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Original Article

In Vitro Inhibitory Effects of Tea Polyphenols on the Proliferation of *Chlamydia trachomatis* and *Chlamydia pneumoniae*

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SUMMARY: In vitro inhibitory effects of tea polyphenols on *Chlamydia trachomatis* and *C. pneumoniae* were investigated. A product of tea polyphenols, Polyphenon 70S was used. Chlamydial strains used were *C. trachomatis* D/UW-3/Cx and L_2/434/Bu, and *C. pneumoniae* AR-39 and AC-43 strains. HeLa229 cells and HL cells were used for cultivation of *C. trachomatis* and *C. pneumoniae*, respectively. In the post-inoculation method, no inclusions of *C. trachomatis* were observed at 0.5 mg/ml of Polyphenon 70S. However, the toxicity of Polyphenon 70S was noted in HeLa229 cells and HL cells at a concentration of 0.25 mg/ml. In the pre-inoculation method, no toxic effects of Polyphenon 70S on the cells were noted. Complete inhibition of *C. trachomatis* D and L_2 was noted at concentrations of 1.6 and 0.4 mg/ml, respectively. With *C. pneumoniae* strains, the end points were 0.8 and 1.6 mg/ml for AR-39 and AC-43, respectively. Our findings encouraged the application of tea polyphenols for topical usage.

INTRODUCTION

Chlamydia trachomatis and *C.* (*Chlamydophila*) *pneumoniae* are ubiquitous pathogens that cause various infections in humans. *C. trachomatis* is the most frequent cause of sexually transmitted diseases (1). *C. pneumoniae* is a common microorganism that causes respiratory tract and other infections (2). Antibiotics such as tetracyclines, macrolides, and fluoroquinolones are effective against both *C. trachomatis* and *C. pneumoniae*. However, considering these drugs' economic aspects and possible drug resistance (3,4), we should have several approaches to control chlamydial infections. Polyphenols are contained in various types of tea and reportedly have inhibitory effects on certain pathogens (5-11). The objective of this study was to clarify the in vitro effects of tea polyphenols on *C. trachomatis* and *C. pneumoniae*.

MATERIALS AND METHODS

Polyphenols: A product of tea polyphenols called Polyphenon 70S (Mitsui Norin Co., Ltd., Tokyo), which contains (–)-epigallocatechin (18.3% wt), (–)-epicatechin 8.6%, (–)-epigallocatechin gallate 35.9%, (–)-epicatechin gallate 11.2% and (–)-gallocatechin gallate 3.5%, was used.

Chlamydial strains and cells: The chlamydial strains used were *C. trachomatis* D/UW-3/Cx and L_2/434/Bu, and *C. pneumoniae* AR-39 and AC-43 strains. HeLa229 cells and HL cells were used for cultivation of *C. trachomatis* and *C. pneumoniae*, respectively.

Drug susceptibility test in cell cultures: Two methods, a post-inoculation and a pre-inoculation method were used to test the in vitro susceptibility of *C. trachomatis* and *C. pneumoniae* to Polyphenon 70S. In the post-inoculation method, the minimum inhibitory concentration (MIC) assay was used to determine susceptibility to an antimicrobial agent (12,13). Briefly, approximately 1.0×10^4 inclusion forming units (IFU) of *C. trachomatis* or *C. pneumoniae* were added to each well of a microtiter plate containing a monolayer of HeLa229 cells or HL cells. The inoculum was then centrifuged onto the cells at 1,500 rpm for 60 min. After the inoculum was removed, culture medium with serial dilutions of Polyphenon 70S was added and incubated for 72 h. The culture medium used was the Eagle's minimum essential medium containing 10% fetal calf serum and 0.6 μg/ml cycloheximide. The cells were then fixed with methanol and stained with FITC-conjugated anti-chlamydial monoclonal antibody (Denka Seiken Co., Ltd., Tokyo). Inclusions were counted by fluorescent microscope and the condition of the cells were noted. At least 3 wells/dilution were tested and the average inclusion counts calculated. The numbers of inclusions were compared with the control wells containing no Polyphenon 70S and the ratio was determined. If there was no effect on chlamydial proliferation, the ratio was 1.0. Alternately, the ratio was expected to be 0 in the case of complete inhibition.

In the pre-inoculation method, 1.0×10^4 IFU of *C. trachomatis* or *C. pneumoniae* were incubated at 35°C for 30, 60, and 90 min with dilution of Polyphenon 70S. Controls were incubated with the same amount of sucrose phosphate glutamate (SPG) solution for the corresponding time. Pretreated inocula were centrifuged onto cells, followed by replacement with culture media without Polyphenon 70S. The same culture medium was used in the pre-inoculation and the post-inoculation methods. The cells were fixed and stained, and the ratio of inclusions against control was determined.

In both the post-inoculation and the pre-inoculation methods, each experiment was repeated more than three times

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and the average of each ratio was recorded.

RESULTS

In the post-inoculation method, 0.25 mg/ml Polyphenon 70S reduced the ratios of inclusion counts for *C. trachomatis*, and no inclusions were observed at 0.5 and 1.0 mg/ml (Table 1). However, the toxicity of Polyphenon 70S was noted in HeLa229 cells at a concentration of 0.25 mg/ml. The same phenomenon was observed in HL cells.

In the pre-inoculation method, no toxic effects of Polyphenon 70S to HeLa229 cells or HL cells were noted. The ratio of inclusion counts decreased according to incubation time and concentration (Table 2). Complete inhibition of *C. trachomatis* was noted at 90 min incubation. There was no inclusion of serovar D at a concentration of 1.6 mg/ml. Based on this result, 90 min incubation was adopted for the pre-inoculation method. Complete inhibition was noted at a 0.4 mg/ml concentration after 90 min incubation with *C. trachomatis* L_2 (Table 3). With *C. pneumoniae* strains, the end points were 0.8 and 1.6 mg/ml for AR-39 and AC-43, respectively (Table 3).

Table 1. Inhibitory effect of Polyphenon 70S on serovar D/UW-3/Cx of *C. trachomatis* using the post-inoculation method

	Concentration of Polyphenon 70S (mg/ml)			
	0.125	0.25	0.5	1.0
Ratio of inclusion counts	1.08	0.38	0	0

Ratio of inclusion counts: See text.

Table 2. Inhibitory effect of Polyphenon 70S on serovar D/UW-3/Cx of *C. trachomatis* using the pre-inoculation method

Incubation time (min)	Concentration of Polyphenon 70S (mg/ml)					
	0.2	0.4	0.8	1.6	3.2	6.4
30	1.00	0.58	0.34	0.18	0.10	0.05
60	0.87	0.41	0.18	0.06	0.02	0.02
90	0.55	0.07	0.03	0	0	0

Ratios of inclusion counts are recorded.

Table 3. Inhibitory effects of Polyphenon 70S on serovars D and L_2 of *C. trachomatis*, and AR-39 and AC-43 strains of *C. pneumoniae* using the pre-inoculation method

Strains	Concentration of Polyphenon 70S (mg/ml)					
	0.1	0.2	0.4	0.8	1.6	3.2
D	nd	0.55	0.77	0.33	0	0
L_2	nd	nd	0	0	0	0
AR-39	nd	0.20	0.04	0	0	nd
AC-43	0.66	0.34	0.15	0.01	0	0

nd: not determined
Ratios of inclusion counts are recorded.

DISCUSSION

Tea polyphenols have been shown to have in vitro antimicrobial effects on the influenza virus (5, 9), *Vibrio cholerae* (7), *Staphylococcus aureus* (10), *Campylobacter jejuni*, *C. coli* (6), and others. Our data demonstrate that tea polyphenols have an in vitro inhibitory effect on *C. trachomatis* and *C. pneumoniae*. However, the inhibitory concentrations are relatively high compared to the MICs of antibiotics such as tetracyclines.

Ikigai et al. indicated that catechin damages the lipid bilayer, which partly explains the greater bactericidal effect of catechin to Gram-positive bacteria than Gram-negative bacteria (8). Although both *C. trachomatis* and *C. pneumoniae* are recognized as Gram-negative bacteria, mechanisms of the inhibitory effect by treatment with tea polyphenols should be investigated further.

Polyphenols are contained in green tea at an approximate concentration of 10-15% and 5% in black tea. Tea leaves usually contain 7 to 8% of epigallocatechin gallate (EGCg), which is the dominant constituent of Polyphenon 70S and is recognized to play a major role in anti-microbial effects. Estimating the concentration in a cup of green tea at 3%, the expected concentration of EGCg would be 2.1 to 2.4 mg/ml. Some reports indicate a possible clinical usage of tea extracts; they suggest oral administration for prevention of food poisoning (14).

It would be difficult to expect clinical effects by oral intake of tea polyphenols on infections other than intestinal infections. However, Lee et al. indicated that plasma concentration of EGCg after ingestion of 1.2 g of decaffeinated green tea was only 46-268 ng/ml (15). Tea polyphenols have inhibitory effects on human papilloma virus, and a cooperative study between China and Japan indicated that ointment containing tea polyphenol was effective against condyloma acuminata (11). These findings encourage the application of polyphenols for topical usage because teas are available worldwide. Considering that *C. trachomatis* causes endocervicitis, urethritis, conjunctivitis and afebrile pneumonia in infants, and that *C. pneumoniae* is a respiratory pathogen, which causes pneumonia and bronchitis, topical usage of polyphenols should be beneficial. For example, washing with solutions containing tea polyphenols could have potential clinical use for cervical infection caused by *C. trachomatis* and inhalation therapy using a nebulizer for respiratory tract infections caused by *C. pneumoniae*.

This study indicates that a component of Polyphenon 70S has an inhibitory effect on the proliferation of both *C. trachomatis* and *C. pneumoniae*. In order to more specifically determine which components have inhibitory effects, each constituent should be examined further.

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