THE RELATIONSHIP BETWEEN DIOXIN AND STEROID HORMONES IN SERUM OF VIETNAMESE MEN

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Introduction

Between 1962 and 1971, the United States Air Force sprayed approximately 107 million pounds of herbicides in South Vietnam for the purpose of defoliation and crop destruction in a program code-named Operation Ranch Hand[1]. During the course of this operation, hundreds of thousands of U.S. service personnel and millions of Vietnamese were exposed to the chemicals in the air, water, and soil and through food raised on contaminated farms[2]. The most widely used defoliants were 2,4-dichlorophenoxyacetic acids (2,4-D) and 2,4,5-trichlorophenoxyacetic acids (2,4,5-T)[3]. The best-known mixture was known as Agent Orange, a 50:50 mixture of the aforementioned herbicides. Agent Orange is known to affect immune system, reproduction, nervous system, and changing steroid hormone levels[4, 5, 6].

Most studies of the relationship between dioxin and steroid hormone have focused on Vietnamese women, whereas there are few studies concerning the relationship between dioxin and steroid hormones in Vietnamese men. The aim of this study was therefore to explore the impact of Agent Orange on serum steroid hormones in Vietnamese men with comparison of the steroid hormones levels between hotspot area and non-sprayed area.

Materials and methods

1.study sample

We focused our study in Phu Cat district of Binh Dinh province and Kim Bang district of Ha Nam province. Phu Cat airbase is one of three main dioxin hotspots in southern Vietnam, and the study subjects were known to have been living in and around the airbase prior to the war. Kim Bang district is located in northern Vietnam and did not experience herbicide operations during the war, which is why it was selected as the non-sprayed area.

In August 2009—2011 blood samples (5—10mL) were collected from Phu Cat 48 men and Kim Bang 36 men aged over 50 years old. All samples were frozen immediately after collection for transport to Japan. Consenting subjects were required to complete a health status questionnaire to gain individual information. Prior to beginning the study, we obtained permission from the Medical Ethics Committee of Kanazawa University (approval no.: 89(2007) and no.:326(2011)), and informed consent was obtained from all the participants in written form.

2.statistical analysis

Statistical analyses were performed using the JMP statistical software package, version 9.0 (SAS Institute Japan). Logarithmic transformation of the measured values of dioxins, dihydrotestosterone (DHT), progesterone, testosterone, and androstenedione was performed to improve normality. Appropriate statistical methods, including a chi-square test, Welch test, student's t-test, linear regression analysis and multivariate analysis for testosterone, dehydroepiandrosterone, and estradiol after adjusted for age, BMI, present job, and smoking habit.

Results and discussion

The results obtained are provided in seven tables.

Table 1 shows the mean age, smoking habit, and present job were significant differences between the hotspot and non-sprayed areas, whereas BMI and alcohol habit did not differ significantly.

Table 1 Demographic characteristics of participants in the hotspot and non-sprayed areas

		Hot spot area			Non-sprayed area			
Characteristics		N	Mean±SD number	%	N	Mean±SD number	%	P-value
Age	(years)	48	67.8±6.4(59-81)		35	64.8±4.4(56-77)		0.013 [1)]
Height	(cm)	48	156.6±4.2		36	159.8±5.0		0.002 [2)]
Weight	(kg)	48	49.3±7.8		36	52.4±7.4		0.072 [2)]
BMI	(kg/m^2)	48	20.1±2.8		36	20.5±2.4		0.490 [2)]
Alcohol habit	(Yes)	48	21	43.8	36	16	44.4	0.949 [3)]
Smoking habit	(Yes)	48	27	56.3	36	29	80.6	0.019 [3)]
Present Job	(Yes)	48	32	66.7	36	14	38.9	0.011 [3)]
Kind of present job								
Multiple-choice	(Yes)	32	2	6.3	14	0	0	
Farmer	(Yes)	30	24	80	14	7	50	
Worker	(Yes)	30	0	0	14	2	14.3	
Fisher	(Yes)	30	0	0	14	1	7.1	
Teacher	(Yes)	30	0	0	14	0	0	
Other job	(Yes)	30	6	20	14	4	28.6	

[1)]Welch test, [2)]Student's t-test, [3)]Chi-squared test
SD: standard deviation, BMI: body mass index

Table 2 shows the testosterone, dehydroepiandrosterone (DHEA), and estradiol levels were significant differences between the hotspot and non-sprayed areas, whereas cortisol, cortisone, progesterone, dihydrotestosterone (DHT), androstenedione and estrone were not significant differences between the two areas.

Table 2 A comparison of serum steroid hormone in male between hotspot and non-sprayed areas

Characteristics		Hotspot area (n=48)	Non-sprayed area (n=36)	p-value
Cortisol(Mean±SD)	(ng/mL)	81.8 ± 31.9	81.3 ± 31.3	0.941
Cortisone(Mean±SD)	(ng/mL)	15.4 ± 4.6	15.6 ± 3.3	0.784
Progesterone(GM GSD)	(pg/mL)	38.0 1.8	41.9 1.7	0.575
Dihydrotestosterone(GM GSD)	(pg/mL)	615.2 1.5	548.3 1.4	0.179
Testosterone(GM GSD)	(pg/mL)	6309.6 1.4	5395.1 1.4	0.029
Dehydroepiandrosterone(Mean±SD)	(pg/mL)	1388.2 ± 550.0	1727.6 ± 717.6	0.016
Androstenedione(GM GSD)	(pg/mL)	1552.4 1.5	1671.1 1.4	0.358
Estradiol(Mean±SD)	(Pg/mL)	12.5 ± 4.1	10.8 ± 3.3	0.042
Estrone(Mean±SD)	(Pg/mL)	28.4 ± 8.2	30.1 ± 9.2	0.366

Student's t-test
SD: standard deviation, GM: geometric mean, GSM: geometric standard deviation

The mean concentration of PCDDs, PCDFs, PCDDs+PCDFs, PCBs, and PCDD/DFs+PCBs toxic equivalents (TEQ) in the serum in the hotspot area was significantly higher than in the non-sprayed area (Table 3).

Table 3 A comparison of dioxins levels in the serum between hotspot and non-sprayed areas

Dioxins		Hotspot area n=48	on-sprayed area n=36	p-value
PCDDs(GM GSD)	(pg-TEQ/g lipid)	16.6 1.9	4.8 1.5	<0.001[1)]
PCDFs(GM GSD)	(pg-TEQ/g lipid)	11.9 1.7	3.6 1.5	<0.001[1)]
PCDDs+PCDFs(GM GSD)	(pg-TEQ/g lipid)	29.0 1.7	8.4 1.5	<0.001[1)]
PCBs(GM GSD)	(pg-TEQ/g lipid)	7.7 2.0	3.4 1.9	<0.001[2)]
PCDD/DFs+PCBs(GM GSD)	(pg-TEQ/g lipid)	37.2 1.8	12.2 1.5	<0.001[1)]

[1)]Welch test, [2)]Student's t-test
GM: geometric mean, GSM: geometric standard deviation

This was a significant correlation between estradiol and PCDDs, PCDDs+PCDFs, and PCDD/DFs+PCBsTEQ levels in the hotspot and non-sprayed areas (Table 4).

Table 4 Linear regression analysis for estradiol

Variable	r	95% CI	p-value
PCDDs	0.2345	0.02—0.43	0.0318
PCDFs	0.1839	-0.03—0.38	0.0940
PCDDs+PCDFs	0.2226	0.01—0.42	0.0418
PCBs	0.1351	-0.08—0.34	0.2204
PCDD/DFs+PCBs	0.2165	0.01—0.41	0.0480

r: correlation coefficient, CI: confidence interval

There was not significant correlation between testosterone, and TEQ dioxins levels, after adjusted for age, BMI, present job, and smoking habit in the hotspot+non-sprayed area (Table 5).

Table 5 Multivariate analysis for testosterone

Variable	t Ratio	95% CI	p-value
PCDDs	0.75	-0.06－0.11	0.5763
PCDFs	-0.06	-0.10－0.09	0.9538
PCDDs+PCDFs	0.35	-0.08－0.11	0.7261
PCBs	0.01	-0.08－0.09	0.9931
PCDD/DFs+PCBs	0.30	-0.08－0.11	0.7673

CI: confidence interval
All analyses were adjusted for age, BMI, present job and smoking habit
There was not significant correlation between dehydroepiandrosterone (DHEA) and TEQ dioxins levels, after adjusted for age, BMI, present job, and smoking habit in the hotspot+non-sprayed area (Table 6)

Table 6 Multivariate analysis for dehydroepiandrosterone

Variable	t Ratio	95% CI	p-value
PCDDs	-1.08	-674.0－190.9	0.2818
PCDFs	-1.12	-727.1－202.5	0.2645
PCDDs+PCDFs	-1.14	-704.1－192.6	0.2595
PCBs	-1.43	-718.4－117.0	0.1558
PCDD/DFs+PCBs	-1.34	-778.0－153.0	0.1853

CI: confidence interval
All analyses were adjusted for age, BMI, present job and smoking habit

There was not significant correlation between estradiol and TEQ dioxins levels, after adjusted for age, BMI, present job, and smoking habit in the hotspot+non-sprayed area (Table 7)

Table 7 Multivariate analysis for estradiol

Variable	t Ratio	95% CI	p-value
PCDDs	1.13	-1.09 – 3.95	0.2625
PCDFs	0.42	-2.22 – 3.41	0.6743
PCDDs+PCDFs	0.90	-1.48 – 3.93	0.3700
PCBs	0.62	-1.75 – 3.33	0.5394
PCDD/DFs+PCBs	0.94	-1.49 – 4.15	0.3517

CI: confidence interval
All analyses were adjusted for age, BMI, present job and smoking habit

This is the first study concerning the relationship between dioxins and steroid hormones in Vietnamese men. All of TEQ dioxins levels in the hotspot area were significantly higher than those in the non-sprayed area. In addition, testosterone, dehydroepiandrosterone (DHEA), and estradiol levels were significant differences between the hotspot and non-sprayed areas. There was a significant correlation between estradiol and PCDDs, PCDDs+PCDFs, or PCDD/DFs+PCBsTEQ levels in the hotspot and non-sprayed areas. Our previous study reported that salivary cortisol and cortisone were associated with breast milk dioxin levels in Vietnamese primipara[7]. However, this present study found no significant correlation between testosterone, dehydroepiandrosterone (DHEA), and estradiol and dioxins levels, after adjusted for age, BMI, present job, and smoking habit in the hotspot+non-sprayed area. The steroidogenesis is influenced by dioxin more likely to the primipara than man. We also found a strong correlation between steroid hormone and BMI rather than dioxin. Since this study was based on a small number of Vietnamese men further studies will be needed to be verified with a larger sample size in the future.

Acknowledgements
This study was supported by the grants from the Japan Society for the promotion of science (Grant-in-Aid for Scientific Research, (B) No. 23406018 and Grant-in-Aid Scientific Research, (A) No. 19209021). We would like to thank all the participants in Phu Cat and Kim Bang.

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