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Synergistic effects of flavonoids and ascorbate on enhancement in DNA degradation induced by a bleomycin–Fe complex

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Abstract

Flavonoids were examined for synergistic effects with ascorbate on enhancement of DNA degradation induced by a bleomycin(BLM)–Fe complex. The synergistic effects of flavonoids and ascorbate on DNA degradation induced by the BLM–Fe complex were observed to be greater with flavonoids such as isorhamnetin, kaempferol and morin, which accelerated oxidation more markedly in the presence, than in the absence of BLM. Conversely, myricetin and fisetin, which showed oxidation barely accelerated by the addition of BLM, inhibited DNA degradation promoted by ascorbate. Consequently, there was a good correlation between oxidation of flavonoids accelerated by BLM and the extent of DNA degradation promoted synergistically with ascorbate. Our previous studies indicated that oxidation of flavonoids accelerated by BLM and DNA degradation promoted by flavonoids were not correlated with Fe(III)-reducing activity of flavonoids. Those results suggest that Fe(III)-reducing activity of flavonoids is not the only factor determining DNA degradation-promoting activity induced by the BLM–Fe complex. On the other hand, in a Fenton reaction, degradation of 2-deoxy-D-ribose promoted by flavonoids was correlated to the Fe(III)-reducing activity of flavonoids. However, there was not a synergistic interaction between flavonoids and ascorbate in the degradation of 2-deoxy-D-ribose. Therefore, it is suggested that the synergistic DNA degradation caused by flavonoids and ascorbate in the BLM–Fe redox cycle arose from the difference in the reductive processes in which flavonoids and ascorbate mainly act.

Keywords: *Flavonoids, bleomycin, DNA degradation, Fe(III)-reducing activity, ascorbate, pro-oxidant*

Introduction

The dietary intake of flavonoids is believed to be very important for preventing a wide variety of diseases, including allergies, cardiovascular disease and certain forms of cancer, that involve free radical-mediated damage in pathological processes [1–4]. In the body, antioxidant capabilities of flavonoids are considered responsible for health benefits. However, flavonoids are known to behave as pro-oxidants under some conditions [5–9]. One such example was the finding that flavonoids accelerated DNA degradation induced by the bleomycin(BLM)–Fe complex [10].

BLM exerts antitumor activity by causing the oxidative cleavage of DNA strands. Fe ion, oxygen and a suitable reducing agent are essential cofactors for BLM-mediated DNA degradation [11–13]. Flavonoids play a role as a reducing agent in the redox cycle of the BLM–Fe complex. Previous studies indicated that flavonoids need the following three hydroxyl groups in the flavonoidal nucleus as crucial structures for effectively promoting DNA degradation [14]: (1) the C7-hydroxyl substitution in the A-ring; (2) the C4′-hydroxyl substitution in the B-ring; (3) the C3-hydroxyl substitution in the C-ring. Flavonoids, lacking even one of these hydroxyl substitutions, have

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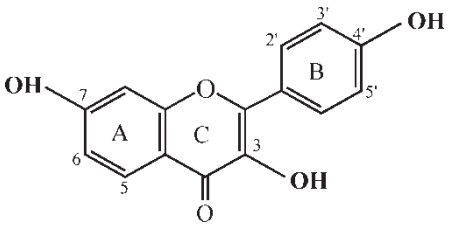

Figure 1. Basic structures of flavonoids for effectively promoting DNA degradation induced by the BLM–Fe complex [14].

markedly diminished promoting activity (Figure 1). The mechanism underlying the degradation of DNA promoted by flavonoids was considered to be their ability to reduce metal ions such as Fe(III) and Cu(II) [10,15–17]. However, no correlation between Fe(III)-reducing activity and DNA degradation-promoting activity was found among these flavonoids [14] (Table I).

The process of oxidative cleavage of DNA strands caused by BLM–Fe complex is represented by the following formula [11,18,19].

$$\mathrm{DNA} + \mathrm{BLM{-}Fe(III)} \rightarrow \mathrm{DNA{-}BLM{-}Fe(III)} \quad (1)$$

$$\mathrm{DNA{-}BLM{-}Fe(III)} + e^- \rightarrow \mathrm{DNA{-}BLM{-}Fe(II)} \quad (2)$$

$$\mathrm{DNA{-}BLM{-}Fe(II)} + O_2 \rightarrow \mathrm{DNA{-}BLM{-}Fe(II){-}O_2} \quad (3)$$

$$\mathrm{DNA{-}BLM{-}Fe(II){-}O_2} + e^- + H^+ \rightarrow \mathrm{DNA{-}BLM{-}Fe(III){-}OOH} \quad (4)$$

$$\mathrm{DNA{-}BLM{-}Fe(III){-}OOH} \rightarrow \mathrm{DNA\ damage} + \mathrm{BLM{-}Fe(III)} \quad (5)$$

Reactions (2) and (4) are redox processes with unspecified reducing agents. The formation of "activated BLM", which is detected as the last intermediate involved in DNA degradation, requires two steps of electron transfer [19,20]. Therefore, flavonoids might be mainly related to another reduction process, the reduction process of DNA–BLM–Fe(II)–O_2 to "activated BLM", but not Fe(III) reduction, in the redox cycle of the BLM–Fe complex. It is well known that ascorbate accelerates DNA degradation induced by the BLM–Fe complex owing to its ability to reduce metal ions [12,21]. Reductants such as ascorbate, vitamin E and glutathione are considered not to act in isolation but to be part of an intricate antioxidant network [22–25].

Table I. Variation in the number and arrangement of the hydroxyl substitutions among flavonoids with the basic structures required for effectively promoting DNA degradation.

	Substitutions			
Compound	C5	C2′	C3′	C5′
Myricetin	OH	H	OH	OH
Quercetin	OH	H	OH	H
Fisetin	H	H	OH	H
Morin	OH	OH	H	H
Kaempferol	OH	H	H	H
Isorhamnetin	OH	H	OCH3	H
5-deoxy Kaempferol	H	H	H	H
Geraldol	H	H	OCH3	H

There are some reports on the cooperative effects of flavonoids and ascorbate [22,26–28]. Hence, in the present study, the cooperative effects of flavonoids, which include crucial structures for effectively promoting DNA degradation, and ascorbate in promoting DNA degradation induced by the BLM–Fe complex, were investigated and compared with the Fenton reaction. Flavonoids and ascorbate may be frequently taken as supplements. It may be beneficial for cancer patients under treatment with BLM to take both agents. The interaction between these chemicals should be further investigated to develop a safe and effective medical regimen.

Materials and methods

Materials

Materials and chemical reagents were obtained from the following companies: Flavonoids from Funakoshi Co. (Tokyo, Japan); ascorbic acid, 2-deoxy-D ribose, bleomycin hydrochloride (BLM), salmon sperm DNA (sodium salt), and ferric chloride from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). All chemicals used were of the highest purity available.

BLM–Fe complex-induced DNA degradation

The reaction mixtures consisted of flavonoids ranging from 5 to 100 μM: 0.5 mg of DNA, 10 mM $MgCl_2$, 5 μg of BLM, 50 μM $FeCl_3$ in a final volume of 1.0 ml of 50 mM Tris-HCl buffer, pH 7.4. Flavonoids were dissolved in dimethyl sulfoxide. The final concentration of dimethyl sulfoxide was less than 1%, which had no detectable effect on DNA degradation. Incubations were started with the addition of $FeCl_3$ to reaction mixtures and continued for 1 h at 37°C. The DNA degradation-promoting activity of flavonoids was evaluated as malondialdehyde (MDA) equivalents. Thiobarbituric reactive substances which arose from deoxyribose degradation of DNA were assessed by a modification of the Uchiyama and Mihara method [29]. Briefly, to 1.0 ml of the reaction

mixture in a 12 ml glass tube, 2.5 ml of 1% phosphoric acid and 1 ml of 0.67% thiobarbituric acid were added. The tube was capped with a screw cap and heated at 100°C for 35 min. After cooling in ice water, 3 ml of n-butanol was added. The mixture was then shaken and centrifuged to separate the organic layer. The fluorescence intensities in the butanol layer were measured at excitation and emission wavelengths of 515 and 553 nm, respectively. Flavonoids tested in this study did not influence the detection of MDA equivalents by the interaction with thiobarbituric acid.

2-Deoxy-D-ribose degradation induced by the Fenton reaction

The reaction mixtures consisted of flavonoids 20 μM: 2.8 mM of 2-deoxy-D-ribose, 50 μM $FeCl_3$, 200 μM $EDTANa_2$, 1.14 mM H_2O_2 in a final volume of 1.0 ml of 50 mM phosphate buffer, pH 7.4. Flavonoids were dissolved in acetonitrile because of the ·OH radical scavenging effect of DMSO [30]. Only myricetin, quercetin, fisetin, morin, kaempferol and 5-deoxy-kaempferol were tested in this reaction owing to the insolubility of isorhamnetin and geraldol. The final concentration of acetonitrile was less than 1%, which had little influence on 2-deoxy-D-ribose degradation induced by the Fenton reaction. Incubations were started with the addition of $FeCl_3$ to reaction mixtures and continued for 1 h at 37°C. The 2-deoxy-D-ribose degradation-promoting activity of flavonoids was evaluated as MDA equivalents, as stated above.

Statistical analysis

The data in figures are given as the mean ± S.D. of three to five experiments. Differences between treatment groups and a control group were determined by Dunnett's test using Stat-100 (BIOSOFT, UK) or Student's *t*-test. A p value of < 0.05 was considered significant. Correlations in Figures 5 and 7 were assessed by least-squares linear regression analysis.

Results

Effects of flavonoids and ascorbate on DNA degradation induced by the BLM–Fe complex

The ability of the tested flavonoids and ascorbate to promote DNA degradation induced by the BLM–Fe complex are shown in Figure 2. Flavonoids, except for morin, promoted the DNA degradation in a dose-dependent manner up to 20 or 50 μM and decreased it at the higher concentration. On the other hand, DNA degradation promoted by ascorbate and morin increased in a dose-dependent manner up to 100 μM. Ascorbate at 20 or 100 μM, which showed 57.2 or 95.2%, respectively, in terms of Fe-reducing activity, promoted 0.66 or 4.98 nmol/h, respectively, of DNA degradation.

Synergistic effects of flavonoids and ascorbate on enhancement in DNA degradation induced by BLM–Fe

Eight flavonoids possessing the three hydroxyl groups necessary for promoting DNA degradation induced by the BLM–Fe complex were examined for cooperation with ascorbate on the enhancement of DNA degradation. As shown in Figure 3, the columns on the left represent total amounts of DNA degradation promoted by 20 μM of tested flavonoids and 100 μM ascorbate separately. Also, the amounts promoted by using both flavonoids and ascorbate together are shown in the right columns. Flavonoids, except for myricetin and fisetin, exhibited a synergistic effect on the enhancement of DNA degradation with ascorbate. DNA degradation synergistically promoted by quercetin, morin, kaempferol, isorhamnetin, 5-deoxy kaempferol and geraldol increased 45.6, 84.0, 96.1, 101.6, 53.5 and 32.4%, respectively, in comparison with total amounts of DNA degradation promoted by using flavonoids or ascorbate separately.

The influence of ascorbate concentration on the synergistic DNA degradation with flavonoids

The concentration of ascorbate influenced the synergism between the flavonoids and ascorbate in enhancing DNA degradation. Figure 4 shows the amounts of DNA degradation obtained by subtracting the total amounts of DNA degradation promoted by using flavonoids or ascorbate separately from the amounts of DNA degradation promoted by using them together. Synergistic effects of flavonoids and ascorbate were observed with all flavonoids, except for myricetin and fisetin, at a concentration of 20 μM ascorbate. Despite the increase from 20 to 100 μM in ascorbate, no effect was observed with fisetin. Myricetin rather inhibited the DNA degradation promoted by 100 μM ascorbate. Other flavonoids tested in this study greatly increased the synergistic effects on DNA degradation, especially morin, kaempferol and isorhamnetin. Synergistic effects on DNA degradation were decreased by increasing ascorbate to 200 μM in the case of all flavonoids which increased synergistic effects at 100 μM ascorbate compared with 20 μM. In contrast to the effect with 100 μM ascorbate, with 200 μM ascorbate quercetin also tended to inhibit DNA degradation. DNA degradation promoted by ascorbate was inhibited more by myricetin and fisetin than by quercetin.

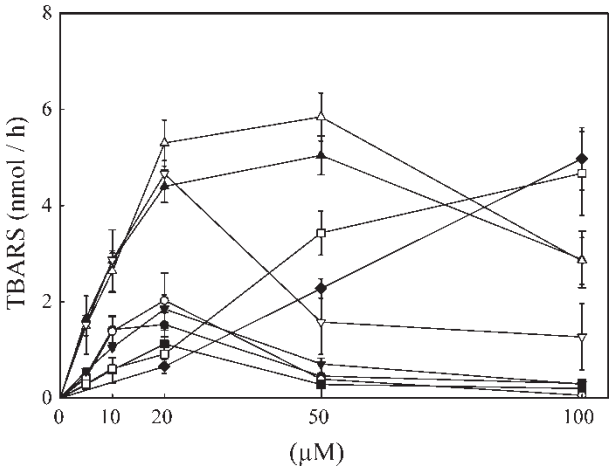

Figure 2. Effects of flavonoids and ascorbate on DNA degradation induced by the BLM–Fe complex. The DNA degradation was evaluated based on the amount of MDA produced by deoxyribose degradation. The amount of MDA produced by BLM–Fe without flavonoids or ascorbate was about 0.5 nmol. The values represent the mean ± S.D. of at least three separate experiments. Flavonoids: Myricetin(•), quercetin(○), fisetin(■), morin(□), kaempferol(▲), Isorhamnetin(△), 5-deoxykaempferol(▼), geraldol(▽) and ascorbate(◆).

The correlation between the oxidation of flavonoids enhanced by BLM, and the synergistic effects of flavonoids and ascorbate on the increase in DNA degradation induced by BLM–Fe

The oxidation of flavonoids was evaluated as the decrease in spectral absorption in the long wavelength area (330 ~ 380 nm) of flavonoids induced by the addition of Fe(III) in the presence and absence of BLM. The decrease in the absorption of flavonoids was enhanced by the addition of BLM into the oxidative reaction mixtures. The order in terms of the enhancement of flavonoid oxidation induced by the addition of BLM was as follows: Isorhamnetin > kaempferol > morin > geraldol > 5-deoxy-kaempferol > quercetin > fisetin > myricetin. Thus, flavonoids which showed a greater enhancement of oxidation on the addition of BLM exhibited greater synergistic effects on DNA degradation when combined with ascorbate. As shown in Figure 5, there was a significant correlation between the extent of oxidation of flavonoids enhanced by BLM and the synergistic effects on DNA degradation induced by BLM–Fe with ascorbate (20 μM ascorbate, $r = 0.841$, $p < 0.01$; 100 μM, $r = 0.938$, $p < 0.001$; 200 μM, $r = 0.854$, $p < 0.01$).

The promoting activity of flavonoids on 2-deoxy-D-ribose degradation in the Fenton reaction

Myricetin, quercetin and fisetin promoted 2-deoxy-D-ribose degradation by ·OH, generated by the addition of Fe^{3+}-EDTA to H_2O_2, at 3.45, 1.37 and 1.48 nmol/h, respectively (Figure 6). However, morin, kaempferol and 5-deoxy-kaempferol tended to inhibit 2-deoxy-D-ribose degradation. The order in terms of the Fe(III)-reducing activity of the flavonoids was as follows: Myricetin > quercetin > fisetin > kaempferol > 5-deoxy-kaempferol > morin. As shown in Figure 7, a significant correlation was seen between the Fe(III)-reducing activity and 2-deoxy-D-ribose degradation-activity among these flavonoids ($r = 0.976, p < 0.001$).

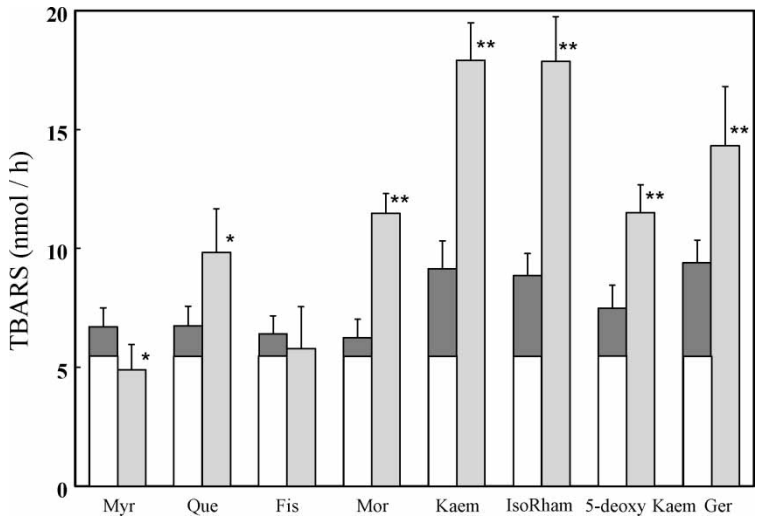

Figure 3. Synergistic effects of flavonoids and ascorbate on enhancement of DNA degradation induced by BLM–Fe. The left and right columns are, respectively, total amounts of DNA degradation promoted by using flavonoids 20 μM (■) or ascorbate 100 μM (□) separately, and the amounts promoted by using them together (▣). The values represent the mean ± S.D. of at least four separate experiments. Significantly different from the total amounts of DNA degradation promoted by using flavonoids or ascorbate separately: *$p < 0.05$, **$p < 0.01$. Flavonoids: Myricetin (Myr), quercetin (Que), fisetin (Fis), morin (Mor), kaempferol (Kaem). Isorhamnetin (IsoRham), 5-deoxykaempferol (5-deoxy Kaem), geraldol(Ger).

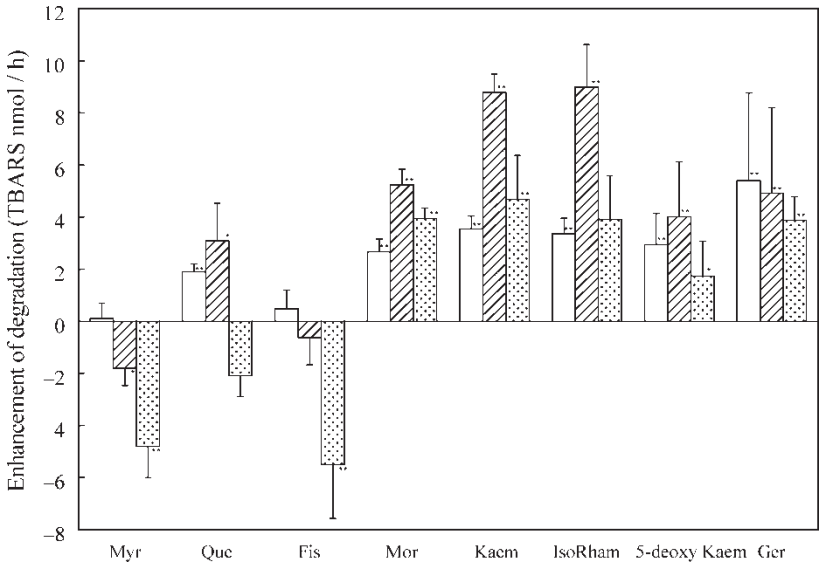

Figure 4. The influence of ascorbate concentration on the synergistic DNA degradation with flavonoids. Each column shows the amounts of DNA degradation calculated by subtracting total amounts of DNA degradation promoted by using flavonoids or ascorbate separately from the amounts of DNA degradation promoted by using them together. The values represent the mean ± S.D. of at least four separate experiments. Significantly different between the total amounts of DNA degradation promoted by using flavonoids 20 μM or ascorbate (▭, 20 μM; ▨, 100 μM; ▭, 200 μM) separately and the amounts promoted by using them together: $*p < 0.05$, $**p < 0.01$. Flavonoids: Myricetin (Myr), quercetin (Que), fisetin (Fis), morin (Mor), kaempferol (Kaem), Isorhamnetin (IsoRham), 5-deoxykaempferol (5-deoxy Kaem), geraldol (Ger).

The effects of flavonoids on 2-deoxy-D-ribose degradation promoted by ascorbate

In Figure 8, the columns on the left show total amounts of 2-deoxy-D-ribose degradation promoted by using flavonoids at 20 μM or ascorbate at 100 μM separately, respectively, while the columns on the right show the amounts promoted by using flavonoids and ascorbate together. The addition of myricetin, quercetin and fisetin to reaction mixtures enhanced degradation 30.0, 15.3 and 12.9%, respectively compared to 2-deoxy-D-ribose degradation promoted by ascorbate. However, the amounts of 2-deoxy-D-ribose degradation enhanced by using flavonoids and ascorbate combined were lower than total amounts of 2-deoxy-D-ribose degradation promoted by using flavonoids or ascorbate separately. In morin, kaempferol and 5-deoxy-kaempferol, 2-deoxy-D-ribose degradation induced by the combination of these flavonoids and ascorbate was similar to that promoted by ascorbate alone. No synergy or additive effects were found in the tested flavonoids when combined with ascorbate in Fenton's reaction.

Discussion

The oxidation of flavonoids with three crucial hydroxyl substitutions, in a DNA degradation reaction catalysed by Fe(III), was markedly greater in the presence than in the absence of BLM [14]. Hence, these flavonoids seemed to promote DNA degradation by acting as a reducing agent in DNA degradation induced by the BLM–Fe complex. The ability of flavonoids to reduce Fe(III), which forms a complex with BLM, had been considered to be responsible for DNA degradation promoted by flavonoids [10,15–17]. However, Fe(III)-reducing activity of these flavonoids correlated neither with DNA degradation, nor their level of oxidation, in the presence of BLM [14].

In the present study, these flavonoids were found to exhibit synergistic effects with ascorbate in promoting DNA degradation induced by the BLM–Fe complex. The synergistic activities of flavonoids with ascorbate were larger in flavonoids such as kaemferol or isorhamnetin, which exhibited more marked enhancement of oxidation in the presence, than in the absence, of BLM. There was a significant correlation between the extent of oxidation enhanced by the addition of BLM and the synergistic effects with ascorbate among these flavonoids, as shown in Figure 5.

Ascorbate is known to promote DNA degradation induced by the BLM–Fe complex owing to its ability to reduce Fe(III) [12,21]. The present study similarly indicated that Fe(III)-reducing activity was responsible for promoting DNA degradation induced by the BLM–Fe complex in ascorbate. Hence, the reducing process in the redox-cycle of the BLM–Fe complex of ascorbate and these flavonoids seemed to be the reduction of Fe(III) and the reduction process of DNA–BLM–Fe(II)–O_2 to "activated BLM", respectively. In addition, it appears that the degree to which each flavonoid acts on the reductive process to "activated BLM", can be deduced from the augmentation of oxidation promoted by the addition of BLM.

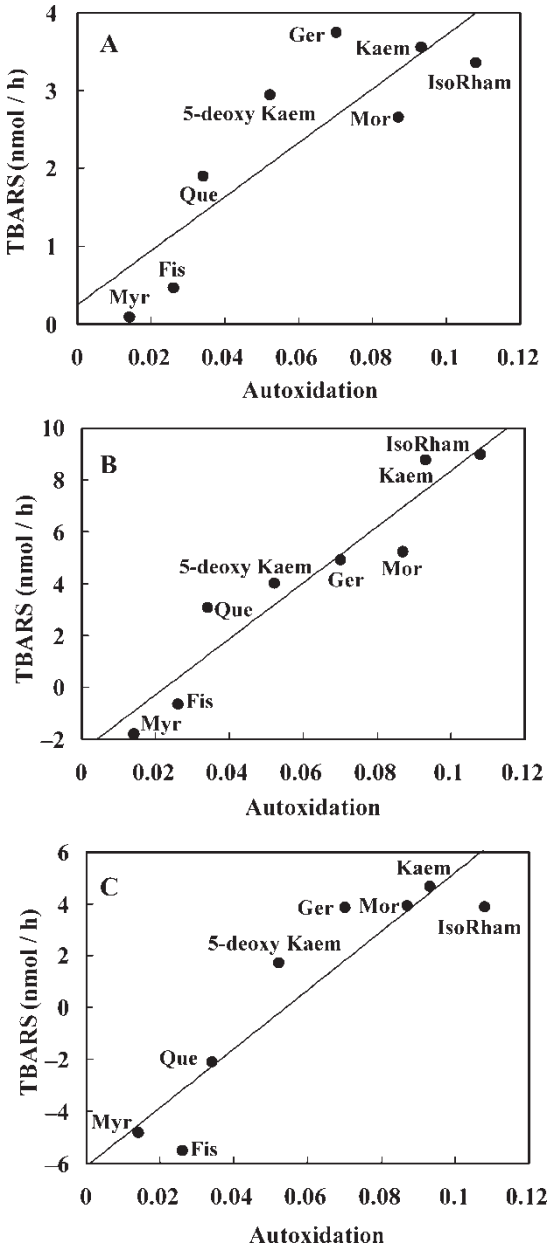

Figure 5. The correlation between the oxidation of flavonoids enhanced by BLM, and the synergistic effects of flavonoids and ascorbate on the increase in DNA degradation induced by BLM–Fe. The values are for the oxidation of flavonoids increased by BLM and the amounts of DNA degradation promoted by synergistic effects of flavnoids and ascorbate indicated in Figure 4 ($r = 0.841$, $p < 0.01$ for ascorbate 20 μM (A), $r = 0.938$, $p < 0.001$ for 100 μM (B) and $r = 0.854$, $p < 0.01$ for 200 μM (C)). Flavonoids: Myricetin (Myr), quercetin (Que), fisetin (Fis), morin (Mor), kaempferol (Kaem), Isorhamnetin (IsoRham), 5-deoxykaempferol (5-deoxy Kaem), geraldol (Ger).

The synergistic DNA degradation between flavonoids and ascorbate in the BLM–Fe redox cycle might be caused by the difference in the reductive process on which they act. However, there was a possibility that the interaction between flavonoids and ascorbate was the mechanism of the synergistic action [22,26–28]. This possibility was investigated using

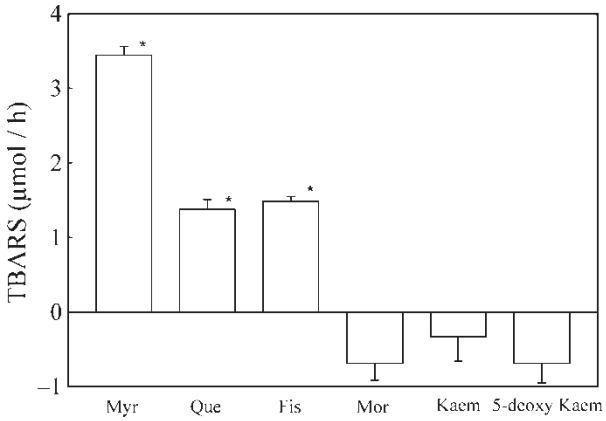

Figure 6. The promoting activity of 2-deoxy-D-ribose degradation of flavonoids in the Fenton reaction. The reaction mixtures consisted of flavonoids 20 μM: 2.8 mM of 2-deoxy-D-ribose, 50 μM $FeCl_3$, 200 μM $EDTANa_2$, 1.14 mM H_2O_2 in a final volume of 1.0 ml of 50 mM phosphate buffer, pH 7.4. After the incubation for 1 h at 37°C, the 2-deoxy-D-ribose degradation was evaluated based on the amount of MDA. The amount of MDA produced by BLM–Fe without flavonoids was about 1.11 nmol. The values represent the mean ± S.D. of at least four separate experiments. Significantly different from the value without flavonoids: $^{\star}p < 0.05$. Flavonoids: Myricetin (Myr), quercetin (Que), fisetin (Fis), morin (Mor), kaempferol (Kaem), 5-deoxykaempferol (5-deoxy Kaem).

Fenton's reaction, which is accelerated by reducing Fe(III) with a reducing agent such as ascorbate or flavonoids. In this experiment, ·OH generated by the addition of Fe^{3+}-EDTA to H_2O_2 causes the degradation of 2-deoxy-D-ribose. As shown in Figure 6, myricetin, quercetin and fisetin, which had strong Fe(III)-reducing activity, enhanced the degradation of 2-deoxy-D-ribose by promoting the reduction of Fe(III). However, morin, kaempferol and 5-deoxy-

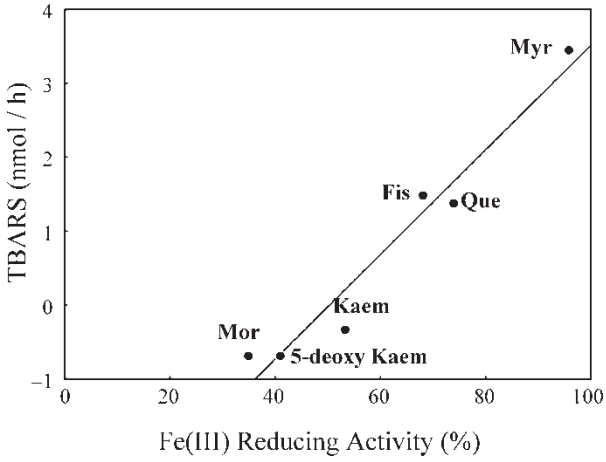

Figure 7. The correlation between the Fe(III)-reducing activity and 2-deoxy-D-ribose degradation-activity in the Fenton reaction among the flavonoids tested in the present study. The values are for the Fe(III)-reducing activity and 2-deoxy-D-ribose degradation-activity of flavonoids in the Fenton reaction ($r = 0.976$, $p < 0.001$). Flavonoids: Myricetin (Myr), quercetin (Que), fisetin (Fis), morin (Mor), kaempferol (Kaem), 5-deoxykaempferol (5-deoxy Kaem).

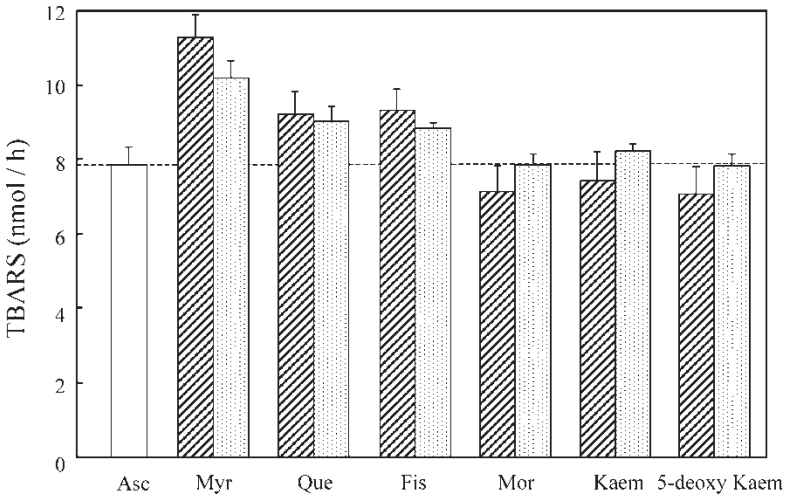

Figure 8. Effects of flaovnoids on 2-deoxy-D-ribose degradation promoted by ascorbate. The left end column (▭) and dotted line are the amount of 2-deoxy-D-ribose degradation promoted by ascorbate alone. The left (▨) and right (⬚) in each column are, respectively, total amounts of 2-deoxy-D-ribose degradation promoted by using flavonoids 20 μM or ascorbate 100 μseparately, and the amounts promoted by using them together. The values represent the mean ± S.D. of at least four separate experiments. Flavonoids: Myricetin (Myr), quercetin (Que), fisetin (Fis), morin (Mor), kaempferol (Kaem), 5-deoxykaempferol (5-deoxy Kaem).

kaempferol, which had weak Fe(III)-reducing activity, rather protected the 2-deoxy-D-ribose degradation caused by ·OH generation. As a result, a significant correlation was recognized between Fe(III)-reducing activity and 2-deoxy-D-ribose degradation-activity among these flavonoids. In Fenton's reaction promoted by reducing Fe(III), no flavonoids acted synergistically with ascorbate to promote 2-deoxy-D-ribose degradation as shown in Figure 8. When flavonoids work on reaction activity via the same mechanism as ascorbate, they are predicted to show additive or small effects with ascorbate. Furthermore, these results suggested that the direct interaction between flavonoids and ascorbate, which was involved in the synergistic effects, did not occur.

Morin at a concentration of 20 μM, which had the weakest Fe(III)-reducing activity among flavonoids with three crucial hydroxyl substitutions, exhibited much lower DNA degradation promoting activity compared with other flavonoids, although it showed high oxidizability in the presence of BLM. The weak Fe(III)-reducing activity of morin might reduce its ability to promote DNA degradation. In addition, as shown in Figure 4, the synergistic effects between flavonoids and ascorbate were lower at a concentration of 200 μM ascorbate than that at 100 μM. Ascorbate might accelerate the reductive process, which was affected mainly by flavonoids, at a concentration of 200 μM, besides the maximum promotion of reducing Fe(III) induced at 100 μM ascorbate. Therefore, the ability to accelerate both reductive processes seemed to be a necessary factor to promote DNA degradation induced by the BLM–Fe complex.

Myricetin and fisetin reduced DNA degradation promoted by ascorbate, as shown in Figure 4. Quercetin also tended to inhibit DNA degradation promoted by ascorbate at 200 μM. These flavonoids have a pyrogallol or catechol moiety in the B-ring on their structures. These moieties are reported to be the most effective in metal chelating and radical scavenging, which are responsibility for antioxidant capability of flavonoids [31,32]. Hence, the ability of antioxidant in these flavonoids is considered to be involved in the reduction of DNA degradation promoted by ascorbate. The other flavonoids without a pyrogallol or catechol moiety are also known to exhibit antioxidant activity through the number and pattern of free hydroxyl substitution in the flavonoidal nucleus [33–35]. The decline in DNA degradation-promoting activity at the higher concentrations might be ascribed to the anti-oxidative property of flavnoids except for morin, as shown in Figure 2. The most desired effect of flavonoids expected for patients who take BLM is considered in the improvement of cancer treatment, not only due to promoting DNA degradation in cancer cells but also by protecting DNA in normal cells from oxidative stress. Further studies would be required to determine the actual clinical effects of flavonoids.

In the present study, the synergistic action between flavonoids and ascorbate was exhibited in DNA degradation induced by the BLM–Fe complex. Furthermore, we propose that this synergistic action between flavonoids and ascorbate arose from the difference in the reductive process in which flavonoids and ascorbate mainly act.

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