

## 비용조직에서 산화질소합성효소들의 발현

박성국<sup>1</sup> · 김동균<sup>3</sup> · 허경욱<sup>1</sup> · 양영일<sup>2</sup>

## Expression of Nitric Oxide Synthases in Nasal Polyp

Seong Kook Park, MD<sup>1</sup>, Dong Kyoon Kim, MD<sup>3</sup>, Kyung Wook Heo, MD<sup>1</sup> and Young Il Yang, MD<sup>2</sup><sup>1</sup>Department of Otorhinolaryngology-Head & Neck Surgery and <sup>2</sup>Pathology, College of Medicine, Inje University, Pusan Paik Hospital, Busan, <sup>3</sup>Dong Gyoon Kim ENT Clinic, Busan, Korea

## -ABSTRACT-

**Background and Objective** : The pathogenesis of nasal polyp is poorly understood. Nitric oxide (NO) plays a major role in a number of physiologic function and may be cytotoxic in high concentrations. NO is formed by the oxidative deamination of L-arginine by nitric oxide synthases (NOS). Three NOS isoforms have been identified in human. Constitutive forms of NOS are present in vascular endothelial cells (Type III NOS, eNOS) and neurons in the brain and the peripheral nervous system (Type I NOS, nNOS) whereas the inducible form (Type II NOS, iNOS) is transcriptionally induced by cytokines in macrophages, neutrophils, mast cells, smooth-muscle cells, and fibroblasts. The aim of this study was to detect and localize three NOS isoforms expression in nasal polyp tissues, and compare these findings with inferior nasal turbinate tissues. **Materials and Methods** : The authors examined the expression and localization of three NOS isoforms in nasal mucosal specimens from patients undergone elective nasal turbinectomy (n=10) and nasal polypectomy (n=23). The mRNA expressions of three NOS isoforms were determined by semi-quantitative reverse transcription-polymerase chain reaction (RT-PCR) followed by Southern hybridization. The protein expression of three NOS isoforms were examined by immunohistochemistry. Statistics were analysed using Wilcoxon rank sum test. **Results** : Semi-quantitative RT-PCR Southern analysis of RNA obtained from 23 surgical specimens of nasal polyps demonstrated that the mRNA expressions of iNOS and eNOS were significantly increased in nasal polyps compared with inferior turbinates. The nNOS mRNA was similarly expressed in nasal polyps and inferior turbinates. The immunohistochemical studies revealed that the immunoreactivity to iNOS protein was mainly localized to epithelium, whereas eNOS protein to vascular endothelium, and nNOS protein to inflammatory cell, epithelium and vascular endothelium in all specimens reviewed. The high level of expression of three NOS isoforms in the nasal polyps were demonstrated in this study. **Conclusion** : The authors suggest that iNOS mRNA, eNOS mRNA and their product, nitric oxide may play an important role in the formation and growth of nasal polyps. (J Clinical Otolaryngol 2002;13:85-92)

**KEY WORDS** : Nitric oxide synthase · Nasal polyp · RT-PCR/southern blot · Immunohistochemistry.

## 서 론

가

(NO)

: 2001 12 10

: 2002 3 18

: , 614 - 735

2 633 - 165

: (051) 890 - 6379 ·

: (051) 892 - 3831

E - mail : sinus4@chollian.net

(Nitric oxide synthase, NOS) L - arginine .<sup>8)</sup> iNOS cNOS

1)

Ca<sup>2+</sup> calmodulin (constitutive NOS, cNOS) 가

Ca<sup>2+</sup> calmodulin (inducible NOS, iNOS)

2) 3가 가 . cNOS neuronal NOS (nNOS NOS1)

endothelial NOS(eNOS NOS3)

iNOS, NOS2) interferon - , tumor necrosis factor - , lipopolysaccharide , iNOS cNOS

100 1000<sup>3)</sup>

4)

5)

### 재료 및 방법

#### 재 료

MAST(MAST Immunosystem, CA, USA)

23 ( 12 , 11 , 14 76 37.3 )

10 ( , 15 27.5 )

42

70

10%

#### 방 법

Total RNA first strand cDNA

Qiagen total RNeasy kit(Germany)

7) total RNA . 1% mercaptoethanol 가 lysis buffer 1 ml 가 (Biospec product, USA) QIAshredder (Qiagen, Germany)

70%

가 RNeasy loading wash buffer 3 3 RNA . 0.8%

agarose RNA actin agarose southern hybridization  
 2 µg total RNA oligo(dT)<sub>15-18</sub> 2 µg  
 70 10 1 mM dNTP(Takara, Japan), 200 unit Moloney murine leukemia virus(MMLV) (50 mM Tris - HCl, pH 8.3, 75 mM KCl, 3 mM MgCl<sub>2</sub>, 10 mM DTT) 25 µl 42  
 1 95 5  
 primer (Table 1) 20 pmol cDNA 2 µl Premix PCR kit (Perkin - Elmer corp., USA) 20 µl가 Perkin Elmer 2400 predenaturation 96 5 94 20 , 62 20 72 1 cycle 35 72 7 postelongation (target sequence) 1.5% agarose total RNA  
 Southern hybridization - actin 1.5% agarose TBE (50 mM Tris - borate, 1 mM EDTA, pH 8.0) 3.3 V/cm 1 , ultraviolet illuminator(UVP Inc, USA) depurination buffer(0.25 M HCl) denaturation buffer(1.5 M NaCl, 0.5 N NaOH) DNA 가 (TransVac - TE80 ; Scientific Instrument Inc, USA) Hybond - N+ membrane(Amersham, England) tranfer DNA가 Hybond - N+ membrane prehybridization (6 SSC ; 0.01 M sodium phosphate, pH 6.8, 1 mM EDTA, pH 6.8 ; , 0.5% SDS, 100 µg/ml salmon sperm DNA) hybridization chamber 68 blocking . [ -<sup>32</sup>P] ATP end - labelling probe 1 × 10<sup>6</sup> cpm/ml 가 62 hybridization Geiger counter background noise가 2 × SSC, 0.1% SDS , me-

**Table 1.** Primer sequences in nasal polyp and inferior turbinate tissues of RT-PCR for the detection of nitric oxide synthases

	Oligonucleotide primer and probe sequence	Product size	Annealing temperature
-actin	s 5'-AGA AAA TCT GGC ACC ACA CC-3'	544 bp	62
	as 5'-AGG AAG GAA GGC TGG AAG AG-3'		
	p 5'-GGC CCC CCT GAA CCC CAA GGC CAA-3'		
eNOS	s 5'-AAG CCG CAT ACG CAC CCA GAG-3'	345 bp	62
	as 5'-TGG GGT ACC GCT GCT GGG AGG-3'		
	p 5'-GGG AGA GCG GCT GCC CGA CTC AGG TCC-3'		
iNOS	s 5'-CTC TAT GTT TGC GGG GAT GTG CG-3'	334 bp	62
	as 5'-GGA TAT CAC TTT CCT CCA TCT CCC CA-3'		
	p 5'-GGG TGG CGG TGC AGC CCA GCA GCC TGG-3'		
nNOS	s 5'-AAA GCG ACC ATC CTC TAT GCC-3'	225 bp	62
	as 5'-CAT TTC CAT CAA AGC ACA GCC-3'		
	p 5'-GGA AGA ATA TGA CAT TGT GCA CCT GGA-3'		

s : sense, as : antisense, p : probe, iNOS : inducible nitric oxide synthase, eNOS : endothelial nitric oxide synthase, nNOS : neuronal nitric oxide synthase

mbrane Kodak X - AR(Kodak, USA) - 70  
 18 .  
 NIH image analysis(Scion Inc. USA) USA) 30 . TBS  
 , - actin band 3 horseradish peroxidase  
 (density ratio) mRNA (HRP) - conjugated streptavidin(Dako, CA, USA)  
 . 30 TBS 3  
 . 0.05% 3 - amino - 9 - ethyl carbazole(AEC,  
 Sigma Chemical Co., SL, USA)/0.01% H<sub>2</sub>O<sub>2</sub>가  
 TBS 10  
 TBS  
 . Mayer 's hem-atoxylin 가  
 silane(Sigma Chemical Co., SL, USA)  
 USA)  
 60 1 , 100% xylene , , , , ,  
 10 2 .  
 90%, 85%, 80%, 70%, 60%, 50% .  
 . peroxi-  
 dase 30% SAS(release 6.12)  
 가 9 : 1 10 tris Wilcoxon rank sum test  
 buffered saline(TBS, pH 7.4) 3 p 0.05  
 1% zinc sulfate(Sigma Chemical Co., SL, USA)가 10 mM citrate buffer(pH 6.0) microwave 5  
 3 가  
 0.5% 0.5%  
 (Dako, CA, USA) TBS 30  
 .  
 가 4  
 .  
 Table 2  
 . 가 1% Tween  
 20(Bio - Rad, CA, USA) TBS 10 3 iNOS 10 4 가 eNOS

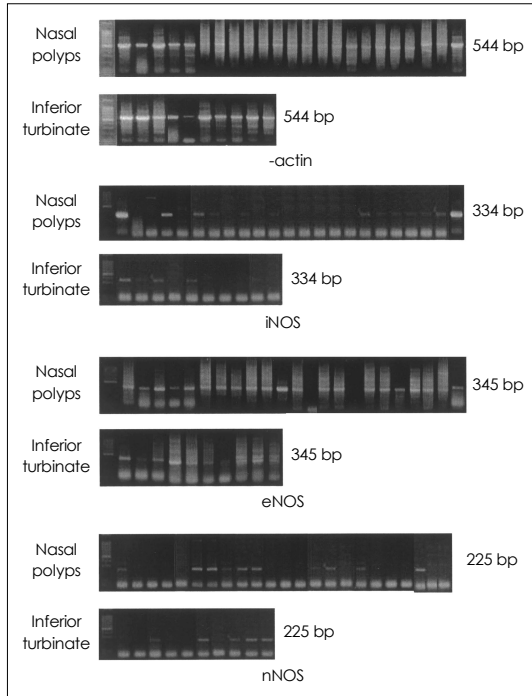
**결 과**

**역전사중합효소 연쇄반응**  
 - actin RT - PCR product 544  
 bp , iNOS 334 bp, eNOS 345 bp, nNOS 225  
 bp RT - PCR PCR product  
 (Fig. 1). RT - PCR Southern hybridization  
 - actin PCR  
 iNOS 23 14 ,  
 eNOS

**Table 2.** Primary antibodies used for immunohistochemical study

Antibody name	Source	Isotype	Dilution fold	Production company
iNOS/NOS Type II	Mouse Monoclonal	IgG2a	1 : 250	Transduction Laboratories
eNOS/NOS Type III	Mouse Monoclonal	IgG1	1 : 100	Transduction Laboratories
nNOS/NOS Type I	Mouse Monoclonal	IgG2a	1 : 250	Transduction Laboratories

iNOS : inducible nitric oxide synthase, eNOS : endothelial nitric oxide synthase, nNOS : neuronal nitric oxide synthase



**Fig. 1.** Results of RT-PCR of  $\beta$ -actin and nitric oxide synthase isoenzymes in nasal polyps and inferior turbinate tissues. iNOS : inducible nitric oxide synthase, eNOS : endothelial nitric oxide synthase, nNOS : neuronal nitric oxide synthase.

**Table 3.** Detection of nitric oxide synthases in nasal polyps and inferior turbinates

	Nasal polyps (n=23) (%)	Inferior turbinates (n=10) (%)
iNOS	14 (61)	4 (40)
eNOS	19 (91)	10 (100)
nNOS	11 (48)	5 (50)

iNOS : inducible nitric oxide synthase, eNOS : endothelial nitric oxide synthase, nNOS : neuronal nitric oxide synthase

**Table 4.** Mean value of density ratio of nitric oxide synthases

	Nasal polyps (n=23)	Inferior turbinates (n=10)
iNOS*	0.5627 $\pm$ 0.5931	0.3278 $\pm$ 0.4012
eNOS*	2.6370 $\pm$ 0.9578	1.8713 $\pm$ 0.6208
nNOS	0.4036 $\pm$ 0.4549	0.4608 $\pm$ 0.6326

\* : p<0.05, iNOS : inducible nitric oxide synthase, eNOS : endothelial nitric oxide synthase, nNOS : neuronal nitric oxide synthase

23 19 , 10  
nNOS 23 11 ,  
10 5 가 (Table 3). iNOS  
- actin 0.5627  $\pm$   
0.5931, 0.3278  $\pm$  0.4012  
(p<0.05),  
eNOS 2.6370  $\pm$  0.9578,  
1.8713  $\pm$  0.6208  
(p<0.05), nNOS  
0.4036  $\pm$  0.4549, 0.4608  $\pm$  0.6326  
(Table 4).

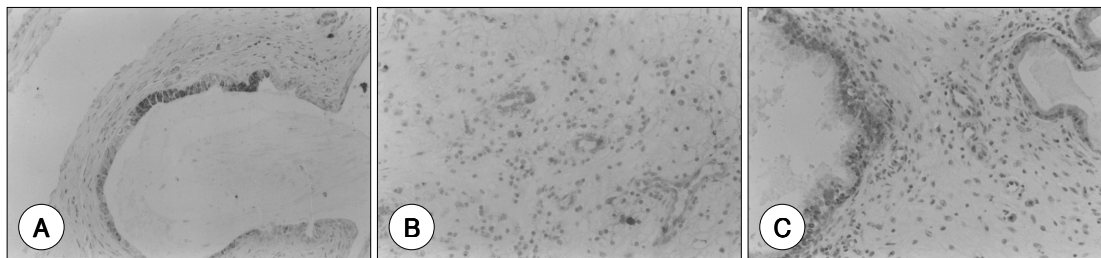
**면역조직화학적 검사**

3가 가  
iNOS ,

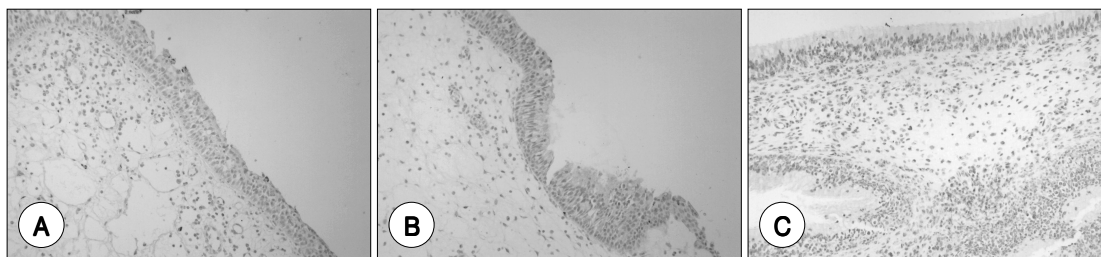
, eNOS ,  
nNOS  
(Figs. 2  
and 3).

**고 찰**

Ca<sup>2+</sup>/  
calmodulin  
(constitutive form) Ca<sup>2+</sup>/calmodulin  
(inducible form)  
2) Lundberg 9) iNOS  
가 (inducible form) (constitutive form)  
iNOS ,<sup>10)</sup> EMT - 6  
가  
iNOS  
.<sup>11)</sup> eNOS



**Fig. 2.** Immunohistochemical staining for Nitric Oxide Synthases in the nasal polyp tissues. A : iNOS was mainly expressed in mucosal epithelial cells. B : eNOS was mainly expressed in endothelial cells, and occasionally in inflammatory cells. C : nNOS was diffusely and highly expressed in surface epithelial cells, inflammatory cells, and endothelial cells (streptavidin-biotin-peroxidase method,  $\times 200$ ).



**Fig. 3.** Immunohistochemical staining for Nitric Oxide Synthases in the inferior turbinate tissues. INOS (A) and eNOS (B) were markedly decreased than nasal polyp tissues. nNOS (C) was mainly expressed in epithelial cells, and occasionally in inflammatory cells (streptavidin-biotin-peroxidase method,  $\times 200$ ).

eNOS (reactive nitrogen intermediate)

가<sup>3)</sup>

nNOS<sup>9)</sup> 가 tumor necrosis factor - , interleukin - 1 Interferon - superoxide(O<sub>2</sub><sup>-</sup>) (reactive oxygen species) 가

nNOS

12) nitrite nitrate 가 , superoxide 가

13) peroxynitrite(ONOO<sup>-</sup>) tyrosine (nitration) sulfahydryl

substance P, epinephrine<sup>14)</sup>

8) ribonucleotide reductase

aconitase<sup>3)</sup> superoxide가

SOD(superoxide dismutase)

가 superoxide diaphorase  
peroxynitrite 8)  
가 , cNOS  
iNOS  
15)  
, FeS , DNA Watkins 20) iNOS mRNA  
가 iNOS mRNA 가  
16) 가  
가 iNOS  
RT - PCR iNOS mRNA  
가 , 가  
neuropeptides, iNOS, eNOS, nNOS mRNA  
가 , iNOS mRNA  
가 eNOS mRNA  
Kim 18) nNOS mRNA  
eNOS nNOS  
iNOS eNOS가 eNOS ,  
iNOS , eNOS  
Jung 19) iNOS  
eNOS가  
, ,  
mNOS(macrophage NOS, 결론  
type NOS)가 eNOS, iNOS mRNA, eNOS  
mNOS mRNA가  
eNOS mRNA, mNOS mRNA가 가  
mNOS mRNA densitometer . iNOS , eNOS  
가 가 nNOS ,  
Ramis 3) 7  
9  
citrulline release assay 중심 단어 :  
/southern blot .

iNOS cNOS  
cNOS iNOS  
NADPH -

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