Clinical Research

Med Sci Monit, 2000; 6(6): 1085-1092

Helicobacter pylori, gastrin and cyclooxygenase-2 in lung cancer

Wojciech Gocyk[1], Tomasz Nikliński, Henryk Olechnowicz, Aleksandra Duda[2], Władysław Bielański[2], Peter C. Konturek[3], Stanisław J. Konturek[2]

[1] Department of Thoracosurgery, District Hospital, Cracow, Poland
[2] Department of Physiology, Jagiellonian University Medical College, Cracow, Poland
[3] Department of Medicine, Erlangen-Nuremberg University, Erlangen, Germany

key words: cancer, lung, pathology, Helicobacter pylori, gastrin

SUMMARY

Introduction: *Tumors arising in the lungs are in over 90% bronchogenic carcinomas that have been attributed predominantly to tobacco smoking, asbestos or air pollution but little is known about endogenous factors that could facilitate their development and invasiveness. The lungs originate embriologically from the same endoderm cells which form the epithelia lining the digestive tract, where gastrin is the major proliferative stimulus. Aims: Since lung cancer patients were recruited mostly among smokers, who also have been found to exhibit significantly higher infection rate of Helicobacter pylori (HP) infection than non-smokers and, as since the HP-infected subjects show enhanced plasma levels of gastrin, we decided 1) to compare the seroprevalence of HP and the expression of its cytotoxin, CagA, in lung cancer patients with those in the age- and gender-matched controls without cancer; 2) to determine the gene expression for gastrin and its receptors (CCK_B-R) in lung cancer, 3) to assess the gastrin levels in plasma bronchial lavage and in tumor tissue and 4) to examine the expression of cyclooxygenase (COX)-1 and COX-2 in cancer tissue resection margin and intact bronchial mucosa.*
Material and methods: *The trial material included 50 patients with lung carcinoma and 100 age- and gender-matched controls. Anti-HP and anti-CagA IgG seroprevalence was estimated by specific antisera using ELISA tests. Gene expression of gastrin, CCK_B-R, COX-1 and COX-2 was examined using RT-PCR, while gastrin was measured by specific RIA.*
Results: *The seroprevalence of HP, especially that expressing CagA, is significantly higher in lung cancers than in healthy controls. Both gastrin and CCK_B-R mRNA were detected in the cancer tissue and at the resection margin and similarly COX-2 mRNA was expressed in most cancers and resection margin but not in bronchial mucosa where only COX-1 was found. The lung cancer tissue and resection margin contained many folds larger amounts of immunoreactive gastrin than intact bronchial mucosa.*

INTRODUCTION

Numerous epidemiological studies demonstrated that malignant tumors in the lungs are predominantly bronchiogenic carcinomas, being the most common visceral malignancies accounting for about one third of all cancer deaths in men and more than 7% of all deaths in both sexes [1,2]. Statistical evidence established a positive relationship between tobacco smoking and lung cancer and a variety of substances present in cigarette smoke have been considered as potential carcinogens but efforts to reproduce the lung cancer by exposing animals to smoke and its ingredients have not been successful [3].

As in other cancers, various genetic mutations have been detected by the time the lung tumor becomes clinically apparent. Furthermore, an overexpression of cyclooxygenase-2 was reported in lung cancer by numerous investigators suggesting that

Received: 2000.11.22
Accepted: 2000.12.02
Correspondence address: Prof. Dr Stanisław J. Konturek, Department of Physiology, Jagiellonian University Medical College, ul. Grzegorzecka 16, 31-531 Kraków, Poland

this enzyme could be responsible for abundant release of eicosanoids, cell proliferation, angiogenesis and reduction in apoptosis [4–8], similarly as in gastric cancer [9].

It is well known that lungs arise embriologically from the same endoderm cells [10] that form the lining of the gastrointestinal tract and possess similar neuro-endocrine and paracrine cells releasing various hormonal peptides and their receptors including gastrin releasing peptide (GRP) and gastrin [11–15].

Helicobacter pylori (HP) infection in the stomach is known to be accompanied by a markedly enhanced and prolonged release of gastrin [16] that has been suggested to account for the development of gastric cancer [16–18] and has been shown to coexpress with COX-2 [5–9,19,20]. Since HP infection was found to reach higher rate in smokers than in non-smokers [21], we decided to assess the relationship between HP infection and related gastrin release and COX-2 expression in lung cancerogenesis. As cyclooxygenase-derived prostaglandins (PG) have been suggested to stimulate angiogenesis decrease apoptosis and tumor growth [20,23], it was rationale to determine the expression of both COX-1 and COX-2 in the lung carcinoma.

Therefore, the aims of this study were following: 1) to compare the seroprevalence of HP and its cytotoxic protein, CagA, in lung cancer patients with these in age- and gender-matched healthy controls; 2) to determine the gene expression of gastrin and its receptors (CCK_B-R) in lung cancer; 3) to assess the gastrin content in the plasma and bronchial lavage as well as in the lung tumor and 4) to examine the coexpression of COX-1 and COX-2 in the lung cancer tissue.

We found that the lung cancer tissue contains higher amounts of gastrin than the intact bronchial mucosa and that cancer is capable of expressing mRNA for gastrin and gastrin-receptors as well as for COX-1 and COX-2 and to release large amounts of the immunoreactive gastrin that could stimulate locally (by autocrine and paracrine pathways) the tumor cell proliferation and possibly also to induce COX-2 expression in cancer tissue.

MATERIAL AND METHODS

The studies were carried out on 50 histologically verified lung carcinoma (45 men and 5 women) with the median age of 64 years (range 47 to 82 years) and 100 controls (90 men, 10 women) with the median age of 63 years (range from 47 to 82 years). Patients symptoms, age, gender, tumor site and its histology were recorded at the Department of Cardiosurgery of District Hospital of Cracow, Poland and the laboratory tests were performed at the Department of Physiology, Jagiellonian University Medical College, Cracow, Poland. Before the surgery, gastroscopy was performed in some patients to obtain mucosal biopsy from corpus and antral mucosa for RT-PCR analysis for the comparison of the expression of gastrin and its receptors (CCK_B-R). During the surgery, the large biopsy samples were taken from the tumor, the resection margin and intact bronchial mucosa at the remote site from the cancer for by routine histological examination to determine the stage and histological type of the tumor. The tumors were identified histologically as quamous cell carcinoma (80%) or adenocarcinoma (20%) [1].

The HP infection status was assessed by our modification of capsulated minidose ^{13}C-Urea Breath Test (UBT) as described earlier [24] and/or by determination of IgG antibodies to HP by enzyme linked immunosorbent assay (ELISA) using commercially available kit (EIAGEN HPIgG, Clone Systems, Italy. Titers higher than 15 AU/ml were considered positive (following the manufacturer recommendations). IgG antibodies against CagA were detected by ELISA using recombinant CagA kindly provided Ora Vax Cambridge, USA as previously [16].

The samples of the tumor, the resection margin and macroscopically intact bronchial mucosa taken at the remote site from the tumor during surgery were used for determination of the mRNA expression of gastrin, CCK_B-R, COX-1 and COX-2 by reverse transcription-polymerase chain reaction (RT-PCR) as well as to assess the tissue content of gastrin using RIA. For this purpose two larger tumor (~100 mg), two tumor resection margin (~100 mg) and two bronchial mucosa samples were taken and frozen immediately in the liquid nitrogen for the detection of the signals for gastrin and gastrin receptors as well as COX-1 and COX-2 using reverse transcriptase-polymerase chain reaction (RT-PCR) as described earlier [25]. For measurement of gastrin content, the tumor, the resection margin and the intact mucosa samples were homogenized in phosphate buffer at pH 7.7 and 0°C for 10 s with Ultra-Turrax T-25, Ika Labortechnik, Staufen, Germany. The homogenized samples were processed as for plasma gastrin RIA using gas-

trin antiserum No 4562 (kindly donated by Professor J. E. Rehfeldt of Copenhagen, Denmark) in the final dilution of 1:140 000. The antibody used recognized G-17 and G-34 equally. The sensitivity of the present assay was 2.5 pmol/mL serum equivalent to human G-17 [15]. Blood samples were collected preoperatively from peripheral vein under basal conditions and after separation of the plasma they were stored at –70°C for gastrin RIA as described before [15,16]. Also the samples of bronchial lavage were obtained after instillation of about 50 ml of sterile saline into the bronchial tree involved in the cancer, then succeed from the lung and immediately centrifuged to separate the cellular components and stored and stored at –80°C for gastrin RIA.

RT-PCR and detection of amplified gene products were performed using the above mentioned large biopsy specimens obtained during surgery as described before [15]. For this purpose, total cellular RNA was isolated from these specimens using TRIzol Reagent (Gibco BRL, UK). Briefly, tissue samples placed in the liquid nitrogen and then transferred to TRIzol Reagent and homogenized. After addition of chloroform the aqueous phase was separated by 13000 rpm spin in 4°C for 10 min. RNA was precipitated using isopropyl alcohol, washed with 75% ethanol and resuspended in DEPC treated water. 1 mg of total cellular RNA was used for the synthesis of cDNA in RT-PCR reaction (Promega Reverse Transcription System). The final concentration of components in 20 ml of total reaction volume was as follows: 5 mM $MgCl_2$, 1x Reverse Transcription Buffer (10 mM Tries-HCl, 50 mM KCl, 0.1% Triton X-100), 1 ml each dNTP 1U/ml Recombinant RNasin Ribonuclease Inhibitor, 15 U/mg AMV Reverse Transcriptase 0.5 mg Oligo $(dT)_{15}$ Primer per microgram RNA. Samples were incubated at 42°C for 15 minutes and heated at 99°C for 5 minutes followed by a 5 min incubation at 0–4°C.

The PCR reactions were prepared in 1 x Dynazyme PCR buffer (Flowgen) with 40 mmol dNTPs (Pharmacia) and 10 mmol of upper and lower primers. The following primer pairs for PCR reaction were used: 5′-GCC CAG CCT CTC ATC ATC as a sense primer 3′-GGG GAC AGG GCT GAA GTG - antisense for gastrin; 5′-TCT CGC GAG CTC TAC TTA GGG as sense primer and 3′-GAA GTT GCA CGT AGC AGC CA - antisense for CCK_B; 5′-AGC GGG AAA TCG TGC GTG as sense primer and 3′-GGG TAC ATG GTG GTG CCG as antisense for β-actin as well as 5′-GCAACACTGGAACATGGCTA-3′ as a sense primer and 5′ACGCCACCATTCTGTCTTTG-3′ as an antisense primer for COX-1; and 5′-TCATTCACCAGGCAAATTGCTGGCAGGG-3′ as a sense primer and 5′-ACAGTTCA GTCGAACGTTCTTTTAGTAGTAC-3′ as an antisense primer for COX-2.

The thermal profile for all these reactions was the same; 95°C for 5 min before 40 cycles or 95°C for 45 sec, 60°C for 90 sec, and 72°C for 90 sec. This was followed by a single stage of 60°C for 120 sec and 72°C for 180 sec. PCR products were analyzed by agarose gel electrophoresis and abundance of cDNA in each sample was estimated by video densitometry analysis (Fotodyne, USA) using Gel-Pro Analyzer program.

Statistical analysis

The major groups were the HP-, CagA-seropositive and serum and bronchial lavage gastrin concentrations in patients with gastric cancer and controls. In general, rank sum test, Sperman's rank test order correlation was used for relation between independent variables. A P value of less than 0.05 was accepted as significant.

RESULTS

Figure 1 shows the HP seropositivity and CagA seropositivity in 50 lung cancer patients and in 100 age- and gender-matched control subjects. The prevalence of HP seropositivity reached ~90% in lung cancer but only 64% in controls and this difference in HP seropositivity between cancers and controls was statistically significant. The CagA seropositivity in lung cancer patients was about

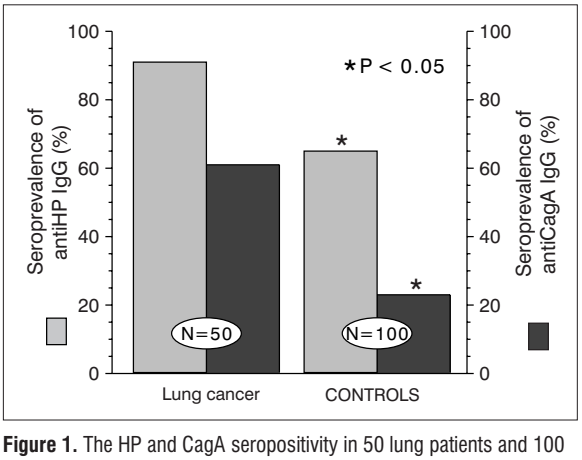

Figure 1. The HP and CagA seropositivity in 50 lung patients and 100 controls. Mean±SEM of 20 tests in 50 lung cancer and 100 controls. Asterisk indicates significant (P<0.05) change as compared to controls.

thrice as high as in controls and this difference was also statistically significant (Figure 2).

Plasma gastrin level in lung cancer patients was about 73±14 pM and this was about three folds higher than that in controls (22±7 pM). Similar difference in gastrin concentrations was found between plasma and bronchial lavage (Figure 1). The gastrin content in lung cancer tissue was nearly 3 ng/g of wet tissue weight and it was about twice higher than at the resection margin and about six times more than in the macroscopically normal bronchial mucosa (Figure 2). This difference between gastrin concentration in lung cancer plasma and in healthy controls as well as between the gastrin content in tumor tissue, resection margin and intact bronchial mucosa was highly statistically significant.

The tumor tissue of all cancer patients except one (patients No 1) showed mRNA expression for gastrin and similar expression was detected in antral biopsy samples taken from some of these patients (Figure 3). The mRNA expression for gastrin receptor (CCK_B) signal was observed in seven out of ten tested patients and it was usually less than that in oxyntic mucosa of the same patients. The ratio of gastrin mRNA to β-actin mRNA varied from case to case and in one patients (No 10) it was similar in the tumor tissue to that in antral mucosa but was negligible in the corpus mucosa (data not shown). The ratio of CCK_B-receptor mRNA to β-actin mRNA in lung tumor tissue or resection margin reached the values smaller than that of intact gastric corpus mucosa which is major target tissue of gastrin and varied from case to case (Figure 3). This ratio was negligible in the antrum mucosa and these results have been omitted for the sake of clarity.

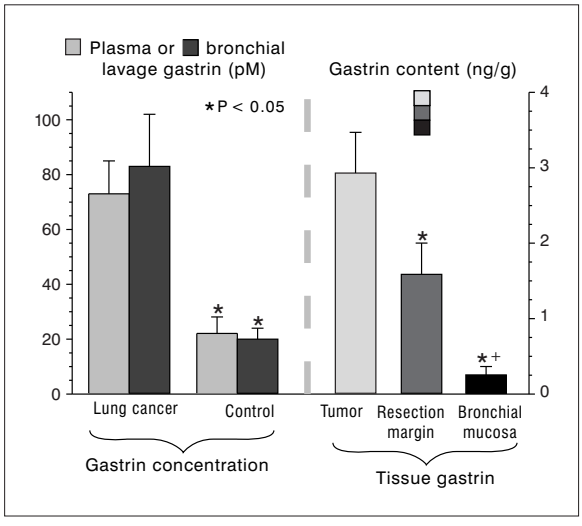

Figure 2. Plasma and bronchial lavage levels of gastrin in lung cancer and controls. Gastrin contents in lung tumor, resection margin and intact bronchial mucosa. Mean±SEM of 50 tests on 50 lung cancer and 100 controls.

Figure 4 shows the mRNA expression for COX-1 and COX-2 in the same cases as shown in Figure 3. The COX-1 mRNA was expressed both in the cancer tissue, the resection margin as well as in intact bronchial mucosa. COX-2 mRNA expression in all tissue samples of lung cancer and its resection margin but in none of the intact bronchial mucosa. In seven out of ten cases, the COX-2 mRNA expression was higher in the tumor tissue than in resection margin.

DISCUSSION

This study demonstrates for the first time that that lung cancer is strongly associated with CagA-positive HP infection and shows that gastric HP infection in lung cancer patients is accompanied by a significant increase in gastrin plasma and bronchial lavage levels as well as by in increased mRNA expression for gastrin and its receptors, as well as for COX-1 and COX-2 in the tumor tissue.

The reason for the higher prevalence of HP infection in the lung cancer patients is not apparent but the fact that the majority of cancer patients were smokers, who are known to have higher HP infection rate [21], at least in heavily smoking Polish population, indicates that smoking could be the major factor of higher HP infection rate in lung cancer patients.

The question remains whether this higher HP infection rate plays any role in the pathogenesis of lung cancer but the fact that this infection is accompanied by an increased plasma level of gastrin [15,16], suggests that this hormone could contribute to the lung cancerogenesis by inducing higher mucosal cell proliferation of bronchial epithelium leading to atrophy and induction of COX-2 as it happens in most of gastric cancers [25,26]. Previous studies showed that all classes of bronchogenic carcinoma exhibit increased local gastrin gene expression [12] and that gastrin concentration is elevated in serum and bronchoalveolar lavage (BAL) fluid in patients with lung cancer [27] and correlates with the disease extension [28] though this was not confirmed by others [29]. In addition, other studies indicate that gastrin is capable of mobilization of calcium ions and stimulation

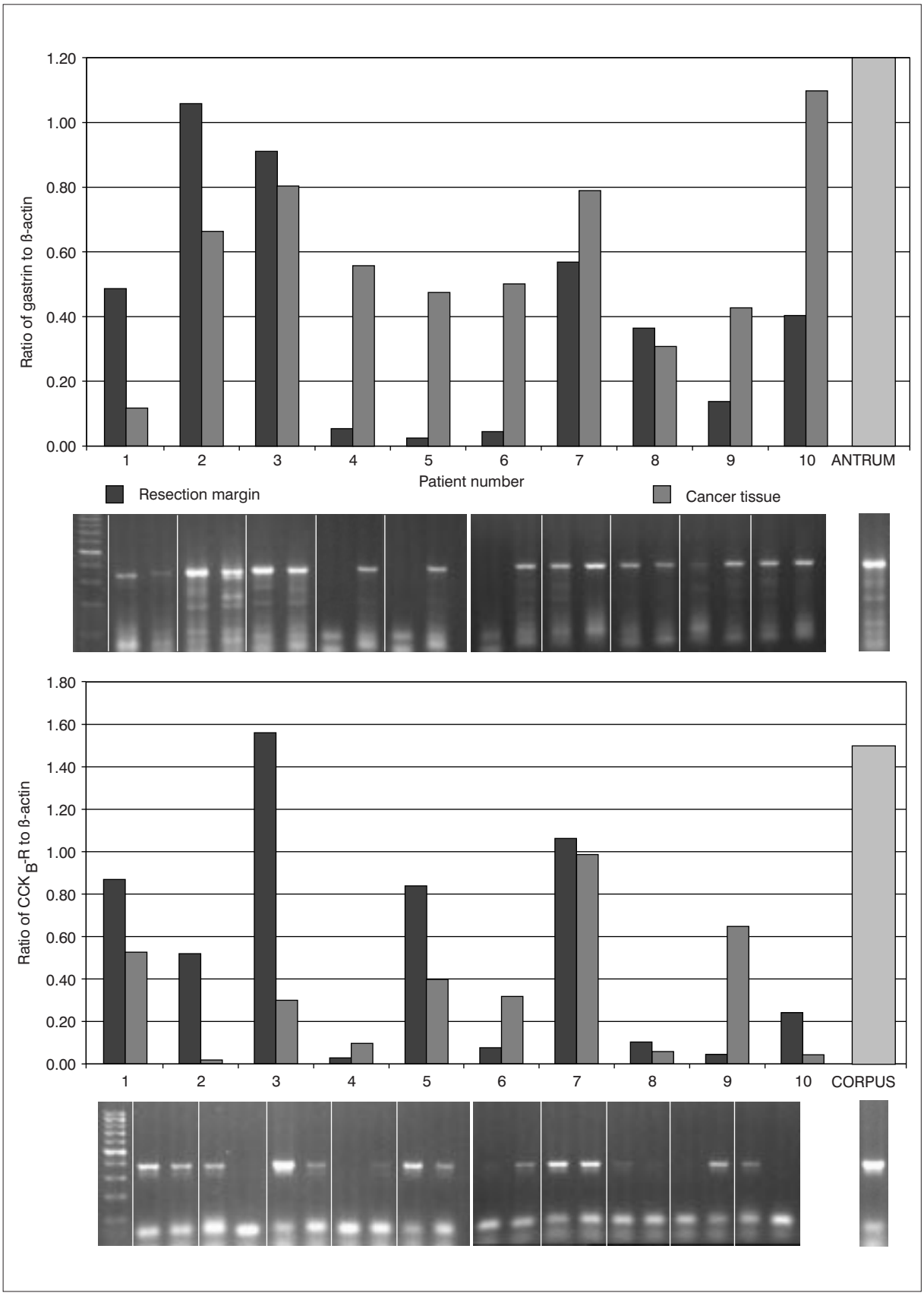

Figure 3. Ratio of gastrin and its receptor (CCK_{13}-R) to β-actin in cancer tissue and resection margin, for comparison gastrin and its receptor ratio is also given for antral mucosa and corpus mucosa, respectively.

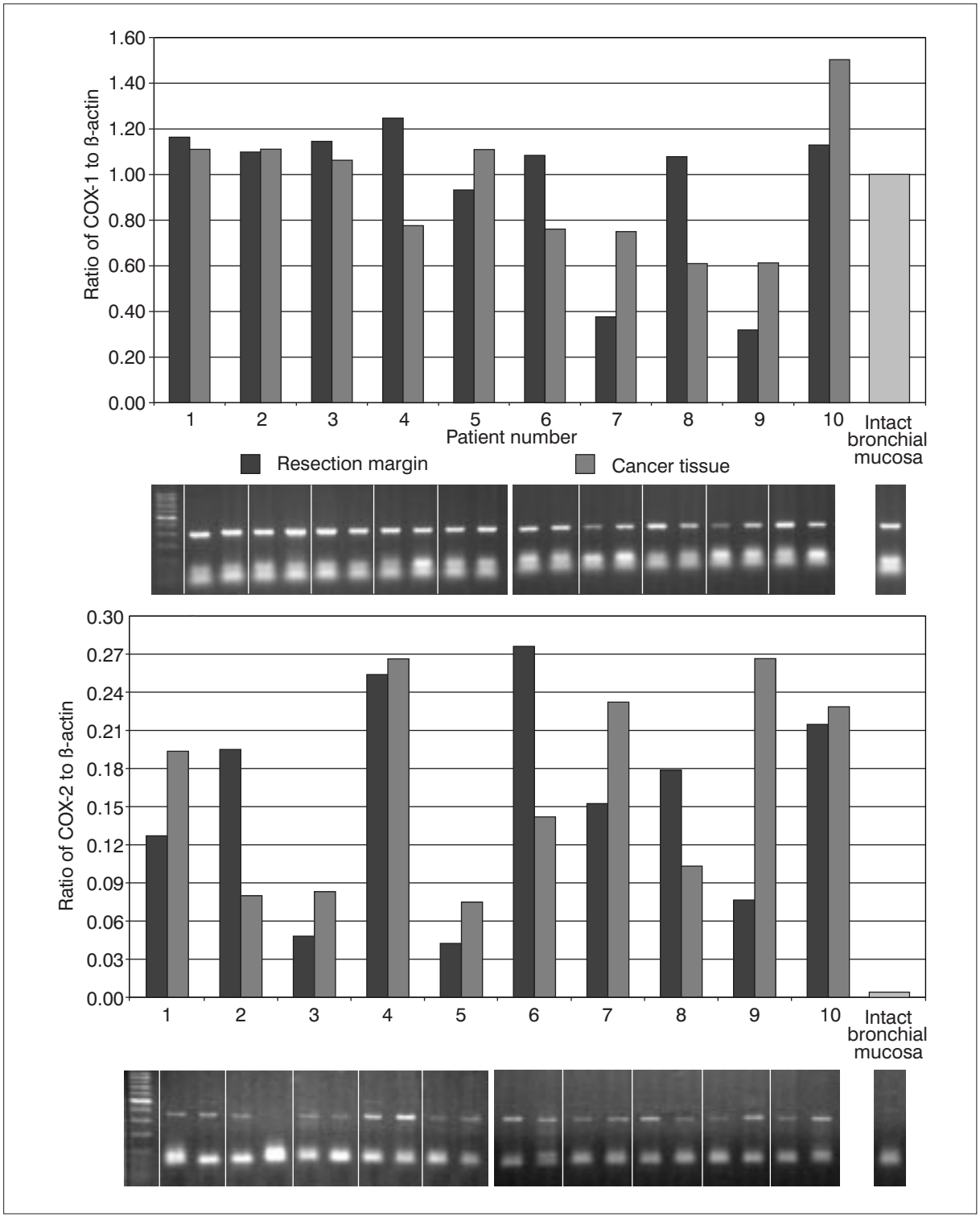

Figure 4. Ratio of COX-1 and COX-2 to β-actin in cancer tissue and resection margin as in studies shown on Figure 3.

of clonal growth of small cell lung cancer cells [30]. Our results confirm that, indeed, the plasma and bronchial lavage fluid obtained from patients with lung cancer exhibit significantly higher gastrin levels than those in control subjects.

The main finding of the present study is the observation that the lung cancer tissue and resection margin express mRNA for gastrin and CCK_B-R and contain several folds higher content of immunoreactive gastrin than the intact bronchial mucosa. This suggests that the gastrin present in lung cancer could contribute to the increased plasma levels of this hormone, though the HP infection by itself could explain, at least in part, the enhanced plasma levels of this hormone originating from gastric G cells as documented previously [15,17]. Our finding that lung cancers exhibit higher expression and content of gastrin and its receptors reminds similar upregulation of gastrin biosynthesis already described for gastric cancers [25] and colorectal cancers [31]. As gastrin is known to be the most potent mucosal growth promoting factor in gastrointestinal tract [32] (and possibly also in the bronchial mucosa), it is tempting to assume that this hormone could play a key role in the initiation and the progression of cancer disease both in the gastrointestinal tract and the lungs that embriologically originating from the same endoderm tube. This is supported by recent report that α-amidated gastrin peptides and their receptors are invariably coexpressed in the adenocarcinoma of the pancreas that also originates from the same endoderm as alimentary tract and the lungs [33]. This suggests, again, that gastrin may be an autocrine factor in carcinoma arising also from the pancreatic acinar cells. This study is of particular interest because it showed that in addition to amidated gastrin, glycine extended gastrins as well as N-terminal progastrins were detected in the pancreatic cancer as it was also found in colorectal cancer [31].

Epidemiological studies indicate that the use of aspirin and other NSAID, whose major target is cyclooxygenase, a rate-limiting enzyme converting arachidonic acid to prostanoids, decreases the incidence and mortality from gastrointestinal cancers [23]. COX-2 has been found to occur in the lung cancer [34] where it was held responsible for the production of extensive amounts of prostaglandins, tumor cell proliferation, angiogenesis, reduction in apoptosis and tumor invasiveness. Assuming that HP and gastrin are initiators of lung carcinogenesis, the excessive production of prostanoids might be implicated in promotion of this process and should be a target for the chemotherapy using specific COX-2 inhibitors [35]. Our results confirm the gene expression of both COX-1 and COX-2 in the lung cancer and thus, support the notion that, indeed, COX-2 and COX-2 derived prostanoids may play a role in the development of lung cancer and should be a target of treatment using specific COX-2 inhibitors. As the elimination of the initiator of lung cancerogenesis such as increased gastrin production seems to be possible, simply by eradication of HP, we propose, based on our present data, that the HP infection in lung cancer patients should be eliminated to reduce hypergastrinemia and COX-2 expression, both factors that might be responsible for the promotion of lung cancer.

CONCLUSIONS

This study provide an evidence that lung cancer is capable of expressing gastrin and its receptors as well as COX-2 that may be implicated in colonic cancerogenesis.

1. Lung cancer tissue and its resection margin exhibit higher contents of gastrin and coexpress more gastrin, its receptors and COX-2 than intact bronchial mucosa.

2. HP infection may contribute to lung cancerogenesis via upregulation of gastrin and COX-2 that may account for the stimulation of tumor growth and angiogenesis, and

3. HP positive patients developing lung cancer should be considered for the HP eradication to reduce the HP provoked hypergastrinemia and COX-2 expression.

REFERENCES:

1. *Kobzik L: The lung. In: Cotran RS, Kumar V, Collins T eds: Pathologic Basis of Disease. WB Saunders Co, Philadelphia, 1999; 697-755*
2. *Samet JM et al: Epidemiology of Lung Cancer. New York, Marcel Dekker, 1994*
3. *Marchevsky AM: Pathogenesis and experimental models of lung cancer. In Marchevsky AM eds: Surgical Pathology of Lung Neoplasms. New York, Marcel Dekker, 1990; 7*
4. *Salgia R, Skarin AT: Molecular abnormalities in lung cancer. J Clin Oncol, 1998; 16: 1207*
5. *Achiwa H, Yatabe Y, Hida T et al: Prognostic significance of elevated cyclooxygenase 2 expression in primary, resected lung adenocarcinomas. Clin Cancer Res, 1999; 5(5): 1001-5*
6. *Wolff H, Saukkonen K, Antiila S et al: Expression of cyclooxygenase-2 in human lung carcinoma. Cancer Res, 1998; 58(22): 4995-5001*

7. *Hida T, Yatabe Y, Achiwa H et al: Increased expression of cyclooxygenase 2 occurs frequently in human lung cancers, specifically in adenocarcinomas. Cancer Res, 1998; 58(17): 3761-4*
8. *Fosslien E: Molecular pathology of cyclooxygenase-2 in neoplasia. Ann Clin Lab Sci, 2000; 30(1): 3-21*
9. *Yamamoto H, Itoh F, Fukushima H et al: Overexpression of cyclooxygenase-2 protein is less frequent in gastric cancers with microsatellite instability. Int J Cancer, 1999; 84(4): 400-403*
10. *Larsen WJ: Human Embryology. Churchill Livingstone, New York 1997*
11. *DeMichele MA, Davis AL, Hunt JD et al: Expression of mRNA for three bombesin receptor subtypes in human bronchial epithelial cells. Am J Respir Cell Mol Biol, 1994; 11(1): 66-74*
12. *Rehfeld JF, Bardram L, Hilsted L: Gastrin in bronchogenic carcinomas: constant expression but variable processing of progastrin. Cancer Res, 1989; 49: 2840-3*
13. *Frankel A, Tsao MS, Viallet J: Receptor subtype expression and responsiveness to bombesin in cultured human bronchial epithelial cells. Cancer Res, 1994; 54(7): 1613-6*
14. *Siegfried JM, Guentert PJ, Gaither AL: Effects of bombesin and gastrin-releasing peptide on human bronchial epithelial cells from a series of donors: individual variation and modulation by bombesin analogs. Anat Rec, 1993; 235(1) 241-7*
15. *DeJong M, Bakker WH, Bernard BF et al: Preclinical and initial clinical evaluation of 111In-labeled nonsulfated CCK8 analog: a peptide for CCK-B receptor-targeted scintigraphy and radionuclide therapy. J Nucl Med, 1999; 40(12): 2081-7*
16. *Konturek JW, Bielański W, Konturek SJ et al: Eradication of Helicobacter pylori and gastrin-somatostatin link in duodenal ulcer patients. J Physiol Pharmacol 1996; 47: 161-76*
17. *Konturek PC, Bielański W, Bobrzyński A et al: Gastric mucosal expression and luminal release of growth factors in gastrin carcinoma and duodenal ulcer patients before and after eradication of Helicobacter pylori. J Physiol Pharmacol, 1997; 48: 375-82*
18. *Danesh J: Helicobacter pylori infection and gastric cancer: systematic review of the epidemiological studies. Aliment Pharmacol Ther, 1999; 13: 851-6*
19. *Uefuji K, Ichikura T, Mochizuki H: Cyclooxygenase-2 expression is related to prostaglandin biosynthesis and angiogenesis in human gastric cancer. Clin Cancer Res, 2000; 6(1): 135-8*
20. *Lipsky PE: Role of cyclooxygenase-1 and –2 in health and diseases. Am J Orthop, 1999; 28: 8-12*
21. *Bielanski W: Epidemiological study on Helicobacter pylori infection and extragastroduodenal disorders in Polish population. J Physiol Pharmacol, 1999; 50: 723-33*
22. *Lim HY, Joo HJ, Yi JW et al: Increased expression of cyclooxygenase-2 protein in human gastrin carcinoma. Clin Cancer Res, 2000; 6: 519-29*
23. *Fosslien E: Molecular pathology of cyclooxygenase-2 in neoplasia. Ann Clin Lab Sci, 2000; 30: 3-21*
24. *Bielański W, Konturek SJ: New approach to ^{13}C urea breath test: capsule-based modification with low-dosed of ^{13}C-urea breath test in the diagnostic of Helicobacter pylori infection. J Physiol Pharmacol, 1996; 47: 545-53*
25. *Konturek PC, Konturek SJ, Bielański W et al: Role of gastrin in gastric cancerogenesis in Helicobacter pylori infected humans. J Physiol Pharmacol, 1999; 50: 857-73*
26. *Moss SF: The carcinogenic effect of H. pylori on the epithelial cells. J Physiol Pharmacol, 1999; 50: 847-56*
27. *Zhon Q, Yang Z, Yang J et al: The diagnostic significance of gastrin measurement of bronchioalveolar lavage fluid for lung cancer. J Surg Oncol, 1992; 50: 121-4*
28. *Zhou Q, Zhang H, Pang X et al: Pre- and postoperative sequential study on the serum gastrin level in patients with lung cancer. J Surg Oncol, 1992; 51: 22-5*
29. *Dowlati A, Bury T, Corhay J-L et al: Gastrin levels in serum and bronchioalveolar lavage of patients with lung cancer: comparison with chronic obstructive pulmonary disease. Thorax, 1996; 51: 1270-2*
30. *Sethi T, Rozengurt E: Gastrin stimulates Ca^{++} mobilization and clonal growth in small cell lung cancer cells. Cancer Res, 1992; 52: 6031-5*
31. *Singh P, Dai B, Wu H, Owlia A: Role of autocrine and endocrine gastrin-like peptides in colonic carcinogenesis. Cur Opinion Gastroenterol, 2000; 16: 28-77*
32. *Johnson LR: Regulation of gastrointestinal growth. In Johnson LR, Christensen J, Grossman MI, Jacobson ED, Schults SG eds: Physiology of the Gastrointestinal Tract. New York USA, Raven, 1981; 169-196*
33. *Goetze JP, Nielsen FC, Burcharth F, Rehfeld JF: Closing the gastrin loop in pancreatic carcinoma. Coexpression of gastrin and its receptor in solid human pancreatic adenocarcinoma. Cancer, 2000; 88: 2487-94*
34. *Ochiai M, Oguri T, Isobe T et al: Cyclooxygenase-2 (COX-2) mRNA expression levels in normal lung tissues and non-small cell lung cancers. Jpn J Cancer Res, 1999; 90(12): 1338-43*
35. *Masferrer JL, Leahy KM, Koki AT et al: Antiangiogenic and antitumor activities of cyclooxygenase-2 inhibitors. Cancer Res, 2000; 60(5): 1306-11*