PERGAMON

Phytochemistry 53 (2000) 391–394

PHYTOCHEMISTRY

www.elsevier.com/locate/phytochem

Importance of a pyrogallol-type structure in catechin compounds for apoptosis-inducing activity

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Received 13 July 1999; accepted 20 September 1999

Abstract

Several catechin compounds were examined for their ability to induce apoptosis in human hystiocytic lymphoma U937 cells. Catechins with a pyrogallol-type structure in a B-ring induced apoptosis and a 3-*O*-gallate group in *cis*-relationship to the B ring enhanced the activity. Catechins without a pyrogallol-type structure in a molecule lacked activity. These data suggest the important role of the 5′(3′)-hydroxyl group in the B-ring and that a pyrogallol-type structure in a molecule is a minimum requirement for apoptosis induction by catechin compounds.

Keywords: *Camellia sinensis*; Theaceae; Apoptosis; Tea catechins; Polyphenol structure

1. Introduction

Catechins are a group of polyphenolic compounds which occur naturally in certain species of plants including tea (*Camellia sinensis*) and are major ingredients in the green tea infusion (Yoshizawa, Horiuchi, Fujiki, Yoshida, Okuda & Sugimura, 1987; Fujita et al., 1989). Recently, green tea catechins have been shown to induce apoptosis in human lymphoblastic leukaemia Molt 4B cells (Hibasami, Achiwa, Fujikawa & Komiya, 1996; Achiwa, Hibasami, Katsuzaki, Imai & Komiya, 1997), promyelocytic leukaemia HL-60 cells (Zhao, Cao, Ma & Liu, 1997), stomach cancer KATO III cells (Hibasami et al., 1998), and other human cancer cell lines (Yang, Liao, Kim & Yurkow, 1998). (−)-Epigallocatechin gallate (EGCg) induced apoptosis effectively, while the induction was very weak by (+)-catechin and (−)-epicatechin which lack a galloyl group (Hibasami et al., 1996; Achiwa et al., 1997), suggesting a certain structure-function relationship in apoptosis-inducing activity. Furthermore, it is noteworthy that (−)-epigallocatechin, a 5′-(or 3′-) hydroxyl derivative of (−)-epicatechin is active (Hibasami et al., 1996). Therefore, it is suggested that a pyrogallol-type structure in a B-ring may contribute to the apoptosis-inducing activity.

In the present work, we examined several polyphenolic compounds derived from tea catechins (Fig. 1) for apoptosis-inducing activity in human hystiocytic lymphoma U937 cells.

2. Results and discussion

When U937 cells were incubated with EGCg, DNA fragmentation in a nucleosome unit was induced (Fig. 2) as reported previously (Saeki et al., 1999). This fragmentation was inhibited by a caspase inhibitor Z-Asp-CH_2-DCB (Mashima, Naito, Kataoka, Kawai & Tsuruo, 1995) (Fig. 2). Caspase is known to play an essential role in apoptosis (Mashima et al., 1995). We have reported that EGCg also induced a chromatin condensation (Saeki et al., 1999), which is one of the

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0031-9422/00/$ - see front matter
PII: S0031-9422(99)00513-0

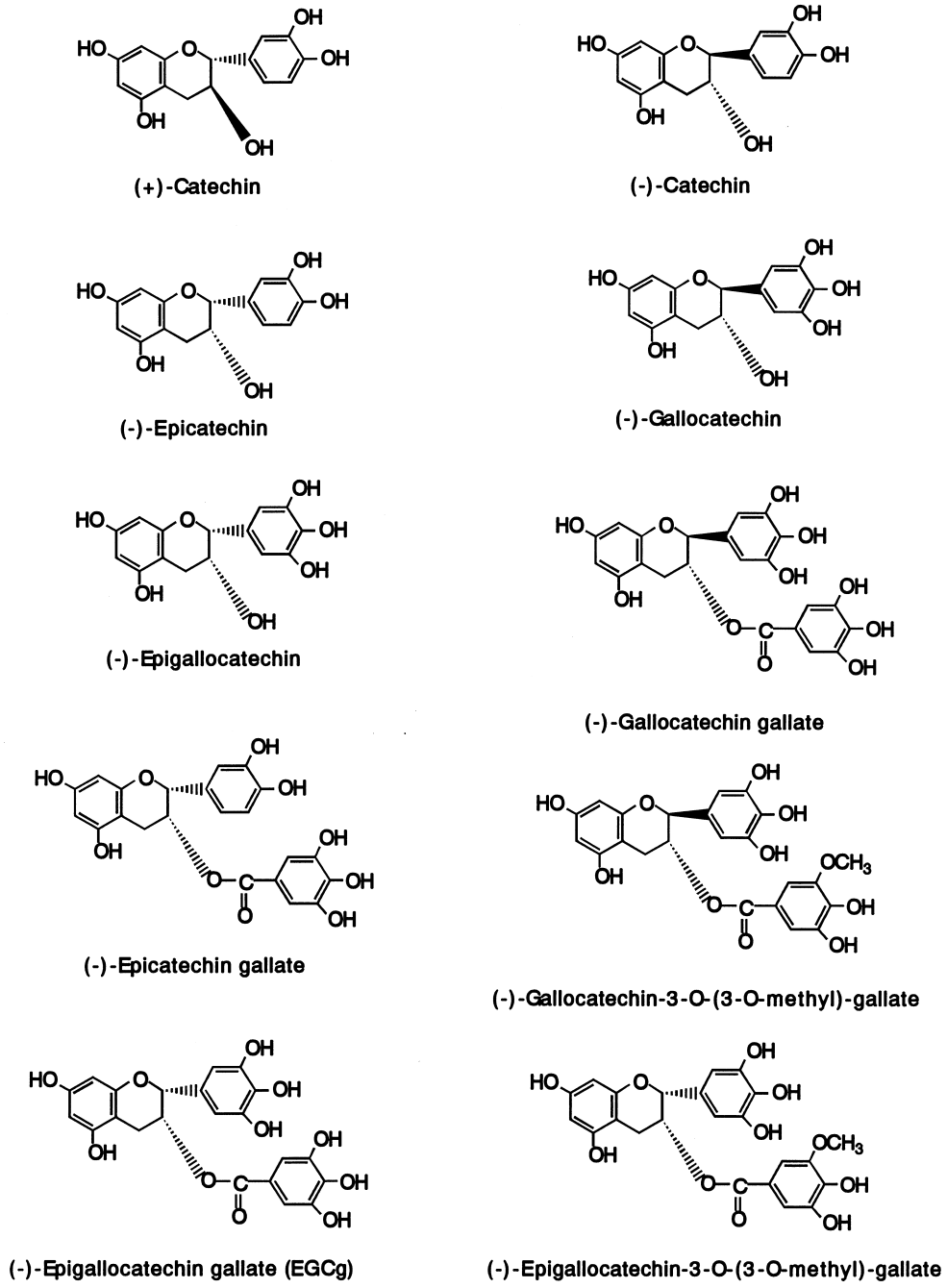

Fig. 1. Chemical structures of catechin compounds.

characteristic features of apoptosis (Darzynkiewicz, Li & Gong, 1994). None of (+)-catechin, (−)- catechin and (−)-epicatechin induced DNA fragmentation, but other catechin compounds tested exhibited the activity (Fig. 2). The DNA fragmentation by these compounds was inhibited by Z-Asp-CH_2-DCB (Fig. 2), indicating that they induced apoptosis in U937 cells.

The fluoroimaging analysis indicated that the degree of DNA fragmentation was concentration-dependent (Fig. 3). The data indicated that catechins with a pyrogallol-type structure in a B ring exhibited positive activity, but that those without a pyrogallol-type structure in any position of the catechin structure did not. (−)-Epicatechin-3-*O*-gallate showed apoptosis-inducing activity (Figs. 2 and 3), though weakly, as has been shown (Hibasami et al., 1996; Achiwa et al., 1997). Thus, a pyrogallol-type structure either in a B-ring or in 3-*O*-ester appears to be a minimum requirement for apoptosis induction.

A 3-*O*-gallate residue with *cis*-relationship to the B-

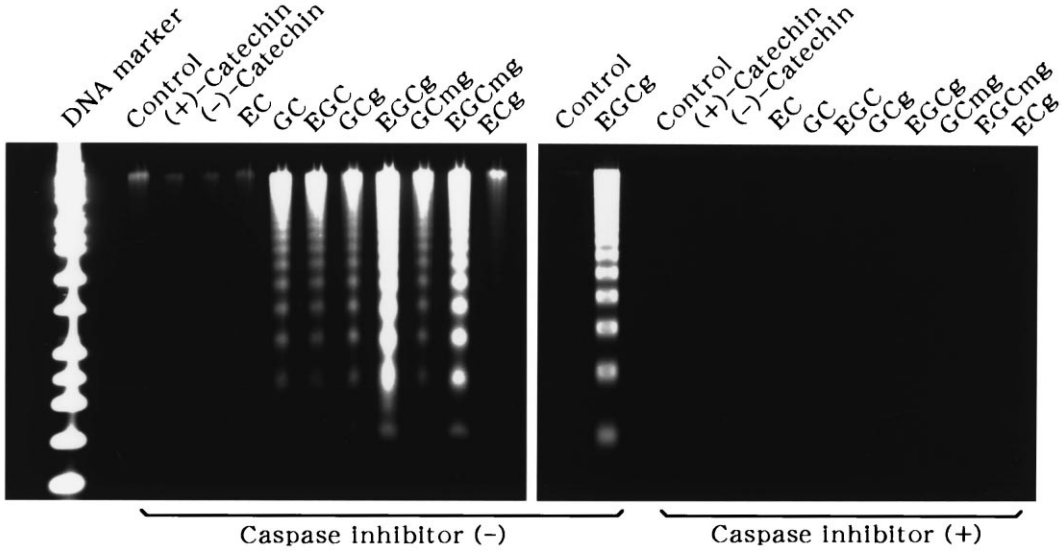

Fig. 2. Fragmentation of DNA from U937 cells treated with catechin compounds. U937 cells were incubated with 400 μM of catechin compounds at 37°C for 16 h in the absence (−) or presence (+) of caspase inhibitor Z-Asp-CH_2-DCB (200 μM) and results were compared with those from untreated cells (control). EC, (−)-epicatechin; GC, (−)-gallocatechin; EGC, (−)-epigallocatechin; GCg, (−)-gallocatechin gallate; GCmg, (−)-gallocatechin-(3-*O*-methyl)-gallate; EGCmg, (−)-epigallocatechin-(3-*O*-methyl)-gallate; ECg, (−)-epicatechin gallate.

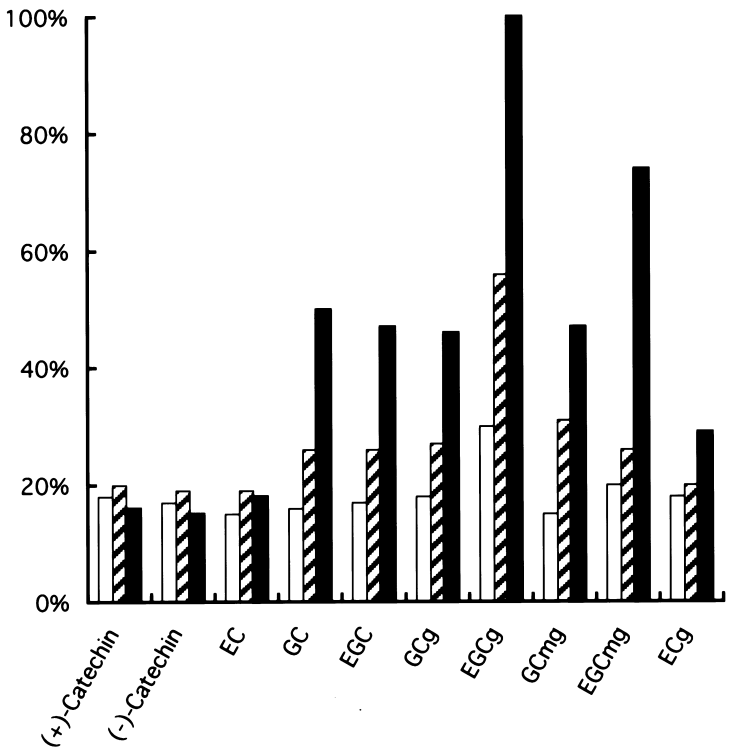

Fig. 3. Concentration-dependent induction of DNA fragmentation by catechin compounds. Degrees of DNA fragmentation were determined by FluorImager which measured fluorescence intensity of each lane in agarose gels stained with SYBR™ Green I. Results are expressed as percentage relative to the value for EGCg at 400 μM (ordinate). For abbreviations used for catechin compounds see Fig. 2. White bars, 100 μM; Latched bars, 200 μM; black bars, 400 μM.

ring enhanced the activity and the destruction of a pyrogallol-type structure by its methylation reduced this effect. In contrast, a 3-*O*-gallate residue in *trans*-relationship to the B-ring had little effect.

Several chemotherapeutic compounds have been reported to induce apoptosis and apoptosis may be a primary mechanism of their anti-cancer activity (Gunji, Kharbanda & Kufe, 1991). Thus, the present data would provide useful information in developing anti-cancer agents.

3. Experimental

3.1. Reagents

U937 cells were obtained from Health Service Research Resources Bank, Osaka, Japan, and cultured in 10% fetal bovine serum in RPMI 1640 medium (Iwaki Glass Co., Chiba, Japan) with 50 U/ml penicillin, 50 μg/ml streptomycin, 2.5 μg/ml amphotericin B, and 50 μg/ml gentamycin at 37°C under 5% CO_2. A caspase inhibitor Z-Asp-CH_2-DCB was obtained from Peptide Institute, Osaka, Japan. Hoechst 33342 (bisbenzimide H 33342 Fluorochrome) was obtained from Calbiochem–Novabiochem. Co., CA, USA. SYBR® Green I was obtained from Molecular Probes, OR, USA. (−)-Epigallocatechin-3-*O*-(3-*O*-methyl)-gallate was prepared from green tea as described previously (Sano, Suzuki, Miyase, Yoshino & Yamamoto, 1999). (−)-Gallocatechin-3-*O*-(3-*O*-methyl)-gallate was isolated from the polyphenolic fraction of oolong tea made from *Benihomare* (*Camellia sinensis*) by preparative HPLC (column, Develosil ODS 5 × 50 cm; solvent, CH_3CN–H_2O (12 : 88), UV, 280 nm). Amorphous powder. $[\alpha]_D$ −58° (*c* 0.15, MeOH). FABMS *m/z*: 473 $[M + H]^+$. ^{1}H-NMR (acetone-d_6): δ 2.77 (1H, *dd*, *J* = 17, 6 Hz, H-4β), 2.98 (1H, *dd*, *J* = 17, 5.5 Hz, H-4α), 3.83 (3H, *s*, OMe), 5.07 (1H, *d*, *J* = 6 Hz, H-2), 5.32 (1H, *ddd*, *J* = 6, 6, 5.5 Hz, H-3), 5.99 (1H, *d*, *J* = 2 Hz, H-8), 6.08 (1H, *d*, *J* = 2 Hz, H-6), 6.54 (2H, *s*, H-2′, H-6′), 7.07 (1H, *d*, *J* = 2 Hz, H-2″), 7.16 (1H, *d*, *J* = 2 Hz, H-6″). ^{13}C-NMR (acetone-d_6): δ 24.7 (C-4), 56.6 (OMe), 71.0 (C-3), 79.1 (C-2), 95.6 (C-8), 96.5 (C-6), 99.3 (C-4a), 106.0 (C-2″), 106.6 (C-2′, C-6′), 111.7 (C-6″), 121.7 (C-1″), 130.8 (C-1′), 133.4 (C-4′), 139.9 (C-4″), 145.8 (C-5″), 146.5 (C-3′, C-5′), 148.5 (C-3″), 156.4 (C-8a), 157.2 (C-5), 158.0 (C-7), 166.1 (C-7″). Other catechin compounds tested were purchased from Funakoshi Co., Tokyo, Japan.

3.2. DNA fragmentation

U937 cells were incubated in a culture medium in the presence or absence of test compounds for 6 h at 37°C in a CO_2 incubation. For DNA fragmentation analysis, 5×10^5 cells were pelleted by centrifugation and DNA was isolated from the cell pellets as described by Sellins & Cohen (1987). DNA was subjected to electrophoresis in 2% agarose gels, stained with SYBR® Green I, and imaged and calculated by using FluorImager (Molecular Dynamics Japan, Tokyo, Japan).

3.3. Effects of a caspase inhibitor

In order to confirm the apoptosis-associated DNA fragmentation, cells were incubated in the presence of test compounds with a caspase inhibitor Z-Asp-CH_2-DCB (200 μM) at 37°C for 16 h, and DNA fragmentation was examined.

Acknowledgements

This study was supported in part by the Program for Promotion of Basic Research Activities for Innovative Biosciences from the Japan Science Society.

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