ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, June 2005, p. 2501–2503 Vol. 49, No. 6
0066-4804/05/$08.00+0 doi:10.1128/AAC.49.6.2501–2503.2005

Biosynthesized Tea Polyphenols Inactivate *Chlamydia trachomatis* In Vitro

Tsutomu Yamazaki,[1,2]* Toshio Kishimoto,[1] Sadashi Shiga,[1] Kozue Sato,[1] Toshikatsu Hagiwara,[1] Miyuki Inoue,[2] Nozomu Sasaki,[2] Kazunobu Ouchi,[3] and Yukihiko Hara[4]

Department of Virology 1, National Institute of Infectious Diseases, Tokyo,[1] Department of Pediatrics, Saitama Medical School, Saitama,[2] Department of Pediatrics 2, Kawasaki Medical School, Kurashiki,[3] and Food Research Laboratories, Mitsui Norin Co., Shizuoka,[4] Japan

Received 2 September 2004/Returned for modification 23 November 2004/Accepted 4 February 2005

Biosynthesized tea polyphenols showed antichlamydial activity against *Chlamydia trachomatis* D/UW-3/Cx and L_2/434/Bu using cell culture. The most active compounds were (−)-epigallocatechin gallate and (−)-epicatechin gallate, followed by (−)-epicatechin (EC). (+)-Epicatechin and (−)-epigallocatechin were intermediate. EC was the least toxic. These results warrant evaluation of tea polyphenols as topical antichlamydial agents.

Chlamydia trachomatis is one of the most common causes of sexually transmitted diseases (5). Results obtained with topical antichlamydial agents such as nonoxynol-9 (3, 8, 10), monocaprin (4), C31G (14), cecropin peptides (1), and protegrins (17) have been reported. Disinfectants containing products such as chlorhexidine gluconate gel (9) and the spermicide benzalkonium chloride (2) have also been shown to inactivate *C. trachomatis*. We have previously reported that tea extracts have antichlamydial activity in vitro and that the active compounds are polyphenols (16).

Five tea polyphenols (Fig. 1), (+)-catechin (Catech), (−)-epicatechin (EC), (−)-epigallocatechin (EGC), (−)-epicatechin gallate (ECg), and (−)-epigallocatechin gallate (EGCg), were tested in this study. These chemicals were biosynthesized naturally and supplied by Mitsui Norin Co., Ltd. (Tokyo, Japan).

The chlamydial strains tested were *C. trachomatis* D/UW-3/Cx and L_2/434/Bu. HeLa 229 cells were used for growing *C. trachomatis*. A preinoculation minimal cidal concentration method, previously described by Lampe et al. (9), was used to test the in vitro susceptibility of *C. trachomatis* to tea polyphenols. A total of 1 ml of culture medium containing 2×10^5 HeLa 229 cells per ml was dispensed into each well of a plastic 24-well culture plate and incubated in 5% CO_2 at 37°C for 24 h to form a confluent monolayer. Then, 1.0×10^4 inclusion-forming units of *C. trachomatis* were incubated with serial dilutions of tea polyphenols at 35°C for 90 min. Controls were incubated with sucrose phosphate glutamate (SPG) buffer containing no test compounds. Pretreated inocula were centrifuged onto the cells at 1,500 rpm for 60 min. After centrifugation, the inocula were removed. Culture medium containing no tea polyphenols was added and incubated for 72 h. Eagle's minimum essential medium containing 10% fetal calf serum and 0.6 μg/ml cycloheximide was used as the culture medium. The cells were fixed with methanol and stained with fluorescein isothiocyanate-conjugated antichlamydial monoclonal antibody (Denka Seiken Co., Ltd., Tokyo, Japan). Inclusions were counted by a fluorescent microscope. At least three wells per dilution were tested, and each experiment was repeated at least three times.

The toxic effects of EC, ECg, and EGCg on HeLa 229 cells were examined by using a CK01 cell counting kit (Dojindo Laboratory Co., Ltd., Kumamoto, Japan), a colorimetric assay for cell proliferation and viability. Cell activity was determined according to the manufacturer's instructions. Briefly, serial dilutions of tea polyphenols in SPG buffer were dispensed into 96-well microtiter plates containing a monolayer of HeLa 229

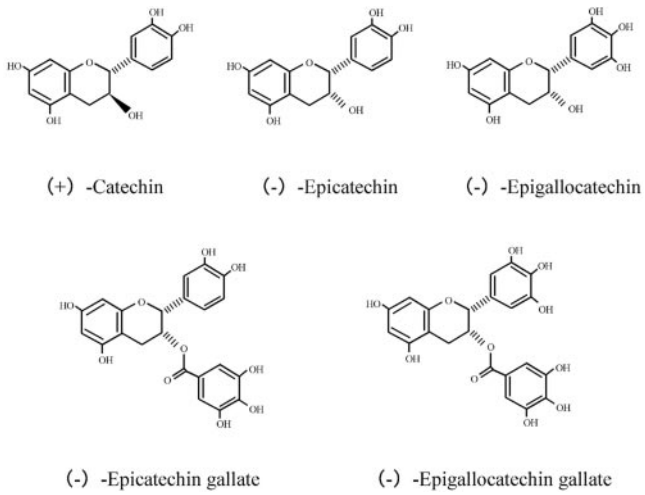

FIG. 1. Structural formula of the biosynthesized tea polyphenols. Five biosynthesized compounds, (+)-catechin, (−)-epicatechin, (−)-epigallocatechin, (−)-epicatechin gallate, and (−)-epigallocatechin gallate, were evaluated. (−)-Epigallocatechin is produced by hydroxylation of (−)-epicatechin. (−)-Epicatechin gallate and (−)-epigallocatechin gallate are synthesized by esterification with gallic acid.

* Corresponding author. Mailing address: Department of Virology 1, National Institute of Infectious Diseases, 1-23-1, Toyama, Shinjuku, Tokyo, Japan 162-8640. Phone: 81-3-5285-1111. Fax: 81-3-5285-1208. E-mail: tochaben@gray.plala.or.jp.

TABLE 1. Inhibitory effect of tea polyphenols on serovars D/UW-3/Cx and L_2/434/Bu of *C. trachomatis* in the preinoculation minimal cidal concentration method

Compound[a]	Drug concn (mg/ml) for complete (100%) inhibition	
	D/UW-3/Cx	L_2/434/Bu
Catech	>6.4[b]	>3.2[b]
EC	>6.4[b]	0.4
EGC	>6.4[b]	>6.4[b]
ECg	1.6	0.4
EGCg	0.8	0.8

[a] Catech, (+)-catechin; EC, (−)-epicatechin; EGC, (−)-epigallocatechin; ECg, (−)-epicatechin gallate; EGCg, (−)-epigallocatechin gallate.
[b] > indicates the highest concentration tested.

cells and incubated for 60 min at 35°C. After removal of tea polyphenols, cell activity was determined. After being fixed with methanol and stained using Giemsa stain, the condition of the cells was assessed using a microscope. The integrity of the cell monolayer and morphological changes in the cells, such as a round shape, were evaluated.

All tea polyphenols tested had an inhibitory effect on chlamydial proliferation (Table 1). ECg and EGCg completely inhibited the proliferation of *C. trachomatis* serovar D at concentrations of 1.6 and 0.8 mg/ml, respectively. Complete inactivation was also noted for *C. trachomatis* serovar L_2 after incubation with EC, ECg, and EGCg at concentrations of 0.4, 0.4, and 0.8 mg/ml, respectively.

The activity of HeLa 229 cells incubated with EC, ECg, and EGCg is shown in Fig. 2. After 60 min of incubation with 0.4 mg/ml EC, cell activity did not decrease and no differences in the condition of the cells were observed after Giemsa staining. By contrast, after 60 min of incubation with increasing concentrations of ECg and EGCg, the activity of HeLa 229 cells decreased and at high concentrations of ECg and EGCg the cells had a round shape.

Tea polyphenols have been shown to have in vitro antimicrobial effects (6, 11-13, 15). We previously reported that Polyphenon 70S, a mixed compound of tea polyphenols, had an in vitro inhibitory effect on *C. trachomatis* (16). Polyphenon 70S is composed of EGCg, EGC, ECg, EC, and (−)-gallocatechin gallate. Each individual constituent had an inhibitory effect at a lower concentration than that in Polyphenon 70S: the endpoint for *C. trachomatis* serovar D was 1.6 mg/ml when incubated with Polyphenon 70S and 0.8 mg/ml when incubated with EGCg. These data suggest that the individual constituents of tea polyphenols, such as EC, are potential candidates for antichlamydial drugs.

The concentration of tea polyphenols required for complete inhibition of chlamydial proliferation is relatively high compared with the MIC of antibiotics such as tetracyclines, macrolides, and fluoroquinolones (16). Therefore, oral administration of tea polyphenols is not suitable for treating systemic infection. Each tea polyphenol constituent might be used topically. The development of more-effective drugs for systemic use by modifying the structures of EC, ECg, and EGCg is expected in the future.

Nonoxynol-9 is an active spermicidal ingredient used in a wide variety of vaginal contraceptive preparations. Products containing nonoxynol-9 also inhibit the growth of *C. trachoma-*

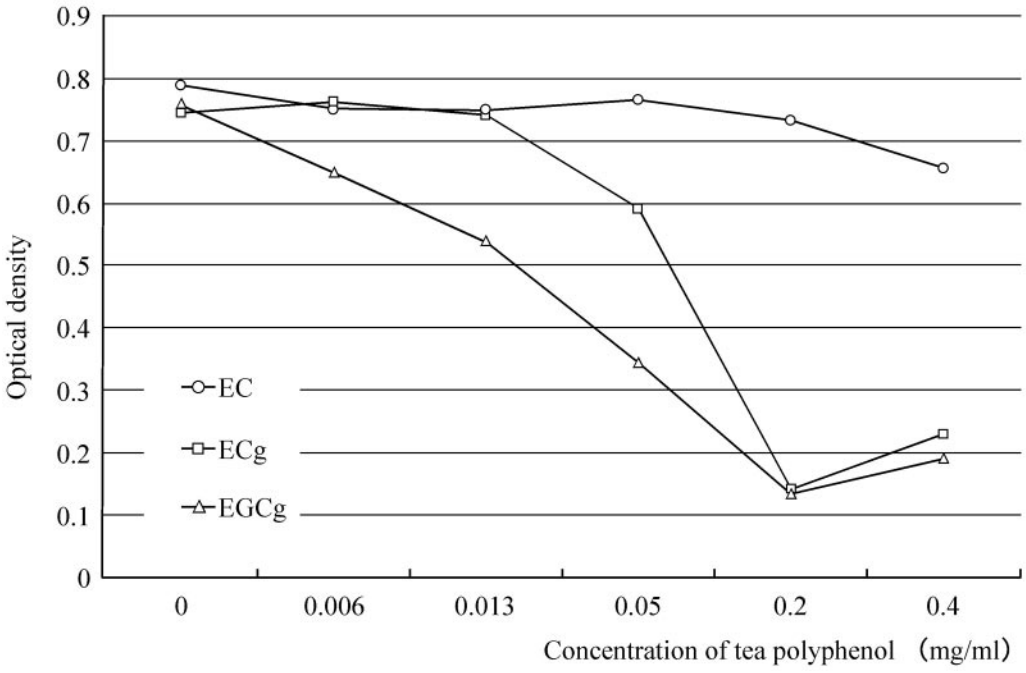

FIG. 2. Activity of HeLa 229 cells incubated with (−)-epicatechin (EC), (−)-epicatechin gallate (ECg), and (−)-epigallocatechin gallate (EGCg) evaluated by a CK01 cell counting kit, a colorimetric assay for cell proliferation. Serial dilutions of tea polyphenols in SPG buffer were incubated with HeLa 229 cells for 60 min, and the cell activity was determined. Cell activity was assessed by the optical density. The activity of the HeLa 229 cells did not decrease after 60 min of incubation with 0.4 mg/ml EC but did decrease after 60 min of incubation with ECg and EGCg at the concentrations used.

tis. Lampe et al. indicated that a chlorhexidine gluconate gel could remain in the vagina for hours after topical application and provided protection against *C. trachomatis* infection for that time period (9). Addition of tea polyphenols to contraceptive jelly could have potential clinical use in the prevention of cervical infection caused by *C. trachomatis*. Of note, EC was the least toxic among the tea polyphenols that inactivated chlamydial strains. Further studies are required to clarify the safety of tea polyphenols for clinical usage.

Ikigai et al. indicated that EGCg damaged bacterial membranes (7). The inhibitory mechanisms of tea polyphenols against *C. trachomatis* are unknown and should be investigated further.

REFERENCES

1. **Ballweber, L. M., J. E. Jaynes, W. E. Stamm, and M. F. Lampe.** 2002. In vitro microbicidal activities of cecropin peptides D2A21 and D4E1 and gel formulations containing 0.1 to 2% D2A21 against *Chlamydia trachomatis*. Antimicrob. Agents Chemother. **46:**34–41.
2. **Bélec, L., C. Tevi-Benissan, A. Bianchi, S. Cotigny, M. Beumont-Mauviel, A. Si-Mohamed, and J.-E. Malkin.** 2000. In vitro inactivation of *Chlamydia trachomatis* and of a panel of DNA (HSV-2, CMV, adenovirus, BK virus) and RNA (RSV, enterovirus) viruses by the spermicide benzalkonium chloride. J. Antimicrob. Chemother. **46:**685–693.
3. **Benes, S., and W. M. McCormack.** 1985. Inhibition of growth of *Chlamydia trachomatis* by nonoxynol-9 in vitro. Antimicrob. Agents Chemother. **27:**724–726.
4. **Bergsson, G., J. Arnfinnsson, S. M. Karlsson, Ó. Steingrímsson, and H. Thormar.** 1998. In vitro inactivation of *Chlamydia trachomatis* by fatty acids and monoglycerides. Antimicrob. Agents Chemother. **42:**2290–2294.
5. **Centers for Disease Control and Prevention.** 1997. *Chlamydia trachomatis* genital infections—United States, 1995. Morb. Mortal. Wkly. Rep. **46:**193–198.
6. **Diker, K. S., M. Akan, G. Hascelik, and M. Yurdakök.** 1991. The bactericidal activity of tea against *Campylobacter jejuni* and *Campylobacter coli*. Lett. Appl. Microbiol. **12:**34–35.
7. **Ikigai, H., T. Nakae, Y. Hara, and T. Shimamura.** 1993. Bactericidal catechins damage the lipid bilayer. Biochim. Biophys. Acta **1147:**132–136.
8. **Kelly, J. P., R. B. Reynolds, S. Stagno, W. C. Louv, and W. J. Alexander.** 1985. In vitro activity of the spermicide nonoxynol-9 against *Chlamydia trachomatis*. Antimicrob. Agents Chemother. **27:**760–762.
9. **Lampe, M. F., L. M. Ballweber, and W. E. Stamm.** 1998. Susceptibility of *Chlamydia trachomatis* to chlorhexidine gluconate gel. Antimicrob. Agents Chemother. **42:**1726–1730.
10. **Lyons, J. M., and J. I. Ito, Jr.** 1995. Reducing the risk of *Chlamydia trachomatis* genital tract infection by evaluating the prophylactic potential of vaginally applied chemicals. Clin. Infect. Dis. **21**(Suppl. 2)**:**S174–S177.
11. **Nakayama, M., M. Toda, S. Okubo, and T. Shimamura.** 1990. Inhibition of influenza virus infection by tea. Lett. Appl. Microbiol. **11:**38–40.
12. **Nakayama, M., K. Suzuki, M. Toda, S. Okubo, Y. Hara, and T. Shimamura.** 1993. Inhibition of the infectivity of influenza virus by tea polyphenols. Antivir. Res. **21:**289–299.
13. **Toda, M., S. Okubo, H. Ikigai, T. Suzuki, Y. Suzuki, and T. Shimamura.** 1991. The protective activity of tea against *Vibrio cholerae* O1. J. Appl. Bacteriol. **70:**109–112.
14. **Wyrick, P. B., S. T. Knight, D. G. Gerbig, Jr., J. E. Raulston, C. H. Davis, T. R. Paul, and D. Malamud.** 1997. The microbicidal agent C31G inhibits *Chlamydia trachomatis* infectivity in vitro. Antimicrob. Agents Chemother. **41:**1335–1344.
15. **Yam, T. S., J. M. T. Hamilton-Miller, and S. Shah.** 1998. The effect of a component of tea (*Camellia sinensis*) on methicillin resistance, PBP2′ synthesis, and β-lactamase production in *Staphylococcus aureus*. J. Antimicrob. Chemother. **42:**211–216.
16. **Yamazaki, T., M. Inoue, N. Sasaki, T. Hagiwara, T. Kishimoto, S. Shiga, M. Ogawa, Y. Hara, and T. Matsumoto.** 2003. In vitro inhibitory effects of tea polyphenols on the proliferation of *Chlamydia trachomatis* and *Chlamydia pneumoniae*. Jpn. J. Infect. Dis. **56:**143–145.
17. **Yasin, B., S. S. L. Harwig, R. I. Lehrer, and E. A. Wagar.** 1996. Susceptibility of *Chlamydia trachomatis* to protegrins and defensins. Infect. Immun. **64:**709–713.