RESISTANCE-CONFERRING MUTATIONS OF *MYCOBACTERIUM TUBERCULOSIS* STRAINS WITH LOW LEVEL RESISTANCE TO ISONIAZID

Hiromi ANO, Tomoshige MATSUMOTO, Takayuki NAGAI, Yoshitaka TAMURA, Hiroko YOSHIDA, Kunimitsu KAWAHARA, Isamu TAKAMATSU, Izuo TSUYUGUCHI, and Tetsuya TAKASHIMA

Abstract [Objective] We investigated the prevalence of isoniazid (INH) resistance-conferring mutations in the INH-indeterminate *Mycobacterium tuberculosis* (MTB) strains.

[Materials and Methods] We initially selected a sample of 47 clinical isolates of MTB from patients, who visited the Osaka Prefectural Medical Center for Respiratory and Allergic Diseases from 2000 to 2005. Strains resistant to the concentration of 1～2 μg/m*l* were defined as "indeterminate". INH resistance-conferring mutations were determined by DNA microarray.

[Results] Of 47 INH-indeterminate strains, only 13 (27.7%) were found to have no resistance mutations, 23 (48.9%) had mutation within the *inhA* regulatory region at -15 C to T, and 2 (4.3%) had mutation within the *inhA* regulatory region at -8 T to A, 6 (12.8%) had mutation within the *katG* gene at 1778 G to A, and 3 (6.4%) had mutations within the *katG* gene both at 1778 G to A and at 982 T to G.

[Conclusions] We showed that the majority of INH-indeterminate strains have resistance-conferring mutations, which were mainly detected within the *inhA* regulatory region.

Key words: *Mycobacterium tuberculosis*, Minimal inhibitory concentration, DNA microarray, INH resistance-conferring mutation

Osaka Prefectural Medical Center for Respiratory and Allergic Diseases

Correspondence to: Hiromi Ano, Osaka Prefectural Medical Center for Respiratory and Allergic Diseases, 3−7−1, Habikino, Habikino-shi, Osaka 583−8588 Japan.
(E-mail: ano@zeus.eonet.ne.jp)

Original Article

EFFECTS OF NEW DISCHARGE CRITERIA INCORPORATING DOTS ON TREATMENT OUTCOME OF PATIENTS WITH SMEAR-POSITIVE TUBERCULOSIS

[1]Eriko MORINO, [2]Takashi ASAKAWA, [1]Emiko TOYOTA, [3]Naoki ISHIZUKA, [1]Gou NAKA, [1]Shinyu IZUMI, [1]Yasuyuki KATO, [1]Atsuto YOSHIZAWA, [1]Masaaki HOJO, [1]Yuichiro TAKEDA, [1]Akihiko KAWANA, [1]Haruhito SUGIYAMA, [1]Nobuyuki KOBAYASHI, and [1]Koichiro KUDO

Abstract [Background] Some problems remain in the treatment of tuberculosis (TB) in Japan, with a higher prevalence of TB, low percentages of completed therapy and cases given DOTS, and longer admission period compared to the United States. We defined our own new criteria for discharge as sputum smear negativity instead of culture negativity, modified according to CDC criteria with shortened admission periods. However, the effects on treatment outcome have not been evaluated.

[Objectives] The aim of this study was to ensure the effectiveness of the new criteria, including DOTS undertaken after discharge.

[Patients/Methods] Group I comprised 459 cases hospitalized between January 2000 and December 2002 that were discharged under the old criteria, while Group II comprised 259 cases hospitalized between January 2003 and April 2004 that were discharged under the new criteria. We tried to undertake DOTS in cooperation with local health centers. The main outcome measures were admission period, treatment completion and relapse rates at 1 year after the completion of treatment.

[Results] The new criteria enabled median admission period to be shortened from 84 days to 69 days, although patients in Group II were older and displayed more severe tuberculosis lesions compared to Group I. DOTS coverage rate increased significantly from 5.9% to 40.5%, and treatment completion rate, percentage of lost cases and relapse rate for completed cases at 1 year changed from 83.0% to 86.6%, 6.3% to 3.9%, and 2.5% to 2.5%, respectively. No significant differences in these 3 rates were noted between Groups I and II.

[Conclusion] The new criteria incorporating DOTS enabled shortened admission period without any adverse effect on treatment outcomes.

Key words: Tuberculosis, Criteria for discharge, Admission periods, DOTS, Outcome of treatments

[1]Department of Respiratory Medicine, International Medical Center of Japan, [2]Tokyo University of Science Graduate School, [3]Research Institute, International Medical Center of Japan

Correspondence to: Eriko Morino, Department of Respiratory Medicine, International Medical Center of Japan, 1−21−1, Toyama, Shinjuku-ku, Tokyo 162−8655 Japan.
(E-mail: eada-iws@cj8.so-net.ne.jp)

Review Article

CRITERIA OF HOSPITALIZATION AND DISCHARGE FOR TUBERCULOSIS IN THE WESTERN DEVELOPED COUNTRIES AND COMPARISON WITH THAT OF JAPAN

[1]Kunihiko ITO and [2]Emiko TOYOTA

Abstract We studied the criteria of hospitalization and discharge in western developed countries, and compared with the criteria of Japan. For 9 regions of USA, New York, Canada, EU, UK, Germany, France, Spain, and Italy we investigated using inter-net search, Pub-Med and other electrical document search webs, official documents expressing opinion on infectiousness after starting chemotherapy and criteria for discontinuing isolation, hospitalization and discharge. In western developed countries, it is the standard opinion that time interval to lose infectiousness after starting chemotherapy is unknown. In many countries there are still remained the hospitalizations for short-term isolation or secure treatment adherence. In criteria for discontinuing isolation or discharge from hospitals, integrated risk of contact with tuberculosis patients (risk of progression to disease when infected and of severe form of tuberculosis such as drug-resistant cases and disseminated or meningitis) is more important determinant than infectiousness itself of tuberculosis patients. In western developed countries, they did not insist on outpatient treatment but adopted flexible policy for hospitalization. In the USA too, inpatient treatment for early treatment phase is frequently seen, and for necessary cases long-term hospitalization is made. The criteria of Japan were thought to focus too strictly on the infectiousness itself of tuberculosis patients.

Key words: Tuberculosis, Infectiousness, Hospitalization, Discharge, Isolation

[1]Department of Research, Research Institute of Tuberculosis, Japan Anti-Tuberculosis Association, [2]Departmet of Respiratory Medicine, International Medical Center of Japan

Correspondence to: Kunihiko Ito, Department of Research, Research Institute of Tuberculosis, Japan Anti-Tuberculosis Association, 3−1−24, Matsuyama, Kiyose-shi, Tokyo 204−8533 Japan. (E-mail: ito@jata.or.jp)

A CASE OF SIADH CAUSED BY ETHIONAMIDE IN A PATIENT WITH PULMONARY TUBERCULOSIS

Tamao NAKASHITA and Shinji MOTOJIMA

Abstract Cases of syndrome of inappropriate secretion of antidiuretic hormone (SIADH) associated with tuberculosis have been reported, however, in most of these cases, tuberculosis disease is miliary or severe. Here we report the first case of SIADH induced by ethionamide (TH). The case is a 76 year-old woman. She noticed cough in April 2004 and chest X-ray showed infiltrative shadows on the right upper lung field. Sputum examination revealed positive for TB-PCR, and she was referred to our hospital. Treatment was started with the combination of isoniazid (INH), rifampicin and ethambutol, however susceptibility test showed the bacilli were resistant to INH, then INH was replaced by TH on day 59. Loss of appetite developed 4 days later, the level of consciousness dropped to Japan Coma Scale II-20, and the Na concentration decreased to 113 mEq/*l* 6 days later. We made the diagnosis of SIADH based on the diagnostic criteria. She recovered from SIADH by the replacement of TH with SM, the restriction of water intake, and the loading of Na. Judging from the coincidence of the administration of TH and the onset of SIADH, no recurrence of SIADH after the cessation of TH, the mildness of tuberculosis, and the onset of SIADH in an already recovered case, we thought that SIADH in this case was causedly TH. Not only adrenal insufficiency but also SIADH should be considered when patients with tuberculosis show hyponatremia, and drugs on use should be reviewed as the possible cause of SIADH.

Key words: Tuberculosis, SIADH, Ethionamide

Department of Respiratory Medicine, National Hospital Organization Utsunomiya National Hospital

Correspondence to: Tamao Nakashita, Department of Rheumatology and Allergy, Kameda Medical Center, 929 Higashi-cho, Kamogawa-shi, Chiba 296–8602 Japan.
(E-mail: tamao0129@kameda.jp)

THE TUBERCULOSIS CONTROL LAW OF JAPAN: CURRENT ISSUES AND PROSPECT OF TUBERCULOSIS CONTROL PLAN

Tarou TSUKAHARA

Abstract Prevention and control measures against tuberculosis still remain a contemporary issue in Japan. In April 2005, the Tuberculosis Control Law was revised, which has newly been with particular emphasis on medical screening. However, the present law has been indicated to have issues in the fields of such as public health, human rights, and legislation. Although the Tuberculosis Control Law will be integrated into the Infectious Diseases Law on the basis of those issues, the aim of the integration of these laws are mainly for the establishment of pathogen control system to prevent biological terrorism and the accidental spread of infectious diseases and for the comprehensive control of infectious diseases based on the latest medical knowledge. In March 2006, the draft for the revised the Infectious Diseases Law was approved by the Cabinet of government. The combination of the two laws is expected to improve the program quality for the control of tuberculosis and infectious diseases but some issues remains to be resolved. This paper will review the combination of the Tuberculosis Control Law and the Infectious Diseases Law in light of what has been done, what will be intended, and what will change after the combination.

Key words: Tuberculosis Control Law, Infectious Diseases Law, *Mycobacterium tuberculosis*

Health Service Bureau, Organization of the Ministry of Health, Labour and Welfare

Correspondence to: Tarou Tsukahara, Health Service Bureau, Organization of the Ministry of Health, Labour and Welfare

NOVEL VACCINES AGAINST *M. TUBERCULOSIS*

Masaji OKADA

Abstract CDC and ACET in U.S.A. reported that novel vaccines instead of BCG are required for the protection against infection of *Mycobacterium tuberculosis* worldwide. However, no novel vaccine for clinical use has not yet been developed in the world including U.S.A. and Europe.

We have developed two novel tuberculosis (TB) vaccines; a DNA vaccine combination expressing mycobacterial heat shock protein 65 (HSP 65) and interleukin-12 (IL-12) by using the hemagglutinating virus of Japan (HVJ)-liposome (HSP 65＋IL-12/HVJ). A mouse IL-12 expression vector (mIL-12 DNA) encoding single-chain IL-12 proteins comorised of p40 and p35 subunits were constructed. In a mouse model, a single gene gun vaccination with the combination of HSP 65 DNA and mIL-12 DNA provided a remarkably high degree of protection against challenge with virulent *Mycobacterium tuberculosis*; bacterial numbers were 100 fold lower in the lungs compared to BCG-vaccinated mice. To explore the clinical use of the DNA vaccines, we evaluated HVJ-liposome encapsulated HAP 65 DNA and mIL-12 DNA (HSP 65＋mIL-12/HVJ). The HVJ-liposome method improved the protective efficacy of the HSP 65 DNA vaccine compared to gene gun vaccination. This vaccine provide remarkable protective efficacy in mouse and guinea pig models, as compared to the current by available BCG vaccine. HSP 65＋IL-12/HVJ vaccine induced CD8＋cytoxic T lymphocyte activity against HSP 65 antigen. Protective efficacy of this vaccine was associated with the emergence of IFN-γ- secreting T cells and activation of proliferative T cells as well as CTL induction upon stimulation with the HSP 65 and antigens from *M. tuberculosis*. Furthermore, we extended our studies to a cynomolgus monkey model, which is currently the best animal model of human tuberculosis, to evaluate the HSP 65＋IL-12/HVJ vaccine. Vaccination with HSP 65＋IL-12/HVJ provided better protective efficacy as assessed by the Erythrocyte Sedimentation Rate, chest X-ray findings, and immune responses than BCG. Most importantly, HSP 65＋IL-12/HVJ resulted in an increased survival for over a year. This is the first report of successful DNA vaccination against *M. tuber-*

culosis in the monkey model. Novel TB vaccines using the monkey model will be discussed in this issue.

The development of novel vaccines against tuberculosis was also studied in murine and cynomolgus monkey systems. Four distinct methods ; DNA vaccination (1. plasmid, 2. adenovirus vector, 3. adenoassouated virus), 4. recombinant BCG, and 5. subunit (recombinant protein) were used for the development of novel vaccines.

Genes (HSP 65 gene, IL-12 gene as well as Ag 85A-, 85B-, MPB51-gene) and IL-6 related genes (IL-6 gene＋IL-6R gene ＋gp130 gene) were administered into the Balb/c mice infected (i.v. or intra-tracheal injection) with *Mycobacterium tuberculosis* (*M. tuberculosis*). Elimination of *M. tuberculosis* in lungs, liver, and spleen of these mice and survival were studied in these models. HSP 65 gene＋IL-12 gene vaccination, or recombinant BCG (BA51 : Antigen 85B-＋Antigen 85A-＋MPB51-gene recombinant BCG) were more prophylactically efficient than parental BCG Tokyo vaccination. In contrast, IL-6 related genes vaccination using adenovirus vector showed therapeutic effect on *M. tuberculosis* infected mice. Cytotoxic T cells (CTL) activity against *M. tuberculosis* in the spleen cells from mice treated with IL-6 related genes vaccination were significantly augmented.

Furthermore, NOD-SCID-PBL/hu mice treated with anti-IL-2 receptor β-chain antibody provide an useful tool for analyzing *in vivo* human T cell immunity against tuberculosis.

In conclusion, we demonstrate the development of a novel HVJ-liposome DNA vaccine encapsulating HSP 65 DNA plus IL-12 DNA. These results suggest that HSP 65＋IL-12/HVJ could be a promising candidate for a new tuberculosis DNA vaccine, which is superior to the currently available BCG vaccine. The goal of our study is to develop a new tuberculosis vaccine superior to BCG. To this aim, we believe that the protective efficacy and protective immune responses for vaccine candidates should be addressed in larger animals, such as non-human primates, before proceeding to human clinical trials. Although other DNA vaccine candidates that appear to protect against virulent *M. tuberculosis* in mice better than BCG have failed to provide better protection than BCG in guinea pigs against aerosol challenge of a low dose of virulent *M. tuberculosis*, some of them are being prepared to enter early human clinical trials. More recently, we evaluated the HSP 65＋hIL-12/HVJ vaccine in the cynomolgus monkey model, which is currently the best non-human primate animal model of human tuberculosis. Monkeys were subsequently challenged with virulent *M. tuberculosis* by the intra-tracheal route after the third vaccination. This challenge dose normally causes death from acute respiratory infection within 4–6 months. In this particular experiment, monkeys vaccinated with HSP 65＋hIL-12/HVJ induced HSP 65-specific T-cell proliferation and improvement of chest X-P findings, resulting in an increased survival for over a year, superior to BCG group. Thus, we are taking advantage of the availability of multiple animal models (mouse, guinea pig, and monkey) to accumulate essential data of the HVJ-liposome DNA vaccine, including the vaccine efficacy and safety, for up-coming Phase I clinical trials.

Key words: TB vaccine, HSP 65 DNA＋IL-12 DNA vaccine, Recombinant BCG vaccine, Clinical application, Cytotoxic T cells, Cynomolgus monkey

Clinical Research Center, National Hospital Organization Kinki-Chuo Chest Medical Center

Correspondence to: Masaji Okada, Clinical Research Center, National Hospital Organization Kinki-Chuo Chest Medical Center, 1180 Nagasone-cho, Kita-ku, Sakai-shi, Osaka 591–8555 Japan. (E-mail: okm@kch.hosp.go.jp)

DEVELOPMENT OF ANTITUBERCULOUS DRUGS: CURRENT STATUS AND FUTURE PROSPECTS

Chairpersons: [1]Haruaki TOMIOKA and [2]Kenji NAMBA

Abstract Worldwide, tuberculosis (TB) remains the most frequent and important infectious disease causing morbidity and death. One-third of the world's population is infected with *Mycobacterium tuberculosis* (MTB), the etiologic agent of TB. The World Health Organization estimates that about eight to ten million new TB cases occur annually worldwide and the incidence of TB is currently increasing. In this context, TB is in the top three, with malaria and HIV being the leading causes of death from a single infectious agent, and approximately two million deaths are attributable to TB annually. In particular, pulmonary TB, the most common form of TB, is a highly contagious and life-threatening infection. Moreover, enhanced susceptibility to TB in HIV-infected populations is another serious health problem throughout the world. In addition, multidrug-resistant TB (MDR-TB) has been increasing in incidence in many areas, not only in developing countries but industrialized countries as well, during the past decade. These situations, particularly the global resurgence of TB and the rapid emergence of MDR-TB, underscore the importance of the development of new antituberculous drugs and new protocols for efficacious clinical control of TB patients using ordinary antimycobacterial drugs. Concerning the development of new antituberculous drugs, the following points are of particular importance. (1) Development of drugs which display lasting antimycobacterial activity *in vivo* is desirable, since they can be administered with long intervals and consequently facilitate directly observed therapy and enhance patient compliance. (2) Development of novel antituberculosis compounds to combat MDR-TB is urgently needed. (3) The eradication of slowly metabolizing and, if possible, dormant populations of MTB organisms that cause relapse, using new classes of anti-TB drugs is very promising for prevention of TB incidence, because it will markedly reduce the incidence of active TB from persons who are latently infected with MTB. Unfortunately, no new drugs except rifabutin and rifapentine has been marketed for TB in the US and other countries during the 40 years after release of rifampicin.

There are a number of constraints that have deterred companies from investing in new anti-TB drugs. The research is expensive, slow and difficult, and requires specialized facilities for handling MTB. There are few animal models that closely mimic the human TB disease. Development time of any anti-TB drug will be long. In fact, clinical trials will require the minimum six-month therapy, with a follow-up period of one year or more. In addition, it is hard to demonstrate obvious benefit of a new anti-TB agents over pre-existing drugs, since clinical trials involve multidrug combination therapy using highly effective ordinary anti-TB drugs. Finaly, there is the perceived lack of commercial return to companies engaged in the development of new anti-TB drugs, because over 95% of TB cases worldwide are in developing countries.

In this symposium, we reviewed the following areas.

1. Critical new information on the entire genome of MTB recently obtained and increasing knowledge of various mycobacterial virulence genes are greatly promoting the identification of genes that code for new drug targets. In this context, Dr. Namba reviewed the status of new types of compounds which are being developed as anti-TB drug. He also discussed the development of new antimycobacterial drugs according to new and potential pharmacological targets and the best clinical development plans for new-TB drugs in relation to corporate strategy.

2. Using such findings for mycobacterial genomes, bioinformatics/genomics/proteomics-based drug design and drug development using quantitative structure-activity relationships may be possible in the near future. In this context, Dr. Suwa and Dr. Suzuki reviewed the usefulness of chemical genomics in searching novel drug targets for development of new antituberculous drugs. The authors reviewed (1) the history and present status of chemical genomics that is defined as the

systemic search for a selective small molecular modulator for each function of all gene products, (2) recent studies of the authors on profiles of the interactions between various kinds of human proteins and small molecule modulators using the new technology devised by Reverse Proteomics Research Institute, and (3) future prospects of the development of new antituberculous drugs based on chemical genomics.

3. It appears also promising to develop new types of drug administration systems using drug vehicles, which enable efficacious drug delivery to their target *in vivo*. Dr. Izumikawa, Dr. Ohno and Dr. Kohno reviewed the usefulness of liposome- and polymer-based technologies, which enable efficacious delivery of encapsulated drugs at required doses for prolonged periods of time with only a single shot without toxicity, and also enable highly targeted delivery of drugs to their target *in vivo*. They indicated that the applications of drug delivery system using conventional anti-mycobacterial agents are challenging to improve the compliance of treatment and better clinical outcome.

4. Immunoadjunctive therapy appears to be promising in improving outcome of clinical control of refractory mycobacterial infections, including MDR-TB and *M.avium* complex infection. Dr. Shimizu, Dr. Sato and Dr. Tomioka reviewed the present status of immunotherapy of mycobacterial infections in combination with antimycobacterial drugs. They indicated that the development of new classes of immunomodulators other than cytokines (IL-2, IFN-γ, GM-CSF, IL-12, etc.) particularly those with no severe side-effects, are urgently needed. Their review dealed with some promising immunoadjunctive agents, especially ATP and its analogues, which potentiate macrophage antimycobacterial activity via purinergic P2 receptors.

The aim of this symposium is to address the future prospects of the development of new drugs and drug regimens for anti-TB chemotherapy. There are a number of difficulties in drug-design for the development of new drug formulations with increased potential for antimycobacterial effects, excellent pharmacokinetics, and tolerability. It should be emphasized that the most urgent goal of chemotherapy of TB and MAC infections, especially that associated with HIV infection, is to develop highly active, low-cost drugs which can be used not only in industrialized countries but also in developing countries, since the incidences of AIDS-associated intractable TB and MAC infections are rapidly increasing in the latter. We strongly wish a great advance of fundametal and practical studies in developing such kinds of new anti-TB drugs in the near future.

1. Prospects for non-clinical or clinical development of new antituberculous drugs in relation to corporate strategy: Kenji NAMBA (New Product Research Laboratories I, Daiichi Pharmaceutical Co., Ltd.)

Tuberculosis (TB) remains one of the deadliest threats to public health. No new anti-TB drugs have been brought into the clinic in the past 40 years. Current non-clinical works with progressed technology and Global Alliance for TB Drug Development, a non-profit organization established in 2000, accelerate research and development of faster-acting anti-TB compounds. We reviewed the status of new types of compounds which are being developed as anti-TB drug, such as diarylquinoline (TMC 207), nitroimidazole (PA-824 & OPC-67683), and moxifloxacin (MFLX). We also discussed the best clinical development plans for new-TB drugs in relation to corporate strategy.

2. Exploring novel drug targets through the chemical genomics approach and its possible application to the development of anti-tuberculosis drugs: Yorimasa SUWA (Reverse Proteomics Research Institute Co., Ltd.), Yohji SUZUKI (Teijin Ltd.)

Recently, chemical genomics approach has been focused as an emerging technology for the drug discovery. In advance to a very large scale national project in US started last year, Reverse Proteomics Research Institute Co., Ltd. (REPRORI) has developed the core technologies for chemical genomics. Here we describe the outline of chemical genomics study, especially that of REPRORI, and discuss about its possible application to the development of anti-tuberculosis drugs.

3. Anti-mycobacterial agents and drug delivery: Koichi IZUMIKAWA, Hideaki OHNO, Shigeru KOHNO (Second Department of Internal Medicine, Nagasaki University School of Medicine)

Mycobacterium infection is a major clinical concern in whole world. Since the newly developed anti-mycobacterial agents are few and still unavailable in clinical settings, the applications of drug delivery system using conventional anti-mycobacterial agents are challenging to improve the compliance of treatment and better efficacy. The efficacy of anti-mycobacterial agents modified by liposome or polymer based technology have been investigated and reported using various animal models. Drug delivery system increased and prolonged the drug concentrations at the blood and targeted organs and the duration of sustained drug release, respectively. These effects lead to decrease in the frequency of drug administrations dramatically and better efficacy rates. The studies, however, were performed only in animal models, the further investigations and evaluations in human are required for practical use.

4. Adjunctive immunotherapy of mycobacterial infections: Toshiaki SHIMIZU, Katsumasa SATO, Haruaki TOMIOKA (Department of Microbiology and Immunology, Shimane University School of Medicine)

There is an urgent need to develop new antimicrobials and protocols for the administration of drugs that are potently efficacious against intractable mycobacterial infections. Unfortunately, development of the new drugs for solving this

problem is not progressing. One promising strategy is to devise regimens to treat infected patients with ordinary antimycobacterial agents in combination with appropriate immunomodulators such as immunomodulating cytokines, inhibitors of immunosuppressive cytokines and new classes of immunomodulators other than cytokines. Therefore, it is important and urgently necessary to synthesize or screen out new low-cost and safe drugs with mild immunopotentiating activity which do not induce immunosuppressing cytokines during administration for long periods of time.

Key words: Antituberculous drugs, Drug targets, Bioinformatics, Drug delivery, Immunoadjunctive therapy

[1]Department of Microbiology and Immunology, Shimane University School of Medicine, [2]New Product Research Laboratories I, Daiichi Pharmaceutical Co., Ltd.

Correspondence to: Haruaki Tomioka, Department of Microbiology and Immunology, Shimane University School of Medicine, 89−1, Enya-cho, Izumo-shi, Shimane 693−8501 Japan. (E-mail: tomioka@med.shimane-u.ac.jp)