COMPARSION OF METHOXYCHLOR-INDUCED WEIGHT CHANGE AND CALBINDIN-D9k mRNA EXPRESSION IN RAT UTERUS BY THE ROUTE OF ADMINISTRATION

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Introduction

Exposure to some synthetic environmental chemicals and their metabolites cause reproductive problems in a variety of vertebrate via endocrine mechanisms. However, in most cases, the link between these compounds and adverse effects on humans, fish, and wildlife has not been established, which necessitates a closer look at the molecular, functional, and clinical implications of these chemicals in the environment. Since a considerable number of environmental chemicals which cause serious effects on reproductive organs, have been reported to have possible endocrine disrupting activity in humans and animals, the Organization for Economic Cooperation and Development (OECD) has proposed the uterotrophic assay as a useful and sensitive method to detect adverse activities in female rats. Environmental estrogens are defined as compounds that bind the estrogen receptors, and elicit or modulate an ER-mediated response. Examples of suspected environmental estrogenic chemicals include polychlorinated hydroxybiphenyls, DDT and derivatives, certain insecticides and herbicides as Kepone and methoxychlor (MC), plastic components as BPA and some components of detergents and their biodegradation products as alkylphenols[1,2]. MC is a chlorinated hydrocarbon pesticide, which is metabolized to 2,2-bis (p-hydroxyphenyl)-1,1,1-trichloroethane (HPTE), a compound that possesses estrogenic and antiandrogenic activity[2,3]. For cell-based endogenous gene induction assays, there are several biomarkers of estrogenicity including pS2, glucose-6-phosphate dehydrogenase, ornithine decarboxylase, and vitellogenin. However, these genes have some disadvantages due to the difficulty of detection because of low expression of these genes. Calbindin-D9k (CaBP-9k) is a member of a large family of intracellular calcium binding proteins that have high affinities for calcium[4]. It was reported that the estrogen level of uterus affected the expression of the CaBP-9k gene in rat uterus. Estrogen injection to 21-day old rats increased the expression of CaBP-9k mRNA when compared with non-treated rats and CaBP-9k reached to the highest level at diesturs when the level of estrogen was high in the uterus[5].

In the present study, we hypothesized that the CaBP-9K gene has the possibility as a biomarker for estrogenic response of the environmental estrogens because of its strict regulation by estrogen. We have analyzed the dose-dependent CaBP-9K gene expression in the uterus for three-days injection of methoxychlor in the overectomized immature rats and examined the relation with uterotrophic response of the compounds. Furthermore, we compared the responses induced by methoxychlor according to the route of administration.

Methods and Materials

Immature female Sprague-Dawley rats (18 days of age) were acquired from Laboratory Animal Resources, National Institute of Toxicological Research (NITR), Korea FDA (Seoul, Korea). All animals with control animals used in this experiment were handled in an accredited Korea FDA animal facility in accordance with the guidelines for animal experiment of the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC) International Animal Care Policies (Accredited Unit-Korea Food and Drug Administration: Unit Number-000996). MC and corn oil were obtained from Sigma (St. Louis, MO. USA). The test compound was dissolved in corn oil for administration. The test compound was prepared daily before treatment.

At 20 days of age rats were assigned to each group by randomization method before the treatment, so no significant difference in mean body weight was observed among the groups at the beginning. The essential procedure was based on the OECD protocol[6]. They were observed daily for clinical signs. Rats were treated once daily by oral gavage (PO) or subcutaneous injection (SC) with MC (50, 100 and 200mg/kg/day) and the vehicle control group was administered only with corn oil. The dose volume was 4ml/kg body weight in PO and 0.4ml/kg in SC. Rats were killed 1 day after the last administration in the same sequence as the test substance was administered. The uterus was carefully dissected and trimmed of fascia and fat to avoid loss of luminal contents. And then, the vagina was removed from the uterus at the level of the uterine cervix and weighed. The uterus with the luminal contents (wet weight) was promptly weighed to the nearest 0.1 mg. The CaBP-9k mRNA expression by MC was assessed by Northern blot analysis. For Northern blot analysis, the excised uterus was washed in cold sterile 0.9% NaCl solution. Total RNA was extracted with Trizol (Life Tech., Rockville, MD) according to manufacturer's suggested procedure. The means were compared using Dunnett’s test after one-way analysis of variance using computer program (SigmaStat V 2.03, SPSS Inc., IL). Significant differences between values are indicated by $P<0.05$.

Results and Discussion

There were no significant differences in body weight at necropsy and the weight of liver among the treated groups and control (Table 1). A dose-dependent uterotrophic response to the oral administration or subcutaneous injection of MC was shown in the present three-day uterotrophic assay (Table 2). A significant increase in the uterine wet weights was observed when treated orally with 50 mg/kg/day and above, but when treated subcutaneously only the highest group showed a significant increase of uterine weight. The weight of vagina showed the same change as that of uterus. The weights of ovaries and liver of treated rats were not significantly different from those of control group, regardless of the route of treatment. In order to correlate the increase in the uterine wet weight after following administration of MC with their ability to modulate the expression of the estrogen-sensitive gene, the CaBP-9k mRNA expression was analyzed using the same uterus from the uterotrophic assay. A significant increase in CaBP-9k mRNA expression was observed when treated with EE for 3 days when administered by PO or by SC (Figure 1). Treatment with MC showed different CaBP-9k mRNA expression by the route of treatment. When injected by SC, the expression of MC-treated group was slightly increased but significantly different from that of control. However, a dramatic increase of CaBP-9k mRNA expression was observed when treated with MC orally. The CaBP-9k mRNA expression was increased in a dose-dependent manner from 50mg/kg/day. The CaBP-9k mRNA expression in uterus was consistent with the weight changes of uterus, specifically by the route of administration.

The present study demonstrates that CaBP-9k mRNA is induced by MC and the weight of uterus is increased. And the expression of mRNA and the weight are different according to the route of administration. When MC was administered orally, the estrogenic response in terms of the weight of uterus and the expression of CaBP-9k mRNA was much stronger than SC treatment. It was supposed that MC has its estrogenic activity after changed to HPTE, an estrogenic metabolite. Usually thought, the metabolite is much more produced in PO route than in SC route, but further study is needed. Since CaBP-9k gene is regulated through the binding of ER/estrogen complex to estrogen response element (ERE), the expression level of ERα mRNA were related with the expression of CaBP-9k mRNA in terms of dose- and time-dependent response. It was reported that environmental estrogens may regulate CaBP-9k mRNA expression like the endogenous steroid through modulating transcriptional activity of estrogen receptor[7]. In summary, the estrogenicity measured in CaBP-9k mRNA expression was correlated with in vivo uterotrophic assay. Our data suggest that CaBP-9k mRNA assay in the rat uterus may be used as a tool to identify substances with estrogenic activity when used in combination with the classical assay.

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Table 1. The Weight Changes of Body and Liver

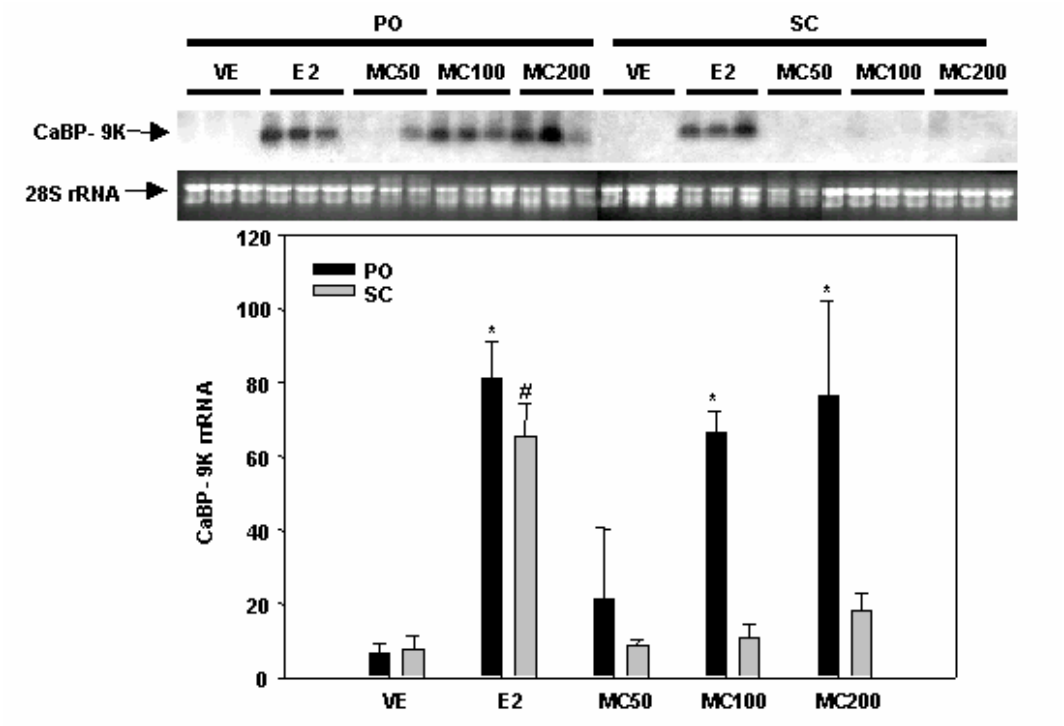
Route	Chemicals		Initial B.W. (g)	Final B.W. (g)	Liver (g)	
					Absolute	Relative
PO	EE	3ug	51.0±5.29	66.8±6.59	2.80±0.35	4.19±0.21
	MC (mg)	0	50.0±5.87	66.5±6.32	2.90±0.41	4.34±0.26
		50	51.0±5.83	65.5±6.53	2.70±0.33	4.11±0.19
		100	51.0±10.18	66.5±10.89	2.83±0.54	4.25±0.28
		200	51.7±8.24	63.7±9.00	2.75±0.31	4.34±0.33
SC	EE	1ug	51.0±6.16	67.8±8.01	2.86±0.32	4.22±0.24
	MC (mg)	0	51.0±6.78	67.2±8.35	2.98±0.48	4.43±0.21
		50	50.8±5.60	68.7±6.95	2.92±0.40	4.24±0.22
		100	50.2±6.08	68.0±8.37	2.92±0.42	4.30±0.20
		200	51.0±4.43	67.3±5.47	2.90±0.35	4.30±0.22

Table 2. The Weight Changes of Female Reproductive Organs

Route	Chemicals		Uterus (mg)		Vagina (mg)		Ovaries (mg)	
			Absolute	Relative	Absolute	Relative	Absolute	Relative
PO	EE	3ug	115.3±30.66*	174.1±54.16*	73.6±12.05*	110.0±13.04*	16.7±3.00	25.1±3.99
	MC (mg)	0	53.6±7.33	81.2±13.86	42.6±5.13	64.9±12.34	19.0±2.14	28.8±4.67
		50	83.0±12.73*	126.8±15.48*	67.2±20.70*	101.9±26.38*	17.7±3.43	26.9±4.42
		100	90.7±14.14*	138.7±28.80*	61.5±15.86	92.3±16.72*	18.0±3.43	27.0±2.55
		200	82.3±14.75*	129.7±20.73*	61.6±10.92	97.0±14.62*	17.9±3.78	28.7±7.54
SC	EE	1ug	255.9±58.50*	385.4±112.61*	71.7±7.63*	107.0±16.63*	20.2±3.42	29.8±3.45
	MC (mg)	0	52.8±15.98	78.1±18.80	45.1±13.09	66.8±14.52	19.4±6.12	28.7±7.32
		50	54.8±14.42	79.2±16.89	38.6±6.91	56.3±8.99	21.5±4.61	32.0±10.24
		100	63.6±13.11	94.7±22.78	43.6±6.80	64.7±11.29	15.6±2.42	23.4±5.77
		200	83.6±29.70*	125.4±46.30*	60.0±10.03*	89.4±15.08*	17.2±2.42	25.9±5.85

The values represent means ± SD.
**: Significantly different from vehicle control, p<0.05.*

Figure 1. Northern Blot Analysis for CaBP-9k mRNA Expression



*#. *: Significantly different from each of vehicle control, p<0.05.*