



Evaluation of Embryotoxic Potential of Olaquinox and Vitamin A in Micromass Culture and in Rats

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Limb bud (LB) and central nerve system (CNS) cells were prepared from 12.5 day old pregnant female Crj:CD (SD) rats and treated with olaquinox and vitamin A. Cytotoxicity and inhibition on differentiation were measured in each cell. Three doses of olaquinox (4, 21 and 100 mg/kg), and 0.2 and 75 mg/kg of vitamin A were administered to pregnant rat for 11 days from 6th to 16th of pregnancy. IC₅₀ values of olaquinox for proliferation and differentiation in CNS cell were 22.74 and 28.32 µg/ml and 79.34 and 23.29 µg/ml in LB cell and those values of vitamin A were 8.13 and 5.94 µg/ml in CNS cell and 0.81 and 0.05 µg/ml in LB cell, respectively. Mean body weights of pregnant rats were decreased at high dose of olaquinox (110 mg/kg) but relative ovary weight, number of corpus lutea, and number of implantation were not changed. Resorption and dead fetus were increased at high dose of olaquinox, and relative ovary weight, the number of corpus lutea and implantation, and sex ratio of male to female were not significantly changed in all dose of olaquinox. Mean fetal and placenta weights were significantly ($p < 0.01$) decreased in rats of high group. Seven fetuses out of 103 showed external anomaly like bent tail, and 10 out of 114 fetuses showed visceral anomalies at high group. The ossification of sternebrae and metacarpals were significantly ($p < 0.01$) increased by low and middle dose of olaquinox but it was significantly ($p < 0.01$) prohibited by high dose of olaquinox. In rats treated with vitamin A, the resorption and dead fetus were increased by high dose. Mean fetal weights were significantly ($p < 0.01$) increased by low dose but significantly ($p < 0.01$) decreased by high dose. Thirty four fetuses out of 52 showed external anomaly; bent tail (1), cranioarchschisis (14), exencephaly (14), dome shaped head (22), anophthalmia (15), brachynathia (10) and others (19). Forty five fetuses out of 52 showed soft tissue anomaly; cleft palate (42/52) and anophthalmia (22/52) by high dose of vitamin A. Sixty one fetuses out of 61 (85.2%) showed skull anomaly; defect of frontal, partial and occipital bone (21/61), defect of palatine bone (52/61) and others (50/61). In summary, we support that vitamin A is strong teratogen based on our micromass and in vivo data, and olaquinox has a weak teratogenic potential in LB cell but not in CNS cell. We provide the in vivo evidence that a high dose of olaquinox could have weak embryotoxic potential in rats.

Key words: Olaquinox, Vitamin A, Teratogenicity, Rat, Micromass

INTRODUCTION

Olaquinox, a synthetic antimicrobial compound, was used as growth promoting feed additives in animal industry (Bronsch *et al.*, 1976; Schneider *et al.*, 1976). Although it has been abandoned in most developed countries but it still was used in some developing countries for growth promotion (Chen *et al.*, 2009). Olaquinox, N-(2-hydroxyethyl)-3-

methyl-2-quinoxalinecarboxamide-1,4-di-N-oxide, is one of the quinoxaline-dioxides used widely as an antimicrobial growth promoter in pig production. Its toxicities were reported to be closely related to the formation of N-oxide reductive metabolites (Liu *et al.*, 2010).

Olaquinox increased the DNA fragmentation in Vero cells, but DNA damage was decreased after incubation with S₉ mix (Chen *et al.*, 2009). Olaquinox or some of its metabolite showed mutagenic activity (Beutin *et al.*, 1981; Ding *et al.*, 2006; Hao *et al.*, 2006). Olaquinox increased genotoxic effects in HepG2 cells through the ROS-induced oxidative DNA damage (Chen *et al.*, 2009). It brought out photo allergic contact dermatitis (Belhadjali *et al.*, 2002).

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Owing to its genotoxicity no threshold can be established for the safe usage of olaquinox, and the Commission of the European Community forbade the usage of olaquinox as an animal growth promoter in 1998 (Commission of the European Communities, 1998). However, it is still broadly used in some developing countries for promoting animal growth and its toxic effects need careful evaluation.

Although traditional toxicology tests have relied on whole animal test to provide information on the evaluation of toxicity; these tests are quite expensive and time consuming. Micromass using LB and CNS cell has been used as an effective tool to screen teratogenicity (Kistler, 1987; Kistler and Howard, 1990; Flint, 1993) and was demonstrated as a potential short term screen for teratogenic potency (Flint and Orton, 1984; Flint, 1986). Lim bud mesenchymal cells when they grown in high-density can proliferate and differentiate into a number of cell types such as cartilage and muscle. So, this cell was used to study of *in vitro* chondrogenesis as shown by the occurrence of cartilaginous foci made with chondrocytes to secrete proteoglycans and collagen (Zanetti and Solursh, 1984; Paulsen and Solursh, 1988). Using Alcian blue, a stain specific for cartilage proteoglycans, the degree of chondrogenesis can be visualized in the micromass cultures as well as quantified by extraction of the stain and spectrophotometric determination of its absorbance. Several studies have shown a fairly good correlation between inhibition of chondrogenesis *in vitro* and teratogenic activity *in vivo* (Flint and Orton, 1984; Guntakatta *et al.*, 1984; Wise *et al.*, 1990; Aulthouse and Hitt, 1994).

In present study, we evaluated the teratogenicity of olaquinox and vitamin A using micromass culture of limb bud and midbrain cell from 12.5 days of fetus and examined teratogenic and embryonic toxicity potential of those compounds in rats to compare with those result in micromass study.

Materials and methods

Animals. Eight week old female and male Crj:CD (SD) rats were provided by National Veterinary Research and Quarantine Service (Anyang, Republic of Korea). Rats were provided with tap water and a commercial diet *ad libitum*. The animal room was maintained at a temperature of $24 \pm 2^\circ\text{C}$, a relative humidity of $50 \pm 20\%$, and a 12 h light/dark cycle. All animals were cared for according to the Code of Laboratory Animal Welfare and Ethics of the NVRQS. Experimental design was approved by the NVRQS Animal Welfare Committee.

Preparation and culture of limb bud and CNS cells. Female rats were mated overnight and the appearance of sperms in vaginal smear was regarded as 0 day of gestation. On the day of 12 uteri were removed and embryo were separated for the micromass culture. Forelimb bud and mid-

brain were separated and LB and CNS cell were prepared by Flint and Orton (1984) method with modification by Kidney and Faustman (1995). Briefly, after trypsinization of midbrain and forelimb bud, medium was added to produce 5×10^6 cells/ml for CNS cell and 2×10^7 cells/ml for LB cell.

Chemical exposure in micromass. Stock solution of vitamin A (Sigma, Republic of Korea) and olaquinox (Dahee, Republic of Korea) were dissolved in dimethylsulfoxide. Dilutions were prepared by adding medium not exceeding 1% (v/v). Plated cells were cultured for 2 h at 37°C , 5% CO_2 incubator for the attachment of cells, after which medium containing test compound was replaced. Exposure concentrations were 6, 14, 32, 73, 166 $\mu\text{g/ml}$ in both LB and CNS for olaquinox. Concentration of vitamin A in CNS cell were 2.3, 5, 10.8, 23.3 and 50.0 $\mu\text{g/ml}$ for cytotoxicity and differentiation, and 0.16, 0.38, 0.89, 2.12 and 5.0 $\mu\text{g/ml}$ for cytotoxicity and 0.003, 0.011, 0.144 and 0.50 $\mu\text{g/ml}$ for inhibition of differentiation in LB cells.

Determination of cytotoxicity and inhibition on differentiation. Assessment of cytotoxicity and inhibition of differentiation was followed by the method of Kidney and Faustman (Kidney and Faustman, 1995) with some modification. For the assessment of cytotoxicity, 8 μl drops of CNS or LB cell suspension were plated in the well of collagen coated, warmed 96-well microplate. Medium was aspirated and cell was fixed in 4.5% glutaraldehyde for 20 min after 5 day culture. Cells were washed two times with PBS, and 200 μl of freshly prepared neutral red in PBS (0.05% w/v) was added to each well. After 1 h, cells were rinsed with PBS twice, and 200 μl of 0.5% (v/v) acetic acid in ethanol was added to solve the dye. Absorbance was determined at 540 nm on microplate reader.

For the assessment of inhibition on differentiation on LB and CNS cells, 20 μl drops of CNS or LB cell suspension were plated in the well of primaria coated, warmed 6-well microplate. The differentiation of CNS cells was quantified by hematoxylin staining and by Alcian blue staining of proteoglycans for LB cells. Briefly, LB cells were fixed with 10% (v/v) formaldehyde and 0.5% (w/v) cetylpyridinium chloride solution for 20 min. After washing with tap water, each well was treated with 3% acetic acid in 1 N HCl for 1 h and then stained overnight with 1% (w/v) Alcian blue. Each well was washed with 3% acetic acid in 1 N HCl. Small foci of cells which differentiated within each micromass island were counted. CNS cells were stained with Delafield's hematoxylin for 3 min and immersed in tap water 20 min to develop the color.

Embryotoxicity of olaquinox and vitamin A in rats. Vitamin A was dissolved in corn oil and olaquinox was dissolved in 2% CMC. Pregnant rats were exposed to 4 mg/

kg (low group), 21 mg/kg (middle group) and 110 mg/kg bw (high group) of olaquinox and 0.2 mg and 75 mg/kg bw of vitamin A, respectively, for 11 days from 6th to 16th of pregnancy. Rats were scarified 1 day before delivery and resorption, fetal weight and other indexes were observed to determine developmental toxicity. Gross external and internal malformations were examined first. Then, skeletal malformation were observed using a clearing technique by Staple and Schnell (1964) consisting of maceration in 1.0% KOH and staining with Alizarin red S. To observe the malformation of head, heads were soaked into Bouin's fixative for 10 day.

Statistical analysis. IC₅₀ values and 95% confidence limit were calculated with Litchfield and Wilcoxon II method using Pharm/PCS (ver 4.1, USA). Data were ana-

lyzed by break down & one-way ANOVA, followed by Duncan test as post-hoc comparison using Statistica (Ver. 5.5, USA).

RESULTS

Effect of olaquinox and vitamin A on the proliferation and differentiation in the limb bud and midbrain cells.

Olaquinox and vitamin A inhibited dose-dependently the proliferation and differentiation of LB and CNS cell, respectively (Fig. 1, 2). IC₅₀ values of olaquinox for the proliferation and differentiation in CNS cell were 22.74 and 28.32 µg/ml, and 79.34 and 23.29 µg/ml in LB cell, respectively (Table 1). Regarding vitamin A, IC₅₀ values of proliferation and differentiation in CNS cell were 8.13 and 5.94 µg/ml, and 0.81 and 0.05 µg/ml in LB cell, respec-

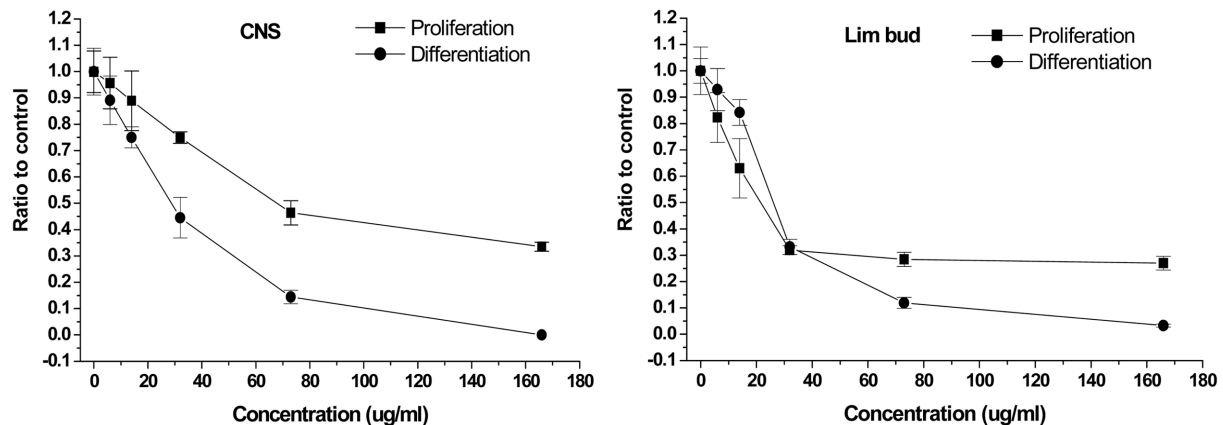


Fig. 1. Dose-response of olaquinox to micromass culture of CNS and LB cells from 12.5 day old rat embryo. Cell proliferation is measured as spectrophotometric estimation of neutral red uptake at 560 nm and differentiation as the number of foci per island which stained with hematoxylin for CNS and Alcian blue for LB cells after 5 days of culture. All points represent mean of 4~6 experiments with standard deviation. Where no bars are observed, they fall within the plot symbol.

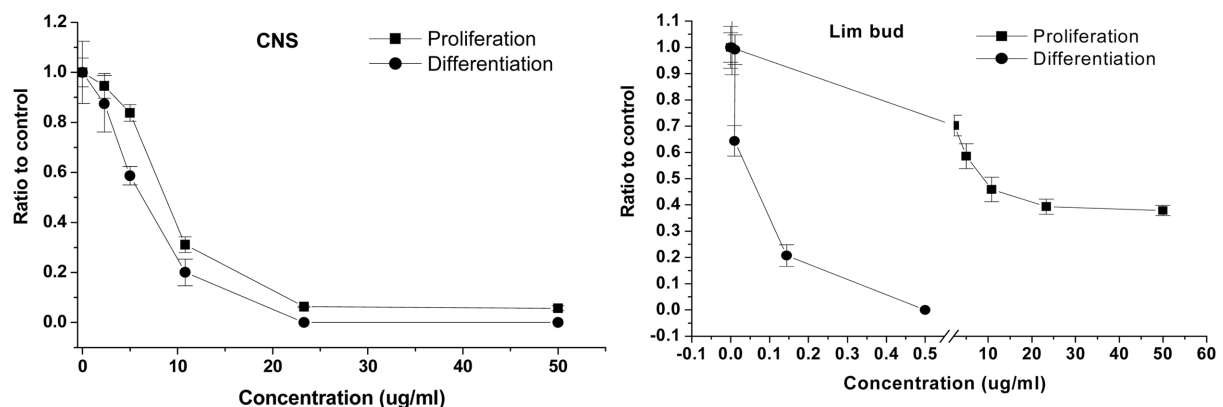


Fig. 2. Dose-response of vitamin A acetate to micromass culture of CNS and LB cells from 12.5 day old rat embryo. Cell proliferation is measured as spectrophotometric estimation of neutral red uptake at 560 nm and differentiation as the number of foci per island which stained with hematoxylin for CNS and Alcian blue for LB cells after 5 days of culture. All points represent mean of 4~6 experiments with standard deviation. Where no bars are observed, they fall within the plot symbol.

Table 1. Inhibitory effects of olaquinox on proliferation and differentiation of CNS and LB cells

Cells ^a		IC ₅₀ (μg/ml)	95% Confidence limits
CNS	Proliferation	22.74	18.31~28.24
	Differentiation	28.32	23.62~33.95
LB	Proliferation	79.34	63.16~99.67
	Differentiation	23.29	19.28~28.13

^aCells were isolated from midbrain and limb bud of 12.5 days old rat embryo.

Table 2. Inhibitory effects of vitamin A acetate on proliferation and differentiation of CNS and LB cells

Cell type ^a		IC ₅₀ (μg/ml)	95% Confidence limits
CNS	Proliferation	8.13	7.10~9.31
	Differentiation	5.94	5.10~6.93
LB	Proliferation	0.81	0.51~1.27
	Differentiation	0.05	0.04~0.07

^aCells were isolated from midbrain and limb bud of 12.5 day old rat embryo.

tively (Table 2).

Effect of olaquinox on embryonic development in rats. In rats given with olaquinox, mean body weight of

mother rats was decreased and the rate of resorption and percentage of dead fetus was increased only at high group, whereas relative ovary weight, the number of corpus lutea and implantation, and sex ratio of male to female were not significantly changed by all dose of olaquinox (Table 3). Mean fetal weights were significantly ($p < 0.05$) increased in female and male rats of middle group but significantly ($p < 0.01$) decreased in rats of high group. Mean placenta weights were decreased only in rats of high group (Table 4). Seven fetuses out of 103 showed external anomaly like bent tail and it was increased compared to that of vehicle control (2/114; number of anomaly to examined fetuses). Regarding visceral anomalies, they were 6/114 in control group and 10/103 in high group. Two fetuses out of 110 showed dilation of lateral ventricle at high group (Table 5). Skeletal anomaly was not observed at all group. The ossification of sternebrae and metacarpals were significantly ($p < 0.01$) increased by low and middle dose of olaquinox but it was significantly ($p < 0.01$) prohibited at high group (Table 6).

Effect of vitamin A on embryonic development in rats.

In rats treated with vitamin A, mean body weight of mother was significantly ($p < 0.01$) decreased only at high group. The percentage of resorption and dead fetus were increased at high dose of vitamin A but sex ratio and number of implantation were not changed by the treatment of vitamin

Table 3. Effect of olaquinox and vitamin A on rat embryonic development

Indices		Dose (mg/kg)					
						Vitamin A	
		0	4	21	110	0.2	75
No. of dams		20	20	20	20	19	17
Mean B.W. of maternal rats (g)		370.83 ± 30.24	380.75 ± 20.77	369.28 ± 26.88	333.12 ± 21.82	352.67 ± 21.06	260.95** ± 52.01
Relative ovary weight of maternal rat (g)	Left	0.018 ± 0.005	0.019 ± 0.006	0.018 ± 0.003	0.017 ± 0.004	0.018 ± 0.004	0.021 ± 0.006
		0.020 ± 0.000	0.020 ± 0.010	0.020 ± 0.010	0.020 ± 0.010	0.017 ± 0.006	0.021 ± 0.006
	Right	0.018 ± 0.005	0.019 ± 0.006	0.018 ± 0.003	0.017 ± 0.004	0.018 ± 0.004	0.021 ± 0.006
		0.020 ± 0.000	0.020 ± 0.010	0.020 ± 0.010	0.020 ± 0.010	0.017 ± 0.006	0.021 ± 0.006
No. of copora lutea		15.0 ± 2.4	15.0 ± 1.9	14.0 ± 1.4	15.0 ± 2.3	14.0 ± 2.0	15.0 ± 2.0
No. of implantations		13.0 ± 3.1	15.0 ± 1.7	13.0 ± 3	14.0 ± 2.6	13.0 ± 2.1	14.0 ± 3.9
Implantation % ^a		95.9	95.7	94.3	92.9	96.0	99.0
No. of resorptions		1.1 ± 1.3	0.9 ± 0.9	0.9 ± 0.9	1.7 ± 2.0	1.0 ± 1.3	7.7 ± 6.2
Resorption % ^b		8.2	5.9	6.3	12.3	6.8	51.0
No. of dead fetus		0.1 ± 0.2	0.0 ± 0.0	0.0 ± 0.0	0.3 ± 0.7	0.1 ± 0.3	0.4 ± 0.8
Dead fetuses % ^c		0.4	0.0	0.0	2.1	0.7	3.1
No. of live fetuses		12.3 ± 3.0	13.8 ± 1.6	12.3 ± 2.9	12.0 ± 3.1	12.2 ± 2.2	6.5 ± 5.0
Live fetuses % ^d		93.8	94.1	93.8	85.7	92.0	49.0
No. of sex	Male	6.1 ± 1.9	7.0 ± 2.4	5.6 ± 2.1	5.5 ± 2.1	6.5 ± 2.0	3.8 ± 2.8
	Female	5.2 ± 1.9	6.8 ± 1.8	6.7 ± 2.5	6.5 ± 2.3	5.7 ± 2.7	3.3 ± 2.7
Sex ratio (male/female)		1.1	1.2	0.9	1.0	1.6	1.3

^a: values are percent of No. of implantations per No. of copora lutea.

^b: values are percent of No. of resorptions per No. of implantations.

^c: values are percent of No. of dead fetuses per No. of implantations.

^d: values are percent of No. of live fetuses per No. of implantations.

Table 4. Effect of olaquinox and vitamin A on fetal and placental weight

Indices		Dose (mg/kg)					
		Vehicle	Olaquinox			Vitamin A	
			4	21	110	0.2	75
No. of dams		20	19	20	20	19	18
No. of fetuses observed		245	263	245	240	232	110
Mean fetal weight (g)	male	3.68 ± 0.29	3.76 ± 0.39	3.86 ± 0.33*	3.05 ± 0.33**	4.01 ± 0.33**	2.74 ± 0.66**
	female	3.49 ± 0.31	3.59 ± 0.36	3.65 ± 0.25*	2.86 ± 0.37**	3.82 ± 0.26**	2.60 ± 0.57**
Mean placenta weight (g)	male	0.54 ± 0.06	0.54 ± 0.04	0.54 ± 0.05	0.40 ± 0.08**	0.54 ± 0.06	0.54 ± 0.15
	female	0.52 ± 0.04	0.51 ± 0.04	0.51 ± 0.05	0.38 ± 0.08**	0.55 ± 0.07	0.54 ± 0.11

Values are represented as mean ± SD for No. of fetuses observed.

*, **, significantly different from control at $p < 0.05$ and $p < 0.01$, respectively.

Table 5. Effect of olaquinox and vitamin A on external, visceral and soft tissue development in fetal rats

(No. of fetuses)

Indices		Dose (mg/kg)					
		Vehicle	Olaquinox			Vitamin A	
			4	21	110	0.2	75
No. of fetuses observed		114	120	114	103	103	52
External anomaly		2	1	ND	7	ND	34
Bent tail		1	1	ND	3	ND	1
Craniorachschisis		ND	ND	ND	ND	ND	14
Exencephaly		ND	ND	ND	ND	ND	14
Dome shaped head		ND	ND	ND	ND	ND	22
Anophthalmia		ND	ND	ND	ND	ND	15
Brachygnathia		ND	ND	ND	ND	ND	10
Others		1	ND	ND	4	ND	19
External anomaly % ^a		1.8	0.8	0	6.8	0	55.7
Visceral anomaly		6	7	4	10	8	7
Dilatation of renal pelvis		3	4	2	6	4	4
Renal displacement		2	3	1	3	4	0
Dilatation of ureter		1	ND	1	2	ND	6
Others		ND	ND	ND	1	ND	2
Visceral anomaly rate %		5.3	5.8	3.5	9.7	7.8	13.5
Soft tissue anomaly		ND	1	ND	2	ND	45
Cleft palate		ND	ND	ND	ND	ND	42
Anophthalmia		ND	ND	ND	ND	ND	22
Dilatation of lateral ventricle		ND	1	ND	2	ND	ND
Others		ND	ND	ND	ND	ND	ND
Soft tissue anomaly %		0	0.8	0	1.9	0	86.5

^a, Values are percentage of No. of fetuses showing anomaly per No. of fetuses observed.

ND, anomaly not detected.

A (Table 3). Mean fetal weights were significantly ($p < 0.01$) increased by low dose but significantly ($p < 0.01$) decreased in rats treated with high dose of vitamin A. The placenta weight was not changed by the treatment of vitamin A (Table 4). In rats treated with vitamin A, 34 fetuses out of 52 showed external anomaly which were bent tail (1), cranioarchschisis (14), exencephaly (14), dome shaped head (22), anophthalmia (15), brachygnathia (10) and others (19). Visceral anomaly such as dilatation of ureter was increased by high dose of vitamin A, which were 6 out of 52 at high

group but 1 out of 114 fetuses in vehicle group. Forty five fetuses out of 52 showed soft tissue anomaly which were cleft palate (42/52) and anophthalmia (22/52) by high dose of vitamin A (Table 5). Sixty one fetuses out of 61 (85.2%) showed skull anomaly which were defect of frontal, partial and occipital bone (21/61), defect of palatine bone (52/61) and others (50/61). dilatation of lateral ventricle at high group (Table 5). The ossification of sternebrae and metacarpals were significantly ($p < 0.01$) increased by low dose but it was significantly ($p < 0.01$) prohibited by high dose of vita-

Table 6. Influence of olaquinox and vitamin A on skeletal development of fetal rats

(No. of fetuses)

Indices	Dose (mg/kg)					
	Vehicle	Olaquinox			Vitamin A	
		4	21	110	0.2	75
No. of observed fetus	131	149	131	120	117	61
Skull anomaly	ND	ND	ND	ND	ND	52
Defect of frontal, parietal, occipital bone	ND	ND	ND	ND	ND	21
Defect of palatine bone	ND	ND	ND	ND	ND	52
Others	ND	ND	ND	ND	ND	50
Anomaly %	0	0	0	0	0	85.2
Vertebrae anomaly	ND	ND	ND	ND	ND	7
Sternebrae	5.3 ± 0.8 ^a	5.5 ± 0.7**	5.7 ± 0.5**	4.8 ± 1.0**	5.7 ± 0.6**	4.3 ± 0.8**
Forelimb anomaly	ND	ND	ND	ND	ND	ND
Metacarpals	3.6 ± 0.5	3.8 ± 0.4**	3.8 ± 0.4**	3.3 ± 0.5**	3.9 ± 0.3**	3.4 ± 0.5**
Hindlimb anomaly	ND	ND	ND	ND	ND	ND

Values are given as mean ± SD for No. of fetuses observed.

ND, anomaly not detected.

^a, No. of ossified bones.*, **, significantly different from control at $p < 0.05$ and $p < 0.01$, respectively.

min A (Table 6).

DISCUSSION

Micromass assay using LB and CNS cell has a lot of advantages in that these cells are originated from rat embryo, and undergo the differentiation process like embryo, and time of differentiation is very similar to embryo. These cells were suggested as a predictive short term assay for the teratogenicity of chemicals (Flint, 1993). The overall predictive accuracy of the system was approximately 89%, and the false-negative rate was approximately 14.8% and no false positives were observed (Guntakatta, Matthews *et al.*, 1984).

In this experiment for olaquinox, IC_{50} values of proliferation and differentiation for CNS cell were 22.74 and 28.32 $\mu\text{g}/\text{ml}$, and 79.34 and 23.29 $\mu\text{g}/\text{ml}$ for LB cell, respectively. Compounds with IC_{50} below 50–75 $\mu\text{g}/\text{ml}$ on differentiation could be considered as potential of teratogenicity and 7–10 $\mu\text{g}/\text{ml}$ or less with a high solubility may indicate high teratogenic risk (Flint, 1986). *In vivo*, development toxicant produce adverse effects on the fetus at exposure levels that do not induce severe toxicity in the mother such as substantial reduction in weight gain. Strong teratogen has selective effect on inhibition cell differentiation at concentration lower than causing cytotoxicity. Based on this assumption, olaquinox could be classified as a compound having a potential of teratogenicity especially in limb bud because differentiation of LB cell was completely inhibited at non-cytotoxic concentration but differentiation in CNS cells was observed at cytotoxic concentration of olaquinox. Regarding vitamin A, IC_{50} values of proliferation and differentiation for CNS cell were 8.13 and 5.94 $\mu\text{g}/$

ml , and 0.81 and 0.05 $\mu\text{g}/\text{ml}$ for LB cell, respectively. Based on these data, vitamin A should be classified as a compound having a high teratogenic potential. Especially it would be a strong teratogen in LB cell in that the ratio of IC_{50} for cytotoxicity to IC_{50} for differentiation in LB cell about 16 but that in CNS was about 1.36. In the presence of active retinoids chondrogenesis is concentration-dependently inhibited (Kistler, 1987).

Retinoids is a well-known teratogen of limb-skeletal deletions, limb truncation and other skeletal malformations (Paulsen *et al.*, 1988). Retinoid bind to the cellular retinoic acid bind protein and control the cell differentiation and embryonic morphogenesis (Lehmann *et al.*, 1992; Willhite *et al.*, 1992; Soprano *et al.*, 1993). *In vivo* experiment 5 mg/kg of all-trans-retinoic acid was highly embryotoxic when administered subcutaneously, but not following oral administration. The most pronounced embryotoxic endpoints were embryolethality and defect of skull, and the thorax, and the sternum (Tzimas *et al.*, 1997). In this experiment, 56% of external anomaly such as craniorachschisis, exencephaly, dome shaped head, anophthalmia and brachygnathia and 87% of soft tissue anomaly such as cleft palate and anophthalmia were showed by high dose of vitamin A. High dose of vitamin A also produced 85% of skull anomaly and inhibited the ossifications of sternebrae and metacarpals. Conclusively our *in vivo* and *in vitro* data for vitamin A are very similar to previous results as a strong teratogen.

In NMRI mice, olaquinox (180 mg/kg) decreased the maternal body weight gain, without changing of the number of implantations, live fetuses, reabsorption and the incidence of malformations whereas rats given 180 mg/kg of olaquinox showed reduction in body weight gain, higher incidence of resorptions, lower numbers of live fetus and

reduction of fetal weights. Five fetuses out of 20 (25%) showed malformation at a dose level of 180 mg/kg (WHO, 1991). In this study, rats given 110 mg/kg showed lower mean body weight and increased rate of implantation and increased number of fetuses, but did not change in relative ovary weight, number of corpus lutea, and number of implantation. External anomaly such as bent tail was increased by 110 mg/kg of olaquinox. Dilatation of renal pelvis and lateral ventricle were shown at a dose level of 110 mg/kg. In summary, olaquinox showed 6.8% of external anomaly and 1.9% of soft tissue anomaly without any skeletal anomaly at a dose level of 110 mg/kg. Our present data suggest that a high dose of olaquinox could act as a teratogen in rats which might provoke external anomaly and delay of ossification of sternebrae and metacarpals. But in pork industry it is added to feed at a dose of 25~100 mg/kg in feed so that we could expect little teratogenic effect of olaquinox in the practical application.

In conclusion, this present study supports that vitamin A could be classified as strong teratogen in micromass and in vivo, and olaquinox has a weak teratogenic potential in LB cell but not in CNS cell. The present result provides in vivo evidence that high dose of olaquinox could have weak embryotoxic potential in rats.

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