Original Research Article

Effect of moxifloxacin on oxidative stress, paraoxonase-1 (PON1) activity and efficacy of treatment in patients with multiple drug-resistant tuberculosis

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Abstract

Purpose: To investigate the effect of moxifloxacin on paraoxonase-1 (PON1) activity, and serum oxidative stress in patients with multiple drug-resistant tuberculosis (MDR-TB).

Methods: A total ofof 130 MDR-TB patients who were treated with moxifloxacin from October 2014 to October 2010 in Eastern Medical District of Linyi People's Hospital of Shandong Province, China were randomly divided into an observation group (65 cases, moxifloxacin group) and control group (65 cases, non-moxifloxacin group). Total oxidant status (TOS), total antioxidant status (TAS), oxidative stress index (OSI), PON1 levels and treatment efficacy for groups were determined.

Results: Compared with pre-treatment levels, TOS (23.3 ± 4.7 vs 13.9 ± 3.3 umol H_2O_2 Eq/L, t = 13.20, p = 0.00) and OSI (17.4 ± 4.8 vs 5.7 ± 1.4 U, t = 18.87, p = 0.00) of the observation group significantly decreased, while TAS (1.4 ± 0.5 vs 3.5 ± 0.7 umol Trolox Eq/L, t = 19.68, p = 0.00) and PON1 (15.5 ± 6.9 vs 31.1 ± 8.3 U/L, t = 11.65, p = 0.00) significantly increased. TOS (23.3 ± 4.7 vs 13.9 ± 3.3 umol H_2O_2 Eq/L, t = 7.73, p < 0.05) and OSI (16.9 ± 5.5 vs 7.4 ± 3.2U, t = 12.04, p = 0.05) reduced significantly in the control group. Moxifloxacin correlated positively with ⊿TAS (r = 0.697, p = 0. 04) and ⊿PON1 (r = 0.785, p = 0.01), but correlation with ⊿TOS (r = -0.625, p = 0.01) was negative. Efficacy was significantly higher in the observation group than that in the control group (81.54 % vs 56.92 %, p = 0.00).

Conclusion: Oxidative stress injury in MDR-TB patients may be effectively managed by combination of moxifloxacin with anti-TB treatment

Keywords: Multiple drug-resistant TB, Moxifloxacin, Paraoxonase, Oxidative stress

INTRODUCTION

Tuberculosis (TB) is one of the global public health problems. In recent years, with greater attention paid to TB prevention and treatment technology, the incidence of TB has declined. However, there are still a large population of patients with TB [1,2]. China ranks second in the world, with respect to the number of TB patients [3]. Due to irregularities in availability of appropriate clinical medications and variations in the causative organism, *Mycobacterium tuberculosis*, the incidence of drug resistance is gradually increasing. Multiple drug-resistant TB (MDR-TB) is that patients have a resistant to two or more anti-TB drugs, such as the conventional first-line anti-TB drugs rifampicin and isoniazid.

Drug resistance greatly increases the treatment cycle and adverse reactions, resulting in a

significant reduction in cure rate [4]. Relevant epidemiological studies have shown that total resistance in TB has reached up to 37.62 % [5]. Results of assessment by World Health Organization showed that the incidence of MDR-TB in TB patients was 20 %. This is a great challenge to clinical treatment of TB because it seriously affects the quality of life and prognosis [6]. Therefore, the search for effective treatment strategies for patients with MDR-TB has become a subject of intensive clinical research.

It has been reported that TB infection and prognosis may have a certain relationship with oxidative stress, leading to disorders in the balance between pro-oxidants and antioxidant system [7,8]. Quinolones have effects on MDR-TB, which may be relevant with oxidative stress imbalance [9].

METHODS

General information

A total of 130 patients with MDR-TB treated in Eastern Medical District of Linyi People's Hospital of Shandong Province, from October 2014 to October 2016 were enrolled. The diagnostic criteria were in accordance with the Guidelines for the Diagnosis and Treatment of Tuberculosis (2013 version) [10] . Inclusion criteria were: patients aged 18 - 60 years; patients who were not taking quinolone antibiotics (including hypnathic acid, pipemidic acid, norfloxacin and gatifloxacin) at least 90 days before enrollment; patients free from cardiovascular disease history (including coronary heart disease and myocardial infarction); absence of significant ECG abnormalities like QRS wide malformations and QT interval prolonged; and absence of neuropsychiatric history like bipolar disorder and schizophrenia.

Exclusion criteria patients with autoimmune diseases; long-term use of immunosuppressive agents; bacteria or fungal infectious diseases like *Streptococcus pneumonia* and large area of yellow ringworm. The ethics committee of hospital approved the study (approval No. LY2014050) whih also followed the guidelines of Declaration of Helsinki, as amended in 1996 [11], and all patients were informed. All the patients of this study gave their informed consent, and were randomly divided into observation group (65 cases) and control group (65 cases). There was no significant difference between the two groups in with regard to their profiles, including gender, age, course of disease, smoking history, MDR-

TB clinical classification, and basic disease and laboratory tests *(p > 0.05, Table 1)*.

The control group was treated with conventional drug regimen, amikacin, dipasic, rifapentine and ethambutol, abbreviated as $6ADL_2E/18DL_2E$; where A, D, L2 and E refer to amikacin, dipasic, rifapentine and ethambutol, respectively, while the figures refer to 6 and 18 months. The observation group was treated with moxifloxacin (Bayer Healthcare Co., Ltd.), specifically $6ADL_2EM/18DL_2EM$, where M refers to moxifloxacin. During the treatment, regular blood routine examination, liver function and kidney function were carried out.

Determination of TAS, TOS, OSI and PON-1 in serum

The xylenol orange method was used to determine the total oxidant status (TOS) by using an automatic biochemical analyzer (HITACHI, Japan). In this method, ferrous ion (Fe^{2+}) is oxidized to ferric ions (Fe^{3+}), which react with xylenol orange. The color intensity is proportional to TOS level, expressed as µmol H_2O_2 Equiv/L.

Total antioxidant status (TAS) was measured by the ABTS method using automatic biochemical analyzer (HITACHI, Japan). The total antioxidants reacted with ABTS (2, 2'-amino-di-3-ethyl-benzothiazolinesulfonic acid-6 ammonium salt). TAS level, which was expressed as mmol Trolox Equiv/L is proportional to the magnitude of color change seen in the reaction. Oxidative Stress Index (OSI) was obtained as in Eq 1.

$$OSI = \{(TOS)/(TAS \times 1000)\}100. \ldots\ldots\ldots\ldots (1)$$

For serum PON-1 activity, venous blood samples (4 %) was drawn after 8h fast and centrifuged at 3000 r/min at 4 ^{o}C for 10 min to collect the supernatant. PONI-1 was assayed by spectrophotometry (SP). Activity was calculated as in Eq 2 [12].

$$PON1 \ activity \ (ku/L) = \{A \times F \times FV/(t \times SV \times L \times \varepsilon)\}10^3 \ldots (2)$$

where *A* is the net absorbance value (i.e. the absorbance value of the specimen minus the absorbance value of the serum control and the substrate control); *F* is the dilution factor of the specimen, *FV* is the final volume of the reaction, *t* is the reaction time (min), *SV* is the sample volume (L), *L* is the optical path (cm), and ε is the molar extinction coefficient. One unit of pon-1 activity was defined as 1µ enzyme required to catalyze the hydrolysis of 1µmol phenylacetate per minute.

Criteria for determination of efficacy

Bacteriological evaluation or X-ray examination was used for efficacy determination. If sputum examination (3 consecutive sputum smears and 1 sputum TB) showed negative results or decreased amounts of inoculum, the treatment was effective, but if it was unchanged or if the amount of inoculum was increased, the treatment was ineffective. X-ray evaluation results were deemed to indicate effective treatment if the lesions were resolved, while deterioration of lesions or absence of significant changes in lesions indicated ineffective treatment [13].

Statistical analysis

All the data in this study were analyzed by SPSS 22.0 Statistical Software. Measurement data of normal distribution are expressed as mean ± SD, and t-test or analysis of variance (ANOVA) was used for group comparison. Enumeration data are presented as {n (%)}, and analyzed by Chi-square test. Pearson or Spearman correlation analysis was employed for correlation analysis, while $p < 0.05$ was considered statistically significant.

RESULTS

General information of the two groups

There were no significant differences in gender, age, duration of disease, smoking history and other general data ($p > 0.05$). The prevalence of hematogenous disseminated TB, infiltrative pulmonary TB, chronic fibrous TB and TB pleurisy were no significant differences ($p > 0.05$). In addition, the proportion of patients with chronic bronchitis and diabetes showed no significant difference ($p > 0.05$), the estimated values of albumin, neutrophils, white blood cell, alanine aminotransferase and serum creatinine had no significant differences ($p > 0.05$, Table 1).

Changes in serum TAS, TOS, OSI and PON1 levels in both groups before and after treatment

There were no significant differences in TOS, OSI and PON1 levels of two groups before treatment ($p > 0.05$). In the observation group, the levels of TOS and OSI were significantly lower than those in the control group, while TAS and PON1 were significantly higher than those in the control group ($p < 0.05$). The levels of TOS and OSI in the observation group were significantly decreased, while the levels of TAS and PON1 were significantly increased after treatment ($p < 0.05$). When compared with those before treatment, the levels of TOS and OSI lowered significantly after treatment in control group ($p < 0.05$) while changes in TAS and PON1 had no statistically significant ($p > 0.05$, Table 2).

Correlation analysis

In the correlation analysis between smoking history/diabetes/moxifloxacin/chronic bronchitis/moxifloxacin and TAS/TOS/OSI/PON1 levels {∆ = (after treatment - before treatment)}, moxifloxacin was positively in correlation with ∆TAS (r = 0.697, $p = 0.04$) and ∆PON1 (r = 0.785, $p = 0.01$), but negatively correlated with ∆TOS (r = -0.625, $p = 0.01$). There were no significant differences among the other indices ($p > 0.05$, Table 3).

Table 1: Demographic and general profile {(mean ± SD), n (%)}

Variable	Observation group (65)	Control group (65)	t or χ^2	*p*-value
Gender (M/F)	37/28	42/23	0.81	0.37
Age	50.2±4.7	51.1±3.9	1.19	0.24
Duration of disease (year)	12.2±4.8	11.9±4.4	0.37	0.71
Smoking history	19 (29.23 %)	17 (26.15 %)	0.15	0.70
Hematogenous disseminated TB	26 (40.00 %)	29 (44.61 %)	0.28	0.59
Infiltrative pulmonary TB	17 (26.15 %)	14 (21.54 %)	0.38	0.54
Chronic fibrous TB	8 (12.31 %)	11 (16.92 %)	0.56	0.46
TB pleurisy	14 (21.54 %)	11 (16.92 %)	0.44	0.50
Diabetes	17 (26.15 %)	20 (30.77 %)	0.34	0.56
Chronic bronchitis	7 (10.77 %)	5 (7.69 %)	0.37	0.55
Albumin (g/L)	29.9±3.1	30.5±2.8	1.16	0.25
White blood cell (10^9/L)	7.67±1.24	7.49±1.33	0.80	0.43
Serum creatinine (umol/L)	77.1±10.4	74.6±12.5	1.24	0.22

Table 2: Changes of serum TAS, TOS, OSI and PON1 levels in both groups before and after treatment (mean ± SD)

Parameter		Observation group (65)	Control group (65)	t or χ^2	*p*-value
TAS (umol Trolox Eq/L)					
	Before	1.4±0.5	1.6±0.3	2.77	0.28
	After	3.5±0.7*	2.0±0.6	13.12	0.00
TOS (umol H_2O_2 Eq/L)					
	Before	23.3±4.7	23.6±4.4*	0.38	0.71
	After	13.9±3.3*	17.5±4.6*	5.13	0.00
OSI (U)					
	Before	17.4±4.8	16.9±5.5	0.55	0.58
	After	5.7±1.4*	7.4±3.2*	3.92	0.01
PON1 (U/L)					
	Before	15.5±6.9	16.1±4.4	0.59	0.57
	After	31.1±8.3*	19.9±7.4	8.12	0.00

Note: Compared to pre- treatment, p < 0.05.

Table 3: Correlation analysis results

Variable	△TAS		△TOS		△OSI		△PON1	
	r	*p-value*	*r*	*p-value*	*r*	*p-value*	*r*	*p-value*
Smoking history	0.01	0.66	-0.01	0.69	-0.02	0.55	-0.03	0.55
Diabetes	-0.04	0.33	-0.03	0.049	0.04	0.26	-0.02	0.59
Chronic bronchitis	0.01	0.61	0.06	0.14	-0.01	0.68	-0.02	0.50
Moxifloxacin	0.70	0.04	-0.63	0.01	0.23	0.09	0.79	0.01
△TAS	-	-	-0.12	0.11	-0.02	0.19	0.24	0.11
△TOS	-0.11	0.15	-	-	-0.04	0.35	-0.19	0.07
△OSI	-0.02	0.18	-0.05	0.30	-	-	0.01	0.41
△PON1	0.25	0.19	-0.19	0.09	0.06	0.41	-	-

Comparison of effectiveness of treatment between the two groups

The treatment efficacy was significantly higher in the observation group than that in the control group *(p* < 0.05, Table 4).

Table 4: Effective rate of treatment for the two groups {n (%)}

Group	Effective	Ineffective
Observation group (n=65)	53 (81.54)	12 (18.46)
Control group (n = 65)	37 (56.92)	28 (43.08)
χ^2	9.24	
P-value	0.00	

DISCUSSION

Studies have shown that *Mycobacterium tuberculosis* gene mutations could occur in the process of growth and reproduction to form resistant strains due to environmental factors. The emergence of clinically resistant strains is closely linked to factors such as insufficient drug dosage, improper medication methods, inadequate course of treatment or intermittent medication and inappropriate order of medication. These factors cause resistance to first-line drugs (isoniazid, rifampicin and pyrazinamide), and second-line drugs (injectables, fluoroquinolones and anti-inflammatory drugs). In MDR-TB, alveolar macrophages and other anti-inflammatory cells accumulate, engulfing MDR-TB bacteria and releasing a large number of reactive oxygen species (ROS), which result in local inflammatory response and damage [14,15] .

Quinolones, also known as pyridine carboxylic acids or pyridine acids, are synthetic 4-quinolone antibacterial drugs. Quinolones are targeted at DNA, thereby blocking DNA gyrase and further causing irreversible damage to DNA. Compared with the first 3 generations of quinolone antibiotics, moxifloxacin as a fourth-generation quinolone accumulates highly in respiratory mucosa, lung tissue, bronchoalveolar lavage fluid and macrophages, effectively killing lung TB mycobacteria [16,17]. In this study, moxifloxacin treatment was used in the observation group, and it greatly improved the effectiveness of MDR-TB. During infections, trauma, and poisoning, large amounts of inflammatory substances are generated, leading to imbalance in antioxidant system known as oxidative stress [9,18]. In MDR-TB patients, excessive activation and aggregation of macrophages and neutrophils

occur, with resultant imbalance between oxidant and antioxidant system, which accounts for high oxidative stress [19]. In this study, total oxidant status and antioxidant status gradually recovered in the two groups of patients after treatment: TAS and PON1 levels significantly increased, while TOS and OSI levels decreased. The degree of improvement was more pronounced in patients with moxifloxacin combination therapy.

Malnutrition is one of the most important causes of high oxidative stress in TB patients. Poor appetite and inadequate intake of vitamin C and other antioxidants by MDR-TB patients may also lead to oxidative stress injury [20]. However, in this study, the albumin levels in the two groups of patients were within normal range, and their difference was not statistically significant. PON1 is a calcium-sensitive lipase with the physiological function of hydrolyzing lipid peroxides *in vivo*. The activity of PON1 is significantly reduced in oxidative stress conditions [21]. In this study, PON1 activity was significantly decreased in both groups before treatment, and increased after treatment, with more significant increase in the observation group. In the correlation analysis, moxifloxacin had a positive correlation with PON1 activity, and its mechanism of action might be related to mitigation of *Mycobacterium tuberculosis*-mediated chronic oxidative stress injury [22,23] .

Limitations of the study

However, this study is a controlled study using a small sample, and the specific mechanism of action of moxifloacin is yet to be elucidated. Thus, further, well-designed studies based on multiple centers are needed in this respect.

CONCLUSION

The results of this study indicate that moxifloxacin can improve the imbalance in oxidant-antioxidant system in patients with MDR-TB, reduce the associated high oxidative stress, and improve the efficiency of treatment.

DECLARATIONS

Acknowledgement

None.

Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

The authors declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by them.

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