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Promotion of Home Terminal Care for Elderly Patients with Cancer

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One half of males and one third of females in our country suffer from malignant tumors. Malignant tumors are also the top cause of death in the breakdown of death rate by cause—keeping the top position, surpassing cardiovascular and cerebrovascular diseases, since 1981. The progress in medical treatment strategies for cancer has been remarkable, through procedures such as surgery, chemotherapy, radiotherapy, and immunotherapy.

Naturally, the positive medical treatments for these malignant tumor patients are performed in hospitals. However, hospitals are also divided into acute term, subacute term, and chronic term hospitals. In an acute term hospital, shortening the number of hospital days is a priority. Therefore, it became regular practice for chemotherapy etc. to be performed not by hospitalization, but by out-patient clinics. Moreover, whether a cancer patient should receive treatment for a terminal stage disease in places like a hospice, or at home also poses a problem.

In the past, deaths at home was far larger in number than deaths at hospitals and other medical institutions. However, the ratio was reversed in 1980. In 2003, 13.0% of all deaths took place at home while 81.6% were at medical institutions. Elderly people desire home death in many cases. How to secure the QOL of elderly people at home is thus a major problem.

Hirakawa and others studied 126 elderly patients with advanced cancer dying at home and examined the differences in symptom experience and care receipt among them for lung, gastric, colon, and liver cancer. This paper revealed that loss of appetite was commonly observed in all four groups. Dyspnea, fever, cough, and sputum were most prevalent among the lung cancer patients. Although stomach cancer had excessive nausea and vomiting followed by cancer of the large intestine, there was no statistical difference regarding frequency by the type of cancer. The pain was divided into the poor control group and the good control group, and was investigated. The poor control group was 45 to 60% as compared to the 10 to 20% of the good control group. Oxygen inhalation is administered in very high frequency of 72.7% for lung cancers. The findings are not surprising. However, the effort to gather and analyze data that is extremely difficult to obtain, namely, cases of elderly cancer patients being cared at home, deserves recognition and respect.

There is another problem that has not been discussed in this paper. It is that the existence of cognitive impairment was 19.2–36.0%. Although it has also been a subject in public long-term care insurance, the problem of dementia is the most important subject of medical care for elderly people. The QOL of elderly people is influenced not only by somatic symptoms, but also their psychological condition. Many of the somatic symptoms are influenced by the psychological condition and environment. In Japan, it is not realistic to administer religious healing. However, as this paper showed, the poverty of psychological support is a huge problem. The doctor should also be concerned about these problems.

Anyway, it is my hope that this paper will create a stir in the state of palliative care for elderly cancer cases at home.

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Symptoms and Care of Elderly Patients Dying at Home of Lung, Gastric, Colon, and Liver Cancer

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Abstract

Backgrounds There is growing concern among health care providers and the public about the quality of home end-of-life care for elderly patients with advanced cancer. Therefore, it is essential to learn more about the distressing symptoms experienced by elderly patients with advanced cancer dying at home. The purpose of the present study was to examine the differences in symptom experience and care receipt among elderly patients dying at home of lung, liver, gastric, and colon cancer.

Methods The present data were obtained from the Dying Elderly at Home (DEATH) project, a multicenter observational study conducted in Japan. The following information was collected: decedent characteristics, observed symptoms, and end-of-life care provided during the last 48 hours of life. A total of 42 gastric, 33 lung, 26 liver, and 25 colorectal cancer decedents were included in the analysis.

Results Anorexia was a common symptom among all four groups. Dyspnea, fever, cough, and sputum were most prevalent among the lung cancer group. Although nausea and vomiting were more prevalent among the gastric cancer group, followed by the colorectal cancer group, the differences were not significant among the four groups. Controlled or uncontrolled pain and anorexia were prevalent among all four groups, but no significant differences were found. Significantly more lung cancer decedents were given oxygen inhalation than other cancer decedents.

Conclusions More elderly lung cancer patients experienced symptom distress such as dyspnea and cough than the other cancer groups. Oxygen inhalation is essential for the end-of-life care of elderly home lung cancer patients.

Key words End-of-life care, Elderly, Cancer, Symptoms, Oxygen

Introduction

Cancer is a common cause of death among elderly people. A trend expected to occur in the near future is a gradual shift in the place where elderly people spend their last years, from hospital to home.[1–3] As a result, there is growing concern among health care providers and the public about the quality of home end-of-life care for elderly patients with advanced cancer.

Successful end-of-life care requires good control of symptoms such as pain. As some researchers have suggested,[4–6] there appears to be substantial heterogeneity in the primary sites of cancer. Therefore, it is vital that we learn more about the distressing symptoms experienced by elderly patients with advanced cancer dying at home.

While a number of studies have examined symptom experience or end-of-life care receipt of elderly patients with advanced cancer at

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Table 1 Summary of symptoms and treatments in the questionnaire

Symptoms
Dyspnea, uncontrolled pain, controlled pain, coma, acute confusion, anxiety, dizziness, nausea and vomiting, anorexia, diarrhea, constipation, fever, urinary and fecal incontinence, hematemesis, hemoptysis, bottom blood, other types of hemorrhage, cough, sputum
End-of-life care
Heart massage, intubation, mechanical ventilation, oxygen inhalation, airway placement, sputum suction, hyperalimentation, intravenous drip injection (except hyperalimentation), antibiotics, vasopressor, blood transfusion, opioids, urinary catheter placement, mental support, religious healing (such as visits by religious leaders), others

hospitals or hospices,[4,5,7–10] to our knowledge, there is still insufficient information regarding the experience of elderly cancer patients dying at home.

The purpose of the present study was to examine the differences in symptom experience and care receipt among elderly patients dying at home of lung, liver, gastric, and colon cancer, which are the most common primary sites of cancer.

Methods

Study design and population

The present data were obtained from the Dying Elderly at Home (DEATH) project, a multicenter observational study. The DEATH project was conducted in collaboration with the Japanese Society of Hospice and Home-care. The society is a non-profit organization consisting of general physicians and other medical and social professionals interested in hospice and home-care. Consecutive decedents aged 65 or older who were using sixteen study clinics belonging to the society with diagnoses of all illnesses including advanced cancer and who died at home from October 2002 to September 2004 were included in the study. Decedents were excluded if they were transferred to a hospital at death. The following information was collected: socio-demographics, ADLs (Japan's Ministry of Health, Labour and Welfare identifies four ranks of ADL of disabled elderly patients as follows:[11] Rank J (independent in ADLs), Rank A (house-bound), Rank B (chair-bound), and Rank C (bed-ridden), cognitive impairment, observed symptoms and provided end-of-life care during the last 48 hours of life. With the approval of the Japanese Society of Hospice and Home-care, we used a questionnaire that included a list of common symptoms and treatments at the end-of-life as shown in Table 1.

Data collection

Immediately after the death of the study patients, general practitioners (GPs) were asked to fill out a questionnaire based on the patients' medical charts and their recollection of the clinical course that followed. Family members or visiting nurses who witnessed the last 48 hours of the patients' lives were asked to provide additional information. The GPs and other information providers were blinded to the study hypothesis or anticipated study results. For ethical reasons, data on all eligible participants obtained from the Japanese Society of Hospice and Home-care remained anonymous. The research protocol was reviewed and approved by the Nagoya University Research Ethics Board.

Statistical analysis

We used data from the DEATH sample of decedents with primary lung, gastric, colon, and liver cancer, with and without metastasis. Thus, a total of 42 gastric, 33 lung, 26 liver, and 25 colorectal cancer decedents were included in the analysis. To assess the differences in characteristics and clinical course among decedents, the survey data were divided into four groups according to cancer type. The data were analyzed using Statview-J5.0. Group differences were compared using the Kruskal-Wallis test and the chi square test. P values of <0.05 were considered to be significant.

Results

The distribution of decedent characteristics is

Table 2 Cancer decedent characteristics

Variables		Gastric (n=42) n (%)	Lung (n=33) n (%)	Liver (n=26) n (%)	Colorectal (n=25) n (%)	*P*
Sex	Female	12 (28.6)	14 (42.4)	13 (50.0)	11 (44.0)	0.31
Age (average±SD)		75.7±1.3	75.8±1.6	76.1±1.2	75.0±2.0	0.99
ADL scale of disabled elderly	J=independent	1 (2.4)	1 (3.0)	3 (11.5)	0 (0.0)	—
	A=house-bound	2 (4.8)	4 (12.1)	2 (7.7)	2 (8.0)	
	B=chair-bound	11 (26.2)	10 (30.3)	7 (26.9)	4 (16.0)	
	C=bed-bound	23 (54.8)	14 (42.4)	13 (50.0)	15 (60.0)	
	Unknown	5 (11.9)	4 (12.1)	1 (3.8)	4 (16.0)	
Cognitive impairment	Present	11 (26.2)	10 (30.3)	5 (19.2)	9 (36.0)	0.58

Table 3 Cancer decedent symptom experience in last two days of life

Symptom	Gastric (n=42) n (%)	Lung (n=33) n (%)	Liver (n=26) n (%)	Colorectal (n=25) n (%)	*P*
Dyspnea	11 (26.2)	27 (81.8)	6 (23.1)	12 (48.0)	<0.01
Pain (uncontrolled)	5 (11.9)	5 (15.2)	5 (19.2)	5 (20.0)	0.79
Pain (controlled)	16 (38.1)	15 (45.5)	15 (57.7)	11 (44.0)	0.47
Coma	15 (35.7)	14 (42.4)	11 (42.3)	12 (48.0)	0.79
Acute confusion	9 (21.4)	5 (15.2)	6 (23.1)	10 (40.0)	0.16
Anxiety	9 (21.4)	7 (21.2)	6 (23.1)	4 (16.0)	0.93
Dizziness	0 (0.0)	1 (3.0)	0 (0.0)	0 (0.0)	0.42
Nausea and vomiting	17 (40.5)	5 (15.2)	7 (26.9)	9 (36.0)	0.10
Anorexia	27 (64.3)	19 (57.6)	17 (65.4)	18 (72.0)	0.73
Diarrhea	4 (9.5)	0 (0.0)	1 (3.8)	2 (8.0)	0.30
Constipation	3 (7.1)	3 (9.1)	1 (3.8)	0 (0.0)	0.46
Fever	5 (11.9)	14 (42.4)	6 (23.1)	5 (20.0)	0.02
Incontinence	5 (11.9)	5 (15.2)	5 (19.2)	6 (24.0)	0.61
Hematemesis	1 (2.4)	1 (3.0)	2 (7.7)	3 (12.0)	0.33
Hemoptysis	0 (0.0)	2 (6.1)	0 (0.0)	0 (0.0)	0.13
Bottom blood	4 (9.5)	0 (0.0)	1 (3.8)	3 (12.0)	0.21
Other hemorrhage	1 (2.4)	0 (0.0)	2 (7.7)	1 (4.0)	0.40
Cough	2 (4.8)	10 (30.3)	1 (3.8)	5 (20.0)	<0.01
Sputum	7 (16.7)	16 (48.5)	5 (19.2)	5 (20.0)	<0.01
Others	10 (23.8)	7 (21.2)	9 (34.6)	9 (36.0)	0.48

shown in Table 2. Except for the gastric cancer group (men : women = 71.4% : 28.6%), the gender ratio was nearly 1 : 1. Most decedents in each group were chair- or bed-bound. The average age ranged from 75.0–76.1. More than half of the decedents in each group had normal cognitive function. No significant differences were found among the four groups in gender, age, ADLs, and cognitive impairment.

The differences in symptom experience among cancer groups are shown in Table 3. Anorexia was a common symptom among all four groups.

Table 4 Cancer decedent care receipt in last two days of life

Care	Gastric (n=42) n (%)	Lung (n=33) n (%)	Liver (n=26) n (%)	Colorectal (n=25) n (%)	*P*
Heart massage	2 (4.8)	0 (0.0)	0 (0.0)	0 (0.0)	0.25
Intubation	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	—
Mechanical ventilation	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	—
Oxygen inhalation	5 (11.9)	24 (72.7)	6 (23.1)	6 (24.0)	<0.01
Airway placement	0 (0.0)	2 (6.1)	1 (3.8)	0 (0.0)	0.28
Sputum suction	7 (16.7)	11 (33.3)	3 (11.5)	7 (28.0)	0.15
Hyperalimentation	4 (9.5)	5 (15.2)	2 (7.7)	3 (12.0)	0.81
Antibiotics	2 (4.8)	5 (15.2)	3 (11.5)	3 (12.0)	0.50
Vasopressor	0 (0.0)	0 (0.0)	1 (3.8)	0 (0.0)	0.28
Blood transfusion	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	—
Intravenous drip injection	17 (40.5)	9 (27.3)	11 (42.3)	9 (36.0)	0.60
Volume (average ± SD)					
24–48 hours before death	518.75	568.75	295	555.56	0.16
0–24 hours before death	304.17	514.29	371.82	350	0.89
Opioids	17 (40.5)	22 (66.7)	13 (50.0)	10 (40.0)	0.10
Urinary catheter placement	6 (14.3)	7 (21.2)	5 (19.2)	6 (24.0)	0.77
Mental support	1 (2.4)	0 (0.0)	2 (7.7)	1 (4.0)	0.40
Religious healing	1 (2.4)	0 (0.0)	1 (3.8)	0 (0.0)	0.58
Others	3 (7.1)	1 (3.0)	2 (7.7)	1 (4.0)	0.81

Dyspnea, fever, cough, and sputum were most prevalent among the lung cancer group, showing significant differences ($P = <0.01$, 0.02, <0.01, <0.01, respectively).

Although nausea and vomiting were more prevalent among the gastric cancer group, followed by the colorectal cancer group, the differences were not significant among the four groups. Controlled or uncontrolled pain and anorexia were prevalent among all four groups, but no significant differences were found.

The differences in end-of-life care receipt among the cancer groups are shown in Table 4. Significantly more lung cancer decedents were given oxygen inhalation than the other cancer decedents. Few life-sustaining treatments such as heart massage, intubation, mechanical ventilation, and vasopressor were administered to any of the groups. Similarly, little mental health support or religious healing (such as visits by religious leaders) were given to any of the groups. Although more lung cancer decedents were given opioids, no significant differences were found between the four groups.

Discussion

One way to palliate dyspnea in the last days of life is to give oxygen therapy. Eighty percent of the lung cancer group presented with dyspnea, and 70% of elderly patients in this group were given home oxygen therapy. These results suggest that oxygen inhalation is essential to end-of-life care for home elderly lung cancer patients. Moreover, according to Kuebler,[12] dyspnea is described as one of the most devastating symptoms for patients and families, and opioids are the most widely used form of relief. Better knowledge and better recognition of opioids may also contribute to good palliative treatment for dyspnea.

Cough was also prevalent among the lung cancer group in our study. This may be a natural occurrence, since cough is a common effect of cardiopulmonary diseases. Although opioids have antitussive activity and constitute one of

the most effective treatments for refractory cough,[13] as mentioned above, it appears that they were not often used for the lung cancer patients in this study. It may also be helpful for GPs and families to be educated in the proper use of opioids for treatment.

Sputum is also a distressing symptom that causes dyspnea or cough.[4,13] However, the frequency of sputum suction was not significantly higher in the lung cancer group, in which sputum was the most prevalent. Although it is possible that the sputum was not severe enough to require sucking, the reasons behind the low frequency of sputum suction in this group should be analyzed further.

More elderly lung cancer decedents had fever in the last two days of their lives compared to the other cancer groups. Because there were no significant differences in the use of antibiotics among the four groups, it appears that lung cancer may be a more common cause of fever than other cancers. We need to pay attention to this particular symptom among elderly lung cancer patients in order to offer adequate management.

Consistent with Baines' and Griffie's suggestions,[14,15] our results showed that nausea and vomiting were more prevalent among the gastrointestinal cancer groups such as the gastric and colorectal cancer groups.

Anorexia was the most prevalent symptom among all four cancer groups, regardless of gastrointestinal illness. Some researchers have suggested that anorexia is one of the most common symptoms in advanced cancer patients.[4,9,16,17] Because it is so closely associated with declining physical, emotional, and social function,[16] anorexia can be a source of discomfort for patients and their families. It is important to educate patients and families on how to cope with their distress.

Study limitations

This study partly relied on family reports of patient symptoms, because the settings were the communities. Because there was a lack of information about the degree of symptom experience and metastatic sites, our results of symptom experiences must be interpreted with caution.

There was also a lack of information about end-of-life interventions such as steroids and non-opioids. Therefore, we were unable to perform a thorough symptom management evaluation.

We enlisted each clinic to perform evaluations because of the large number of settings. This may have biased the assessors' evaluation and limited the validity of the results, because it is possible that the data-collecting procedures and quality varied depending on the GPs in charge of data collection.

The reduced number of patients and limited study settings also impeded generalization. Selection bias is also possible, because the Japanese Society of Hospice and Home-care is interested in hospice and home-care. Larger studies in other groups of patients are needed for a better understanding of the actual situation of elderly cancer patients dying at home in Japan.

Conclusions

In conclusion, more elderly lung cancer patients experienced symptom distress such as dyspnea and cough than other cancer groups. Oxygen inhalation is essential to end-of-life care for elderly home lung cancer patients.

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References

1. Hashimoto H. Establishment of terminal care systems on the basis of patients' will. In: Iryokeizaikikou ed. Iryohakusyo. Tokyo: Nihon Iryokikaku; 2001:47–58. (in Japanese)
2. Sauvaget C, Tsuji I, Li JH, Hosokawa T, Fukao A, Hisamichi S. Factors affecting death at home in Japan. Tohoku J Exp Med. 1996;180:87–98.
3. Tilden VP, Drach LL, Tolle SW. Complementary and alternative therapy use at end-of-life in community settings. J Altern Complement Med. 2000;8:897–906.
4. Morita T, Tsunoda J, Inoue S, Chihara S, Ichiki, T. Symptom prevalence and risk factors in terminally ill cancer patients. Jpn J Cancer Clin. 1998;44:879–884. (in Japanese)

5. McCarthy EP, Phillips RS, Zhong Z, Drews RE, Lynn J. Dying with cancer: patients' function, symptoms, and care preferences as death approaches. J Am Geriatr Soc. 2000;48:S110–S121.
6. Heidrich DE. Malignancy. In: Kuebler KK, Berry PH, Heidrich DE ed. End-of-Life Care: Clinical Practice Guidelines. Philadelphia: WB Saunders; 2002:165–179.
7. Mercadante S, Casuccio A, Fulfaro F. The course of symptom frequency and intensity in advanced cancer patients followed at home. J Pain Symptom Manage. 2000;20:104–112.
8. Ahronheim JC, Morrison RS, Baskin SA, Morris J, Meier DE. Treatment of the dying in the acute care hospital. Arch Intern Med. 1996;156:2094–2100.
9. Tranmer JE, Heyland D, Dudgeon D, Groll D, Squires-Graham M, Coulson K. Measuring the symptom experience of seriously ill cancer and noncancer hospitalized patients near the end of life with the Memorial Symptom Assessment Scale. J Pain Symptom Manage. 2003;25:420–429.
10. Morita T, Tsunoda J, Inoue S, Chihara S. Characteristics of palliative care for elderly cancer patients—a pilot study at our hospital. Geriat Med. 1997;35:1505–1511. (in Japanese)
11. Hirakawa Y, Masuda Y, Kimata T, Uemura K, Kuzuya M, Iguchi A. Effects of home massage-rehabilitation therapy for the bed-ridden elderly: a pilot trial with a three-month follow-up. Clin Rehabil. 2004;18:1–8.
12. Kuebler KK. Dyspnea. In: Kuebler KK, Berry PH, Heidrich DE ed. End-of-Life Care: Clinical Practice Guidelines. Philadelphia: WB Saunders; 2002:301–315.
13. Berry PH. Cough. In: Kuebler KK, Berry PH, Heidrich DE ed. End-of-Life Care: Clinical Practice Guidelines. Philadelphia: WB Saunders; 2002:235–241.
14. Baines MJ. Nausea, vomiting, and intestinal obstruction. In: Fallon M, O'Neill B ed. ABC of Palliative Care. London: BMJ Books; 1999:16–18.
15. Griffie J, Mckinnon S. Nausea and vomiting. In: Kuebler KK, Berry PH, Heidrich DE ed. End-of-Life Care: Clinical Practice Guidelines. Philadelphia: WB Saunders; 2002:333–343.
16. Mckinnon S, Kuebler KK. Cachexia and anorexia. In: Kuebler KK, Berry PH, Heidrich DE ed. End-of-Life Care: Clinical Practice Guidelines. Philadelphia: WB Saunders; 2002:213–220.
17. Oi-Ling K, Man-Wah DT, Kam-Hung DN. Symptom distress as rated by advanced cancer patients, caregivers and physicians in the last week of life. Palliat Med. 2005;19:228–233.

Vascular Endothelial Growth Factor-A Expression Is Associated with Subsequent Recurrence in the Liver During Long-Term Follow-Up of Colorectal Cancer Patients in Dukes C

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Abstract

Background We aimed to determine the subgroup of patients with colorectal cancer, in which expression of vascular endothelial growth factor-A (VEGF-A) affects their prognosis strongly.

Methods 119 paraffin embedded specimens of colorectal cancer were investigated by staining with a monoclonal antibody against VEGF-A as well as basic fibroblast growth factor (FGF2), CD34 and p53, which was compared with the pattern of liver metastasis or recurrence and overall survival.

Results VEGF-A positive ratio was higher in patients with liver metastasis at diagnosis (45% of 46) or recurrence in the liver during follow-up (39% of 33) than without liver metastasis or recurrence in the liver (16% of 40 patients) ($P<0.001$). Moreover, the risk of recurrence in the liver during long-term follow-up was significantly increased by VEGF-A production only in patients with Dukes C (N = 46) [odds ratio (OR) = 22.4; 95% confidence interval (CI): 4.6–108], but not in Dukes B (N = 24). In multiple logistic regression analysis using variables of age, gender, expression of FGF2, CD34, p53, stage of lymph node metastasis, and lymph/vascular vessel invasion in pathological specimen, expression of VEGF-A was sole significant factor (OR = 43.5; 95%CI: 4.2–448). In Kaplan-Meier survival curves, 5-year survival in VEGF-A positive and negative patients belong to Dukes C was 68% and 93% (log-rank test: $P<0.05$), respectively. Hazard ratio of VEGF-A adjusted by age and gender was 4.3; 95%CI: 1.2–15.5.

Conclusions These results suggest that patients with Dukes C colorectal cancer and VEGF-A positive in tumor specimen may have a higher risk of recurrence in the liver during long term follow-up.

Key words Angiogenesis, Pathology, Colorectal, Cancer, Prognosis

Introduction

Colorectal cancer is the third most common cancer and the fourth most frequent cause of cancer deaths worldwide.[1] Approximately, 30% of patients diagnosed with colorectal cancer already have metastasis at first presentation, whose only less than 10% can survive beyond 5 years.[1] Recently, for these patients with metastatic colorectal cancer, the addition of bevacizumab, an antibody against vascular endothelial growth factor (VEGF), to fluorouracil-based combination chemotherapy was proved to improve their

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survival.[2–4] Even without metastasis at diagnosis, the prognosis of patients with colorectal cancer is not satisfactory: 25–35% of patients without regional lymph node involvement and approximately 40–75% of patients with positive lymph nodes will not live 5 years after curative resection.[1] Therefore, the next targets of anti-VEGF antibody in addition to standard treatments are these patients without metastasis at diagnosis. However, the relationship between VEGF protein expression and the prognosis of patients with colorectal cancer remains controversial: some have indicated VEGF expression as an independent factor in predicting patients' prognosis;[5–11] while others reported no such associations.[12–14] Therefore, we hypothesized that VEGF may affect the prognosis of a specific subgroup in colorectal cancer patients. In particular, since liver metastasis/recurrence can be a key issue in colorectal cancer,[15–17] we aimed to determine the subgroup, of which VEGF expression associates with prognosis, by stratifying with either no recurrent (Group I) or subsequent liver recurrence during follow-up (Group II) or liver metastasis at diagnosis (Group III) and further by stratifying with Dukes stages, simultaneously p53 and FGF2 as other known angiogenic factors as well as CD34 as a marker for neovascularisation were also stained for comparison.

Patients and Methods

Patients

Retrospectively, 33 patients with delayed liver metastasis were selected as cases. For case comparison, 40 patients without liver metastasis and 46 patients with simultaneous liver metastasis were chosen from the list in the department of surgery matched by age within 5 years independent of survival. As a result, this study included 119 patients with primary colon adenocarcinoma diagnosed and treated either by potentially curable surgery (N = 71) defined as the removal of all of the macroscopic tumoral tissue, free resection margins, and lymphadenoectomy extended beyond involved nodes at postoperative pathological examination or by non-curable surgery (N = 48) at the Department of Surgery, Jikei University Hospital, between January 1976 and September 1992. Liver metastasis was evaluated with CT and ultrasonography. Patients aged 28 to 80 years (mean ± SD: 58 ± 11 years) at diagnosis and there were more men (N = 82) than women (N = 37). No major complications were observed and all of the patients could be discharged from the hospital. Although 3 patients who moved to different hospitals after discharge were deleted from this study due to a short term follow up period of less than 90 days, all 119 patients were followed up at the outpatient clinic of Jikei University Hospital periodically. The primary endpoint was set as death due to any cause and the secondary endpoint was set as liver recurrence during follow-up. Patients who did not develop to the primary endpoint were counted as censored cases on the final day of the outpatient clinic. Paraffin-embedded specimens and information regarding the pattern of liver metastasis were available for all 119 patients.

The following parameters were recorded in all patients: cancer site (right and left colon by using the middle transverse colon as partition), kinds of surgical resection, Dukes' stage, number of resected nodes, number of metastatic nodes, tumor size, macroscopic findings of tumor, postoperative complications, and recurrences after potentially curative resections.

All patients and their families were informed about the possible risks and benefits of operations as well as the usage of clinical data for research purposes, and written consent was obtained.

Pathologic specimens

Tumor specimens were obtained by surgery. Formalin-fixed, paraffin-embedded specimens of colon cancer were retrieved from the Department of Pathology and processed for conventional histological assessment by hematoxylin and eosin (H&E) staining. Only patients with adenocarcinoma of the colon and rectum confirmed by two or more board-certified pathologists were included. Histologic features of the extent of the lesions, depth of tumor invasion, invasion into lymphatic (Ly) or blood vessels metastasis (V), and lymph node metastasis (N) as well as histologic differentiation (well, moderate, or poor) were also evaluated.

Immunohistochemical staining for VEGF-A, fibroblast growth factor-2 (FGF2), CD34 and p53

Immunohistological staining was performed by standard methods as follows: 1) Deparaffinize and hydrate sections; 2) Wash in deionized water;

3) Block endogenous peroxidase with 3% hydrogen peroxide for 5 minutes; 4) Wash in deionized water; 5) Block non-specific staining by incubation with 10% porcine serum (Bio-Products, Woodland, CA, USA) in phosphate buffered saline (PBS) for 10 minutes at room temperature; 6) Incubate with diluted primary antibody overnight at 4°C: VEGF-A (A-20: sc-152) that is considered to detect precursor/mature and all kinds of VEGF-A of human (Santa Cruz Biotechnology, Inc., Santa Cruz, California, USA) at the dilution of 1:50; FGF-2 (Santa Cruz Biotechnology, Inc.) at the dilution of 1:800; CD34 (BD Biosciences. San Jose. California USA) at the dilution of 1:30; p53 mouse monoclonal antibody (Novocastra, Newcastle, UK) at the dilution of 1:100; 7) Rinse in PBS; 8) Incubate with diluted biotinylated antibody (DAKO Cytomation, Glostrup, Denmark) at the dilution of 1:500 for 30 minutes at room temperature; 9) Rinse in PBS; 10) Incubate with diluted peroxidase-conjugated streptavidin (DAKO Cytomation) at the dilution of 1:500 for 30 minutes at room temperature; 11) Rinse in PBS; 12) Incubate with 20 mg 3,3'-Diaminobenzidine, tetrahydrochloride and 20 μl 30% hydrogen peroxide in 100 ml PBS for 5 minutes at room temperature; 13) Wash in water; 14) Counter-stain with hematoxylin for 1 minute; 15) Wash in water; 16) Dehydrate and clear sections; 17) Mount with permount.

Two investigators (K.E. and M.O.) who were blinded to the clinical information of each patient evaluated the staining levels independently, after which discordant evaluations were adjusted by connected microscopes.

Statistical Analysis

Chi-square test and the analysis of variance were used to evaluate the associations between VEGF-A expression and clinicopathologic parameters. Survival curves of the patients were compared using the Kaplan-Meier method and analyzed using log-rank test. Cox proportional hazards models were fitted for multivariate analysis.

Results

Patients' characteristics and liver metastasis patterns

After surgery, patients were followed from 126 days to 19 years (median: 5.7 years). According to the pattern of liver metastasis or recurrence, 119 patients were divided into 3 groups: Group I: no metastasis at diagnosis and during follow-up (N = 40; median follow-up: 8.1 years); Group II: no metastasis at diagnosis but liver metastasis emerged during follow-up (N = 33; median follow-up: 5.7 years); Group III: liver metastasis at diagnosis (N = 46; median follow-up: 2.5 years). By these patterns of liver metastasis, 5 year survival curves were compared to find significant differences: Group I: 98%; Group II: 69%; Group III: 39% (log rank test: $P<0.0001$) (Fig. 1). Associations between patients' characteristics and these three liver metastasis patterns are shown in Table 1. Invasion of tumor cells into vascular vessel was observed more in Group I.

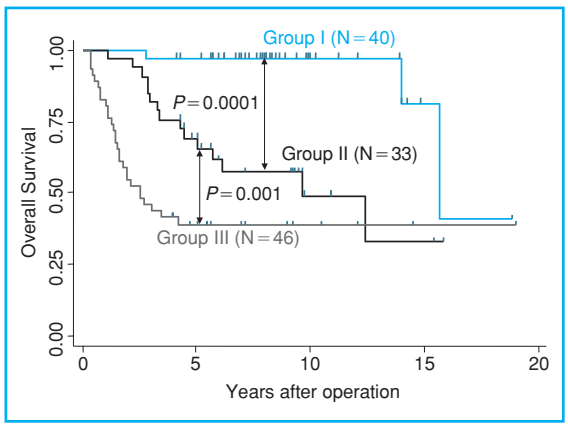

Fig. 1 Kaplan-Meier survival curves by pattern of liver metastasis

Group I: no metastasis at diagnosis and during follow-up. Group II: no metastasis at diagnosis but liver metastasis emerged during follow-up. Group III: Liver metastasis at diagnosis. Statistical differences were analyzed using log-rank test.

Protein expressions of VEGF-A, FGF2, CD34 and p53 and liver metastasis pattern

Typical histologic pictures of VEGF-A, FGF2, CD34 and p53 staining of paraffin-embedded specimens are demonstrated in Fig. 2. Although VEGF-A was stained lightly or moderately, the staining pattern was clearly discriminate as positive or negative. In contrast, FGF2 was positive in parts of tumor cells. Vascular cells were clearly stained with CD34. Positive staining pattern of p53 was sufficiently clear to between discriminate positive and negative. The positive ratio of VEGF-A, FGF2, CD34 and p53 was 69 (58%), 53 (45%), 57 (49%), 84 (71%) in 119 patients,

Table 1 Patients' characteristics by pattern of liver metastasis

	Group I	Group II	Group III	*P*-value
	Non-liver metastasis (N=40)	Delayed liver metastasis (N=33)	Simultaneous liver metastasis (N=46)	
Age (years old): mean±sd	57±12	57±11	60±10	NS*1
Male/Female	27 (33%)	24 (29%)	31 (38)	NS*2
Dukes B/C/D	11/29/0	13/17/3	0/0/46	<0.001*2
HPN classification*3				
H: 0/1/2/3	40/0/0/0	33/0/0/0	0/29/5/12	<0.001*2
P: 0/1/2/3	40/0/0/0	30/1/1/1	38/6/2/0	NS*2
N: 0/1/2/3	14/16/7/3	11/16/6/0	9/26/8/3	NS*2
Vessel invasion*3				
Lymphatic: 0/1/2/3	0/34/5/1	1/24/6/2	0/31/9/6	NS*2
Vascular: 0/1/2/3	2/34/4/0	12/19/1/1	5/32/8/1	0.004*2
Differentiation				
well/moderate/poor	35/4/1	22/10/0	38/5/2	NS*2

Group I: no metastasis at diagnosis and during follow-up. Group II: no metastasis at diagnosis but liver metastasis emerged during follow-up. Group III: liver metastasis at diagnosis. *1: Statistical difference was analyzed with ANOVA. *2: Statistical difference was analyzed with chi-square test. *3: According to the Japanese classification of colorectal carcinoma.

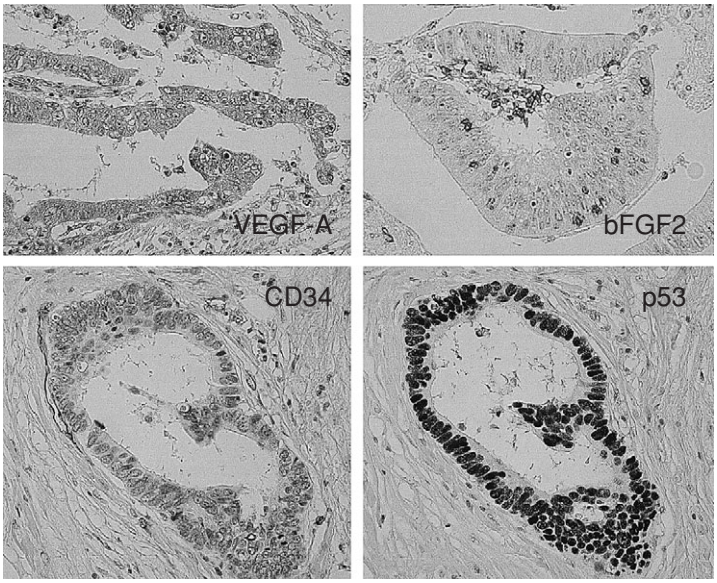

Fig. 2 Typical positive staining of CD34, p53, bFGF2 and VEGF

Slides were examined by two independent examiners blinded to each other's work and with no prior knowledge of clinical and pathological parameters. For each colorectal cancer, staining was evaluated at the invasive edge of the tumor. Slides were examined at ×400 (40×objective, 10×ocular).

respectively. Positive VEGF-A expression was associated with the positive expressions of both CD34 ($P=0.017$) and p53 ($P=0.003$). Moreover, FGF2 had positive associations with expressions of CD34 ($P<0.001$) and p53 ($P<0.001$). Similarly, CD34 showed positive association with p53 ($P<0.001$). In association with the pattern of liver metastasis, the expression of VEGF-A protein was significant, but not FGF2, CD34 nor p53 (Table 2).

Table 2 Expression of VEGF, FGF2, CD34 and p53 and their deviation by pattern of liver metastasis

	Group I	Group II	Group III	*P*-value
	Non-liver metastasis (N = 40)	Delayed liver metastasis (N = 33)	Simultaneous liver metastasis (N = 46)	
VEGF: positive (%)	11 (16)	27 (39)	31 (45)	<0.001*
FGF2: positive (%)	13 (25)	16 (30)	24 (45)	NS*
CD34: positive (%)	14 (25)	18 (32)	25 (44)	NS*
p53: positive (%)	23 (27)	25 (30)	36 (43)	NS*

Group I: no metastasis at diagnosis and during follow-up. Group II: no metastasis at diagnosis but liver metastasis emerged during follow-up. Group III: liver metastasis at diagnosis. *: Statistical difference was analyzed with chi-square test.

Table 3 Risk of delayed liver metastasis by expression of VEGF*1

	Dukes B (N = 24)		Dukes C (N = 46)	
	Crude	Adjusted*2	Crude	Adjusted*2
OR (95%CI)	1.3 (0.1–15.0)	18.2 (0.4–483)	22.4 (4.6–108)	43.5 (4.2–448)

*1: Only patients without metastasis at diagnosis (N = 71) were focused in this analysis. Three patients belong to Dukes D had no liver metastasis at diagnosis. *2: Multivariate analysis adjusted by H, P, N, Ly, V as well as age and gender.

Risk of liver metastasis during follow-up evaluated by VEGF-A expression

Since emerging liver metastasis during follow-up is a strong prognostic factor, next we focused only on patients without liver metastasis at diagnosis (N = 73) (Table 3). The risk of delayed liver metastasis was significantly increased by the presence of VEGF-A in tumor tissue in focusing on patients belongs to Dukes C (N = 46) alone [odds ratio (OR) = 22.4; 95% confidence interval (CI): 4.6–108] ($P<0.001$), but not in Dukes B (N = 24). In multiple logistic regression analysis with age, gender, stages of hepatic, peritoneal and lymph node metastasis, and lymph/vascular vessel invasion in pathological specimen, expression of VEGF-A was the sole significant factor (OR = 43.5; 95%CI: 4.2–448) ($P = 0.002$).

Survival of patients evaluated by VEGF-A expression stratified by Dukes classification

Kaplan-Meier survival curves of patients by VEGF-A expression are shown stratified by Dukes B (Fig. 3A), Dukes C (Fig. 3B) and Dukes D (Fig. 3C). Significant difference was detected only in Dukes C (log-rank test: $P<0.05$), but not in Dukes B and not in Dukes D. In Dukes C, 5-year survival rate in VEGF-A positive and negative patients was 68% and 93%, respectively. The hazard ratio of VEGF-A adjusted by age and gender in Cox regression analysis was 4.3; 95%CI: 1.2–15.5 ($P = 0.027$) in restricting to Dukes C.

Discussion

We demonstrated that VEGF-A production by primary colorectal cancer might be associated with liver metastasis/recurrence. Our data support previous articles suggesting that VEGF may associate with the prognosis of patients with colorectal cancer.[5–11] Neovascularization is a key process in the growth of solid tumours, as these tumours will not grow beyond a few cubic millimetres unless a vascular network is established.[18,19] To stimulate neovascularization, the tumor cells produce a variety of angiogenic factors, such as VEGF.[20,21] Thus, VEGF produced by primary tumor induced neovascularization to make cancer cells shed into the circulation and colonize distant sites as well.

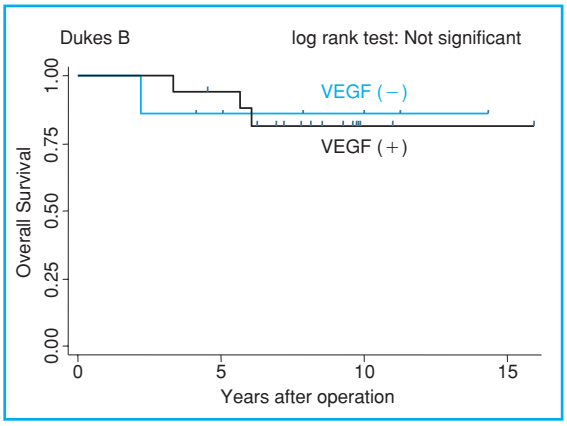

Fig. 3A Kaplan-Meier survival curves of patients by VEGF expression are shown stratified by Dukes B

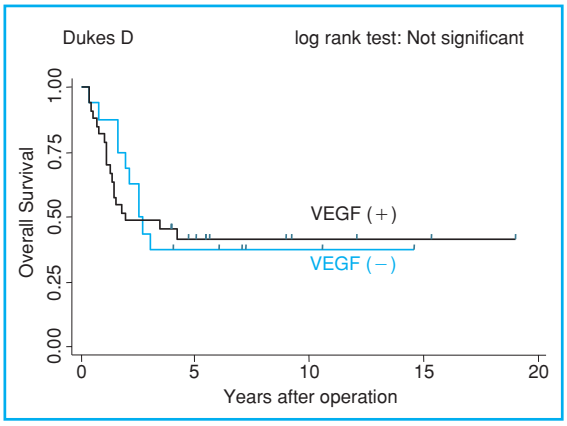

Fig. 3C Kaplan-Meier survival curves of patients by VEGF expression are shown stratified by Dukes D

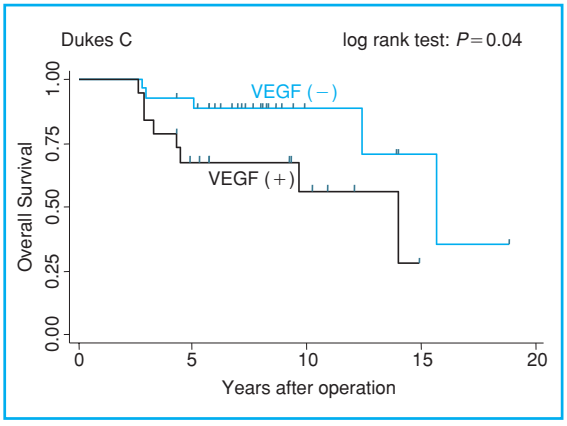

Fig. 3B Kaplan-Meier survival curves of patients by VEGF expression are shown stratified by Dukes C

In this study, no association between liver metastasis pattern and other markers: FGF2, CD34 and p53 was detected in multivariate analysis. A predictive value of serum FGF, an angiogenic factor, was not as clear as VEGF,[22,23] as in our research. CD34 was used to monitor neovascularization around the tumor tissue, which was demonstrated to predict the prognosis of patients with colorectal cancer.[24] Theoretically, increase of micro-vessels detected with CD34 can be a result of VEGF production, thus both have similar meaning regarding the progression of tumor and prognosis of patients.[25,26] Mutations of p53 and activation of the Ras/MAPK pathway plays a role in the induction of VEGF expression in human colorectal cancer.[27] This evidence is consistent with out results in that positive VEGF expression is associated with positive expression of both CD34 and p53.

VEGF is reported to associate with the prognosis of patients with Dukes A, B in colorectal cancer.[28] On the other hand, the clinical effectiveness of anti-VEGF on patients with Dukes D colorectal cancer was demonstrated by randomized clinical trial[2–4] as already mentioned in 'Introduction'. Of interest, an association between VEGF production and liver metastasis was observed only in Dukes C but not in Dukes B and not in Dukes D in this study. Previous morphmetric studies of colon cancer suggest that neovascularisation reaches its maximal level early in the malignant process.[29,30] Indeed, transcription levels of VEGF isoform show an inverse relationship with Dukes stage.[31] Others indicate that a tumor area with a low microvascular count is associated with VEGF expression up-regulated in response to hypoxia, induced by a lack of a functional vasculature.[32] Therefore, we hypothesized that neovascularisation is already established in tumor of Dukes D through VEGF and/or other angiogenic factors and thus production of VEGF may not discriminate the prognosis of patients with this stage anymore.

In conclusion, patients with Dukes C colorectal cancer and VEGF-A positive in tumor specimen may have a higher risk of recurrence in the liver during follow-up.

References

1. Weitz J, Koch M, Debus J, Hohler T, Galle PR, Buchler MW. Colorectal cancer. Lancet. 2005;365:153–165.
2. Hurwitz H, Fehrenbacher L, Novotny W, et al. Bevacizumab plus irinotecan, fluorouracil, and leucovorin for metastatic colorectal cancer. N Engl J Med. 2004;350:2335–2342.
3. Hurwitz HI, Fehrenbacher L, Hainsworth JD, et al. Bevacizumab in combination with fluorouracil and leucovorin: an active regimen for first-line metastatic colorectal cancer. J Clin Oncol. 2005;23:3502–3508.
4. Kabbinavar FF, Hambleton J, Mass RD, Hurwitz HI, Bergsland E, Sarkar S. Combined analysis of efficacy: the addition of bevacizumab to fluorouracil/leucovorin improves survival for patients with metastatic colorectal cancer. J Clin Oncol. 2005; 23:3706–3712.
5. Takahashi Y, Kitadai Y, Bucana CD, Cleary KR, Ellis LM. Expression of vascular endothelial growth factor and its receptor, KDR, correlates with vascularity, metastasis, and proliferation of human colon cancer. Cancer Res. 1995;55:3964–3968.
6. Chin KF, Greenman J, Reusch P, Gardiner E, Marme D, Monson JR. Vascular endothelial growth factor and soluble Tie-2 receptor in colorectal cancer: associations with disease recurrence. Eur J Surg Oncol. 2003;29:497–505.
7. Werther K, Christensen IJ, Brunner N, Nielsen HJ. Soluble vascular endothelial growth factor levels in patients with primary colorectal carcinoma. The Danish RANX05 Colorectal Cancer Study Group. Eur J Surg Oncol. 2000;26:657–662.
8. Broll R, Erdmann H, Duchrow M, et al. Vascular endothelial growth factor (VEGF)—a valuable serum tumour marker in patients with colorectal cancer? Eur J Surg Oncol. 2001;27: 37–42.
9. Akbulut H, Altuntas F, Akbulut KG, et al. Prognostic role of serum vascular endothelial growth factor, basic fibroblast growth factor and nitric oxide in patients with colorectal carcinoma. Cytokine. 2002;20:184–190.
10. Kang SM, Maeda K, Onoda N, et al. Combined analysis of p53 and vascular endothelial growth factor expression in colorectal carcinoma for determination of tumor vascularity and liver metastasis. Int J Cancer. 1997;74:502–507.
11. De Vita F, Orditura M, Lieto E, et al. Elevated perioperative serum vascular endothelial growth factor levels in patients with colon carcinoma. Cancer. 2004;100:270–278.
12. Khorana AA, Ryan CK, Cox C, Eberly S, Sahasrabudhe DM. Vascular endothelial growth factor, CD68, and epidermal growth factor receptor expression and survival in patients with Stage II and Stage III colon carcinoma: a role for the host response in prognosis. Cancer. 2003;97:960–968.
13. Lee JC, Chow NH, Wang ST, Huang SM. Prognostic value of vascular endothelial growth factor expression in colorectal cancer patients. Eur J Cancer. 2000;36:748–753.
14. Roumen RM, Slooter GD, Croiset van Uchelen FA, Huib LV. Preoperative serum vascular endothelial growth factor is not a marker for subsequent recurrence during long-term follow-up of colorectal cancer patients. Dis Colon Rectum. 2005;48:1070–1075.
15. Choti MA, Sitzmann JV, Tiburi MF, et al. Trends in long-term survival following liver resection for hepatic colorectal metastases. Ann Surg. 2002;235:759–766.
16. Bolton JS, Fuhrman GM. Survival after resection of multiple bilobar hepatic metastases from colorectal carcinoma. Ann Surg. 2000;231:743–751.
17. Minagawa M, Makuuchi M, Torzilli G, et al. Extension of the frontiers of surgical indications in the treatment of liver metastases from colorectal cancer: long-term results. Ann Surg. 2000;231:487–499.
18. Weidner N, Semple JP, Welch WR, Folkman J. Tumor angiogenesis and metastasis: correlation in invasive breast carcinoma. N Engl J Med. 1991;324:1–8.
19. Carmeliet P, Jain RK. Angiogenesis in cancer and other diseases. Nature. 2000;407:249–257.
20. Shweiki D, Itin A, Soffer D, Keshet E. Vascular endothelial growth factor induced by hypoxia may mediate hypoxia-initiated angiogenesis. Nature. 1992;359:843–845.
21. Plate KH, Breier G, Weich HA, Risau W. Vascular endothelial growth factor is a potential tumour angiogenesis factor in human gliomas in vivo. Nature. 1992;359:845–848.
22. Dirix LY, Vermeulen PB, Hubens G, et al. Serum basic fibroblast growth factor and vascular endothelial growth factor and tumour growth kinetics in advanced colorectal cancer. Ann Oncol. 1996; 7:843–848.
23. Akbulut H, Altuntas F, Akbulut KG, et al. Prognostic role of serum vascular endothelial growth factor, basic fibroblast growth factor and nitric oxide in patients with colorectal carcinoma. Cytokine. 2002;20:184–190.
24. Tanigawa N, Amaya H, Matsumura M, et al. Tumor angiogenesis and mode of metastasis in patients with colorectal cancer. Cancer Res. 1997;57:1043–1046.
25. Galindo Gallego M, Fernandez Acenero MJ, Sanz Ortega J, Aljama A. Vascular enumeration as a prognosticator for colorectal carcinoma. Eur J Cancer. 2000;36:55–60.
26. Zheng S, Han MY, Xiao ZX, Peng JP, Dong Q. Clinical significance of vascular endothelial growth factor expression and neovascularization in colorectal carcinoma. World J Gastroenterol. 2003;9:1227–1230.
27. Cassano A, Bagala C, Battelli C, et al. Expression of vascular endothelial growth factor, mitogen-activated protein kinase and p53 in human colorectal cancer. Anticancer Res. 2002;22:2179–2184.
28. Galizia G, Ferraraccio F, Lieto E, et al. Prognostic value of p27, p53, and vascular endothelial growth factor in Dukes A and B colon cancer patients undergoing potentially curative surgery. Dis Colon Rectum. 2004;47:1904–1914.
29. Bossi P, Viale G, Lee AK, Alfano R, Coggi G, Bosari S. Angiogenesis in colorectal tumors: microvessel quantitation in adenomas and carcinomas with clinicopathological correlations. Cancer Res. 1995;55:5049–5053.
30. Pavlopoulos PM, Konstantinidou AE, Agapitos E, Kavantzas N, Nikolopoulou P, Davaris P. A morphometric study of neovascularization in colorectal carcinoma. Cancer. 1998;83:2067–2075.
31. Hollingsworth SJ, Drye ER, Tou SI, Boulos PB. Expression of angiogenic VEGF-A (soluble isoforms 121, 165) and lymphangiogenic VEGF-C in colorectal cancers with micro-satellite instability. J Surg Oncol. 2005;92:317–325.
32. Boxer GM, Tsiompanou E, Levine T, Watson R, Begent RH. Immunohistochemical expression of vascular endothelial growth factor and microvessel counting as prognostic indicators in node-negative colorectal cancer. Tumour Biol. 2005;26:1–8.

Review Article

Autonomic Dysfunction in Patients with Vertigo

JMAJ 49(4): 153–157, 2006

Noriaki Takeda*1

Abstract

Autonomic function and vertebral blood flow were measured in patients with vertigo. Based on our findings obtained in a series of studies, we propose the following hypothesis of sympatho-vascular mechanisms of vertigo. Hyporesponse of the sympathetic nervous system to stress observed in patients with vertigo induces asymmetrical blood flow of the vertebral artery. Asymmetrical activity of the sympathetic nervous system observed in patients with vertigo also induces asymmetrical blood flow of the vertebral artery. The asymmetrical vertebral blood flow induces asymmetrical activities of the inner ear and/or the vestibular nuclei, resulting in the development of vertigo.

Key words Vertigo, Sympathetic nervous system, Parasympathetic nervous system, Vertebral artery, Stress

Introduction

Autonomic dysfunction has been proposed to be an underlying mechanism of the development of vertigo, although there is little evidence.[1,2] In the present review, a series of our studies on autonomic nervous activity and the vertebral blood flow in patients with vertigo is summarized and how the autonomic dysfunction triggers the onset of vertigo is discussed.

Systemic Autonomic Dysfunction in Patients with Vertigo

Systemic autonomic nervous function was examined in patients with vertigo including Meniere's patients by means of power spectral analysis of heart rate variability.[3] The power spectral density (PSD) of beat-to-beat heart rate intervals was analyzed using a continuous non-invasive finger blood pressure recording system. A fast-Fourier transform algorithm was used to compute the PSD. In frequencies up to 5 Hz, the PSD of heart rate variability contains three major components: a low frequency (P1), a middle frequency (P2) and a high frequency (P3). Each component was normalized by dividing the absolute values of total power (T). P2 and P3 were used as an index of sympathetic and parasympathetic nervous functions, respectively. In patients with vertigo at rest, the normalized power of P2 slightly increased and the normalized power of P3 significantly decreased, in comparison with healthy subjects at rest (Fig. 1). These findings indicated that the parasympathetic nervous activity at rest was suppressed in patients with vertigo. Yamada et al. used power spectral analysis of heart rate variability and reported that parasympathetic hypofunction in patients with Meniere's disease.[4]

Then, the effects of passive tilt up on the autonomic nervous activity were examined in patients with vertigo. In healthy subjects, the normalized power of P2 increased and the normalized power of P3 decreased with passive tilt up. These findings indicated that the sympathetic nervous activity was stimulated and the parasympathetic nervous activity was suppressed in response to passive tilt up. On the contrary, in spite of a decrease of the normalized power of P3, passive tilt up did not increase the normalized power of P2 in patients with vertigo (Fig. 2). These find-

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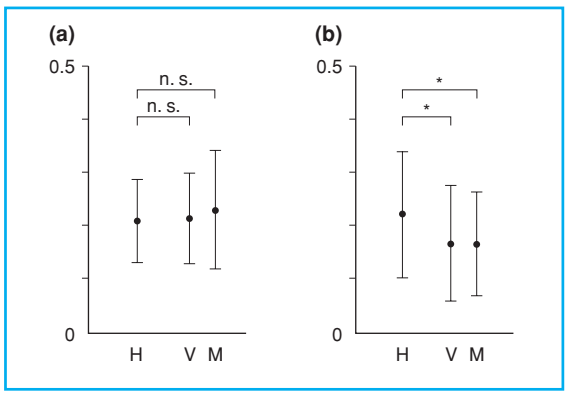

Fig. 1 Normalized power of P2 as an index of sympathetic activity (a) and normalized power of P3 as an index of parasympathetic activity (b) in health subjects (H), patients with vertigo (V) and patients with Meniere's disease (M) at rest

Points are means ± SD. *$P<0.05$.

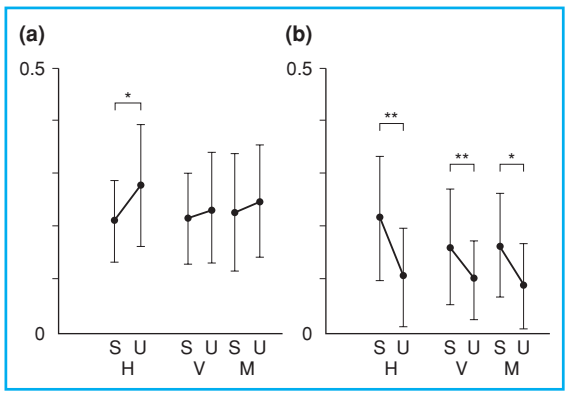

Fig. 2 Normalized power of P2 as an index of sympathetic activity (a) and normalized power of P3 as an index of parasympathetic activity (b) in health subjects (H), patients with vertigo (V) and patients with Meniere's disease (M) in response to passive tilt up

Points are means ± SD. S: supine, U; upright. *$P<0.01$, **$P<0.05$.

ings indicated that response of the sympathetic nervous system to passive tilt up was impaired. Yamada et al. also reported that sympathetic response to postural changes was suppressed in patients with Meniere's disease.[4]

It has been speculated that both mental and physical stress played an important role in the onset of vertigo.[5] On the other hand, because of neurotic personality in patients with vertigo, vertigo itself can be a stress.[6] Therefore, it is suggested that a reciprocal causal relationship between vertigo and stress induced the suppression of the parasympathetic nervous activity and the repeated stimulation of the sympathetic nervous activity. The present finding of the impaired response of the sympathetic nervous system to passive tilt up might be explained by the desensitization of the sympathetic nervous system after repeated activations in patients with vertigo. Accordingly, it is suggested that the response of the sympathetic nervous system to stress is also desensitized in dizzy patients.

Asymmetrical Sympathetic Activity in Patients with Vertigo

The right-left differences of sympathetic nervous activity were examined in patients with vertigo including Meniere's patients by means of recovery curve of palm skin temperature after cold exposure.[7] A contact skin thermometer was attached to the palm of both hands. Both hands were immersed in iced water for 30 sec. Subsequently, palm skin temperature of both hands was recorded at 1 min intervals. Figs. 3 and 4 represent the thermal recordings of both hands in a healthy subject and a Meniere's patient, respectively. There is no difference in the recovery of palm skin temperature between right and left hands of the healthy subject (Fig. 3). However, asymmetrical recovery of palm skin temperature was observed in patients with Meniere's disease during an active spell. After immersion in iced water, the patient showed an abnormal slow recovery time in the side affected by Meniere's disease. Then, the asymmetrical recovery of palm skin temperature disappeared in the interval between active spells in the same patient (Fig. 4). During active spells, a significantly high proportion of Meniere's patients had an anisothermal recovery of palm skin temperature after immersion in iced water, in comparison with that of healthy subjects. However, during intervals between active spells, the frequency of anisothermal recovery of palm skin temperature in Meniere's patients did not differ from that of healthy subjects (Table 1). Since the palm skin temperature is regulated by anastomotic skin blood flow, and its blood flow through arteriovenous anastomoses is controlled by efferent sympathetic nerve fibers,[8] these findings indi-

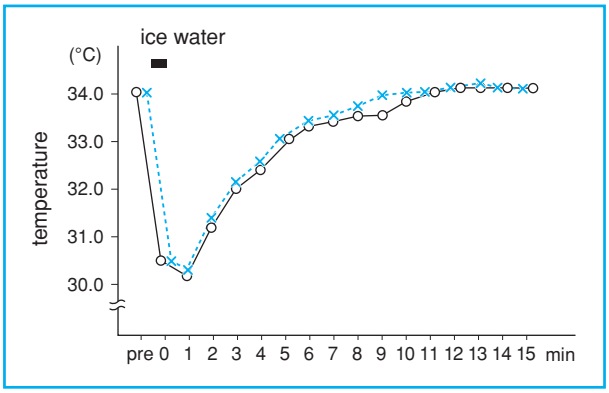

Fig. 3 Changes in palm skin temperature in a healthy subject after hand being immersed in iced water

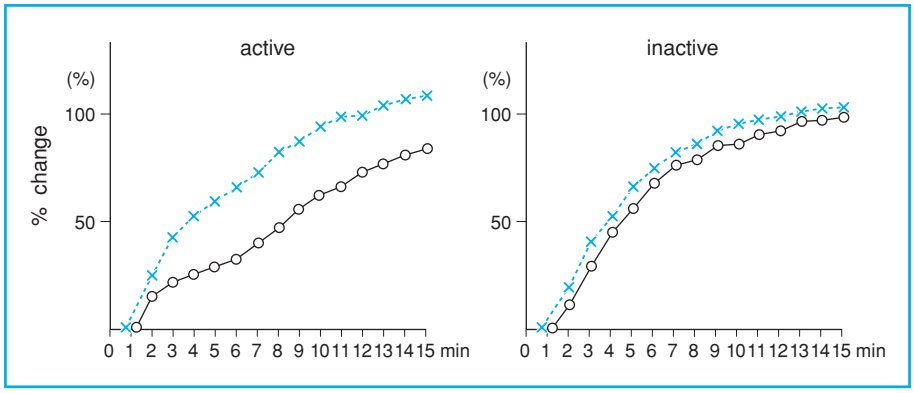

Fig. 4 Changes in palm skin temperature in a patient with Meniere's disease after hand being immersed in iced water

Active: during active spells, inactive: during intervals between active spells. Points are percentages of the palm skin temperature before immersion in iced water

Table 1 Appearance of anisothermal recovery of palm skin temperature after hand immersed in iced water

Healthy	Meniere's disease	
	active	inactive
18.2% (6/33)	60.0%* (12/20)	19.2% (5/26)

*$P<0.05$

cated the asymmetrical activity of the sympathetic nervous activity in patients with vertigo. Because of the correlation between the sympathetic asymmetry and active spells, it is suggested that the asymmetrical activity of the sympathetic nervous activity contributed to vertigo attacks. Uemura et al. reported autonomic dysfunction revealed by mecholyl test on the affected side of Meniere's patients.[9] Yamada et al. also reported sympathetic hypofunction in Meniere's patients at the attack stage, but not at the interval stage.[4]

Asymmetrical Vertebral Blood Flow in Patients with Vertigo

The blood supply of both inner ear and vestibular nuclei in the brain stem originates from the vertebral artery, of which blood flow is mainly controlled by the sympathetic nervous system. A question arises whether the asymmetrical activity of the sympathetic nervous activity in patients

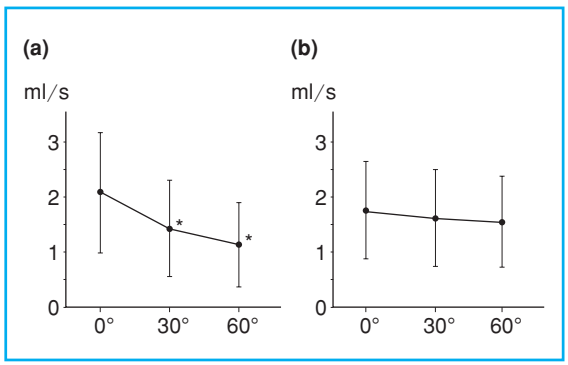

Fig. 5 Blood flow of the vertebral artery in patients with vertigo (a) and in healthy subjects (b)

Points are means ± SD. *$P<0.01$ vs. 0 degree.

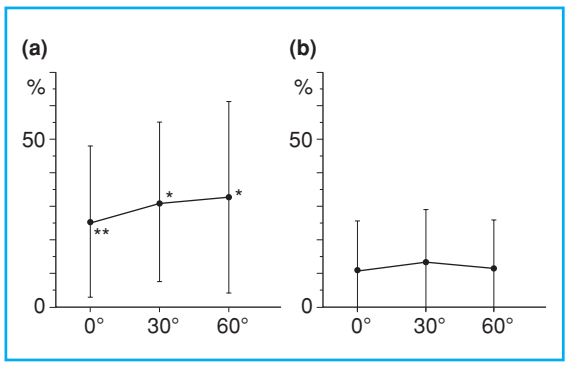

Fig. 6 Right-left differences of the vertebral blood flow in patients with vertigo (a) and in healthy subjects (b)

Points and bars means ± SD of asymmetrical percentage calculated as follows:

$$\%\text{Asymmetry} = \frac{|\text{Right blood flow} - \text{Left blood flow}|}{\text{Right blood flow} + \text{Left blood flow}} \times 100$$

*$P<0.05$ vs. 0 degree,
**$P<0.01$ vs. healthy subjects at 0 degree

with vertigo may affect the blood flow of the vertebral artery. To clarify the question, the effects of passive tilt up on the vertebral blood flow were examined in patients with vertigo.[10] The blood flow of the vertebral artery was measured at the level of C2-C3 by means of Doppler spectral analysis. There were no differences of vertebral blood flow at rest between patients with vertigo and healthy subjects at rest. In healthy subjects, the vertebral blood flow did not change with passive tilt up. By contrast, the vertebral blood flow significantly decreased in response to passive tilt up in patients with vertigo (Fig. 5).

Then, the right-left differences of the vertebral blood flow were analyzed. The right-left differences of the vertebral blood flow in patients with vertigo at rest were significantly more than those in healthy subjects at rest. In response to passive tilt up, the right-left differences of the vertebral blood flow were significantly higher in dizzy patients. However, the asymmetry of vertebral blood flow did not increase with passive tilt up in healthy subjects (Fig. 6). Since the vertebral blood flow is mainly regulated by the sympathetic nervous system, it is suggested that in patients with vertigo, both poor response of the sympathetic nervous system to stress and asymmetrical activity of the sympathetic nervous activity induce asymmetrical vertebral blood flow.

Furthermore, changes in the right-left differences of the vertebral blood flow were examined in patients with Meniere's disease. The right-left differences of the vertebral blood flow were significantly higher during active spells, in comparison with those during intervals between active spells (Table 2). These findings suggested that the asymmetrical vertebral blood flow contributed to the onset of vertigo in patients. Asymmetrical vertebral blood flow might induce asymmetrical activities of the inner ear and/or the vestibular nuclei, resulting in the development of vertigo.

Table 2 %Asymmetry of vertebral blood flow in healthy subjects and patients with Meniere's disease

Healthy	Meniere's disease	
	active	inactive
2.9 ± 3.8%	36.8 ± 6.7%*	18.1 ± 4.3%

Values are means ± SD, *$P<0.05$

Hypothesis of Sympatho-Vascular Mechanisms of Vertigo

In conclusion, in patients with vertigo, parasympathetic nervous activity at rest was suppressed and the response of the sympathetic nervous system to passive tilt up was impaired. These findings suggested that a reciprocal causal relationship between vertigo and stress induced the suppression of parasympathetic nervous activity and

desensitized sympathetic nervous activity in response to stress. Patients with vertigo also have asymmetrical sympathetic nervous activity. Because of the correlation between the sympathetic asymmetry and active spells, it is suggested that asymmetrical sympathetic nervous activity contributed to vertigo attacks. In addition to the above mentioned autonomic dysfunction in patients with vertigo, their vertebral blood flow was significantly lower in response to passive tilt up. In response to passive tilt up, the right-left differences of the vertebral blood flow significantly increased in these dizzy patients. Since vertebral blood flow is mainly regulated by the sympathetic nervous system, it is suggested that in patients with vertigo, both poor response of the sympathetic nervous system to stress and asymmetrical activity of the sympathetic nervous system induce asymmetrical vertebral blood flow. Because of the correlation between asymmetry of the vertebral blood flow and active spells, it is suggested that asymmetrical vertebral blood flow induces asymmetrical activities of the inner ear and/or the vestibular nuclei, resulting in the development of vertigo. In an animal study, it was reported that unilateral electrical stimulation of the cervical sympathetic ganglion induced directional preponderance of per-rotatory nystagmus in rabbits, suggesting that asymmetrical sympathetic activity causes asymmetric vestibular activities.[11]

Based on our findings, we proposed the following hypothesis of sympatho-vascular mechanisms of vertigo: Hyporesponse of the sympathetic nervous system to stress observed in patients with vertigo induces asymmetrical blood flow of the vertebral artery. Asymmetrical activity of the sympathetic nervous system observed in patients with vertigo also induces asymmetrical blood flow of the vertebral artery. The asymetrical vertebral blood flow induces asymmetrical activities of the inner ear and/or the vestibular nuclei, resulting in the development of vertigo.

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I would like to dedicate this review to the memory of Dr. Toru Matsunaga, Professor Emeritus of Osaka University.

References

1. Pappas DG, Crawford W, Coghlan HC. Dizziness and the autonomic dysfunction syndrome. Otolaryngol Head Neck Surg. 1986;94:186–194.
2. Pappas DG. Autonomic related vertigo. Laryngoscope. 2003; 113:1658–1671.
3. Kawasaki Y. Autonomic nervous function of vertiginous patients: assessment by spectral analysis of heart rate variability. Nippon Jibiinkoka Gakkai Kaiho. 1993;96:444–456.
4. Yamda M, Mizuta K, Ito Y, Furuta M, Sawai S, Miyata M. Autonomic nervous functions in patients with Meniere's disease evaluated by power spectral analysis of heart rate variability. Auris Nasus Larynx 1999;26:419–426.
5. Hallam RS, Stephens SD. Vestibular disorder and emotional distress. J Psychosom Res. 1985;29:407–413.
6. Yardley L, Luxon LM, Haacke NP. A longitudinal study of symptoms, anxiety and subjective well-being in patients with vertigo. Clin Otolaryngol Allied Sci. 1994;19:109–116.
7. Matsunaga T, Yamamoto K, Kubo T, Ogino H, Ochiai K, Takeda N. Sympatho-vascular mechanism of vertigo attack in Meniere's disease. In: Filipo R, Barbara M, ed. Meniere's Disease: Perspectives in the 90's. Amusterdam: Kulger Publications; 1994: 77–80.
8. Delius W, Hagbarth KE, Hongell A, Willin BC. Maneuvers affecting sympathetic outflow in human skin nerves. Acta Physiol Scand. 1972;84:177–186.
9. Uemura T, Itoh M, Kikuchi N. Autonomic dysfunction on the affected side in Meniere's disease. Acta Otolaryngol. 1980;89: 109–117.
10. Taya N. Effects of the head up tilting and the cold pressor test on vertebral blood flow in patients with vertigo or dizziness. Medical Journal of Osaka University. 1993;45:63–73.
11. Yamamoto K, Matsunaga T, Kubo T. Effects of cervical stimulation on vestibulo-ocular reflex of rabbits with normal, low and high blood pressure. Pract Otol. (Kyoto) 1983;76(Suppl 4): 2317–2323.

Review Article

COPD and Macrolide

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Abstract

Respiratory virus including rhinovirus (RV) induces exacerbations of chronic obstructive pulmonary disease (COPD). RV infection stimulates various cells in the airways such as epithelial cells, mast cells and eosinophils, and produces a variety of proinflammatory cytokines such as interleukin (IL)-6 and IL-8, mucin and chemical mediators including histamine. These factors may be associated with airway inflammation with leukocyte accumulation, mucus hypersecretion, airway smooth muscle contraction and subsequent COPD exacerbations with airway narrowing. Macrolide antibiotics bafilomycin A_1 and erythromycin inhibit RV infection by reducing the expression of ICAM-1, the major RVs receptor, and by blocking RV entry. Furthermore, erythromycin reduced the frequency of common colds and COPD exacerbations. Erythromycin increases bactericidal activity of airway surface liquid in human airway epithelial cells through human beta-defensin production. Herein, we review the pathogenesis of RV infection-induced exacerbations of COPD. Furthermore, we describe the mechanisms of the inhibitory effects of erythromycin on COPD exacerbations.

Key words Macrolide, Rhinovirus, Cytokine, COPD, Defensin, Mucin

Clinical Importance of Rhinovirus Infection on COPD Exacerbation

Human rhinoviruses (RVs) are the most commonly implicated pathogens of common colds.[1] The importance of RV infection in the exacerbation of bronchial asthma has been recognized.[2,3] Respiratory virus infection, including RVs, influenza viruses, and respiratory syncytial viruses, is associated with the exacerbation of chronic obstructive pulmonary diseases (COPD).[4] After the establishment of RT-PCR methods for RVs,[2] the important role of RV infection on exacerbations of COPD has also been reported.[4,5] In a report by Seemungal et al.,[4] 39% of 168 COPD exacerbations were associated with viral infection. RV was the most common respiratory virus detected, and was detected in 58% of viral infections. Coronavirus, influenza A and B, parainfluenza, adenovirus and respiratory syncytial (RS) virus were also detected in COPD exacerbations. Rohde et al. also found viruses in 56% of COPD exacerbations, and RV was the most common virus detected in 36% of virus-associated COPD exacerbations.[5]

Effects of Rhinovirus Infection on the Airway Epithelial Cells

In order to understand the mechanisms of airway inflammation after RV infection related to acute exacerbations of COPD and bronchial asthma, various studies have been performed on the production of pro-inflammatory substances, adhesion molecules and chemical mediators from the cells in the lung. RV infection increases the production of various pro-inflammatory substances

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including interleukin (IL)-1α, IL-1β, IL-6, IL-8, IL-11, tumor necrosis factor-α (TNF-α), RANTES, and granulocyte-macrophage colony-stimulating factor (GM-CSF) in the epithelial cells, primary cultures of epithelial cells or cell lines (Table 1).[6–14] Subauste et al.[6] demonstrated that RV14 infection induced the release of IL-6, IL-8 and TNF-α, and that pre-exposure of a human bronchial epithelial cell line (BEAS-2B) to TNF-α increased susceptibility to RV14 infection. They suggested that inflammatory cytokines produced by RV infection may increase the susceptibility to RV infection. IL-6 induces antibody production in B cells as well as T cell activation and differentiation. IL-8 is a major chemoattractant for neutrophils and stimulates neutrophils, causing enzyme release and production of reactive oxygen species. The number of neutrophils increases in the airway during the acute stage of a cold,[15] and in the sputum in COPD.[16] IL-8 increase in sputum of COPD patients at a stable condition[17] and during exacerbations.[18,19] Similarly, GM-CSF can prime both neutrophils and eosinophils for enhanced activation to chemical stimuli. RV infection increases the production of eotaxin and RANTES, which activates eosinophils, in the bronchial epithelial cells.[7,8] IL-11 is suggested to have direct effects on bronchial hyperresponsiveness.[13] We also demonstrated that primary cultures of human tracheal epithelial cells and submucosal gland cells can be infected with RV14, a major type RV, and RV2, a minor type RV, through binding to intercellular adhesion molecule (ICAM)-1 and low density lipoprotein (LDL) receptors, respectively, and produce pro-inflammatory cytokines, including IL-1α, IL-1β IL-6, IL-8, TNF-α, and GM-CSF, and ICAM-1 and LDL receptor.[9–11,20] Activation of the transcription factor, nuclear factor-kappa (NF-κ) B, is associated with the production of pro-inflammatory cytokines and ICAM-1,[12,21,22] and the endogenous production of IL-1β is also associated with ICAM-1 expression after RV infection.[9]

Thus, these proinflammatory cytokines may be partly associated with the accumulation of inflammatory leukocytes[18,23–25] and with subsequent airway thickness and inflammation as reported by Hogg et al.[26] The proinflammatory cytokines may relate to the upregulation of the inducible form of heme oxygenase (HO-1),[27] and the increased concentrations of carboxyhemoglobin concentrations in patients with COPD at exacerbations.[28,29]

The up-regulation of ICAM-1 could increase susceptibility to major group RVs[30] and could lead cells adjacent to infected cells to infec-tion when viruses are released from the cells originally infected. Inflammatory conditions such as asthma, smoking, ozone exposure, and production reactive oxygen species in COPD[31] in which ICAM-1 expression is increased on respiratory epithelial surfaces, may cause a predisposition to RV infection by increasing the expression of the major group of RV receptors. The RV infection would enhance airway inflammation by recruiting neutrophils, and, potentially, other inflammatory cells, causing increased mediator release and exacerbation of COPD.

Furthermore, we demonstrated that hydrogen peroxide increases the transepithelial influx of

Table 1 Effects of rhinovirus infection on the function of cells in airways

Cell	Effects of rhinovirus infection	References
Epithelial cells	1. Cytokines production IL-1, IL-6, IL-8, TNF-alpha, GM-CSF, RANTES	6–14, 51
	2. ICAM-1 expression	9, 21
	2. Mucin secretion	34, 35
	3. Affected barrier function	32
	4. Increased bacterial adhesion	37
Mast cells	1. Histamine release	45
	2. Cytokines production: IL-4, IL-6, IL-8	45
Eosinophils	Chemotaxis	51
Smooth muscle	Contraction	53

mannitol in cultured human tracheal epithelial layers, and RV infection further increases the mannitol influx in cells treated with IL-1β.[32] These findings suggest that RV infection may affect the integrity of airway epithelial cells, although RV infection does not induce airway epithelial cell damage[33] as induced by influenza virus infection. Furthermore, RV infection induces mucin release, including MUC5AC, into the mucosal side of airway epithelial cells.[34,35] Mucus hypersecretion[36] and airway epithelial-hyperpermeability may induce mucous hypersecretion and airway narrowing in COPD.[26] Infection with *Streptococcus pneumoniae* after respiratory infection is associated with severity of illness and more frequent hospitalization. RV infection also increases the adherence of *Streptococcus pneumoniae* to human tracheal epithelial cells via increases in PAF receptors,[37] suggesting that increased adherence of *Streptococcus pneumoniae* may be one of the reasons that pneumonia develops after RV infection.[38]

Effects of Rhinovirus Infection on Mast Cells and Cells Other than Airway Epithelial Cells

Cells other than lung epithelial cells also have been reported to produce pro-inflammatory substances and chemical mediators such as histamine, and to be associated with the exacerbation of COPD and asthma.[16,39] Infection with respiratory viruses including RVs activates histamine release from the basophils of peripheral blood,[40] and the plasma histamine content increases after RV infection.[41] Virus infection including RV increases histamine release in basophils stimulated with anti-IgE and calcium ionophore after RV infection.[42] Response to histamine increases in human small airway smooth muscle from COPD.[43] Mast cells are major sources of histamine release in airways and are associated with the pathogenesis of bronchial asthma[44] and COPD.[16,39] We demonstrated that RV infection primes the production of IL-4, IL-6, IL-8, GM-CSF, and histamine in response to stimuli including IgE in both the human mast cell line and the human basophilic leukocyte cell line (Table 1).[45]

The number of macrophages increases in the airway epithelium in patients with COPD,[25,39] and airway macrophages secrete TNF-α after RV infection.[46] Increased levels of TNF-α in sputum may suggest the role of TNF-α in the pathogenesis of COPD.[18,19] Similarly, eosinophil accumulation is observed in airway mucosa after experimental RV infection.[47] Eosinophil granular proteins including eosinophil cationic protein (ECP) have also been detected in the nasal secretions of children with wheezing illness caused by RV infection,[48] and sputum in asthmatic patients experimentally infected with RV type 16,[49] and in patients with COPD.[16,18] On the other hand, RV 16 did not induce superoxide production from peripheral blood eosinophils as shown by Handzel et al.[50] Therefore, inflammatory mediators such as RANTES and GM-CSF[7,8,10,45] released from cells including airway submucosal cells and mast cells may activate eosinophils after RV infection. In fact, we have demonstrated that eosinophil migration through the airway epithelial cell layers increases in response to the addition of supernatants of human tracheal submucosal glands infected with RV14, through the GM-CSF and RANTES in the supernatants.[51]

RV stimulates lymphocytes to induce interferon (IFN)-γ production and T cell proliferation through eosinophil and monocyte activation.[52] Experimental RV infection revealed the accumulation of lymphocytes and monocytes in the airway mucosa and submucosa.[47] Activated lymphocytes may also be associated with the exacerbation of bronchial asthma and COPD.[26] Furthermore, the direct effects of RV on airway smooth muscle contraction was demonstrated by Hakonarson et al.[53]

Inhibition of Rhinovirus Infection by Bafilomycin

In contrast to influenza virus, an effective vaccination for RVs has not been developed because there are more than 100 serotypes of RVs. Although a variety of antiviral agents has been studied on the inhibition of RV infection or common colds, soluble ICAM-1 is the only possible agent that may be useful in alleviating the symptoms of the common cold.[54] On the other hand, other WIN compounds[55] and a RV proteinase enzyme inhibitor[56] are undergoing clinical trials. Two viral proteases designated 2A and 3C have been viewed as excellent targets for antiviral intervention for the picornavirus family including human RV.[57]

The vacuolar (H^+)-ATPases (V-ATPases) are a

family of ATP-driven proton pumps responsible for the acidification of a variety of intracellular compartments in eukaryotic cells.[58] V-ATPases provide the acidic environment required for the dissociation of internalized ligand-receptor complexes within endosomes.[58] Furthermore, exposure of influenza virus to a low pH within endosomes by V-ATPases induces the formation of a fusion pore between the viral and endosomal membranes that permits entry of the viral RNA.[59] The specific V-ATPases inhibitor bafilomycin[60] blocks infection with influenza virus and RV in HeLa cells and Madin-Darby canine kidney (MDCK) cells,[61–63] and inhibits the uncoating of RV type 2 and type 14 from late endosomes.[64,65] However, the role of V-ATPases in RV infection of human airway epithelial cells, the primary target for respiratory viruses, has not been elucidated.

To examine the effects of bafilomycin A_1 on RV infection in the airway epithelium, primary cultures of human tracheal epithelial cells were infected with RV14.[22] Viral infection was confirmed by showing that viral RNA in the infected cells and viral titers of the supernatants and lysates from infected cells increased with time. RV14 infection upregulated the expression of mRNA of ICAM-1, the major RV receptor, on epithelial cells, and it increased the production of IL-1β, IL-6, IL-8 and TNF-α in supernatants. Treatment with bafilomycin A_1 after viral infection reduced viral titers of RV14 in supernatants and cell lysates in association with the inhibition of cytokine production and ICAM-1 induction after viral infection. Furthermore, preincubation with bafilomycin A_1 reduced ICAM-1 mRNA expression and cytokine production before RV14 infection, and reduced susceptibility to RV14 infection of epithelial cells. RV14 increased activated NF-κB in cultured human tracheal epithelial cells, and bafilomycin A_1 reduced the activated NF-κB before and after RV14 infection. Bafilomycin A_1 inhibited this acidification of intracellular pH and decreased the number of acidic endosomes in the epithelial cells. These results suggest that bafilomycin A_1 may inhibit infection with RV14 by not only blocking the RV RNA entry in the endosomes but also reducing ICAM-1 expression in cultured human tracheal epithelial cells.

Inhibition of Rhinovirus Infection by Erythromycin

Macrolide antibiotics inhibit the production of ICAM-1,[66] which plays a vital role in the accumulation of immune effector cells to sites of local

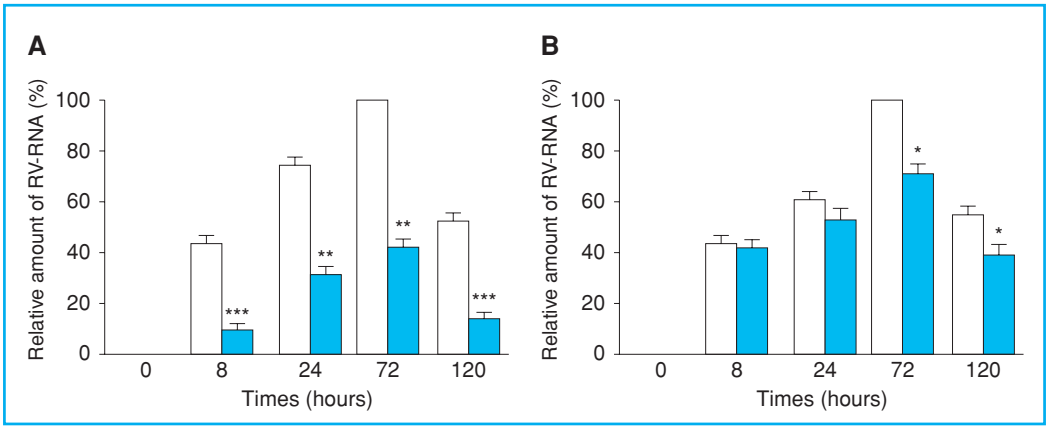

Fig. 1 Time course of replication of RV RNA from human tracheal epithelial cells after infections of either RV14 (A) or RV2 (B) in the presence of erythromycin (10 μM; blue columns) or ethanol (0.1%) as a vehicle of erythromycin (control; white columns) as detected by real-time quantitative amounts of RT-PCR

Results are expressed as relative amounts of RNA expression (%) compared with those of maximal RV RNA at day 3, and reported as means ±SE from 7 samples. Significant differences from treatment with a vehicle of erythromycin (control) at each time are indicated by $^{*}P<0.05$, $^{**}P<0.01$ and $^{***}P<0.001$. To examine the effects of erythromycin on viral RNA in the cells, the cells were treated with erythromycin or a vehicle of erythromycin from 3 days before RV infection to the RNA extraction after RV infection. The RNA extraction was performed at either 0, 8, 24, 72 or 120 h after RV infection. Reproduced with permission from Suzuki et al.[20]

inflammation, and is also known as a receptor for a major subgroup of RVs such as RV14.[30] Because glucocorticoid-induced reductions in ICAM-1 expression inhibit RV14 infection,[67] macrolide antibiotics may also inhibit RV14 infection. Furthermore, a macrolide antibiotic bafilomycin A_1 inhibits RV14 infection.[22] However, the effects of erythromycin, a clinically used macrolide antibiotic, on RV infection have not been investigated.

To examine the effects of erythromycin on RV infection in the airway epithelium, primary cultures of human tracheal epithelial cells were infected with the RV major subgroup, RV14, and the minor subgroup, RV2.[20] Infection was confirmed by increases in viral RNA of the infected cells and viral titers of the supernatants. RV14 upregulated the expression of the mRNA and protein of ICAM-1, the major RV receptor, and it increased cytokine production. Erythromycin reduced the supernatant RV14 titers, RV14 RNA, susceptibility to RV14 infection, and the production of ICAM-1 and cytokines (Fig. 1). Erythromycin also reduced the supernatant RV2 titers, RV2 RNA, susceptibility to RV2 infection, and cytokine production, although the inhibitory effects of erythromycin on the expression of the LDL receptor, the minor RV receptor, were small. Erythromycin reduced NF-κB activation by RV14, and decreased the number of acidic endosomes in the epithelial cells. These results suggest that erythromycin inhibits infection by the major RV subgroup by reducing ICAM-1 and infection by both RV subgroups by blocking RV RNA entry into the endosomes. Erythromycin may also modulate airway inflammation by reducing the production of proinflammatory cytokines and ICAM-1 induced by RV infection.

Prevention of Common Colds and Exacerbations in COPD: Effects of Erythromycin

RV infection is associated with exacerbation of COPD, and erythromycin inhibits RV infection in airway epithelial cells. Low-dose, long-term erythromycin therapy has been reported to be effective in treating patients with diffuse panbronchiolitis (DPB) or bronchiectasis via mechanisms other than antibacterial activity.[68] However, the inhibitory effects of erythromycin on common colds and exacerbations in COPD have not been studied.

To examine whether erythromycin therapy lowers the frequency of the common cold and subsequent exacerbations in patients with COPD, a prospective, randomized, controlled, but not blinded trial was performed.[69] One hundred and nine patients with COPD were enrolled on the study. The patients were randomly assigned to erythromycin therapy or to no active treatment in September 1997. The patients were then observed for 12 months, starting in October, during which time the risk and frequency of catching common colds and COPD exacerbations were investigated. Fifty-five patients received erythromycin at study entry (erythromycin group). The remaining 54 patients received no active treatment (control group). The number of common colds and COPD exacerbations for 12 months was significantly lower in the erythromycin group than that in the control group (Table 2). Furthermore, significantly more patients were hospitalized due to exacerbations in the control group than in the erythromycin group. These findings suggest that erythromycin has beneficial

Table 2 Effects of erythromycin on the prevention of common colds and exacerbations in COPD patients

Measures	Control group (n = 54)	Erythromycin group (n = 55)	*P* Value
Total common colds, No.	245	67	0.0002
Total number of exacerbations, No.	64	14	<0.0001
Total patients with exacerbations, No.	30	6	<0.0001
Total patients with severe exacerbations, No.	10	0	0.0004

Reproduced with permission from Suzuki et al.[69]

effects on the prevention of common colds and exacerbations in COPD patients. However, this intervention should be restricted to patients who are at high risk of exacerbations of COPD because of the potential risk for the emergence of erythromycin-resistant pathogens.

Increased Bactericidal Activity of Surface Liquid in Human Airway Epithelial Cells by Erythromycin

As already described in this article, erythromycin reduces the frequency of common colds and exacerbations in COPD, and erythromycin inhibits RV infection, and reduces the production of ICAM-1 and proinflammatory cytokines before and after RV infection. To further investigate the mechanisms of the inhibition of COPD exacerbations, we studied the effects of erythromycin on the bactericidal activity of surface liquid in human airway epithelial cells.

Defensins, one of the most intensively studied classes of antimicrobial peptides, are identified in a wide distribution of animals including humans.[70] It is suggested that the main function of defensins is to kill bacteria and fungi either on the surfaces of the epithelial cells or within phagolysosomes of phagocytes. Defensins are small cationic peptides containing arginine-rich 29–47 amino acids with three disulfide bonds, which can be divided into the α- and β-defensin subfamilies in human subjects.[70] Of the β-defensins, airway epithelial cells produce human β-defensin (HBD)-1, HBD-2, and HBD-3.[70–75] Recent studies have demonstrated that human airway epithelial cells produce sodium-sensitive antimicrobial peptides into the apical side of the surface liquid, suggesting a major role of HBDs in host defense against bacterial infections.[72,75]

Macrolide antibiotics have clinical benefits in patients with DPB and in patients with cystic fibrosis (CF). Although many mechanisms have been proposed, the precise mechanisms are still uncertain. We examined the effects of erythromycin on the bactericidal activity of airway surface liquid (ASL) secreted by cultured human tracheal epithelial cells.[76] ASL was collected by washing the surface of the cells with a sodium solution (40 mEq/L). Methicillin-resistant *Staphylococcus aureus* and *Pseudomonas aeruginosa* were incubated with airway surface liquid and the number of surviving bacteria was examined. The number of bacteria in ASL from the cells cultured in medium alone was significantly lower than that in the sodium solution. Furthermore, the number of bacteria in ASL from the cells treated with erythromycin was significantly lower than that in ASL from the cells treated with solvent alone. The production of messenger ribonucleic acid and protein of human beta-defensin-1 and human beta-defensin-2 were significantly increased by erythromycin. The bactericidal activity of ASL was observed at low concentrations (40 mEq/L) of sodium but not at higher concentrations (≥80 mEq/L). ASL did not contain significant amounts of antibiotics supplemented in the culture medium. Erythromycin at the levels in ASL and in the culture medium did not inhibit bacterial growth. These results suggest that erythromycin may increase the bactericidal activity of ASL in human airway epithelial cells through human beta-defensin

Table 3 Biologic functions of erythromycin other than antibacterial activities in airways

Target cell or site	Function	References
Neutrophil	Reduced intrapulmonary influx, Reduced chemotaxis	79
Airway epithelial cell	Reduced production of IL-6, IL-8 and ICAM-1	20, 66
	Improvement of sputum mucosity	80
	Inhibition of goblet cell hypersecretion	81
	Increased production of human-beta defensin	76
Biofilm	Reduced biofilm formation	82
Pseudomonas	Direct anti-pseudomonal activity	83
Rhinovirus	Inhibition of rhinovirus infection	20

production, and reduce the susceptibility of the airway to bacterial infection.

Biologic Functions of Erythromycin in Airways

Macrolide antibiotics improve survival in patients with DPB,[77] and have clinical benefits in patients with CF.[78] Many effects of macrolides have been proposed (Table 3), including the effects on neutrophil function,[79] reduced IL-8 production,[66] improvement of sputum mucosity,[80] modulation of goblet cell hypersecretion,[81] inhibition of the alginate biofilm produced by *Pseudomonas aeruginosa* (*P. aeruginosa*),[82] and direct anti-pseudomonal activity (Table 3).[83] We demonstrated that macrolides inhibit RV infection and reduce the production of ICAM-1 and cytokines.[20] Because bacterial infections are also associated with the exacerbation of COPD,[18] the increased production of antimicrobial peptide including HBDs[76] may also be associated with the prevention of acute exacerbations of COPD.

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References

1. Racaniello VR. Picornaviridae: The viruses and their replication. In: Knipe DM, et al. ed. Fields Virology, Fourth Edition. Philadelphia: Lippincott-Williams and Wilkins; 2001:685–722.
2. Johnston SL, Pattemore PK, Sanderson G, et al. Community study of role of viral infections in exacerbations of asthma in 9–111 year old children. Br Med J. 1995;310:1225–1229.
3. Nicholson KG, Kent J, Ireland DC. Respiratory viruses and exacerbations of asthma in adults. Br Med J. 1993;307:982–986.
4. Seemungal T, Harper-Owen R, Brownmik A, et al. Respiratory viruses, symptoms, and inflammatory markers in acute exacerbations and stable chronic obstructive pulmonary disease. Am J Respir Crit Care Med. 2001;164:1618–1623.
5. Rohde G, Wiethege A, Borg I, et al. Respiratory viruses in exacerbations of chronic obstructive pulmonary disease requiring hospitalisation: a case-control study. Thorax. 2003;58:37–42.
6. Subauste MC, Jacoby DB, Richards SM, Proud D. Infection of a human respiratory epithelial cell line with rhinovirus. Induction of cytokine release and modulation of susceptibility to infection by cytokine exposure. J Clin Invest. 1995;96:549–557.
7. Papadopoulos NG, Papi A, Meyer J, et al. Rhinovirus infection up-regulates eotaxin and eotaxin-2 expression in bronchial epithelial cells. Clin Exp Allergy. 2001;31:1060–1066.
8. Schroth MK, Grimm E, Frindt P, et al. Rhinovirus replication causes RANTES production in primary bronchial epithelial cells. Am J Respir Cell Mol Biol. 1999;20:1220–1228.
9. Terajima M, Yamaya M, Sekizawa K, et al. Rhinovirus infection of primary cultures of human tracheal epithelium: role of ICAM-1 and IL-1β. Am J Physiol. 1997;273:L749–L759.
10. Yamaya M, Sekizawa K, Suzuki T, et al. Infection of human respiratory submucosal glands with rhinovirus: effects on cytokine and ICAM-1 production. Am J Physiol. 1999;277:L362–L371.
11. Suzuki T, Yamaya M, Kamanaka M, et al. Type 2 rhinovirus infection of cultured human tracheal epithelial cells: role of LDL receptor. Am J Physiol. 2001;280:L409–L420.
12. Zhu Z, Tang W, Ray A, et al. Rhinovirus stimulation of interleukin-6 in vivo and in vitro. Evidence for nuclear factor κB-dependent transcriptional activation. J Clin Invest. 1996;97:421–430.
13. Einarsson O, Geba GP, Zhu Z, Landry M, Elias JA. Interleukin-11: stimulation in vivo and in vitro by respiratory viruses and induction of airways hyperresponsiveness. J Clin Invest. 1996;97:915–924.
14. Johnston SL, Papi A, Bates PJ, Mastronarde JG, Monick MM, Hunninghake GW. Low grade rhinovirus infection induces a prolonged release of IL-8 in pulmonary epithelium. J Immunol. 1998;160:6172–6181.
15. Turner RB. The role of neutrophils in the pathogenesis of rhinovirus infections. Prdiatr Infect Dis. 1990;9:832–835.
16. Louis RE, Cataldo D, Buckley MG, et al. Evidence of mast-cell activation in a subset of patients with eosinophilic chronic obstructive pulmonary disease. Eur Respir J. 2002;20:325–331.
17. Betz R, Kohlhaufl M, Kassner G, et al. Increased sputum IL-8 and IL-5 in asymptomatic nonspecific airway hyperresponsiveness. Lung. 2001;179:119–133.
18. Sethi S. New developments in the pathogenesis of acute exacerbations of chronic obstructive pulmonary disease. Curr Opin Infect Dis. 2004;17:113–119.
19. Chung KF. Cytokines in chronic obstructive pulmonary disease. Eur Respir J. 2001;34:50s–59s.
20. Suzuki T, Yamaya M, Sekizawa K, et al. Erythromycin inhibits rhinovirus infection in cultured human tracheal epithelial cells. Am J Respir Crit Care Med. 2002;165:1113–1118.
21. Papi A, Johnston SL. Rhinovirus infection induces expression of its own receptor intercellular adhesion molecule 1 (ICAM-1) via increased NF-kappa B-mediated transcription. J Biol Chem. 1999;274:9707–9720.
22. Suzuki T, Yamaya M, Sekizawa K, et al. Bafilomycin A1 inhibits rhinovirus infection in human airway epithelium: effects on endosome and ICAM-1. Am J Physiol. 2001;280:L1115–L1127.
23. Pearse JE, Sabroe I. The role of interleukin-8 and its receptors in inflammatory lung disease: implications for therapy. Am J Respir Med. 2002;1:19–25.
24. Wedzicha JA. Exacerbations: etiology and pathophysiologic mechanisms. Chest. 2002;121:136S–141S.
25. de Boer WI, van Schadewijk A, Sont JK, et al. Transforming growth factor beta 1 and recruitment of macrophages and mast cells in airway in chronic obstructive pulmonary disease. Am J Respir Crit Care Med. 1998;158:1951–1957.
26. Hogg JC, Chu F, Utokaparch S, et al. The nature of small-airway obstruction in chronic obstructive pulmonary disease. New Engl J Med. 2004;350:2645–2653.
27. Maines MD. The heme oxygenase system: a regulator of second messenger gases. Annu Rev Pharmacol Toxicol. 1997;37;517–554.
28. Yasuda H, Yamaya M, Nakayama K, et al. Increased arterial

carboxyhemoglobin concentrations in chronic obstructive pulmonary disease. Am J Respir Crit Care Med. 2005;171: 1246–1251.
29. Montuschi P, Kharitonov SA, Barnes PJ. Exhaled carbon monoxide and nitric oxide in COPD. Chest. 2001;120;496–501.
30. Greve JM, Davis G, Meyer AM, et al. The major human rhinovirus receptor is ICAM-1. Cell. 1989;56:839–847.
31. Dekhuiizen PNR, Aben KK, Dekker I, et al. Increased exhalation of hydrogen peroxide in patients with stable and unstable chronic obstructive pulmonary disease. Am J Respir Crit Care Med. 1996;154:813–816.
32. Ohrui T, Yamaya M, Sekizawa K, et al. Effects of rhinovirus infection on hydrogen peroxide-induced alterations of barrier function in the cultured human tracheal epithelium. Am J Respir Crit Care Med. 1998;158:241–248.
33. Winther B, Gwaltney JM, Hendley JO. Respiratory virus infection of monolayer cultures of human nasal epithelial cells. Am Rev Respir Dis. 1990;141:839–845.
34. Inoue D, Yamaya M, Kubo,H, et al. Mechanisms of mucin production by rhinovirus infection in cultured human airway epithelial cells. Respir Physiol Neurobiol. (in press)
35. He SH, Zheng J, Duan MK. Induction of mucin secretion from human bronchial tissue and epithelial cells by rhinovirus and lipopolysaccharide. Acta Pharmacol Sin. 2004;25:1176–1181.
36. Yuta A, Doyle WJ, Gaumond E, et al. Rhinovirus infection induces mucus hypersecretion. Am J Physiol. 1998;274:L1017–L1023.
37. Ishizuka S, Yamaya M, Suzuki T, et al. Effects of rhinovirus infection on the adherence of *Streptococcus pneumoniae* to cultured human airway epithelial cells. J Infect Dis. 2003;188:1928–1939.
38. Juven T, Mertsola J, Waris M, et al. Etiology of community-acquired pneumonia in 254 hospitalized children. Pediatr Infect Dis J. 2000;19:293–298.
39. Grashoff WF, Sont JK, Sterk PJ,et al. Chronic obstructive pulmonary disease: role of bronchiolar mast cells and macrophages. Am J Pathol. 1997;151:1785–1790.
40. Chonmaitree T, Lett-Brown MA, Tsong Y, Goldman AS, Baron S. Role of interferon in leukocyte histamine release caused by common respiratory viruses. J Infect Dis. 1988;157:127–132.
41. Calhoun WJ, Swenson CA, Dick EC, Schwartz LB, Lemanske RF Jr, Busse WW. Experimental rhinovirus 16 infection potentiates histamine release after antigen bronchoprovocation in allergic subjects. Am Rev Respir Dis. 1991;144:1267–1273.
42. Ida S, Hooks JJ, Siraganian RP, Notkins AL. Enhancement of IgE-mediated histamine release from human basophils by viruses: role of interferon. J Exp Med. 1977;145:892–905.
43. De Jongste JC, Hons H, Block R, Bonta IL, Frederiksz AP, Kerrebijn KF. Increased in vitro histamine responses in human small airways smooth muscle from patients with chronic obstructive pulmonary disease. Am Rev Respir Dis. 1987;135:549–553.
44. Galli SJ. New concepts about the mast cell. N Engl J Med. 1993;328:257–265.
45. Hosoda M, Yamaya M, Suzuki T, et al. Effects of rhinovirus infection on histamine and cytokine production by cell lines from human mast cells and basophils. J Immunol. 2002;169:1482–1491.
46. Gern JE, EC Dick, WM Lee, et al. Rhinovirus enters but does not replicate inside monocytes and airway macrophages. J Immunol. 1996;156:621–627.
47. Fraenkel DJ, Bardin PG, Sanderson G, Lampe F, Johnston SL, Holgate ST. Lower airways inflammation during rhinovirus colds in normal and in asthmatic subjects. Am J Respir Crit Care Med. 1995;151:879–886.
48. Heymann PW, Rakes GP, Hogan AD, Ingram JM, Hoover GE, Platts-Mills TA. Assessment of eosinophils, viruses and IgE antibody in wheezing infants and children. Int Arch Allergy Immunol. 1995;107:380–382.
49. Grunberg K, Smits HH, Timmers MC, et al. Experimental rhinovirus 16 infection. Effects on cell differentials and soluble markers in sputum in asthmatic subjects. Am J Respir Crit Care Med. 1997;156:609–616.
50. Handzel ZT, Busse WW, Sedgwick JB, et al. Eosinophils bind rhinovirus and activate virus-specific T cells. J Immunol. 1998; 160:1279–1284.
51. Furukawa E, Ohrui T, Yamaya M, et al. Human airway submucosal glands augment eosinophil chemotaxis during rhinovirus infection. Clin Exp Allergy 2004;34:704–711.
52. Gern JE, Vrtis R, Kelly EAB, Dick EC, Busse WW. Rhinovirus produces nonspecific activation of lymphocytes through a monocyte-dependent mechanism. J Immunol. 1996;157:1605–1612.
53. Hakonarson H, Maskeri N, Carter C, Hodinka RL, Campbell D, Grunstein MM. Mechanism of rhinovirus-induced changes in airway smooth muscle responsiveness. J Clin Invest. 1998; 102:1732–1741.
54. Turner RB, Wecker MT, Pohl G, et al. Efficacy of tremacamra, a soluble intercellular adhesion molecule 1, for experimental rhinovirus infection: a randomized clinical trial. JAMA 1999; 281:1797–1804.
55. Hadfield AT, Diana GD, Rossmann MG. Analysis of three structurally related antivial compounds in complex with human rhinovirus 16. Proc Natl Acad Sci USA. 1999;96:14730–14735.
56. Zalman LS, Brothers MA, Patick AK, et al. Inhibition of human rhinovirus-induced cytokine production by AG7088, a human rhinovirus 3C protease inhibitor. Antimicrob Agents Chemother. 2000;44:1236–1241.
57. Binford SL, Maldonado F, Brothers MA, et al. Conservation of amino acids in human rhinovirus 3C protease correlates with broad-spectrum antiviral activity of rupintrivir, a novel human rhinovirus 3C protease inhibitor. Antimicrob Agents Chemother 2005;49:619–626.
58. Forgac, M. Structure, function and regulation of the vacuolar (H^+)-ATPases. FEBS Letters. 1998;440:258–263.
59. White JM. Membrane fusion. Science. 1992;258:917–924.
60. Bowman EJ, Siebers A, Altendorf K. Bafilomycins: A class of inhibitors of membrane ATPases from microorganisms, animal cells, and plant cells. Proc Natl Acad Sci USA. 1988;85:7972–7976.
61. Bayer N, Schober D, Prchla E, Murphy RF, Blaas D, Fuchs R. Effect of bafilomycin A1 and nocodazole on endocytic transport in HeLa cells: implications for viral uncoating and infection. J Virol. 1998;72:9645–9655.
62. Ochiai H, Sakai S, Hirabayashi T, Shimizu Y, Terasawa K. Inhibitory effect of bafilomycin A_1, a specific inhibitor of vacuolar-type proton pump, on the growth of influenza A and B viruses in MDCK cells. Antiviral Res. 1995;27:425–430.
63. Pérez L, Carrasco L. Entry of poliovirus into cells does not require a low-pH step. J Virol. 1993;67:4543–4548.
64. Bayer N, Prchla E, Schwab M, Baas D, Fuchs R. Human rhinovirus HRV14 uncoats from early endosomes in the presence of bafilomycin. FEBS Lett. 1999;463:175–178.
65. Prchla E, Kuechler E, Blaas D, Fuchs R. Uncoating of human rhinovirus serotype 2 from late endosomes. J Virol. 1994;68: 3713–3723.
66. Khair OA, Devalia JL, Abdelaziz MM, Sapsford RJ, Davies RJ. Effect of erythromycin on Haemophilus influenzae endotoxin-induced release of IL-6, IL-8 and sICAM-1 by cultured human bronchial epithelial cells. Eur Respir J. 1995;8:1451–1457.
67. Suzuki T, Yamaya M, Sekizawa K, et al. Effects of dexamethasone on rhinovirus infection in cultured human tracheal epithelial cells. Am J Physiol. 2000;278:L560–L571.
68. Kudoh S. Erythromycin treatment in diffuse panbronchiolitis. Curr Opin Pulm Med. 1998;4:116–121.
69. Suzuki T, Yanai M, Yamaya M, et al. Erythromycin and common cold in COPD. Chest. 2001;120:730–733.
70. Van Wetering S, Sterk PJ, Rabe KF, Hiemstra PS. Defensins: key players or bystanders in infection, injury, and repair in the lung? J Allergy Clin Immunol. 1999;104:1131–1138.
71. Duits LA, Rademaker M, Ravensbergen B, et al. Inhibition of hBD-3, but not hBD-1 and hBD-2, mRNA expression by corticosteroids. Biochem Biophys Res Comm. 2001;280:522–525.
72. Goldman MJ, Anderson GM, Stolzenberg ED, Kari UP, Zasloff M, Wilson JM. Human β-defensin-1 is a salt-sensitive antibiotic in lung that is inactivated in cystic fibrosis. Cell. 1997;88:553–

560.
73. Harder J, Bartels J, Christophers E, Schroder JM. Isolation and characterization of human beta-defensin-3, a novel human inducible peptide antibiotic. J Biol Chem. 2001;276:5707–5713.
74. Jia HP, Schutte BC, Schudy A, et al. Discovery of new human beta-defensins using genomics-based approach. Gene. 2001; 263:211–218.
75. Smith JJ, Travis SM, Greenberg EP, Welsh MJ. Cystic fibrosis airway epithelia fail to kill bacteria because of abnormal airway surface fluid. Cell. 1996;85:229–236.
76. Ishizawa K, Suzuki T, Yamaya M, et al. Erythromycin increases bactericidal activity of surface liquid in human airway epithelial cells. Am J Physiol. 2005;289:L565–L573.
77. Kudoh S, Azuma A, Yamamoto M, Izumi T, Ando M. Improvement of survival in patients with diffuse panbronchiolitis treated with low-dose erythromycin. Am J Respir Crit Care Med. 1998;157:1829–1832.
78. Equi A, Balfour-Lynn IM, Bush A, Rosenthal M. Long term azithromycin in children with cystic fibrosis: a randomized, placebo-controlled crossover trial. Lancet. 2002;360:978–984.
79. Kadota J, Sakito O, Kohno S, et al. A mechanism of erythromycin treatment in patients with diffuse panbronchiolitis. Am Rev Respir Dis. 1993;147:153–159.
80. Dupont MJ, Lapointe JR. Quantitative effect of roxithromycin and rifampicin on mucoid cultures from directly plated sputum of cystic fibrosis patients chronically colonized with Pseudomonas aeruginosa. Drugs Exp Clin Res. 1990;16:597–605.
81. Tamaoki J, Takeyama K, Yamawaki I, Kondo M, Konno K. Lipopolysaccharide-induced goblet cell hypersecretion in the guinea pig trachea: inhibition by macrolides. Am J Physiol. 1997;272:L15–L19.
82. Kobayashi H. Biofilm disease: its clinical manifestation and therapeutic possibilities of macrolides. Am J Med. 1995;99: 26S–30S.
83. Tateda K, Ishii Y, Matsumoto T, et al. Direct evidence for antipseudomonal activity of macrolides: exposure-dependent bactericidal activity and inhibition of protein synthesis by erythromycin, clarithromycin, and azithromycin. Antimicrob Agents Ch. 1996;40:2271–2275.

A Case of Prolonged Depression with Tamoxifen

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Abstract

Tamoxifen is known to trigger depressive symptoms as a result of its anti-estrogenic activity. We experienced a patient with a history of depression, who received tamoxifen (an estrogen partial agonist) as a postoperative therapy for breast cancer and developed an relapse of depressive symptoms after about 10 months of treatment. The symptoms aggravated to the state of psychotic depression. This case was poorly responsive to antidepressants and resistant to treatment, but the symptoms improved after interruption of tamoxifen. This observation suggests that tamoxifen may aggravate depression in severe and refractory conditions.

Key words Depression, Tamoxifen, Estrogen, Breast cancer

Introduction

Depressive symptoms accompanying malignant tumors have recently been studied extensively from the standpoint of liaison psychiatry. In particular, several cases of depression have been reported in association with breast cancer, which is a common disease in females.[1,2] Although the etiology is not clear, suspected causes include the lowering of self-esteem due to the alteration of appearance and the psychogenic reaction to the disease and to surgical treatment, in addition to the influence of hormonal changes.[1] While anti-estrogens such as tamoxifen are often used to prevent cancer recurrence, this treatment may increase the risk of depressive symptoms.[1] In this paper, we report our experience with a patient who had a history of depression, received tamoxifen after surgery for breast cancer, and then developed severe depression with psychotic symptoms, which became prolonged. To ensure anonymity, details of the patient's personal information have been modified as necessary.

The Case

Patient: Female, 63 years old.
Genetic factors: Mother committed suicide. Younger sister had depression.
Past history: Tuberculosis at the age of 42, hypertension at the age of 51, breast cancer surgery at the age of 62.
Personality before illness: Meticulous and worrisome.
Life history: The patient was born as the 3rd daughter in a family of 5 children. After graduating from a local high school, she worked for an insurance company as a clerk. After marrying at the age of 23, she mothered 2 children while working part-time.
History of present illness: The patient first visited our department 19 years ago (44 years old) presenting with depressive mood, insomnia, appetite loss, anxiety, thirst, and palpitations. No abnormality was noted in blood counts, biochemistry, etc. Depression was suspected. The symptoms were relieved by treatment with antidepressants for several months, and treatment

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was terminated after about 1 year. Recurrence of depression occurred several times thereafter, but each episode was improved by medication.

Two years ago, the patient received regular screening for breast cancer in the insurance system, and was found to have breast cancer of the left breast. Total mammectomy and axillary dissection were performed in August (61 years old). Histopathological study proved invasive ductal carcinoma, n0, G2, ER(+), PR(+), pT = 0.8 cm. Administration of tamoxifen 20 mg was commenced shortly after surgery, and the progress of treatment was good. Her mental state before and after surgery was stable.

About 1 year ago, in January (62 years old, 5 months from the beginning of tamoxifen therapy), the patient voluntarily stopped taking psychotropic drugs because she felt her condition was good. Outpatient treatment at our department was terminated, ending with the visit in March. She continued to use tamoxifen and hypotensive medication.

In May, the younger sister of the patient was admitted to a hospital for the treatment of depression, and the patient took care of her sister. In June (10 months after the beginning of tamoxifen therapy), the sister was discharged from the hospital. The patient then gradually started to complain depressive mood, loss of motivation, thirst, hypogeusia, lack of concentration, etc., and she revisited our department in early July. Blood counts, biochemistry, and head CT (Fig. 1) revealed no clear abnormality, and the relapse of depression was suspected. EEG was irregular with few alpha waves, suggesting a strained state. The patient remained irregular in the use of drugs. In addition to the above-mentioned symptoms, she showed panic attacks, insomnia, appetite loss, and perplexity. Two weeks later, persecutory delusions developed, including the belief that the police and fire fighters would come to collect her debt. The patient was hospitalized for the second time in August.

On admission, the patient was very agitated and perplexed. While her breast cancer had been monitored regularly on an outpatient basis, no recurrence had been noted. Because tamoxifen had been continued, we continued administration after admission. Blood sampling at the time of admission showed slight liver dysfunction (GTP 49 and GPT 61). CK was as high as 1099. The patient was considered to have severe depression, and treatment was started with a drip infusion of clomipramine, an antidepressant. Because delirium was noted and drug-induced delirium due to clomipramine was suspected, the dose was reduced and then stopped. Thereafter, the patient gradually came to show longer lucid intervals.

In mid September, the patient mainly showed depressive symptoms, including depressive mood, appetite loss, and malaise, while abnormal speech and behavior almost disappeared. Blood analysis became largely normal (GOT 20, GPT 21, CK 31). The Hamilton Depression Rating Score was 32. The brain MRI conducted after improvement of disturbance of consciousness demonstrated no abnormality relative to the condition before admission (Fig. 2). EEG indicated improvement, showing a slight increase in the frequency of alpha waves.

Three months later, the patient strongly complained about insomnia. Blood analyses were stable, and the condition was considered to be an aggravation of depression. The Hamilton score was 22, and the persistence of severe depression without psychotic symptoms was inferred. Her condition aggravated further when the patient was temporarily released for regular monitoring of breast cancer. In addition to guilt and suicidal feeling, she started to have delusion of poverty. No recurrence of breast cancer was noted, and tamoxifen was continued.

The condition did not improve for 4 months, and the complaints involving persecution and self-accusation continued. The Hamilton score was 22. The antidepressant was switched to milnacipran 100 mg, and the patient was kept under careful observation.

In January of this year, the patient started to smile occasionally when she was with her family. However, she stayed mostly in bed during the daytime. Her facial expression was stiff, and she expressed suicidal feelings. The Hamilton score was 22, and no improvement of depression was seen.

Because tamoxifen had been suggested to be a cause of depression, we considered the possibility that tamoxifen might affect for the prolonged presence of depression. Following the discussion with the surgeon, tamoxifen was stopped 5 months later. No other changes were made to the regimen, and the patient was kept under careful observation. Within about 2 weeks

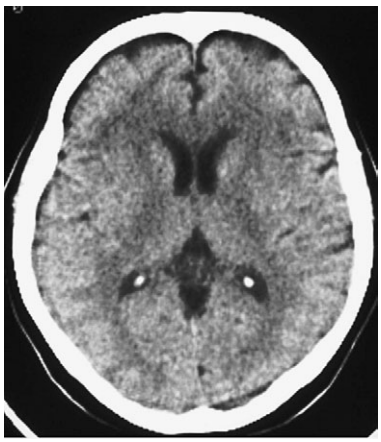

Fig. 1 Head CT
Slight brain atrophy predominantly in the region from the frontal to the temporal lobe. No remarkable change as compared with the condition 5 years previously.

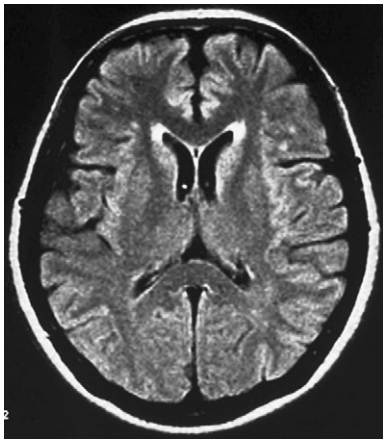

Fig. 2 Head MRI
Slight atrophy in the temporal and parietal lobes and multiple lacunar infarction are noted, but no organic changes that may cause disturbance of consciousness were found.

after stopping tamoxifen, the patient started to talk more with nurses. Although she described that her condition was "not improving and not good," she stopped complaining about insomnia. She started to smile during interviews with the physician. Her facial expression improved in about one month, and the patient started to show active behavior, such as walking in the ward. She became able to take a walk with her husband, and to eat meals regularly. Although the patient's self-evaluation remained poor, evaluation by family members improved and the Hamilton score dropped to 7. The condition clearly improved (Fig. 3).

A different physician was later appointed to take charge of this patient. While aggravation was not seen, remission did not occur and the symptoms became prolonged. Although the main drug was changed to amoxapine and maprotiline, no remarkable changes were observed. Exacerbation did not occur in the course of about one year including several stays at home, and the patient was discharged from the hospital. No recurrence of breast cancer has since been noted, and no abnormality has been shown by monthly blood tests. The brain MRI conducted in October of this year showed no remarkable changes (Fig. 2).

Laboratory test results:

- Blood analyses on admission
  WBC 5800, Hb 12.0, Plt 196,000, TP 5.6, GOT 49, GPT 61, γGTP 84, Na 140, K 3.2, Cl 103, CRP 1.25
  f-T3 2.67, f-T4 0.90, TSH 3.29, ACTH 25.2, cortisol 29.1
- EEG
  July, last year: Irregular basic pattern with marked presence of beta waves.
  September, last year: Irregular basic pattern. Appearance of 9–10 Hz alpha waves as compared with the previous data.
  October, last year: Slow alpha rhythm. Improved appearance of 9–10 Hz alpha waves as compared with the previous data.

The Treatment course is shown in Fig. 3.

Discussion

Tamoxifen is a partial agonist of estrogen, which is used as an adjuvant therapy for breast cancer based on the fact that breast cancer cells often have estrogen receptors and the cancer cells are activated by estrogen. Because the partial agonist binds to estrogen receptors in competition with estrogen and its estrogenic effect is weaker than that of estrogen, it exerts an antiestrogenic effect (Fig. 4). In addition, tamoxifen has various effects such as promoting translation of denatured RNA, reducing cell proliferation, promoting apoptosis of malignant cells, reducing circulation of insulin-like growth factor-I, and increasing circulation of serum hormone binding

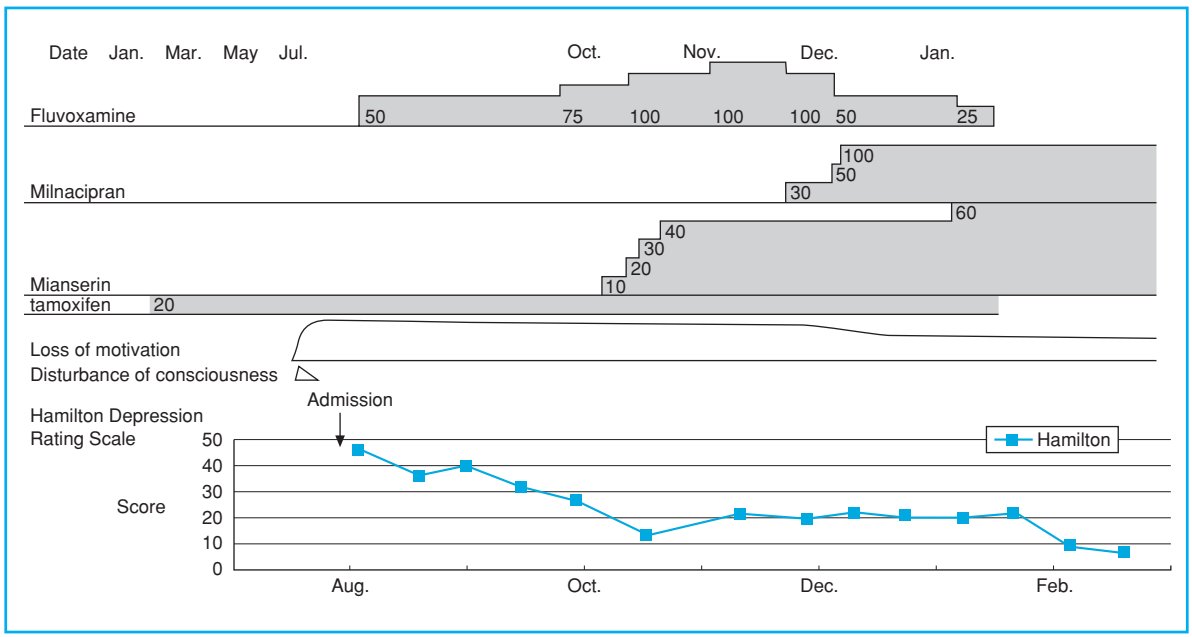

Fig. 3 Treatment course

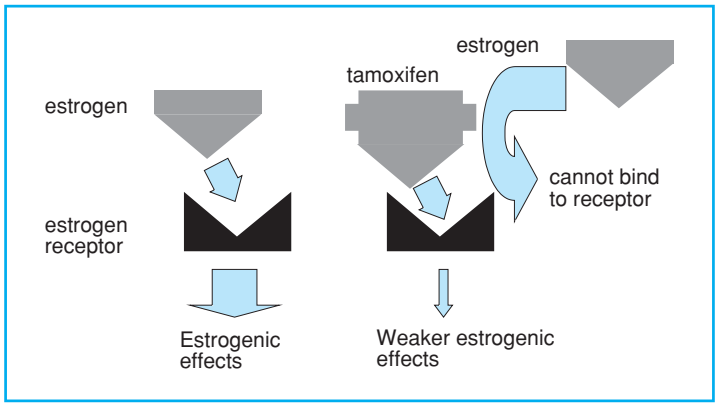

Fig. 4 Antagonism of estrogenic effects by tamoxifen
Tamoxifen binds to the same receptor as estrogen but causes weaker effects

globulin (SHBG) (Fig. 5).[1] These effects are considered to derive from the action of tamoxifen itself, in addition to its anti-estrogenic activity. The possibility that tamoxifen as an anti-estrogen may promote development, severity, and prolongation of depression has not been discussed much in Japan, but this possibility has been pointed out by several authors[2–6] based on the facts that a decrease in estrogen may cause depression and women often develop depression during the menopause. While there is clinical evidence that estrogen may counter the development of depression, this effect is considered to derive from the positive effect of estrogen on serotonin and noradrenaline.[4] Tamoxifen passes through the blood-brain barrier (BBB) and inhibits these effects of estrogen. It has been reported that depression in patients receiving tamoxifen most frequently occurs within 12 months from the beginning of treatment, in

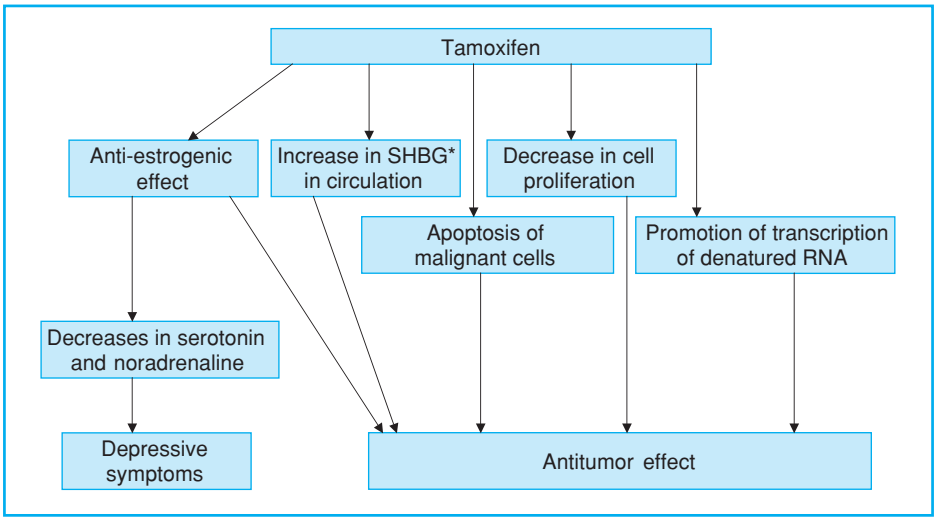

Fig. 5 Effects of tamoxifen
*: Serum hormone binding globulin

particular during the period from 3 to 6 months, and dose reduction or termination of tamoxifen usually results in alleviation of symptoms within weeks. The depression related to tamoxifen does not always coincide with climacteric symptoms, which are also major side effects of tamoxifen, and patients with severe depression often lack symptoms such as flushing of the face.[2] These clinical features also suggest the possibility that the development and prolongation of depression related to tamoxifen may not be a result of simple anti-estrogenic activity. The development of depression probably results from the action of tamoxifen itself, in addition to the action mediated by estrogen. The reported incidence rate of depression ranges widely from 1 to 15%. Our search of the literature indicated only 1 paper (by Love[3]) that reported the low level of 1%, and the other 3 studies provided incidence rates in the range from 12 to 16%. The depression in these patients usually improved after dose reduction or termination of tamoxifen in 2 to 4 weeks.[2]

The patient reported here is considered to be a case of recurrent depressive disorder, in view of her past history. Because recurrence of depression did not occur directly following the use of tamoxifen after breast cancer surgery, this case is considered to be an endogenous episode triggered by the psychological pressure caused by hospitalization and home care of her younger sister. As reported by Ishige[7] and Takao et al.,[8] aggravation to psychotic depression may occur even 16 months after drug administration. Therefore, the direct involvement of tamoxifen in the pathogenesis of this case may not be ruled out. Past episodes in this patient were characterized by prompt response to antidepressants even when there was severe perplexity. The present episode was unprecedentedly severe, involving psychotic symptoms during the acute phase. Physical illness was excluded by blood counts, biochemistry, thyroid hormone, brain CT, EEG, and other data, and depression improved after the termination of tamoxifen. Because of these facts, it is considered probable that tamoxifen may have affected the severity and prolongation of this episode.

In this case, the dose of antidepressant was not changed during observation for one month after termination of tamoxifen. While depression improved gradually, the improvement was not sufficient to stop administration of antidepressant, and complete remission did not take place. As depression gradually exacerbated 2 months later, the dose of antidepressant had to be increased again. In many previous reports, termination of tamoxifen after development of depression led to improvement in a reversible manner. However, it is not clear whether there are irre-

versible factors. Ishige reported a case that required antidepressant therapy after termination of tamoxifen and continued to have symptoms. We cannot rule out the possibility that the drug was involved in the prolongation of depression after termination of tamoxifen in our case.

Our patient had previously been showing prompt response to tricyclic antidepressants without remarkable side effects. However, in this episode, she developed severe delirium during the use of a tricyclic antidepressant. This could be related to the changes in organic factors, and there has been no report suggesting the involvement of tamoxifen in such alteration of pharmacological effects. However, in view of the timing, such involvement should also be considered as a possibility. More detailed study is needed concerning the use of tamoxifen in patients with depression. Because it may be involved in the severity and prolongation of symptoms, we should be careful regarding the use of tamoxifen in patients with a history of depression.

References

1. Thompson DS, Spanier CA, Vogel VG. The relationship between tamoxifen, estrogen, and depressive symptoms. The Breast Journal. 1999;5:375–382.
2. Cathacart CK, Jones SE, Pumroy CS, et al. Clinical recognition and management of depression in node negative breast cancer patient treated with tamoxifen. Breast Cancer Res Treat. 1993; 27:277–281.
3. Love RRL, Cameron L, Connell BL, et al. Symptoms associated with tamoxifen treatment in postmenopausal women. Arch Intern Med. 1993;151:1842–1847.
4. Anelli TF, Anelli A, Tran KN, et al. Tamoxifen administration is associated with a high rate of treatment-limiting symptoms in male breast cancer patients. Cancer. 1994;74:74–77.
5. Shariff S, Cumming CE, Lees A, et al. Mood disorder in women with early breast cancer taking tamoxifen, an estradiol receptor antagonist: an expected or unexpected effect? Ann NY Acad Sci. 1995;761:365–368.
6. Breuer B, Anderson R. The relationship of tamoxifen with dementia, depression, and dependence in activities of daily living in elderly nursing home residents. Women & Health. 2000;31:71–85.
7. Ishige H. Nyuugan shujutsu go tamoxifen touyochuuni hasshoushita utsubyou. Japanese Journal of Breast Cancer. 1997;12:727–729. (in Japanese)
8. Takao S, Nishimura H, Sagayama K, et al. Heikeizen nyuugan kanjano jutsugo Tamoxifen＋Goserelin touyochuuni hasshoushita utsubyouno nirei. Japanese Journal of Breast Cancer. 2000;15:589–592. (in Japanese)

Brachial Artery Aneurysm

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Abstract

The brachial artery is not a common site for peripheral arterial aneurysms, and little data on the causes and management of these aneurysms is currently available. We present the case of a patient admitted to the outpatients' clinic of the South Surgical Unit based in Mayo Hospital Lahore.

Key words Brachial artery, Aneurysm, Atherosclerotic aneurysm

Introduction

Aneurysms are abnormal dilatations of the vessel wall caused by a number of factors including atherosclerosis, trauma, infection and vasculitis etc. Peripheral arterial aneurysms include popliteal (most common), femoral etc. but brachial artery aneurysms are rare entities. Only few cases have been reported in medical literature to date. We present a case of a brachial artery aneurysm in a patient from a rural area of Pakistan.

Case Report

A 27-year-old female, married, mother of 3 children, resident of Peeroshah (Gujrat) visited our outdoor clinic with a painless swelling on the medial side of her right arm that she had been carrying for 6 years. The patient noticed it herself, and her apprehension about the nature of the swelling brought her to the hospital. It slowly progressed in size, and did not cause any impairment in the functional capacity of the limb. The patient reported no pain, numbness, tingling sensation, wasting, or colour changes in the forearm and hand. There was no history of trauma to the arm, multiple venepunctures (therapeutic or diagnostic), arteriography, dialysis, intravenous drug abuse, or any surgery in the affected area. There were no complaints such as palpitations, shortness of breadth or dizzy spells (which would suggest a central aneurysm). The patient was not a known diabetic, was not hypertensive and had no family history of similar swellings.

Examination showed a healthy looking young female with no evidence of anemia, having an ovoid swelling of 4 × 2 cm on the medial aspect of her right arm 12 cm above the medial epicondyle, with no visible pulsations, scar marks, pigmentation or prominent veins, and the colour of the skin overlying the swelling was same as that of the surrounding skin. Palpation revealed a 4-×-2-cm non-tender, pulsatile, expansile, non-fluctuant mass having same temperature as that of the surrounding skin, which was compressible, non-reducible, not blanching on pressure, not attached to the overlying skin or underlying muscle or bone, and more mobile in the longitudinal plane than horizontal plane. Axillary and supra clavicular lymph nodes were not palpable bilaterally. Distal neuro-vascular status was intact. On auscultation, no bruit was audible over the swelling. Examination of precordium revealed no added heart sounds or murmurs suggestive of valvular heart disease. The remainder of the systemic examination was unremarkable. Lab investigations showed hemoglobin of 11 g/dL, ESR 18 mm at

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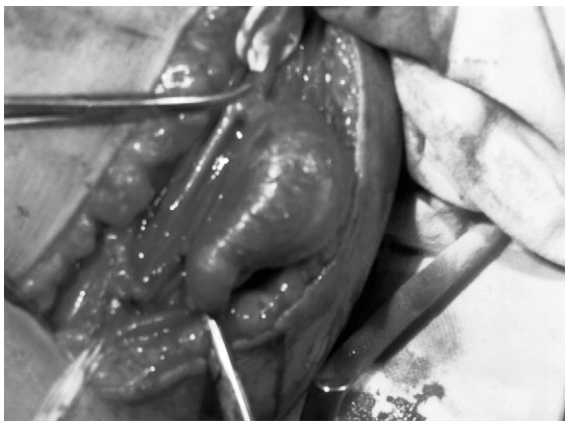

Fig. 1 Brachial artery aneurysm during dissection

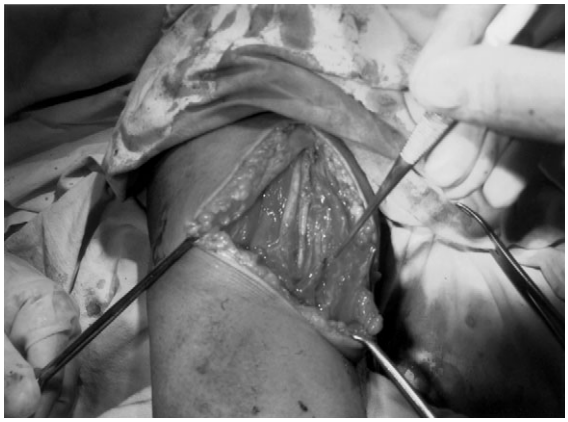

Fig. 2 After resection and anastomosis

the end of first hour, and TLC 4,800 mm^3. The plasma homocysteine level was 9.0 micromol/l. (normal = 4–12 micromol/l for an adult female.) There was no evidence of bacterial or viral infection, so bacterial cultures were not performed. Doppler's examination revealed loud flow at the site of swelling. Ankle brachial index was 1.0 bilaterally. An ultrasonography report documented a pulsatile anechoic mass measuring 3.2 × 2 × 2 cm seen along the right brachial artery, while abdominal aorta was normal. Color Doppler showed an anechoic pulsatile area measuring 3.2 × 2 × 2 cm seen along right brachial artery, which had arterial pattern & turbulent flow. The impression was of a right brachial artery aneurysm.

Resection and anastomosis was performed under general anesthesia (Figs. 1 and 2). There was no invasion of the surrounding muscles or nerves. The surrounding muscles were normal in bulk and color. Since the aneurysmal segment was short (3 × 2 cm), we preferred end-to-end vascular anastomosis. The muscular branches of the brachial artery to the biceps brachii were higher than the proximal end of the aneurysm. The median nerve was identified and preserved. The ulnar and radial nerves were not in close proximity to the aneurysm. There was no neurological deficit pre- and post-operatively.

Histopathology showed a dilated arterial segment with thinning of the tunica media. There was evidence of mural thrombus formation consisting of fat with mild fibrosis, which had caused the arterial wall to balloon out.

Conclusion: Brachial artery aneurysm due to atherosclerosis.

Pre-operative and post-operative Doppler exam showed normal distal arterial blood flow, and sensation was intact. The post-operative course was smooth and uneventful. The patient was discharged on the third post-operative day. Follow-up at 2-month intervals showed no symptoms or signs of recurrence of swelling.

Discussion

Dilatation of local segments of the arterial system is called aneurysm. If all three layers of the arterial wall are involved in the aneurismal sac, it is a true aneurysm, while if it has only single layer of fibrous tissue as the wall of the sac, it is called a false aneurysm. They can be either congenital or acquired (mycotic, syphilitic, traumatic, collagen disease). They can be central (aortic, carotid) or peripheral (femoral, popliteal, brachial). Most peripheral aneurysms are pseudo aneurysms produced due to local arterial damage either by diagnostic and therapeutic arterial catheterizations or by direct trauma to the arterial site, both leading to disruption of wall continuity and bleeding into the surrounding tissue where the circulating blood is held by the adjacent tissues, fascia and thrombus and not by the normal arterial wall. The usual presentation is a painless, pulsatile, asymptomatic mass usually incidentally diagnosed. However, it does become symptomatic when complications arise, such as disruption causing profuse bleeding and vascular collapse or thrombosis in the sac, which throws

emboli into peripheral circulation.

Differential diagnosis includes hematomas, pulsating tumors (such as bone sarcomas, osteoclastomas), arterio venous malformations, lymphadenopathy, lipomas, and abscesses. Investigations of choice are ultrasonography, colour Doppler studies and subtraction image angiography. Treatment options include observation, ultrasound guided compression, thrombin injection and operative repair. The operative approach involves initial and distal control, followed by direct dissection of the aneurismal portion of the vessel itself. Heparin is administered and the aneurysm sac opened. The defect in the artery is often small and is most easily identified by transient release of the proximal clamp. The false aneurysm tissue is debrided, and the arterial defect is closed with interrupted 5/0 or 6/0 polypropylene sutures, taking care to traverse the entire arterial wall. Ultrasound guided compression begins by identifying the high-velocity jet of blood entering the false aneurysm by a standard 5 MHz. Pressure is applied with the transducer-headed flow within the false aneurysm. Throughout the compression, the native artery is visualized to preserve flow in this vessel. Compression continues for 10 minutes and is released. Continued flow through the false aneurysm mandates repeat compression cycles for up to 1 hour. Re-imaging the following day to confirm obliteration is mandatory. If persistent flow is noted, the compression therapy can be reapplied. Although non-invasive, it is associated with significant disadvantages, can be painful, and is less effective in anticoagulated patients. For thrombin injection therapy, a solution of thrombin is prepared by reconstituting 1,000 units of thrombin powder in 1 ml of normal saline. A 22–25-gauge needle (spinal needle for the obese) is used. Under ultrasound guidance, the tip of the needle in inserted into the sac, and 0.5 to 1.0 ml of thrombin is injected. Repeat duplex is performed to confirm aneurismal thrombosis within several days. Of all three treatment options, thrombin injection is the least invasive method.

References

1. Messina LM, Brothers TE, Wakefield TW, et al. Clinical characteristics and surgical management of vascular complications in patients undergoing cardiac catheterization: interventional versus diagnostic procedures. J Vasc Surg. 1991;13:593–600.
2. Kent CK, McArdle CR, Kennedy B, et al. A prospective study of the clinical outcome of femoral pseudoaneurysm and arteriovenous fistulas induced by arterial puncture. J Vasc Surg. 1993; 17:125–133.
3. Kang SS, Labropoulos N, Mansour MA, et al. (Division of Vascular Surgery, Loyola University Medical Center, Maywood, IL 60153, USA). Expanded indications for ultrasound-guided thrombin injection of pseudoaneurysms. J Vasc Surg. 2000 Feb;31(2): 289–298.
4. Dzepina I, Unusic J, Mijaovic D, Balic K. Pseudoaneurysms of the brachial artery following venipuncture in infants. Pediatr Surg Int. 2004 Aug;20(8):594–597. Epub 2004 Aug 25.
5. Ishmoto T, Shindo S, Santoshi N, et al. Aneurysm formation of a dorsal superficial antebrachial artery due to sports injury: a case report. Vasc Endovascular Surg. 2003 Mar–Apr;37(2):141–143.
6. Tobias AM, Chang B. A rare brachial artery pseudoaneurysm 13 years after excision of a humeral osteochondroma. Ann Plast Surg. 2004 Apr; 52(4):419–422.

Straight Back Syndrome and Respiratory Failure

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Key words Straight back syndrome, Respiratory failure, Mitral valve plolapse syndrome, Pulmonary function, Chest X-ray, ECG

Definition of Straight Back Syndrome

What is straight back syndrome? As the name suggests, it is characterized by straightness of the backbone. This syndrome was first proposed by Rawlings in 1960 in the Am J Cardiol. According to his paper,[1] straight back syndrome is the absence of normal dorsal curvature (physiological kyphosis) in the thoracic part of the spine, resulting in the reduced anteroposterior diameter of the thorax (the space formed by the ribs, thoracic vertebrae, and diaphragm) and presenting symptoms such as palpitation, chest pain, and shortness of breath, and abnormal clinical findings such as cardiac murmurs and radiographic cardiomegaly.

Diagnosis of Straight Back Syndrome

Diagnosis of straight back syndrome is relatively simple. It only requires frontal and lateral chest radiographs, based on which the physician examines the condition of the thoracic spine (whether it is straight or curved dorsally) and the size of the thorax. However, there are 2 sets of diagnostic criteria, one formulated by Davies et al. and the other by De Leon et al.[2,3]

In the criteria by Davies et al., we use the lateral chest radiograph and measure the distance *a* from the middle of the anterior border of T8 to the line connecting T4 (top of the anterior border) and T12 (bottom of the anterior border). Straight back syndrome is diagnosed when this distance *a* is smaller than 1.2 cm (Fig. 1). This method focuses on the straightness of the thoracic spine.

On the other hand, in the diagnostic criteria by De Leon et al., the anteroposterior diameter *a* is defined as the distance from the anterior border of T8 to the posterior border of the sternum on the lateral radiograph, and the lateral diameter *b* is defined at the level of the diaphragm on the frontal radiograph. Straight back syndrome is diagnosed when a/b is $1/3$ or less (Fig. 2). This method is considered to detect the reduced anteroposterior diameter of the thorax.

Relationship between Straight Back Syndrome and Respiratory Failure

A case of straight back syndrome developing respiratory failure

The literature (Ref. 4) states that patients with straight back syndrome rarely bear the risk of respiratory failure. However, the possibility of respiratory failure is not zero, as my colleagues experienced 3 patients diagnosed with straight back syndrome who developed respiratory failure. Here we briefly look at one of the 3 cases experienced by my colleagues.

The patient was a 52-year-old female with a height of 156 cm and weighing 38.9 kg. The chief complaint was breathing difficulty.

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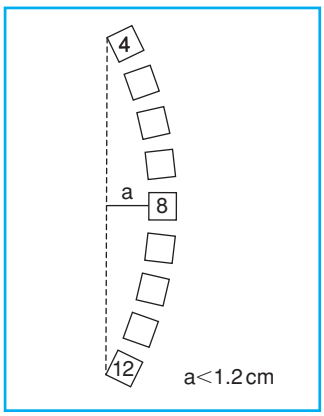

Fig. 1 Diagnostic criteria by Davies et al.
On the lateral chest radiograph, the distance *a* is measured from the middle of the anterior border of T8 to the line connecting T4 (top of the anterior border) and T12 (bottom of the anterior border). Straight back syndrome is diagnosed when this distance *a* is smaller than 1.2 cm.

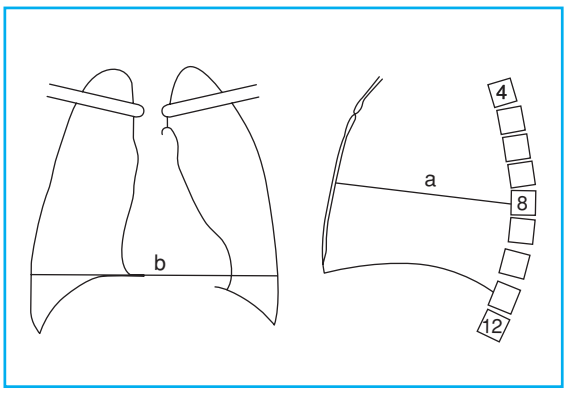

Fig. 2 Diagnostic criteria by De Leon et al.
The anteroposterior diameter *a* is defined as the distance from the anterior border of T8 to the posterior border of the sternum on the lateral radiograph, and the lateral diameter *b* is defined at the level of the diaphragm on the frontal radiograph. Straight back syndrome is diagnosed when *a*/*b* is 1/3 or less.

The most remarkable feature of this patient was her slender frame. The physician diagnosed straight back syndrome according to the above diagnostic criteria, and noted the presence of right scoliosis. Dyspnea began when she was about 47 years old, and the patient was receiving medication including expectorants. Elevation of arterial pCO_2 was noted, and this probably was the cause of headache in the early morning. The patient developed pneumonia when she was 48 years old and 50 years old.

When the patient was hospitalized at the age of 52, pulmonary function tests showed that the %VC was 44.1% and the one-second forced expiratory volume rate (FEV1.0%) was 93.3%. Thus, she showed signs of restrictive ventilatory impairment similar to that in other types of thoracic cage abnormalities. Arterial oxygen partial pressure (PaO_2) was 65.1 Torr. Although this did not meet the criterion for chronic respiratory failure (PaO_2 of 60 Torr or less; see below), the decrease in PaO_2 was somewhat abnormal and $PaCO_2$ was abnormally high at 68.5 Torr. $PaCO_2$ further increased to 77.6 mmHg after walking. The pH was 7.357, indicating slight acidosis. Differing from the type of respiratory failure seen with common thoracic cage abnormalities, this patient showed a condition resembling type-2 respiratory failure, in which the lowering of PaO_2 occurs concomitantly with the elevation of $PaCO_2$.

Reminiscent of the sleep apnea syndrome, oxygen saturation measured with a pulse oximeter during sleep was as low as 85% on average and 59% at the minimum. ECG in II, III, and aVF showed ST depression, negative T, and incomplete right bundle branch block.

Echocardiography, lung perfusion scintigraphy, and Ga scintigraphy showed no abnormality.

Based on these observations, this patient with a condition seemingly (though not exactly) consistent with type-2 respiratory failure was considered to have developed this condition as a consequence of straight back syndrome. The deformation of the spine due to this syndrome, as well as right scoliosis and the small size of the thorax, indirectly affected respiratory function over a long period and caused respiratory insufficiency probably in the form of alveolar ventilation (VA) impairment, which often develops to type-2 respiratory failure. The possibility that other respiratory disorders or hidden heart disease might be the cause respiratory failure, however, could not be ruled out.

Features of respiratory insufficiency in straight back syndrome

Unfortunately, the data from our respiratory function laboratory for the previous years were found to include no information concerning

respiratory insufficiency in straight back syndrome. Probably because we did not realize the need, we have not performed respiratory function tests in patients with this syndrome.

We apologize for not being able to provide our own data, but the literature (Ref. 5) states that no significant pulmonary insufficiency was observed, although the total lung capacity (TLC) was somewhat low. We can safely say that the absence of physiological kyphosis in itself does not affect the ventilatory movement of the thoracic cage, despite the potentially reduced size of the lungs. This means that, unlike other types of thoracic cage abnormalities, this syndrome does not imply the risk of respiratory failure. Some patients, however, may actually develop respiratory failure. In such cases, impairment of ventilatory function is considered to result from the small size of the lungs and the abnormality of the thoracic cage containing the lungs.

Patients with common types of thoracic cage abnormalities other than straight back syndrome, such as funnel chest, usually show respiratory function test results indicating restrictive ventilatory impairment characterized by reduced vital capacity. While there are 2 types of respiratory failure, patients with chest deformation are more likely to develop type-1 respiratory failure than the other type. In type 1 respiratory failure, PaO_2 decreases, but $PaCO_2$ does not increase.

Features of cardiac insufficiency in straight back syndrome

Because of the reduced anteroposterior diameter of the thorax, straight back syndrome is more likely to affect the heart than to cause respiratory insufficiency. This section outlines the effect on the heart.

The cardiologists in our hospital have occasionally encountered cases suspected of having straight back syndrome. According to their accounts and the literature (Ref. 4), frontal chest radiographs of such patients usually show a cardiothoracic ratio (CTR) of 40% or less. The reduced anteroposterior diameter of the thorax often results in protrusion of the left second arc of the heart shadow due to compression of the heart and great vessels, as well as flattening of the heart shadow in about half of all cases. The lateral chest radiograph characteristically shows straight thoracic spine, shortening of the anteroposterior diameter of the thorax, and forward displacement of the heart shadow, often described as so-called small heart syndrome.

ECG is normal in most cases, although right axis deviation, rSr in VI, and small terminal r in aVR have been reported.

Straight back syndrome and mitral valve prolapse

However, we need to summarize certain facts about mitral valve prolapse, as it is detected by echocardiography in about half of all cases.

When mitral valve prolapse is detected by echocardiography in straight back syndrome, the anterior cusp of the mitral valve is displaced along the posterior cusp in such a manner that it pushes the posterior cusp slightly toward the left ventricle. The sail-like part of the valve leaflet does not protrude toward the left atrium, and prolapse is localized to the area inside of the center of the anterior cusp.[6]

The mechanism for mitral valve prolapse in these cases has been reported to involve the twisting of the mitral valve during the contraction of the left ventricle, which has been deformed to an oval shape as a result of the reduced anteroposterior diameter of the thorax. The twisting occurs because the anterolateral papillary muscle mainly moves from the lateral to the medial direction, while the posteromedial papillary muscle mainly moves from the posterior to the anterior direction during contraction. When mitral valve prolapse is present, late systolic murmur can be heard at the apex of the heart.

The mitral valve prolapse syndrome can be primary, secondary, or functional. Although the prognosis is generally good, precautions should be taken to prevent the possibility of sudden death.

Etiology and Prognosis of Straight Back Syndrome

As for the etiology of straight back syndrome, Bon Tempo et al.[7] reported a theory based on abnormality in the embryonic period. However, because the manifestations of mitral valve prolapse may lessen or disappear with the postnatal growth of the thoracic cage, recent discussion tends to support the importance of a postnatal acquired condition.

The prognosis of straight back syndrome is

usually good, even if complicated with mitral valve prolapse. While specific treatment is generally not required, rare cases developing chronic respiratory failure require treatment for this condition.

Issues requiring future investigation include changes with the growth of the thoracic cage and the positioning of this syndrome as a cause of arrhythmias.

Classification of Respiratory Failure

To provide additional information, this section discusses some facts about respiratory failure. The concept of respiratory failure that is the most widely accepted at present characterizes this condition by the abnormal levels of arterial blood gases, in particular O_2 and CO_2, resulting in the inability of the body to function normally.

However, there is some difficulty with this concept in that arterial blood gases simply reflect the overall functional impairment of the lungs as the gas exchange apparatus, but they do not necessarily detect the condition hindering normal functioning of the body.

Because the ability of the body to function normally is reflected in the gas exchange function at the tissue level, we should measure the mixed venous blood oxygen partial pressure (PvO_2) in the capillary bed of the lungs. While PvO_2 can be measured using a Swan-Ganz catheter, this method is not practical, and the presence of respiratory failure is usually diagnosed based on the analysis of arterial blood gases.

The diagnostic criteria for respiratory failure based on arterial blood gases have been formulated by Filley et al. and Campbell et al. While these criteria developed in other countries are also widely used in Japan, in 1978 the Respiratory Failure Study Team of the Ministry of Health and Welfare proposed the diagnostic criteria for respiratory failure based on arterial blood. The Japanese criteria have also been used in clinical practice recently.[8]

The Japanese diagnostic criteria define respiratory failure as an abnormal condition causing respiratory impairment presenting a PaO_2 (arterial O_2 partial pressure) of 60 Torr or less, or equivalent respiratory impairment. In addition, the criteria classify respiratory failure into type 1, which shows normal $PaCO_2$ (arterial CO_2 partial pressure), and type 2, which shows abnormal $PaCO_2$ exceeding 45 Torr. Furthermore, respiratory failure may sometimes be classified into acute respiratory failure and chronic respiratory failure. It seems that acute respiratory failure tends to have causes other than respiratory diseases, while chronic respiratory failure is more likely to be caused by respiratory diseases.

Finally, let us reemphasize the above-mentioned fact that the diagnostic criteria for respiratory failure are based on arterial blood gases, and a PaO_2 (arterial O_2 partial pressure) of 60 Torr or less and a $PaCO_2$ (arterial CO_2 partial pressure) of 45 Torr or more are important markers.

Because straight back syndrome is asymptomatic in many cases, it is likely to be overlooked. Patients with this syndrome are often diagnosed by general practitioners. When a slender person (particularly a slender female) presents with indefinite complaints such as palpitation, chest pain, and shortness of breath, the physician is recommended to consider the possibility of straight back syndrome even in the absence of specific symptoms. Although we have not confirmed the prevalence in the literature, it is considered to be high.

References

1. Rawlings MS. The “straight back” syndrome, a new cause of pseudoheart disease. Am J Cardol. 1960;5:333–338.
2. Davies MK, et al. The straight back syndrome. QJ Med. 1980;49:443–460.
3. De Leon AC, et al. The Straight Back Syndrome. Clinical cardiovascular manifestation . Circulation 1956;32:193–203.
4. Ishihara T. Straight back shoukougun. Igaku no Ayumi—Respiratory Diseases. 1995;3(Suppl):579–580. (in Japanese)
5. Gould KG, et al. Pulmonary function and work capacity in the absence of physiologic dorsal kyphosis of the spine. Dis Chest. 1969;55:405–411.
6. Murakami H. Straight back syndrome to tokuhatsusei soubouben itsudatsu ni okeru soubouben itudatsu kikou no dansou sin ekozuhou niyoru kentou. The Sapporo Medical Journal 1987;56:519–531. (in Japanese)
7. Bon Tempo CP, et al. Radiographic appearance of the thorax in systolic click-late systolic murmur syndrome. Am J Cardiol. 1975;36:27–31.
8. Fukuchi Y. Kokyufuzen. In: The Lung Function Seminar ed. Respiratory Function Tests. 1994:272–270. (in Japanese)

Evaluation and Management of Chronic Heart Failure

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Key words Heart failure, Diagnosis, Therapy, Evidence-based medicine, Renin-angiotensin system, Beta blockers

Introduction

Heart failure (HF) is a leading cause of hospitalization and it is associated with a poor prognosis. HF occurs when the cardiac function fails to pump a supply of blood adequate to meet the needs of metabolizing tissues. Development of HF can result in various signs and symptoms, such as dyspnea and fatigue, which may limit exercise tolerance along with fluid retention, pulmonary congestion, peripheral edema and body weight gain. As patients with HF face a very high risk of hospitalization and mortality, optimal therapies based on the results from large scale clinical trials should be selected according to an appropriate grading of the HF.

Acute and Chronic HF

Several forms of HF are known—right-sided versus left-sided, acute versus chronic, low-output versus high-output, and systolic versus diastolic. The therapeutic strategy and goal may slightly differ between acute and chronic HF. For example, the therapeutic goals in the management of acute HF are to relieve symptoms and to ameliorate the hemodynamic abnormalities: these goals are achieved by diuretics and inotropes, and sometimes, by mechanical circulatory support (LVAD, IABP and PCPS). By contrast, chronic HF patients should be treated by the appropriate applications of evidence-based, guideline-recommended medical therapies to retard progression of the disease and thereby decrease the risk of hospitalization and death.

Assessing the Causes and Severity of HF

HF is a principal complication of virtually all forms of heart disease. Among them, coronary artery disease, hypertension, and dilated cardiomyopathy are the main causes of HF in a substantial proportion. Other underlying disorders include arrythmias, congenital heart disorders, and other myocardial and pericardial diseases.

For many years, HF was considered to be synonymous with diminished contractility of the left ventricle (LV). However, it has been increasingly recognized that a large number of HF patients, as many as 20% to 60%, have a relatively (or near) normal LV ejection fraction (LVEF).

How can we diagnose whether the symptom is due to HF when LV systolic function is preserved? Measurement of brain natriuretic peptide (BNP) and Doppler echocardiography can help estimate the severity of HF. It has recently become evident that the heart is not just a pump but is also an endocrine organ, which may play a crucial role in controlling the circulating blood volume. BNP, a member of a family

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of the natriuretic peptides, is mainly secreted from the ventricles.[1] BNP measurements can help in diagnosis by providing information on the severity of HF,[2] and thus provide an objective guide for optimizing the treatment of HF. Doppler echocardiography is an useful noninvasive diagnostic modality that enables us to evaluate the diastolic performance of LV, by assessing the mitral valve inflow pattern, and to estimate pulmonary artery pressure, by evaluating the tricuspid valve regurgitant gradient.

Treatment for Chronic HF

Previous large scale clinical trials have demonstrated that several drug classes are effective in decreasing rates of hospitalization and mortality in HF patients; these include angiotensin-converting enzyme inhibitors (ACEIs), angiotensin II receptor blockers (ARBs), beta blockers, and aldosterone antagonists.

ACEIs and ARBs

Activation of the neurohumoral system, including the renin-angiotensin-aldosterone system (RAAS), may act as a compensatory mechanism against HF; however, it may also aggravate the syndrome of HF by accelerating structural changes in the heart, and by modulating body-fluid homeostasis. Many clinical trials have demonstrated that ACEIs can reduce mortality within a wide range of HF. For example, the Cooperative North Scandinavian Enalapril Survival Study (CONSENSUS) was one of the first double-blind, placebo controlled clinical trials to assess the efficacy of ACEIs in HF. In this study, crude mortality rate of the patients with severe HF was reduced by 40% at the end of six months in patients receiving enalapril treatment compared to placebo treatment.[3] Many other clinical trials, such as V-HeFT II[4] and SOLVD,[5] have also support the proposition that ACEIs can reduce mortality of patients within a wide range of HF.

By blocking the renin-angiotensin system (RAS) at the receptor level, ARBs may provide more thorough RAS blockade than ACEIs. However, in the clinical trials where the effectiveness ACEIs and ARBs was compared (ELITE II, OPTIMAAL), these two drug classes were found to have similar efficacy,[6,7] although in general, ARBs were better tolerated than ACEIs. The Candesartan in HF Assessment of Reduction in Mortality and Morbidity (CHARM)-Alternative Study has shown that ARBs decrease the risk of cardiovascular death or hospital admission for HF in patients who are ACEI-intolerant.[8] The CHARM-Added trial has shown the possibility that the combined use of ARBs and ACEIs may lead to a further reduction in relevant cardiovascular events in patients with HF and reduced LVEF.[9]

Beta-Blockers

Beta-blocking agents were previously considered to be specifically contraindicated for the HF patients owing to their intrinsic negative inotropic activity. However, recent large controlled studies, however, have shown that beta-blockers are, on the contrary, effective in reducing the rate of mortality and sudden cardiac deaths in patients within a wide range of HF, like ACEIs and ARBs. In the US Carvedilol Study, carvedirol treatment resulted in a 48% reduction in the progression of HF as compared with the placebo treatment in patients who had a LVEF≤0.35% and were receiving optimal standard therapy, including ACEIs.[10]

Notably, in the Carvedilol Prospective Randomized Cumulative Survival (COPERNICUS) Trial, beta-blocker therapy was also found to be effective in reducing morbidity and mortality in patients with severe HF, which was defined by the occurrence of dyspnea or fatigue at rest or on minimal exertion and a LVEF<25% despite appropriate conventional therapy.[11]

Several further clinical trials, such as CIBIS-II[12] and MERIT-HF,[13] have shown that other beta-blocking agents, bisoprolol and metropolol, are also effective in decreasing the hospitalization rate due to HF and in improving the functional status and survival rate of HF patients. It is worth keeping in mind, however, that beta-blockers are not always safe. They may worsen the bronchial asthma, bradycardia, hypotension, and atrio-ventricular block as well as HF symptoms. Beta-blockers should be administered at a low initial dose after 2 or 4 weeks of clinical stability. In-hospital initiation of beta-blockers is recommended, especially when patients are within moderate to severe HF. In-hospital initiation of beta-blockers also provides positive effects

on long-term patient compliance and clinical outcomes.

Aldosterone Antagonists

Spironolactone is a competitive aldosterone receptor antagonist. Aldosterone is considered to aggravate HF by promoting the sodium retention and subsequent intravascular volume expansion, sympathetic activation, and myocardial fibrosis. The Randomized Aldactone Evaluation Study (RALES) has demonstrated that the blockade of aldosterone receptors by spironolactone in subjects receiving standard therapy significantly reduces the risk of both morbidity and death among patients with severe HF.[14] In the RALES trial, treatment of HF patients with this "old" diuretic surprisingly resulted in a 30% reduction in the risk of death as compared with the placebo treatment. Although aldosterone receptor antagonists are such effective agents in the treatment of HF, we have to keep in mind that hyperkalemia can occur especially in older patients treated with ACEIs. Indeed, the publication of RALES was associated with an abrupt increase in hyperkalemia-associated morbidity and mortality.[15] Gynecomastia or breast pain was reported in 10 percent of men who were treated with spironolactone. This side-effect may be weakened by Eplerenone,[16] a next generation aldosterone receptor antagonist that is selective for aldosterone receptors. Eplerenone has also been shown to reduce the morbidity and mortality of HF patients who are already treated with ACEIs and beta-blockers; nevertheless, hyperkalemia must still be given due attention.

Conclusions

Recently the AHA/ACC guidelines for the diagnosis and management of CHF have been updated.[17] These guidelines recommend that most patients with current or prior symptoms of HF should be routinely managed with a combination of three types of drugs: a diuretic, an ACEI or an ARB, and a beta-blocker. In addition, even when the patient responds favorably to the diuretic, treatment with both an ACEI and a beta-blocker should be initiated and main tained in patients who can tolerate them. Besides selecting evidence-based guideline-recommended therapies, it is advisable to be aware of the possible adverse effects of the above-mentioned drugs. Hence, successful treatment of HF demands close cooperation between general physicians and cardiologists.

References

1. Sudoh T, Kangawa K, Minamino N, et al. A new natriuretic peptide in porcine brain. Nature. 1988 Mar 3;332(6159):78–81.
2. Mukoyama M, Nakao K, Saito Y, et al. Increased human brain natriuretic peptide in congestive heart failure. N Engl J Med. 1990;323(11):757–758.
3. The CONSENSUS Trial Study Group. Effects of enalapril on mortality in severe congestive heart failure. Results of the Cooperative North Scandinavian Enalapril Survival Study (CONSENSUS). N Engl J Med. 1987;316(23):1429–1435.
4. Cohn JN, Johnson G, Ziesche S, et al. A comparison of enalapril with hydralazine-isosorbide dinitrate in the treatment of chronic congestive heart failure. N Engl J Med. 1991;325(5):303–310.
5. The SOLVD Investigators. Effect of enalapril on survival in patients with reduced left ventricular ejection fractions and congestive heart failure. N Engl J Med. 1991;325(5):293–302.
6. Pitt B, Poole-Wilson PA, Segal R, et al. Effect of losartan compared with captopril on mortality in patients with symptomatic heart failure: randomised trial—the Losartan Heart Failure Survival Study ELITE II. Lancet. 2000;355(9215):1582–1587.
7. Dickstein K, Kjekshus J. Effects of losartan and captopril on mortality and morbidity in high-risk patients after acute myocardial infarction: the OPTIMAAL randomised trial. Optimal Trial in Myocardial Infarction with Angiotensin II Antagonist Losartan. Lancet. 2002;360(9335):752–760.
8. Granger CB, McMurray JJ, Yusuf S, et al. Effects of candesartan in patients with chronic heart failure and reduced left-ventricular systolic function intolerant to angiotensin-converting-enzyme inhibitors: the CHARM-Alternative trial. Lancet. 2003;362(9386):772–776.
9. McMurray JJ, Ostergren J, Swedberg K, et al. Effects of candesartan in patients with chronic heart failure and reduced left-ventricular systolic function taking angiotensin-converting-enzyme inhibitors: the CHARM-Added trial. Lancet. 2003;362(9386):767–771.
10. Colucci WS, Packer M, Bristow MR, et al. Carvedilol inhibits clinical progression in patients with mild symptoms of heart failure. US Carvedilol Heart Failure Study Group. Circulation. 1996;94(11):2800–2806.
11. Packer M, Coats AJ, Fowler MB, et al. Effect of carvedilol on survival in severe chronic heart failure. N Engl J Med. 2001;344(22):1651–1658.
12. The Cardiac Insufficiency Bisoprolol Study II (CIBIS-II): a randomised trial. Lancet. 1999;353(9146):9–13.
13. Effect of metoprolol CR/XL in chronic heart failure: Metoprolol CR/XL Randomised Intervention Trial in Congestive Heart Failure (MERIT-HF). Lancet. 1999;353(9169):2001–2007.
14. Pitt B, Zannad F, Remme WJ, et al. The effect of spironolactone on morbidity and mortality in patients with severe heart failure. Randomized Aldactone Evaluation Study Investigators. N Engl J Med. 1999;341(10):709–717.
15. Juurlink DN, Mamdani MM, Lee DS, et al. Rates of hyperkalemia after publication of the Randomized Aldactone Evaluation Study. N Engl J Med. 2004;351(6):543–551.
16. Pitt B, Remme W, Zannad F, et al. Eplerenone, a selective

aldosterone blocker, in patients with left ventricular dysfunction after myocardial infarction. N Engl J Med. 2003;348(14):1309–1321.

17. Hunt SA, Abraham WT, Chin MH, et al. ACC/AHA 2005 guideline update for the diagnosis and management of chronic heart failure in the adult—Summary article: A report of the American College of Cardiology/American Heart Association task force on practice guidelines (Writing Committee to update the 2001 guidelines for the evaluation and management of heart failure): Developed in collaboration with the American College of Chest Physicians and the International Society for Heart and Lung Transplantation: Endorsed by the Heart Rhythm Society. Circulation. 2005;112(12):1825–1852.