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Short Communication

Serum anti-tuberculous activity of a novel antibiotic

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Serum anti-tuberculous activity of a novel antibiotic indicated that the *in vivo* anti-tuberculous activity of the antibiotic was raised and decreased as time was passing after it was injected into rat body with two activity peaks which were around 1 and 6 h respectively. With *in vivo* anti-tuberculous activities being promising, the antibiotic could be an anti-tuberculous drug candidate.

Key word: Antibiotic, *in vivo*, anti-tuberculous, serum.

INTRODUCTION

In the process of screening bacteria that can antagonize *Xanthomonas oryzae* pv. *oryzae* (the pathogen of rice) we obtained a bacterium that could produce a novel antibiotic (Chen-Xiaoxi et al., 2000) . In a previous paper, we had reported that the antibiotic had a potent in vitro anti-tuberculous activity, even if the *Mycobacterium tuberculosis* belonged to multi-drug resistant tuberculosis (MDR-TB) stains (Chen-Xiaoxi and Yue, 2010). This finding is of significance because the world is facing the danger of the resurgence of TB and the rapid emergence of MDR-TB and because no new drugs except rifabutin and rifapentine have been marketed for TB during the 40 years after release of rifampicin due to some constraints that have deterred companies from investing in new anti-TB drugs (Joas da Silva et al., 2009) . With the in vitro anti-tuberculous activity being so promising, we wondered whether the antibiotic had a good *in vivo* anti-tuberculous performance.

MATERIALS AND METHODS

Fermentation, extraction and purification of the antibiotic

The antibiotic-producing bacteria (*Bacillus subtilis*) was cultured on surface of the quartz sand that was immersed in KMB culture medium at 37°C for ten days, the surface of the quartz sand not being covered with liquid culture medium (previous work had shown that the bacteria produced more antibiotic if it was cultured on solid medium) . Thereafter, the quartz sand, which absorbed antibiotic secreted by the bacteria, was immersed in water in order to be distilled. The condensed water was collected and passed through an active carbon chromatographic column, which was then eluted with ether. The eluted ether was left at room temperature of 25 to 30°C overnight to evaporate the ether. The remainder was chromatographed on silica gel column which was eluted with ether. The fraction with the greatest activity was further chromatographed on silica gel column and then eluted with normal pentane ether=2:8. The normal pentane and the ether were both evaporated at room temperature of 25 to 30°C. Anti -bacterial activity was confirmed by agar diffusion test (the test cell was *Escherichia coli* and the agar diffusion test was carried out on LB agar plates).

Animal and bacterium

Sprague-Dawley rat, half male and half female, 6 to 8 weeks old, 300 to 350 g, were purchased from National Rodent Laboratory Animal Resources, Shanghai Branch, P. R. China. All animal experiments were carried out in the animal centre of Zhejiang Chinese Medicine University. *M. tuberculosis* was H37RV strain, which was available in the Key Laboratory *M. tuberculosis,* Shamghai Pulmonary Hospital affiliated to Tongji University School of Medicine.

Anti-tuberculous activity of rat-derived-serum that contained the antibiotic or the metabolite of the antibiotic

The animal experiment was approved by the Committee on Laboratory Animals, Zhejiang Chinese Traditional Medicine University. Eleven rats was anesthetized using coelio-injection of pentobarbitone (10 mg/kg). Nine rats were respectively injected with the antibiotic (2 mg/kg) through tail vein. Other two rats were injected with same volume of physiological saline (the control). The time that the antibiotic or physiological saline was injected was set as 0.0 h. Carotid artery was revealed by ophthalmology scissors. About 0.8 ml blood was drawn by 1 ml syringe at different time interval (0.5, 1.0, 1.50, 2.0, 2.5, 3, 3.5, 4.0 and 5.5 h, etc.) from each rat through carotid artery. In order to prevent hematopexis, certain amount of haemostatic (disebrin) was added into syringe/

Table 1. Anti-tuberculous activities of the antibiotic-contained rat serum, which was derived at different time intervals from rats that had been i.v. injected with the antibiotic(the control or CK was injected with normal saline).

C	0.5	1.0	1.5	2	2.5	3	3.5	4	5	5.5			
K	0	0	0	0	0	0	0	0	0	0			
1	0.5	1.0	1.5	2	2.5	3	3.5	4	5	5.5	6	6.5	7
	no	no								—	—	—	—
2	0.5	1.0	1.5	2	2.5	3	3.5	4	5	5.5			
			—	—					—	—			
3	0.5	1.0	1.5	2	2.5	3	3.5	4	5				
	—	—							—				
4	0.5	1.0	1.5	2	2.5	3	3.5	4	5				
	—							—	—				
5	0.5	1.0	1.5	2	2.5	3	3.5	4	5	5.5			
	—	—	—						—	—			

C	5.5	6.5	7.5	8.5	9.5	10.5	11.5	12.5
K	0	0	0	0	0	0	0	0
A	5.5	6.5	7.5	8.5	9.5	10.5	11.5	12.5
	—	—						
B	5.5	6.5	7.5	8.5	9.5	10.5		
	—	—	—	—				
C	5.5	6.5	7.5	8.5	9.5	10.5	11.5	12.5	13.5
					—	—	—		
D	5.5	6.5	7.5	8.5	9.5	10.5	11.5	12.5	13.5
	—	—	—						

(i) 0, 5, 1.0, 1.5, 2.0, 3.0, 3.5 etc. means time (h) to drew blood from carotid artery. The time that the antibiotic was injected was set as 0.0 h, (ii) "0"was results from control serum (that is antibiotic-free-serum or injected with normal saline) and "0"means cells of *M. tuberculosis* could be freely living in the control serum, "+" means the growth of cells of the *M. tuberculosis* was inhibited to a certain degree by the antibiotic-contained-serum, "—" means the growth was completely inhibited by antibiotic-contained-serum, (iii) CK were serial codes, which were assigned to the control rats that is rats that were injected with normal saline, 1, 2, 3, 4, 5 as well as A, B, C, D were also serial codes, which were respectively assigned to different individual rat that was injected with the antibiotic, ( ) "no" means no datum was available because of some reasons, ( ) For the first group (rat 1, 2, 3, 4, 5 or the CK), no more serum could be drawn from blood vessel after 5.5 , 6.0 or 7.5 h; while for the second group (rat A, B, C and D or the CD), the starting time to draw blood was 5.5 h.

carotid artery. After having been prepared all the blood samples were centrifuged at 3000 rpm for 5min and the eleven sets of serum (the supernatant) were thus obtained. Complement system, which could have some antibacterial activity, was inactivated by incubating the serum at 56°C for 30 min. The eleven sets of serum were tested for activity against a model strain of *M. tuberculosis* (H37RV) in Lownstein- Jenson media at 37°C for 4 weeks (activity tests were carried out in Key Laboratory *M. tuberculosis*, Shamghai Pulmonary Hospital affiliated to Tongji University School of Medicine).

RESULTS

The antibiotic-containing rat serum had shown anti-tuberculous activity. The anti-tuberculous activities are summarized in Table 1. From Table 1, it could be seen that after the antibiotic having been injected into rat bodies, the *in vivo* anti-tuberculous activities of the antibiotic were dramatically raised and decreased within the rat bodies as time was passing, with two activity peaks which were around 1 and 6 h respectively.

DISCUSSION

Our previous *in vitro* anti-tuberculous test had shown that the MICs for the antibiotic against *M. tuberculosis*, including MDR strains, were 0.025 µg/ml (Chen-Xiaoxi and Yue, 2010). The antibiotic -producing bacterium had been identified to be a member of *B. subtilis* (Chen- Xiaoxi et al., 2010). To the best of our knowledge, it was first reported that a strain of *B. subtilis* could produce such a potent anti-tuberculous antibiotic. This paper's experiments

experiments had confirmed that the antibiotic's in vivo anti-tuberculous activity was also very good. The paper's novelty was that when the antibiotic existed within rat body, its anti-tuberculous activity was up and down dramatically as time went on. To the best of our knowledge, there is no anti-tuberculous antibiotic or anti-tuberculous drug whose in vivo activity fluctuated so greatly. Undoubtedly, it is the antibiotic's metabolite rather than the antibiotic itself that was responsible for second *in vivo* activity peak and it was the antibiotic itself that was mainly responsible for the first in vivo activity peak.

M. tuberculosis, the etiological agent of tuberculosis, kills more than 2 million people every year worldwide in concurrence with HIV-related infections. Moreover, appearance of multi- drug resistant strains of *M. tuberculosis* to many, if not all, of the existing drugs has been noted. This has necessitated the development of novel anti-tubercular agents (Ducati et al., 2006; Tomioka, 2006; Tomioka and Namba, 2007; Coleman et al., 2001; Lourenco et al., 2008; Biava et al., 2008). This paper's research had provided a new evidence or in vivo experimental data that the antibiotic produced by the *B. subtilis* had a good potential as a drug candidate in the fight against *M. tuberculosis*.

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