Urinary 17-KS and 17-OHCS as Markers of Endocrine Disruption by Air Pollution in Primary School Children

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Abstract

The present study was undertaken to determine the effects of air pollution on urinary excretion of 17-ketosteroids (17-KS) and 17-hydroxycorticosteroids (17-OHCS), which are markers of adrenal cortex functions, in primary school children from five schools with different levels of air pollution. One hundred and twenty-nine fourth-grade children were included in the study. Urinary excretion of 17-KS and 17-OHCS were significantly lower in male children living in polluted areas than in children living in clean areas. We conclude that adrenal cortex functions before puberty can serve as practical markers of endocrine disruption by environmental factors in epidemiological studies.

Key words: air pollution, 17-ketosteroids, 17-hydroxycorticosteroids, school children, endocrine disruption

Introduction

Diesel exhaust emission, one of the major sources of air pollution, comprises thousands of chemical components, including nitrogen oxides, dioxin-like compounds and polycyclic aromatic hydrocarbons^{1, 2, 3)}. Although it has been hypothesized that such chemicals may contribute directly or indirectly to increases in disorders of the reproductive tract^{4, 5, 6)}, there are few biological studies of endocrine disruption by diesel engine exhaust.

In a previous study, it was shown that inhaling diesel engine exhaust affects spermatogenesis in rats⁷⁾. The study indicated that there was a correlation between reduced spermatogenesis and adrenal secretion of sex hormones. It has also been suggested that urinary 17-ketosteroids (17-KS) excretion levels could be useful markers for evaluating the effects of diesel exhaust on endocrine systems⁸⁾.

The present study was undertaken to determine whether 17-KS could serve as a practical marker of endocrine disruption by environmental pollution in primary school children. The levels of another marker for adrenal cortex functions, 17-hydroxycorticosteroids (17-OHCS), were also measured in order to confirm the functional changes of the adrenal gland.

Materials and Methods

Study Areas

The present study was part of an extended epidemiological study to assess the effect of air pollution on the health of school children. Five areas of investigation were selected in the Greater Tokyo Area on the basis of cumulative NO_x and particulate matter concentrations during the past 10 years as measured at the general environmental monitoring stations in these areas. Studies were conducted in 1996 and 1997 with parental permission. The average annual mean values of nitrogen dioxide (NO₂), nitrogen oxide (NO) and the particulate matter (SPM) concentrations for the past 10 years at the studied sites are summarized in Table 1.

Subjects and Study Period

The periods of study 1 and study 2 for the fourth-grade male children (ages 9-10) were November, 1996, October, 1997, respectively. Urine samples from the children were collected.

Table 1 Annual average levels of nitrogen dioxide (NO₂), nitrogen oxide (NO) and particulate matter (SPM) for the past 10 years in each area

	NO ₂ (ppb)	NO (ppb)	SPM (μ g/m ³)
Study 1			
Adachi Ward	31.8	28.4	57.2
Higashiyamato City	23.2	17.2	49.2
Study 2			
Itabashi Ward	42.7	41.1	29.3
Oume City	26.5	16.3	30.6
Hachijyo Island	6.0	7.0	12.4

Received May 10 1999/Accepted Sep. 22 1999

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Samples from those who had been living for more than 3 years in the same area were analyzed for 17-KS and 17-OHCS.

Urine Sampling

The urine samples analyzed in this study consisted of spot urine usually collected in the morning. The samples were then frozen in 30-ml aliquots and kept at -20°C until analysis. The concentrations of 17-KS and 17-OHCS in individual urine samples were corrected for creatinine content to take into consideration fluctuations of the urine concentration due to diuresis.

Measurement of 17-KS and 17-OHCS of Urine Samples

Urinary 17-KS and 17-OHCS concentrations, markers of adrenal cortex function, were measured using diagnostic kits, OS-KIT and Oha-KIT (Kanto Chemicals, Tokyo, Japan). Creatinine (CRE) levels in urine were measured using diagnostic kits (Daiichi Pure Chemicals, Tokyo, Japan) and an automatic analyzer (Model Hitachi 705, Hitachi, Tokyo, Japan). The Jaffe's method was used to measure the levels of CRE. The results were expressed as 17-KS/CRE and 17-OHCS/CRE ratios ($\mu\text{g/ml/CRE}$) in spontaneous urine samples.

All values were expressed as mean $\pm$ standard deviation (SD). Student's t-test was used to determine the significance of differences between any two groups. P values less than 0.05 were considered significant.

Results

Study 1.

Figure 1 shows the urinary 17-KS and 17-OHCS concentrations in male school children living in Adachi Ward and Higashiyamato City. The 17-KS values were lower in the group living in Adachi Ward than in the group living in Higashiyamato City. The 17-KS values were 0.86 ± 0.17 and 0.99 ± 0.23 ($\mu\text{g/ml/CRE}$) for the groups living in the polluted area and clean area, respectively. The difference between the two groups was significant ($p < 0.05$). The 17-OHCS values were 4.20 ± 0.84 and 5.73 ± 0.86 ($\mu\text{g/ml/CRE}$) for the groups living in Adachi Ward and Higashiyamato City, respectively. The 17-OHCS values differed significantly ($p < 0.01$) between these two groups. There were no significant differences of CRE levels.

Fig. 1 Urinary excretion of 17-KS and 17-OHCS in school boys living in areas with different levels of pollution. The data are expressed as $\mu\text{g/ml/creatinine}$ (means $\pm$ SD). * $p < 0.05$ and ** $p < 0.01$ compared to Higashiyamato City.

Study 2.

The levels of urinary 17-KS and 17-OHCS of three areas, Itabashi Ward, Oume City and Hachijyo Island, are shown in Figure 2. The 17-KS values were 0.56 ± 0.13 , 1.08 ± 0.32 and 2.21 ± 0.58 ($\mu\text{g/ml/CRE}$) for the groups living in Itabashi Ward, Oume City and Hachijyo Island, respectively. Levels of 17-KS were lowest in the group living in Itabashi Ward. There were significant differences between the levels in the group living in Itabashi Ward and the levels in the groups living in Oume and Hachijyo Island ($p < 0.01$, $p < 0.001$, respectively). There was also a significant difference between the group living in Oume City and the group living in Hachijyo Island ($p < 0.01$). The 17-OHCS value was also lowest in the group living in the most polluted area, Itabashi Ward; the values were 4.52 ± 0.90 $\mu\text{g/ml/CRE}$ in Itabashi Ward; 5.70 ± 1.25 $\mu\text{g/ml/CRE}$ in Oume City; and 9.06 ± 1.81 $\mu\text{g/ml/CRE}$ in Hachijyo Island. The 17-OHCS values were significantly different between Itabashi Ward and Oume City, Itabashi Ward and Hachijyo Island, and Oume City and Hachijyo Island ($p < 0.01$, $p < 0.001$, $p < 0.01$, respectively). There were no significant differences of CRE levels among these three groups.

Fig. 2 Urinary excretion of 17-KS and 17-OHCS in school boys living in three areas with different pollution levels (means $\pm$ SD). ** $p < 0.01$ compared to Oume City. ## $p < 0.01$ and ### $p < 0.001$ compared to Hachijyo Island.

Discussion

This study clearly demonstrated that urinary excretion of 17-KS and 17-OHCS was significantly lower in the male school children who had lived in polluted areas for more than 3 years than in the children from cleaner areas. These results are quite similar to the results observed in rats exposed to diesel exhaust⁽⁸⁾. The NO_2 concentration was higher and the SPM concentration was lower in Itabashi Ward than in Oume City, and the 17-KS and 17-OHCS levels were lower. Altogether, this suggests that the gaseous phase of air pollution contains agents responsible for endocrine disruption.

17-KS is a metabolite of androgens and a marker of adrenal cortex and testis functions^(9, 10, 11). The urinary excretion of 17-KS increases with age, especially it is elevated drastically at the time of puberty⁽¹¹⁾. The age of the school children examined in the present study was 9-10 years, and the children were still juveniles with respect to sexual development. Thus it is not likely that

androgen from testis influenced the urinary 17-KS levels in these subjects, although the serum levels of adrenal androgen were not measured. Urinary 17-KS is mainly derived from dehydroepiandrosterone sulfate, which is one of the adrenal androgens, and is associated with bone metabolism, especially mineral accumulation^{12, 13)}. In a previous study conducted using the same samples as those analyzed in the present study, urinary markers of bone resorption were significantly higher in polluted areas¹⁴⁾. Therefore, it is reasonable to assume that the values of urinary 17-KS measured in the present study mainly reflect adrenal cortex secretion of androgen, and that the low levels of 17-KS indicate depressed or delayed development of adrenal cortex functions. Adrenal insufficiency might have affected bone metabolism. This hypothesis does not necessarily indicate that the depressed adrenal functions detected in the present study would continue in the same way after puberty. Because in inhalation experiments in rats, increased secretion of adrenal androgen was observed around the stage of puberty in the diesel exhaust-exposed group, and may have caused a negative feedback

effect on gonadotropin-releasing-hormone in the pituitary gland and depressed spermatogenesis in the testis shortly after sexual maturity^{7, 8)}. Spermatogenesis of children who live in polluted areas may be affected in later stages of sexual development: postpuberty and sexual maturity.

Depressed or delayed development of adrenal cortex functions was also shown by the fact that urinary 17-OHCS was also significantly lower in school children from polluted areas than in those from clean areas. 17-OHCS has been used in the clinical field as a practical marker of cortisol secretion from the adrenal cortex^{10, 11)}. No adverse effects with a close connection to the decreased or increased cortisol secretion from the adrenal cortex have been observed in rats exposed to diesel exhaust, as has been observed in the case of androgen secretion from the adrenal cortex, suggesting a possible ability to adapt to environmental changes. Further studies to find useful markers, in addition to 17-KS and 17-OHCS, of endocrine disruption by air pollution will be necessary to provide useful estimates of the human risks of such pollution.

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