Effects of UVA Irradiation on the Concentration of Folate in Human Blood

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Although it is well known that ultraviolet A (UVA) irradiation destroys folate, no definite conclusion for the biological degradation has yet been drawn. In the present study, we determined the effects of UVA exposure on the blood folate concentration in vitro and in vivo. UVA irradiation reduced the synthesized folate pteroylmonoglutamic acid (PGA) content in the blood, but not 5-methyltetrahydrofolate, a major folate form in the blood stream. Exposure to sunlight also decreased the plasma folate concentration in human subjects who took PGA prior to the exposure, but not in subjects who did not take PGA. These results suggest that UVA exposure destroyed PGA but not 5-methyltetrahydrofolate in human blood in vivo.

Key words: folate; ultraviolet A (UVA); pteroylmonoglutamic acid; 5-methyltetrahydrofolate; humans

Folate is a vitamin; therefore, folate deficiency, or impairment of the folate metabolism in humans, leads to several diseases such as megaloblastic anemia,1 neural tube defects,2 and increasing the risk of development of cardiovascular diseases.3 It is well known that the requirement of folate increases in pregnant women.4 Supplementation with folate during pregnancy is strongly recommended in many countries. Pteroylmonoglutamic acid (PGA) is a synthetic form of folate (Fig. 1) and is the oxidized and most stable form of the folates. Therefore, PGA is commonly used as a folate supplement. However, there is a deficit in masking vitamin B12 deficiency by PGA supplementation which is a more severe deficiency than folate deficiency.5 Vorobey et al.6 have reported that PGA in an aqueous solution was degraded by UVA. Furthermore, Der-Petrossian et al.7 have reported that extracorporeal exposure of plasma to UVA during extracorporeal photopheresis led to photodegradation of folate. We have reported that the folate level in serum was higher in young Japanese women than in young men who had been given a vitamin mixture containing PGA.8 We discussed that the phenomenon that a lower folate level in men would be a result of bathing in a lot of sunlight compared with women. On the other hand, the serum folate concentration of subjects who did not take PGA was measured by an HPLC method and microbiological assay recently described.

Materials and Methods

Subjects. Healthy Japanese college students aged from 21 to 24 years old participated in the present experiments. They did not have regular use of medications or dietary supplements, or any habitual alcohol or cigarette consumption. This study was reviewed and approved by The Ethical Committee of the University of Shiga Prefecture.

Chemicals. PGA and 5-MTHF calcium salt were purchased from Wako Pure Chemical Industries (Osaka, Japan), and from Schircks Laboratories (Jona, Switzerland), respectively.

Experiment 1 (Change of PGA in an aqueous solution by UVA irradiation). An aqueous solution of 49 μM PGA was made. 200 μl of the solution was put into the wells of a microtiter plate (Sumilon multiwell plate, MS-8496F, 0.4 ml × 96 wells, flat bottom), and the plate was irradiated with UVA light (EBF-140L/1, Spectronics Corporation; the wavelength was 365 nm) for 0, 30, 60, 90, or 120 min at room temperature. The UVA dose in this study was 0, 800, 1600, 2400, and 3200 mJ/cm², respectively. The respective residual amount of PGA was measured by an HPLC method and microbiological assay recently described.

Experiment 2 (Changes of 5-MTHF in an aqueous solution by UVA or UVB irradiation). An aqueous solution of 20 μM 5-MTHF was made, 200 μl of the solution was put into wells of a microtiter plate

Key words: folate; ultraviolet A (UVA); pteroylmonoglutamic acid; 5-MTHF, 5-methyltetrahydrofolic acid; UVA, ultraviolet A; UVB, ultraviolet B; UVC, ultraviolet C

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Abbreviations: PGA, pteroylmonoglutamic acid; 5-MTHF, 5-methyltetrahydrofolic acid; UVA, ultraviolet A; UVB, ultraviolet B; UVC, ultraviolet C
Experiment 1: Change of PGA in an Aqueous Solution by UVA Irradiation (Experiment 1). Blood was taken from the venous vein at 09:00 before breakfast from Japanese college students (7 males and 4 females), who ate freely, but had not taken any vitamin supplements, by using a syringe coated with EDTA. One milliliter of 110 μM PGA was added to 6 ml of the blood taken and mixed well. Three milliliters of each sample was withdrawn from the PGA- or 5-MTHF-added blood, and the sample in a plate (Sumilon dish, φ60 × 15 mm) was irradiated with UVA light (EBF-140L/J, Spectronics Corporation; the wavelength was 365 nm) for 120 min at room temperature. The UVA dose was 3200 mJ/cm². As a control, the dish was placed in the dark. The folate was measured by a microbioassay. To correct the vaporization under processing, the amount of protein in the blood was measured.

Experiment 2: Change of the folate concentration in blood by UVA irradiation (in vitro experiment). Blood was taken from the venous vein at 09:00 before breakfast from Japanese college students (4 males and 4 females), who ate freely, but had not taken any vitamin supplements, by using a syringe coated with EDTA. A 3-ml amount of the blood in a plate (Sumilon dish, φ60 × 15 mm) was irradiated with UVA light (EBF-140L/J, Spectronics Corporation; the wavelength was 365 nm) for 120 min at room temperature. The UVA dose was 3200 mJ/cm². As a control, the dish was placed in the dark. The folate was measured by a microbioassay. To correct the vaporization under processing, the amount of protein in the blood was measured.

Experiment 3: Change of the folate concentration in blood by UVA irradiation (in vitro experiment). Blood was taken from the venous vein at 09:00 before breakfast from Japanese college students (7 males and 4 females), who ate freely, but had not taken any vitamin supplements, by using a syringe coated with EDTA. One milliliter of 110 μM PGA was added to 6 ml of the blood taken and mixed well. Three milliliters of each sample was withdrawn from the PGA- or 5-MTHF-added blood, and the sample in a plate (Sumilon dish, φ60 × 15 mm) was irradiated with UVA light (EBF-140L/J, Spectronics Corporation; the wavelength was 365 nm) for 120 min at room temperature. The UVA dose was 3200 mJ/cm². As a control, the dish was placed in the dark. The folate was measured by a microbioassay. To correct the vaporization under processing, the amount of protein in the blood was measured.

Experiment 4: Change of the folate concentration in blood, to which PGA or 5-MTHF has been added, by UVA irradiation (in vitro experiment). Blood was taken from the venous vein at 09:00 before breakfast from Japanese college students (7 males and 4 females), who ate freely, but had not taken any vitamin supplements, by using a syringe coated with EDTA. One milliliter of 110 μM PGA or 110 μM 5-MTHF was added to 6 ml of the blood taken and mixed well. Three milliliters of each sample was withdrawn from the PGA- or 5-MTHF-added blood, and the sample in a plate (Sumilon dish, φ60 × 15 mm) was irradiated with UVA light (EBF-140L/J, Spectronics Corporation; the wavelength was 365 nm) for 120 min at room temperature. The UVA dose was 3200 mJ/cm². As a control, the dish was placed in the dark. The folate was measured by a microbioassay. To correct the vaporization under processing, the amount of protein in the blood was measured.

Experiment 5: Comparison of the folate concentrations in blood between male and female young adults who ate freely. The subjects were 23 male and 32 female students who ate freely. Blood was taken from the venous vein before lunch at around 12:00. The folate concentration was measured by a microbioassay.

Experiment 6: Change of the folate concentration in blood, withdrawn from the subjects who took no PGA supplements, by sunlight exposure (in vivo experiment). The subjects were 9 male (average (± SD) age, height, body weight, and BMI were 23.6 ± 2.7 years, 173.7 ± 4.6 cm, 69.2 ± 8.7 kg, and 22.9 ± 2.7 kg/m²) and 14 female (average (± SD) age, height, body weight, and BMI were 21.8 ± 2.4 years, 160.0 ± 4.0 cm, 51.7 ± 4.4 kg, and 20.0 ± 1.3 kg/m²) students who ate freely. Blood was taken from the venous vein before and after sunlight exposure at 11:00 and 13:00, respectively. The subjects with short trousers and the tank tops were exposed to sunlight from 11:00 to 13:00 in the summer. The dose of UVA was about 19,000 mJ/cm². The folate concentration was measured by a microbioassay.

Experiment 7: Change of the folate concentration in blood, withdrawn from the subjects who took PGA, by sunlight exposure (in vivo experiment). The subjects were 7 female students. Their
of a degassed solution of 20 mM phosphoric acid containing 5 mM EDTA and 5 mM folic acid. The solution was loaded into a Shimadzu Chromatopac C-R8A instrument for data processing. The UV detector was set at 280 nm. The HPLC system was interfaced with a Shimadzu auto-injector, a column oven CTO-10A, and an Shiseido Superiorex ODS (3 μm, 4.6 mm x 250 mm) column. The mobile phase was a mixture of a degassed solution of 20 mM phosphoric acid containing 5 mM hexanesulfonate-acetonitrile (9:1, v/v) and was used at a flow rate of 1.0 mL/min. The column temperature was maintained at 40℃, and the UV detector was set at 280 nm. The HPLC system was interfaced with a Shimadzu Chromatopac C-R8A instrument for data processing.

Microbioassay. Plasma was obtained from EDTA-treated blood by centrifuging at 3,000 x g for 5 min at 4℃. The plasma obtained was directly used for a microbioassay using Lactobacillus rhamnosus ATCC 27773.13)

Protein determination. Protein concentration was determined by a BioRad protein assay, with bovine serum albumin as the standard.

Statistical analysis. The computer program, GraphPad Prism version 4.03 (GraphPad Software, San Diego, USA) was used for data analysis. The D’agostino and Pearson omnibus normality test showed that the blood folate concentration in experiments 3, 4, 5 and 6 was normally distributed, and the Shapiro-Wilk normality test showed this in experiment 7. Statistical significance was assessed by two-tailed paired Student’s t test in experiments 3, 4, 6 and 7, and by a two-tailed unpaired Student’s t test in experiment 5.

Results

Change of PGA in an aqueous solution by UVA irradiation (Experiment 1)

When the PGA solution was irradiated with UVA, the amount of PGA decreased according to the exposure time as shown in Fig. 2. This phenomenon was observed when the assay method was changed from HPLC to a microbioassay, using Lactobacillus rhamnosus which needs folate for growing. This result means that the degraded PGA did not have folate activity.

Change of 5-MTHF in an aqueous solution by UVA or UVB irradiation (Experiment 2)

Even when the 5-MTHF solution was irradiated with UVA or UVB, the amount of 5-MTHF was not decreased as shown in Fig. 3.

Change of the folate concentration in human blood with UVA irradiation in vitro (Experiment 3)

The blood was withdrawn from college students, and the EDTA-treated blood was directly exposed to UVA for 120 min. However, the folate concentra-
tion did not change with UVA irradiation as shown in Fig. 4.

**Change of the folate concentration in human blood which PGA or 5-MTHF added to the blood with UVA irradiation in vitro (Experiment 4)**

PGA or 5-MTHF was directly added to human blood, and the blood was exposed to UVA. As is shown in Fig. 5, the folate concentration in the PGA-added blood was significantly decreased with UVA irradiation, while that in the 5-MTHF-added blood did not decrease.

**Comparison of the human blood folate concentrations between males and females who ate freely (Experiment 5)**

The concentrations of blood folate in humans who ate freely, but had not taken folate as a supplement, were measured. We have previously reported that the concentration was higher in females than in males. In the present experiment, the difference between males and females was not significant as shown in Table 1.

**Fig. 5. Change of the Folate Concentration in Human Blood, to Which PGA or 5-MTHF Had Been Added, by UVA Irradiation (Experiment 4, in vitro experiment).**

Blood was taken from the venous vein. One milliliter of 110 μM PGA or 110 μM 5-MTHF was added to 6 ml of the blood taken and mixed well. Three milliliters of the sample was withdrawn from the PGA- or 5-MTHF-added blood and irradiated with UVA light for 120 min. The UVA dose was 3,200 mJ/cm². As a control, the blood in a dish was placed in the dark. The folate was measured by a microbioassay. Circles indicate individual values, and the horizontal line in the figure is the mean. *Statistically significant at p < 0.05.

**Fig. 6. Change of the Folate Concentration in Human Blood, Which Was Withdrawn from the Subjects Who Had Not Taken a PGA Supplement, by UVA Irradiation (Experiment 6, in vivo experiment).**

The subjects were 9 male and 14 female students who ate freely. The blood was taken from the venous vein before and after sunlight exposure at 11:00 and 13:00, respectively. The folate concentration was measured by a microbioassay.

**Table 1. Comparison of the Serum Folate Concentration between Male and Female Japanese Young Adults Who Ate Freely (Experiment 5)**

<table>
<thead>
<tr>
<th>Gender</th>
<th>Mean (pmol/ml)</th>
<th>SD</th>
<th>Minimum</th>
<th>Maximum</th>
<th>Medium</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male (n = 23)</td>
<td>15.0</td>
<td>5.8</td>
<td>7.2</td>
<td>29.2</td>
<td>13.8</td>
</tr>
<tr>
<td>Female (n = 32)</td>
<td>17.7</td>
<td>5.9</td>
<td>9.5</td>
<td>31.5</td>
<td>15.7</td>
</tr>
</tbody>
</table>

The subjects were 23 male and 32 female students, who ate freely. Blood was taken from the venous vein before lunch at around 12:00. The folate concentration was measured by a microbioassay.
After exposure, the concentration was decreased by 74% with sunlight vs. sunlight exposure including UVA.

Change of the folate concentration in blood, withdrawn from those subjects who had taken no PGA supplements, by sunlight exposure (in vivo experiment)

The subjects who had not taken a folate supplement were exposed to sunlight. As is shown in Fig. 6, the concentration of blood folate did not decrease with sunlight exposure including UVA.

Change of the folate concentration in blood, withdrawn from those subjects who had taken PGA, by sunlight exposure (in vivo experiment)

The subjects who had been administered with PGA were exposed to sunlight. As is shown in Fig. 7, the concentration of blood folate significantly decreased with sunlight exposure (38.0 ± 7.2 pmol/ml vs. 28.1 ± 4.6 pmol/ml). On the contrary, the blood folate concentration in the subjects not exposed to sunlight was not altered (38.6 ± 3.3 pmol/ml vs. 38.2 ± 2.3 pmol/ml in 11:00 vs. 13:00). In other words, the blood folate concentration was decreased by 74% with sunlight exposure.

Discussion

Folate, a B-group vitamin, is the essential cofactor in the biosynthesis of a de novo purine base. So, the rate of folate catabolism progressively increases during pregnancy and the folate demand increases. For a example, Higgins et al. have reported that the requirements of folate in the second trimester and in the third trimester were 430 and 540 μg/d, while that in nonpregnant women was 250 μg/d. Some investigators have also advised women to consume a folate supplement for the duration of pregnancy and lactation. The main folate supplement that is available on the market is PGA, which is a synthetic form of folate, and is the oxidized and most stable form of folates. However, the demerit of PGA is that it is destroyed by UVA exposure which partly penetrates into the blood stream. Therefore, there is the possibility of a reduction of the blood folate level with sunlight exposure. In fact, Der-Petrossian et al. have reported that extracorporeal exposure of plasma to UVA during extracorporeal photophoresis led to the photodegradation of folate. Furthermore, it has been proposed that folate deficiency may result from intense solar exposure, and that sun-induced folate degradation may play a key role in the evolution of human skin color. On the other hand, Gambichler et al. have reported that the serum folate concentration was not decreased by exposure to UVA; their data suggest that UVA exposure do not significantly influence the serum folate level of healthy subjects and they concluded that the neural tube defects claimed to occur after per conceptual UVA exposure were probably not due to UVA-induced folate deficiency. Therefore, no definite conclusion about the biological significance of folate photodegradation in vivo can yet be drawn. We carried out the present experiment to elucidate this controversy.

Some investigators have already shown that PGA was degraded by UVA exposure; however, these experiments just showed the detection of the degraded compounds. No quantitative experiment had been reported.

In experiment 1, we showed that PGA in an aqueous solution was completely destroyed by exposure to 3,200 mJ/cm² UVA, as determined by the methods of a microbioassay and HPLC. However, we showed that 5-MTHF was not destroyed by exposure to UVA in experiment 2. In experiments 3 and 4, the blood folate concentration did not decrease, even when the blood was directly exposed to UVA, while the folate concentration in blood with added PGA decreased and that with added 5-MTHF did not. 5-MTHF is the major form of blood folate when humans eat an ordinary food. From these findings, it is suggested that the blood folate level decreased when the blood contained PGA, while the blood folate level did not decrease when the blood did not contain PGA. We have already reported that the female blood folate level was higher than that of males when the subjects were fed on a semi-purified diet containing PGA. However, as shown in Table 1 (experiment 5), the blood folate levels between males and females were no different when the subjects ate freely and had not taken PGA. From these data, we hypothesize that PGA in the blood is destroyed, but not 5-MTHF, by exposure of sunlight. If humans do not take PGA, the blood folate concentration would not be decreased by exposure to sunlight, because the form of blood folate is mainly 5-MTHF. On the contrary, if humans take PGA, the blood concentration would be decreased by exposure to sunlight, because the PGA taken appears in the blood stream as PGA itself. Thus, we carried out experiments 6 and 7. As was anticipated, when the subjects who had taken no PGA supplement were exposed to sunlight, the blood folate level did not decrease, while when the subjects who had taken PGA were exposed to sunlight, the folate level was significantly decreased (Fig. 8).
In conclusion, only PGA, a synthetic form of folate in the blood stream was destroyed by UVA, but not 5-MTHF. We recommend that 5-MTHF is superior to PGA as a folate supplement.

Acknowledgment

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References