Effects of green tea extract administration on the pharmacokinetics of clozapine in rats

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Abstract

The pharmacokinetic interaction between clozapine, an atypical antipsychotic with metabolic complications, including weight gain, and green tea consumption has not been evaluated, although green tea is responsible for beneficial effects, including weight reduction, and is widely consumed in the world. Commercial green tea extract (175 mg kg^{-1}) or saline was administered orally for 4 days before the oral administration of clozapine (20 mg kg^{-1}) to rats. Plasma concentrations of clozapine were measured up to 5 h after clozapine administration, and then hepatic CYP1A2 expression and activity were determined. There was no significant difference in the elimination half-life of clozapine between the green tea extract and saline groups. However, the time to reach peak concentration (T_{max}) was significantly increased by green tea extract. The mean total area under the plasma concentration–time curve ($AUC_{0-\infty}$) and maximal peak plasma concentration (C_{max}) of clozapine in the green tea extract group were significantly lower than those of controls. Green tea extract induced a ∼2-fold increase in hepatic CYP1A2 levels, while the activity increased slightly (by 10% of control). Because of this reduction in AUC and T_{max} of clozapine by green tea extract pretreatment, we suggest that both the rate and amount of absorption of clozapine may be reduced by green tea extract, although the hepatic elimination phase may not be significantly altered. Therefore, the clinical implications of the effects of green tea on the bioavailability of clozapine in patients should be further evaluated.

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Introduction

Clozapine is an atypical antipsychotic with antagonistic effects not only on D_2 receptor, but also on serotonin (5-HT) receptors, histamine receptors and α-adrenoceptors (Richelson 1984; Richelson & Souder 2000). Because of the blockade of these multiple receptors and the low D_2 receptor potency in the mesostriatal pathway, clozapine lacks the extrapyramidal side effects of conventional antipsychotics. Although there is the potentially fatal complication of agranulocytosis in clozapine-treated patients, taking regular white blood cell counts can minimize the risk (Miller 2000). Furthermore, as it has a broad spectrum of efficacy in patients who are resistant to conventional antipsychotics, clozapine is widely used in psychiatric therapy, particularly in patients showing intolerable complications or resistance to conventional pharmacotherapy. However, it appears to cause weight gain, leading to obesity. This side effect is more pronounced than with conventional antipsychotics and with the other atypical antipsychotics: risperidone, ziprasidone and quertiapine (Allison et al 1999a; Nasrallah 2003). Clozapine-induced obesity in turn leads to cardiovascular, endocrine and orthopaedic complications (Allison et al 1999b; Henderson et al 2000). Therefore, agents that could reduce these complications promise significant medical benefits. At clinically relevant dosages, clozapine is metabolized primarily to desmethylclozapine (DMCLZ) and to the N-oxide form by hepatic cytochrome P450 1A2 (CYP1A2), and is then conjugated by glucuronidation. In a pharmacokinetic study, the elimination half-life of clozapine was about 1.5 h in rats (Sun & Lau 2000). In smokers who show elevated CYP1A2 activity, the dose of clozapine to obtain effective plasma concentration thus needs to be increased. Other CYP isozymes have minor roles in clozapine metabolism at therapeutic doses (for review see Prior & Baker 2002).

Green tea is now widely consumed around the world, and it has been suggested to have various beneficial effects, such as chemopreventiveness (Fujiki 2002 et al; Chung et al 2003) and activity against cardiovascular disease (Riemersma et al 2001; Sueoka et al 2001) and neurodegeneration (Mandel & Youdim 2004), although these beneficial effects have not been conclusively proven. A number of investigators have suggested that the beneficial effects of green tea might be from its polyphenols, such as (−)-epigallocatechin gallate (EGCG), (−)-epigallocatechin (EGC), (−)-epicatechin gallate and (−)-epicatechin. Furthermore, the standardized green tea extract used in this study, or green tea consumption itself, promotes weight reduction when used with exercise and body-weight maintenance programs (Chantre & Lairon 2002; Kovacs et al 2004). As numerous preparations of green tea extract are currently commercially available, we selected a specific green tea extract product in this study that is relatively rich in catechins, including EGCG, and has been reported to reduce body weight gain (Chantre & Lairon 2002).

In connection with these beneficial effects of green tea, it may offer benefits for treating drug-induced weight gain as well as diet-induced obesity. However, there is little information about the effects of green tea consumption on the pharmacokinetics of the antipsychotics that cause weight gain, including clozapine. We therefore evaluated the pharmacokinetic interaction between green tea extract and clozapine in rats using commercially available standardized green tea extract and clinically relevant doses of clozapine.

Materials and Methods

Materials

A commercially available standardized green tea extract (Exolise) was purchased from Alkopharma (Carros, France). Clozapine, caffeine, paraxanthine and EGCG were purchased from Sigma (St Louis, MO). All other solvents and chemicals were of either analytical or HPLC grade.

Treatment of animals and sample preparation

Male Sprague-Dawley rats, 270–320 g, were housed five per cage and were given free access to food and water in a temperature-controlled room (23°C) with a 12-h lighting cycle. All procedures were performed in accordance with the Animal Care Guidelines published by the National Institute of Toxicological Research (NITR) in Korea (2000) and our institutional animal care committee approved care of the animals and all procedures. Rats received intragastric doses of green tea extract at $175 \, \text{mg kg}^{-1}$ ($175 \, \text{mg}$ of green tea extract contained $22 \, \text{mg}$ of EGCG and $9 \, \text{mg}$ of caffeine) or saline (control group) at 12-h intervals for four successive days. One capsule of Exolise contained $350 \, \text{mg}$ of green tea extract with $44 \, \text{mg}$ of EGCG as a major constituent , as measured by high-pressure liquid chromatography (HPLC) analysis

(data not shown). The measured contents of EGCG and other catechins in green tea extract used in this study were similar to those in the green tea preparations (tea-leaf extracts or decaffeinated green tea solids) used in other studies (Chen et al 1996; Ohe et al 2001). After fasting for 12 h since the last dose of green tea extract or saline, a single oral dose of clozapine ($20 \, \text{mg kg}^{-1}$), which produced clinically effective serum concentrations (Table 1; $C_{max} > 300 \, \text{ng mL}^{-1}$), was given. At each time point (0, 10, 20, 30, 45, 90, 180 and 300 min after clozapine administration), $300\text{-}\mu\text{L}$ samples of tail blood were collected for the determination of plasma concentrations of clozapine. Finally, the liver was removed under secobarbital-induced anaesthesia and immediately frozen in liquid nitrogen. Because $175 \, \text{mg}$ of green tea extract contains $9 \, \text{mg}$ of caffeine, liver samples of rats treated with caffeine without clozapine were removed and stored immediately.

Sample preparation and HPLC analysis of clozapine

Blood samples were centrifuged at $3000 \, g$ for $10 \, \text{min}$ and plasma samples were stored at $-70°C$ until analysis. One-hundred microlitres of plasma was spiked with the same volume of $50 \, \text{mM}$ phosphate buffer, pH 6.9–acetonitrile (82:18, v/v; preparation buffer). Following centrifugation ($13\,000 \, g$, $5 \, \text{min}$), the supernatant was injected after membrane filtration (Millex-GV, $0.45 \, \mu\text{m}$ pores; Millipore) into an HPLC system with column switching (Nanospace SI-2 model; Shiseido, Japan). On-line sample clean up was performed on a pre-treatment CAPCELL PAK MF column (Shiseido, Japan) using preparation buffer. After column switching, the sample was separated on an analytical column (C_{18} CAPCELL PAK, $250 \times 1.5 \, \text{mm}$ i.d.; Shiseido) using an analytical mobile phase consisting of $50 \, \text{mM}$ phosphate buffer, pH 6.9–acetonitrile (60:40), adjusted to pH

Table 1 Pharmacokinetic parameters of clozapine after oral administration of $20 \, \text{mg kg}^{-1}$, with or without green tea extract, to rats

Parameter	Group	
	Control	Green tea extract
C_{max} (ng mL^{-1})	323.9 ± 43.6	$184.70 \pm 56.18**$
T_{max} (min)	28.0 ± 4.5	$51.0 \pm 22.8*$
$AUC_{0\text{-}300}$ clozapine (min μg mL^{-1})	38.90 ± 10.25	$20.30 \pm 5.65**$
$AUC_{0\text{-}\infty}$ clozapine (min μg mL^{-1})	52.80 ± 9.26	$26.60 \pm 7.85**$
Half-life (min)	99.6 ± 23.1	86.1 ± 25.8
$AUC_{0\text{-}300}$ desmethylclozapine (min μg mL^{-1})	15.70 ± 6.45	$6.90 \pm 2.51**$
$AUC_{0\text{-}300}$ desmethylclozapine/ clozapine ratio	0.42 ± 0.15	0.36 ± 0.12

$AUC_{0\text{-}\infty}$ is the area under the plasma concentration–time curve from time zero to infinity. Values are means $\pm$ s.d., n = 5. $*P < 0.05$, $**P < 0.01$ compared with control (Mann–Whitney U test).

3.5 with phosphoric acid. The signal was detected using an ultraviolet detector at 252 nm and the determinations exhibited linearity in the range 5–500 ng mL^{-1}.

Pharmacokinetic and statistical analyses

The area under the concentration–time curves from the time of dosing to the infinite time point (AUC$_{0-\infty}$) was estimated using the WinNonlin program (Pharsight Corp., Mountain View, CA). Statistical and graphical analyses were accomplished using commonly available commercial software packages (Prism; GraphPad Software Inc., San Diego, CA). Data are presented as means ± standard deviations (s.d.) or standard error of mean (s.e.m.). Two-tailed Mann–Whitney U-tests or Kruskal–Wallis tests with multiple comparisons were used to test differences and statistical significance was assumed at $P < 0.05$. Friedmans test with Dunnett's comparison was used to compare the plasma concentration–time profile.

Western blot analysis

After quantitative analysis of microsomal proteins, rat liver microsomal proteins (40 µg) were separated by SDS–polyacrylamide gel electrophoresis using 8–16% Tris-glycine polyacrylamide gradient gels (Invitrogen, Carlsbad, CA) and transferred to a polyvinylidene difluoride membrane (Millipore, Milford, MA). After overnight blocking with Tris-buffered saline (TBS) containing 0.1% Tween 20 and 5% non-fat dry milk, the membrane was incubated with a polyclonal antibody for cytochrome P450 (CYP1A2, diluted 1:2000; Serotec Ltd, Kidlington, UK) and anti-rabbit IgG conjugated with horseradish peroxidase (1:5000; Pierce) for 1 h at room temperature. Target bands were detected using the enhanced chemiluminescence (ECL) western blotting system (Amersham Biosciences, Aylesbury, UK).

Measurement of CYP1A2 activity

For the measurement of hepatic CYP1A2 activity, hepatic microsomes were prepared by differential centrifugation. After determination of protein content, 0.5 mg of microsomal protein was incubated with caffeine (10 mM) in 0.1 mM of potassium phosphate buffer (pH 7.4 containing 1 mM of NADPH) for 1 h at 37°C. Incubation of the microsome fraction with buffer instead of caffeine as a blank excluded the presence of residual caffeine in green tea extract-treated rats (data not shown). The reaction mixture was centrifuged at 100 000 g for 30 min and 20 µL of supernatant was injected into a Waters HPLC system. Caffeine and metabolites were monitored at 274 nm and were separated on a Waters Nova-Pak C$_{18}$ column (150 × 4.6 mm). The mobile phase consisted of 15% methanol in 25 mM sodium acetate buffer, pH 4.0. CYP1A2 activity was determined from the ratio of paraxanthine to caffeine.

Results and Discussion

Changes in plasma concentrations over time of clozapine and its CYP1A2-mediated metabolite, desmethylclozapine, in controls and green tea extract-treated rats are shown in Figure 1. During the elimination phases of clozapine and desmethylclozapine, no differences in half-life or elimination pattern were observed between groups. The mean elimination half-life of clozapine in the control and green tea extract-treated groups was 99.6 ± 23.1 min (range 70–122 min) and 86.1 ± 25.8 min (range 58–118 min), respectively ($P > 0.05$). The change in plasma concentration of desmethylclozapine over time was similar to that of clozapine. However, the mean plasma concentration of clozapine at the first sampling point (10 min) in the control group was already 10 times higher (about 161 ng mL^{-1}) than in the green tea extract group (16 ng mL^{-1}), indicating rapid absorption of clozapine in the controls. Furthermore, the mean time for a peak plasma concentration (T$_{max}$) in the control group was 28 min, consistent with previous reports
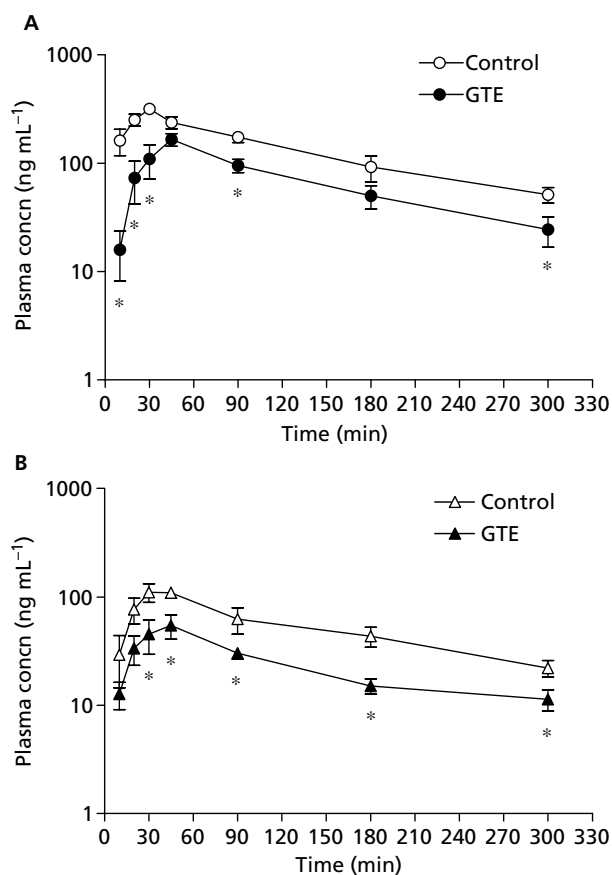

Figure 1 Plasma concentration versus time profiles (mean ± s.e.m., n = 5) of clozapine (A) and desmethylclozapine (B) after oral administration of a single dose of 20 mg kg^{-1} clozapine to rats receiving intragastric doses of green tea extract (GTE) at 175 mg kg^{-1} or saline (control group) at 12-h intervals for four successive days. The profiles of clozapine and desmethylclozapine were similar. *$P < 0.05$ vs control by Friedmans test with Dunnett's comparison.

(Baldessarini et al 1993; Sun & Lau 2000). However, because the mean T_{max} of clozapine in the green tea extract group was 51 min, green tea extract may have interfered with the intestinal absorption process of clozapine. In addition to the delaying effects of green tea extract for T_{max}, the peak clozapine concentration (C_{max}) of the green tea extract group was significantly lower than that of controls. Thus, there was significant interaction between green tea extract and clozapine, particularly during the absorption of clozapine. As well as C_{max}, the $AUC_{0-\infty}$ in the green tea extract-treated group was also significantly lower than that of controls ($P < 0.01$), indicating the reduction of systemic exposure to clozapine by oral green tea extract pretreatment. Delayed gastric emptying of clozapine caused by green tea extract may be involved in the delayed absorption and reduced relative oral bioavailability of clozapine through a reduction in the rate and degree of absorption of clozapine in the gastrointestinal tract. Because clozapine was given 12 h after the last dose of green tea extract, direct physicochemical interaction between the ingredients of the extract and clozapine is unlikely to have occurred. However, we could not exclude other possibilities (e.g. alteration of intra-gastrointestinal pH or interactions between transport mechanisms for clozapine after green tea extract administration). Therefore, human studies based on green tea drinking or green tea extract capsule intake in various administration protocols should be performed to resolve this point.

Hepatic microsomal CYP1A2 proteins were examined by western blotting to determine whether the production of this major enzyme contributing to clozapine metabolism was affected by green tea extract or caffeine. As shown in Figure 2, when green tea extract or caffeine (9 mg, equivalent to the content in 175 mg of green tea extract) was given, CYP1A2 levels were, respectively, 2.0 and 3.4 times those of controls. Our results support a previous study that repeated administration of caffeine induces the synthesis of CYP1A2 in the liver (Chen et al 1996). However, the effects of green tea extract on this process are unlikely to contribute to pharmacokinetic interactions between clozapine and green tea extract, because the rate of elimination of clozapine in the green tea extract-treated group was very similar to that in the controls. To confirm whether the effects of caffeine present in the green tea extract preparation had little effect on clozapine elimination, despite the higher levels of CYP1A2 in rats treated with green tea extract, CYP1A2 activity was determined by the rate of conversion of caffeine to paraxanthine in microsomal preparations in-vitro. Treatment with green tea extract containing 9 mg kg^{-1} caffeine induced CYP1A2 activity to only 10% higher than that of the control. Consistent with these results, the AUC_{0-300} of the desmethylclozapine-to-clozapine ratio, as an indicator of CYP1A2 activity, was not significantly different between the green tea extract and the control group (Table 1). Thus, consumption of green tea extract is unlikely to alter the elimination of clozapine via CYP1A2-mediated metabolism. Moreover, clozapine is extensively metabolized in the human liver (Choc et al 1990; Dain et al 1997) and has low oral bioavailability (5–6%) in rats (Sun & Lau 2000), which indicates that clozapine undergoes extensive first-pass
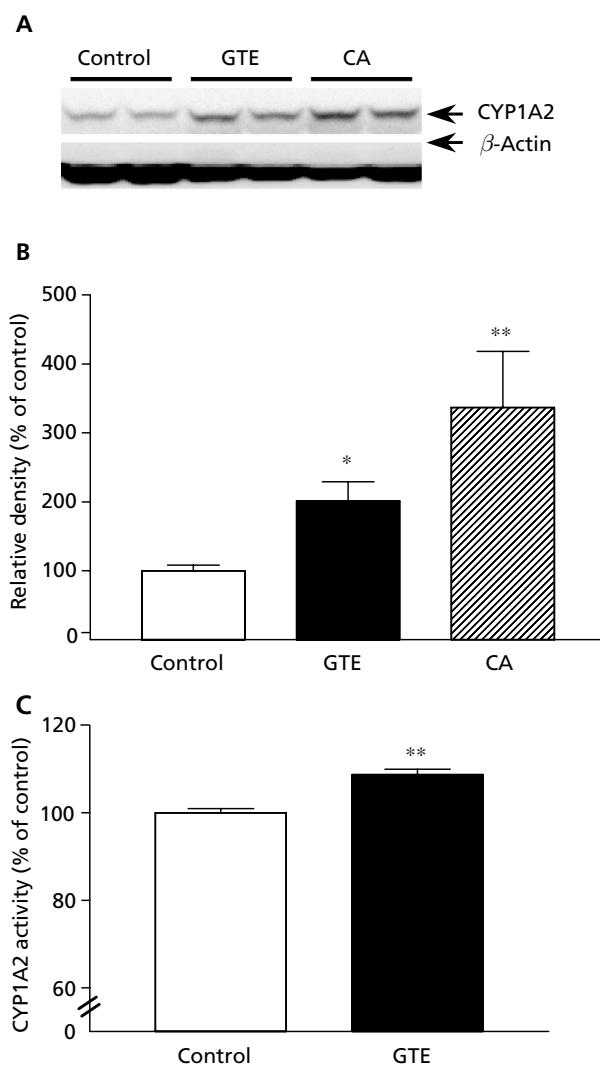

Figure 2 Hepatic cytochrome P450 (CYP1A2) expression (A), concentration (B) and activity (C) after treatment of rats with green tea extract (GTE) or caffeine (CA) for four successive days (means ± s.e.m.). Both GTE and CA significantly increased CYP1A2 expression compared with controls (A, B); *$P < 0.05$, **$P < 0.01$ versus control by Kruskal–Wallis tests with Dunn's post-hoc test. CYP1A2 activity was slightly increased (~110% of control) by GTE (C); *$P < 0.05$ versus control by Mann–Whitney U-test (n = 6).

metabolism and that systemic clearance of clozapine might depend on hepatic blood flow rather than intrahepatic CYP1A2 activity. Direct metabolic competition between green tea flavonoids (e.g. EGCG) and clozapine is thus unlikely to occur because of the escape of EGCG from first-pass hepatic metabolism (Cai et al 2002). One recent report claimed that CYP1A2 activity was decreased by the consumption of several kinds of tea, whereas it was increased by caffeine (Maliakal & Wanwimolruk 2001). However, Niwattisaiwong et al (2004) reported that green tea consumption for four weeks did not alter CYP1A2 activity. Although the green tea formulation we used here differed from previous studies, the consumption of 175 mg

of green tea extract for four days produced a slight but significant increase in CYP1A2 activity. Several factors, such as different doses or formulation (tea solutions vs extracts) and different treatment periods in consuming tea or caffeine might contribute to this discrepancy between studies. By contrast, Chen et al (1996) reported that the increase in CYP1A2 activity following green tea consumption was not caused by the polyphenol, but by the caffeine content. Other studies also suggested that caffeine could be a dietary factor for induction of hepatic CYP1A2 production (Goasduff et al 1996; Carrillo et al 1998; Hägg et al 2000). However, relatively higher doses of caffeine were used in those studies than in our study. For animal studies in particular, the dose of caffeine used to induce CYP1A2 activity ($> 50 \, \text{mg kg}^{-1}$ daily) was much higher than in this study ($18 \, \text{mg kg}^{-1}$ daily). Furthermore, other variable components of green tea extract, including catechins (Muto et al 2001), may inhibit CYP1A2 activity independently from gene expression, via inhibition of CYP reductase. Therefore, the role of catechins in the caffeine-mediated induction of CYP1A2 should be further investigated. The clinical implications of the effects of concomitant consumption of green tea may be trivial, at least for the hepatic elimination of clozapine, although further clinical pharmacokinetic studies on the interactions between green tea and clozapine are clearly warranted. In addition, the clinical implications of our results should be further evaluated in clozapine-treated patients, as humans show high variation in their beverage consumption.

Conclusion

Oral administration of green tea extract for four days was found to decrease both the rate and amount of absorption of clozapine and to decrease the systemic exposure to clozapine in rats. In addition, a short period of green tea extract consumption produced a slight elevation in CYP1A2 activity, although the effect of this on the elimination of clozapine is probably negligible in our study. Thus, the pharmacokinetic interactions between green tea consumption and clozapine administration need to be studied, especially regarding their absorption processes in man.

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