Kor. J. Food Hygiene 5(4), 229-236 (1990)

Vitamin A의 최기형성에 미치는 Ethanol의 영향

조대현 · 진강 · 조태순*
국립보건안전연구원
*성균관대학교 약학대학

Effects of Ethanol on the Teratogenicity of Hypervitaminosis A in Rats

Dae-Hyun Cho, Kang Chin and Tae-Soon Cho*
National Institute of Safety Research, Seoul, 122-020, Korea.
**College of Pharmacy, Sung Kyun Kwan University*

ABSTRACT—The effects of ethanol (ET) on the teratogenicity of the fat soluble drug, Vitamin A (VA) were examined in SPF Wistar rats. VA and ET were orally administered with sonde. The drugs were administered for 3 days of day 9-11 of gestation.

Four groups were made; G-I control (sesame oil + saline), G-II VA 40×10^4 (I.U/kg/day), G-III ET 2 (g/kg/day), G-IV 40×10^4 + ET 2. Congenital malformations were found G-II, G-III and G-IV. All fetuses in G-IV combination had malforamtions. Main malformation in G-IV combination were microstomia, disposition of ear, open eyelids brachygnathia and cleft plate. Accordingly it might be demonstrated that the teratogenicity of hypervitaminosis A was pontentiated by concurrent ethanol in rats.

Keywords□Hypervitaminosis A, Ethanol, Teratogenicity, Rat.

INTRODUCTION

Vitamin A (VA), a drug for treatment of VA deficiency and its prophylactic administration during periods of increased requirement, such as infancy, pregnancy and lactation, is required for growth, especially of bone, reproduction, and embryonic development[1]. There are many reports on VA fetal toxicity and congenitial malformations in rats, mice and rabbits[2-6].

Numerous studies have described physical growth retardation and a pattern of craniofacial abnormalities (collectively termed fatal alcohol syndrome) in children born to women who had consumed large amounts of alcohol during pregnancy[7]. Although retarded growth and development have been reported in the progeny of alcohol-fed rats, mice, rabbits, and dogs[8-10], the evidence for a teratogenic action of alcohol in animals is more controversial. Some investigator have reported no malformations in fetuses of alcohol-treated animals[8, 9, 11], whereas others have reported a wide spectrum of craniofacial, limb, and other defects[12-15]. Some differences in results most likely reflect species and strain differences in susceptibility to alcohol, whereas others may be due to the different amounts of alcohol ad-

Received for Publication 27 November, 1990
Reprint request: Dr. D.H. Cho at the above address

ministered and to variety of routes and schedules of administraion.

However, it is also possible that some of the adverse effects of alcohol may be due to the synergism of other agents (nutritional and non-nutritional) with alcohol. Little attention has been given to the possibility that some features of the fetal alcohol syndrome may be due to such interaction.

Therefore, the study reported here was designed to investigate the possibility that alcohol consumtion during pregnancy can potentiated the recognized teratogenic agents.

In the present paper, we investigated the effects of ethanol on the teratogenicity of VA in rats.

MATERIALS AND METHODS

Animal and Drugs—SPF Wistar rats, about 14 weeks of age, raised in our vivarium (barrier system), were used in this study, Vitamin A (Retinol acetate, Roche) and ethanol (99.8% up, Merck) were used.

Experimental procedures—All animals were mated overnight by placing two females with one male in a cage. The morning upon which a vaginal plug was observed or sperms detected in the vaginal smear was designated day 0 of gestation. At that time the females were transferred to plastic cages and given commercial chow and tap water ad libitum until the day of sacrifice. Retinol acetate and ethanol were orally administered by stomach tube for 3 days of day 9-11 of gestation.

Retinol acetate was dissolved in sesame oil at a concentration of 40,000 IU/*ml* (dosage volume; 10 m*l*/kg). Ethanol was mixed in saline at a concentration 25 w/v% (dosage volume; 8 m*l*/kg).

Dams were devided into 4 groups—G-I Control (sesame oil+saline), G-II VA40 (Vitamin A 40×10^4 IU/kg), G-III E2 (ethanol 2g/kg), G-IV VA+E2 (Vitamin A 40×10^4 IU/kg+ethanol 2g/kg).

Examination—Dams were killed by exsanguination under ether anesthesia. The uterus was exposed and the number of inplants, intrauterine death and living fetuses were recorded. Living fetuses were removed from the uterus, examined for the external malformations and weighed. Fetal body weight was analyzed by students's t-test to compare the mean of each group with the mean of control group, VA alone group.

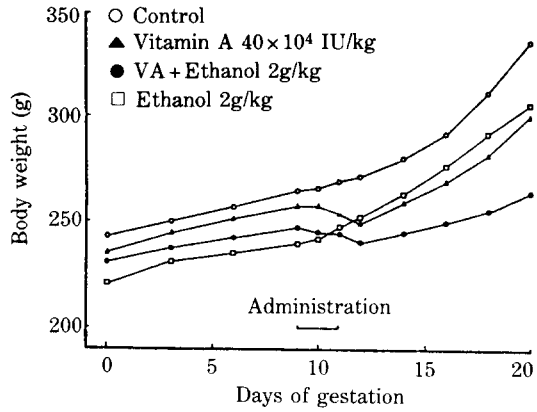

Fig. 1. Effect of treatment on body weight changes of pregnant rats.

The embryolethality was represented by precentage of dead implants, which was calculated from (the numbers of intrauterine death/the number of implant)×100. The average percentage of dead implants of each group was the sum of the percentage of dead implants divided by the numbers of litters. The statistical analysis was performed by wilcoxon's rank sum test to compare the percentage of dead implants and incidence of malformed fetuses of treatment groups with one of control group, VA alone group.

RESULTS

The body weight change during gestation was shown in Fig. 1. The maternal weight gain was significant suppressed after the first administration of G-II (VA 40×10^4 IU/kg), G-IV (VA 40×10^4 IU/kg+E 2g/kg) and a significant inhibition was still observed on day 20 of gestation in G-IV ($p<0.05$, $p<0.01$).

Table 1 showed treatment effects on reproductive performance of pregnant rats. In an analysis of variance and covariance, no significant differ-

Table 1. Effect of treatment on reproductive performance of pergnant rats.

Treatment group	Control	VA 40	E2	VA+E2
No. of dams	13	12	12	12
No. of dead dams	0	0	0	0
(Mortality %)	(0)	(0)	(0)	(0)
No. of dams with live fetuses	13	9	12	6
No. of dams with totally dead fetuses	0	13	0	6
No. of corpora	216	188	168	203
lutea (mean±S.D.)	(16.6±1.6)	(15.7±1.7)	(14.0±1.3)*	(16.9±2.9)
No. of implants	196	167	162	162
(mean±S.D.)	(15.1±2.5)	(13.9±2.0)	(13.5±1.3)	(13.5±2.1)
Implantation ratio (%)	91.3±8.3	89.0±9.6	96.3±5.9	81.3±16.1

* $p<0.05$: Significant difference from control

Table 2. Effect of treatment on fetal development of pregnant rat

Treatment group	Control	VA 40	E2	VA+E2
No. of dams	13	12	12	12
No. of implants	196	167	162	162
No. of lieve fetuses	171	93	145	40
(mean±S.D.)	(13.2±2.9)	(7.8±6.4)**	(12.1±1.1)	(3.3±4.0)**
Sex ratio (male/female)	91/80	51/42	66/79	25/15
Body weight (g)				
male (M±S.D.)	3.03±0.18	2.92±0.68	2.64±0.22*	2.42±0.43**+
female (M±S.D.)	2.89±0.21	2.67±0.68	2.45±0.25*	2.15±0.41**+
Placental weight (g) (mean±S.D.)	0.43±0.04	0.43±0.06	0.42±0.06	0.42±0.08
No. of dead implants	25	74	17	122
Early death	20	17	14	58
Late death	5	50	3	64
Mortality (%)	12.0	47.6**	10.4	74.8**+

*; $p<0.05$ **; $p<$: Significant difference from control

+; $p<0.05$, ++; $p<0.01$: Significant difference from vitamin A (VA 40) alone

ence was found in the number of implants and implantation ratio among treatment groups, but significant difference of the number of corpora lutea were found control and E2 group ($p<0.05$).

Table 2 showed the number of live fetuses, the fetal body weight and the average percentage of dead implants significant difference of the average number of live fetuses were found between control and VA 40, VA+E2 ($p<0.01$). Fetal body weight was significantly low in E2 ($p<0.05$), VA+E2 ($p<0.01$) against control and in VA+E2 ($p<0.05$) against VA 40, but not singificant different between control and VA 40. Singificant difference of the average percentage of the dead

Table 3. Effect of treatment on external malformations

Treatment group	Control	VA 40	E2	VA+E2
No. of dams examined	13	9	12	6
No. of fetuses examined	171	93	145	40
No. of fetuses with external malformations (%)	2 (1.0)	88 (94.8)	39 (29.2)	40 (100)
Exencephaly	0	3 (13.0)	0	2 (6.7)
Encephalocele	0	8 (7.4)	0	1 (3.3)
Microtia	0	22 (35.9)	0	12 (38.2)
Accessory auricle	0	32 (40.7)	0	33 (76.8)**
Disposition of ear	0	34 (61.6)	0	40 (100)**
Open eyelids	0	55 (65.1)	0	31 (75.6)*
Exophthalmia	0	31 (43.9)	0	19 (48.2)
Microstomia	0	59 (53.7)	0	36 (85.0)**
Pseudomacroglossia	0	14 (24.6)	0	11 (25.1)
Brachygnathia	0	2 (14.8)	0	19 (50.8)**
Mandibular fissure	0	1 (3.7)	0	5 (14.5)*
Cleft palate	0	78 (87.5)	0	15 (33.3)**
Hypoplastic palate	0	0	0	18 (33.3)
Anury	1 (0.5)	0	0	0
Brachyury	0	0	0	8 (18.5)
Kinky tail	1 (0.5)	1 (0.9)	36 (26.9)	16 (41.8)**
Hematoma	0	2 (1.9)	8 (6.1)	4 (13.3)

Occurence rate (%) in parentheses is the average of the incidence in each litter
*; $p<0.05$, **; $p<0.01$: Significant difference from vitamin A (VA 40) alone

Fig. 2. Vitamin A + ethanol-treated 20-day-old Wistar rat fetus with disposition of ear and accessoty auricle.

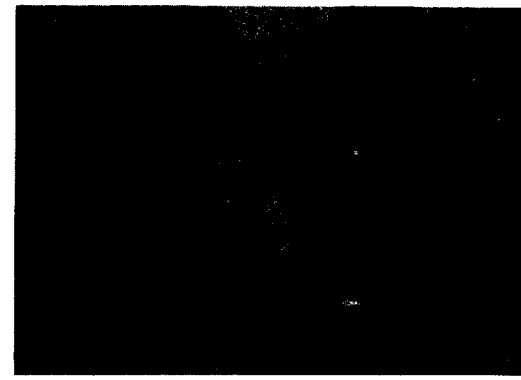

Fig. 3. Vitamin A + ethanol-treated 20-day-old Wistar rat fetuses with microstomia. Note exophthalmia (right) in affected fetus.

implants was high in VA 40 ($p<0.01$) and VA 40+E2 ($p<0.01$) against control and in VA+E2 ($p<0.05$) against VA 40.

As shown in Table 3, the incidence of malformation were in 100% in VA+E2, whereas the incidence were 94.8% in VA 40, 29.2% in E2, and 1.0% in control. The most frequent external malformation of VA+E2 were disposition of ear

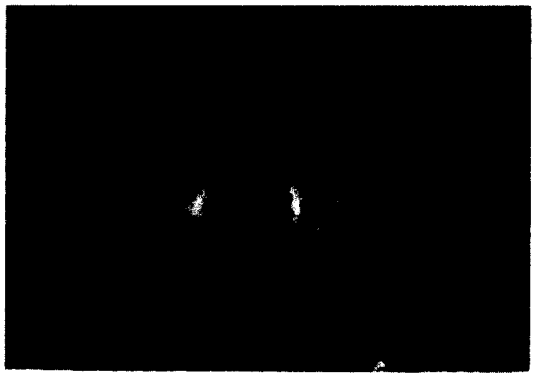

Fig. 4. Vitamin A + ethanol-treated 20-day-old Wistar rat fetus with open eyelids and encephalocele.

Fig. 5. Vitamin A + ethanol-treated 20-day-old Wistar rat fetus with brachygnathia. Note pseudomacroglossia, exencephaly and exophthalmia in affected fetus.

(100%) (Fig. 2), microstomia (85.0%) (Fig. 3), accessory auricle (76.8%) (Fig. 2), open eyelids (15.6%) (Fig. 4) and brachygnathia (50.8%) (Fig. 5).

The others were kinky tail cleft, palate exophthalmia and so on. Signififcant difference were found in the incidences and types of malformation between VA 40 and VA + E2.

DISCUSSION

The present result suggests that the teratogenic effect of vitamin A (retinol acetate) was potentiated by concurrent ethanol. No report have described the effect of ethanol on fetal toxicity of vitamin A.

Excessive intake of vitamin A results in c ongenital malformation in many speces if animals[2-6]. But, the mechanism of action of excess vitamin A in the experimental production of teratogenic effects is not yet understood. Intraamniotic administration of vitamin A[16] or *in vitro* treatment of rat embryos with retinoic acid[17] produced the same type of malformations as *in vivo* treatment[18]. From this, the conclusion was drawn that excess vitamin A acts directly on the embryo.

The effects of vitamin A on DNA, protein, glyoprotein, and sulfated mucopolysaccharide synthesis has been investigated in fetuses[19, 20], but no convincing evidence was reported that those parameters directly induced the aberrations of development. On the other hand, there is evidence that retinoic acid may act like a hormone[21].

Under the conditions employed in these experimennts, alcohol induced in the incidence of kinky tail, hematoma, although others have reported a cleft palate of alcohol in mice[12]. The most striking effect of alcohol consumption during gestation in experimental animals is the reduction in fetal weight. It is possible that this alteration in growth may have an adverse effect of the various anomalies. However, alcohol alone did not cause the various malformations, despite the marked reduction in fetal weight, and vitamin A did not further reduce fetal weight when compared to alcohol alone, although they induced a significfant incidence of malformation. Another possible mechanism of action jof alcohol may be thought it retardation of fetal development.

The manner in which alcohol potentiates vitamin A teratogenicity is not clear. Therefore, the observation that alcohol consumption potentiates teratogenic effects (the incidences of dead and abnormal fetuses) of vitamin A is of importance. An analog of vitamin A (13-cis-retinoic acid) has become a popular agent for the treatment of acne and is prescribed in relatively large daily doses, several cases of congenital malformations, including microstomia, cleft palate have been reported infants born to women who had used this vitamin analog during early stages of pregnancy[22, 23], and

concern has been expressed regarding its use by teenagers who may become pregnant.

If these individuals also consume alcohol, the data presented here suggest that the alcohol may act to potentiate the effects of 13-cis-retinoic acid.

Threshold level of potentiation of vitamin A teratogenicity by ethanol is currently under investigation.

CONCLUSION

The effects of ethanol (ET) on the teratogenicity of the fat soluble drug vitamin A (VA) were examined in SPF Wistar rats. VA and ET were orally administered with stomach tube. The drugs were administered for 3 days of day 9-11 of gestation.

Four groups were made; G-I control (sesame oil + saline), G-II VA 40×10^4 (IU/kg/day), G-III E2 (g/kg/day), G-IV VA 40×10^4 + E2, Congenital malformations were found G-II, G-III and G-IV. All fetuses in G-IV combination had malformations. Main malformation in G-IV combination were microstomia, disposition of ear, open eyelids, brachygnathia, abd cleft palate.

Accordingly it might be demonstrated that the teratogenicity of hypervitaminosis A was pontentiated by concurrent ethanol in rats.

국문요약

SPF Wistar계 랫트를 이용하여 Vitamin A(VA)의 과잉투여(40×10^4IU/kg)에 의한 최기형성에 대하여 ethanol(E) 2g/kg을 임신 9일부터 임신 11일까지 3일간 경구로 병용투여한 결과 다음과 같다.

1. 임신모체의 영향에 있어서 VA×E 병용투여군이 대조군 및 VA 단독투여군에 비해 유의한 체중증가의 억제를 보였다.

2. 태자사망율은 VA+E 병용투여군이 VA 단독투여군에 비해 유의하게 증가되었고($p<0.05$), 태자의 평균 체중은 VA+E 병용투여군이 대조군에 비해 유의하게 감소되었으며($p<0.01$), VA 단독투여군과의 비교에서도 유의한 감소를 보였다($p<0.05$).

3. 외표기형의 발생율은 VA 단독투여군에 비해 VA+E 병용투여군이 증가되어 나타났다. 발현된 기형의 특징은 귀위치 이상, 부이(복이), 안검개존, 소구증, 단악증, 구개열 등이다.

이상의 경과에서 Vitamin A의 과잉투여에 의한 최기형성에 ethanol이 상승적으로 작용함을 알 수 있었다.

REFERENCES

1. Wasserman, R.H. and Corradino, R.H.: Metabolic role of vitamin A AND D.A. *Rev. Biochem,* **40,** 501-532 (1971).
2. Cohlan, S.Q., Excessive intake of vitamin A as a cause of congestinal anomalies in the rat. *Science* **117,** 535-536 (1953).
3. Giroud, A. et Martinet, M., Teratogenese Par hypervitaminose A Chez la rat, la Souis, le cobaye et le lapin. *Arch. Franc. Pediot.* **16,** 1-5 (1959).
4. Kalter, H. and Warkany, J., Experimental Procuction of congenital malformation in stratins of Inbred mice by maternal treatment with hypervitaminosis A. *Amer. J. Pathol.* **38,** 1-21, (1961).
5. Hayashi, Y., Malformations of the ear in teh mouse fetus caused by meternal hypervitaminosis A during pergnancy, *Cong. Anom.* **12**(12), 73-80 (1972).
6. Kistler, A., Teratogenesis of retinotic acid in rats, *Teratology* **23,** 25-31 (1981).
7. Streissguth A.P., Landesman-Dwyer S., Martin J.C., Smith D.W., Teratogenic effects of alcohol in humans and laboratoty animals. *Science* **209,** 353-361 (1980).
8. Lee M., Leichter, J., Effect of matenal thanol adminstration on physical growth of the offspring in rats. *Growth* **43,** 288-297 (1978).

9. Schwetz, B.A., Smith, F.A., Staples R.E., Teratogenic potenialof ethanol in mice, rat and rabbits, *Teratology* **18,** 385-392 (1978)
10. Ellis, F.W., Pik J.R., An animal model of the fettal alcohol syndrome in Beagles. *Alcoholism clin Exp* **4,** 123-134 (1980).
11. Hood, R.D., Lary J.M., Balacklock, J.B., Lack of prenatal effects of maternal ethanol eonsumption in CD-1 mice. *Toxicol. Lett* **4,** 79-82 (1979).
12. Chernoff, G.F., The fetal alcohol syndrome in mice: An animal model. *Teratology* **15,** 223-230 (1977).
13. Kronik, J.B., Teratogenic effects of ethyl alcohol adminstered to pregnant mice. *Am. J. Obstet. Gynecol.* **124,** 676-680 (1976).
14. Randall, C.R., Taylor W,J., Prenetal ethanol exposure in mice: teratognic effects. *Teratology* **19,** 305-312 (1979).
15. West, J.R., Black, A.C., Reimann, P.C., Alkana, R.L., Polydactyly and Polysydactyly induced by prenetal exposure to alcohol. *Teratology* **24,** 13-18 (1981).
16. Nanda, R. and D. Rome: Effect of intraamniotic administration of vitamin A on rat fetueses. *Teratology,* **16,** 35-40 (1977).
17. Morriss, G.M. and C.E. Steele: The effect of excesss vitamin A on the development of rat embryos in culture. *J. Embryol. Exp. Morphol.* **32,** 505-514 (1974).
18. Geelen, J.A.,G.: Hupervitaminosis a induced teratogenesis. *Critical Rev. Toxicol.,* **6,** 351-375 (1979).
19. Lorente, C.A. and S.A. Miller: The effect of hyperviatminosis A on rat palatal development. *Teratology,* **18,** 277-284 (1978).
20. Eckhert, C.D. and L.S. Hurley: Influence of various levels of hypervitaminosis A and Zinc deficiency on teratogenesis and DNA synthesis in the rat. *Teratology,* **19,** 279-284 (1979).
21. Takase, S., D.E. Ong and F. Chytil: Cellular retinol binding protein allows specific interation of retinol with the nucleus *in Vitro. Proc. Natl. Acad. Sci. USA.,* **76,** 2204-2208 (1979).
22. Fernoff, P.M., Lammer, E.J.: Craniofacial features of isotretinoin embryopathy, *J. Pediatr* **105,** 595-597 (1984).
23. Lott, I.T., Bocian, M. Pribram, H.W.: Fetal hydrocephalus and ear anomalies associated with maternal use of isotretinoin. *J. Pediatr* **1,** 597-600 (1984).