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Short Communication

In vivo activities of Baicalin against *Chlamydia trachomatis*

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Our previous studies have shown that Baicalin could effectively inhibit *Chlamydia trachomatis in vitro*. In this study, Baicalin was tested for potential antichlamydial activity using a murine genital *Chlamydia trachomatis* infection model. It was demonstrated that Baicalin significantly reduced *C. trachomatis* loading in BALB/c mice that were vaginally infected with the pathogen. On the basis of these data and our previous observations, we concluded that further evaluation of Baicalin for prevention and treatment of sexually transmitted chlamydial infection is warranted.

Key words: *Chlamydia trachomatis*, Baicalin, vaginally infected.

INTRODUCTION

Chlamydia trachomatis is primarily a human pathogen associated with common sexually transmitted diseases and trachoma. Most developing countries that have the highest burden of chlamydial infections have limited capacity to effectively screen for chlamydial infections and treatment is thus largely based on symptomatic case ascertainment (Schachter, 1999; Behets et al., 2001).

Studies demonstrated that the majority of infected individuals do not seek treatment because they have no or very mild symptoms (Westrom and Mardh, 1983). Without proper treatment, about one-third of infected individuals develop long-term, devastating complications, such as infertility and chronic pelvic inflammatory pain syndrome (Laga et al., 1994). Infected individuals are also at increased risk of HIV acquisition, owing to ulcerative damages that occur in the epithelial tissues. The medical and financial burdens of these conditions call for the development of new strategies to effectively prevent *C. trachomatis* infection (Ridgway, 1997).

Although effective antimicrobial treatment is available, this has been largely unsuccessful in halting the spread of infection, most likely due to the high rate of asymptomatic infections which may persist for months to years. Multiple-antibiotic resistant strains of *Chlamydia* have also been reported recently (Somani et al., 2000). Furthermore, it has been suggested that antibiotic treatment can result in persistent infections with aberrant forms of *C. trachomatis* that may be reactivated at a later date.

We have reported previously that Baicalin can effectively inhibit *C. trachomatis in vitro* (Huang et al., 2009). In the present investigation, we studied the *in vivo* therapeutic effects of Baicalin using a mouse experimental model of *C. trachomatis* -caused chlamydial genital infection.

MATERIALS AND METHODS

C. trachomatis serovar D (Department of Pathogenic Biology, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China) was cultured in HeLa cells as described previously (Rasmussen et al., 1997). The infectivity of the chlamydia was expressed as the number of inclusion forming units. Baicalin (HPLC Content > 98.0%) from Chongqing Green Valley Bio-tech Co. LTD. (Chongqing, China). Medroxyprogesterone was used to make mice susceptible to infection (Amit et al., 2009). Eight-week-old Balb/c female mice were injected subcutaneously with the hormone (2.5 mg/mouse) 2 weeks before infection. The injection was repeated 7 days before infection. The progesterone-treated animals were given intravaginally either 15 ml of 1 mM Baicalin prepared in 50 mM Hepes buffer (pH 7.0) or the vehicle Hepes buffer (10 mice in each group). One hour later, the animals were infected intravaginally with *C. trachomatis* (2×10^7 IFUs per mouse). After infection, intravaginal administration of Baicalin or vehicle was repeated three times per day. 5, 11, 16 and 19 days after infection, vaginal swabs were taken. Infectious elementary

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Abbreviations: HIV, Human immunodeficiency virus; **HPLC,** high performance liquid chromatography.

Table 1. Summary statistics for EB counts of vaginal swabs.

	Mean (standard deviation)		Geometric mean		Percent reduction of Chlamydial load	
	Baicalin	**vehicle**	**Baicalin**	**vehicle**	**Baicalin**	**vehicle**
Day 5	169,478.0 (104,163.4)	784,962.0 (879.145.2)	107,579.6	684,647.3		
Day 11	511.9 (648.7)	812,254.7 (956,553,2)	321.4	75,691.8	99.7	88.9
Day 16	97.5 (165.3)	97,563.7 (103,478,1)	18.7	11,568.9	99.8	98.3
Day 19	5.5 (11.2)	12,457.4 (11,236,9)	2.9	1673.5	99.9	99.8

bodies on the swabs were eluted into 1.0 ml sucrose phosphate-glutamate buffer, serially diluted and inoculated onto HeLa cells grown on coverslips. After 30 h of culture, coverslips were fixed with methanol. Immunostaining and fluorescence microscopy were performed.

RESULTS AND DISCUSSION

It is now widely accepted that *C. trachomatis* is the most prevalent sexually transmitted pathogen. Classical antibiotics are undesirable for long-term, prophylactic use because they frequently disrupt normal microflora and consequently increase the risk for bacterial vaginosis. Among non antibiotic reagents tested against *C. trachomatis*, only one offered partial protection *in vivo*; others were either ineffective or even worsened the infection *in vivo* although, *in vitro*, they demonstrated adverse effects on the pathogen.

Baicalin, being a medicinal plant traditionally used in Oriental medicine, is a flavonoid derived from Scutellariae Radix and known to have various biological functions, including antimicrobial activities including antimicrobial, anti-inflammatory activities, and also shown to have the therapeutic potential for the treatment of atherosclerosis and restenosis (Gao et al.,1999). Previous studies showed that Baicalin can inhibit infection *in vitro* by several strains of *Chlamydia pneumoniae* (Liu et al., 2006). We previously also characterized the inhibition of *C. trachomatis* in cell culture by Baicalin (Huang et al., 2009).

The objective of the present study is to investigate whether Baicalin would also inhibit chlamydial infection *in vivo*. Here, we tested the effects of Baicalin on *C. trachomatis* infection *in vivo* using a MoPn vaginal infection model previously established in BALB/c mice. Table 1 apparently revealed that the majority of control vehicle-treated mice had higher bacterial loadings as compared with Baicalin-treated animals. The findings from this study further confirmed that Baicalin significantly reduced *C. trachomatis* loading in BALB/c mice that were vaginally infected with the pathogen and suggested that Baicalin deserved further evaluation as antichlamydial candidates for the prevention of sexually transmitted chlamydial infection.

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