Original Article

COMPARISON OF INHIBITORY EFFECT OF RIFALAZIL AND RIFAMPICIN AGAINST *MYCOBACTERIUM ULCERANS* INFECTION INDUCED IN MICE

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Abstract [Purpose] Buruli ulcer is a human skin disease caused by *Mycobacterium ulcerans* infection, which is characterized by massive skin ulceration and persistent necrotic change. In recent years Buruli ulcer has rapidly emerged as an increasingly important cause of human morbidity around the world. The disease is endemic at least 32 countries in Africa, Western Pacific, Asia and South America, and it is considered the third most common mycobacterial infection of humans after tuberculosis and leprosy. An effective chemotherapeutic regimen against Buruli ulcer disease has not been established to date. In this study, the inhibitory effect of rifalazil (RLZ) against *M. ulcerans* was assessed in experimentally infected mice and compared to that of rifampicin (RFP).

[Materials and Methods] Five-week-old BALB/c female mice were challenged with 25 μl (CFU $= 4 \times 10^4$) of *M. ulcerans* cultured in Middlebrook 7H9 broth in bilateral hind footpads. Mice were administered *per os* with a suspension of RLZ or RFP at 2.5, 5, or 10 mg/kg once daily 5 times per week starting from one day up to 6 weeks after infection. During the treatment, mice were observed weekly for footpad skin lesions and examined for footpad swelling. In addition, CFU enumeration was done on both hind footpads and spleen at 2, 4, and 6 weeks after initiating treatment.

[Results] In the infected control mice group, slightly erythematous lesions and moderate swelling of footpads were observed 4 weeks after the infection. Ulcerative lesion was observed 6 weeks after the infection. Mean $\log_{10}$ CFU/footpad (FP) was 5.22 on day 1 after the infection and increased to 5.56, 6.29, and 7.33 at 2, 4, and 6 weeks after treatment was initiated in the treated groups. On the other hand, no visible erythema, swelling or ulcerative lesion in footpads were observed in RLZ-administered groups. Furthermore, $\log_{10}$ CFU/FP decreased to 4.14 after only 2 weeks of initiating treatment in 2.5 mg/kg administered group, i.e. the lowest dose employed group. Log_{10} CFU/FP decreased to <2.1 in 6 weeks in the 10 mg/kg administered group, which was close to the detection limit (<1.7) of the CFU assay. By contrast, inhibitory effect on disease progression and reduction of CFU were observed only in the group of mice given 10 mg/kg among RFP-administered groups ; the reduction of CFU was not observed in the early period but 6 weeks after initiating treatment.

[Conclusion] These results clearly demonstrate that the *in vivo* anti-*M.ulcerans* activity of RLZ is much higher than RFP. RLZ activity against *M.ulcerans* can be expected to control the disease progression in the clinical applications.

Key words : *Mycobacterium ulcerans*, Rifalazil, Rifampicin, Murine infection model, Inhibitory effect

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LEUKOPENIA DUE TO ANTI-TUBERCULOUS CHEMOTHERAPY INCLUDING RIFAMPICIN AND ISONIAZID

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Abstract [Objectives] To examine the incidence rate by age and gender of leukopenia caused by chemotherapy including rifampicin (RFP) and isoniazid (INH), and to study the relationships between the leukopenia and the hepatic side effect or other haematological disorders such as thrombocytopenia.

[Subjects] Out of the tuberculosis patients who were admitted to our hospital in 1987−88, 1991−92, and 1996−2000, 1,525 patients (1,153 men, 372 women) were chosen for our study who had the white blood cell counts (WBC) in the peripheral blood more than 3,000/mm^3 before chemotherapy, and underwent haematologic examination at least twice within 3 months after starting chemotherapy.

[Methods] The definition of leukopenia was as follows:

1) WBC became less than 3,000/mm^3 during chemotherapy

for patients with pre-treatment WBC more than 4,000/mm^3, or

2) WBC decreased more than 1,000/mm^3 in patients with pre-treatment WBC between 3,000 and 4,000/mm^3.

The incidence rates of leukopenia by age, gender, and regimens of chemotherapy were calculated.

The case-control study was done between the control and the leukopenia groups excluding patients suffered from agranulocytosis to clarify the hematological and biochemical characteristics of the leukopenia group. The control patients were chosen in the following way. For each patient with leukopenia, a patient with the same admission year, same gender, same regimen of chemotherapy, and the nearest age was chosen as a control patient. The changes in counts of white blood cell, granulocyte, and platelet, in hemoglobin concentration, and in hepatic enzyme levels before and during chemotherapy were compared between the leukopenia and the control groups. Thrombocytopenia was defined as platelet count less than 15×10^4/mm^3 and hepatic dysfunction as either aspartate aminotransferase (AST) higher than 31 IU/*l* or alanine aminotransferase (ALT) higher than 34 IU/*l*.

［Results］

(1) Incidence rate of leukopenia

The leukopenia appeared in 36 patients (14 men, 22 women), two (one man, one woman) of whom showed agranulocytosis. The incidence rate was 1.2% (14/1,153) for men and 5.9% (22/372) for women. The incidence rate of women was higher than that of men in the age groups between 20 to 79 y.o., but no difference was seen in the age groups elder than 80 y.o. There were no differences in the incidence rate among groups treated with HRE (E: ethambutol), HRS (S: streptomycin), and HREZ (Z: pyrazinamide). The chemotherapy was continued in 30 patients after the appearance of leukopenia, and the natural recovery from leukopenia was seen in 19 patients, while the leukopenic state lasted during the chemotherapy in the remaining 11 patients. In two patients who exhibited agranulocytosis all drugs were discontinued. In the remaining 4 patients one or more drugs were discontinued.

(2) Case-control study between leukopenia (N＝34) and the control (N＝34) groups

There were no differences in age, sputum culture positivity on admission, degree of roentgenographic extent of the disease, ratio of cavity formation, and quantity of daily doses between the two groups. There was also no difference between the days until leukopenia appeared after starting chemotherapy (47.6±29.5 days) in the leukopenia group, and the days until WBC count became minimum within 3 months after starting chemotherapy (41.7±21.0 days) in the control group. The negativity of tuberculin skin testing was higher in the leukopenia group [7/14 (50%)] than in the control group [1/10 (10%)], however, the difference was statistically not significant due to rather small size of cases. Before the starting chemotherapy, the counts of WBC (7,230±1,530 vs 5,500±1,510/mm^3, $p<0.001$), neutrophil (5,230±1,450 vs 4,320±1,620/mm^3, $p<0.05$), lymphocyte (1,440±830 vs 830±440/mm^3, $p<0.001$) and platelet (34.9±12.2 vs 24.1±6.4 $\times10^4$/mm^3, $p<0.001$) in the peripheral blood and the globulin level (3.71±0.61 vs 3.35±0.61 g/d*l*, $p<0.05$) in the serum were significantly higher in the control group than in the leukopenia group. The decrements in the counts of WBC and granulocyte during chemotherapy were larger in the leukopenia group than in the control group (ΔWBC: 2,880±1,530 vs 1,910±1,520/mm^3, and ΔNeut: 2,840±1,510 vs 1,820±1,380/mm^3, $p=0.01$, respectively), but the counts of lymphocyte were similar in both groups. The platelet counts also decreased in both groups, but to the mid-normal level in the control group, and to the lowerest normal level in the leukopenia group, in which 15 out of 34 patients (44%) showed thrombocytopenia. The levels in the serum of hepatic enzymes such as AST, ALT, and γ-GTP (γ-glutamyl aminotransferase) increased during chemotherapy in the leukopenia group, while decreased in the control group, and the facts indicate that in the former not only bone marrow cells but also hepatic cells were impaired by anti-tuberculosis drugs.

[Considerations] Leukopenia may occur in the course of treatment with anti-tuberculosis drugs, but it is not necessary to stop the chemotherapy immediately, because the WBC count recovers spontaneously or remains under stable leukopenic state during chemotherapy in most cases. But when leukopenia appears, the peripheral blood counts must be checked cautiously, and the chemotherapy should be stopped if the WBC count progressively decreases. The patients who showed leukopenia due to anti-tuberculosis drugs may have had weaker natural and acquired (cell-mediated) immunologic response to tuberculosis infection, and more vulnerable bone marrow cells and hepatic cells to anti-tuberculosis drugs than the control.

Key words: Leukopenia, Rifampicin, Thrombocytopenia, Tuberculosis, Immunity, Hepatic dysfunction

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TUBERCULOUS LYMPHADENITIS: A CLINICAL STUDY OF 23 CASES

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Abstract [Introduction] Tuberculous lymphadenitis is a relatively rare disease in adults. In the absence of pulmonary tuberculosis, tuberculous lymphadenitis is very difficult to differentiate from other diseases. We described our experiences of patients with tuberculous lymphadenitis.

[Results] We diagnosed 23 patients with tuberculous lymphadenitis out of 207 patients with tuberculosis. Their ages ranged from 18 to 99 years (mean, 45.7 years), and the male-to-female ratio was 7:16. The most common complaints were cervical mass and fever. With the exception of two patients, all diagnosed patients had a strong positive skin test to tuberculin. Observing the site of affected lymph nodes, 16 patients had cervical node involvement, 3 patients had axillary node involvement, 7 patients had mediastinal node involvement, 3 patients had hilar node involvement, 3 patients had abdominal node involvement, and 1 patient had inguinal node involvement. Fifteen patients had neither hilar nor mediastinal node involvement. Eleven patients had no tuberculous lesions other than lymphadenitis. Seven patients underwent biopsy of the lymph nodes. Four of these patients had the evidence of acid-fast bacilli. The remaining three patients were also diagnosed histologically. Five patients underwent fine needle aspiration. Two of them had the evidence of acid-fast bacilli. Acid-fast bacilli were detected in 10 out of 16 sputum samples and in 1 out of 2 pleural effusion samples. Five patients were diagnosed clinically by image (Computed tomography etc.) and by therapeutic effect. Eleven cases underwent contrast-enhanced computed tomography (CT) of the lymph nodes. Seven cases showed central low attenuation with peripheral rim enhancement, whereas the other four cases showed homogeneous attenuation. All patients received chemotherapy for a mean duration of 14.5 months (range, 6–30 years) with apparent improvement, but 1 patient relapsed.

[Conclusion] Tuberculous lymphadenitis remains one of important targets for the differential diagnosis of lymphadenopathy. It is essential that a peripheral lymph node biopsy be performed and examined either histologically and/or microbiologically. A tuberculin skin test and contrast-enhanced CT imaging should also be performed.

Key words: Tuberculous lymphadenitis, Diagnosis, Computed tomography, Treatment

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A STUDY ON CASES DEVELOPED PULMONARY TUBERCULOSIS AFTER RECEIVING GASTRECTOMY

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Ayako FUJIKAWA, Meiji KUGA, and Go ISHIMARU

Abstract [Objective] Patients who had undergone gastric resection are considered to be high risk of developing tuberculosis. We investigated the factors leading to pulmonary tuberculosis after gastrectomy.

[Materials and Methods] We retrospectively examined 654 pulmonary tuberculosis patients discharged from Chiba-East National Hospital from January 1999 to December 2001.

[Results] Fifty-five patients (31−84 years old, mean 63.5 ± 12.5 years, 48 males and 7 females) had the history of gastric resection. The proportion of patients receiving gastrectomy among patients with pulmonary tuberculosis was 8.4 percent. The mean age of patients received gastric resection was 50.2 ± 16.6 years, and the mean interval from gastrectomy to the development of pulmonary tuberculosis was 13.6±11.0 years. On admission to our hospital, 34 out of 55 cases were smear positive by sputum examination for acid-fast bacilli and 39 cases had cavitary lesions on chest X-ray. Gastrectomy was done due to carcinoma of the stomach in 31 cases, peptic ulcer in 21 cases, adenomatous polyp in two cases, and accidental injury in one case. Out of total 55 cases, 52 patients improved, but three cases died of pulmonary tuberculosis. None had the recurrence of carcinoma of the stomach.

Body weight, Body mass index, Prognostic nutritional index (PNI ; 10 × serum albumin concentration + 0.005 × peripheral lymphocyte count) which was proposed by Onodera, serum albumin level and serum total cholesterol level were lower in the gastrectomy group than in the non-gastrectomy group.

The odds ratio of developing tuberculosis among gastrectomy patients compared with the appropriate controls in 30 to 59 year-old-men was 3.8.

[Conclusion] This study confirms that gastrectomy is one of the risk factors of developing tuberculosis in 30 to 59 year-old-men. However, whether gastrectomy in itself is a risk factor or whether it is secondarily associated with another risk factor such as underweight status and/or inadequate nutrition following surgery remains unclear.

Key words : Tuberculosis, Gastrectomy, Peptic ulcer, Risk factor, High risk group, Odds ratio

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PYOTHORAX ASSOCIATED LYMPHOMA WITH INCREASED NEURON-SPECIFIC ENOLASE LEVEL IN SERUM AND PLEURAL EFFUSION: A CASE REPORT

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Abstract A 72-year-old male with a past history of artificial pneumothorax for pulmonary tuberculosis at the age of 25 was referred to our hospital for the clinical signs of pain and swelling of the back. His neuron-specific enolase values were high in both serum and pleural effusion. The computed tomography image showed a tumor mass arising from the wall of chronic pyothorax. The tumor was resected including the wall of the chronic pyothrax and right chest wall with several ribs. The tumor was 7.2 × 7.0 × 3.0 cm in size and the pathological diagnosis was non-Hodgkin's lymphoma diffuse large cell, B-cell type. Postoperative chemotherapy and radiation therapy were performed but he died of recurrence and metastasis of the tumor 5 months later after the operation.

Key words : Pyothorax-associated lymphoma, Tuberculous chronic pyothorax, Malignant lymphoma, NSE (neuron-specific enolase), Epstein-Barr virus

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