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Drug-induced lymphocyte stimulation test in the prediction of drug-induced hypersensitivity to antituberculosis drugs

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ABSTRACT

Antituberculosis (TB) chemotherapeutic drugs may cause a variety of adverse drug reactions (ADRs). To assess the potential of drug-induced lymphocyte stimulation test (DLST) in screening ADRs in patients treated with anti-TB drugs, we performed DLST in 272 TB patients (176 cases with ADRs and 96 controls without ADRs) treated with anti-TB drugs isoniazid (INH), rifampicin (RFP), ethambutol (EMB), and pyrazinamide (PZA). The ADRs were diagnosed by drug provocation test based on clinical and laboratory examinations. The sensitivities of DLST in the diagnosis of INH-, RFP-, EMB-, or PZA-induced ADRs were 57.8%, 37.1%, 42.4%, and 23.1%, respectively, with the corresponding specificities being 93.4%, 94.0%, 97.5%, and 98.8%. DLST has high specificity and limited sensitivity in the diagnosis of anti-TB drug-induced ADRs. In combination with clinical observation and drug use history, DLST could have a predictive validity of ADRs, especially when a positive result is obtained.

1. Introduction

Tuberculosis (TB) is 1 of the leading infectious diseases in the world, with worldwide incidence of 9.4 million in 2009, resulting in an estimated 1.7 million deaths per year (Lawn and Zumla, 2011). Currently, the standard treatment regimens consist of isoniazid (INH), rifampicin (RFP), ethambutol (EMB), and pyrazinamide (PZA), effective in treating most patients with previously untreated TB. However, those chemotherapeutic drugs can cause a variety of adverse drug reactions (ADRs). Hypersensitivity or drug allergy is one of the most common adverse reactions in the clinic, often impacting on the quality of life in affected patients. In severe cases, drug hypersensitivity results in reduced adherence to treatment regimens and even discontinuation of the medication. The irregular and low-dose use of the TB medication may lead to therapeutic failure, disease recurrence, and drug-resistant *Mycobacterium tuberculosis* (Nanashima et al., 2012).

Currently, the drug provocation test (DPT) is considered the "gold standard" for identification of drug hypersensitivity (Aberer et al., 2003). Labor and time consuming, small doses of anti-TB drugs are applied one by one to the patient with hypersensitivity, and the offending drug can be identified when same adverse reactions occur. However, when DPT is conducted, severe and even life-threatening allergic reactions may occur, even at low dosages. In addition, DPT is not recommended for use in pregnant women or patients at increased risk due to comorbidities such as acute infections, uncontrolled asthma, or other diseases, where exposure may provoke a situation beyond medical control (Aberer et al., 2003).

The T-cell–mediated delayed hypersensitivity is responsible for the pathogenesis of severe ADRs (Chung et al., 2008; Hanafusa et al., 2012; Nishio et al., 2007; Takahashi et al., 2006). The in vitro drug-induced lymphocyte stimulation test (DLST) has been extensively used to diagnose immunity-mediated drug hypersensitivity (Hanafusa et al., 2012; Matsuno, 2012; Nyfeler and Pichler, 1997). DLST is a laboratory-based in vitro method that measures the uptake of a DNA precursor, tritiated [^{3}H] thymidine, by lymphocytes after exposure to an antigen in vitro. DLST has certain advantages over DPT, with less patient risk. A single blood sample can be used to test for multiple drugs simultaneously. In Japan, DLST has been widely used to examine drug-induced pneumonitis and liver injury (Ikegame et al., 2011; Kawakami et al., 2007; Matsuno et al., 2007). However, the use of DLST in detection of hypersensitivity of anti-TB drugs remains controversial and has only been reported in a few studies to date (Ikegame et al., 2011; Matsuno et al., 2007; Miwa et al., 2012; Nyfeler and Pichler, 1997; Suzuki et al., 2008). In the present study, we assessed the potential of DLST for determining drug-induced ADRs in a large number of Chinese patients with newly diagnosed TB by comparing the results between DLST and DPT.

2. Study population and methods

This prospective study was reviewed and approved by the Ethics Committee of Shanghai Pulmonary Hospital in accordance with the Declaration of Helsinki, and informed consent was obtained from each patient. The methods were carried out in accordance with the approved American Thoracic Society guidelines (Blumberg et al., 2003).

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2.1. Study population

We enrolled patients with previously untreated pulmonary TB who were admitted to Shanghai Pulmonary Hospital for TB treatment between February 2010 and January 2013. The patients who developed ADRs were selected as case group, and the patients without ADRs during the treatment were selected as control group.

Inclusion criteria were 1) being ≥18 years of age; 2) being positive for sputum culture for *M. tuberculosis* for at least 2 times; 3) having initially received first-line standard anti-TB therapy, including INH (5 mg/kg), RFP (10 mg/kg), EMB (15 mg/kg), and PZA (25 mg/kg); 4) manifesting hypersensitivity after anti-TB therapy in case group. The adverse events were categorized as reported previously (Miwa et al., 2012; Suzuki et al., 2008: A) Drug eruption: generalized measles-like rash with itching following anti-TB therapy. Other possible causes were excluded and rash disappeared after withdrawal of therapy. B) Drug-induced liver injury: alanine aminotransferase (ALT) and total bilirubin (TBIL) levels more than 3 times of the normal limits in 2 months after anti-TB therapy, regardless the presence of symptoms such as nausea, vomiting, and abdominal pain. Liver injury caused by other possible causes including viral hepatitis, cirrhosis, liver cancer, and other drug-induced injury were excluded. C) Drug fever: recurrence of fever (>39.0 °C) despite microbiological and radiographic improvement by anti-TB therapy for several weeks. Fever due to infection, including TB, was excluded. Drug-related fever spontaneously resolved after withdrawal of drugs. The patients of control group showed none of the above ADRs throughout anti-TB treatment and had no history of hypersensitivity to any other drug.

Exclusion criteria were 1) being HIV positive; 2) having autoimmune diseases such as systemic lupus erythematosus, Sjogren's syndrome, and Rheumatoid arthritis; 3) having long-term use of hormones and/or immunosuppressive agents; 4) having diabetes mellitus; 5) use of nonstandard treatment regimens; and 6) no positive drug being identified with DPT test.

2.2. Identification of drug allergy with DPT in case group

When ADRs were observed, all drugs were temporarily stopped. After all symptoms resolved and the laboratory parameters became normal, DPT was performed with careful observation. The anti-TB drugs were restarted one by one, starting with low dosage, and gradually increasing to the usual dosage in accordance with American Thoracic Society guidelines (Blumberg et al., 2003). For patients with rash and drug fever, RFP was the first drug for test. We used a low dosage of 150 mg (1 capsule) per day as a starting dose and gradually increased the dosage to 300 mg, 450 mg and 600 mg per day at intervals of 2–3 days. If there was symptom provoked, the test was stopped and interpreted as positive. After all symptoms had resolved, INH (100 mg, 200 mg, and 300 mg per day), EMB (250 mg, 500 mg, and 750 mg), and PZA (250 mg, 500 mg, 1000 mg, and 1500 mg) were then tested sequentially. For patients with liver injury, a test plan with longer observation duration of at least 1 week was scheduled. ALT and TBIL levels were strictly monitored.

Table 1
Clinical characteristics of the subjects enrolled in the present study.

Characteristics	Case group	Control group
Total number	176	96
Male/female	109:67	61:35
Mean age (year) with range	45 ± 17 (21–71)	47 ± 15(19–73)
Complicated with		
Lymph node TB	23	12
Miliary TB	5	1
Endobronchial TB	26	18
Pulmonary TB only	122	65
Drug sensitive test		
Sensitive to 4 drugs	164	89
Resistant to anti-TB drugs, not MDR	12	7
MDR or XDR	0	0
History of other diseases		
Chronic respiratory disease	11	4
Hypertension	24	14
Cardiovascular disease	8	5
Duodenal/gastric disease	14	6
Hyperthyroidism	2	0
None	117	67
Outcomes		
Survival	176	96
Death	0	0

MDR = multidrug-resistant tuberculosis; XDR = extensively drug-resistant tuberculosis.

Table 2
Adverse reactions of 176 patients of case group dosed with anti-TB drugs.

Events	Number (n)	Percentage (%)	Time of onset (day)[a]
Eruption	57	28.1	20.5 ± 19.2
Liver injury	104	51.2	28.3 ± 16.1
Fever	42	20.7	24.2 ± 18.4

[a] Mean ± SE.

2.3. Identification of drug allergy with DLST in case and control groups

DLST was performed in patients within 2 weeks after the presentation of ADRs. None of the patients were treated with glucocorticoids before completion of DLST. DLST was performed, as previously described (Lopez et al., 2009; Martin et al., 2010; Nyfeler and Pichler, 1997). Twenty milliliters of heparinized peripheral venous blood were obtained from each patient and centrifuged to obtain autologous plasma. The lymphocytes were isolated via density gradient centrifugation and resuspended to a cell density of 10^6 cells/mL in RPMI 1640 medium (Gibco, Carlsbad, CA, USA), in the presence of 20% autologous plasma and penicillin (10,000 units/mL)–streptomycin (10 mg/mL). The lymphocytes were seeded in 96-well dishes (200 μL per well) and cultured with each anti-TB drug (INH, RFP, EMB, and PZA), at concentrations of 1 μg/mL, 10 μg/mL, and 100 μg/mL. The positive control was phytohemagglutinin, and the negative control was vehicle. After 5 days of incubation at 5% CO_2, [^{3}H] thymidine was added for an additional 18 h. The lymphocytes were harvested, and mitogenic activity was quantified by [^{3}H] thymidine incorporation using a scintillation counter (SN-6930, Shanghai Nucleus Research Institute, Shanghai, China). Experiments were performed in triplicate. The stimulation index (S.I.) is defined as count per minute (cpm) of the stimulation/cpm of the negative control. The DLST negative controls (cpm) are 367 ± 84 and 356 ± 72, for the case group and control group, respectively. DLST findings were considered positive if the S.I. was >1.8.

Table 3
Comparison of S.I. between DPT-positive and DPT-negative cases with anti-TB drugs.

Events	Drug	DPT-positive S.I. value	DPT-negative S.I. value	*U* value	*P* value
Total, n = 176	INH	3.13 ± 2.33	1.28 ± 0.73	−5.740	0.001
	RFP	2.24 ± 1.85	1.32 ± 0.66	−2.039	0.041
	EMB	2.12 ± 1.37	1.16 ± 0.45	−3.357	0.001
	PZA	1.59 ± 1.07	1.12 ± 0.36	−1.561	0.118
Drug eruption, n = 57	INH	3.71 ± 2.57	1.29 ± 0.77	−3.655-	0.001
	RFP	2.47 ± 2.12	1.35 ± 0.88	−1.918	0.055
	EMB	2.26 ± 1.48	1.19 ± 0.34	−2.212	0.027
	PZA				
Drug-induced liver injury, n = 104	INH	2.61 ± 2.08	1.24 ± 0.82	−3.752	0.001
	RFP	2.09 ± 1.68	1.29 ± 0.86	−2.165	0.030
	EMB	2.00 ± 1.24	1.16 ± 0.59	−2.917	0.004
	PZA	1.70 ± 0.91	1.14 ± 0.32	−1.047	0.295
Fever, n = 42	INH	3.61 ± 2.56	1.36 ± 0.74	−2.446	0.014
	RFP	2.34 ± 1.76	1.41 ± 0.78	−0.302	0.762
	EMB	2.82 ± 1.46	1.12 ± 0.23	−2.515	0.012
	PZA	1.55 ± 0.91	1.06 ± 0.22	−0.1.474	0.141