EFFECTS OF CHLORPYRIFOS-METHYL ON ESTROGENIC AND ANDROGENIC ACTIVITY IN RATS

Hwan-goo Kang, Sang-hee Jeong, Dong-gyu Kim, Joon-hyoung Cho

National Veterinary Research & Quarantine Service, Anyang 430-016, Republic of Korea

Introduction

Chlorpyrifos-methyl, organophosphate insecticide, is widely used on stored grain and commonly founded in mixed feed in USA[1]. Organophosphates, acetylcholine estrase inhibitor, were shown to modify central hormonal axis and to affect on the prolactin level in serum [2]. Some pesticide like vincozolin, p,p'-DDE and linuron are reported to alter differentiation of androgen dependent tissue in the female rats by acting as androgen receptor antagonist[3,4,5]. Others like toxaphene, endosulfan, lindane interact with human estrogen receptor in vitro[6,7]. Chlorpyrifos, an analogy of chlorpyrifos-methyl, is reported to be potential endocrine disrupter by lowering the serum thyroxine level in ewes[8]. The estrogenic and adrogenic effects of chlorpyrifos-methyl has not been reported yet. So, this study is to investigate the estrogenicity and androgenicity in vivo using immature rat uterotrophic assay and rat Hershberger assay.

Method and Materials

Immature rat uterotrophic assay

Immature Spraque-Daeley female rats (PND 19) were randomly assigned to each groups 1 day before treatment. Vehicle control (corn oil), positive control (17 b-estradiol, 2 ug/kg bw/day), chlorpyrifos-methyl (2, 10, 50, 250 mg/kg bw/day) and chlorpyrifos-methyl (10 or 250 mg/kg bw/day) with 17 b-estradiol (2 ug/kg bw/day) were administered via subcutaneous injection for 3 consecutive days. Animals were sacrificed 6 hours after the last treatment. Uteri and other organ were removed, trimmed and weighed. Uteri were stained with H&E and measured uterine epithelial cell height. Uterine cell proliferation was measured according to Cho *et al*[9].

Rat Hershberger assay

Pubertal Spraque-Daeley female rats (PND 42) were castrated and recovered for 8 days. Vehicle control (corn oil), positive control (Testosterone propionate, 0.4mg/kg bw/day), chlorpyrifos-methyl (2, 10, 50, 250 mg/kg bw/day) alone and chlorpyrifos-methyl (50mg/kg bw/day) with Testosterone propionate (0.4 mg/kg bw/day) were administered via gavage (chlorpyrifos-methyl and vehicle) or subcutaneously (Testosterone propionate) for 10 consecutive days. Animals were sacrificed 24 hours after the last treatment. Androgen dependent accessory sex organs, ventral prostate (VP), seminal vesicle with coagulating glands (w/wo fluid, SV/CG), glans penis (GP), Cowper's gland (CG) and Levator *ani plus* balbocavenosus muscle (LABC), were removed and weighed. Serum hormone values were measured with immunofluorescence kit (DELFIA)

Statistics

Statistical analysis was performed using one-way analysis of variance (ANOVA) and Duncan' test ; p values of <0.05, <0.01 were considered to be significant.

Results and Discussion

Effect of chlorpyrifos-methyl on uterine weight and uterine epithelial cell height and cell proliferation

Relative uterine and vagina weight and uterine cell proliferation were not affected by the treatment with chlorpyrifos-methyl of 2, 10, 50 and 250 mg/kg bw/day alone (Table 1). Table 2 showed that chlorpyrifos-methyl (10 or 250 mg/kg bw/day) administration also did not significantly change the uterine weight and epithelial cell height increased by 17 b-estradiol (2 ug/kg bw/day). Theses result suggest that chlorpyrifos-methyl be less likely to interact with estrogen dependent process although it may be able to bind to estrogen receptor in vitro transformed yeast system[10].

Table 1. Relative uterine and other organ weight, body weight gain and labeling index after the treatment of CPM and 17ß-estradiol in BrdU treated immature female rats

Group	Labeling index	Body weight gain(g)	Uterus (wet, g/100g)	Vagina (wet, g/100g)	Liver (g/100g)	Ovary (g/100g)		Adrenal gland (g/100g)	
						Right	Left	Right	Left
Vehicle	75.1 ±21.2	5.3 ±0.8	0.061 ±0.005	0.053 ±0.009	4.33 ±0.25	0.017 ±0.002	0.015 ±0.001	0.018 ±0.001	0.019 ±0.002
17ß-estradiol (2/kg bw)	**138.3 ±42.0	6.1 ±2.0	**0.171 ±0.012	**0.133 ±0.012	4.36 ±0.13	0.0016 ±0.004	0.015 ±0.003	0.020 ±0.002	0.021 ±0.002
CPM (2/kg bw)	67.7 ±15.3	5.8 ±1.5	0.071 ±0.007	0.055 ±0.008	4.12 ±0.19	0.015 ±0.003	0.014 ±0.002	0.018 ±0.003	0.020 ±0.001
CPM (10/k bw)	77.5 ±2.10	6.0 ±2.0	0.067 ±0.008	0.052 ±0.008	4.35 ±0.26	0.015 ±0.003	0.014 ±0.002	0.018 ±0.003	0.020 ±0.001
CPM (50/kg bw)	75.2 ±23.4	6.0 ±1.8	0.069 ±0.005	0.058 ±0.003	4.39 ±0.19	0.016 ±0.001	0.017 ±0.002	0.020 ±0.002	0.020 ±0.001
CPM (250/kg bw)	84.4 ±33.3	5.1 ±1.8	0.064 ±0.005	0.051 ±0.008	**4.99 ±0.26	0.016 ±0.002	0.020 ±0.011	*0.024 ±0.008	*0.022 ±0.003

BrdU (25mg/kg bw) was administered to all rats by intraperitoneal route for 4 consecutive days at 19 days of age. Values are the Mean±SD (n=7). *, ** Significantly different from Vehicle at $p<0.05$, $p<0.01$, espectively.

Table 2. Relative uterine and liver weight, body weight gain and uterine epithelial cell height after the treatment of 17ß-estradiol with or without CPM in immature female rats

Group	Initial weight(g)	Body weight gain(g)	Uterus (wet, g/100g)	Liver (g/100g)	Uterine epithelial cell Height(um)
Vehicle	35.8±2.6	7.8±1.1	0.082±0.010	4.37±0.26	7.38±0.47
E2(2?/kg bw)	36.2±2.0	8.1±1.2	**0.182±0.017	4.31±0.31	**12.60±1.21
E2+CPM (2?/kg bw+10?/kg bw)	36.0±3.0	8.4±1.1	**0.171±0.007	4.40±0.16	**11.48±1.61
E2+CPM (2?/kg bw+250?/kg bw)	35.7±2.1	7.6±0.8	**0.178±0.010	**4.83±0.12+	*12.46±1.00

17ß-estradiol (E2) were administered by sc injection within 10 minutes after the injection of CPM or vehicle. Values are the Mean±SD (n=8). *, ** Significantly different from Vehicle at $p<0.05$, $p<0.01$, espectively.
+, Significantly different from E2 at $p<0.05$.

Effect of chlorpyrifos methyl on the weights of androgen-dependent accessory sex organs

Relative accessory sex organ's weight (VP, SV/GC, GP, CG and LABC) were not affected by the treatment with chlorpyrifos-methyl of 2, 10, 50 and 250 mg/kg bw/day alone. Relative weight of adrenal gland was significantly increased by the treatment of chlorpyrifos-methyl treatment. Chlorpyrifos-methyl 50 mg/kg/day administration significantly attenuated the Testosterone-propionate (0.4 mg/kg/day) induced increase of the weights of VP, SV/CG, LABC, CG, respectively. This result suggest that chlorpyrifos-methyl has an inhibitory effect on the function of androgen. Our previous result showed that chlorpyrifos-methyl had an affinity for androgen receptor in androgen receptor transformed yeast system[10]. It is also reported that ACTH, prolactin levels were significantly high and FSH were low during organophosphate insecticide poisoning and organophosphate insecticide, fenitrothion, blocked didhydrotestosterone-dependent AR activity in vitro[2,3]. But the inhibitory mechanism remains to be determined whether chlorprifos-methyl blocks the androgen receptor or affects on the hyothalamo-pituitary-gonadal axis or other synthesis related enzyme such as 5a-reductase.

Conclusion

In the present study, chlorpyrifos-methyl showed an inhibitory effect on the function of testosterone propionate in castrated but no effect on estrogen dependent uterine response in immature rat. Therefore, we suggest that chlorpyrifos-methyl be an endocrine disrupter through anti-androgenic activity. But the mechanism of inhibitory effect of chlorpyrifos-methyl on the androgen system in vivo needs to be determined.

References

1. Lovell RA, MacChesney DG. And Price WD. (1996) J. AOAC. international 79(12): 544-548.
2. Guven M., Bayra F., Unluhizarchi K, and Kele TF. (1999) Human & Exp. Toxicol. 18(10): 598-601.
3. Tammura H., Maness SC., Reischmann K. Dorman K., Gray DC. and Gaido KW. (2001) Toxicol. Sci. 60: 56-62.
4. Lambright C., Ostby J. and Bobesine K. (2001) Toxicol. Sci. 56: 389-399.
5. Kelc WR., Lambright C. and Gray LE. Jr. (1997) Toxicol. Appl. Pharmacol. 142: 192-200.
6. Schrader TJ. and Cooke GM. (2000). Toxicol. Sci. 53: 278-288.
7. Andersen HR., Vinggaard AM., Rasmussen TH, Gjermandsen IM. And Boderfeld-Jorgensen EC. (2002) Toxicol. Appl. Pharmacol. 179 : 1-12
8. Rawlings NC., Cook SJ. And Waldbillig D. (1998) J. Toxicol. Environ. Hlth. 54: 21-36.
9. Cho JH., Jeong SH. and Ku HO. (1994) Kor. J. Vet. Publ. Hlth. 18(3): 287-299.
10. NVRQS. (1999) Annual report of National Veterinary Research and Quarantine Service.

Table 3. Relative organ weight, body weight gain and level of serum hormones after the treatment of CPM alone and with TP in castrated male rats.

Group Indexs	Vehicle	TP	CPM 2	CPM 10	CPM 50	CPM 250	CPM 50 +TP
SV/CG	0.1246	**2.2333	0.1214	0.1267	0.1297	0.1298	**1.7439^{++}
(mg/g)	±0.0083	±0.3188	±0.0135	±0.0131	±0.0219	±0.0135	±0.3185
SV/CG (w/o)	0.1138	**1.2043	0.1132	0.1117	0.1199	0.1144	**1.0376^{++}
(mg/g)	±0.0088	±0.1465	±0.0137	±0.0126	±0.0227	±0.0123	±0.1042
Ventral prostate	0.0402	**0.6723	0.0393	0.0374	0.0379	0.0419	**0.5476^{++}
(mg/g)	±0.0052	±0.0715	±0.0064	±0.0064	±0.0072	±0.0082	±0.1065
Glans penis	0.1213	**0.2585	0.1162	0.1182	0.1197	0.1114	**0.2561
(mg/g)	±0.0169	±0.0168	±0.0156	±0.0184	±0.0103	±0.0098	±0.0190
LABC	0.6351	**1.9482	0.6397	0.6345	0.6411	0.5944	**1.7872^{++}
(mg/g)	±0.0740	±0.0862	±0.0556	±0.0979	±0.0664	±0.0474	±0.0822
Cowper's gland	0.0113	**0.1276	0.0124	0.0128	0.0120	0.0126	**0.1113^{++}
(mg/g)	±0.0026	±0.0166	±0.0034	±0.0015	±0.0021	±0.0018	±0.0143
Body weight	54.4	**70.5	54.5	53.0	51.6	*45.6	**66.4
Gain(g)	±5.8	±3.8	±6.4	±5.1	±3.7	±5.4	±6.8
T4	7.19	8.53	7.26	7.80	7.52	6.84	6.85
	±1.53	±2.96	±1.47	±2.20	±1.57	±1.39	±2.36
Testosterone	ND	2.16	ND	ND	ND	ND	1.24
		±1.26					±0.68
Liver(g/100g)	3.91	*4.21	4.09	4.14	*4.20	**4.60	*4.33
	±0.33	±0.23	±0.13	±0.35	±0.28	±0.30	±0.13
Kidney(right)	3.29	3.44	$^{+}$3.22	$^{+}$3.21	3.32	3.38	3.33
(mg/g)	±0.23	±0.20	±0.17	±0.18	±0.20	±0.16	±0.09
Kidney(left)	3.16	*3.33	3.19	$^{+}$3.13	3.19	3.28	3.25
(mg/g)	±0.14	±0.12	±0.13	±0.13	±0.14	±0.21	±0.14
Adrenal gland	0.0813	**0.0678	*0.0908^{++}	*0.0889^{++}	**0.1003^{++}	**0.0981^{++}	0.0835^{++}
(right)(mg/g)	±0.0056	±0.0058	±0.0048	±0.0093	±0.0065	±0.0049	±0.0060
Adrenal gland	0.0874	**0.0705	0.0936^{+}	0.0941^{++}	**0.1059^{++}	**0.1033^{++}	0.0893^{++}
(left)(mg/g)	±0.0117	±0.0060	±0.0061	±0.0083	±0.0090	±0.0075	±0.0070
Thyroid gland	0.0451	0.0422	0.0456	0.0456	0.0433	0.0464	0.0436
(mg/g)	±0.0061	±0.0048	±0.0080	±0.0080	±0.0058	±0.0069	±0.0032

Values are the Mean±SD (n=10). *,**Significantly different from Vehicle at $p<0.05$, $p<0.01$, respectively.

$^{+}$, $^{++}$, Significantly different from TP at $p<0.05$, $p<0.01$, respectively.