Activity-Guided Fractionation of Green Tea Extract with Antiproliferative Activity against Human Stomach Cancer Cells

Junei Kinjo,* a Tsuneatsu Nagao, a Takashi Tanaka, a Gen ichiro Nonaka, a Masafumi Okawa, a Toshihiro Nohara, a and Hikaru Okabe a

a Faculty of Pharmaceutical Sciences, Fukuoka University; 8–19–1 Nanakuma, Jonan-ku, Fukuoka 814–0180, Japan; b School of Pharmaceutical Sciences, Nagasaki University; 1–14 Bunkyomachi, Nagasaki 852–8521, Japan; c Usaien Pharm. Co., Ltd.; 1–3–17 Zaimoku, Saga 840–0055, Japan; and d Faculty of Pharmaceutical Sciences, Kumamoto University; 5–1 Oe-honmachi, Kumamoto 862–0973, Japan. Received March 4, 2002; accepted May 16, 2002

Epidemiological studies have suggested that the consumption of green tea provides protection against stomach cancer. Fractionation of green tea extract, guided by antiproliferative activity against human stomach cancer (MK-1) cells, has resulted in the isolation of six active flavan-3-ols, epicatechin (EC), epigallocatechin (EGC), epigallocatechin gallate (EGCg), gallochatechin (GC), epicatechin gallate (ECg), gallochatechin gallate (GCg), together with inactive glycosides of kaempferol and quercetin. Among the six active flavan-3-ols, EGCg and GCg showed the highest activity, EGC, GC, ECg followed next, and the activity of EC was lowest. These data suggest that the presence of the three adjacent hydroxyl groups (pyrogallol or galloyl group) in the molecule would be a key factor for enhancing the activity. Since reactive oxygen species play an important role in cell death induction, radical scavenging activity was evaluated using the DPPH (1,1-diphenyl-2-picrylhydrazyl) radical. The order of scavenging activity was ECg ª EGCg ª EGC ª GCg ª GC ª EC. The compounds having a galloyl moiety showed more potent activity. The contribution of the pyrogallol moiety in the B-ring to the scavenging activity seemed to be less than that of the galloyl moiety.

Key words  green tea; human stomach cancer; flavan-3-ol; antiproliferative activity; radical scavenging activity

Green tea [Camellia sinensis (L.) O. Kuntze, Theaceae] has a longstanding reputation in Asia for its health-promoting properties. Epidemiological studies have suggested that the consumption of green tea provides protection against stomach cancer. In a rural area of northern Kyushu, Japan, a decreased risk of stomach cancer was also noted among cases reporting a high consumption of green tea. Numerous in vitro and in vivo studies on green tea preparations have demonstrated the antmutagenic, antitumorigenic, and antioxidant properties of the flavan-3-ols which form the majority of soluble tea constituents. However, although other ingredients such as caffeine and flavonol glycosides are also contained in green tea, there is no report in regard to antiproliferative activity-guided fractionation using human stomach cancer cells.

In the course of our continuing studies on the antiproliferative activity in plants, we found that a hot water extract of green tea leaves inhibits the growth of human stomach cancer (MK-1) cells. This paper deals with the isolation of the active principles by activity-guided fractionation and the structure–activity relationship. Further, since reactive oxygen species play an important role in cell death induction, radical scavenging activity of the isolated antiproliferative compounds were also tested and compared to determine the correlation between the two activities.

MATERIALS AND METHODS

Materials The processed green tea leaves (Camellia sinensis, Theaceae) were supplied by Saga Tea Experimental Station, Ureshino, Saga Prefecture, Japan.

Extraction and Isolation The processed green tea leaves (200 g) were extracted with hot water. The detailed procedure for the fractionation of the extract and isolation of the constituents is shown in Chart 1.
and kaempferol 3-O-glucosyl-(1→3)-rhamnosyl-(1→6)-glucoside, respectively, by comparison of their physical data with those reported. Compound 9 was identified as a mixture of quercetin 3-O-glucosyl-(1→3)-rhamnosyl-(1→6)-galactoside and quercetin 3-O-glucosyl-(1→3)-rhamnosyl-(1→6)-glucoside.

The antiproliferative activity of 1—9 was determined by MTT assay, and their GI50 values are listed in Table 1. All flavan-3-ols (1—6) showed potent activity. Compounds 3 and 6 showed the highest activity of all, and 2, 4 and 5 showed moderate activity. Compound 1 showed the lowest activity. Flavonol glycosides (7—9) did not show any antiproliferative activity. Although there are some structural similarities (the substitution patterns of the hydroxy groups in A- and B-rings) between 1 and 9, compound 9 did not show any activity. Therefore, the saturated C-ring, like flavan-3-ols, might influence the antiproliferative activity against MK-1 cells.

In spite of a configurational difference at C-3, the GI50 value of 2 was the same as that of 4, and the GI50 values of 3 and 6 were equal, indicating that the configuration at C-3 would not affect the antiproliferative activity. The GI50 values of 2, 4 and 5 (gallate of 1) were about one-third that of 1. These data suggest that the presence of three adjacent hydroxyl groups (pyrogallol or galloyl group) in the molecule would be a key factor for enhancing the activity. Finally, the activity of 3, having a pyrogallol moiety in the B-ring and galloyl moiety at C-3, was five times higher than that of 1.

Inoue et al. demonstrated that three adjacent phenolic hydroxyl groups of gallic acid should be essential to cytotoxicity. They also disclosed that the cell death induced by gallic acid was accompanied by internucleosomal DNA fragmentation characteristic of apoptosis. Hibasami et al. also reported that the green tea extract and 3 showed both growth inhibition and the induction of programmed cell death (apoptosis) against human stomach cancer KATO III cells. Since reactive oxygen species play an important role in cell death induction, the DPPH radical scavenging activity of some active flavan-3-ols (1—5) was measured by ESR (Table 1). Although 1—5 showed DPPH radical scavenging activity, their potencies were different. Unexpectedly, the number of hydroxyl group was not directly proportional to the activity. The order of scavenging activity was 5 > 3 > 2 > 4 > 1. The compounds having a galloyl moiety showed more potent activity. The contribution of the pyrogallol moiety in B-ring to the scavenging activity seemed to be less than that of the galloyl moiety at C-3, although both moieties were a key factor in enhancing the antiproliferative activity.

In conclusion, the water extract of green tea showed antiproliferative activity against human stomach cancer (MK-1)
cells. By activity-guided fractionation, six flavan-3-ols (1—6) were isolated as active principles. The structure–antiproliferative activity relationship was slightly correlated with that of radical scavenging activity.

Acknowledgements The authors express their gratitude to Mr. M. Muraoka and Mr. N. Tanaka of Saga Tea Experimental Station for supplying the processed green tea leaves. This work was supported by a Grant-in-Aid for Scientific Research (C) (No. 12672080) from the Ministry of Education, Culture, Sports, Science and Technology, Japan and by funds (No. 006006) from the General Research Institute of Fukuoka University.

REFERENCES AND NOTES