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Changes of NOS Activity and NO Content in Rectal Cancer Tissues

Hyon Chol Jo[1]*, Song Guk Han[1], To Myong Kim[1], and Hak Song Jo[1]
[1]*Department of Pathology, Pyongyang University of Medical Sciences, DPR Korea.*
***e-mail:** k.h.rim58@star-co.net.kp*

ABSTRACT

Nitric Oxide (NO) plays an important role in many pathological processes (inflammation, infection, cell death, neoplastic disease, cirrhosis, diabetes, intestinal disease) and normal physiological processes (blood pressure regulation, neurotransmission, wound healing, host defense mechanisms). We aimed to investigate the relationship between changes in NOS activity and changes in NO content in cancer tissues according to the histopathological classification of rectal cancer. The study population included 102 rectal tissue from rectal cancer patients over the past three years and 33 rectal autopsy specimens from those who had not experienced any inflammatory or neoplastic disease in the past. The activity of NOS in rectal tissue was determined by the Griess' method and the citrulline assay for NO production, and the NO content was determined by the Griess' method. Histopathological examination of histotype, depth and differentiation was performed on microscopic images, and the histopathological parameters of rectal cancer were compared with the changes in NOS activity and NO content in the cancer tissues and t-tests were performed. The changes in NOS activity and NO content in rectal cancer tissues are as follows. In rectal cancer, NOS activity and NO content in cancer tissues were significantly higher than those in healthy controls (0.076±0.002μmol/min and 5.84±0.17μmol/g, significantly) with 0.16±0.01μmol/min and 15.63±0.64μmol/g, significantly. In the histopathological classifications (macroscopic pattern, histotype, depth, and differentiation) in rectal cancer, NOS activity and NO content decreased according to the classification.

Keywords: Rectal cancer, NOS activity, NO content.

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INTRODUCTION

In 1980, Furchgott found that endothelial cells secrete very potent vasodilator(EDRF; Endothelium derived relaxing factor), which was found to be NO in 1986.

Nitric Oxide (NO) plays important role in many pathological processes (inflammation, infection, cell death, neoplastic disease, cirrhosis, diabetes, intestinal disease) and normal physiological processes (blood pressure regulation, neurotransmission, wound healing, host defense mechanisms)[4,5,6].

NO is product produced from L -arginine by nitric oxide synthase (NOS). Recently, there has been growing body of research in various countries to explore the relationship between NO and tumor development and apoptosis, especially in the study of NOS activity and NO content in serum and cancer tissues of cancer patients, showing important clinical significance in tumor invasion, metastasis, therapeutic effects and prognostic judgment of patients with malignancy[1,2,3,7,8,9].

We aimed to investigate the relationship between changes in NOS activity and changes in NO content in cancer tissues according to the histopathological classification of rectal cancer.

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SUBJECTS AND METHODS

The study population included 102 rectal tissue from rectal cancer patients operated at the Department of Anorectal Surgery of Pyongyang University Hospital of Medical Scienses over the past three years and 33 rectal autopsy specimens from those who had not experienced any inflammatory or neoplastic disease in the past. The sex and age group of the study population showed mean age of 51.8±1.0 years, with significant 50-year age for both sexes and 1:1.6-year sex ratio.

The activity of NOS in rectal tissue was determined by the Griess' method and the citrulline assay for NO production, and the NO content was determined by the Griess' method.

After macroscopic examination of the rectal cancer surgical material, the tissue sections were taken to reveal the full-thickness structure of the rectum in the center of the cancer lesion and H-E stained specimens were prepared according to the conventional tissue preparation method.

Histopathological examination of histotype, depth and differentiation was performed on microscopic images, and the histopathological parameters of rectal cancer were compared with the changes in NOS activity and NO content in the cancer tissues and t-tests were performed.

The clinical and pathological diagnosis of rectal cancer were made according to clinicopathological diagnosis and treatment guidelines published by the Ministry of Public Health, DPRK.

RESULTS

Table 1. Changes of NOS activity and NO content in rectal cancer tissues($\overline{X}$ ±SE).

Classification	Cases	NOS activity (μ mol/min)	Increasing ratio	NO content (μ mol/g)	Increasing ratio
Healthy	33	0.076±0.001		5.84±0.03	
Rectal cancer	102	0.16±0.01*	2.11	15.63±0.64*	2.68

*; p<0.05 (compared with healthy)

As shown in Table 1, there was a significant increase in NOS activity and NO content in rectal cancer tissues compared with healthy.

Table 2. Changes of NOS activity and NO content according to macroscopic pattern in rectal cancer tissues ($\overline{X}$ ±SE).

Classification	Cases	NOS activity (μ mol/min)	Increasing ratio	NO content (μ mol/g)	Increasing ratio
Healthy	33	0.076±0.001		5.84±0.03	
Polyps	16	0.25±0.06*	3.29	17.94±1.76*	3.07
ulcer localization type	49	0.14±0.01*	1.84	16.34±0.85*	2.80
ulcer infiltration type	26	0.13±0.01*	1.84	15.67±1.24*	2.68
diffuse infiltration type	11	0.11±0.02	1.44	11.06±1.15*	1.89

*; p<0.05 (compared with diffuse infiltration type)

As shown in Table 2, the increase in NOS activity and NO content in rectal cancer tissues compared with healthy was significantly higher in other macroscopic types compared with diffuse infiltration type.

Table 3. Changes of NOS activity and NO content according to histotype in rectal cancer tissues ($\overline{X}$ ±SE).

Classification	Cases	NOS activity (μ mol/min)	Increasing ratio	NO content (μ mol/g)	Increasing ratio
Healthy	33	0.076±0.001		5.84±0.03	
Papillary adenocarcinoma	27	0.19±0.02*	2.50	18.09±0.81*	3.10
Tubular adenocarcinoma	58	0.18±0.03*	2.37	17.86±1.14*	3.06
Mucinous adenocarcinoma	13	0.17±0.03	2.25	13.52±0.89	2.32
Undifferentiated carcinoma	4	0.11±0.01	1.45	10.79±2.25	1.85

*; p<0.05 (compared with undifferentiated carcinoma)

As shown in Table 3, the increased NOS activity and NO content in rectal cancer tissues compared with healthy control were significantly higher in papillary and tubular adenocarcinoma compared with undifferentiated carcinoma.

Table 4. Changes of NOS activity and NO content according to depth in rectal cancer tissues($\overline{X}$ ±SE)Y.

Classification	Cases	NOS activity (μ mol/min)	Increasing ratio	NO content (μ mol/g)	Increasing ratio
Healthy	33	0.076±0.001		5.84±0.03	
Mucosa	2	0.44±0.03*	5.79	21.20±0.03*	3.63
Submucosa	3	0.36±0.03*	4.74	19.23±2.28*	3.29
Muscle layer	35	0.17±0.01*	2.24	17.87±1.15*	3.06
Serosa	48	0.15±0.02	1.97	14.03±0.83	2.40
Subserosa	14	0.12±0.01	1.58	13.49±0.30	2.31

*; p<0.05 (compared with subserosa)

As shown in Table 4, compared with healthy, the levels of NOS activity and NO in rectal cancer tissues increased significantly in the mucosa, submucosa and muscle layer compared with the subserosa.

Table 5. Changes of NOS activity and NO content according to differentiation in rectal cancer tissues($\overline{X}$ ±SE).

Classification	Cases	NOS activity (μ mol/min)	Increasing ratio	NO content (μ mol/g)	Increasing ratio
Healthy	33	0.076±0.001		5.84±0.03	
Well differentiated carcinoma	31	0.23±0.04*	2.63	18.53±1.27*	3.17
Moderately differentiated carcinoma	53	0.19±0.03*	2.50	15.72±0.94*	2.69
Poorly differentiated carcinoma	14	0.15±0.01*	1.97	15.67±0.39*	2.68
Undifferentiated carcinoma	4	0.11±0.01	1.45	10.79±2.25	1.85

*; p<0.05 (compared with undifferentiated carcinoma.

As shown in Table 5, compared with healthy controls, the levels of NOS activity and NO in rectal cancer tissues increased significantly in other differentiated cancers compared with undifferentiated cancers.

DISCUSSION

The NOS activity and NO content of healthy control were 0.076±0.002μmol/min and 5.84±0.17 μmol/g, significantly, while the NOS activity of rectal cancer patients was 0.16±0.01 μmol/min and NO content was 15.63±0.64 μmol/g (Table 1). According to the literature, the decreased activity of iNOS in cancer cells in gastrointestinal tumors leads to decreased NO synthesis, which leads to reduced NO-mediated antitumor activity, creating favorable environment for the development of tumors. The NOS activity of rectal cancer tissues was significantly different from that of polyps, ulcer localization type, ulcer infiltration type and diffuse infiltration types, 0.25±0.06 μmol/min, 0.14±0.01 μmol/min, 0.13±0.01 μmol/min, 0.11±0.02 μmol/min, significantly, and NO content, 17.94±1.76μmol/g, 16.34±0.85μmol/g, 15.67±1.24μmol/g, 11.06±1.15μmol/g, significantly (Table 2).

The increased NOS activity by macroscopic type of rectal cancer was gradually decreased by 3.29, 1.84, 1.84 and 1.44 for polyps, ulcer localization type, ulcer infiltration type and diffuse infiltration types, significantly, when referring to healthy controls, and increased ratio of NO content was gradually decreased by 3.07, 2.80, 2.68, and 1.89, significantly (Table 2). This indicates that the NO content of rectal tissue can reflect the NOS activity of rectal cancer tissue and may also help in the macroscopic classification of rectal cancer. The NOS activity was significantly different in papillary and tubular carcinomas ($p<0.05$) compared with undifferentiated carcinomas ($p<0.05$) and in mucinous adenocarcinomas ($p>0.05$), although no significant difference was found in papillary and tubular carcinomas ($p<0.05$) compared with non-differentiated carcinomas ($p<0.05$).

NO content was 18.09±0.81 μmol/g, 17.86±1.14 μmol/g, 13.52±0.89 μmol/g, 10.79±2.25 μmol/g, significantly, which showed significant differences in papillary and tubular carcinomas ($p<0.05$) but not in mucinous adenocarcinomas ($p>0.05$ Table 3). The increased NOS activity by histotype of rectal cancer, which was defined as 2.50, 2.37, 2.25, and 1.45 in papillary, tubular, mucinous, and undifferentiated cancers, significantly, was gradually reduced by the folding effects of 3.10, 3.06, 2.32, and 1.85, significantly (Table 3).

These results show that the long-term serum levels of iNOS in immune cells and tumor cells are almost consistent with the long-term serum levels of NO induced by iNOS in patients.
Therefore, it is likely that NO produced by iNOS of tumor cells during rectal carcinogenesis and the large amount of NO produced by macrophages play important functions such as immune function, direct killing of tumor cells, repair of the damaged mucosa, and gradually decreases with increasing malignancy.

According to literature data, serum NO levels in patients with esophageal cancer have been measured according to the degree of depth, and they have shown a decreasing trend with increasing depth in submucosal, muscle layer, and esophageal epithelial invasive cancers, which is significantly lower than healthy controls.
The NOS activity was 0.44±0.03μmol/min, 0.36±0.03μmol/min, 0.17±0.01μmol/min, 0.15±0.02μmol/min, 0.12±0.01μmol/min in the mucosa, submucosa, muscle layer, serosa, and subserosa, significantly, but no significant difference was found in the serosa ($p > 0.05$ Table 4). NO content was 21.20±0.03 μmol/g, 19.23±2.28 μmol/g, 17.87±1.15 μmol/g, 14.03±0.83 μmol/g, 13.49±0.30 μmol/g, significantly, which showed significant differences in the mucosa, submucosa, and muscle layer ($p<0.05$) but not significant differences ($p>0.05$) in the subserosal area (Table 4).

The rate of increase in NOS activity with the degree of depth of rectal cancer was gradually decreased in turn, with 5.97, 4.74, 2.24, 1.97 and 1.58 for mucosa, submucosa, muscle layer, serosa, and subserosa, significantly, as the healthy, and the rate of increase in NO content was gradually decreased as 3.63, 3.29, 3.06, 2.40 and 2.31 (Table 4). As shown in the experimental data, the changes in nitric oxide synthase activity and nitric oxide content with the degree of adenocarcinoma of the rectum were found to be

lower in NOS activity and lower in NO content with the increasing cancer stage.

The NOS activity was significantly different from that of well differentiated, moderately differentiated, poorly differentiated and undifferentiated carcinomas, 0.23±0.04 μmol/min, 0.19±0.03 μmol/min, 0.15±0.01 μmol/min, and 0.11±0.01μmol/min, significantly, in well differentiated, moderately differentiated, poorly differentiated carcinomas compared with undifferentiated carcinomas($p<0.05$ Table 5). The rate of increase in NOS activity with the degree of differentiation of rectal cancer was gradually decreased by 2.63, 2.50, 1.97, and 1.45 for well differentiated, moderately differentiated, poorly differentiated and undifferentiated cancers, significantly, when the healthy controls were used, and the rate of increase in NO content was gradually decreased by 3.17, 2.69, 2.68 and 1.85 (Table 5).

We believe that NO content measurements of rectal cancer tissues can infer the pathological and morphological classification of rectal cancer.

CONCLUSION

The changes in NOS activity and NO content in rectal cancer tissues are as follows.

1) In rectal cancer, NOS activity and NO content in cancer tissues were significantly higher than those in healthy (0.076±0.002μmol/min and 5.84±0.17μmol/g, significantly) with 0.16±0.01μmol/min and 15.63±0.64μmol/g significantly.

2) In the histopathological classifications (macroscopic pattern, histotype, depth, and differentiation) in rectal cancer, NOS activity and NO content decreased according to the classification.

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