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Effect of vitamin B_{12} on cleft palate induced by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin and dexamethasone in mice

Key words: Cleft palate, Transforming growth factor-β3 (TGF-β3), Activin receptor-like kinase 5 (ALK5), Vitamin B_{12}, 2, 3, 7, 8-Tetrachlorodibenzo-*p*-dioxin, Dexamethasone

Introduction

Cleft palate is one of the most common birth deficits in humans. It is considered a multifactorial disease and both genetic and environmental factors play a role in its development. In our previous study, we have established that 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) and dexamethasone (DEX) induced cleft palate in mice However, the molecular mechanism of TCDD and DEX induced cleft palate were largely unknown. Meanwhile, an effect of vitamin B 12 on the combined toxicity of TCDD and DEX for palatal development has not been investigated.

Objective

Investigate the effect of vitamin B 12 on palatal development by co-administration of TCDD and DEX.

Methods

To examine the morphological and histological features of the palatal shelf and expression levels of key signaling molecules (trans-forming growth factor-β3 (TGF-β3) and TGF-β type I receptor (activin receptor-like kinase 5, ALK5)) during pala-togenesis among a control group (Group A), TCDD+DEX exposed group (Group B), and TCDD+DEX+vitamin B 12
exposed group (Group C).

Results

TCDD+DEX expose-induced cleft palate morphology deficit failed to be prevented by vitamin B 12

Table 1 Fetal mice development and incidence of cleft palate among different groups

Group	Pregnant mice	Live births	Fetuses with clefts	Frequency of clefts (%)
A	6	36	1	2.8
B	6	37	37	100
C	6	34	34	100

Split χ^2 test showed that there was no difference between Groups B and C (P>0.05)

Table 2 Average distance between the two cut-off points of opposing palates among different groups

Group	Distance (μm)		
	GD 13.5	GD 14.5	GD 15.5
A	313.3 ± 8.0^{ac}	0^{ad}	0^{ad}
B	328.1 ± 10.3^{ac}	241.5 ± 7.1^{bd}	154.1 ± 47.5^{be}
C	323.7 ± 9.0^{ac}	225.9 ± 12.8^{bd}	100.0 ± 13.9^{be}

Data are expressed as mean±SD. $^{a-b}$The same letter denoted values that are not significantly different within the same time period in different groups (P>0.05). $^{c-e}$The same letter denoted values that are not significantly different at different time periods in the same groups (P>0.05)

Results

Vitamin B 12 partially restored the altered epithelium differentiation

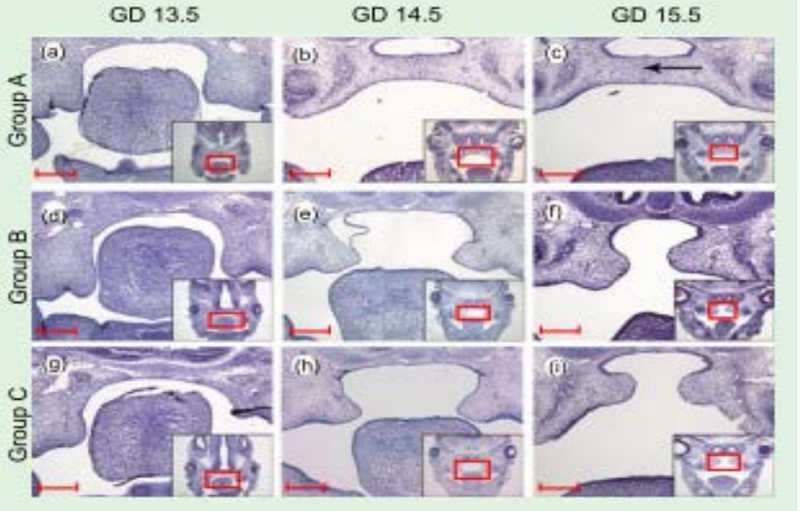

Fig. 2 Histological sections of embryonic palate in three groups

(a–c) Group A on GD 13.5, GD 14.5, and GD 15.5, respectively; (d–f) and (g–i) Groups B and C, respectively, at different periods. On GD 13.5, the size and morphology of palate among three groups were similar (a, d, and g). On GD 14.5, the palate shelves in Group A were elevated and already fused. While in Groups B and C, the size was smaller and there was no contact between opposing palate shelves though they could be elevated to normal position (b, e, and h). At the time of GD 15.5, the fused palate was further developed in Group A, but in Groups B and C the two opposing shelves remained separated (c, f, and i). Each picture (100×) was an enlarged view of the frame in the inset (40×). Black arrow indicates fused palate. Scan bar=100 μm

Results

Vitamin B 12 partially restored the altered epithelium differentiation

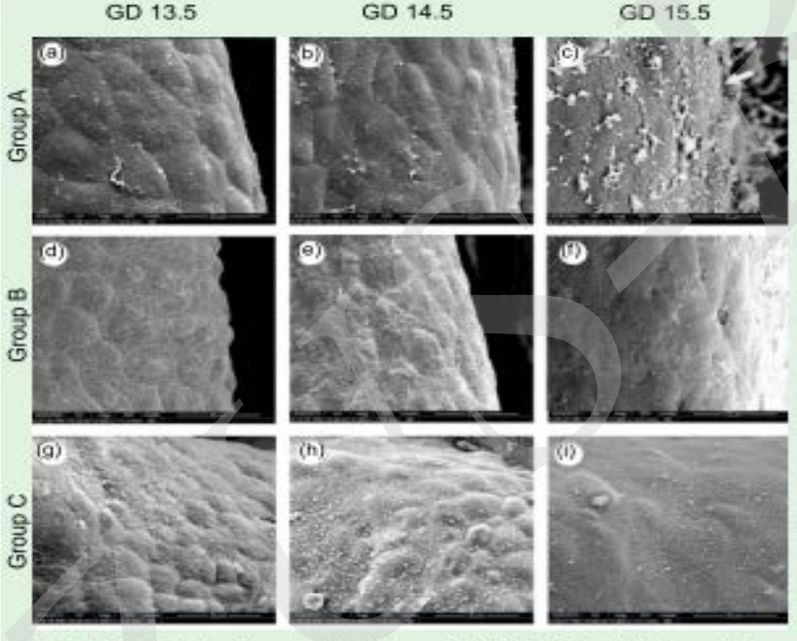

Fig. 3 Medial edge epithelium (MEE) of palatal palates among the three groups at different times using scanning electron microscopy (SEM)

(a–c) Normal cell morphology throughout the palatogenesis in control group embryos. (d–f) The epithelial cell transdifferentiated, underwent hyperplasia, and formed abnormal epithelium in TCDD+DEX exposed embryos. (g–i) There were some differences from that of Group B in the antagonistic group with a few of the bulging cells on the epithelium on GD 13.5 and no obvious damage and desquamated epithelium on GD 14.5 and GD 15.5 (5000×). White arrow indicates fused palate

Results

Vitamin B 12 could partly rescue the altered expression levels of TGF-β3

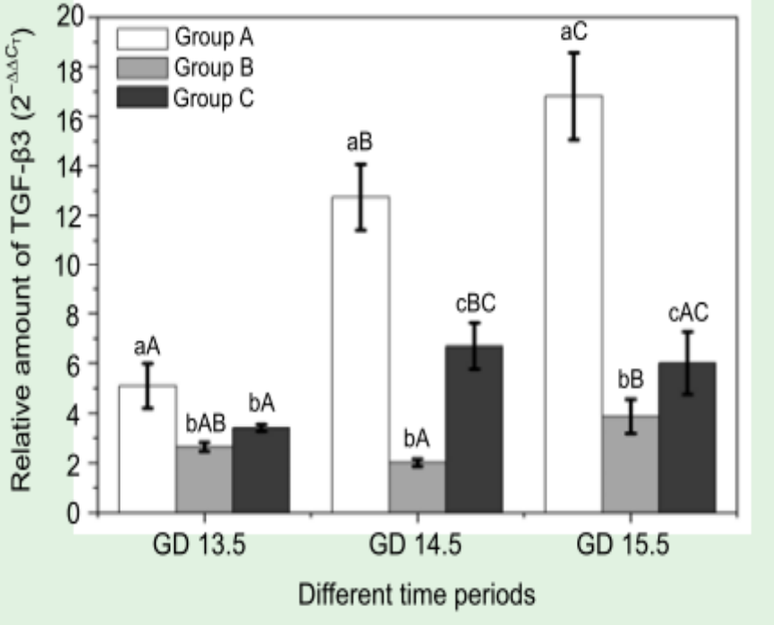

Fig. 4 Time-course of TGF-β3 expression in palatal shelves among three groups

$^{a-c}$ The same letter denoted values that were not significantly different within the same time period in different groups (P>0.05). $^{A-C}$ The same letter denoted values that were not significantly different at different time periods in the same groups (P>0.05)

Results

Vitamin B 12 could partly rescue the altered expression levels of ALK5

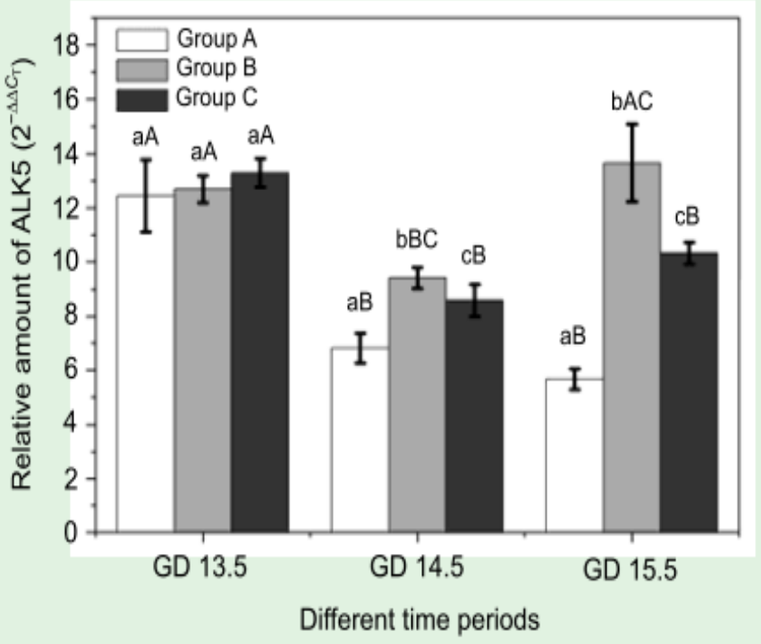

Fig. 5 Time-course of ALK5 expression in palatal shelves among three groups

[a–c] The same letter denoted values that were not significantly different within the same time period in different groups (P>0.05). [A–C] The same letter denoted values that were not significantly different at different time periods in the same group (P>0.05)

Conclusions

Vitamin B 12 may inhibit the expression of some cleft palate inducers such as TGF-β3 and ALK5 in DEX+TCDD exposed mice, which may be beneficial against palatogenesis to some degree

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