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Note

A dosage design of mitomycin C tablets containing finely powdered green tea

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Abstract

We previously reported a method of preparing finely powdered green tea (PT), powder characteristics and release profiles of green tea components from PT. In this study, we performed formulation studies of PT tablets containing mitomycin C (MMC), expecting its combined antitumor effects with mitomycin C and green tea components. The hardness of PT tablets was low (22–50 N) and the disintegration time was about 180 min regardless of hardness or tabletting pressure (15–200 MPa). Perfiller®-101 improved tablet characteristics practically into 90 N of hardness and 18.5 min of disintegration time. Release rates of MMC, caffeine and EGCG from the tablets were similar, and depended on the disintegration time. PT and epigallocatechin gallate (EGCG) increased significantly in MMC uptake in Ehrlich ascites carcinoma cells as compared with the control dose-dependently in vitro.

Keywords: Green tea; Epigallocatechin gallate; Caffeine; Mitomycin C; Tablet characteristics; Ehrlich ascites carcinoma cells

We previously reported a method of preparing finely powdered green tea and its powder characteristics, in view of its possible application to solid preparations (Kurita et al., 2003). It is well known that green tea contains various biologically active components (Stagg and Millin, 1975; Mitscher et al., 1997), and it has also been reported green tea has cancer prevention and antitumor effects (Ohno et al., 1995; Mitscher et al., 1997; Fujiki et al., 2002). The most characteristic components, epigallocatechin gallate (EGCG), has anti-carcinogenic and anti-tumor effects (Wang et al., 1992; Nishida et al., 1994; Hasegawa et al., 1995; Roy et al., 2003), and theanine has a reinforcing effect on the anticancer drug doxorubicin by influx acceleration and efflux depression of drug in Ehrlich ascites carcinoma cells (Sadzuka et al., 2000). Therefore, a combination therapy of antitumor drugs with these tea components is expected to improve the clinical performance of cancer chemotherapy. In this study, we designed and prepared tablets containing finely powdered green tea, and their disintegration and the dissolution of active components were evaluated. In addition, we have investigated effects of green tea components on mitomycin C (MMC) uptake in Ehrlich ascites carcinoma cells in vitro.

Commercial green tea of medium quality was precrushed with a speed mill grinder (SA-100®: Okada Seiko Co., Ltd.) and the section passing through

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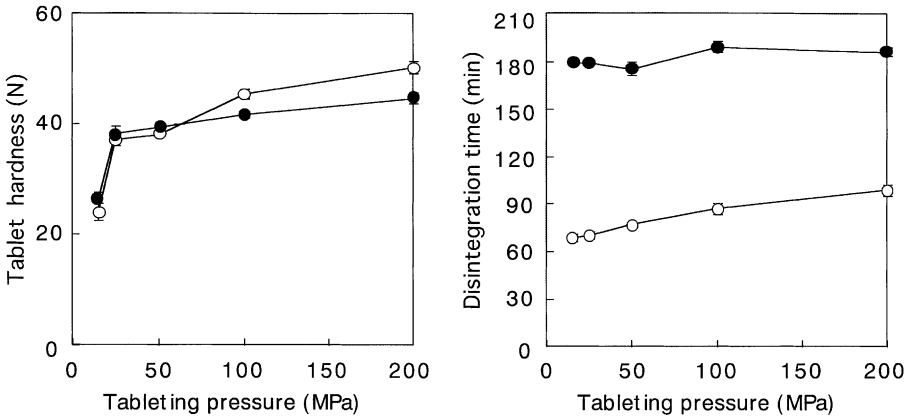

Fig. 1. Tablet hardness and disintegration time of finely powdered green tea (PT) tablets. Tablets were prepared by the direct compressing method. (●) PT (Rp.1), (○) PT with 10% CMC-Ca (Rp.2). Each point represents the mean of six determinations ± S.D.

a 1 mm sieve was collected. The precrushed green tea crushed to a fine powder with a ceramic mortar (Teafine®: Mutow Co., Ltd.) by 2 g/min. The crushed powder was sieved and the section passing through a 105-μm sieve was defined as finely powdered green tea (PT).

Fig. 1 shows the relationship between tableting pressure and the hardness or disintegration time of PT tablets. PT tablets were prepared by the direct compressing method. With an increase in the tableting pressure from 15 to 25 MPa, the hardness of PT tablets increased from 22 to 37 N. With an increase in tableting pressure from 25 to 200 MPa, the hardness of PT tablet remained at about 50 N. A further increase in tableting pressure did not increase tablet hardness.

Disintegration tests were performed according to the JP XIV (General Test Methods; Disintegration Test Methods). Distilled water was used as test fluid. The disintegration time of PT tablet remained nearly constant (about 180 min) irrespective of tableting pressure (or tablet hardness). PT became wet with the test fluid and changed to paste, covering the tablet surface, which prevented the test fluid from entering the inner core of the tablet. Therefore, PT tablets were gradually disintegrated from the surface while maintaining their shape. After addition of 10% carboxymethyl cellulose calcium (CMC-Ca, E.C.G®-505: Gotoku Chemical Co., Ltd.) as a disintegrator to PT, the disintegration time markedly decreased (about 70–90 min) to about 50% of that of PT tablet, but still not sufficient for practical use.

Addition of trehalose or lactose-corn starch mixture to PT improved the hardness and disintegration of the tablets to some extent.

Fig. 2 shows the relationship between tableting pressure and tablet hardness or disintegration time of PT tablets containing a mixture of synthetic aluminum silicate, hydroxypropyl starch and crystalline cellulose (Perfiller®-101 (PF), Japan Pharmaceutical Additive Specification, Freund Co., Ltd.). Tablet hardness of PF-added PT tablets was 46–80 N at a tableting pressure of 25 MPa, showing practical hardness. Differences in tablet hardness among PF component ratios were negligible at a tableting pressure of 25 MPa but became marked at high tableting pressures. In particular, tablet hardness sharply increased with a PF component ratio of 50% or more. This marked change in tablet hardness with tableting pressure may be due to crystalline cellulose in PF.

The disintegration time of PF-added PT tablets decreased with an increase in the PF component ratio, markedly decreasing with a PF component ratio of 50% or more. This improvement in disintegration may be due to hydroxylpropylstarch in PF and synthetic aluminium silicate. In particular, tablets with a high PF component ratio showed disintegration behavior by immediate water absorption and an increase in tablet volume. Hydroxylpropylstarch appears to be involved in the improvement of disintegration in at least tablets with a high PF component ratio. Tablets with a low PF component ratio as with PT tablets were gradually disintegrated from their circumferential core while

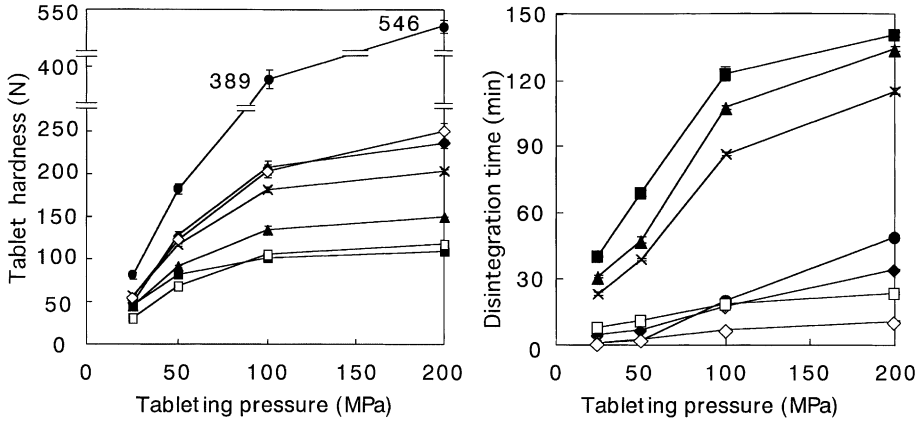

Fig. 2. Tablet hardness and disintegration time of Perfiller®-101 (PF)-added tablets. (■) 30% PF (Rp.13), (▲) 40% PF (Rp.15), (×) 45% PF (Rp.16), (◆) 50% PF (Rp.17), (●) 70% PF (Rp.19), (□) 30% PF with 10% CMC-Ca (Rp.14), (◇) 50% PF with 10% CMC-Ca (Rp.18). Each point represents the mean of six determinations ± S.D.

maintaining their shapes. At each PF component ratio, disintegration time increased with tableting pressure. In the high tableting pressure range, differences among the component ratios increased. 10% CMC-Ca-added tablets showed a marked decrease in disintegration time (from 40–140 to 8–24 min and from 5–24 to 1–11 min, respectively), with negligible changes in tablet hardness. These results suggest that the disintegration time of PF-added PT tablets depends on the amount of PF incorporated as well as tableting pressure (or tablet hardness).

Fig. 3 shows release profile of EGCG and caffeine from PF-added PT tablets (panel A) and release profile of mitomycin C (MMC), EGCG and caffeine from MMC-added PT tablet (panel B). Tablets were prepared by direct tableting (50 MPa). Release test was

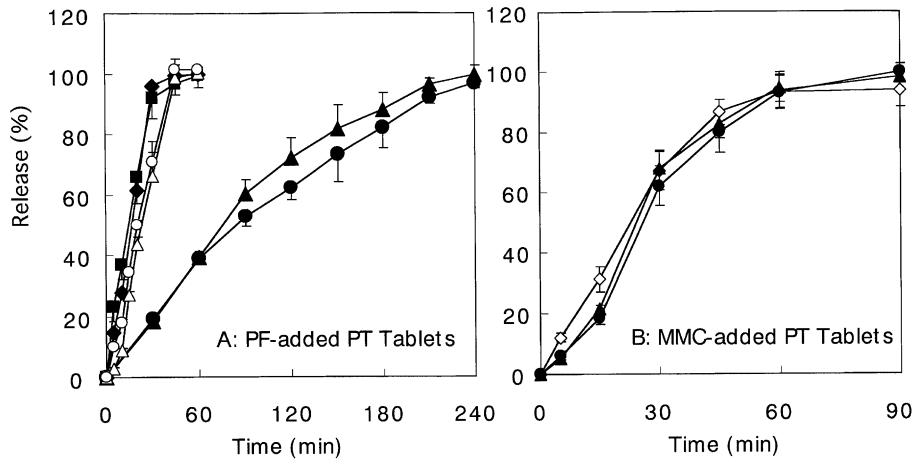

Fig. 3. Release profile of EGCG and caffeine from PF-added PT tablets (panel A) and release profile of MMC, EGCG and caffeine from MMC-added PT tablet (panel B) in distilled water at 37 °C (panel A). (●) EGCG released from 30% PF-added PT tablets, (○) EGCG released from 30% PF and 10% CMC-Ca-added PT tablets, (■) EGCG released from 50% PF-added PT tablets, (▲) caffeine released from 30% PF-added PT tablets, (△) caffeine released from 30% PF and 10% CMC-Ca-added PT tablets, (◆) caffeine released from 50% PF-added PT tablets. Each point represents the mean of four determinations ± S.D (panel B). (◇) MMC, (●) EGCG, (▲) caffeine. Each point represents the mean of four determinations ± S.D.

carried out according to JP XIV (Dissolution Test, second method). Distilled water were used for test medium and the paddle rotation speed was 100 rpm. We previously reported release of final amounts of 4.98 mg EGCG and 2.7 mg caffeine from 100 mg PT in dissolution tests using water as the test fluid (Kurita et al., 2003). The release of EGCG and caffeine from PF-added PT tablets to water (panel A) depended on the disintegration time of each type of tablets. For 50% PF-added PT tablets with a disintegration time of 18.5 min, the 50% release time of EGCG or caffeine was about 15 min. For 30% PF-added PT tablets with a disintegration time of about 125 min, the 50% release time of EGCG or caffeine was about 90 min. For 30% PF- and 10% CMC-Ca-added tablets (disintegration time; 16 min), the 50% release time was about 20 min. Thus, the release time of EGCG or caffeine can be controlled by setting the disintegration time of PT tablets.

MMC-added PT tablet (panel B) contains 0.4% MMC (Kyowa Hakko Kogyo Co., Ltd.), 49.4% PF and 50% PT (1 mg MMC/250mg tablet), and were prepared by direct tableting (50 MPa). From the MMC-added PT tablet, MMC was also released similar to EGCG and caffeine. Fifty percent release time of MMC was 18 min, and 96% MMC was released after 60 min. Release of EGCG and caffeine from the MMC-added PT tablet was almost equivalent to those from the MMC-absence PT tablet (50% PF-added tablets). This result shows that release of MMC can be controlled simultaneously with those of EGCG and caffeine.

To examine the MMC influx into Ehrlich ascites carcinoma cells, cells (1×10^7 cells/ml Eagle's MEM medium containing 10% fetal bovine serum) were incubated with 10 μg/ml MMC at 37 °C for 60 min in the presence or absence of test drugs. PT was suspended in saline and agitated for 15 min, and filtered fluid was used for the test as PT extracts. After incubation, the medium was cooled on ice and then centrifuged at $150 \times g$ for 3 min. The cells were washed and resuspended in ice-cold phosphate buffer (10 mM, pH 7.8). The suspension was mixed for 30 s with 5.0 ml of chloroform–methanol (4:1, v/v) and then centrifuged ($1200 \times g$, 15 min). The concentration of MMC in the water phase was determined with HPLC.

Fig. 4 shows the effects of PT extracts (panel A) and EGCG (panel B) on mitomycin (MMC) uptake in Ehrlich ascites carcinoma cells in vitro. The PT extracts of 5, 50 and 500 μg/ml contained about 0.55, 5.5 and 55 μM EGCG, and 0.7, 7 and 70 μM caffeine, respectively. PT extracts combined group tended to show an increase as compared with the control (saline combined group) dose-dependently, i.e., 5, 50 and 500 μg/ml PT increased the MMC concentration in cells by 12.6% ($P < 0.05$), 14.9% ($P < 0.05$)

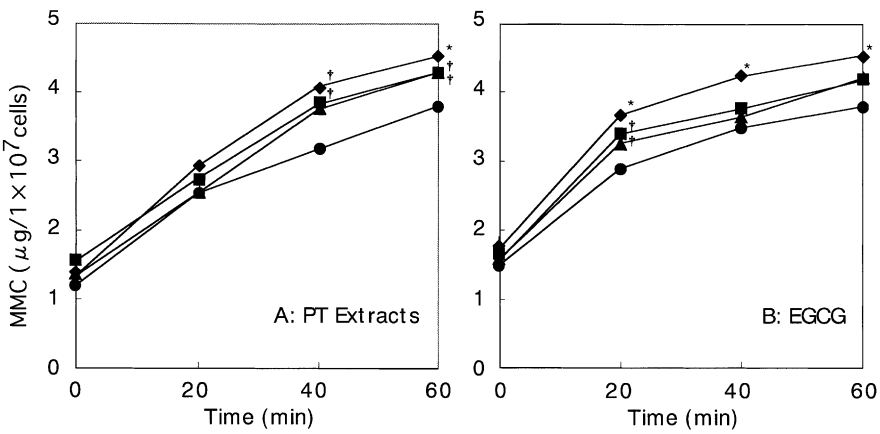

Fig. 4. Effect of green tea components on mitomycin C uptake in ehrlich ascites carcinoma cells. Ehrlich ascites carcinoma cells were incubated with 10 μg/ml mitomycin C (MMC) at 37 °C in the absence or presence of PT extracts; (●) control (saline), (▲) 5 μg/ml, (■) 50 μg/ml, (◆) 500 μg/ml (panel A), or epigallocatechin gallate (EGCG); (●) control (saline), (▲) 1 μM, (■)10 μM, (◆) 100 μM (panel B). Each point represents the mean of three or four determinations, and each S.D. is less than 10%. Significant differences from the level of control are indicated by (∗) $P < 0.01$ and (†) $P < 0.05$.

and 19.2% ($P < 0.01$), respectively after 60-min incubation (see panel A). It was unclear what component was effective or effectiveless in MMC uptake. We have found that EGCG, the most characteristic green tea components, combined group showed an increase dose-dependently, i.e., 1, 10 and 100 μM EGCG increased the MMC concentration in cells by 10.5, 12.5 and 19.3% ($P < 0.01$), respectively after 60 min incubation (see the panel B).

But caffeine, another one of the most characteristic green tea components, combined group (1, 10, and 100 μM) did not increase the MMC concentration significantly as compared with the control (data not shown). This result supports that caffeine did not increase doxorubicin uptake, however, might inhibit efflux of doxorubicin in Ehrlich ascites carcinoma cells, resultantly, enhances the efficacy of doxorubicin (Sadzuka et al., 2000).

Additionally, poorly water-soluble green tea components such as flavonoids (Ferrara et al., 2001) and saponins (Sagesaka et al., 1994) may elute from PT. Especially, poorly water-soluble flavonoids such as apigenin, kaempferol and quercetin have several activities on a cancer cell line in vitro and in vivo (Stavric, 1984; Marchand, 2002). These poorly water-soluble components exist hardly in hot water extraction of green tea leaves, that is, usual green tea beverage. Therefore, PT might be more advantageous than usual green tea beverage on combination therapy of antitumor drugs. Recently it has been reported that EGCG or other catechins are transported by multidrug resistance protein (MRP1 and MRP2) (Hong et al., 2003), and MMC is also transported by MRP2. Affector of green tea components in MRPs, and enhanced antitumor effects of MMC combined with green tea components should be resolved in continued study.

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