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Alterations in Palatal Ruga Patterns in Jcl:ICR Mouse Fetuses from Dams Treated with All-*trans*-retinoic Acid

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ABSTRACT

Pregnant Jcl:ICR mice were orally given all-*trans*-retinoic acid (RA) at dose levels from 0.08 to 80 mg/kg once at days 10.5, 11.5, or 12.5 (vaginal plug = day 0) of gestation. The dams were sacrificed at day 18.5 of gestation, and the fetuses were dissected. After fixation in Bouin's solution, the fetal palates were observed under a dissecting microscope. Pattern alterations rare in the control fetuses were shortness, fusion, maldirection, trirugal malalignment, modified cross, and cross. These alterations were defined as abnormalities. Cleft palate was induced at dose levels of 20 mg/kg and above. At each treatment day, the total incidence of abnormal rugae increased from the dose of 0.3 or 0.6 mg/kg, dose-dependently. Although missing ruga-8 was a common pattern alteration in vehicle controls, its incidence increased dose-dependently. The incidence of supernumerary posterior to ruga 3 increased dose-dependently after treatment at day 11.5 or day 12.5 of gestation, however the increase was not observed after treatment at day 10.5 of gestation. These findings indicate that the abnormalities of ruga, missing ruga-8 and supernumerary posterior to ruga 3 are very sensitive indicators of teratogenicity of RA.

Key words: *Retinoic acid, Palatal ruga, Mouse, Cleft palate*

In developmental toxicity studies, some fetuses exposed to test substances may exhibit minor morphological alterations. Among these "variations," some were reported to be good indicators for detection of possible teratogens. For example, Yasuda and Maeda[22] and Kimmel and Wilson[6] noted that the lumbar rib or supernumerary 14th rib occurred dose-relatedly in fetuses exposed to vitamin A or actinomycine D. Recently, Mori et al[10] found abnormal pad patterns on volar skin of mouse fetuses trans-placentally exposed to retinoic acid at levels below those that caused skeletal malformations.

Variant patterns of palatal rugae in mice and rats are also candidates for such "warning signs." Palatal rugae are transverse ridges on the oral surface of the hard palate. Rogers et al[16] reported a dose-related increase in incidence of irregular palatal rugae in fetuses from rats and mice treated with cacodylic acid. Thereafter variant palatal rugae have been described in fetuses from rats treated with phenytoin[9], retinoic acid[3], and mice treated with corticosteroid[19], methyl mercury[12], cadmium[11], methoxy acetic acid[23,24], and dioxins[23–25]. Variant rugae have also been noted in transgenic or spontaneous mutant mice[1,2], and in fetuses from dams treated with intra-amniotic administration of physiological saline[13]. These factors are known to be related to cleft palate.

Among the above factors, retinoic acid is an extensively studied teratogen which induces various types of major malformations and minor variations[8]. Ikemi et al[3,4] induced variant patterns of palatal rugae in rats by all-*trans*-retinoic acid (RA), classified these patterns into anomalies and variations, and analyzed stage-specificity and dose-response relationship. They concluded that the observation of palatal rugae may provide useful information on the malformation potential of chemicals.

The normal pattern of palatal rugae in mice is somewhat different from that in rats. Therefore terms for description of aberrant patterns of rugae in mice should be different from those used for rats. Mice are more frequently used than rats in the field of genetics and developmental biology. In addition, the palatal development in mouse and rat fetuses is chronologically different[18,20]. Therefore detailed descriptions of aberrant patterns of palatal rugae induced by a well-known teratogen in mice, and comparisons between mice and rats are of special significance.

In this study, we described and classified pattern alterations of palatal rugae induced by RA in detail, and examined stage-specificity and dose-response relationship.

MATERIALS AND METHODS

Sexually mature (over 9 weeks old) females of Jcl:ICR mice (Japan CLEA, Tokyo, Japan) were used in this study. The animal room was generally maintained at 23 ± 2°C with 50 ± 10% humidity in a 12-hour light-dark cycle. After confirmation of copulation, two or three females were kept in a plastic cage. The animals were offered pellet feed (CE-2, Japan CLEA, Tokyo, Japan) and tap water *ad libitum*. For mating, one female was placed together with one male overnight. The day of observation of vaginal plugs was designated as gestation day (GD) 0. Five or six copulated animals were allotted to each group.

To examine dose-response relationship and stage-specificity in induction of altered patterns of palatal rugae, we performed three experiments. In the first and second experiments, pregnant mice were orally given RA at dose levels 0, 0.08, 0.3, 1.2, 5, 20, 40, and 80 mg/kg once at GD10.5 or GD11.5. In the third experiment, pregnant mice were orally given RA at dose levels 0, 0.6, 1.2, 5, 20, 40, and 80 mg/kg at GD12.5. The dosage volume (5 ml/kg) was determined based on body weight measured at the time of administration.

RA (Sigma Chemical, St. Louis, MO) was suspended in corn oil (Katayama Pharmaceutical Co., Ltd., Osaka, Japan). The dosing solution was prepared by an ultrasonic homogenizer (Model UR-200P, Tomy Seiko Co., Ltd., Tokyo, Japan) under a shading condition just before dosing. Corn oil alone was administered to the control animals. Maternal body weight was periodically measured. Dams were sacrificed by cervical dislocation at GD18.5. The uterus was examined for numbers of implantations, viable and dead fetuses. Distribution of implantations was also observed. Each live fetus was sexed, weighed and examined for external abnormalities (including the oral cavity). After fixation in Bouin's solution, fetal palates were observed under a dissecting microscope by dissecting away the lower jaw and tongue, and the pattern of palatal rugae was recorded excluding fetuses with cleft palate and inadvertently damaged palate. The normal pattern of palatal rugae in a near term fetus is illustrated in Fig. 1. Alterations of the ruga pattern, including those rarely found in controls, are schematically shown in Fig. 2. Definitions of these variant types were originally given by Sakamoto et al[19]. We modified these definitions and divided the alterations into two classes, variations and abnormalities, according to the incidence in our historical control data and the grade of alterations. Definitions of these alterations are as follows:

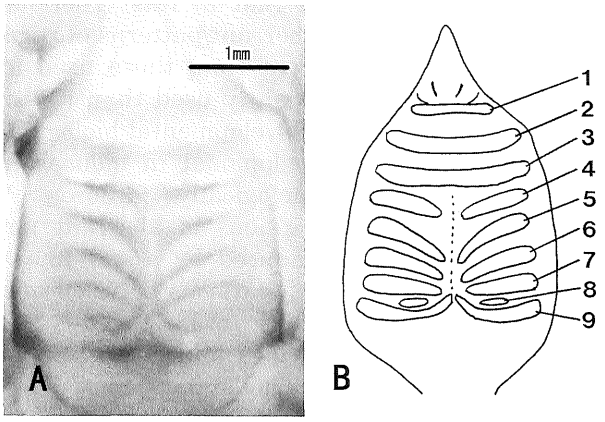

Fig. 1. (A) Normal pattern of palatal rugae in a day 18 mouse fetus. (B) Line drawing of (A). Rugae are numbered anterior to posterior.

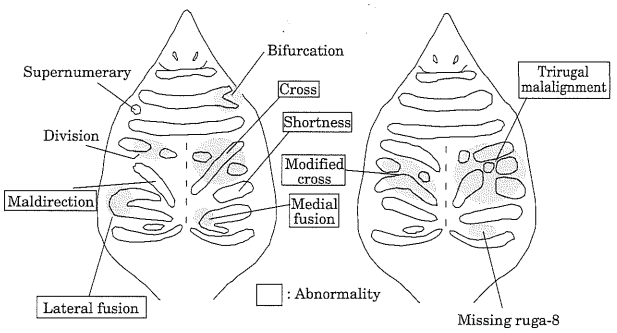

Fig. 2. Schematic line drawing of variation and abnormality of ruga pattern

1. Bifurcation: The lateral extremity of ruga branched off into two.
2. Division: Ruga separated into two or more parts.
3. Supernumerary: A small ruga or localized protrusion of the palatal mucous membrane in addition to the eight or nine rugae defined above.
4. Missing ruga-8: Absence of the small eighth ruga.
5. Shortness: Rugae with lengths less than two-thirds the usual length.
6. Fusion: Two adjacent rugae fused at the lateral or medial end.
7. Maldirection: An obliquely running ruga.
8. Trirugal malalignment: Disrupted three rugae with malaligned medial fragments.
9. Modified cross: A flexed ruga accompanied by two short ridges rostrally.
10. Cross: An obliquely running ruga accompanied by a short ridge rostrally and another short one caudally.

Pattern alterations common in our historical control data were bifurcation, division, supernumerary, and missing ruga-8. Pattern alterations rare in our historical control data were shortness, fusion, maldirection, trirugal malalignment, modified cross, and cross. The former were defined as "variation," and the latter as "abnormality".

RESULTS

No dam died during gestation in any group. There was no significant difference in maternal

body weight changes between the treated and control groups (data not shown). As results of the examination of fetuses at GD18.5, no significant difference was observed among the groups in number of implantation and live fetuses, fetal mortality, and body weight of live fetuses (Tables 1–3). Incidence of cleft palates increased at 20 mg/kg and above on each treatment day. Threshold dose for cleft palate was estimated to be less than 20 mg/kg. Incidence of limb deformities increased from the dosage of 20 or 40 mg/kg at each treatment day. Threshold dose for limb deformities was less than 20–40 mg/kg.

Almost all fetuses in the 80 mg/kg groups exhibited cleft palate on every treatment day. In the 40 mg/kg groups, the number of examined fetuses was half of the control group at GD10.5, one third at GD11.5, and three quarters at GD12.5. Notably there were two litters in which all fetuses had cleft palate at GD11.5.

Fig.1 illustrates the normal pattern of palatal rugae in a mouse fetus. The oral surface of the mouse palate has nine pairs of transverse ridges, or rugae. The rugae are numbered from the most anterior (rostral) ruga. The most posterior (caudal) ruga is defined as ruga 9 irrespective of presence or absence of a small eighth ruga between ruga 7 and the most posterior one. Rugae 1, 2, 3 run parallel to the frontal plane and extend across the midline, whereas rugae 4 to 9 are separated at the midline. Rugae 4 to 7 are slanted medio-caudally. The medial end of ruga 9 curves slightly anteriorly.

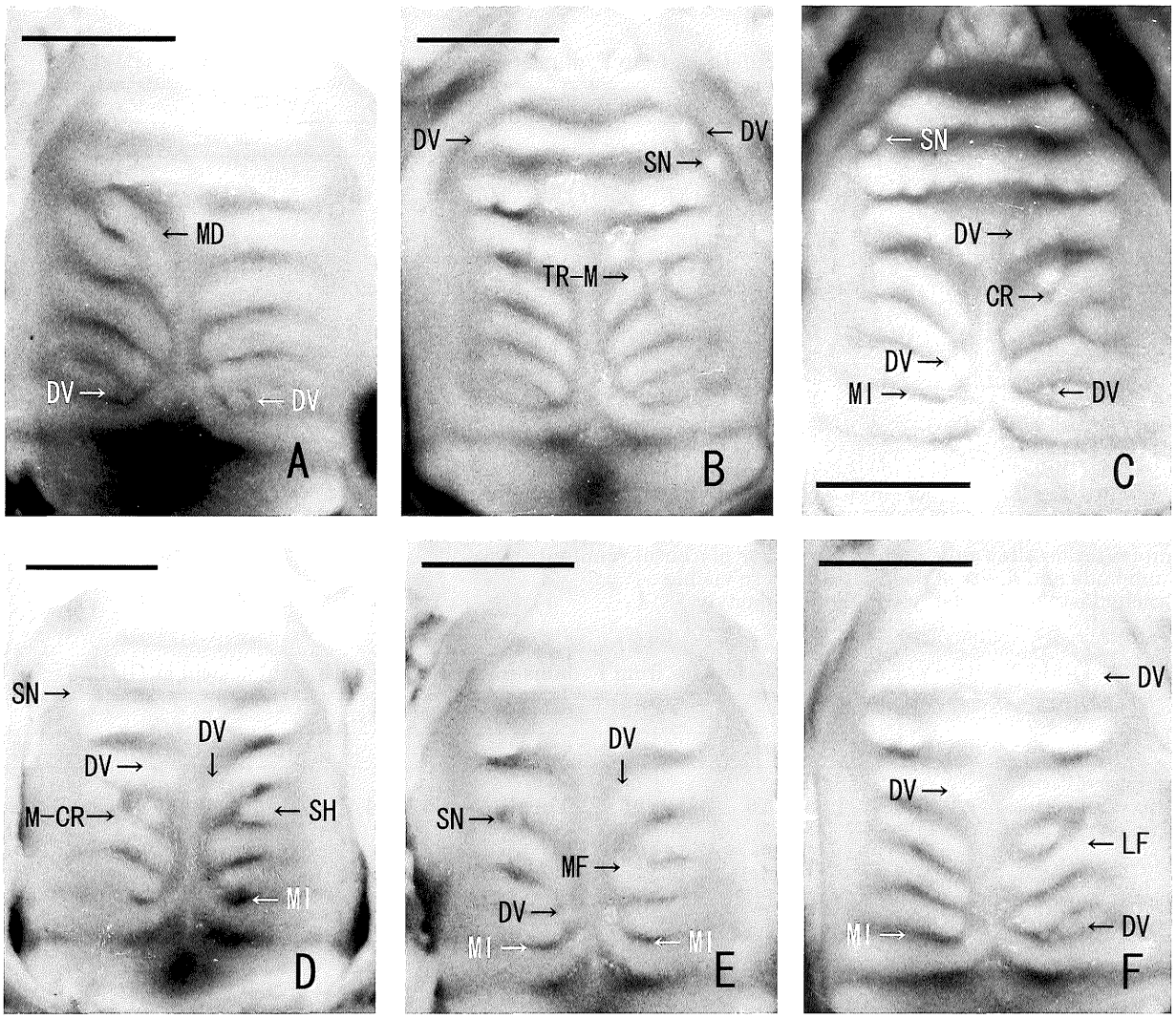

Fig. 3. The day-18 fetal palate with typical abnormal patterns and some ruga variations. (A) A fetus from a litter treated with RA (20 mg/kg) at GD10.5. MD: maldirection, DV: division. (B) A fetus from a litter treated with RA (5 mg/kg) at GD10.5. TR-M: trirugal malalignment, DV: division, SN: supernumerary. (C) A fetus from a litter treated with RA (0.3 mg/kg) at GD10.5. CR: cross, DV: division, SN: supernumerary, MI: missing ruga-8. (D) A fetus from a litter treated with RA (20 mg/kg) at GD10.5. SH: shortness, M-CR: modified cross, DV: division, SN: supernumerary, MI: missing ruga-8. (E) A fetus from a litter treated with RA (20 mg/kg) at GD10.5. MF: medial fusion, DV: division, SN: supernumerary, MI: missing ruga-8. (F) A fetus from a litter treated with RA (5 mg/kg) at GD10.5. LF: lateral fusion, DV: division, MI: missing ruga-8. Bar = 1 mm.

Table 1. Litter parameters in F_1 fetuses from F_0 dams treated orally with retinoic acid at GD10.5

	Dose (mg/kg)							
	Control	0.08	0.3	1.2	5	20	40	80
No. of examined dams	5	5	5	5	5	5	5	5
Mean no. of implantations	15.2 ± 2.4	15.2 ± 1.6	15.8 ± 2.6	14.8 ± 1.6	15.0 ± 0.7	14.2 ± 1.3	14.6 ± 0.9	13.0 ± 3.2
Dead fetuses Total (Mean%)[a]	8 (11.0)	2 (2.8)	6 (8.2)	4 (6.0)	3 (4.0)	4 (5.3)	9 (12.4)	5 (8.1)
Early[b] (Mean%)	6 (8.0)	2 (2.8)	4 (5.5)	2 (3.3)	2 (2.7)	1 (1.3)	6 (8.4)	3 (5.6)
Late[c] (Mean%)	2 (3.0)	0 (0.0)	2 (2.7)	2 (2.7)	1 (1.3)	3 (3.9)	3 (4.0)	2 (2.5)
Mean no. of live fetuses (Total)	13.6 ± 2.8 (68)	14.8 ± 1.9 (74)	14.6 ± 3.3 (73)	14.0 ± 2.6 (70)	14.4 ± 1.1 (72)	13.4 ± 1.1 (67)	12.8 ± 1.3 (64)	12.0 ± 3.2 (60)
No. of fetuses with abnormalities (Mean %)								
Cleft palate	0	0	1 (1.1)	0	0	9 (12.8)	31 (49.7)	56 (94.0)
Brachymelia/fore limb	0	0	0	0	0	35 (50.0)	40 (64.5)	57 (96.0)
/hind limb	0	0	0	0	0	0	11 (18.8)	29 (51.9)
Deformity of finger[d]	0	0	0	0	0	21 (30.2)	48 (76.7)	54 (90.9)
Deformity of toe[d]	0	0	0	0	0	0	6 (10.9)	13 (27.4)
Sex ratio males/females	1.27	1.31	1.09	1.12	1.00	1.16	1.00	1.00
Mean live fetal Male	1.52 ± 0.07	1.49 ± 0.04	1.56 ± 0.08	1.53 ± 0.11	1.49 ± 0.08	1.45 ± 0.06	1.55 ± 0.17	1.56 ± 0.10
body weight (g) Female	1.50 ± 0.06	1.41 ± 0.06	1.47 ± 0.09	1.46 ± 0.06	1.39 ± 0.09	1.40 ± 0.07	1.49 ± 0.14	1.48 ± 0.09

a): Mean% ((No. of dead fetuses/No. of implantations) × 100) was calculated on the basis of the litter as sample unit.
b): Implantation sites only, embryonic and/or placental remnants.
c): Limb buds and digits of dead fetuses were visible.
d): Brachydactyly, oligodactyly and/or syndactyly.

Table 2. Litter parameters in F_1 fetuses from F_0 dams treated orally with retinoic acid at GD11.5

	Dose (mg/kg)							
	Control	0.08	0.3	1.2	5	20	40	80
No. of examined dams	5	5	5	5	5	5	5	5
Mean no. of implantations	14.0 ± 1.9	12.8 ± 3.7	13.0 ± 1.6	14.6 ± 2.1	11.4 ± 2.7	13.8 ± 1.6	14.2 ± 1.1	15.4 ± 0.5
Dead fetuses Total (Mean%) [a]	3 (3.9)	1 (1.5)	2 (3.0)	2 (2.5)	3 (7.5)	4 (5.9)	3 (4.1)	3 (3.8)
Early[b] (Mean%)	2 (2.5)	0 (0.0)	1 (1.5)	2 (2.5)	2 (5.0)	3 (4.3)	2 (2.9)	2 (2.5)
Late[c] (Mean%)	1 (1.3)	1 (1.5)	1 (1.4)	0 (0.0)	1 (2.5)	1 (1.5)	1 (1.3)	1 (1.3)
Mean no. of live fetuses (Total)	13.4 ± 1.3 (67)	12.6 ± 3.7 (63)	12.6 ± 1.5 (63)	14.2 ± 1.8 (71)	10.8 ± 3.8 (54)	13.0 ± 1.9 (65)	13.6 ± 1.1 (68)	14.8 ± 0.5 (74)
No. of fetuses with abnormalities (Mean %)								
Cleft palate	0	1 (1.4)	0	0	1 (1.4)	5 (8.7)	42 (62.4)	72 (97.3)
Brachymelia/fore limb	0	0	0	0	0	0	23 (33.3)	31 (41.3)
/hind limb	0	0	0	0	0	0	15 (20.0)	21 (28.0)
Deformity of finger[d]	0	0	0	0	0	0	15 (20.0)	38 (50.7)
Deformity of toe[d]	0	0	0	0	0	2 (3.3)	21 (29.2)	45 (60.0)
Sex ratio males/females	0.91	0.80	1.03	0.87	1.08	1.17	0.94	0.64
Mean live fetal Male	1.45 ± 0.11	1.69 ± 0.13	1.43 ± 0.10	1.37 ± 0.11	1.59 ± 0.25	1.42 ± 0.11	1.50 ± 0.09	1.43 ± 0.16
body weight (g) Female	1.39 ± 0.12	1.63 ± 0.20	1.35 ± 0.15	1.31 ± 0.12	1.50 ± 0.23	1.33 ± 0.08	1.41 ± 0.12	1.39 ± 0.14

a): Mean% ((No. of dead fetuses/No. of implantations) × 100) was calculated on the basis of the litter as sample unit.
b): Implantation sites only, embryonic and/or placental remnants.
c): Limb buds and digits of dead fetuses were visible.
d): Brachydactyly, oligodactyly and/or syndactyly.

Table 3. Litter parameters in F_1 fetuses from F_0 dams treated orally with retinoic acid at GD12.5

	Dose (mg/kg)						
	Control	0. 6	1.2	5	20	40	80
No. of examined dams	6	4	5	6	6	6	6
Mean no. of implantations	13.5 ± 1.6	14.8 ± 0.5	15.0 ± 1.6	13.2 ± 2.1	14.8 ± 1.5	13.0 ± 4.0	14.5 ± 2.3
Dead fetuses Total (Mean%) [a]	8 (9.3)	2 (3.3)	4 (5.5)	3 (3.7)	7 (8.1)	4 (7.6)	7 (8.1)
Early[b] (Mean%)	7 (8.0)	1 (1.7)	1 (1.4)	3 (3.7)	5 (5.9)	2 (5.6)	4 (4.5)
Late[c] (Mean%)	1 (1.3)	1 (1.7)	3 (4.1)	0 (0.0)	2 (2.2)	2 (2.1)	3 (3.6)
Mean no. of live fetuses (Total)	12.2 ± 1.7 (73)	14.3 ± 0.5 (57)	14.2 ± 2.3 (71)	12.7 ± 2.0 (76)	13.7 ± 2.1 (82)	12.3 ± 4.5 (74)	13.3 ± 2.3 (80)
No. of fetuses with abnormalities (Mean %)							
Cleft palate	0	0	0	0	4 (5.4)	21 (23.4)	41 (51.7)
Brachymelia/fore limb	0	0	0	0	1 (1.4)	0	1 (1.0)
/hind limb	0	0	0	0	16 (16.7)	74 (100)	80 (100)
Deformity of finger[d]	0	0	0	0	0	0	1 (1.0)
Deformity of toe[d]	0	0	0	0	21 (16.7)	66 (90.5)	74 (98.3)
Omphalocele with agnathia	0	0	0	1 (1.7)	0	0	0
Sex ratio males/females	1.15	1.38	0.92	1.11	0.82	1.31	091
Mean live fetal Male	1.60 ± 0.10	1.31 ± 0.21	1.39 ± 0.15	1.49 ± 0.07	1.58 ± 0.05	1.59 ± 0.20	1.54 ± 0.11
body weight (g) Female	1.53 ± 0.10	1.25 ± 0.17	1.31 ± 0.13	1.43 ± 0.05	1.50 ± 0.04	1.41 ± 0.10	1.47 ± 0.09

a): Mean%((No. of dead fetuses/No. of implantations) × 100) was calculated on the basis of the litter as sample unit.
b): Implantation sites only, embryonic and/or placental remnants.
c): Limb buds and digits of dead fetuses were visible.
d): Brachydactyly, oligodactyly and/or syndactyly.

Table 4. Variations and abnormalities of palatal rugae in F_1 fetuses from F_0 dams treated orally with retinoic acid at GD10.5, GD11.5 or GD12.5

	Dose (mg/kg)								
	Control	0.08	0.3	0.6	1.2	5	20	40	80
GD10.5									
No. of litters (fetuses) examined[b]	5 (68)	5 (74)	5 (72)	—	5 (70)	5 (72)	5 (58)	5 (33)	2 (4)
No. of fetuses with abnormalities (Mean%)[a]	1 (1.5)	2 (2.5)	4 (6.4)	—	5 (6.5)	11 (15.6)	12 (25.8)	7 (22.5)	2 (33.3)
No. of fetuses with variations (Mean%)[a]				—					
Supernumerary (SN)	38 (59.3)	37 (49.5)	33 (45.0)	—	26 (35.9)	25 (34.5)	25 (41.7)	14 (45.8)	4 (100)
Division (DV)	65 (94.7)	66 (89.2)	66 (89.6)	—	58 (84.1)	63 (86.9)	44 (78.5)	28 (80.8)	4 (100)
Bifurcation (BI)	2 (3.6)	0	4 (4.8)	—	1 (1.5)	3 (4.3)	1 (1.5)	0	0
Missing ruga-8 (MI)	10 (15.3)	18 (23.4)	18 (22.5)	—	16 (21.1)	27 (38.6)	25 (40.0)	33 (46.7)	4 (100)
GD11.5									
No. of litters (fetuses) examined[b]	5 (63)[c]	5 (56)[e]	5 (63)	—	5 (70)[g]	5 (53)	5 (53)[h]	3 (22)[c]	2 (2)
No. of fetuses with abnormalities (Mean%)[a]	1 (1.5)	1 (2.9)	5 (8.0)	—	10 (15.5)	17 (31.1)	24 (48.4)	15 (73.2)	1 (50)
No. of fetuses with variations (Mean%)[a]									
Supernumerary (SN)	31 (50.9)	25 (47.3)	24 (38.2)	—	33 (49.2)	40 (61.9)	42 (81.3)	15 (71.2)	2 (100)
Division (DV)	43 (68.9)	52 (89.8)	59 (93.7)	—	62 (89.1)	27 (51.2)	28 (53.0)	7 (26.7)	0
Bifurcation (BI)	1 (1.4)	1 (2.9)	3 (4.7)	—	4 (6.0)	1 (3.3)	10 (24.9)	3 (7.7)	0
Missing ruga-8 (MI)	14 (22.7)	7 (13.7)	9 (14.7)	—	30 (42.3)	32 (59.5)	19 (31.9)	13 (42.1)	1 (50)
GD12.5									
No. of litters (fetuses) examined[b]	6 (70)[d]	—	—	4 (55)[f]	5 (71)	6 (75)	6 (78)	6 (53)	5 (36)[d]
No. of fetuses with abnormalities (Mean%)[a]	0	—	—	5 (9.8)	13 (18.3)	24 (31.3)	27 (33.1)	18 (31.0)	29 (83.9)
No. of fetuses with variations (Mean%)[a]									
Supernumerary (SN)	26 (36.7)	—	—	36 (65.4)	36 (49.7)	35 (43.9)	44 (57.0)	39 (78.2)	25 (70.6)
Division (DV)	62 (89.2)	—	—	39 (70.1)	58 (81.4)	51 (68.6)	68 (87.5)	46 (90.7)	15 (48.2)
Bifurcation (BI)	1 (1.2)	—	—	5 (9.3)	4 (5.0)	3 (4.5)	1 (1.7)	0	2 (4.0)
Missing ruga-8 (MI)	6 (8.5)	—	—	7 (13.7)	9 (13.1)	31 (39.8)	27 (34.1)	22 (45.4)	15 (51.7)

a): Mean% was calculated on the basis of the litter as sample unit.
b): Fetuses with cleft palate were excluded from observation.
c): Four fetuses were excluded from observation because of damage or specimen loss.
d): Three fetuses were excluded from observation because of damage or specimen loss.
e): Six fetuses were excluded from observation because of damage or specimen loss.
f): Two fetuses were excluded from observation because of damage or specimen loss.
g): One fetus was excluded from observation because of damage.
h): Seven fetuses were excluded from observation because of damage or specimen loss.

Alterations in palatal ruga patterns in fetuses treated with RA in this study are schematically illustrated in Fig.2. Supernumerary, division, and missing ruga-8 were commonly observed in control fetuses. Fig. 3 shows actual palates with typical abnormal patterns of rugae in the RA treated groups. The frequencies of ruga variation and abnormality of palatal rugae induced by RA are summarized in Table 4. Frequencies of fetuses with a division in the control groups (GD10.5, GD11.5 and GD12.5) ranged from 68.9% to 94.7%. The division that occurred in ruga 3 was not counted as a variation because of the high incidence in controls. Incidences of division at each ruga (except for ruga 3) are shown in Fig. 4, and they were high at ruga 4 and ruga 7 in each dose group. There were no differences among dose groups at each treatment day. Supernumerary occurred mainly in the middle of two adjacent rugae near the lateral ends. Incidence of supernumerary on the anterior part of palate (ruga 1–3) was not dose-dependent, whereas that on the posterior part (ruga 3–7) increased dose-dependently. These dose-dependent increases were observed after treatment at GD11.5 especially between rugae 3 and 4 or GD12.5 between rugae 4 and 5, however none were observed after treatment at GD10.5 (Fig.5). Frequencies of fetuses with missing ruga-8 increased from 1.2 or 5 mg/kg group at each treatment day (Fig. 6). As is shown in Fig.7, the total incidence of abnormal rugae (shortness, fusion, maldirection, trirugal malalignment, modified cross, and cross) increased from 0.3 mg/kg group at each treatment day, whereas RA induced cleft palate at 20 mg/kg and above. Frequencies of fetuses with abnormal rugae at GD10.5 and GD12.5 attained a plateau level (approximately 30%) at 20 mg/kg and 5 mg/kg, respectively, whereas at GD11.5 it increased to 73.2% at 40 mg/kg.

DISCUSSION

This study clearly showed that RA induced various abnormalities in pattern of palatal rugae in mice. In order to verify the usefulness of palatal rugae as a sensitive indicator of developmental

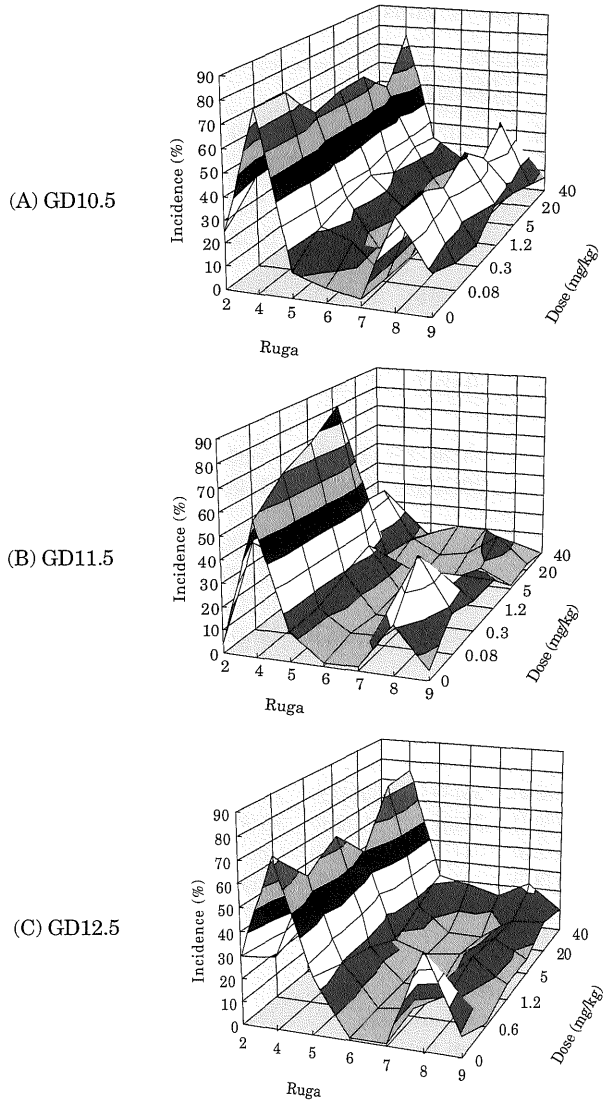

Fig. 4. Incidence of the divisions which were located on each palatal ruga (refer to Fig.1) in day-18.5 mouse fetuses. (A) Treated at GD10.5, (B) Treated at GD11.5, (C) Treated at GD12.5.
Data for 80 mg/kg groups are omitted because of the small number of examined fetuses.

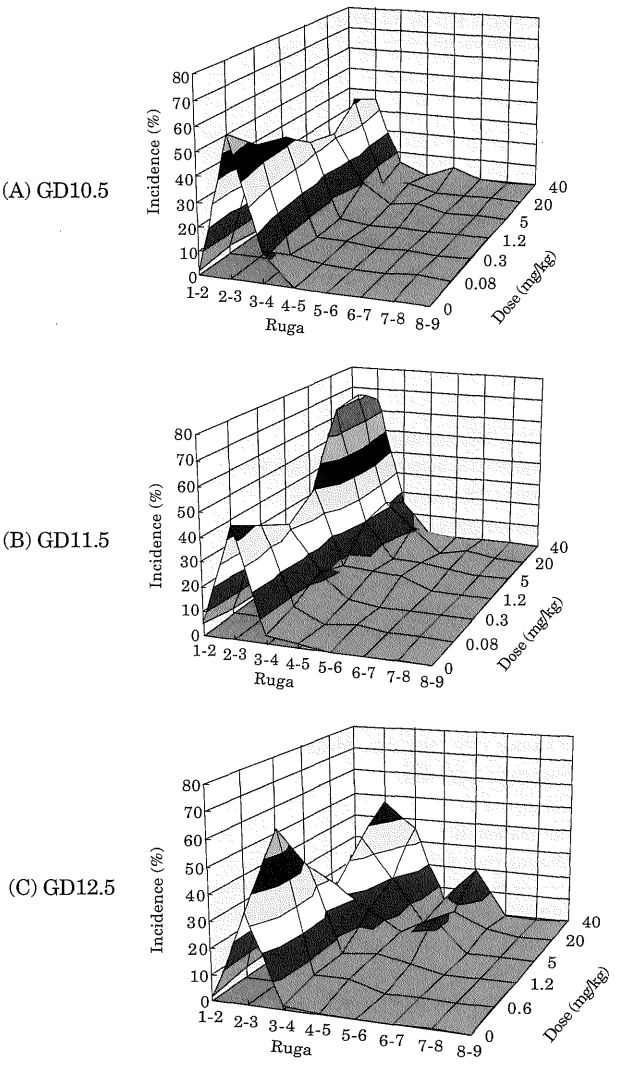

Fig. 5. Incidence of the supernumeraries which were located on each palatal ruga (refer to Fig.1) in day-18.5 mouse fetuses. (A) Treated at GD10.5, (B) Treated at GD11.5, (C) Treated at GD12.5.
Data for 80 mg/kg groups are omitted because of the small number of examined fetuses.

toxicity, we classified palatal ruga patterns into ten types and observed the locations where ruga variations occurred. Sakamoto et al[19] classified palatal ruga patterns into five types (bifurcation, division, supernumerary, shortness, cross) in 14- and 15-day mouse embryos, and Yasuda et al[26] separated fusion into medial and lateral fusion. We added three types (maldirection, modified cross, trirugal malalignment) as abnormal categories in this study. These types had been observed not in control mouse fetuses, but in mouse fetuses from dams treated with RA, so it was presumed that these ruga abnormalities could be indicators of teratogenicity of RA. The total incidence of abnormal rugae (shortness, fusion, maldirection, modified cross, trirugal malalignment, and cross), which occurred at region posterior to ruga 4 increased dose-dependently.

RA is known to induce various malformations other than cleft palate, including phocomelia and other limb reductions, neural tube defects, and urogenital system dysmorphogenesis[8]. Among these malformations, microphthalmia and anophthalmia were induced at 1.25 mg/kg and exencephaly at 2.5 mg/kg by oral gavage on GD7[21]. These dose levels were 50–100-fold less than those that are commonly used to examine the teratogenicity of this compound at later developmental stages in mice. However, the threshold dose level for induction of palatal ruga abnormality was 0.3 or 0.6 mg/kg, still lower than the above dose levels, even though RA was administered at late developmental stages.

Bifurcation, division, supernumerary, and miss-

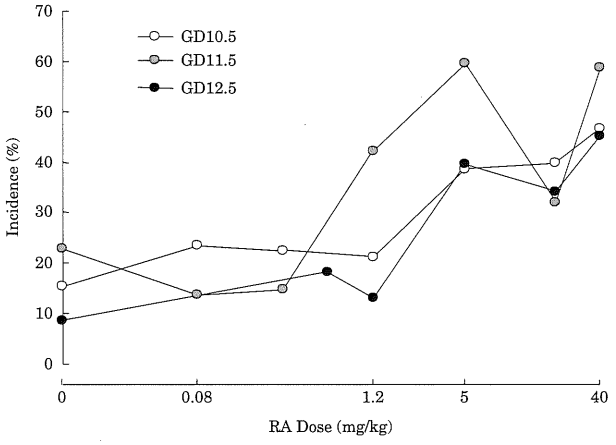

Fig. 6. Incidence of fetuses with missing ruga-8.
Data for 80 mg/kg groups are omitted because of the small number of examined fetuses.

ing ruga-8 are common variations. Incidences of bifurcation were 1.2%–3.6%, division 68.9%–94.7%, supernumerary 36.7%–59.3%, and missing ruga-8 8.5%–22.7% in the control groups, each incidence varied widely. Incidences of bifurcation and division did not increase dose-dependently at any treatment day. Incidence of supernumerary posterior to ruga 3 increased dose-dependently after treatment at GD11.5 and GD12.5, however the increase was not observed after treatment at GD10.5. At GD11.5 incidence of supernumerary increased between rugae 2–4, whereas at GD12.5 it increased between rugae 3–4 and 5–6. As the treatment day was set from GD11.5 to GD12.5, the region where the supernumerary occurred with dose-dependence shifted posteriorly. Because anterior rugae develop earlier than posterior rugae as described below, the above shifting suggests stage-dependency of the location of a supernumerary ruga.

Normal development of palatal rugae was described by Peterková et al[15]. They defined the appearance of vaginal plug as day 1, hence their day 12 corresponds to our GD11. The foremost rugae start to differentiate between GD11 and GD12. Between GD12 and GD13 the number of rugae conspicuously increases in the anterior third of palatal shelf, and by palatal shelf horizontalization (GD14) new rugae originate in the middle third. As stated above, it was presumed that the developmental critical period for the supernumerary on this region was 1 or 2 days before rugae formation. As to the missing ruga-8 and the abnormal rugae that occurred at region posterior to ruga 4, stage-specificity was not observed, but dose-dependency was noted on every treatment day in contrast to the supernumerary. Dose levels of ruga abnormalities induced by RA were lower than those of supernumerary and missing ruga-8. Although no type of abnormality occurred dose-dependently, the total incidence of abnormalities increased dose-dependently from lower dose levels.

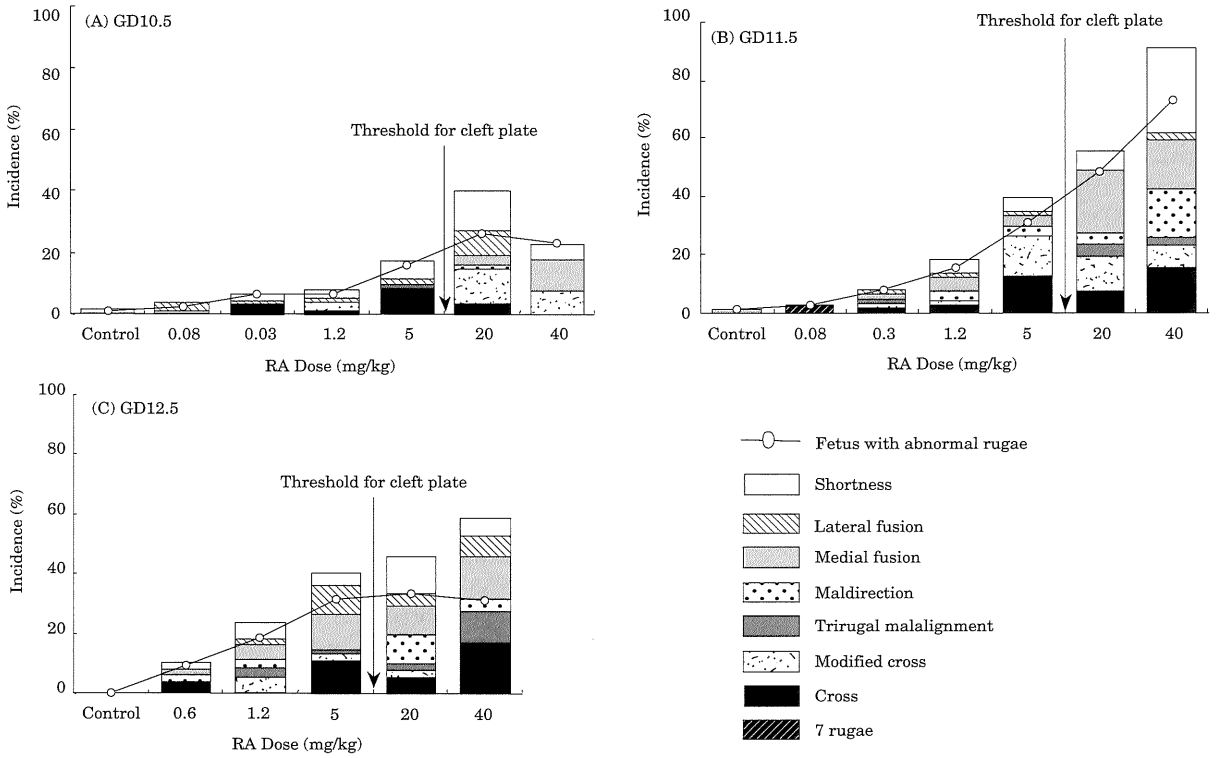

Fig. 7. Incidence of fetuses with ruga abnormalities and total incidence of abnormal pattern of palatal rugae in mouse. (A) Treated at GD10.5, (B) Treated at GD11.5, (C) Treated at GD12.5.
Data for 80 mg/kg groups are omitted because of the small number of examined fetuses.

These differences in stage-specificity and dose-dependence suggest a different pathogenesis for each ruga alteration. Kochhar[7] examined cleft palate from mice treated with RA (80 mg/kg) on the 10th–16th days of gestation (corresponding to our GD9–GD15). He presumed that the developmental changes responsible for cleft palate on GD9 differed from those leading to cleft palate on GD12–GD14. It is conceivable that the earlier produced type resulted only secondarily to the growth retardation of palatal shelves. Cleft palate produced by treatment on GD11–GD14 was considered to result from a primary effect on some factors residing in the palatal shelves themselves, such as changes in the intra- or extracellular components of the mesenchymal or epithelial tissues comprising the shelves. Yasuda and colleagues[27] suggested the size of palatal shelves affects the ruga patterns. Missing ruga-8 might be induced secondarily to the growth retardation of palatal shelves.

When compared with ruga alterations in rats, peakless, extrapeaks, aberrant median continuity were not detected in mice. In contrast, cross, modified cross, maldirection, and trirugal malalignment were not found in rats. These differences are due to differences in the normal pattern of palatal rugae between mice and rats. Rugae in rats are generally continuous in the median portion of the palate, and rugae 4, 5, and 6 curved anteriorly with one peak on the lateral palatal shelf. Rugae lateral to the peaks slant latero-posteriorly. In mice, ruga 4 and posterior ones have no median continuity with contralateral ones, and slant medio-posteriorly. Thus mice can not have ruga alterations resulting in peaks or median continuity.

In this study, the most sensitive period for cleft palate and ruga abnormalities induced by RA in mice was GD11.5, whereas in rats it was GD14[3]. There was approximately a 3 day difference between mice and rats. It was presumed that this difference was due to a different rate of development between mice and rats. Sakamoto et al[18] observed transition from open palate to closed palate in mouse embryos on GD14. Schüpbach et al[20] described occurrence of palatal closure in rats between GD16.0 to 17.5.

Recently, transgenic mice or knock out mice have often been used in developmental research. Balling et al[1] had generated transgenic mice that ectopically express *Hox-1.1* from the chicken β-actin promoter. This phenotype was similar to the effects seen after systemic administration of retinoic acid during gestation. An atypical pattern of rugae was observed in offspring that had a closed palate. This suggests that retinoic acid embryopathy and specific developmental defects caused by ectopic expression of a potential developmental control gene share a common pathogenesis.

In humans, variations in palatal ruga patterns have been described in detail in the field of oral anatomy. Kamijo[5] reported developmental changes of palatal ruga pattern in human fetuses and adults. Roth[17] described sagittally running palatal rugae as a unique abnormal pattern. Park et al[14] observed maxillary dental casts obtained from patients with submucous cleft palate, and found that one or more of the palatal rugae curved towards the region of the bony notch in the posterior border of the hard palate in the majority of cases. Abnormal ruga pattern may indicate disturbed palatal development also in humans.

These studies indicate that altered patterns of palatal rugae may result from disturbed palatal development by environmental and/or genetic factors in several species including humans. Alterations in palatal ruga patterns in mice defined in the present study would be useful for assessing developmental toxicity of environmental factors as well as for detecting slight changes in craniofacial development in mutant mice.

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