

Blood was taken from healthy volunteers into a test tube and was anticoagulated with heparin. Erythrocytes were separated from plasma by centrifugation at 1500 × g for 10 min at 4°C. The cells were washed five times with 10 volumes of phosphate buffered saline (PBS). The buffy coat of white cells was removed with each washing and erythrocytes were finally centrifuged at 1000 × g for 10 min to obtain packed cells.

2.3. Preparation of cigarette tar extract solution

Cigarette tar extract was obtained according to the method of Pryor et al [21]. Briefly, cigarettes (85 mm in the length and 0.9 g in the weight) were obtained commercially and smoked with a metal aspirator connected with a water tap. The tar was collected on glass fiber filter paper (diameter 1.5 cm, Toyo Roshi, Ltd. Japan). The filter containing wet tar was soaked in 5.0 ml of PBS, sonicated in a sonication bath for 10 min and then the solution was filtered with 90 mm filter paper (Toyo Roshi, Ltd. Japan). The sonication and filtration were repeated with another 5 ml of PBS. This tar extract solution was vortexed for 1 min and then used for the experiments within 24 hr.

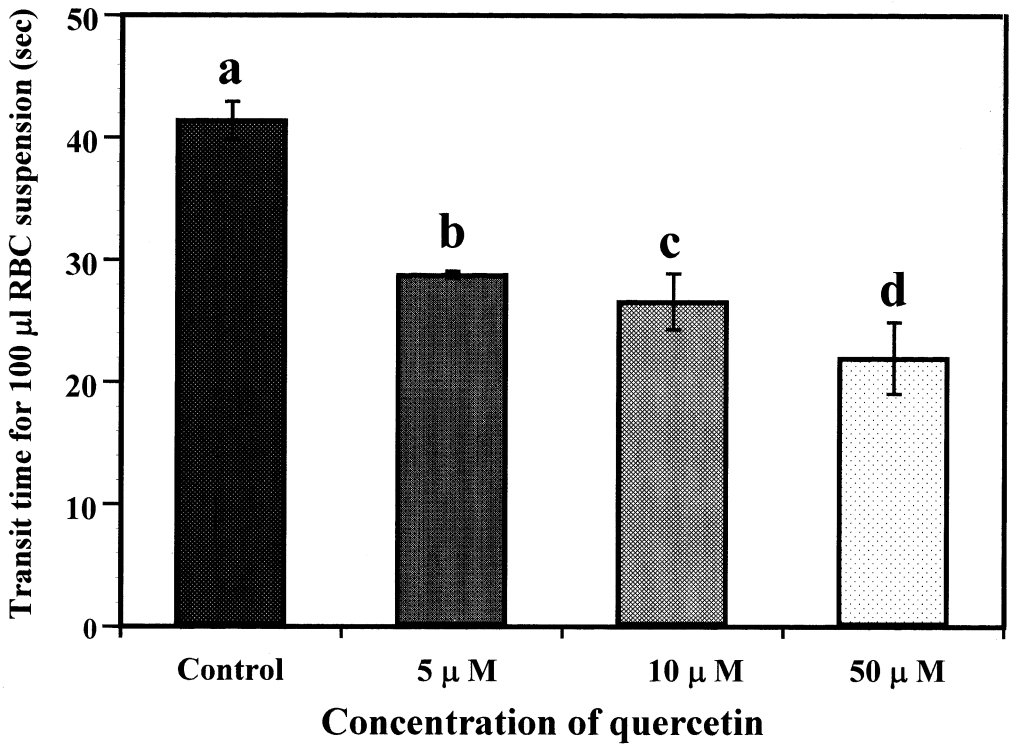

Fig. 2. Effect of quercetin aglycone in different concentration on erythrocyte deformability. Erythrocyte suspension was prepared with 9 vol of 0.85% NaCl at a hematocrit of 10% and incubated for 30 min at 37°C in the absence or presence of quercetin dissolved in ethanol at different concentrations. In each case, ethanol concentration in the suspension was adjusted to 2.5% (v/v). The suspension (100 μl) was then injected to MC-FAN. Each value is the mean ± SD of at least four experiments. Means not sharing a common letter are significantly different ($p < 0.05$).

2.4. Measurement of erythrocyte deformability

Erythrocytes were suspended by 9 vol of 0.85% NaCl or by 9 vol of tar extract solutions. Its hematocrit was adjusted to 10% (Hematocrit KH-120, KUBOTA, Tokyo, Japan). Quercetin, Q3G, Q3GA or flavone was added to the cell suspension as ethanol solution. The final ethanol concentration in the suspension was set at 2.5% (v/v) and the final concentration of each antioxidant was 10 μM unless mentioned differently. The resulting mixture was incubated at 37°C for 30 min. It was then forced to flow through microchannels (equivalent diameter 7 μm, equivalent length 32 μm, 8736 in parallel) by applying a water pressure difference of 20 cm height at room temperature using Micro Channel array Flow ANalyzer (MC-FAN) KH-3 model (Hitachi Haramachi Electronics, Hitachi, Japan). A transit time for 100 μl of erythrocyte suspension was measured to estimate its deformability. Each erythrocyte passed through a microchannel of diameter 7 μm with changes of its shape. Erythrocyte cell shape during the transit was monitored continuously by CCD (Connective Computer Detector) camera equipped with MC-FAN [20]. The transit time range of normal erythrocyte was 40~50 sec. In that case, countable transit time of erythrocyte passing through microchannel array was the index of erythrocyte deformability. All measurements were made using a single silicon chip, which was cleaned between runs by ultrasonic washing with neutral detergent and ethanol. The experiment of deformability was carried out within four days after the collection of erythrocytes.

2.5. Measurement of thiobarbituric acid reacting substances (TBARS)

Degree of lipid peroxidation of erythrocyte exposed to tar extracted solution was determined by TBARS content by the method of Stocks and Dormondy [22]. To 0.4 ml of erythrocyte suspension was added 50 μl of 40 mM butyl hydroxy toluene (BHT) and 1.0 ml of 30% of trichloroacetic acid. The mixtures was vortexed, allowed to stand in ice for 1 hr, and then centrifuged at 3,000 rpm for 15 min. To 1.0 ml of each supernatant was added 0.1 ml of 0.1 M EDTA and 0.35 ml of 1% thiobarbituric acid (TBA). Then the mixture was vortexed and kept in a boiling water bath for 15 min. The reaction mixture was again cooled and 1.5 ml of butanol was added followed by centrifugation at 3,500 rpm for 10 min. Butanol phase was measured with fluoresence intensity at the excitation wavelength of 515 nm and emission wavelength of 553 nm.

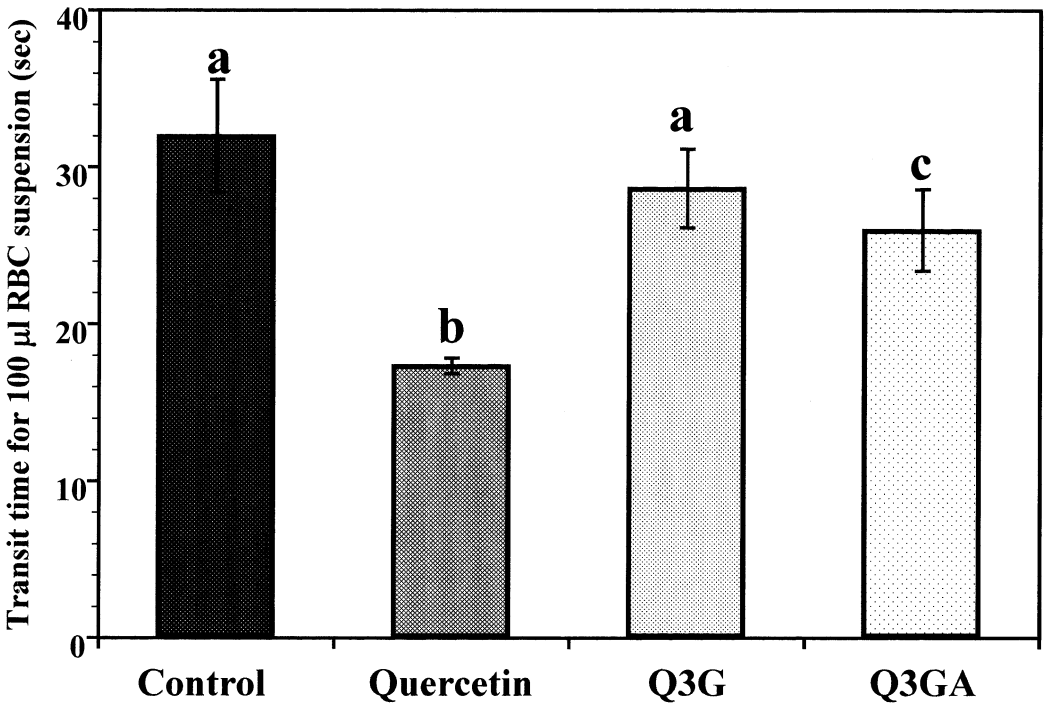

Fig. 3. Effect of quercetin and its related compounds on erythrocyte deformability. The erythrocyte suspension was prepared with 9 vol of 0.85% NaCl at a hematocrit of 10%. The suspension was incubated for 30 min at 37°C in the presence of quercetin or its derivatives at a final concentration of 10 μM. Ethanol concentration was adjusted to 2.5% in each case including control experiment. Then, 100 μl of the suspension was injected to MC-FAN. Each value is the mean ± SD of at least four experiments. Means not sharing a common letter are significantly different ($p < 0.05$).

2.6. Measurement of fatty acid composition

Polyunsaturated fatty acid (PUFA) content in erythrocyte membranes was measured with and without tar extracted solution. The lipids in erythrocyte suspension were extracted by the method of Bligh and Dyer [23]. The lipid portion was evaporated with nitrogen gas and the residue was dissolved in 0.1 ml of 5% HCl-methanol and heated for 2 hrs. Then, the resulting fatty acid methyl ester was extracted by hexane and analyzed by capillary GLC (GC-18A, Shimadzu, Japan) using a column of Supelco^{TM}-2330 stationary phase (0.25 mm ID, 30 m length, 0.2 μm film) equipped with flame ionization detector.

2.7. Statistical analysis

One-way of the analysis of variance followed by the Bonferroni/Dunn's multiple comparison test was used to determine the statistical significance among each group ($p < 0.05$).

3. Results

3.1. Effect of quercetin and its related compounds on erythrocyte deformability

Fig. 2 shows the effect of quercetin aglycone on erythrocyte deformability at different concentrations. Erythrocyte transit time was decreased with the elevation of quercetin aglycone concentration in the range from 5 μM to 50 μM. This result demonstrated that quercetin aglycone increased erythrocyte deformability in concentration dependent manner. Fig. 3 shows that Q3GA at 10 μM slightly but significantly decreased the transit time. However, Q3G at the same concentration did not affect the transit time. Thus, not Q3G but Q3GA elevated erythrocyte deformability, although its effectiveness was weaker than that of quercetin aglycone. We added ethanol solution of quercetin to the erythrocyte suspension at 2.5%. This might affect the erythrocyte deformability, because high concentration of ethanol apparently decreased erythrocyte deformability (data are not shown here). However, a separate experiment using 0.25% ethanol solution in erythrocyte suspension did not show lower transit time (39 ± 4 sec) than that of the control (35 ± 6 sec), whereas quercetin at 10 μM with 0.25% ethanol solution lowered the transit time (25 ± 2 sec). This result confirmed that quercetin possesses a capacity to increase erythrocyte deformability independently from ethanol effect.

3.2. Effect of quercetin and its derivatives on erythrocyte deformability exposed to tar extrtact

The effect of cigarette tar extract in different content on erythrocyte deformability was shown in Fig. 4. The transit time increased with the elevation of cigarette tar extract of

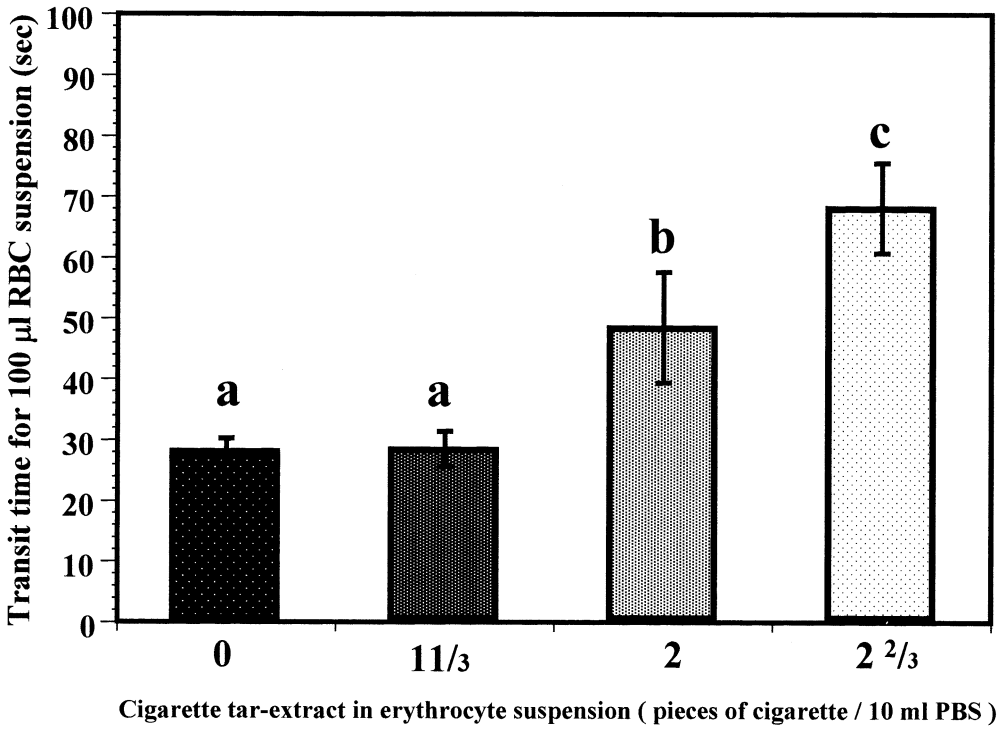

Fig. 4. Effect of cigarette tar extract in different amount on erythrocyte deformability. Aqueous cigarette tar extract solutions were prepared as described in Materials and methods. Tar extract solution with different amount was used to prepare erythrocyte suspension by diluting 9 vol of PBS containing tar extract at a hematocrit of 10%. This suspensions was incubated for 30 min at 37°C. Then 100 μl of the erythrocyte suspension in different amount of tar extract was injected to MC-FAN. Each value is the mean ± SD of at least four experiments. Means not sharing a common letter are significantly different ($p < 0.05$).

two or more pieces indicating that erythrocyte deformability was decreased with increasing the amount of tar extract. In the following experiments, we used the amount of cigarette tar extract from 2⅔ pieces because this amount significantly affected the transit time. Fig. 5 shows that quercetin aglycone and Q3GA, but not Q3G, at 10 μM lowered the transit time of erythrocyte suspension when exposed to tar extract. It is apparent that quercetin aglycone and Q3GA protected erythrocytes from tar extract-induced lowering of its deformability. Photographs shown in Fig. 6 demonstrated the change of size and shape of erythrocyte by exposing to tar extract and its modification with quercetin aglycone in microchannel array. Normal erythrocyte possessed typical biconcave with little change as echinocytes. The treatment of erythrocyte with tar extract only became spherical and swelled. A prominant perforation was characteristic for these cells. By mixing with tar extract solution in erythrocyte treated with quercetin, most of the cells retained the biconcave shape with echinocytes formed.

3.3. Antioxidant activity of quercetin in tar extract-exposed erythrocyte suspension

Table 1 compared the effect of quercetin and that of flavone, a compound composed of basic structure of quercetin without hydroxyl group, on erythrocyte deformability with and without cigarette tar extract. Flavone did not show any effect on the transit time of erythrocyte suspension in both cases as differently from quercetin aglycone. Table 2 shows tar extract-induced lipid peroxidation of erythrocyte suspension during the incubation 30 min before measuring of erythrocyte deformability, as expressed by TBARS and PUFA content. The content of TBARS increased and that of PUFA decreased when erythrocytes were exposed to cigarette tar extract, indicating that the relevant oxidative damage happened in erythrocyte membranes before the measurement of erythrocyte deformability. However, addition of quercetin at 10 μM to the suspension decreased the TBARS and increased PUFA contents significantly. This phenomenon demonstrated that quercetin exerted a protective effect on tar extract-induced oxidative damage of erythrocyte membranes.

4. Discussion

Cigarette smoking is associated with numerous human pathologies including cardiovascular diseases which operates through its complex effects on blood rheology as well as erythocyte deformability [24–26]. In recent years, an increasing body of evidence has supported the hypothesis that dietary consumption of antioxidants including flavonoids favorably influences the incidence of the cigarette smoking-related diseases, in particular, cardiovascular dis-

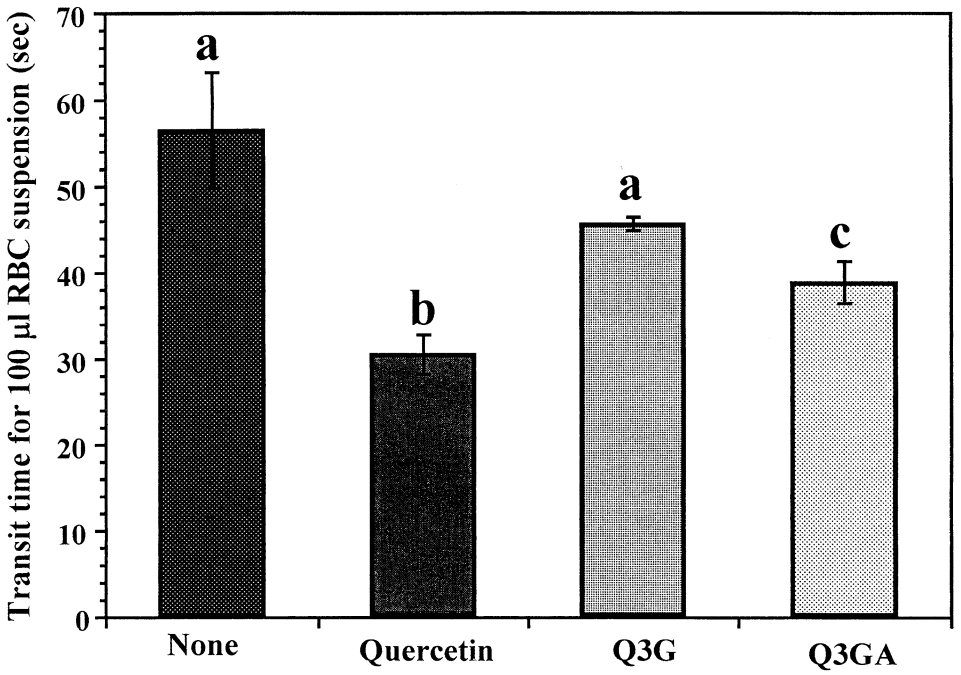

Fig. 5. Effect of quercetin and its derivatives on erythrocyte deformability exposed to cigarette tar extract. Erythrocytes were diluted with 9 vol PBS containing 2⅔ pieces of tar extract with a hematocrit value of 10%. Quercetin or its derivatives was added as ethanol solution at the final concentration of 10 μM in the suspension. The reaction mixture was incubated for 30 min at 37°C. In each case, ethanol content was set at 2.5%. Then 100 μl of erythrocyte suspension was injected to MC-FAN. Each value is the mean ± SD of at least four experiments. Means not sharing a common letter are significantly different ($p < 0.05$).

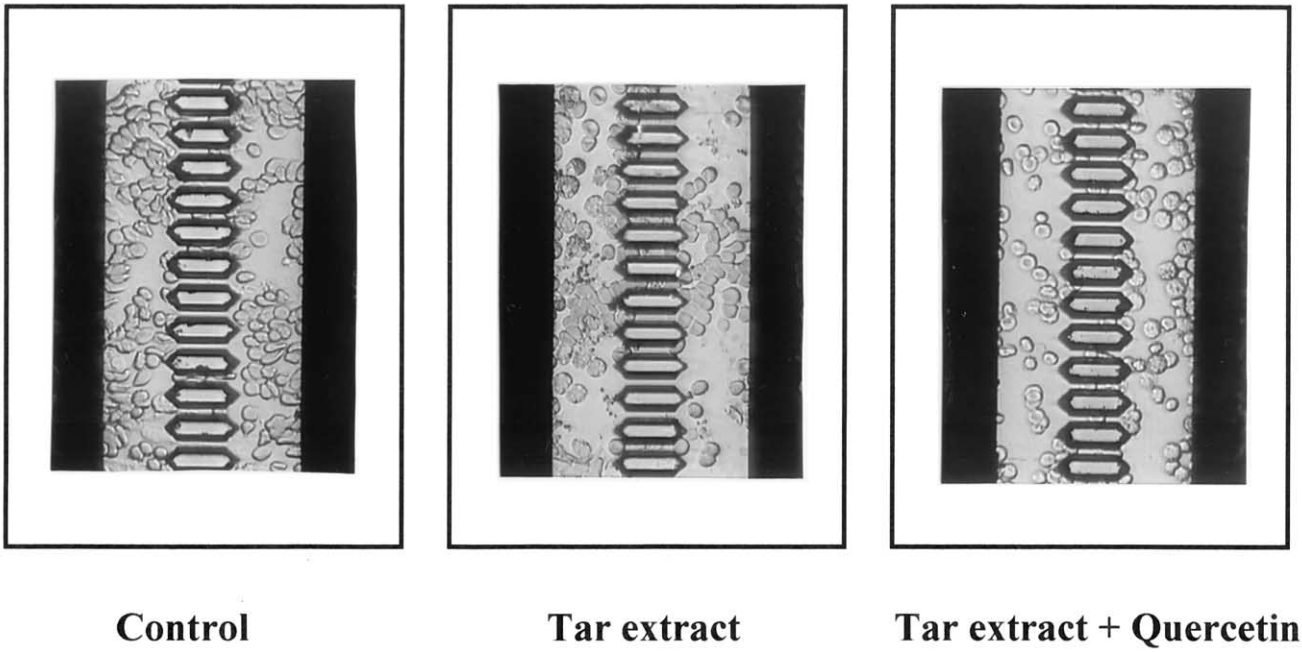

Fig. 6. Photographs of erythrocyte suspension containing quercetin in microchannel array. Erythrocytes were suspended with 9 vol of 0.85% NaCl (A) or tar-extract containing PBS (B). Quercetin was added at the final concentration of 10 μM to the erythrocyte suspension with tar extract contaning PBS (C). Each suspension was incubated for 30 min at 37°C. Transit time of the suspension through microchannel (7 μm diameter) was measured as an index of erythrocyte deformability.

Table 1
Comparison of the effect on erythrocyte deformability between quercetin and flavone

	Transit time for 100μl erythrocyte suspension (sec)	
	Without tar extract	With tar extract
Control	36 ± 3[a]	62 ± 9[a]
Flavone	31 ± 5[a]	76 ± 19[a]
Quercetin	26 ± 3[b]	40 ± 6[b]

Erythrocyte suspension was prepared with or without 9 vol of PBS containing tar extract (2⅔ pieces cigarette/10 ml PBS). Quercetin or flavone was added at the final concentration of 10 μM and then incubated at 37°C for 30 min. Then 100 μl of erythrocyte suspension was injected to MC-FAN apparatus. Each value is the mean ± SD of at least four experiments. Means not sharing a common letter are significantly different ($p < 0.05$).

eases [1]. The aim of the present study was to evaluate the effect of quercetin, one of the most abundant flavonoids in plant foods, on cigarette tar extract induced impairment of erythrocyte deformability. Several recent studies have revealed that cigarette smoke induces lipid peroxidation of erythrocytes resulting in the lowering of its deformability [27,28]. Our results also demonstrated that erythrocyte deformability was decreased in accordance with the occurrence of lipid peroxidation in this blood cell (Fig. 4, Table 2). However, it was noteworthy that quercetin aglycone substantially increased erythrocyte deformability when it was mixed with erythrocyte suspension in the absence of cigarette tar extract (Fig. 2). Bilto and Abdalla [29] also reported that eight selected flavonoids including quercetin increased the filterability of erythrocytes through 5 μm diameter pores. These results imply that quercetin affects the physicochemical property of erythrocytes by hydrophobic interaction with their membranes. We already suggested that quercetin aglycone has strong affinity with liposomal membranes [30]. Nakayama et al. [31] claimed that quercetin aglycone has a planner structure favorable to enter into cell membranes effectively. It is therefore likely that quercetin aglycone is localized in the interface of the membranes, where ROS generated from tar extract induces membranous lipid peroxidation. The phenomenon that quercetin aglycone suppressed cigarette tar extract-induced lipid peroxidation and impairment of erythrocyte deformability (Fig. 5, Table 2) indicates that quercetin aglycone protects erythrocyte membrane from peroxidative attack by scavenging ROS in the interface of erythrocyte membrane. Interestingly, a compound containing no polyhydroxyl structure, flavone, did not exert any significant effect on erythrocyte deformability regardless of cigarette tar extract. Thus, it is reasonable that polyhydroxyl structure is necessary for flavonoids to interact with erythrocyte membrane, as well as to extert antioxidant effect.

Table 2
Antioxidant activity of quercetin on erythrocyte suspension with tar extract during incubation for 30 min

	TBARS (μM)	PUFA (mg/ml erythrocyte suspension)
Control	0.5 ± 0.02[a]	0.71 ± 0.06[a]
Plus tar extract	13.3 ± 0.9[b]	0.40 ± 0.08[b]
Plus tar extract and quercetin	10.7 ± 0.2[c]	0.65 ± 0.05[c]

Erythrocyte suspension containing cigarette tar extract from 2⅔ pieces or no tar extract was used. Quercetin was added to the suspension at the final concentration of 10 μM. Then the reaction mixture was incubated at 37°C for 30 min. Each value is the mean ± SD of at least four experiments. Means not sharing a common letter in rows are significantly different ($p < 0.05$).

In recent years, quercetin metabolites, rather than quercetin aglycone, have been paid much attention concerning the potential pharmacological and physiological function in the circulatory system, because dietary quercetin was found to be present as conjugated metabolites exclusively [15,32]. We recently identified one of the quercetin metabolites possessing antioxidant activity, Q3GA, in rat plasma after oral adminstration [19]. Our present study demonstrated that Q3GA itself increased erythrocyte deformability significantly and also protected erythrocytes from the impairment by cigarette tar extract (Fig. 3, Fig. 5), eventhough its effectiveness was lower than that of quercetin. Quercetin possesses a planner structure with phenolic hydroxyl groups at the 3′- and 4′-position in the B-ring. This catechol group is the site for hydrogen or electron donation for scavenging ROS [33]. Q3GA possesses this *O*-diphenol structure and therefore it can act as an effective scavenger for ROS generated from cigarette tar extract. In addition, Q3GA seems to possess an ability to interact with erythrocyte memebranes because it contains an unique planner structures as similar to quercetin aglycone. Glucuronidation step in quercetin metabolism may retain the effect of its aglycone on erythrocyte membranes. However, it should be noted that Q3G, a quercetin glucoside commonly present in plant foods, did not affect erythrocyte deformability (Figure 3, Figure 5) in spite of its catechol group and planner structure. Not glycoside but carboxyl anion in Q3GA may enhance the interaction with the erythrocyte cell surface composed of carbohydrate chain. Quercetin is present mostly in the form of glycosides in plant foods such as vegetables and fruits. Dietary glycosides are likely to be converted to quercetin metabolites including glucuronide conjugates through hydrolysis by cellular deglucosidation and the glycosidase activity of intestinal bacteria [34,35].

In conclusion, the antioxidant nature of quercetin can extert a protective effect on aqueous cigarette tar extract-induced impairment of erythrocyte deformability. Furthermore, the protective effect of Q3GA against this event implies that quercetin metabolites circulating in blood plasma participate in the antioxidant defense of erythrocyte membranes. Intake of quercetin-rich food may be helpful in the prevention of cigarette tar-induced impairment of erythrocyte deformability, which seems to be related in the lower

incidence of smoking related diseases, e.g. cardiovascular diseases and so on. Further studies are necessary in the experiment *in vivo* to evaluate the physiological effect of dietary quercetin in erythrocyte deformability.

Acknowledgments

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References

[1] M.G. Traber, A. viet Vander, A.Z. Reznick, C.E. Cross, Tobacco-related diseases: Is there a role for antioxidant micronutrient supplementation?, Clinics in Chest Medicine 21 (2000) 173–187.
[2] W.A. Pryor, B.J. Hales, P. Premovic, D.F. Church, The radical in cigarette tar: their nature and suggested physiological implications, Science 220 (1983) 425–427.
[3] T. Nakayama, D.F. Church, W.A. Pryor, Quantitative analysis of hydrogen peroxide formed in aqueous cigarette tar extracts, Free Radic. Biol. Med. 7 (1989) 9–15.
[4] R.W. Carrell, C.C. Winterbourn, F.A. Rechmilewitz, Activated oxygen and hemolysis, Br. J. Haematol. 30 (1975) 259–264.
[5] K.J.A. Davies, A.L. Goldberg, Oxygen radicals stimulate intracellular proteolysis and lipid peroxidation by independent mechanisms in erythrocytes, J. Biol. Chem. 262 (1987) 8220–8226.
[6] I. Tedesco, M. Russo, P. Russo, G. Iacomino, G.L. Russo, A. Carraturo, C. Faruolo, L. Moio, R. Palumbo, Antioxidant effect of red wine polyphenols on red blood cells, J. Nutr. Biochem. 11 (2000) 114–119.
[7] T.W. Chung, C.P. Ho, Changes of viscosity of low shear rates and viscoelastic properties of oxidative erythrocyte suspensions, Clin. Hemorheol. Microcirc. 21 (1999) 99–103.
[8] G. Bekyarova, T. Yankova, I. Kozarev, D. Yankov, Reduced erythrocyte deformability related to activated lipid peroxidation during the early postburn period, Burns 22 (1996) 291–294.
[9] M.A. Srour, Y.Y. Bilto, M. Jama, M.R. Irhimeh, Exposure of human erythrocytes to oxygen radicals causes loss of deformability, increased osmotic fragility, lipid-peroxidation and protein degradation, Clin. Hemorheol. Microcirc. 23 (2000) 13–21.
[10] S.K. Jain, N. Mohandas, M.R. Clark, S.B. Shohet, The effect of malonaldehyde, a lipid peroxidation, on the deformability and ^{51}Cr-survival of erythrocytes, Br. J. Haematol. 53 (1983) 247–255.
[11] R.P. Hebbel, A. Leung, N. Molandas, Oxidation-induced changes in microrheologic properties of the red blood cell membrane, Blood 76 (1990) 1015–1020.
[12] T. Nagai, E. Sekizuka, K. Tsukada, T. Shimizu, T. Kawamura, H. Minamitani, O. Chikara, Shape analysis of red blood cell deformability with microchannel array capillary model, Microcirculation Annual. 15 (1999) 43–44.
[13] E. Ernst, Haemorheological consequences of chronic cigarette smoking, J. Cardiovasc. Risk 2 (1995) 435–439.
[14] G. Galea, R.J. Davidson, Haematological and haemorheological changes associated with cigarette smoking, J. Clin. Pathol. 38 (1985) 978–984.
[15] J.-H. Moon, R. Nakata, S. Oshima, T. Inakuma, J. Terao, Accumulation of quercetin conjugates in blood plasma after the short-term ingestion of onion by women, Am. J. Physiol. 279 (2000) 461–467.
[16] Hertog, E.J.M. Feskens, P.C.H. Hollman, M.B. Katan, D. Kromhout, Dietary antioxidant flavonoids and risk of coronary heart disease: the Zutphen elderly study, Lancet. 342 (1993) 1007–1011.
[17] C. Manach, F. Regerat, O. Taxier, G. Aqulla, C. Demigne, C. Remesy, Bioavailability, metabolism and physiological impact of 4-oxo-flavonoids, Nutr. Res. 16 (1996) 517–544.
[18] S. Chien, Red cell deformability and its relevance to blood flow, Ann. Rev. Physiol. 49 (1987) 177–192.
[19] J.-H. Moon, T. Tsushida, K. Nakahara, J. Terao, Identification of quercetin 3-*O*-*β*-*D*-glucuronide as an antioxidative metabolite in rat plasma after oral adminstration of quercetin, Free Radic. Biol. Med. 30 (2001) 1274–1285.
[20] Y. Kikuchi, K. Sato, Y. Mizuguchi, Modified cell-flow microchannels in a single crystal silicon substrate and flow behaviour of blood cells, Microvasc. Research 47 (1994) 126–139.
[21] W.A. Pryor, Biological effects of cigarette smoke, wood smoke, and the smoker from plastics: the use of electron spin resonance, Free Radic. Biol. Med. 13 (1992) 659–676.
[22] J. Stocks, T.L. Dormandy, The autooxidation of human red cell lipids induced by hydrogen peroxide, Br. J. Haematol. 20 (1971) 95–111.
[23] E.G. Bligh, W.J. Dyer, Rapid method of total lipid extraction and purification, Can. J. Biochem. Physiol. 37 (1959) 911–917.
[24] A. Marazana, R.J. Mehlhorn, Loss of glutathione, ascorbate recycling, and free radical scavenging in human erythrocytes exposed to filtered cigarette smoke, Arch. Biochem. Biophys. 350 (1998) 169–182.
[25] M.A. Srour, Y.Y. Bilto, M. Juma, Susceptibility of erythrocytes from non-insulin-dependent diabetes mellitus and hemodialysis patients, cigarette smokers and normal subjects to in vitro oxidative stress and loss of deformability, Clin. Hemorheol. Microcirc. 22 (2000) 173–180.
[26] K. Salbas, Effect of acute smoking on red blood cell deformability in healthy young and elderly non-smokers, and effect of verapamil on age and acute smoking-induced change in red blood cell deformability, Scand. J. Clin. Lab. Invest. 54 (1994) 411–416.
[27] U. Codandabany, Erythrocyte lipid peroxidation and antioxidants in cigarette smokers, Cell. Biochem. Funct. 18 (2000) 99–102.
[28] E.R. Miller III, L.J. Appel, L. Jiang, T.H. Risby, Association between cigarette smoking and lipid peroxidation in a controlled feeding study, Circulation. 19 (1997) 1097–1101.
[29] Y.Y. Bilto, S.S. Abdalla, Effects of selected flavonoids on deformability, osmotic fragility and aggregation of human erythrocytes, Clin. Hemorrheol. Microcirc. 18 (1998) 165–173.
[30] J. Terao, M.K. Piskula, Flavonoids and membrane lipid peroxidation inhibition, Nutrition 15 (1999) 790–791.
[31] T. Nakayama, K. Ono, K. Hashimoto, Affinity of antioxidative polyphenols for lipid bilayers evaluated with a liposome, Biosci. Biotechnol. Biochem. 62 (1998) 1005–1007.
[32] C. Manach, C. Morand, V. Crespy, C. Demigne, O. Texier, F. Regerat, C. Remesy, Querecetin is recovered in human plasma as conjugated derivatives which retain antioxidant properties, FEBS Lett. 426 (1998) 331–336.
[33] C.A. Rice-Evans, N.J. Miller, G. Paganga, Structure-antioxidant activity relationships of flavonoids and phenolic acids, Free Radic. Biol. Med. 20 (1996) 933–956.
[34] J. Terao, K. Murota, J.-K. Moon, Quercetin glucosides as dietary antioxidants in blood plasma. Modulation of their function by metabolic conversion. Free Radic. Chem. Biol. and Med. (2000) 460–475 (T. Yoshikawa, S. Toyokuni, Y. Yamamoto, Y. Naito, Eds.), OICA International, London.
[35] K. Murota, S. Shimizu, H. Chujo, J.-K. Moon, J. Terao, Efficiency of absorption and metabolic conversion of quercetin and its glucosides in human intestinal cell line Caco-2, Arch. Biochem. Biophys. 384 (2000) 391–397.