

Moderate Alcohol Consumption Reduces Urinary 8-Hydroxydeoxyguanosine by Inducing of Uric Acid

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Abstract: Recent studies suggest that moderate alcohol consumption is associated with a low risk of cancer, coronary heart disease, and other diseases. Most of these diseases are considered to be related to the action of reactive oxygen species (ROS) at certain stages of disease progression. However, considerable evidence exists indicating that ethanol generates ROS *in vivo*. Thus, the reduced risk of disease as a result of alcohol consumption seems to contradict evidence suggesting the induction of ROS by ethanol. In the present study, we investigated whether oxidative stress was induced in moderate alcohol drinkers. We measured the total urinary biopyrrins and 8-hydroxydeoxyguanosine (8-OHdG) levels as a systemic oxidative stress marker and an oxidative DNA damage marker, respectively. Serum uric acid was also measured as an alcohol-induced antioxidant. We compared total urinary biopyrrins and 8-OHdG levels among groups with different alcohol habits. The results showed that total biopyrrins levels increased with the amount of alcohol consumed, but that the level of 8-OHdG significantly decreased with the amount of alcohol consumed. The decrease in 8-OHdG levels seemed to be associated with increasing levels of uric acid. Judging from the increasing level of total biopyrrins, alcohol may induce ROS. ROS may then cause cell damage in liver, as suggested by the positive correlation between the total biopyrrins levels and the serum GOT, GPT, and γ -GTP levels. However, since ROS may be more effectively counteracted by uric acid in organs other than the liver, DNA damage may be suppressed rather than induced. Accordingly, moderate alcohol consumption seems to have the overall effect of reducing DNA damage, as shown by the decrease in urinary 8-OHdG levels observed in our study.

Key words: Alcohol, Oxidative stress, 8-Hydroxydeoxyguanosine, Biopyrrins

Introduction

Educating workers to improve their drinking habits as well as to reduce smoking and engage in regular exercise is an important target in worker health promotion¹, and thus investigating the health effects of alcohol consumption in workers is useful for promoting health objectives.

Recent studies show that moderate alcohol consumption (less than 45 g per day by Japanese and 60 g per day by Westerners), as opposed to no alcohol consumption, reduces the risk of death from coronary heart disease (CHD)^{2–4},

cancer⁵, and other causes^{5–7}. On the other hand, ethanol exposure has been shown to cause lipid peroxidation in rat livers^{8,9} and brains¹⁰, DNA cleavage in isolated hepatocytes¹¹, a reduction in the levels of antioxidant vitamins A and E^{12,13}, and a reduction in the levels of cerebral and hepatic glutathione¹². Further, the hydroxyl radical level in the peripheral blood of human subjects has been shown to increase with alcohol consumption¹⁴. These findings suggest that exposure to ethanol induces reactive oxygen species (ROS), and several possible mechanisms to explain the generation of ROS after ethanol exposure have been presented¹⁵. Since the occurrence of CHD and cancer have been considered to be related to ROS^{16,17}, the data showing reduced risk of death

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Table 1. Results of the analysis of the long-term effects of alcohol

Groups*	Age	Smoking habit				Blood chemistry		
		Male	Female	Non-Smokers	Smokers	GOT	GPT	γ -GTP
Non-drinkers	39.3 \pm 11.3	17	7	13	11	20.8 \pm 5.7 ^a	25.2 \pm 14.1 ^c	27.6 \pm 24.7 ^e
Light-drinkers	33.3 \pm 11.1	17	4	10	11	21.0 \pm 3.6 ^b	22.9 \pm 10.3 ^d	30.0 \pm 33.7 ^f
Moderate-drinkers	44.0 \pm 11.2	21	1	9	13	33.8 \pm 21.0 ^b	41.0 \pm 31.0 ^d	77.68 \pm 65.5 ^f

*Non-drinkers: those who do not consume alcohol; light-drinkers: those who consume 1–15 units of alcohol per week; Moderate-drinkers: those who consume 16–42 units of alcohol per week. ^{a, b, c, d, e, f} $p < 0.05$ significant difference (Bonferroni test).

Table 2. Results of the analysis of the acute effects of alcohol

Groups	Age	Smoking habit				Blood chemistry		
		Male	Female	Non-Smoker	Smoker	GOT	GPT	γ -GTP
Abstaining group	36.6 \pm 11.7	21	4	14	11	22.6 \pm 7.7 ^a	27.6 \pm 16.3	40.4 \pm 45.3
Alcohol-consuming group	41.7 \pm 12.5	17	1	5	13	34.4 \pm 22.1 ^b	38.4 \pm 32.7	73.8 \pm 67.0

^{a, b} $p < 0.05$ significant difference (Bonferroni test).

from CHD and cancer in alcohol drinkers seem to contradict the evidence that suggests induction of ROS by ethanol. Whether the *in vivo* induction of ROS by ethanol contributes to the toxic effects of alcohol remains uncertain.

In the present study, we investigated whether moderate alcohol consumption induces oxidative stress and whether the oxidative stress contributes to cell and DNA damage. We also investigated whether moderate alcohol consumption alters the level of uric acid, an antioxidant that is induced by alcohol. Finally, we discuss the relationship among alcohol consumption, oxidative stress, and DNA damage in view of our results.

Materials and Methods

Subjects

Sixty-eight healthy Japanese, consisting of 58 males (21 to 58 years old, mean 35.9 \pm 13.5) and 10 females (20 to 61 years old, mean 39.7 \pm 11.5) were included in the study. The subjects had not been exposed to workplace chemicals suspected of inducing oxidative stress. All subjects agreed to anonymously donate blood and urine samples and gave their written informed consent. The blood and urine samples were collected at the time of the subject's regular health checks.

Classification of the subjects

An experienced occupational health doctor interviewed the subjects concerning their drinking habits, smoking history, medical history, and other lifestyle factors using a questionnaire (see appendix for the alcohol drinking habit questionnaire). First, the subject's habitual alcohol intake level was reported

as frequency of alcohol consumption. Subjects who were habitual drinkers were also asked to report the amount and type of alcohol beverages usually consumed. The amount of ethanol was calculated in grams as follows¹⁸⁾: 180 ml of sake (rice wine), 27.7 g of ethanol; 180 ml of shochu (white spirits), 45 g of ethanol; 30 ml of whiskey or brandy, 12.9 g of ethanol; 60 ml of wine, 7.2 g of ethanol; and 633 ml of beer, 28.4 g of ethanol. Wannamethee *et al.*⁴⁾, classified levels of alcohol drinking into none, occasional (less than 1 unit per week), light (1–15 units per week), moderate (16–42 units per week), and heavy (more than 6 units per day), and since one UK unit of alcohol (1 drink) was defined as half a pint (570 ml) of beer, a single measure of spirits, or a glass of wine (approximately 8–10 g alcohol)⁴⁾, we defined 1 unit equals 10 g of ethanol as the conversion factor. To evaluate the long-term effects of alcohol consumption, we classified the subjects into three groups: non-drinkers, light drinkers (1–15 units per week), and moderate drinkers (16–42 units per week). To evaluate the acute phase effects (acute effects) of alcohol consumption, we subclassified the light and moderate drinkers into two groups depending on their drinking history on the day before the day of sampling: an abstaining group and an alcohol-consuming group. The characteristics of each group tested for long-term effects of alcohol consumption are shown in Table 1, and the characteristics of each group tested for acute effects are shown in Table 2.

Biological and hematological measurements

Serum levels of glutamic oxaloacetic transaminase (GOT), glutamic pyruvic transaminase (GPT), and gamma-glutamyl

transpeptidase (γ -GTP) activity were selected for estimating cell damage. The total urinary biopyrrin, which are oxidative metabolites of the antioxidant bilirubin¹⁹, were selected as an indicator for estimating levels of systemic oxidative stress. The level of urinary 8-hydroxydeoxyguanosine (8-OHdG), a useful marker of oxidative DNA damage²⁰⁻²², was selected as an indicator for estimating the level of oxidative DNA damage. The serum level of uric acid, which varies with the amount of ethanol consumed^{23, 24}, was measured as an antioxidant in the serum²⁵⁻²⁸. Blood samples were collected from an antecubital vein of each subject in the morning. GOT, GPT, γ -GTP, and uric acid were measured with an automated biochemical analyzer (AU-5200; Olympus, Japan) with Quick Auto II GOT (Shino-Test Corporation, Sagamihara, Japan), Quick Auto II GPT (Shino-Test Corporation), DAIYA- γ -GTP (DAIYA SHIYAKU Co. Ltd, Japan) and Quick Auto Neo UA (Shino-Test Corporation), respectively. Urine samples were collected in the morning and a portion of it was immediately frozen; exposure of the sample to light was kept at a minimum. The level of urinary 8-OHdG was measured with Wellreader sme 3400 (Iwaki, Japan) by using an 8-OHdG Check ELISA kit, which contains the anti-8-OHdG monoclonal antibody N45.1 (Japan Institute for the Control of Aging, Japan). Total urinary biopyrrins levels were measured with a BIOPYRRIN EIA KIT, which contains the anti-bilirubin monoclonal antibody 24G7 (Shino-Test Corporation). The level of creatinine in urine was measured with an automated biochemical analyzer (7150 Automatic Analyzer; Hitachi, Ltd., Japan) and Accuras Auto CRE (Shino-Test Corporation). The urinary 8-OHdG and total biopyrrins values were adjusted for the urinary creatinine value.

Statistical analysis

Analysis of variance was used to test differences among group means and the Bonferroni test was used for multiple comparisons. Spearman's correlation coefficient was used to test relations between categorical variable and numerical variable, and Pearson's correlation coefficient was used to test relations between numerical variables.

Results

Analysis of the long-term effects of alcohol consumption showed that the levels of all cell damage markers GOT, GPT, and γ -GTP significantly increased with moderate alcohol consumption (Table 1) indicating that moderate daily alcohol consumption had induced liver toxicity. The total urinary biopyrrin levels correlated positively ($p < 0.01$) with the activity of GOT ($r = 0.427$), GPT ($r = 0.386$), and γ -GTP

($r = 0.591$) levels. The total biopyrrin level of the moderate-drinker group ($2.3 \pm 1.3 \mu\text{mol/g creatinine}$) was significantly higher than that of the light-drinker group ($1.5 \pm 0.9 \mu\text{mol/g creatinine}$) (Fig. 1A) whereas the level of urinary 8-OHdG tended to be lower in the moderate-drinker group (Fig. 1B). The uric acid level of the moderate-drinker group ($6.0 \pm 1.1 \text{ mg/g creatinine}$) was significantly higher than that of the light drinker group ($5.0 \pm 1.1 \text{ mg/dl}$) and non-drinker group ($4.9 \pm 1.3 \text{ mg/dl}$) (Fig. 1C). The correlation coefficient for the relationship between the uric acid levels and weekly alcohol consumption level was 0.334 ($p < 0.01$).

Analysis of the acute effects of alcohol consumption showed that the GOT level of the alcohol-consuming group was significantly higher than that of the abstaining group (Table 2). The levels of GPT and γ -GTP activities of the alcohol-consuming group also tended to be higher than those of abstaining group. Although not significant, the total urinary biopyrrin level of the alcohol consuming group ($2.1 \pm 1.3 \mu\text{mol/g creatinine}$) was higher than that of the abstaining group ($1.7 \pm 1.1 \mu\text{mol/g creatinine}$) (Fig. 2A). On the contrary, the urinary 8-OHdG level of the alcohol-consuming group ($6.5 \pm 2.2 \mu\text{g/g creatinine}$) was significantly lower than that of the abstaining group ($8.4 \pm 2.7 \mu\text{g/g creatinine}$) (Fig. 2B). The correlation coefficient for the relationship between urinary 8-OHdG levels and the level of alcohol consumed on the day before specimen collection was -0.315 ($p < 0.05$). In addition, the level of serum uric acid of the alcohol-consuming group ($6.1 \pm 1.0 \text{ mg/dl}$) was significantly higher than that of the abstaining group ($5.0 \pm 1.1 \text{ mg/dl}$) (Fig. 2C). The correlation coefficient for the relationship between uric acid levels and the level of alcohol consumed on the day before the specimen collection was 0.456 ($p < 0.01$).

There was no correlation between the level of urinary 8-OHdG and total urinary biopyrrin level (data not shown), but the serum uric acid levels correlated negatively with the urinary 8-OHdG levels ($r = -0.342$, $p < 0.01$).

Since smoking has been reported to induce 8-OHdG production^{17, 29, 30} we investigated whether smoking worked as a confounder to alcohol consumption which lead to the alcohol consumption responsible to the change of 8-OHdG level. We analyzed the level of 8-OHdG as a dependent variable using factorial analysis of variance with daily alcohol consumption (or the amount of alcohol consumed on the day before the sample collection) and smoking habit as two factors. After controlling the smoking habit the effect of the amount of alcohol consumed on the previous day still remained. We also examined whether age influenced the production of total urinary biopyrrins, but no correlation between the level of total urinary biopyrrins and age was found (data not shown).

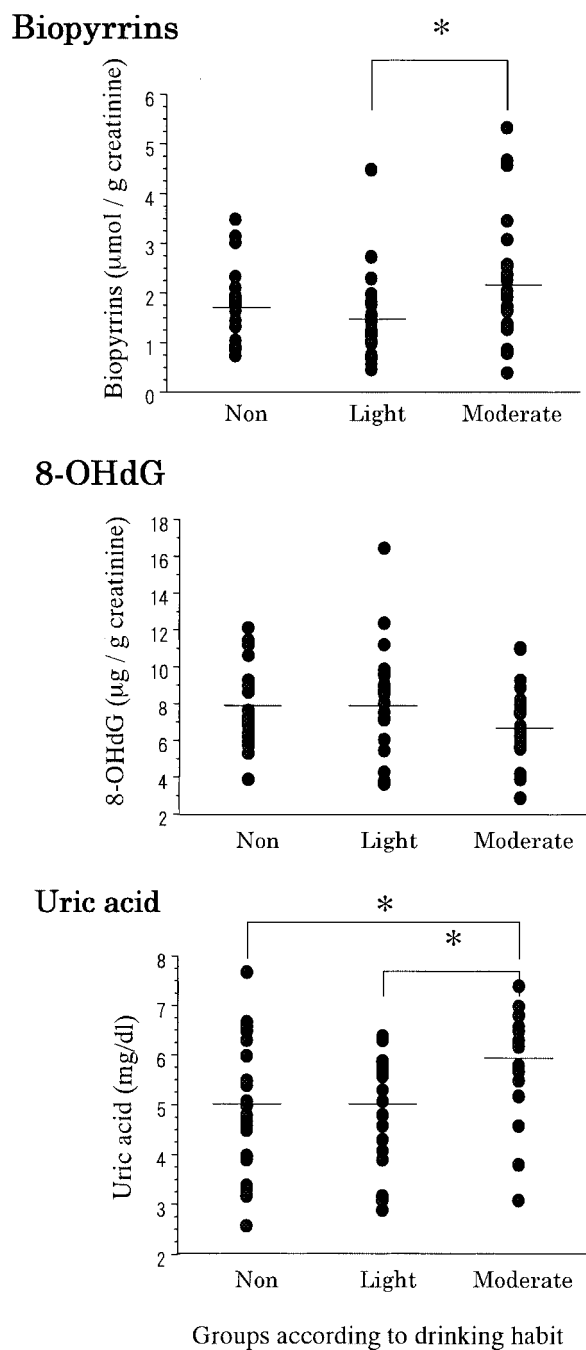


Fig. 1. Results of the assessment of the long-term effects of alcohol consumption.

Subjects have been classified: Non, non-drinkers; Light, light-drinkers (1–15 units per week); and Moderate, moderate-drinkers (16–42 units per week). *P<0.05 (Bonferroni test).

Discussion

The amount of alcohol consumption was determined during an interview with each subject, and the reliability of the self-reported consumption was supported by measuring the liver

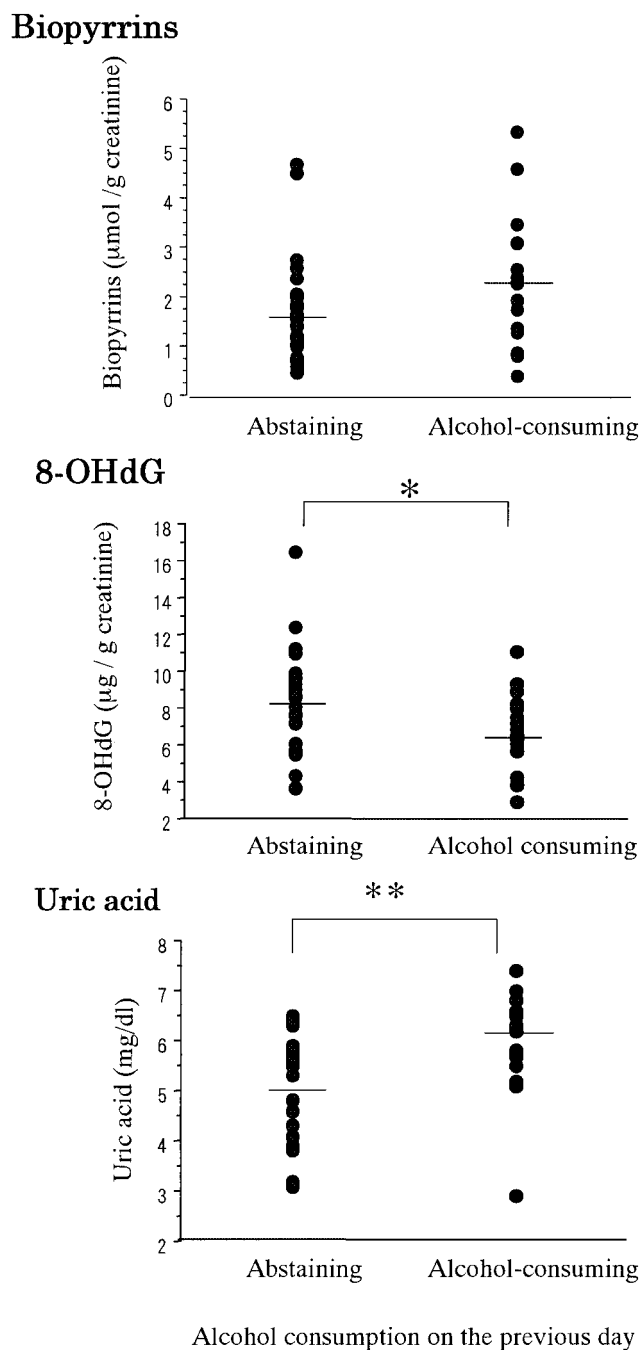


Fig. 2. Results of the assessment of the acute effects of alcohol consumption.

To evaluate the acute effects of alcohol consumption, light and moderate drinkers were subclassified into an abstaining group or an alcohol-consuming group depending on their drinking history on the day prior to specimen collection. *P<0.05, ** P<0.01 (Bonferroni test).

function indicators GOT, GPT, and γ-GTP. The finding that the levels of these indicators in the moderate drinker group were significantly higher than in the light- and non-drinker groups supported the validity of our grouping methods.

We measured total urinary biopyrrins, which are oxidative metabolites of bilirubin, as an oxidative stress marker, because determination of the level of total urinary biopyrrins has been described as a simple screening method for the level of oxidative stress³¹⁾ based on the following findings. Increased urinary excretion of total biopyrrins has been reported after injection of mice with lipopolysaccharide, which is known to induce ROS³²⁾, and also when the peritoneal cavity of mice was exposed to air³³⁾. In both studies, the total biopyrrin levels were associated with the ROS levels. In addition, total biopyrrin have been found to increase in the urine of patients after abdominal operations³¹⁾. Our analysis of the long-term effects of alcohol consumption showed that the total urinary biopyrrin level of the moderate-drinker group was higher than that of the light-drinker group, and the difference was statistical significant. The level in the moderate drinker group was also higher than in the non-drinker group, but the difference was not significant. In view of the earlier studies showing that alcohol drinking induced oxidative stress in alcohol-dependent patients¹⁴⁾, and the results of our own analysis of the acute effects of alcohol consumption showing that the total biopyrrin level in the alcohol-consuming group was higher than in the abstaining group, although not statistically significant, it can be concluded that the total urinary biopyrrin level increased in the moderate drinker group. Although more total biopyrrin have been reported to be excreted by younger than the older subjects³⁴⁾, no correlation was found between total urinary biopyrrin levels and age in our study. In addition, there was no significant age difference between the moderate drinker group and the light or non-drinker groups. Thus, the increasing total urinary biopyrrin levels must be dependent on the amount of daily alcohol consumption. These results suggest that moderate alcohol consumption probably induced ROS, and since there were positive correlations between total urinary biopyrrin levels and the levels of GOT, GPT, and γ -GTP activity, the ROS probably damaged some hepatocytes.

The amount of urinary 8-OHdG decreased significantly with the amount of alcohol consumed on the previous day. As noted in other reports^{17, 29, 30)}, smoking may induce 8-OHdG and may confound with the alcohol consumption. According to the result given by factorial analysis of variance the effect still remained even the smoking habit was controlled.

Since 8-OHdG is known to be the major product of oxidative DNA damage among the many other products of oxidative DNA damage³⁵⁾ and is known to cause mutation^{36, 37)}, it is considered to be an important factor in the development of cancer, and because of this many researchers use the level of 8-OHdG produced as an indicator for cancer risk assessment^{38, 39)}. A decrease of 8-OHdG production seems

to reduce cancer risk related to ROS. Our results are consistent with another study that showed a reduction in the mortality rates for cancer among drinkers who consumed 1–300 g alcohol per week⁵⁾. The amount of alcohol they drank was slightly lower than the amount consumed by our moderate drinkers group, who drank 160–420 g alcohol per week, but the ranges of the levels of alcohol consumption overlapped each other.

The evidence that total urinary biopyrrin increased with the amount of alcohol consumed apparently conflicts with the decrease in urinary 8-OHdG with the amount of alcohol consumed. Recent studies suggest that Kupffer cell stimulation by endotoxin released by gut bacteria affected by alcohol might release free radicals in liver⁴⁰⁾. In addition, the classical pathway for ethanol metabolism in liver appears to be associated with the formation of free radicals⁴¹⁾. Since free radicals induction might reflect induction of heme oxygenase, an enzyme of bilirubin biosynthesis in the liver⁴²⁾, biopyrrins probably increased, as a result of oxidation of bilirubin synthesized in the liver. Accordingly, the total biopyrrin level might directly reflect oxidative stress produced in the liver and correlate well with increase in the levels of GOT, GPT, and γ -GTP. The serum uric acid levels in the present study, also increased with the amount of alcohol consumed on the habitual base and the acute phase base, these findings were compatible with the increase serum uric acid levels as a results of ethanol consumption in previous reports^{23, 24)}. Serum uric acid is known to be increased by ethanol via ethanol-induced activation of adenine nucleotide turnover, which is triggered by the acetate formed from ethanol⁴³⁾. Production of uric acid via adenosine nucleotide turnover may occur in every tissue in the body, and uric acid may be increased throughout the body, because the large amount of acetate formed from ethanol in the liver is probably released and utilized at other tissues⁴³⁾. Uric acid is considered one of the major antioxidants in plasma that inhibits oxidative damage, such as lipid peroxidation^{25–28)}. In our study, the level of 8-OHdG decreased with the level of uric acid, although the total urinary biopyrrin level was not correlated with the 8-OHdG levels. Accordingly, uric acid increased with the amount of alcohol consumed and might have mitigated oxidative DNA damage, at least in organs other than liver, thereby decreasing urinary 8-OHdG.

The difference in uric acid levels between the abstaining group and the alcohol-consuming group based on the analysis of the acute effects was more profound than the difference based on the analysis of the long-term effects. On the other hand, the difference in total biopyrrin levels between the moderate-drinker group and the light-drinker group based on the analysis of the long-term effects was more profound than the difference in the acute effects analysis. These findings

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