The Effect of Dexamethasone and Hypervitaminosis A on the Cell Proliferation of Rat Palatal Processes

RAVINDRA NANDA, B.D.S., M.D.S., Ph.D.
DAVID ROMEO, D.M.D.
Farmington, Connecticut 06032

Excess *vitamin A* and *dexamethasone* were given to *pregnant rats* from day 10 to 13 to determine their effect on the *proliferation activity* of the mesenchymal cells of *palatal processes*. ^{3}*HTdr* was given to the rats on days 14, 15, 16, and 17 of gestation. The animals were killed one hour after injection. Four fetal heads from each rat were prepared for radioautography. The radioautograms were examined microscopically to determine *labeling indices* for various areas and regions of the palatal processes. The results show that dexamethasone disturbs the cell proliferation ability of the mesenchymal cells significantly more severely than does vitamin A. The results suggest that, although both teratogenic agents produce an anomaly of similar morphological nature, their effect on cell proliferation ability of the palatal processes varies significantly.

Introduction

The production of cleft palate in experimental animals has been actively investigated in recent years. Numerous teratogens have been used to induce this anomaly. However, debate still exists concerning the mode of action of the various environmental agents which produce cleft palate.

In a previous study (Nanda, van der Linden, and Jansen, 1970) we reported that hypervitaminosis A and dexamethasone produce cleft palate with similar morphological features in rat fetuses. However, the literature suggests that these two agents have different modes of action.

Several explanations have been offered regarding the mechanism of excess vitamin A teratogenicity. It has been suggested that it has a direct effect on the fetus (Giroud, Gounelle, and Martinet, 1957; Nanda, May, and Lite, 1977), disturbs acid mucopolysaccharide metabolism of the ground substance of the palatal processes (Kochhar and Johnson, 1965; Nanda, 1971), disturbs the blood concentration of thyroid hormone (Takekoshi, 1964), and interferes with carbohydrate metabolism (Woollam and Millen, 1960).

Dexamethasone, a glucocorticoid preparation, is known to be a highly teratogenic agent (Pinsky and DiGeorge, 1965). It has been reported that dexamethasone suppresses the normal adrenals to a low level of activity (Liddle, 1960; Beaven, Espuier, and Hast, 1964) and possibly alters ovarian secretion (Butt et al., 1963).

The histological observations reported on cleft palate fetuses from excess vitamin A and dexamethasone treated rats consistently show short and deformed palatal processes, short nasal septums, precocious cartilage formation and growth disturbance in the maxillo-mandibular area (Cohlan, 1954; Pinsky and DiGeorge, 1965; Nanda, van der Linden, and Jansen, 1970; Nanda, 1970). It has also been suggested that teratogenic agents, such as excess vitamin A and cortisone, disturb the cell proliferation ability of the cells of the palatal processes (Kochhar, 1968; Nanda, 1971; Jelinek and Dostal, 1974). However, none of these studies has made an attempt to investigate cell proliferation levels on different days of gestation prior to and after the normal presumptive fusion time of the palatal processes.

The present study was undertaken to com-

The authors are affiliated with The Department of Orthodontics, University of Connecticut School of Dental Medicine, Farmington, Connecticut.

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pare the effects of excess vitamin A and dexamethasone on the proliferation activity of mesenchymal cells of rat palatal processes on different days of gestation. The normal cell proliferation levels established in our previous study were used as controls (Nanda and Romeo, 1975). This control group consisted of the same sample size as the experimental group

Materials and Methods

Thirty-two female Wistar albino rats were housed in a constant temperature room with a controlled light cycle (12 hours of light and 12 hours of dark). Female rats were kept with male rats from 5:00 P.M. to 8:00 A.M.. At the end of the mating period, vaginal smears were made to determine pregnancy. If the test was positive, it was noted as day 0 of gestation.

The pregnant rats were divided into two equal groups. Group I, comprised of sixteen rats, received 60.000 I.U. of vitamin A palmitate once a day via stomach tube, from day 10 to day 13 of gestation. Group 2 received 0.5 mg of dexamethasone phosphate from day 10 to day 13, twice a day by an intraperitoneal route. On day 14 of gestation, four rats from each group were given 250 μCi/Kg of body weight of tritiated thymidine (^{3}HTdr) via intraperitoneal injection. Similarly, four rats from each group were given the same treatment on days 15, 16, and 17 of gestation. All rats were killed one hour after the administration of ^{3}HTdr. Four fetal heads from each rat were recovered and placed in Bouin's solution for 24 to 48 hours. They were subsequently embedded in paraffin and serially sectioned at a thickness of 5 μm. The sections were coated with Kodak NTB-2 emulsion and exposed for three weeks. The sections were developed and stained with hematoxylin-eosin.

The palatal processes in the frontal plane of the fetal head were studied in three regions, namely, anterior, middle, and posterior. The palatal processes in each region were further divided into three arbitrarily defined areas, namely, tip, middle, and lateral. The method and description of dividing palatal processes into regions and areas has been described in detail in an earlier publication (Nanda and Romeo, 1975).

The labeling indices of the mesenchymal cells of the palatal processes, maxilla, mandible, nasal septum, and tongue on different days were determined by computing the percentage of labelled cells per 1000 cells. Counting was accomplished by the use of a grid in the eyepiece of a Zeiss microscope.

The labeling indices of mesenchymal cells of the different areas and regions were statistically analysed using standard analysis of variance and Duncan's multivariance analysis. The results of the labeling indices of the vitamin A and dexamethasone groups were compared to controls (Nanda and Romeo, 1975) to ascertain if there was any significant difference in cell proliferation activity as indicated by ^{3}HTdr uptake. The control sample also consisted of 16 pregnant rats, and four fetal heads from each rat were used to study cell proliferation.

Results

Gross malformations were noted in both the vitamin A and dexamethasone groups. In the central nervous system, exencephaly and meningo-encephalocele were noted. The eyes demonstrated exopthalmos and ablepharia. The limbs of several fetuses had a short radius, syndactyly, and missing claws. The craniofacial complex demonstrated cleft palate, microstomia, short snout, and occasional absence of the external ear. All experimental groups of fetuses exhibited unfused palatal processes on day 17.

Figure 1 shows cumulative mean labeling index of mesenchymal cells of palatal processes in all groups. The values of the dexamethasone group were significantly lower ($p<0.05$) than the control and vitamin A groups on all four days. In the vitamin A group the mean labeling index was significantly less ($p<0.05$) only on day 14 and 15. No significant drop in the mean labeling index of the vitamin A group was observed from day 15 to 16 as was seen with the control group. Figures 2, 3, and 4 describe the mean labeling index in the different regions of the palate on different days. The mean labeling indices of the dexamethasone group were significantly lower ($p<0.05$) than the control and vitamin A groups on all days in all regions except on day 17 in the middle region, where no significant difference was observed between the two experimental groups (Figure 3). The mean labeling index of the vitamin A group exhibited significantly more increase

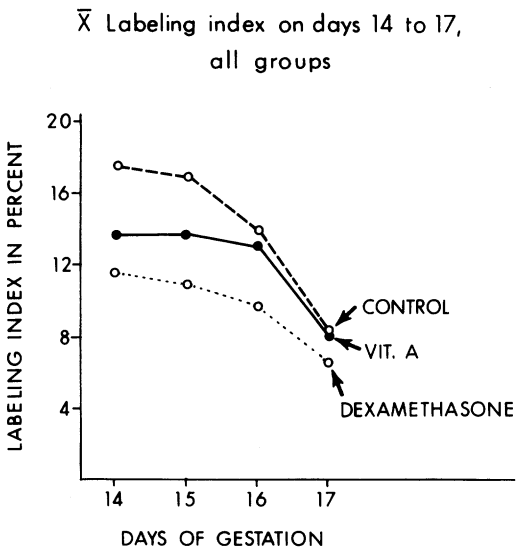

FIGURE 1. Mean labeling index of mesenchymal cells of the palatal processes on different days of gestation in different groups.

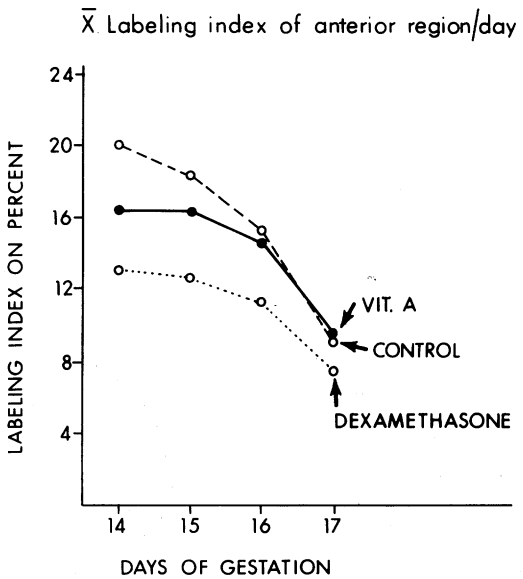

FIGURE 2. Mean labeling index in the anterior region of the palatal processes in different groups on days 14 to 17.

from day 15 to 16 in the posterior region as compared to the other two regions.

Table 1 describes the mean labeling index in different areas of the anterior, middle, and posterior regions of the palate on different days for the control, vitamin A, and dexamethasone groups.

Table 2 describes the mean labeling index in different areas of the head on different days. No significant differences were observed in the mean labeling indices in the tongue on different days between the control and vitamin A groups. In the nasal septum and mandibular areas, the labeling index was

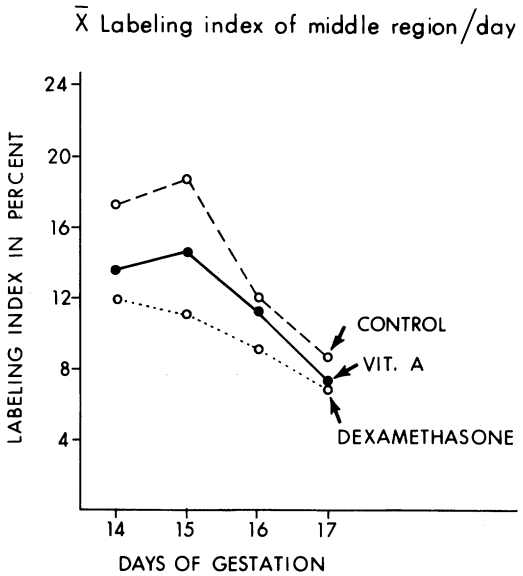

FIGURE 3. Mean labeling index in the middle regions of the palatal processes in the different groups on days 14 to 17.

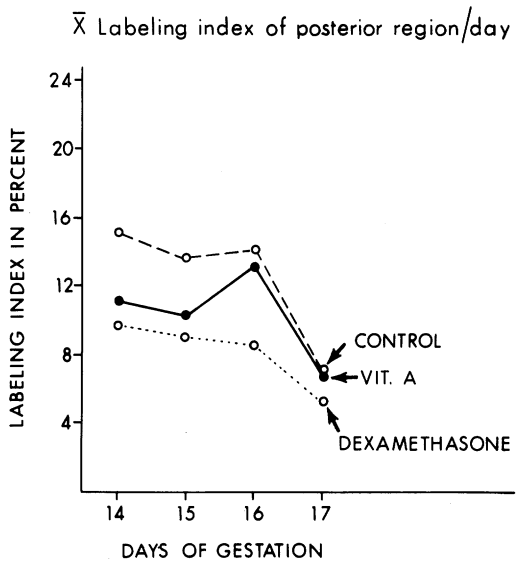

FIGURE 4. Mean labeling index in the posterior region of the palatal processes in the different groups on days 14 to 17.

TABLE 1. A comparison of the mean labeling index of different areas of the palate in the anterior, middle, and posterior regions. (Vitamin A and Dexamethasone were statistically compared to the control utilizing Duncan's multivariant analysis. Those values with an asterisk were *not* found to be significantly different.)

region	*area of palate*	*days*	*labeling indices*			*statistical difference*[1]		
			normal	*vitamin a*	*dexamethasone*	*norm. vs. vit. a*	*norm. vs. dex.*	*vit. a vs. dex.*
	Tip							
		14	28.2	21.0	15.3	**	**	**
		15	22.9*	22.0*	14.8		**	**
		16	19.0	16.1	12.7	*	**	*
		17	7.0*	11.0	7.1*	*		*
Anterior	Middle							
		14	18.0	15.4	13.2	*	**	*
		15	18.4	16.1	13.7	*	**	*
		16	17.2*	18.2*	12.4		**	**
		17	9.9*	9.5*	7.3		*	*
	Lateral							
		14	14.8	12.9	10.8	*	**	*
		15	14.0	11.1*	9.2*	*	**	
		16	9.9*	9.9*	8.9*			
		17	10.1	7.9*	8.0*	*	*	

region	*area of palate*	*days*	*labeling indices*			*statistical difference*[1]		
			normal	*vitamin a*	*dexamethasone*	*norm. vs. vit. a*	*norm. vs. dex.*	*vit. a vs. dex.*
	Tip							
		14	22.4	19.2	14.7	*	**	*
		15	25.8	20.9	12.6	**	**	**
		16	11.3*	12.2*	9.2		*	*
		17	7.2*	7.3*	6.1*			
Middle	Middle							
		14	16.3	11.5*	10.9*	**	**	
		15	17.5	12.2	10.4	**	**	*
		16	14.8	13.0	11.2	*	*	*
		17	10.4	7.8*	7.7*	*	*	
	Lateral							
		14	13.0	10.1*	10.5*	*	*	
		15	12.9	10.6*	10.7*	*	*	
		16	10.3	8.6*	7.1*	*	*	
		17	8.4*	7.0*	6.7*			

region	*area of palate*	*days*	*labeling indices*			*statistical difference*[1]		
			normal	*vitamin a*	*dexamethasone*	*norm. vs. vit. a*	*norm. vs. dex.*	*vit. a vs. dex.*
	Tip							
		14	18.7	12.1*	11.8*	**	**	
		15	18.6	13.8	11.5	**	**	*
		16	14.4*	15.6*	9.7		**	**
		17	6.2*	5.6*	5.1*			
Posterior	Middle							
		14	15.2	12.4	10.2	*	**	*
		15	12.4	9.8*	9.3*	*	*	
		16	17.4	14.8	8.9	*	**	**
		17	7.2*	7.9*	5.6			*
	Lateral							
		14	11.3	8 .8*	7.2*	*	*	
		15	10.2	7.1*	6.3*	*	*	
		16	10.6	9.0	6.9	*	*	*
		17	7.3*	6.6*	5.1*			

[1] Determined by Duncan's multivariate analysis.

* Significant differences at $P < .05$ level.

** Significant differences at $P < .01$ level.

TABLE 2. Meal labeling index in different areas of head on different days. (Comparison of Two Experimental Groups with Control Group)

days	*mandible*			*nasal septum*			*maxillary area*			*tongue*		
	control	*vit. a*	*dexm.*	*control*	*vit. a*	*dexm.*	*control*	*vit. a*	*dexm.*	*control*	*vit. a*	*dexm.*
14	27.1	20.8*	16.3*	18.3	14.2*	13.7*	18.4	13.7*	11.4*	29.7	29.2	21.8*
15	22.5	18.1*	13.7*	19.2	15.9*	12.8*	16.3	14.6*	9.8*	26.5	25.7	20.6*
16	18.2	16.7	10.1*	9.8	7.8**	7.2**	14.2	11.7**	9.2*	23.1	21.9	14.3*
17	11.5	9.5**	7.2**	7.1	6.4	5.5	10.7	9.8	8.0**	19.8	18.4	13.5*

* Significant Differences at $P < 0.01$ Level Utilizing t-Test.
** Significant Differences at $P < 0.05$ Level Utilizing t-Test.

found to be significantly more ($p<.01$) disturbed in dexamethasone group as compared to the vitamin A group ($p<.05$).

Discussion

The present investigation demonstrates that excess vitamin A and dexamethasone inhibit DNA synthesis of mesenchymal cells of palatal processes as revealed by tritiated thymidine uptake. In addition, both teratogens produce anomalies which are similar morphologically and histologically. However, their effect on cellular proliferation differs significatly. The inhibition of DNA synthesis resulting from dexamethasone administration is more severe than that produced by hypervitaminosis A as compared to controls.

Dexamethasone, a glucocorticoid preparation, has been found to be 300 times more potent than hydrocortisone and prednisolone when given to mice in teratogenic doses (Nanda, May, and Lite, 1977). Although cortisone in teratogenic doses does not cause cleft palate in Wistar rats, the administration of dexamethasone in the present study resulted in unfused palatal processes in all 17 day old fetuses (Vannier, Jequier, and Jude 1969; Nanda, 1971). Although no parallel study was found in the literature on the effect of dexamethasone on cellular proliferation of mesenchymal cells of palatal processes, the results support the findings of investigators who have utilized other glucocorticoid preparations, such as cortisone and triamcinolone (Mott et al., 1969; Zimmermann, Andrew, and Kalter, 1970). It has also been shown that glucocorticoids inhibit RNA synthesis and possibly block the translation of proteins, resulting in the production of cleft palate (Zimmermann, Andrew, and Kalter, 1970).

An analysis of ^{3}HTdr uptake in different areas of palatal processes in different regions reveals that the most severe inhibition is in the tip and middle areas of the palate on days 14 and 15. Normally, the palatal processes attain a horizontal position on day 16 in Wistar albino rats. Inhibition of cell growth could disturb this change. However, failure of horizontilization on day 16 observed in dexamethasone treated fetuses cannot be solely attributed to the disturbance in cell proliferation of mesenchymal cells of the palate as similar changes were also seen in mandibular and maxillary areas, the nasal septum, and the tongue on different days of gestation as compared to controls. It has been repeatedly shown that a normal coordinated development of mandible, nasal septum, maxillary area, and tongue play an important role in horizontalization of the palatal processes.

The findings associated with excess vitamin A support previous results of Kochhar (1968) and Nanda (1969) regarding significantly less ^{3}HTdr uptake by mesenchymal cells of the palatal processes. Our data indicate that certain areas and regions of the palate show disturbances which are more severe on days 14 and 15, especially in the tip and middle areas. This decrease in proliferative activity was not as severe as in the dexamethasone group. The most significant finding was a "catch-up" in the ^{3}HTdr uptake by mesenchymal cells on days 16 and 17, an observation not noted in the dexamethasone group. During the four-day observation period, the cell proliferation levels of the vitamin A group improved steadily to almost no significant difference in the majority of the areas and regions on day 17 as compared to controls. This is a significant finding as a previous in vitro study (Nanda, 1974) showed that palatal processes of fetuses of vitamin A treated rats had the potential to fuse when grown in a normal culture medium, whereas palatal explants from the dexamethasone group never

attained fusion (Nanda, 1969). These observations suggest that, as both teratogens were given on day 13 of gestation, their effects are similar and most severe on days 14 and 15. The absence of a "catch-up" phenomenon in the dexamethasone group suggests that it is a more potent teratogenic agent.

In the vitamin A group, significantly less ^{3}HTdr uptake by mandibular and maxillary areas as well as nasal septums is noted as compared with the controls. No differences were found in the tongue. These findings support the hypothesis that vitamin A retards the growth of palatal processes in vivo and that subsequently the processes do not come in contact with each other at a morphologically determined time (Nanda and Romeo, 1975). Moreover, the lateral growth of the head and disturbed growth of the craniofacial areas adjacent to the processes also prevent the subsequent fusion of the palatal processes. The present results suggest that, although various teratogenic agents may produce an anomaly of similar morphological nature, their effect on cell behavior may vary significantly.

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