Sex of Mouse (CDI) Offspring not a Factor in Hydrocortisone Induced Cleft Palate

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Sex differences in the incidence of cleft palate, in man, have been reported repeatedly. Most studies indicate that a higher frequency of cleft palate is found in females (*6, 8, 15*). It has been suggested by Meskin, Pruzansky and Gullen (*15*) that the palatal shelves of female human embryos fuse later in development than males and for this reason females would exhibit a longer period of teratogenic susceptibility or potential disruption of embryonic development.

It was also shown by Burdi and Silvey (*2, 3*) that the human male embryo is more advanced than the female in palatal closure and that both profile reversal and reorientation of the palatal shelves occurs earlier in males than in females. Experimentally induced cleft palate in mice has yielded conflicting reports. Dagg, Schlager and Doerr (*4*) found that mice of the 129/Dg and BALB/tDg strains treated with 5-fluorouracil during pregnancy showed a greater incidence of cleft palate in female offspring than in males. Gebhardt and Shade (*7*) using X-ray, dexamethasone and 6-aminonicotinamide as teratogenic agents observed no sex differences in the incidence of induced cleft palate in Swiss albino mice. Waterman, Meskin and Shapiro (*17*) found that if A/Jax mice were airshipped on the 12th or 13th day of pregnancy, there was a significant increase in the ratio of female offspring with cleft palate. Air shipment on days 14 and 15 of pregnancy produced no sex differences in the offspring with cleft palate.

In a previous paper the author (*14*) did not find a statistically demonstrable difference in percentage of cleft palate in males and females when cortisone was used as the teratogenic agent.

Hydrocortisone is also teratogenic and induces cleft palate in offspring of mice when injected at specific times during pregnancy (*9, 11, 13, 16*). Since this drug can be injected at higher dosage than cortisone without increasing the average resorption rate, it could prove a better tool in evaluating possible sex differences in the incidence of malformed offspring. This is the subject of the present paper.

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Materials and Methods

Mice of the CD_1 (Charles River) strain were used. All animals were fed water and Purina Chow *ad libitum*. Females were kept with males overnight and examined each morning. When a copulation plug was found, the females were separated and this day was counted as 0. The pregnant females were divided into three groups. One group was injected with 5 mg of hydrocortisone, one group with 10 mg of hydrocortisone, and the third group was not injected. Injections of hydrocortisone (Hydrocortone Acetate, Merck) were made on days 10, 11, 12 or 13 of gestation. While the 10th and 12th days have been shown to be critical in cortisone induced cleft palate (*14*) the 13th day was added as a check. Spontaneous clefting in CD_1 mice used is 1%. Animals were sacrificed one day before expected delivery. The uteri were examined for resorption sites and number of offspring, and the fetuses were then removed, weighed and examined for the presence of cleft palate. Sex was determined by laparotomy and examination of the gonads under a dissection microscope. Ovaries were characteristically situated underneath and lateral to the kidneys and are somewhat irregularly shaped. Testes, at this stage of development, are round, shiny and well marked and are situated laterally to the urinary bladder. Random histological examinations confirmed the diagnosis.

Statistical analyses were made of sex distribution and incidence of induced cleft palate in either sex. For detection of differences in sex distribution a comparison of observed binominal frequencies was made using normal approximation with continuity correction. For detection of differences between the sexes of incidence of induced cleft palate, chi square with continuity correction was computed on a 2×2 contingency table (*1*).

Results

The incidence of induced cleft palate according to sex and dose is shown in Table 1 and Figure 1. The female:male ratio in the non-injected animals was not significantly different from 50:50. A very small number of cleft palates were found in this group. Five mg of hydrocortisone did not increase significantly the incidence of resorption sites, but 10 mg hydrocortisone given on the 12th day of pregnancy did so. In none of the groups was the female:male ratio of the offspring significantly different from 50:50. No statistically significant difference between the sexes in the incidence of cleft palate was demonstrable. (All chi square values were below 3.84 with one degree of freedom; p 5%). In the control group, 5 offspring (2 males, 3 females) had exencephaly. In the hydrocortisone injected group, 4 exencephalies were noted in the groups injected with 5 mg: 1 male in the group injected on the 11th day, 1 male in the group injected on the 12th day and 2 females in the group injected on the 13th day.

TABLE 1. Effect of hydrocortisone on the occurrence of the induced cleft palate in CD_1 mice. (The critical value of *chi square* for one degree of freedom associated with 5% probability is 3.84).

CD_1-Hydrocortisone									
dosage of hydrocortisone		*number of mothers*	*offspring*					*sex* χ^2	*cleft palate* χ^2
			males		*females*		*resorptions*		
mg/day	*Day*		*normal*	*cleft palate (%)*	*normal*	*cleft palate (%)*			
0	—	27	153	1	137	2	11	0.768	0.487
5	10	15	41	32 (42)	45	25 (35)	11	0.062	1.051
5	11	15	56	25 (30)	40	27 (40)	17	1.321	1.520
5	12	16	54	29 (34)	58	31 (34)	17	0.209	<0.001
5	13	14	50	8 (13)	47	6 (11)	16	0.225	0.161
10	10	7	2	22 (91)	4	30 (88)	21	1.724	0.242
10	11	13	6	57 (90)	5	44 (89)	17	1.75	0.016
10	12	19	20	65 (76)	17	53 (75)	51	1.452	0.013
10	13	19	47	51 (57)	53	31 (37)	21	1.077	3.772

Discussion

Cleft palate in mice is induced by a number of drugs, some of which produce at the same time several different congenital anomalies, while others, such as cortisone and hydrocortisone produce cleft palate almost exclusively. It is not surprising therefore that certain teratogenic agents may affect the sexes differently. Their selective lethal effects during pregnancy could eliminate more embryos of one sex (for instance male) and

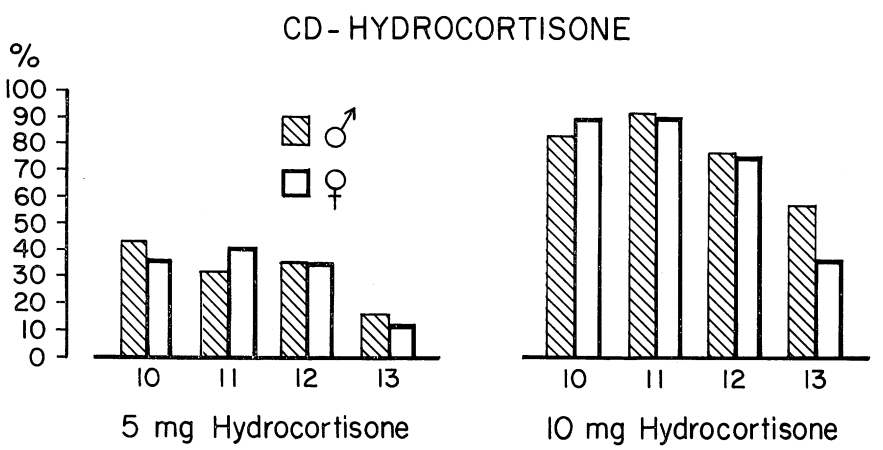

FIGURE 1. Relationship between the dose of hydrocortisone injected in the pregnant CD_1 mice the day of injection and the percentage of induced cleft palate in the offspring.

this could result at term in a larger number of abnormal newborns of the opposite sex. This, however, is not apparent in the effect of hydrocortisone in CD_1 mice.

The present experiments show that the critical time for induction of cleft palate with hydrocortisone using one injection only, is from the 10th to the 12th day of gestation. This is slightly earlier than when cortisone is used as a teratogenic agent. Also, hydrocortisone does not apparently induce as many resorptions as cortisone does. Comparison with the data of Dostal (*5*), who injected 0.2 mg of hydrocortisone directly into the amniotic sac, shows good correlation with our groups which were injected systemically with 10 mg.

Comparing the therapeutic doses of cortisone and hydrocortisone (*12*), the present teratogenic doses of hydrocortisone are much higher than the doses of cortisone normally used. Even so, the number of resorption sites present is not high, in comparison with cortisone injected animals (*14*). With this small percentage of resorptions, selective death of embryos of one sex is not a problem, and the absence of sex-differences in hydrocortisone induced cleft palate was clearly demonstrable with the strain and dose used. Comparing the percentage in induced cleft palate in our group with the results of other investigators, a strain difference can be noticed. While Pinsky and DiGeorge (*16*) showed that A/Jax mice injected during 4 days of development showed 18% of induced cleft palate using 4 mg of hydrocortisone, Lahti and Saxen (*13*) in Swiss random bred mice showed 35% of induced cleft palate, with the same schedule and dosage. A genetic influence on cortisone-induced cleft palate has been described (*10*). The present results confirm the importance of genetic factors in relation to environmental influences. Our experiments confirm those of Ingalls and Curley (*9*) that a single dose of hydrocortisone can be an effective teratogenic agent depending on timing and dose. A slight difference in reported values and those obtained by Ingalls and Curley (*9*) may be due to genetic factors. With the doses used, it is not possible to distinguish higher susceptibility in either sex.

Conclusion

The incidence of hydrocortisone induced cleft palate and sex of the fetus has been studied in CD_1 mice. Doses of 5 mg and 10 mg of hydrocortisone were injected into pregnant females and the sex of the fetuses was determined with laparotomy by the position and appearance of the gonads. Hydrocortisone did not have a statistically significant effect on the sex ratio of the surviving fetuses. No difference was demonstrated between sexes in the CD_1 mouse strain in the incidence of cleft palate following the administration of the teratogen at key stages of palatal closure.

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