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ORIGINAL ARTICLE

Biotin supplementation to pregnant dams prevents cleft palate in biotin-deficient fetal mice

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Summary

To clarify the role of biotin in palatal development, we investigated the effects of biotin supplementation to pregnant dams on the development of palatal processes in biotin-deficient fetal mice. Pregnant mice were administered either a biotin-deficient diet or biotin-supplemented (control) diet from day 0 of gestation (dg 0). Several mice in the biotin-deficient group were administered biotin intraperitoneally at 0900 on dg 10. After biotin administration, they were fed the biotin-supplemented diet during day 10–12, 10–13 or 10–14 of gestation, and then switched to a biotin-deficient diet until dg 15. The incidence of cleft palate was 63.6% in the biotin-deficient group, whereas no cleft palate was noted in the other groups. This suggested that biotin is necessary for palatal development in the initial stage of palatogenesis.

Introduction

Biotin serves as an essential cofactor for carboxylases in fatty acid synthesis, branched-chain amino acids (BCAA) metabolism, and gluconeogenesis[1]. Biotin deficiency rarely occurs in humans, as biotin is well distributed in food. Clinical signs characteristic of biotin deficiency include dermatitis, hair loss, and neurological signs. It has been reported that marginal biotin deficiency is common during normal pregnancy[2,3], which suggested that pregnancy increases the dietary requirement for biotin.

Biotin is essential for reproduction and embryonic development in mammals. Maternal biotin deficiency during gestation causes severe malformations, such as cleft palate, micrognathia, and micromelia, in mouse fetuses[4,5]. In mice, palatal outgrowths are first detectable by embryonic day 11.5 (E 11.5) and palatal fusion is complete by E 15.5[6]. When pregnant mice were fed a biotin-deficient diet before biotin dosing on day 14 of gestation (dg 14, day of plug = dg 0), the biotin concentration in palatal processes on dg 15 was recovered to the same level as that in the control group (biotin-supplemented) with no significance[7]. This suggested that the biotin concentration in the palatal processes rapidly increased to the control level after switching to a biotin-supplemented diet. Although an increase in palatal fusion was observed in the dg 12 supplemented groups, it was significantly lower than that in the control group. Furthermore, the incidence of cleft palate was significantly lower in the dg 11 supplemented group than in the biotin-deficient group[8]. There was no significant difference in the incidence of cleft palate between the dg 11 supplemented group and the control group. These studies suggest that biotin is necessary for the normal development of the palatal process in the mouse fetus at dg 11 and earlier.

The mechanism by which biotin deficiency interferes with palatal development is not yet known. To clarify the role of biotin in palatal development, the present study was designed to examine the effects of biotin supplementation to pregnant dams on palatal development in biotin-deficient fetal mice.

Materials and Methods

Animals

Nulliparous female ICR mice, aged 7 weeks, were obtained from CLEA Japan Inc. (Tokyo, Japan). All animals, including males used for mating, were housed for 2

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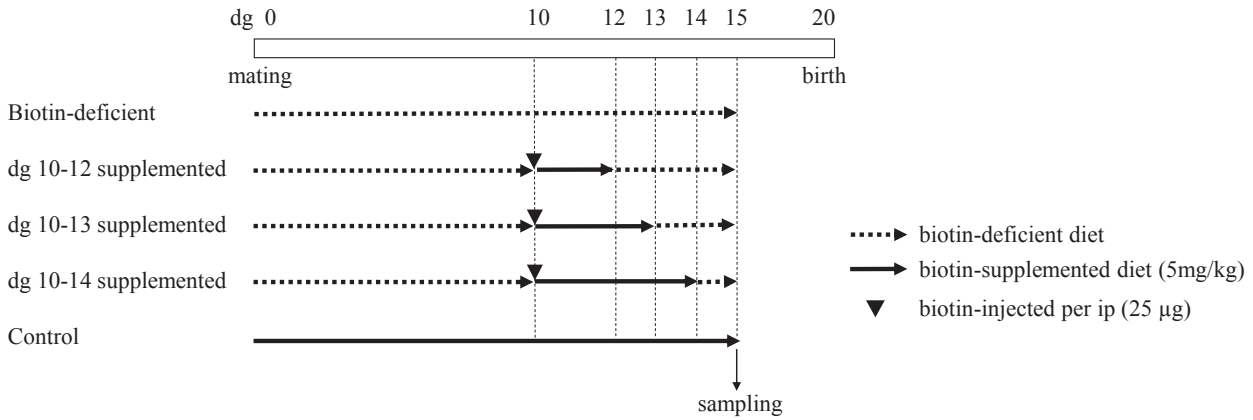

Fig. 1 Experimental protocol

weeks before mating in an animal room maintained under 12-h light-dark cycle conditions of 0900–2100 and at a constant room temperature of 22 ± 1℃. The female mice were mated with healthy males for a short period in the morning (0900–1100). The day when a copulation plug was detected at the end of mating was designated as dg 0 (Fig. 1). Pregnant females were randomly divided into two groups: a biotin-deficient group fed a biotin-deficient diet (Oriental Yeast, Tokyo, Japan) and a control group fed a biotin-supplemented diet (biotin-deficient diet supplemented with 5 mg of biotin/kg). Several mice in the biotin-deficient group were intraperitoneally administrated 250 μL biotin solution (0.1 mg/mL) at 0900 on dg 10. After biotin administration, they were fed the biotin-supplemented diet during day 10–12, 10–13, or 10–14 of gestation. These mice were administered the biotin-deficient diet on dg 12, 13, or 14, which was continued until dg 15. These groups were defined as the dg 10–12 supplemented, dg 10–13 supplemented and dg 10–14 supplemented groups, respectively. Mice were housed in stainless steel cages with a wire-bottomed floor, and given the diets and distilled water ad libitum during experimental periods.

All experimental procedures, including the care and treatment of mice described in this paper, were approved by the Institutional Animal Care and Use Committee of the School of Human Science and Environment, University of Hyogo (#205).

Sample preparation

Pregnant mice were killed on dg 15. Fetuses were collected from the uterus and immersed in phosphate-buffered saline (PBS). The placenta was removed from the fetuses and the number of fetuses was confirmed. Palatal processes were carefully dissected from the head in fetuses under a dissecting microscope using a technique described previously[9]. These samples were immediately stored at −80℃ until analysis.

Biotin determination

Palatal processes were lysed with solubilization buffer (1 % Triton-X100 and 0.02 % protease inhibitor in PBS). These samples were homogenized on ice. The homogenate samples were centrifuged at 21,130 g for 15 min at 4℃ and the supernatant was collected.

The biotin concentration in palatal processes was measured using a microtiter plate adaptation of a microbiological assay with *Lactobacillus plantarum* ATCC 8014[10-12]. This bacterium was obtained from American Type Culture Collection, which is generally used for assessing the quantity of some vitamins and cultured in a microtiter plate for 24 h. The cell density was measured at 610 nm. As biotin in samples partially existed in a protein-binding form, to measure total biotin, the sample solution was pretreated with 2.25 M H_2SO_4 at 121℃ for 60 min and neutralized with 4.5 M NaOH. Total and free-form biotin concentrations in palatal processes are expressed as nmol/g protein.

Statistical analysis

Values are expressed as the mean ± SD. Statistical comparison of means among experimental groups was conducted by one-way ANOVA and Tukey-Kramer tests. Incidences of cleft palate were compared between the groups using the χ^2 test. P values by χ^2 tests were corrected by the Bonferroni method. Statistical analysis of the data was performed using SPSS 24 (IBM Corporation). Differences were considered significant if *P*-values were less than 0.05 in all analyses.

Results

Body weight and fetal growth

The effects of biotin supplementation to pregnant dams on pregnant and fetal mice are presented in Table 1. There was no significant difference in food intake of

Table 1 The effects of biotin supplementation to pregnant dams on pregnant and fetal mice

	Dietary groups				
	Deficient	day 10–12 of gestation supplemented	day 10–13 of gestation supplemented	day 10–14 of gestation supplemented	Control
Dams					
No. dams examined	3	3	3	3	3
Body weight gain during dg 0–15 (g)	18.2 (17–19)[a]	22.2 (22–23)[b]	17.7 (17–19)[a]	19.3 (18–20)[ab]	22.2 (21–23)[b]
Fetuses					
No. fetuses examined	38	47	38	42	43
Mean	12.7 (12–13)	15.7 (13–18)	12.7 (12–13)	14.0 (13–15)	14.3 (12–16)
Body weight on dg 15 (g)	0.39 ± 0.032	0.42 ± 0.043	0.42 ± 0.053	0.43 ± 0.023	0.49 ± 0.022
Cleft palate (%)	63.6[c]	0.0[d]	0.0[d]	0.0[d]	0.0[d]

Values are mean ± SD or mean (min-max).
Different superscript letters indicate significant difference between groups: [ab]$P < 0.05$; [cd]$P < 0.001$.

dams during dg 0–15, body weight of fetal mice, or fetal number. Body weight gain of dams was significantly lower in the biotin-deficient group than in the control group. There were significant differences among the biotin-supplemented groups. There were no clinical signs of biotin deficiency in dams from these groups. Although 63.6 % of fetal mice in the biotin-deficient group had cleft palate, no cleft palate was observed in all biotin-supplemented groups and control group.

Biotin concentration

The biotin concentration of palatal processes in mouse fetuses on dg 15 are presented in Fig. 2. The total biotin concentration in the biotin-deficient palatal processes was significantly lower than those in the biotin-supplemented groups (day 10–12, 10–13, or 10–14 of gestation supplemented) and control group. There were no significant differences in total biotin among the biotin-supplemented groups (day 10–12, 10–13, or 10–14 of gestation supplemented). The free biotin concentration in the biotin-deficient and all biotin-supplemented groups (day 10–12, 10–13, or 10–14 of gestation supplemented) was significantly lower than that in the control group. There were no significant differences in the free biotin in palatal processes among the biotin-supplemented groups (day 10–12, 10–13, or 10–14 of gestation supplemented).

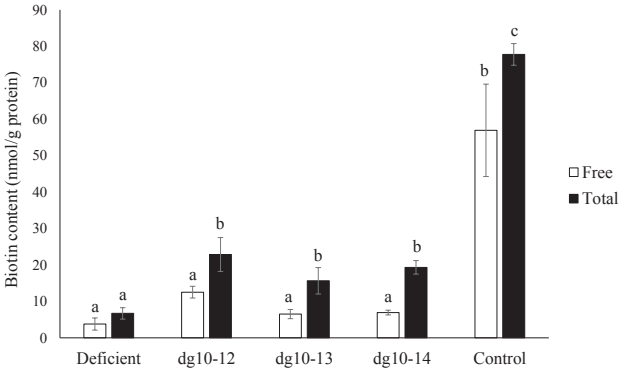

Fig. 2 Biotin concentration in palatal processes on dg 15
Values are mean ± SD.
Different superscript letters indicate significant difference between groups.

Discussion

Maternal biotin deficiency causes severe malformations in mouse fetuses[4,5]. The main malformations caused by biotin deficiency are cleft palate, micrognathia, and micromelia. However, the specific cause has not yet been defined. Although the biotin-deficient state continued until immediately before biotin dosing on dg 14, the biotin concentration in palatal processes on dg 15 recovered to the same level as that in the control group (biotin-supplemented) with no significance[7]. This suggested that the biotin concentration in palatal processes rapidly increases to the control level after switching to a biotin-supplemented diet.

When mouse dams were fed the biotin-deficient diet from dg 0, the incidence of fused palatal processes was less than 10 % in the biotin-deficient mice on dg 15[7]. Although an increase in the incidence of palatal fusion was observed in the day 12–14 of gestation supplemented mice compared with that in the biotin-deficient mice, it was significantly lower than that in the control mice receiving biotin from dg 0. Furthermore, the incidence of cleft palate was significantly lower in the dg 11 supplemented mice than that in biotin-deficient mice[8]. There was no significant difference in the incidence of cleft palate between the dg 11 supplemented mice and the control groups. This suggests that biotin is necessary for the normal development of palatal processes in the mouse fetus at dg 11 and earlier. In the present study, no cleft palate was noted in the biotin-supplemented groups (day 10–12, 10–13, or 10–14 of gestation supplemented). Cleft

palate may prevent by biotin administration to pregnant mice with biotin deficiency at dg 10–12. The total biotin concentration in the biotin-deficient palatal processes was significantly lower than those in the biotin-supplemented groups (day 10–12, 10–13, and 10–14 of gestation supplemented) and control group. There were no significant differences in total biotin among the biotin-supplemented groups (day 10–12, 10–13, and 10–14 of gestation supplemented). This suggested that the length of supplementation period do not have an effect because a sufficient amount of biotin was administered. Further studies are needed on mice fed less biotin than in the present study.

Cleft lip and/or cleft palate are the most common craniofacial malformations, and have incidence rates in the Japanese population than in other ethnic groups[13]. Most cases of cleft lip and cleft palate are caused by multiple genetic and environmental factors. In mice, secondary palate development starts at E 11.5 and palatal process fusion is completed at E 15.5[6]. A recent study demonstrated that environmental factors interfere with histone acetylation, thereby leading to cleft palate[14]. The expression of HCS, which catalyzes the covalent binding of biotin to histones, depends on biotin[15]. These studies suggest that an alternation in histone biotinylation by maternal biotin deficiency affects palatal development.

In conclusion, we demonstrated that biotin administration to pregnant dams from day 10 to 12 of gestation prevents cleft palate in biotin-deficient fetal mice. This suggests that biotin is required in the initial stage of palatogenesis. It should be clarified whether the blood and liver biotin levels are restored by examining the temporal changes in blood and liver biotin levels after biotin administration. Further studies are needed to clarify biotin requirements during midgestation to prevent cleft palate.

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