

ENHANCED LIPID PEROXIDATION AND SERUM ANTIOXIDANT LEVELS IN YUSHO VICTIMS OVER 30 YEARS AFTER ACCIDENTAL POISONING WITH POLYCHLORINATED BIPHENYLS IN NAGASAKI, JAPAN

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Abstract

More than thirty years have passed since the accidental poisoning with polychlorinated biphenyls (PCB) in Japan and yet high concentrations of PCB are still detected in the serum of the victims. PCB produces superoxide and thus victims are considered to be at persistent state of oxidative stress. We measured urinary concentrations of 8-isoprostane (8IP) in Yusho victims and age-matched controls to assess this hypothesis. The mean urinary concentration of 8IP was significantly higher in Yusho victims than that in the controls. It was considered that Yusho is an oxidative stress and 8IP is a useful tool in order to check the oxidative condition in Yusho victims. Furthermore, we measured the serum concentrations of Cu,Zn-superoxide dismutase (SOD) and Mn-SOD in the victims and age-matched controls to examine the antioxidative state. The mean serum concentration of Cu,Zn-SOD was significantly lower and that of Mn-SOD was significantly higher in Yusho victims than in the controls. Such antioxidative state is thought to be indicative of an imbalance of Redox regulation mechanism. Persistence of imbalance could result in serious ailments in the future.

Introduction

Two well-known cases of food poisoning by coplanar PCB or dioxin-like compounds were investigated to determine the effects of these compounds on humans, especially due to the long half-life of these compounds. These two cases included the incidence of Yusho, which was caused by the contamination of rice oil with PCB and polychlorinated dibenzofuran in western Japan in 1968 and a similar poisoning incidence of Yu-cheng that occurred in Taiwan in 1978.¹ The Japanese Government has conducted annual examinations of Yusho victims because of the long half-life of PCB. More than thirty years have passed since the accidental poisoning, yet high and significant concentrations of PCB are still detected in the serum of Yusho victims. Previous studies reported that PCB causes the release of O₂⁻,² suggesting that Yusho is an oxidative stress and Yusho victims have been exposed to oxidative stress for more than 30 years. The present study was designed to examine whether or not Yusho is an oxidative stress checking the conditions of lipid peroxidation (LPO). Ordinarily, antioxidant enzymes, such as Cu,Zn-SOD and Mn-SOD, can convert superoxide to H₂O₂. Furthermore, the present study was also designed to examine the antioxidative state checking the serum concentrations of Cu,Zn-SOD and Mn-SOD in Yusho victims.

Subjects and Methods

The study protocol was approved by the Ministry of Health, Labour and Welfare and a signed consent form was obtained from each subject before enrolment in the present study. Early-morning void urine samples were collected and kept at -80°C until analysis. Urinary excretion level of 8-isoprostane (8IP) as a marker of LPO was measured in 44 Yusho victims who had lived in the T area at Nagasaki Prefecture since 1968 and 46 age-matched healthy individuals living in other areas of Nagasaki Prefecture. The concentrations of urinary 8IP were measured by an enzyme immunoassay (Cayman, Ann Arbor, MI). Results were expressed relative to urinary levels of creatinine. Fresh venous blood samples were centrifuged shortly after clot formation. All serum samples were stored at -70°C prior to use. Serum concentrations of SOD were measured in 38 victims at T area in Nagasaki Prefecture and in 20 age-matched healthy individuals. The serum concentrations of the constitutive type Cu,Zn-SOD and inducible type Mn-SOD were measured by enzyme-linked immunosorbent assay system (RPJ301,302, Amersham K.K., Tokyo, Japan). All data were presented as mean ± SEM. Statistical analyses were performed using Mann-Whitney's U-test and the relationship between parameters was statistically examined by Spearman's rank test.

Results

There was no significant difference between the ages of Yusho victims and age-matched controls. The mean urinary concentration of 8IP relative to urinary creatinine (8IP/cre) was significantly higher in the victims (9.73 ± 0.85 pg/mg creatinine) than in the controls (7.02 ± 0.42) (p < 0.02) (Fig. 1).

The mean serum concentration of Cu,Zn-SOD was 15.4 ± 2.0 ng/ml in Yusho victims and 31.2 ± 3.8 ng/ml in the controls, indicating a significantly lower level in the victims. (p < 0.001) (Fig. 2)

Conversely, the mean serum Mn-SOD concentration was significantly higher in the victims than in the controls (Yusho: 129.4 ± 3.6, control: 110.9 ± 7.9 ng/ml) (p < 0.01) (Fig. 3).

Discussion

We have been involved in the annual examination of Yusho victims and their severe symptoms reported in the early period are not recognized at the present time. However, accumulation of PCB has been noted since the accident, producing superoxide in hepatocytes and adipocytes. Several studies reported LPO in elderly individuals with various chronic conditions, eg diabetes mellitus, hypertension, and hyperlipidemia.³ In the animal experiment exposed by PCB, production of superoxide and LPO were noticed in the liver.⁴ In this study, we recognized high urinary levels of 8IP, suggesting that Yusho is a disorder of oxidative stress. Although the precise mechanism is unclear, it is possible that the persistently high levels of urinary 8IP in Yusho victims are due to failure of the defence system. The severe symptoms during the early period are not evident in Yusho victims who suffered from PCB intoxication more than 30 years ago and still show high serum PCB levels. More than thirty years have passed since the incidence and blood concentrations of PCB are much lower at the present time than these at the early period (Data not shown). Therefore, it was likely that present situation of Yusho victims was close to that of intoxication of parathion at a low concentration.

PCBs induce the release of superoxide during the metabolic process and the resultant oxidative stress is very crucial. Previous studies reported the presence of high concentrations of Mn-SOD in the blood of patients with ovarian cancer and it was thought to be produced by endothelial cells stimulated by tumour necrosis factor or interleukin -1.⁵ Thus, there seems to be a similar scenario affecting the induction of Mn-SOD. It is also possible that superoxide induces, via nuclear factor-kappa B pathway, Mn-SOD in Yusho victims.

The significant decrease in Cu,Zn-SOD concentration was confirmed at the protein level in the serum of Yusho victims. Preliminary studies by the members of the Study Group for the Therapy of "Yusho" at Fukuoka also confirmed a significant decrease of Cu,Zn-SOD activity in the liver of rats treated with coplanar PCB. (Personal communication) It is thought that serum concentration of SOD reflects that of living cells. Although there is no report of liver dysfunction in Yusho victims, it is possible that a large amount of SOD protein in a large organ such as the liver could account for the concentration of SOD protein in the serum.⁶ PCBs can compete for thyroxin receptor and decrease serum T3 and T4 concentrations, as confirmed in rats that received long-term treatment of PCB.⁷ Low blood levels of Cu,Zn-SOD protein associated with high levels of Mn-SOD protein were also reported in untreated patients with Hashimoto disease and with myxedema.⁸ None of the 38 victims examined in the present study had abnormal thyroid function, but there was a positive correlation between Mn-SOD and thyroid stimulating hormone. It is likely that such changes in SOD were confirmed in the serum because the condition of Yusho victims was close to subclinical hypothyroid state.

This is the first report that provides the evidence that Yusho is an oxidative stress based on the high urinary levels of 8IP. This method is a useful tool because of the use of urine. The antioxidative state in Yusho victims reported in the present study indicates an imbalance in the Redox regulation mechanism, and persistence of such imbalance could result in carcinogenesis and organ failure. Therefore, a close follow-up of the Yusho victims is important to their well-being, and it will require the efforts of not only health care individuals but also the support of the government.

Acknowledgements

We thank Professor Yamada (Kyushu University) for critical advices. This work was supported in part by a Grant-in -Aid for Scientific Research from the Ministry of Health Labour and Welfare, Japan.

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Figures

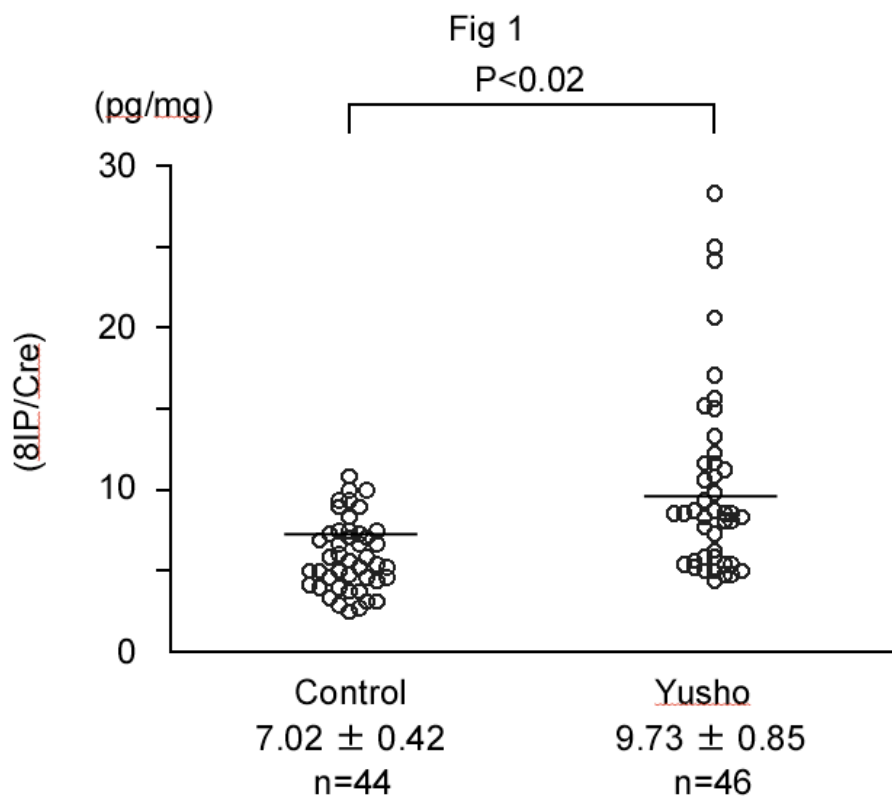


Fig. 2

Cu,Zn-SOD

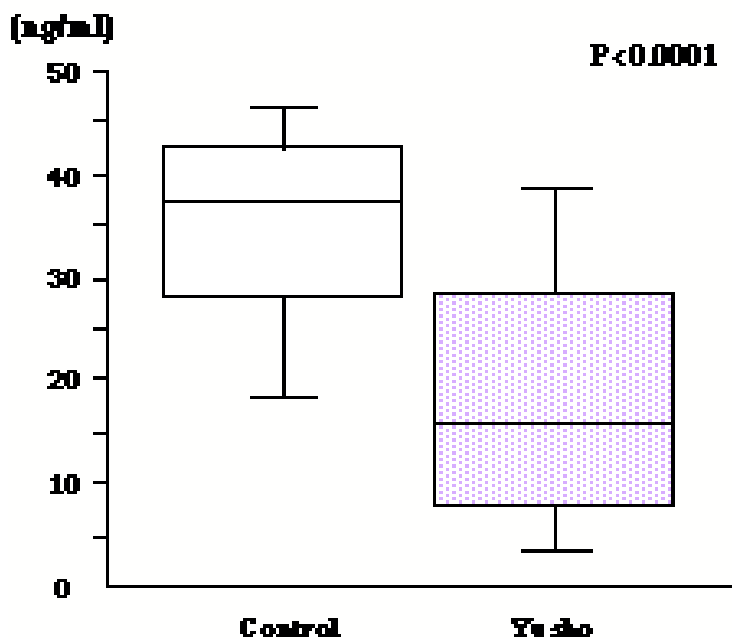


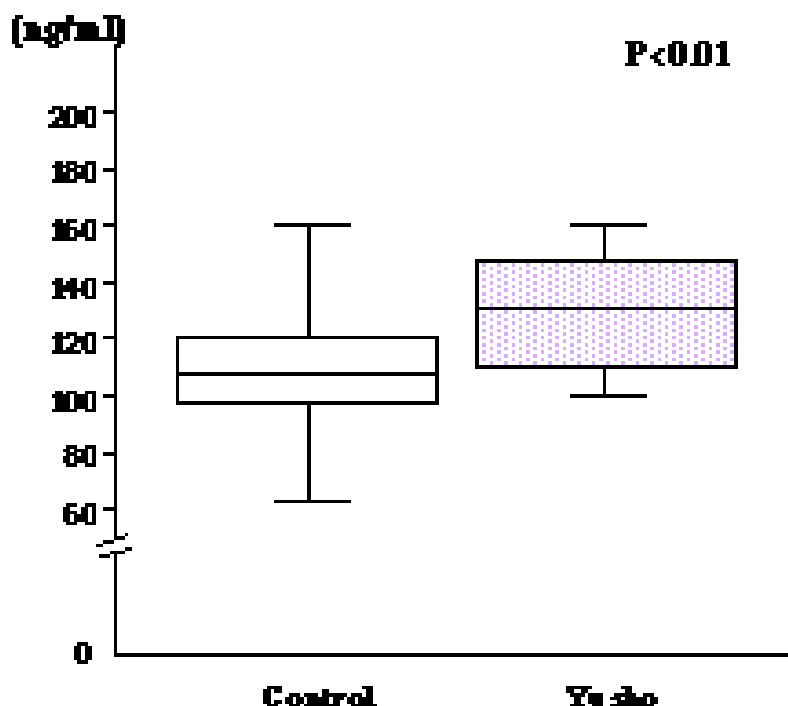
Fig. 3**Mn-SOD****Figure legends**

Figure 1.

Urinary concentrations of 8-isoprostane in Yusho victims and normal subjects. Results are expressed relative to urinary levels of creatinine. The horizontal line indicates the mean value of each group. Urinary 8-isoprostane excretion in Yusho victims is significantly higher than in the control ($P < 0.05$). A statistical analysis was performed using Mann Whitney's U-test.

Figure 2.

Serum concentrations of Cu,Zn-SOD protein in the victims of Yusho accident and normal controls. The mean serum concentration of Cu,Zn-SOD was significantly lower in the victims than in the controls. In the box and whisker plots, the 25th to 75th percentiles are represented by the horizontal lines of the box; the median is indicated by the internal horizontal line across the box, and the whiskers on each box represent the 10th to 90th percentiles.

Figure 3

Serum concentrations of Mn-SOD in Yusho victims and normal controls

The mean serum concentration of Mn-SOD was significantly higher in the victims than in the controls. In the box and whisker plots, the 25th to 75th percentiles are represented by the horizontal lines of the box; the median is indicated by the internal horizontal line across the box, and the whiskers on each box represent the 10th to 90th percentiles.