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Embryotoxic effects of DA-125, a new anthracycline anticancer agent, in rats

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새로운 안트라사이클린계 항암제 DA-125의 랫트에 있어서 태자독성효과

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초 록 : DA-125는 새로운 안트라사이클린계 항암성 항생제로서 아드리아마이신의 유도체이다. Sprague-Dawley 랫트를 이용하여 DA-125의 배아 및 태자독성발현능력을 조사하였다. 교미확인(정충확인일=0일)된 120마리의 랫트를 4개군으로 나눈 후 0, 0.1, 0.3 및 1.0mg/kg의 용량으로 임신 7일 부터 임신 17일까지 1일 1회 연속 정맥투여 하였으며 임신 20일째에 제왕절개를 하여 태자를 적출하였다. 1mg/kg 투여군에서는 모동물의 사료섭취량의 감소, 체중감소 및 비장중량의 감소와 배아 흡수율의 증가 및 태자체중의 감소가 관찰되었다. 또한 여러가지 종류의 외표, 내부장기 및 골격기형들이 각각 11.9, 41.8 및 14.5%의 빈도로 출현했다. 그중 특이 기형소견으로는 뇌탈출증, 복벽파열, 외측 및 제3뇌실의 확장, 늑골유착 등을 들 수 있다. 0.1 및 0.3mg/kg 투여군에서는 어떠한 배아 및 태자 독성증상도 나타나지 않았다. 이상의 결과에서 DA-125는 랫트에 있어서 경미한 모독성 용량에서 배아 및 태자독성효과를 나타냄을 알 수 있었다.

Key words : DA-125, antitumor antibiotic, embryotoxicity study, rats

Introduction

Adriamycin is a widely used and highly valued antineoplastic agent, but its chronic treatment is limited by cardiotoxicity[1,2]. In recent years attempts have been made by researchers to develop new anticancer drugs, which has less side effects than existing ones.

DA-125 is a new anthracycline antitumor antibiotic, which is derived from adriamycin. It was currently synthesized by Dong-A pharmaceutical company. The mechanism of action for this new drug might lie in their ability to inhibit nucleic acid synthesis through intercalation with DNA[3]. Preclinical studies suggest that it may have greater pharmacological activity and less cardiac toxicity and skin irritation than adriamycin[4,5].

It is well known that anticancer drugs are teratogenic or embryotoxic because of their unspecific attack on embryo or fetus showing a very high cell proliferation rate. This study was performed to evaluate the potential of DA-125 to induce fetal dysmorphogenesis, prenatal mortality and intrauterine

growth retardation in the SD rat.

Materials and Methods

Animal maintenance and mating procedure: Sprague-Dawley rats (Korea Research Institute of Chemical Technology, Toxicology Center Breeding Facility) were kept under SPF(specific pathogen free) conditions at a constant day/night cycle (light; 7 to 19 O'clock). Standard laboratory rodent diet(Jeil Fed Co., Daejeon, Korea) and sterilized water were available ad libitum. For mating two females were placed into the cage of one male overnight and the first 24h period following the mating procedure was designated as day 0 of pregnancy when sperms were detected.

Test substance:DA-125 was supplied by Dong-A pharmaceutical Co.(47-1 Sanggal-ri, Kiheung-up, Yongin-gun, Kyunggi-do, South Korea). It has chemical structure of 7-0-(2, 6-dideoxy-2-fluoro-a-talopyranosyl)-adriamycinone-14-b-alaniate HCl and has a molecular weight of 670. DA-125 was dissolved in a solution of 1 mMol lactic acid (in saline, pH 4.0) before administration.

Drug treatment : The application volume was calculated according to the body weight on day 7,10 and 14 of gestation. DA-125 was administered intravenously in the tail vein of rats from day 7 to 17 of gestation.

Experimental groups : Table 1 shows the experimental design of the embryotoxicity study with DA-125 carried out on rats.

Foundation for dosages : Dosages of 0.5, 1.0, 1.5 and 2.0mg/kg were given in a intravenous pilot study to 5 pregnant dams per group. The dose of 0.5mg/kg was well tolerated. At 1.0mg/kg a slight reduction in body weight of dams was observed, while at 1.5 and 2.0mg/kg, the embryolethality was very high(81 and 100%, respectively) and the depression of body weight of dams was extreme. Based on these results, 1mg/kg was selected for the high dose.

Observation of dams : Food consumption, body weight change, and signs of intoxication were examined in the pregnant femals. They were subjected to autopsy at the end of pregnancy and the following organ weights were measured : brain, adrenal gland, liver, spleen, kidney, heart, and ovary.

Caesarian section on day 20 of gestation : On day 20 of gestation all pregnant females were sacrificed by carbon dioxide. The implantation sites, corpora lutea, living fetuses, dead fetuses and resorptions were numbered and registered. All living fetuses were weighed, sexed and evaluated for externally visible abnormalities. Alternate fetuses were selected for either skeletal or visceral examination. The evaluation of skeletal abnormalities was performed after clearing 95% ethanol-fixed fetuses with KOH, staining the skeleton with alizarin red, and dyeing the cartilage with alcian blue[6,7]. Alizarin red stains the calcified bone anlagen[8]. For the visceral examination of Bouin's fluid-fixed fetuses, we adapted Wilson's technique[9] for the head and abdomen, and Nishimura's method[10] for the thorax.

Statistical analylsis of data : The variables such as body weight of dams, implantation rate, litter size, and number of live fetuses were evaluated by Dunnett's[11] or Scheff's[12] test. Fetal deaths were analysed by Kruskal-Wallis test. The sex ratio were analysed using a X^2-test. Fetal weights were analysed separately for each sex using Dunnett's or Scheffe's test. In addition, the incidence of external, visceral and skeletal malformations in fetuses was recorded in percents. A difference was considered statistically significant at $p<0.05$. No letter effect was observed.

Table 1. Experimenral design for embryotoxicity study of DA-125 in rats.

Group	Dose (mg/kg/day)	Volume (ml/kg)	No. of dams
Control	0	1	23
DA-125	0.1	1	25
	0.3	1	28
	1.0	1	24

Results

Effect on dams : No notable changes in behaviour or clinical signs were observed in dams of any group. There were no significant differences in the food consumption of pregnant animals, except that dams of 1mg/kg group consumed significantly less diet on day 8 of gestation. The body weight development of 0.1 and 0.3mg/kg group compared well with the control. At 1mg/kg, the body weight of day 14, 17 and 20 of gestation was significantly low. At autopsy of dams, no treatment related pathologic findings were observed in dams of any group, except that at 1mg/kg, both a decrease in the weight of spleen and

Table 2. Relative organ weights of dams treated with DA-125

Dose(mg/kg)	0	0.1	0.3	1.0
No. of animals	23	25	28	24
Body weight(g)	355.3±34.11	358.5±29.10	349.5±30.78	322.4±23.00
% Body weight				
Brain(g)	0.525±0.052	0.520±0.040	0.533±0.040	0.578±0.043*
Adrenal gland-left (g)	0.009±0.001	0.009±0.002	0.008±0.001	0.009±0.002
Adrenal gland-right (g)	0.008±0.001	0.008±0.001	0.008±0.001	0.008±0.002
Liver(g)	4.084±0.371	4.144±0.277	4.043±0.365	4.011±0.290
Spleen (g)	0.186±0.022	0.179±0.024	0.171±0.024	0.154±0.028**
Kidney-left (g)	0.288±0.038	0.275±0.028	0.283±0.039	0.298±0.041
Kidney-right (g)	0.297±0.038	0.284±0.030	0.289±0.034	0.304±0.044
Heart (g)	0.262±0.028	0.255±0.022	0.265±0.029	0.264±0.025
Ovary-left (g)	0.015±0.004	0.014±0.003	0.015±0.004	0.014±0.003
Ovary-right (g)	0.015±0.004	0.016±0.005	0.015±0.003	0.016±0.003

Values are Mean±S.D.

*and** indicate significant difference at $p<0.05$ and $p<0.01$ levels when compared with control group.

Table 3. Caesarean section data of dams treated with DA-125

Dose(mg/kg)	0	0.1	0.3	1.0
No. of pregnant animals	23	25	28	24
Corpora lutea(Mean±S.D.)	14.83±3.33	15.08±2.56	15.25±2.03	15.21±2.30
Implantations(Mean±S.D.)	12.35±4.76	13.00±3.11	12.36±4.13	11.88±4.82
% to corpora lutea : Mean±S.D.	80.44±22.67	86.20±13.29	80.49±22.58	76.18±24.97
Fetal deaths(resorptions+dead fetuses)	30	29	31	50
Resorptions	30	29	31	48
Early	29	27	28	34
Late	1	2	3	14**
Dead fetuses	0	0	0	2
Live fetuses				
Male/Female	135/119	140/156	169/146	127/108
Litter size(Mean±S.D.)	11.04±4.82	11.84±3.21	11.25±3.93	9.79±4.68
% to implantation : Mean±S.D.	89.09±18.19	91.06±10.72	90.11±10.72	81.18±21.14
Sex ratio(male/female)	1.13	0.90	1.16	1.18
No. of fetuses with external anomalies(%)	3[a](1.2)	3[b](1.0)	4[c](1.3)	28[d](11.9)
Body weight of live fetuses				
Male(Mean±S.D.)	3.75±1.06	3.54±0.84	3.64±0.84	2.99±0.83**
Female(Mean±S.D.)	3.57±0.99	3.40±0.77	3.50±0.71	2.86±0.64**

a) acaudate, agnathia, exencephaly, open eye, protruded tongue

b) acaudate, short snout, short tail

c) micrognathia, open eye, protruded tongue, short snout

d) agnathia, anophthalmia, cleft lip, club foot, crooked snout, curvature of thoracic vertebrae. dome-shaped head. edema, exencephaly, exopthalmus, gastroschisis, hematoma, micrognathia, protruded tongue, short snout

** indicate significant difference at $p<0.01$ levels when compared with control group.

an increase in the weight of brain were seen(Table 2).

Effect of fetuses : Significant differences were observed in late resorptions and fetal weight of 1mg/kg group, compared with those of control (Table 3). Fetuses of 1mg/kg group showed external malformations(11.9%). These include exencephalia, protruded tongue, gastroschisis (Photo 1), cleft lip, edema, club foot (Photo 2), agnathia (Photo 3), among others. Fascial abnormalities were dominating (81.6%). A very low incidence of malformations was observed in the fetuses of the other groups. Micrognathia and exophthalmus (Photo 4) occurred in a fetus of 0.3mg/kg group. The results of visceral examination of the fetuses are shown in Table 4. At 1mg/kg, malformed fetuses exhibit only facial and ocular abnormalities, such as micropthalmia, dilatation of lateral and 3rd ventricle, agenesis of palate etc. at a frequency of 41.4%. No malformations were observed in the fetuses of the other groups, except that at 0.3mg/kg, a multiple abnormality(aglossia and agenesis of palate) occurred. Dilatation of the renal pelvis and ureter was observed in all groups. The results of

Table 4. Visceral findings in fetuses from dams treated with DA-125

Dose(mg/kg)	0	0.1	0.3	1.0
No. of dams	21	25	27	22
No. of fetuses examined (Mean±S.D.)	120(5.7±1.9)	142(5.7±1.6)	152(5.6±2.0)	111(5.0±2.0)
No. of fetuses with malformations(%)	0	0	1[a](0.7)	48[b](41.4)
No. of fetuses with variations(%)	7(5.8)	4(2.8)	13(8.6)	8(7.2)
Dilatation of renal pelvis	5	3	1	1
Dilatation of ureter	1	0	8	7
Dilatation of renal pelvis and ureter	1	1	4	0

[a] Aglossi and agenesis of palate

[b] Anophthalmia, microphthalmia, dilatation of lateral ventricle, dilatation of lateral and 3rd ventricle and agenesis of palate

Table 5. Skeletal findings in fetuses from dams treated with DA-125

Dose(mg/kg)	0	0.1	0.3	1.0
No. of dams	23	25	28	24
No. of fetuses examined (Mean±S.D.)	130(5.8±2.4)	154(6.2±1.6)	163(5.8±2.0)	124(5.2±2.3)
No. of fetuses with malformations(%)	4(3.0)[a]	7(4.5)[b]	3(1.8)[c]	18(14.5)[d]
No. of fetuses with variations(%)	5(3.7)	7(4.5)	13(8.0)	47(37.9)[e]
No. of fetuses with retardations(%)	8(6.0)	5(3.2)	18(11.0)	49(39.5)[f]
No. of ossification centers				
No. of sternebrae	4.2±1.9	4.7±0.9	4.8±0.9	3.9±1.7
No. of metacarpals in both forelimbs	5.9±2.5	6.7±0.9	6.9±0.9	6.2±1.7
No. of lst & 2nd phalanges in both forelimbs	1.7±2.8	1.7±3.2	1.4±2.7	1.6±2.9
No. of 3rd phalanges in both forelimbs	2.6±3.6	2.2±3.3	2.0±3.2	1.9±3.3
No. of metatarsals in both hindlimbs	7.1±2.9	8.0±0.7	8.0±0.7	7.1±2.3
No. of 1st & 2nd phalanges in both hindlimbs	0.6±1.1	0.8±1.8	0.7±1.6	0.7±1.5
No. of 3rd phalanges in both hindlimbs	2.4±3.5	1.7±3.1	1.8±3.1	1.7±3.1
No. of sacral and caudal vertebrae	7.6±2.5	7.7±1.7	7.8±1.6	6.8±2.3

[a] Fused ribs, shortened 13th rib, shortened mandibula, incomplete ossification of cranial bones, absence of lumbar vertebral body

[b] Shortened 13th rib, incomplete ossification of cranial bones

[c] Shortened 13th rib

[d] Fused ribs, flying rib, bifurcated rib, shortened 13th rib, sternal cleft, shortened mandibula, absence of thoracic vertebral body, incomplete ossification of cranial bones

[e] 14th rib, cervical rib, asymmetric sternebrae, lumbarization of sacral vertebrae

[f] Enlarged fontanelle, cleaved sternebrae, cleaved thoracic and/or lumbar vertebral body, dumbbell-shaped thoracic vertebral body, abscence of lumbar vertebral arch.

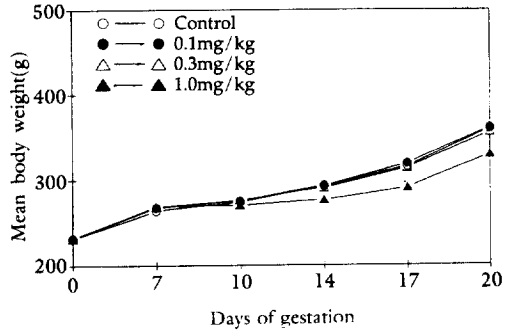

Fig 1. Mean body weight changes during gestation of dams treated with DA-125

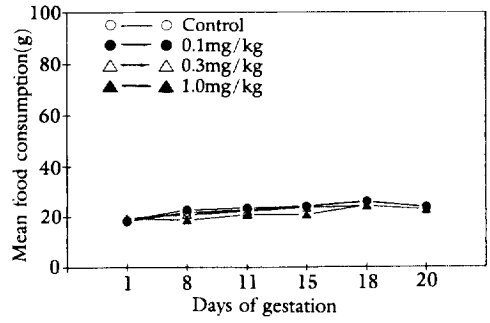

Fig 2. Mean food consumption during gestation of dams treated with DA-125

skeletal examination of the fetuses are shown in Table 5. A high incidence of malformations(14.5%), variations(37.9%) and retardations(39.5%) were observed in the fetuses of 1mg/kg group. Rib abnormalities occurred predominantly (68.2%). A low frequency of malformations, variations and retardations was seen in the fetuses of the other groups. The rate of ossification of evaluated skeletal districts compared well between the groups.

Discussion

Although 0.1 and 0.3mg DA-125/kg had no significant effect on the dams and embryonal development, there were signs of slight maternal toxicity and embrytoxicity at dose level of 1mg/kg. The signs of embryotoxicity concerned increased resorption rate, decreased fetal weight and high incidence of various types of external, visceral and skeletal malformations.

An increase in resorption rate must be called embryocidal effect. A reduction in fetal weight has to be classified as a sign of intrauterine growth retardation. Structural deviations of fetuses found at 1mg/kg can be divided into malformations, retardations or variations, which also occurr in the controls at a lower frequency. The dominating sings of abnormal development observed at 1mg/kg concerned fascial, ocular and rib abnormalities. Indications of retarded ossification, e.g. enlarged fontanelle, dumbbell-shaped thoracic vertebral body, correlated with the finding that fetal weight was reduced. Variations of fetuses found in this study are common and known for the Sprague-Dawley rat[13,14]. The high incidence of variations at 1mg/kg group was of interest.

The embryotoxic effects observed were regarded as the result of a direct or an indirect action(e.g. maternal toxicity), but it was not possible to decide which finding had been indirectly induced. Perhaps in vitro study can help to solve this problem.

From the results mentioned above, it can be concluded that the test agent DA-125 is embryotoxic at maternally subtoxic dose, i.e.at 1mg/kg.

Kurebe et al[15] reported that tetrahydropyranyladriamycin(THP-adriamycin), an anthracycline analogue, caused the embryotoxicity similar to that of DA-125, when given to the rats at dose level of 0.3mg/kg on days 7-17 of gestation. A decrease in body weight of dams and fetuses, a high rate of fetal deaths and an increase in the number of lumbar vertebrate were observed by these experimenters.

Thompson et al reported that adriamycin is teratogenic in rats at 1-2mg/kg, but in contrast to DA-125, it produced visceral anomalies, e.g. esophageal and intestinal atresia, when administered on day 6-15. The embryotoxic effects of adriamycin in rat embryos in vitro, which include malformations involving with the prosencephalic region and embryolethality, were also reported by Fantel et al and Barber and Fantel[16].

Summary

DA-125 is a new anthracycline antitumor antibiotic, which is derived from adriamycin. The potential of DA-125 to induce embryotoxicity was evaluated in the Sprague-Dawley rats. One hundred twenty naturally mated SD rats(sperm in vaginal lavage=day 0) were distributed among three treated groups and a control group. DA-125 was administered intravenously at dose levels of 0. 0.1, 0.3 and 1.0mg/kg/day. Dams were treated from day 7 to 17 of gestation and were subjected to the caesarean section on day 20.

At 1mg/kg, reduced food intake, reduced body weight and decreased weight of spleen were observed in dams. An increase in the resorption rate and a reduction in the fetal weight were also found. In addition, various types of external, visceral and skeletal malformations occurred at an incidence of 11.9, 41.8 and 14.5%, respectively. Characteristic malformations include exencephalia, gastroschisis, cleft lip, dilatation of lateral and 3rd ventricle, fused ribs, among others. There were no signs of maternal toxicity or embryotoxicity at 0.1 and 0.3mg/kg.

The results show that the test agent DA-125 is embryotoxic at maternally subtoxic dose in rats.

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Legends for figures

Photo 1. Exencephalia, protruded tongue, and gastroschisis in a fetus of 1mg/kg group.
Photo 2. Cleft lip, edema and club foot in a fetus of 1mg/kg group.
Photo 3. Agnathia in a fetus of 1mg/kg group.
Photo 4. Micrognathia and exophthalmus in a fetus of 0.3mg/kg group.

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