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Significance of the urinary 8-OHdG level as an oxidative stress marker in lung cancer patients

Tokujiro Yano*, Fumihiro Shoji[1], Hiromitsu Baba, Tadashi Koga, Takeshi Shiraishi, Hiroyuki Orita, Hitoshi Kohno

Department of Surgery, Saga Prefectural Hospital KOSEIKAN, Saga, Japan

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ABSTRACT

Objective: In the present study, we investigated the relationship between the urinary excretion rate of the oxidized nucleoside 8-hydroxydeoxyguanosine (8-OHdG) and clinical factors in lung cancer patients.
Methods: The present study included 100 patients, who underwent a lung surgery. The patients included 62 men and 38 women with a mean age of 65.5 years ranging from 35 to 82. The diagnosis included 81 primary lung cancers, 9 metastatic lung cancers and 10 benign lung diseases. Urine samples collected for 24 h were analyzed for the content of 8-OHdG using an ELISA assay.
Results: The urinary excretion rate of 8-OHdG in smokers was significantly higher than that in never-smokers. Specifically, the 8-OHdG excretion rate of current smokers was higher than that of patients who had quit smoking for longer than 1 month. Excluding current smokers, the urinary excretion rate of 8-OHdG did not relate to age or gender, but to the malignant potential of the disease. The urinary 8-OHdG level increased in the order of metastatic lung cancer, primary lung cancer and benign disease. In lung cancer patients, furthermore, the mean urinary 8-OHdG level of patients with stages II–IV disease was significantly lower than that of patients with stage I disease.
Conclusions: Smoking significantly increased the urinary excretion rate of 8-OHdG, suggesting that smoking causes an increased rate of oxidative DNA modifications. On the other hand, the capacity to repair oxidative DNA modifications might be impaired to some extent in cancer patients.

1. Introduction

Oxidative DNA damage by reactive oxygen species is thought to contribute to carcinogenesis. Among various oxidative DNA damage products, oxidized-nucleoside 8-hydroxydeoxyguanosine (8-OHdG) has been the most studied. 8-OHdG is produced from both oxidized guanine base in DNA and oxidized nucleotides in cellular pools by nucleotide excision repair, nucleotide incision and transcription coupled mechanisms [1]. Since the excised 8-OHdG is water-soluble and readily excreted in the urine without further metabolism, urinary 8-OHdG level has been validated as a biomarker of the rate of oxidative DNA modification [2–4]. Furthermore, the oxidation of guanine bases in replicating DNA lead to G→T transversion mutations [5]. The G→T transversions are found in the activated K-ras oncogene [6] and in the *p* 53 tumor suppressor gene of human lung cancers [7].

Cigarette smoking is a well-known risk factor for cancer of several organs, especially lung cancer [8]. It has been demonstrated that smokers excrete 35–50% more 8-OHdG in urine than non-smokers [9,10], thus indicating that cigarette smoking is a major cause of oxidative DNA damage, and consequently induces various types of cancer. Recently, Gackowski et al. [11] reported that urinary excretion of 8-OHdG was similar among lung cancer patients, healthy smokers, and healthy non-smokers although the level of 8-OHdG in DNA isolated from the leukocytes of lung cancer patients was significantly higher than that in DNA isolated from the two other groups. A deficiency of repair mechanisms was thus suggested in lung cancer patients. In the present study, we examined the urinary level of 8-OHdG as a marker of oxidative stress in lung cancer patients, and evaluated the clinical factors, other than smoking, which affected the oxidative stress state.

* Corresponding author. Present address: Department of Surgery and Science, Graduate School of Medical Sciences, Kyushu University, Maidashi 3-1-1, Higashi-ku, Fukuoka 812-8582, Japan. Tel.: +81 92 642 5462; fax: +81 92 642 5482.
E-mail address: tokujiro@surg2.kyushu-u.ac.jp (T. Yano).
[1] Present address: Department of Surgery and Science, Graduate School of Medical Sciences, Kyushu University, Japan.

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Table 1
Baseline characteristics of the patients

Male:female	62:38
Mean age (range)	65.5 (35–82)
Smokers:never-smokers	62:38
Diseases	
Benign diseases	8
Metastatic lung cancer	9
Primary lung cancer	81
Male:female	49:32
Mean age (range)	67.5 (38–82)
Smokers:never-smokers	52:29
Stage I:II:III–IV	56:4:21

2. Patients and methods

2.1. Patient selection and study design

The present study prospectively included 100 patients, who underwent a lung surgery at the Saga Prefectural Hospital KOSEIKAN from October 2003 to September 2005. Table 1 shows the baseline characteristics of enrolled patients. They included 62 men and 38 women with a mean age of 65.5 years ranging from 35 to 82. The diagnosis included 81 primary lung cancers, 9 metastatic lung cancers (5 colon cancer, 2 breast cancer, 1 hepatocellular cancer, 1 renal cell cancer) and 10 benign lung disease (5 inflammatory disease, 4 giant bulla, and 1 benign tumor). The primary lung cancer patients consisted of 49 men and 32 women with a mean age of 67.5 years ranging from 38 to 82. According to the UICC TNM classification, 56 patients were pathologic stage I disease, 4 were stage II, and 21 were stage III or IV.

All patients collected urine preoperatively for 24 h, and the urine samples were stored −80 °C until use. The urine samples were assayed by ELISA (Japan Institute for the Control of Aging, Shizuoka, Japan) for the concentration of 8-OHdG in all 100 patients and isoprostanes in 48 patients. The detail procedures were described elsewhere [12]. The determination range was 0.5–200 ng/ml for 8-OHdG and 0.05–50 ng/ml for isoprostanes. The intra-assay coefficient of variation (CV) of ELISA for 8-OHdG ranged from 1.8% to 5.5% while the inter-assay CV ranged from 2.8% to 7.9%. For isoprostanes, the intra-assay CV ranged from 4.4% to 11.0% while the inter-assay CV ranged from 5.0% to 11.0%. All samples were assayed in duplicate. The data are presented as the urinary 8-OHdG or isoprostanes (ng/ml)/creatinine (mg/ml) ratio.

The study was conducted in accordance with the Declaration of Helsinki. The study protocol was approved by the Institutional Review Board, and written informed consent form was obtained from all the patients on admission.

2.2. Statistics

A statistical analysis was performed using either Student's *t*-test for comparisons between two groups or an analysis of variance (ANOVA) for comparisons among the three or more groups. When the ANOVA showed that a comparison was statistically significant, then individual pairs of comparison were made using the Fisher and Scheff tests. A multivariate analysis was performed using logistic regression procedures to identify independently significant factors for the low level of urinary 8-OHdG. All results were considered significant at p values of less than 0.05.

3. Results

The urinary excretion rate of 8-OHdG in smokers was significantly higher than that in never-smokers (7.4 ng/(kg h) vs. 5.7 ng/(kg h), $p = 0.006$). Specifically, smoking currently within 2 weeks significantly influenced the oxidative stress levels (Fig. 1). However, a past smoking habit (pack–years) was not associated with the urinary excretion rate of 8-OHdG (Fig. 2). Excluding current smokers (within 4 weeks), the urinary excretion rate of 8-OHdG did not correlate with age or gender, but it was associated with the disease types. The urinary excretion rate of 8-OHdG increased in the order of metastatic lung cancer, primary lung cancer and benign disease (Fig. 3).

In the primary lung cancer patients, the urinary excretion rate of 8-OHdG in smokers was also significantly higher than that in never-smokers, and smoking currently within 2 weeks significantly

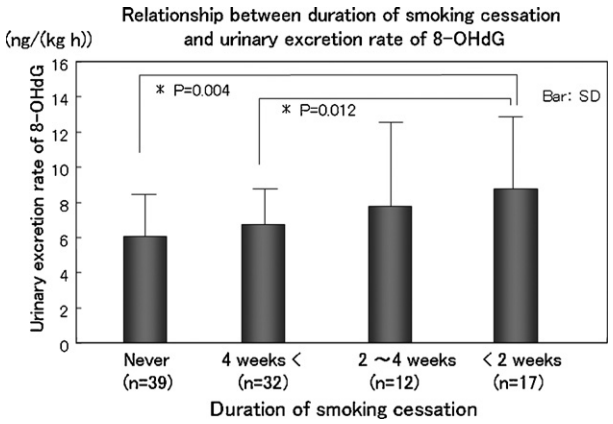

Fig. 1. Relationship between duration of smoking cessation and urinary excretion rate of 8-OHdG.

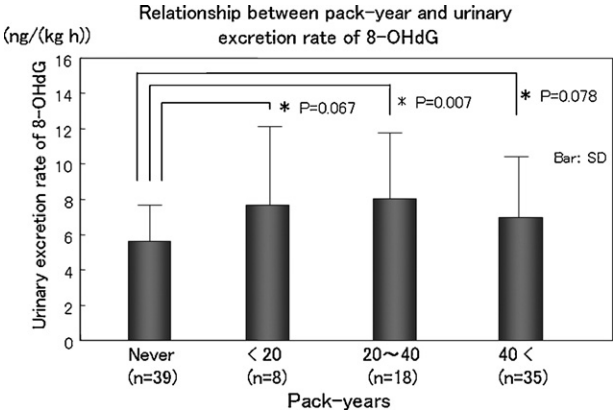

Fig. 2. Relationship between pack–year and urinary excretion rate of 8-OHdG.

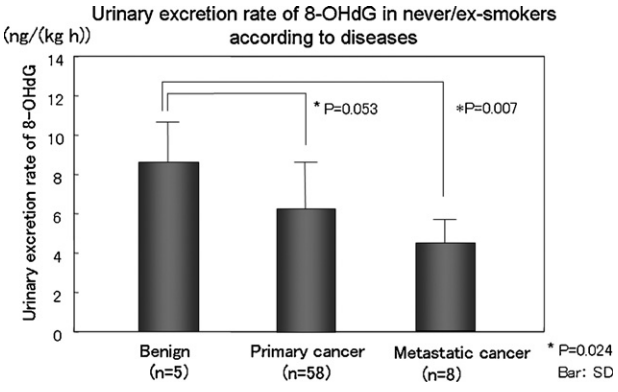

Fig. 3. Urinary excretion rate of 8-OHdG in never/ex-smokers according to diseases.