KOREA J. ORTHOD. 2001 : 31(6) : 579-87

A study of TGF-β Expression Patterns In Cleft Palate Formed Rats Induced by BAPN

Ki-Chul Tae[1] · En-Chel Kim[2]

Cleft palate has been studied with epidemiologic and molecular methods, and many etiologic factors have been examined closely. Among the research methods, biologic molecule research has been the most important method for cleft palate formation study. The TGF-β played an important role in cell migration, epithelial-mesenchymal transdifferentiation, extracellular matrix synthesis and deposition. But there was not much research on the correlation cleft palate induced by beta-aminonitroproprionitrile(BAPN) and TGF-β expression. The purpose of the present study was to examine how TGF-β is expressed in cleft palate rats. 4 Timed-pregnant Sprague-Dawley rats were obtained on the 10th gestation day. On the 13th day of gestation, BAPN-monofumarate salts ($(C_3H_6N_2)_2 \cdot C_4H_4O_4$) were individually, orally administered to 3 pregnant rats at a ratio of 1g/kg body weight. And 4 pregnant rats were sacrificed on day 20 post coitus (p.c.). The TGF-β expression in the cleft formed rats fetuses showed the following patterns :

1. Osteoblast and mesenchymal cells of the cleft palate rats were of low expression compared with those of the control rats.
2. The cleft palate rats didn't show any difference in the TGF-β expression of osteocyte from the control rats.
3. In western blot analysis, the thickness of band of TGF-β in the cleft palate rats was thinner and more diluted than that of the control rats.

Key words : Cleft palate, TGF-β, BAPN, Immunohistostain, Western blot

Craniofacial deformity shows several different patterns and cleft palate, with or without cleft lip, is one of the frequent malformations. Approximately 15 of every 1000 living newborn infants have some form of craniofacial malformation.[1] Cleft lip and/ or palate account for approximately 65% of all congenital anomalies.[1] The clinical treatment of cleft lip and palate usually has poor results, particularly if inheritance patterns for malformations exist or if there has been known exposure to specific teratogens. Generally, cleft lip and/ or palate is caused by pertuvation or insults during embroynic development between the fourth and 10th weeks of gestation in human beings.[3] The etiology of cleft palate is environmental insults, maternal diseases, chemotherapy, radiation, alcohol, excess retinoic acid and anticonvulsant medications or genetic susceptibility transmitted through Mendelian or non-Mendelian inheritance patterns.[4] 8~10% of congenital malformations are thought to be caused by teratogenic agents.[2]

[1] *Assistant professor, Dept. of Orthodontics, Dental school, Wonkwang Dental Institute, WonKwang University*
[2] *Assistant professor, Dept. of Oral Pathology, Dental school, Won kwang University*
(This article was supported by 2001 Wonkwang University granted)

Reprint requests to : Prof. Ki-Chul Tae
344-2 Shinyong-Dong Ik-San Cheon-Buk 570-711
Department of Orthodontics, College of Dentistry, Wonkwang University
82-63-850-1960 / kkojji@wonkwang.ac.kr

Table 1. Number of rat fetuses sacrificed 20 days p.c.

	Group 1	Group 2
Number delivered	12	33
Number of cleft formed fetuses	0	6

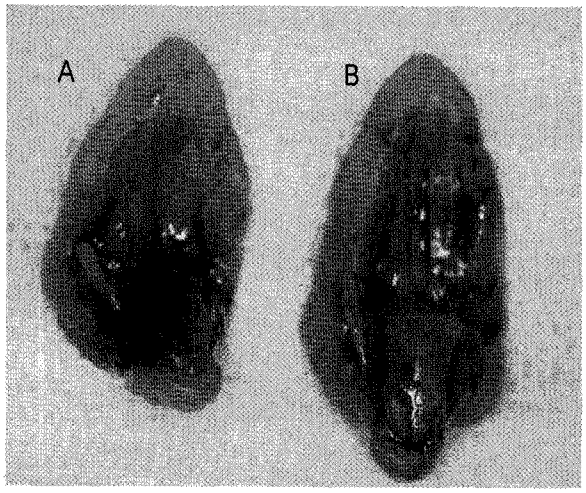

Figure1. Palate feature comparison between control and experimental fetuses
A. Control rat fetus B. Cleft-formed rat fetus

Mammalian palatogenesis, a complex process involving cell proliferation, migration, differentiation, extracellular matrix production, remodeling, epithelial-mesenchymal transdifferentiation and cell death, is accomplished by fusion and merging on the nasal cartilage and on the two horizontal shelves of the maxillary process.[4,5] In the histologic view, the process of fusion and merging happens in epithelial cells and mesenchymal cells. Fusion occurs when epithelial cells and mesenchymal cells of both shelves are in a consolidation process, when mesenchymal cells penetrate to the contact area. Merging creates a tremendous forward and downward growth of the paired shelves.[6] These biological processes are regulated by growth factors, which are polypeptides similar to hormones.[7]

In 1978, DeLarco and Todaro found a polypeptide that was a transformed phenotype by a retrovirus when fibroblast cells were cultured. This peptide was labelled a transforming growth factor (TGF). The TGF was divided into TGF-α and TGF-β.[8] The TGF-β participated in cell migration, epithelial-mesenchymal transdifferentiation, extracellular matrix synthesis and deposition. Previous situ hybridization studies have shown that TGF-β is abundant in fetal bone and megakaryocytes. And it was showed that TGF-β was closely associated with connective tissue, cartilage, bone, and tissue derived from neural crest mesenchyme with immunohistochemical investigation.[9,10] Also, TGF-β was closely involved in palate formation. Failure of any of these cellular activities, due to genetic or environmental factors, may result in the formation of cleft palate, so clinical problems of considerable significance were evoked.[11,12] Many studies have focused on the biologic etiology of cleft palate, but there was little in vitro study that investigated the correlation between cleft palate mice due to teratogenic agents and TGF-β expression patterns.

This study attempts to discover the correlation between cleft palate formed by BAPN administration and TGF-β expression utilizing Immunohistostain and Western blot analysis.

MATERIALS AND METHODS

4 Timed-pregnant Sprague-Dawley rats were obtained on the 10th gestation day (Day 1-vaginal smear) from the Damul Breeding Laboratories(Korea Co.). Rats were housed in lucite cages lined with soft cedar shavings and fed a diet of Rodent Chew and water ad lid. They were divided into 1 control pregnant rat and 3 experimental pregnant rats. At gestation day 13, BAPN monofumarate salts ($(C_3H_6N_2)_2 \cdot C_4H_4O_4$, Sigma Co.) were individually, orally administered to 3 pregnant rats at a ratio of 1g/kg body weight. 4 pregnant rats were sacrificed on day 20 post coitus(p.c.), because palatal closure in rat finishes on day 17 p.c. Total number of fetuses from 4 pregnant rats was 45, and they were divided into 2 groups(Table 1, Figure 1). And 6 cleft fetuses were delivered from 3 BAPN dosed pregnant rats.

Group 1 : fetus group from derived 1 control pregnant rat
Group 2 : fetus group from 3 BAPN dosed pregnant rats

Table 2. Comparison of TGF-β expression in both groups.

	osteoblast	osteocyte	mesenchymal cell	Epithelial cell
Control group	++ ~ +++	++	++	++
Cleft group	+ ~ ++	++	+	+ ~ ++

We have used 3 fetuses for immunohistostain, 3 for western blot analysis, and 3 for control group.

1. Immunohistochemical stain

All fetus skulls were preserved in 10% paraformaldehyde for 24 hours. After fixation, the specimen was embedded in wax. For staining, histostain bulk kits (Zymed Co.) were used. The specimen was sectioned into 4-5μmm, and then stored at 4 °C. The primary antibody was anti-TGF-β, and the secondary antibody was anti-mouse IgG. A staining method was used for the phenomenon of capillary gap action. Mayer hematoxylin processing was done after streptavidine alkaline phosphatase and substrate were processed.

2. Analysis of immunohistochemical stain results

The specimens were classified, according to stain condition, as diffuse or localized at low-grade magnification. At high-grade magnification, we tried to evaluate the degree of TGF-β expression on the cytoplasm of cells.

For qualitative analysis, we classified the staining grades into 4 : negative(-), slight(+), moderate(++) and heavy(+++).

3. Western blot analysis

Protein extraction was done by cutting the palate of the maxilla (0.04g) and mandible (0.04g) separately and then homogenizing them in ice boxes separately. Chloroform and ethanol were added to the tissue sequentially and then they were rotated in a centrifuge. The upper liquid in the tube was collected and isoprophanol was added, and then the sample was re-rotated. 95% ethanol was added and then the upper liquid was removed. The pallet was dried in air. 1% SDS(sodium deoxylsulfate) was added and then the pallet was melted in a 50~55° C water bath. The upper liquid was collected in a new tube.

We used an enzyme-linked immunosorbent assay (ELISA) method and spectrometer for quantification of the protein. Each 20ℓ protein was electroblotted on SDS gel. A primary antibody, anti-TGF-β (Zymed Co.), was added and a secondary antibody, anti-mouse IgG (Zymed Co.), was added to the polyvinylidene difluride (PVDF. Bio-rad Co.) membrane in a blocking buffer solution, sequentially. Finally the membrane was washed with a phosphate buffer solution (PBS) and then developed on a film cassette in a dark room for 30 minutes.

To determine the expression of TGF-β, we examined the strength and width of the band on the membrane and film.

RESULTS

This study observed a contrast in the morphogenesis of cleft palate compared to normal palate with immunohistostain and TGF-β expression in maxilla and mandible with western blot analysis.

1. Immunohistostain

The control groups showed a continuing epithelial line, cell cluster, woven bone, and hyperchromatized nucleus in the cells. Heavy expression of TGF-β was shown at the mesenchymal cell near the sheath of the osteoblast. Osteoblasts were shown at the junction area, which was the middle area in the palate and had a

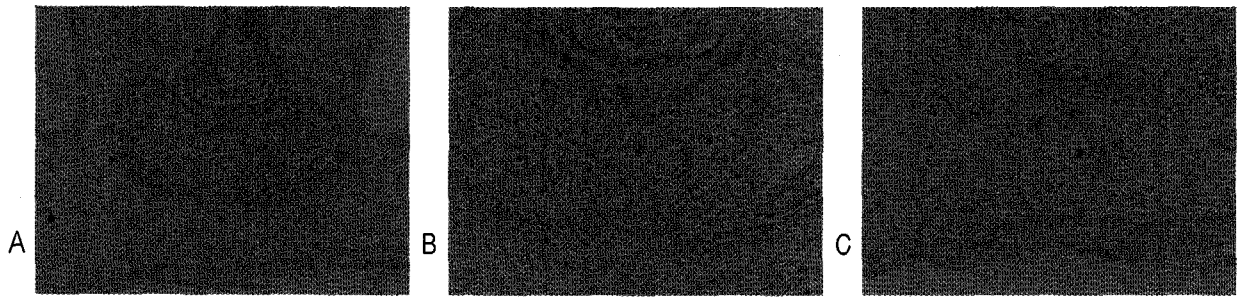

Figure 2. TGF-β expression in the control group
A. TGF-β expression pattern on woven bone.
B. osteoblast and osteocyte showing moderate TGF-β expression
C. Epithelium showing higher TGF-β expression than mesenchymal cells

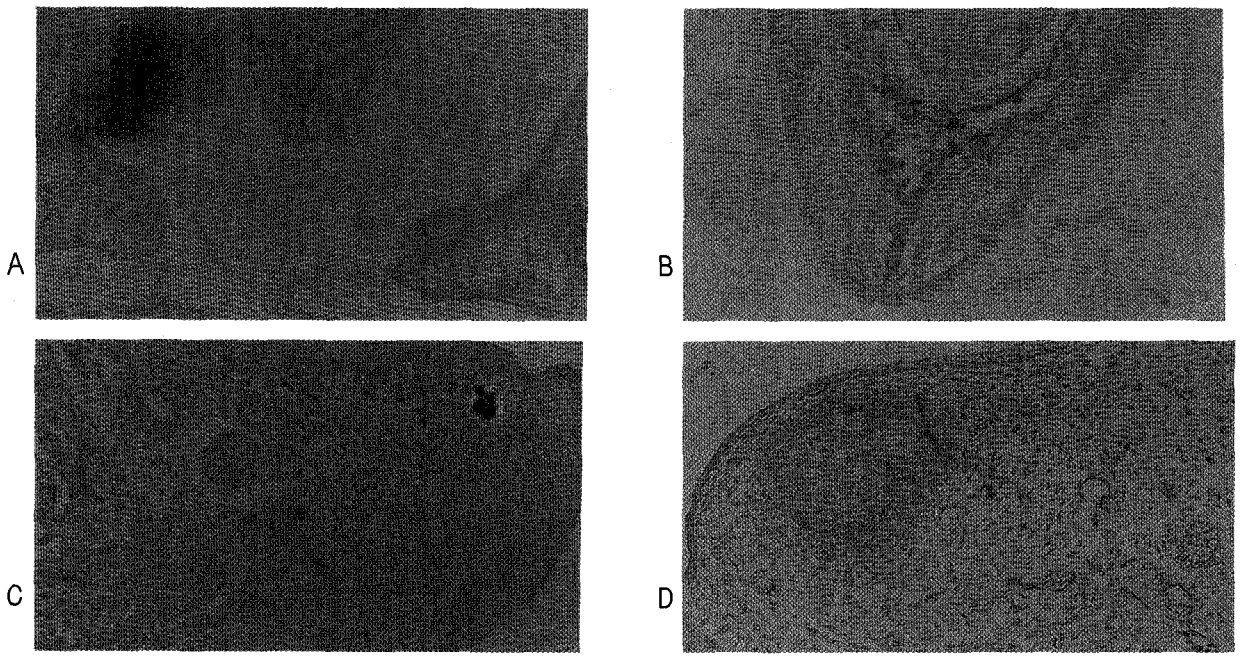

Figure 3. TGF-β expression on cleft formed group
A. overall features of cleft palate mice.
B. TGF-β expression patterns at woven bone area in nasal septum.
C & D. TGF-β expression patterns at focal area in palatal process.

moderate staining. Osteocytes were dispersed throughout woven bone and showed moderate TGF-β expression. Mesenchymal cells that were located around woven bone showed moderate TGF-β expression. But other mesenchymal cells that were located near palatal epithelium showed diffuse TGF-β expression. In the epithelial cells, moderate activity of TGF-β was shown. Sometimes epithelia that were located on the nasal floor showed heavy TGF-β expression (Figure 2-B,C).

By contrast, the cleft groups had a prominent nonunion on the palate area (Figure 3-A). In the nasal process, nasal cartilages had a diffuse expression. Mesenchymal cells that were located around woven bone showed a moderate or diffuse expression. Mesenchymal cells near epithelia showed a diffuse expression.

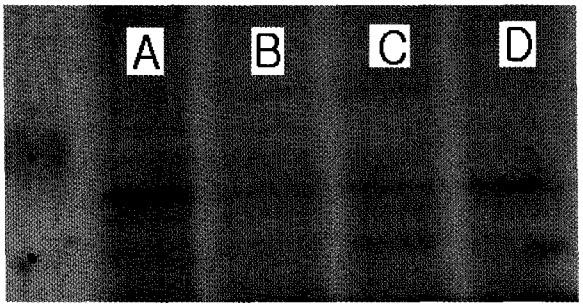

Figure 4. Western analysis of TGF- expression : The expression of TGF-β in the cleft group's maxilla was a thinner and more diffuse band expression compared with the control group's maxilla
A : Maxilla of control group
B : Mandible of control group
C : Maxilla of cleft group
D : Mandible of cleft group

Epithelia on the nasal process showed a diffuse TGF-β expression and the thickness of epithelia revealed even distribution on the nasal process (Figure 3-B). In both palatal processes, the amount of woven bone showed small dispersed compared to the control group. The osteoblasts showed a slight or moderate TGF-β expression, osteocytes showed a moderate TGF-β expression, mesenchymal cells showed a diffuse TGF-β expression and epithelia showed a moderate TGF-β expression in the palatalshelves. The thickness of the medial edge epithelia in the cleft rat showed less density compared to other sites in epithelia of cleft palate rat (Figure 3-C,D).

2. Western blot analysis

Verification for this result was displayed on an Image-pro software program. The results showed that the expression of TGF-β in the cleft group's maxilla was a thinner and more diffuse band expression compared with the control group's maxilla. But the expression of TGF-β in the mandible of the cleft group was thicker compared to control group's mandible. The thickness rank of bands was as followed; maxilla of control group, mandible of cleft group, maxilla of cleft group and mandible of control group (Figure 4).

DISCUSSION

Cleft palate has long been thought to result from a combination of genetic and environmental factors. Implicated in the interactive processes related to cleft palate were the aberrations in homeotic gene expression,[11,13] regulatory factors,[14] a number of ligand receptor molecules,[3] migrations of cranial neural crest cells,[3,15] and rates of mesenchyme cell proliferation leading to hypoplasia of medial process.[3] Other factors have included deficient vascularization and myogenic cell differentiation, bilateral asymmetry resulting from unilateral or bilateral hypoplasia and abnormal medial edge epithelial transformation.[16-18] Yet attempts to identify these components in human populations have so far produced inclusive results.[19] Placental permeability, genetic stability, high number of fetuses, and a limited gestation length were properties that made the rat a practical subject for use in experimental investigations.[15] In experimental studies, there were many teratology-induced drugs that induced cleft palate and many studies for teratogenic factors were reported, but implausible teratogenic factors affected the different groups in divergent manners. Diazooxonorleucine (DON),[20] benzodiazepine,[21,22] 6-aminonicotineamide (6-AN)[20] and cortisone[23] were some of the substances that have been demonstrated to induce cleft formation in rats. Pyridoxal phosphate has a role in the action of those hormones, acting by binding to a nuclear receptor protein, including transcription of DNA, and hence modulating gene expression, leading to new protein synthesis. As well pyridoxal phosphate acts as a cofactor in the release of hormone-receptor complexes from tight nuclear binding, resulting in the release of hormones from the nucleus, and freeing or recycling receptors for future uptake of hormones.[25] Such hormones include androgens, estrogens, progesterone, glucocorticoids, calcitriol, retinal and retinoic acid, and thyroid hormones.[24] For example, glucocorticoids enhance the binding sites per cell and promote proliferation and growth of mesenchymal cells, but high doses of glucocorticoids inhibit mesenchymal cell proliferation and decrease extracellular matrix synthesis.[3,23,25] BAPN, which was used in this study, is a teratogen that inhibits the crosslinking of collagen, so it alters

the shape of the Meckel's cartilage and reduces the length of the mandible. Shortening of the mandible may cause a high and retracted tongue position that interferes with horizontal palatal growth.[27]

Rat palate formation stage was as follows : 13.5 day p.c. was when the shelves grew downward toward the floor of mouth, 14.5 day p.c. was when the shelves elevated and were horizontally opposed, and 15.5 day p.c. was when the shelves came into contact and began to fuse.[12] The rat fetus was delivered at day 20 or 21 p.c.[12] Palatal mesenchyme was derived from the cephalic neural crest and the palatal shelves elevated to horizontal position, grew toward one another and fused at their medial edge, forming a midline seam of epithelial cells.[30] The disruption of this epithelial seam yielded mesenchymal continuity across the newly formed shelf. The epithelial seam disappeared through programmed cell death. Recent detailed studies by Fitchett and Hay have shown that only the outer-most layer, the periderm, actually dies while the medial edge epithelial cells transdifferentiate into mesenchymal cell types and migrate away from the midline.[17] The differentiation of epithelial cells into mesenchymal cell types was a recurrent theme in embryonic development.[31] But small vacules having shown at the mid-palatal area of control group in this study, it was suggested that the epithelial cell might be apoptosis at this area. Decreased activity of the medial epithelium thickness in this study was similar to cleft palate induced by retinoic acids.[32,33] The medial edge epithelium of the palatal process in the cleft palate group showed thin density, but we don't know why this appearance occurred. Palatal mesenchyme in organ culture has been reported to be affected by retinoic acid.[33] Although the mechanism has not been fully described, this study contained evidence that BAPN mimics the effects of retinoic acid to the mesenchyme.

Formation of the palate involved complex interactions of palatal and craniofacial factors. There are numerous theories concerning the mechanism for palatal shelf elevation. Both extrinsic and intrinsic factors are thought to be involved. Examples of the extrinsic factors were position of the tongue, vertical growth of the nasal septum and primary palate, angulation change of the cranial base, and the growth of Meckel's cartilage. The length of the mandible or growth of Meckel's cartilage is essential for making space available for the tongue to move down and forward during embryonic development. If the tongue does not move down and forward, the space for the palatine process is too small to allow fusion.[27,34] Intrinsic factors are those that play an active role in the shelves themselves,[4] and it plays an active role in cell migration, epithelial-mesenchymal transdifferentiation, extracellular matrix synthesis and deposition, and cell proliferation.[35] Diewert suggested that BAPN may also affect the intrinsic capacity for movement in the palatal shelves. In this study, cleft palate formed rats didn't show any elevated tongue posture, which suggests that disturbed intrinsic factors induced fusion failure on palatal shelves[27] and that the mesenchyme and epithelium of embryonic palate required growth factors and hormones for normal progression.[30,36]

As one of the growth factors, the TGF-β family participated in a secondary palate formation. The TGF-β is considered a complex and extensive characterized family among the growth factors. This growth factors family is biologically active as disulfide-linked dimmers and its molecular weight is 25kd.[37] The role of TGF-β was in inducing fibrosis and angiogenesis when injected subcutaneously into newborn mice.[10] Furthermore, TGF-β was shown to reversibly inhibit osteoblast formation and bone reabsorption.[38] Exogenous TGF-β increases the synthesis of the extracellular matrix proteins such as fibronectin and collagen types III, IV, and V, but the inhibits the synthesis of collagen type I and EGF. And TGF-β stimulates collagen and glycosaminoglycan synthesis and proliferation of the palatal mesenchyme.[39] Linask reported that differences between murine and human palatal mesenchyme cells included increased proliferation in human but decreased proliferation in murine cells after application of TGF-β.[40] Whether these differences reflected alterations in a long-established human cell line or real species differences that were obtained in vivo was unclear.[40]

TGF-β was divided into TGF-β1, TGF-β2 and TGF-β3. In most in vitro systems, TGF-β1 and TGF-β2 exert similar effects and appear to be func-

tionally equivalent, but several findings indicate that they are not functionally identical in all systems.[41,42] The most important functions of TGF-β1 and TGF-β2 seemed to be the chemotaxis and mitogenesis of osteoblast precursors and the ability to stimulate their deposition of collagen matrix for connective tissue.[42] TGF-β1 can inhibit colony formation and block the effects of fibroblast growth factor (FGF) on endothelial proliferation. By contrast, TGF-β2 has been reported to be more effective than TGF-β1 as an inducer of mesoderm formation. TGF-β1 & -β3 have been visualized in the murine palate between 11.5 and 15.5 days of gestation. TGF-β3 appears first, initially in the epithelium of the vertical palatal shelves, TGF-β2 appears in the epithelium of the horizontal shelves, followed by TGF-β2 in the mesenchyme under the median epithelium of the fusing shelves.[5,10,12,15,43] Failure of any of these cellular activities, due to either genetic or environmental factors, may result in the formation of cleft palate.[19] In this study, TGF-β expression patterns of mesenchymal cells in cleft palate fetus were diluted in appearance compared to the control group's mesenchyme. It suggests that the differentiation of mesenchymal cells to osteoblast resulted in decreased activity in cleft palate induced by BAPN. TGF-β had a heavier expression in the mandible of the cleft palate rats compared to the control group's mandible with Western blot analysis. It will required more research to discover why the different patterns were shown in the mandible.

CONCLUSIONS

This study attempted to discover the correlation between cleft palate formed by BAPN administration and TGF-β expression utilizing immunohistostain and Western blot analysis. 4 Timed-pregnant Sprague-Dawley rats were obtained on the 10th gestation day. On the 13th day of gestation, BAPN-monofumarate salts ($(C_3H_6N_2)_2 \cdot C_4H_4O_4$) were individually, orally administered to 3 pregnant rats at a ratio of 1g/kg body weight, and 4 pregnant rats were sacrificed on day 20 post coitus (p.c.). The TGF-β expression in the cleft formed rat fetuses showed the following patterns :

1. Osteoblast and mesenchymal cell of the cleft palate rats were of low expression compared to those of the control rats.
2. The cleft palate rats didn't show any difference in the TGF-β expression of osteocyte from the control rats.
3. In western blot analysis, the thickness of band of TGF-β in the cleft palate maxilla rats was thinner and more dilute than that of the control maxilla rats.

REFERENCES

1. Slavkin HC. Congenital craniofacial malformations: identifying individuals at risk. Entechnology J 1979 : 58 : 7-20.
2. Warkany J. Congenital malformation : notes and comments. Chicago : Year book Medical Publishers : 1971.
3. Ferguson MW. The orofacial region. Textbook of fetal and perinatal pathology. London : Blackwell 1991 : 843-50.
4. Ferguson MW. Palate development. Development.1988 : 103 : 41-60.
5. Fitzpatrick DR. Denhez F. Kondaiah P. Akhurst RJ. Differential expression of TGF-β isoforms in murine palatogenesis. development 1990 : 109 : 585-95.
6. Millard R. Cleft craft; the evolution of its surgery ; the unilateral deformity. Little brown and company, Boston. 1976 : 1st Edi. : 3-17.
7. Pisano MM. Green RM. Hormone and growth factor in volvement in craniofacial development. IRCS Med Sci 1986 : 14 : 635-40.
8. DeLarco JE. Todaro GJ. Growth factors from murine sarcoma virus-transformed cells Proc. Natl Acad Sci USA. 1978 : 75 : 4001-5.
9. Rizzino A. Transforming growth factor-β : multiple effects on cell differentiation and extracellular matrices. Dev Biol 1988 : 130 : 411-22.
10. Roberts AB. Sporn MB. et.al Transforming-growth factor type-β rapid induction of fibrosis and angiogenesis in vivo and stimulation of collagen formation in vitro. Proc Nat. Acad Sci USA 1986 : 83 : 4176-71.
11. Hall BK. Ekanayake S. Effects of growth factors on the differentiation of neural crest cells and neural crest cell-derivatives. Int J Dev Biol 1991 : 35 : 367-87.
12. Pelton RW. Hogan BL. Miller DA. Moses HL. Differential expression of genes encoding TGFS-β1, β2, and β3 during murine palate formation. Dev Biol 1990 : 141 : 456-60.
13. Balling R, Mutter G, Gruss P, Kessel M. Craniofacial abnormalities induced by ectopic expression by the homeobox gene Hox-1.1 in transgenic mice. Cell 1989 : 58 : 337-47.
14. Melnick M, Shields ED, Burzynski NJ. Clinical dysmorphology of orofacial structures. Littleton, MA : PSG Publishing : 1982.
15. Slavkin HC. Regulatory issues during early craniofacial development; a summery. Cleft Palate J. 1990 : 27 : 101-9.
16. Carette MJ, Ferguson MW. The fate of medial edge epithelial cells during palatal fusion in vitro : an analysis by DiI labeling and confocal microscopy. Development 1992 : 114 : 379-88.
17. Fitchett JE. Hay ED. Medial edge epithelium transforms to mesenchyme after embryonic palatal shelves fuse. Dev Biol 1989 : 131 : 455-74.
18. Thorp BH. Anderson I. Jakowlew SB. Transforming growth factor-β1, -β2 and -β3 in cartilage and bone cells during endochondral ossifica-

tion in the chick. Development. 1992 : 114 : 907-11.
19. Slavkin HC. Incidence of cleft lips, palates rising. JADA. 1992 : 123 : 61-5.
20. Diewert VM. Pratt RM. Selective inhibition of mandibular growth and induction of cleft palate by diazo-oxo-norleucine(DON) in the rat. Teratology. 1979 : 20 : 37-51.
21. Dolovich LR. Addis A. Vaillancourt JM. et. al. Benzodiazepine use in pregnancy and major malformations or oral cleft ; meta-andysis of cohort and case-control studies. Bio Med J 1998 : 26 : 839-43.
22. Yoneda T. Pratt RM. Vitamin B6 reduces cortisone-induced cleft palate in the mouse. Teratology. 1982 : 26 : 255-8.
23. Montenegro MA. Rojas M. Dominguez S. Rosales CJ. Differences in extracellular matrix components and cell density during normal and dexamethasone-treated secondary palate development in two strains of mice with different susceptibility to glucocorticoid induced-clefting. J Craniofac Genet Dev Biol 1998 : 18 : 100-6.
24. Macrae R. Encyclopedia of food science, food technology and nutrition. Vol 7. London Academic Press. 1993 : 4788-804.
25. Miller TJ. Cleft palate formation : a role for pyridoxine in the closure of the secondary palate in mice. Teratology. 1972 : 6 : 351-6.
26. Shils H. Olson JA. Shike M. Modern nutrition in health and diseases. Vol 1. 8th Edi. Philadelphia : Lea & Febiger, 1994 : 383-92.
27. Diewert VM. Correlation between alterations in Meckel's cartilage and induction of cleft palate with-aminoproprionitrale in the rat. Teratology. 1981 : 24 : 43-52.
28. Jacobsson C. Granstrom G. Effects of vitamin B6 on beta-amino-proprionitrile-induced palatal cleft formation in the rat. Cleft palate J 1997 : 34 : 95-100.
29. Foreman DM. Sharpe PM. Ferguson MW. Comparative biochemistry of mouse and chick secondary palate development in vivo and in vitro with particular emphasis on extracellular matrix molecules and the effects of growth factors on their synthesis. Arch Oral Biol 1991 : 36 : 457-71.
30. Pratt RM. Hormones, growth factors and their receptors in normal and abnormal prenatal development. Teratology. New York : Pienum Publishing Corp 1984 : 189-217.
31. Shuler CF, Guo Y, Majumder A, Luo R. Molecular and morphologic changes during the epithelial-mesenchymal transformation of palatal shelf medial edge epithelium in vitro. Int J Dev Biol 1991 : 35 : 463-72.
32. Abbott BD. Pratt RM. Retinoids and EGF after embryonic mouse palatal epithelial and mesenchymal cell differentiation in organ culture. J Craniofac Gen Dev Biol 1987 : 7 : 219-40.
33. Degitz SJ. Morris D. Foley GL. Francis BM. Role of TGF-b in RA-induced cleft palate in CD-1 mice. Teratology 1998 : 58 : 197-204.
34. Chai Y. Mah A. Crohin C. Groff S. Specific transforming growth factor -subtypes regulate embryonic mouse Meckel's cartilage and tooth development. Dev Biol 1994 : 162 : 85-103.
35. Holland PWH. Homeobox genes and the vertebrate head. Development 1988 : 103 : 17-24.
36. Pratt RM. Role of epidermal growth factor in embryonic development. Curr Topic Dev Biol 1987 : 22 : 175-93.
37. Holley RW. Armour R. Baldwin JH. Density-dependent regulation of growth of BSC-1 cells in cell culture; growth inhibitors formed by cells. Proc Natl Acad Sci USA 1978 : 75 : 1864-6.
38. Sandberg M. Vuorio T. Hirvonen H. Alitalo K. Vuorio E. Enhenced expression of TGF-b and c-fos mRNAs in the growth palate of developing human long bones. Devlopment. 1988 : 102 : 461-70.
39. Linask KK. D'Angelo M. Gehris AL. Greene RM. TGF-receptor profiles of human and murine embryonic palate mesenchymal cells. Exp Cell Res 1991 : 192 : 1-9.
40. Sporn MB. Robert AB. Wakefield LM. De Crombergghe B. Some recent advances in the chemistry and biology of transforming growth factor-beta. J Cell Biol 1987 : 105 : 1039-45.
41. Gehris AL.D'angelo M. Greene RM. Immunodetection of the transforming growth factors 1 and 2 in the developing murine palate. Int J Dev Biol 1991 : 35 : 17-24.
42. Wahl SM. Hunt DA. Wakerfield LM. et. al. Transforming growth factor type induces monocyte chemotaxix and growth factor production. Proc Natl Acad Sci USA 1987 : 263 : 7646-54.
43. Schmid P. Cox D. Bilbe G. Maier R. Differential expression of TGF-1, 2 and 3 genes during mouse embryogenesis. Development 1991 : 111 : 117-30.

국문초록

BAPN으로 유도한 구개열 백서에서 TGF-β 발현 양상에 대한 연구

원광대학교 치과대학 교정학 교실 · 구강병리학교실, 치의학 연구소

태 기 출 · 김 은 철

구개열에 대한 유병률과 분자생물학적 방법으로 연구가 지속되어 왔고, 원인 요소에 많은 연구가 수행되어 왔다. 많은 연구방법중 분자생물학적 방법은 구개열 연구에 중요한 방법으로 고려 된다. 구개 형성에 여러성장 요소가 관여하는데, 그중 TGF-β는 세포이주, 상피-간엽 형질 전환, 세포외 기질 합성과 축적에 관여한다. 구개열 연구중에 beta-aminonitroproprionitrile (BAPN)을 이용하여 구개열 형성 백서와 TGF-β 발현 양상과의 상관성에 대한 연구는 수행

되지 않았다. 이 연구는 임신 10일째 백서 4마리를 구입한 후, 그 중 3마리에 BAPN을 1g/kg body weight비율로 구강 투여하고 임신 20일째 희생하여 구개열 태자와 정상 태자에서 TGF-β 발현 양상을 면역조직화학염색과 Western blot 분석을 시행하여 다음과 같은 결과를 얻었다.

1. 면역조직화학염색 결과 구개열 백서의 골아세포와 간엽조직에서 TGF-β 발현 양상은 대조군과 비교할 때 낮은 활성을 보였다.
2. 구개열 백서의 골세포에서 TGF-β 발현 양상은 대조군과 유의한 차이는 보이지 않았다.
3. Western blot 분석결과 구개열 백서의 상악에서 TGF-β 발현은 대조군의 상악에 비해 밴드가 약하게 발현되었다.

주요 단어 : 구개열, TGF-β, BAPN, 면역조직화학염색, Western blot