□生 理 学□ Reserch note

Preliminary Research on the Excretion of Urinary 8-Hydroxyguanosine (8-OHdG) as a Marker of Protozoan Parasites Infection in Captive Western Lowland Gorillas (*Gorilla gorilla gorilla*)

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動物園で飼育されるニシローランドゴリラ (*Gorilla gorilla gorilla*) の尿中8-OHdG量を指標とした健康評価に関する予備研究－特に原虫感染個体との比較

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ABSTRACT. Urinary 8-hydroxyguanosine (8-OHdG), a common biomarker of oxidative stress, was measured in 9 captive western lowland gorillas (*Gorilla gorilla gorilla*) by ELISA kit. The data showed that urinary 8-OHdG ranged from 4.3 to 193.1 ng/mg creatinine. An individual range of median value was 6.8-52.4ng/mg creatinine. No statistically significant effect was found for infection of protozoan parasites (*Balantidium coli* and *Isospora* sp.) without any symptoms (> 0.05).

Key word : Western lowland gorillas, oxidative stress, 8-OHdG.

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It has been reported that oxidative stress is one index for evaluation of health status because it relates to the development of various chronic diseases such as cerebral apoplexy [1], cancer [2], Alzheimer's diseases [3], diabetes [4], and cataracts [5], in humans. Bacterial and viral infections are also known to induce oxidative stress [6]. The oxidative DNA damage product, 8-hydroxyguanosine (8-OHdG) which is detected in serum and urine after human is exposed to the oxidative stress, is a predominant form of radical-induced lesions in DNA. Therefore, 8-OHdG has been widely used as a biomarker for oxidative stress and is considered an index to evaluate the risk of chronic diseases, as well as a prognostic indicator of lung cancer and lymphoma [7, 8]. It is reported that urinary 8-OHdG shows no statistically significant effect with sex, age, body weight and body mass index [9]; however, it has also been reported as showing a statistically significant effect with sex and age in humans [10,11]. It is also reported that acute and chronic psychological stress can induce oxidative stress in rats and human, with significant correlations between cortisol and 8-OHdG in both urine and saliva in human [12]. This means that oxidative stress markers including 8-OHdG are useful for evaluating the seriousness of disease and psychological stress in human. ELISA is commonly used to measure urine 8-OHdG levels because it is non-invasive and

samples can be analyzed without any purification process [13].

It is usually difficult to evaluate the general health status of wildlife, even under captive condition, because they usually do not show symptoms until clinical conditions are severe. Physical examinations and blood chemistry data can be used for evaluations of general health status. However, manual or chemical immobilizations are often required to conduct these procedures and stress inducted by such immobilization might affect the results of physical examinations and impact on blood chemistry data. Thus, urine samples are useful for an evaluation because such samples can be obtained non-invasively. In animals, it is reported that urinary 8-OHdG levels in asymptomatic Japanese macaques were significantly lower than those in symptomatic macaques with bacterial infection; showing that urinary 8-OHdG might be useful as a noninvasive indicator of bacterial infection [14].

In this study, urinary 8-OHdG was measured in captive western lowland gorillas infected with intestinal protozoan parasites without clinical symptoms using ELISA to determine variations in levels of urinary 8-OHdG excretions. The values of urinary 8-OHdG were applied for a health status index in this species. Urine samples were obtained in 2005 from 9 adult captive western lowland gorillas (two males and four females, aged 26 to 39 years, bred in Ueno Zoological Gardens, Tokyo, and one male and two females, aged 20 to 35 years, bred in Kyoto Municipal Zoo). Four gorillas were infected with parasitic protozoans (*Balantidium coli* and /or *Isospora* sp.) but did not demonstrate any symptoms (Table 1). The gorillas were housed singly in individual indoor rooms at night, and during the daytime were in an outside space with others. They were fed two or three times a day and drunk by bottle twice a day or free from water tub. Forty-two urine samples were collected from clean concrete floors of individual rooms using a 15 ml syringe immediately after excretion or after 15 hours of excretion. And then these urine samples were stored at −20℃ until analysis. The frequency of urine collection was twice a month for one month in three gorillas at Kyoto Municipal Zoo or six months in six gorillas at Ueno Zoological Gardens. Samples were used to determine the range of urinary 8-OHdG values among the asymptomatic individuals. Routine health check was scored the remain of food, fecal condition, appetite, walking position and stick urinalysis by keepers.

Measurements were conducted using a competitive ELISA kit (New 8-OHdG Check, Japan Institute for the control of Aging, Shizuoka, Japan) with a monoclonal antibody specific for 8-OHdG and a determination range of 0.5 to 200 ng/ml. After thawing, urine samples were centrifuged at 2000 g for 10 min and supernatants diluted five times using phosphate buffered saline before analysis. Each sample was measured in triplicate. After measurements of 8-OHdG using the ELISA kit, results were corrected with urinary creatinine and determined according to the picric acid method of Jeffe [15]. Thus data were expressed as the urinary "8-OHdG (ng/ml) / creatinine (mg/ml)" ratio. The line for the serial half dilution with phosphate buffered saline of gorilla urine 8-OHdG showed the linear regression of the dilution ratio (Fig. 1). The slope of absorbance at 450 nm of a serial half dilution of gorilla urine sample did not differ from the regression equation generated by the standard curve of the kit (Fig. 2). The intra- and inter-assay coefficients of variation for a lower concentration standard urine sample (1.2ng/ml) were 28.3% (n=5) and 36.4% (n=5). And the intra- and inter-assay coefficients of variation for a higher concentration standard urine sample (24.0 ng/ml) were 11.5% (n=5) and 15.8% (n=5). The Mann-Whitney U-test was applied to evaluate difference of urinary 8-OHdG levels between a protozoa infected group and a non-infected group. The protozoa infected group included case 1, 2, 4 and 6, namely western lowland gorillas infected by

Table 1 Urinary 8-OHdG excretion of asymptomatic captive western lowland gorillas in Japanese zoos

Case	sex	age	infection of parasites	range of urinary 8-OHdG concentration and median (ng/mgcreatinine)		
1[1]	male	26	*Isospora* sp.	6.0-193.1	36.4	6[2]
2	male	29	*Balantidium coli*	9.6-43.7	20.6	6
3	female	28	non	10.2-19.7	12.6	6
4	female	29	*Balantidium coli* and *Isospora* sp.	15.0-44.0	28.0	6
5	female	36	non	14.0-34.8	18.3	6
6	female	39	*Balantidium coli*	33.9-60.3	52.4	6
7	male	35	non	4.3-12.4	8.3	2
8	female	20	non	5.0-8.6	6.8	2
9	female	33	non	7.2-7.6	7.4	2

[1] 1st to 6th cases obtained from Ueno Zoological Gardens, and 7th to 9th cases obtained from Kyoto Municipal Zoo.
[2] Number of samples examined .

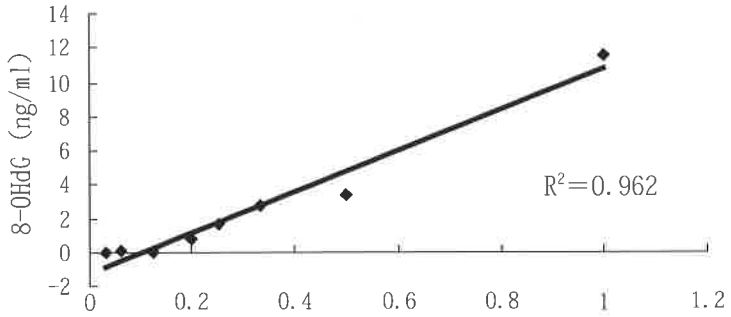

Fig.1 Correlation between the levels of 8-OHdG and the serial dilution of western lowland gorilla urine (◆,from 1:1 to 1:32). Peasons correlation coefficient test, $P<0.01$.

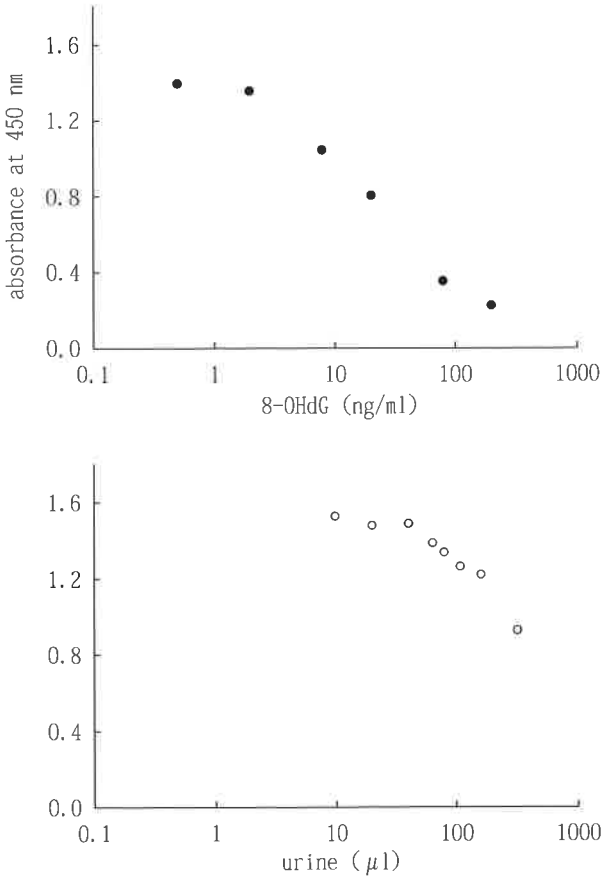

Fig. 2 Parallels between the standard curve of the 8-OHdG kit (●) and the serial dilution of the western lowland gorilla urine (○, from 1: 1 to 1:32). Phosphate buffered saline was used for dilution.

Balantidium coli (number of cysts per gram of feces 0.6-114.6; precipitation method) and/or *Isospora* sp. (number of oocysts per gram of feces 0.2-49.0; flotation method) (Table 1). The non-infected group included case 3, 5, 7, 8 and 9 (Table 1).

Urinary 8-OHdG ranged from 4.3 to 193.1 ng/mg creatinine. An individual range of median value was 6.8-52.4ng/mg creatinine (Table 1). The range of values for western lowland gorillas was similar to the value in asymptomatic individuals of Japanese macaque (*Macaca fuscata*; 6.9-203.2 ng/mg creatinine [14]), rather than those of chimpanzees (*Pan troglodytes*; 0.0-92.9 ng/mg creatinine) and humans (2.0-30.0ng/mg creatinine [16]).

The median value for infected with *B.coli* was 33.9 ng/mg creatinine. The median value for infected with *Isospora* sp. was 28.0 ng/mg creatinine. The median value for non-infected group was 18.3 ng/mg creatinine. There was no statistically significance between the protozoa infected group and the non-infected group ($P>0.05$). The results were thus categorized as asymptomatic values for western lowland gorillas unrelated to intensity levels of protozoan parasites' infection. Due to the low sample number in the present study, it was not possible to evaluate differences in urinary 8-OHdG levels for sex between infected and not.

Our results suggested that urinary 8-OHdG is probably not a useful marker for infection of *B. coli* and *Isospora* sp. in western lowland gorillas. However, as it is reported that urinary 8-OHdG might be a useful non-invasive marker for bacterial infection in Japanese macaque [14], further studies on disease-related variations of urinary 8-OHdG in western lowland gorillas is needed. The present survey was supported in part by the Grant-in-Aid of the Cooperative Project of Rakuno Gakuen University, 2004, the Global Environment Research Fund (F-062, 2006-2008) of the Ministry of the Environment, and the Grant-in-Aid (Nos. 18510205, 20380163) of the Ministry

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要　　約

生体内の酸化ストレスを評価する一般的な生体指標である尿中 8-hydroxyguanosine（以下，8-OHdG）量を国内飼育下の 9 頭のニシローランドゴリラにおいて定量した。検査対象個体に原虫感染が認められたが，臨床症状は観察されなかった。全個体の 8-OHdG 値（ng/mg creafinine）の範囲は 4.3 ～ 193.1，各個体の中央値の幅は 6.8 ～ 52.4 であった。原虫陽性と陰性個体との 8-OHdG 値の比較を行い，有意差は認められなかった（> 0.05）。

キーワード：ニシローランドゴリラ，酸化ストレス，8-OHdG

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