Phytomedicine 20 (2013) 1247–1250

Contents lists available at ScienceDirect

Phytomedicine

journal homepage: www.elsevier.de/phymed

Short communication

Effects of green tea extract and (−)-epigallocatechin-3-gallate on pharmacokinetics of nadolol in rats

S. Misaka[a,*], N. Miyazaki[a], T. Fukushima[b], S. Yamada[c], J. Kimura[a]

[a] Department of Pharmacology, School of Medicine, Fukushima Medical University, Fukushima, Japan
[b] Department of Hygiene and Preventive Medicine, School of Medicine, Fukushima Medical University, Fukushima, Japan
[c] Department of Pharmacokinetics and Pharmacodynamics, School of Pharmaceutical Sciences, University of Shizuoka, Shizuoka, Japan

ARTICLE INFO

Article history:
Received 25 February 2013
Received in revised form 16 May 2013
Accepted 2 July 2013

Keywords:
(−)-Epigallocatechin-3-gallate
Food–drug interaction
Green tea extract
Nadolol
Pharmacokinetics

ABSTRACT

Green tea catechins have been shown to affect the activities of drug transporters *in vitro*, including P-glycoprotein and organic anion transporting polypeptides. However, it remains unclear whether catechins influence the *in vivo* disposition of substrate drugs for these transporters. In the present study, we investigated effects of green tea extract (GTE) and (−)-epigallocatechin-3-gallate (EGCG) on pharmacokinetics of a non-selective hydrophilic β-blocker nadolol, which is reported to be a substrate for several drug transporters and is not metabolized by cytochrome P450 enzymes. Male Sprague-Dawley rats received GTE (400 mg/kg), EGCG (150 mg/kg) or saline (control) by oral gavage, 30 min before a single intragastric administration of 10 mg/kg nadolol. Plasma and urinary concentrations of nadolol were determined using high performance liquid chromatography. Pharmacokinetic parameters were estimated by a noncompartmental analysis. Pretreatment with GTE resulted in marked reductions in the maximum concentration (C_{max}) and area under the time–plasma concentration curve (AUC) of nadolol by 85% and 74%, respectively, as compared with control. In addition, EGCG alone significantly reduced C_{max} and AUC of nadolol. Amounts of nadolol excreted into the urine were decreased by pretreatments with GTE and EGCG, while the terminal half-life of nadolol was not different among groups. These results suggest that the coadministration with green tea catechins, particularly EGCG, causes a significant alteration in the pharmacokinetics of nadolol, possibly through the inhibition of its intestinal absorption mediated by uptake transporters.

Introduction

Green tea (*Camellia sinensis*) has been known to have various beneficial effects on health such as cancer prevention, anti-obesity and anti-infection (Khan and Mukhtar 2007). Catechins, flavanol compounds in green tea, are presumed to play pivotal roles in these actions. In addition to the pharmacological effects, previous *in vitro* studies suggested that catechins may have an impact on the drug disposition by modulating the activities of drug metabolizing enzymes and uptake or efflux transporters (Jodoin et al. 2002; Misaka et al. 2013b; Roth et al. 2011). However, to date, only a few clinical and preclinical studies have been reported regarding pharmacokinetic interactions of drugs with green tea infusion or catechins (Chow et al. 2006; Donovan et al. 2004). With respect to the drug transporter-mediated interactions, (−)-epigallocatechin-3-gallate (EGCG), the most abundant catechin, elevated intracellular accumulation of doxorubicin through the inhibition of P-glycoprotein (P-gp)-mediated efflux in a human multidrug resistant carcinoma cells (Qian et al. 2005). Moreover, Lin et al. (2008) showed that an intravenously administered EGCG inhibited the biliary excretion of irinotecan, and consequently prolonged its half-life in rats, possibly by modulating the P-gp activity in the liver. However, far less is known about whether green tea or catechins induce the pharmacokinetic interaction with other drugs which are influenced by P-gp-mediated efflux transport.

Nadolol is a non-selective β-adrenoceptor antagonist used for the treatment of angina pectoris and hypertension (Heel et al. 1980). Due to its high water solubility, nadolol is not metabolized by cytochrome P450s (CYP) in the body (Meier 1982), and it belongs to the biopharmaceutical classification system (BCS) class III drug (Yang et al. 2007). Similar to the other BCS class III drugs including fexofenadine, nadolol has been shown to be a substrate for efflux

Abbreviations: AUC, area under the time–plasma concentration curve; BCS, biopharmaceutical classification system; BDDCS, biopharmaceutics drug disposition classification system; CYP, cytochrome P450; GTE, green tea extract; EGCG, (−)-epigallocatechin-3-gallate; OATP, organic anion transporting polypeptide; P-gp, P-glycoprotein.

* Corresponding author at: Department of Pharmacology, School of Medicine, Fukushima Medical University, 1 Hikarigaoka, Fukushima 9601295, Japan.
Tel.: +81 24 547 1156; fax: +81 24 548 0575.
E-mail address: misaka@fmu.ac.jp (S. Misaka).

0944-7113/$ – see front matter © 2013 Elsevier GmbH. All rights reserved.
http://dx.doi.org/10.1016/j.phymed.2013.07.003

Table 1
Pharmacokinetic parameters of nadolol after oral administration of 10 mg/kg of nadolol following pretreatment with saline (control), green tea extract (GTE, 400 mg/kg) or epigallocatechin-3-gallate (EGCG, 150 mg/kg) in rats ($n = 5–6$).

	Control	GTE	EGCG
C_{max} (ng/mL)	168 ± 28	26 ± 5[a]	32 ± 15[a]
T_{max} (h)	2.0 (1.0–2.0)	1.0 (0.5–3.0)	2.0 (0.5–3.0)
$AUC_{0-\infty}$ (h ng/mL)	433 ± 69	111 ± 20[a]	115 ± 23[a]
$t_{1/2}$ (h)	2.1 ± 0.4	2.1 ± 0.3	2.7 ± 0.6

Data are expressed as mean ± SEM, except for T_{max} which represent median value with range.
[a] $p < 0.001$, compared with control.

and uptake transporters such as P-gp and organic anion transporting polypeptide (OATP) 1A2 *in vitro* (Kato et al. 2009; Terao et al. 1996). Furthermore, our group recently demonstrated that the plasma concentration of nadolol was significantly increased by the coadministration of itraconazole, a P-gp inhibitor, in rats (Miyazaki et al. 2013). These results suggest that nadolol may be a suitable probe substrate for the evaluation of P-gp activity *in vivo*.

In this study, therefore, we examined whether green tea catechins affect the pharmacokinetics of nadolol in rats. After single oral gavages of green tea extract (GTE) or EGCG, nadolol was intragastrically administered, and then plasma concentrations and urinary excretions of nadolol were determined periodically.

Results and discussion

After overnight fasting, rats were pretreated with GTE at a dose of 400 mg/kg, followed by an intragastric administration of nadolol (10 mg/kg) 30 min later. As previously reported (Misaka et al. 2013a), the plasma concentration of EGCG reached approximately 1.2 μg/mL (2.6 μM) at this dose of GTE, and this value was comparable to the maximum plasma concentration (C_{max}) of EGCG in humans when taking 400–800 mg of green tea mixture (Chow et al. 2005), although the bioavailability of EGCG as well as other catechins has been shown to be extremely low (1.6%) in rats (Chen et al. 1997). The concentration of EGCG in the intestinal lumen could be much higher than that in plasma, and thus EGCG may be enough to inhibit the activity of P-gp expressed in the apical membrane of the intestinal epithelial cells (Jodoin et al. 2002). Fig. 1A and Table 1 show the plasma concentration profile and the pharmacokinetic parameters of nadolol. Unexpectedly, pretreatment with GTE led to marked decreases in C_{max} and area under the time–plasma concentration curve ($AUC_{0-\infty}$) of nadolol compared with control by 85% and 74%, respectively ($p < 0.001$). In previous reports, the inhibition of intestinal P-gp resulted in an increase in plasma concentration of P-gp substrates such as talinolol (de Castro et al. 2008). Also the inhibition of intestinal P-gp by itraconazole significantly increased plasma concentration of nadolol (Miyazaki et al. 2013). The present results are opposite to the result of pharmacokinetic interaction by P-gp inhibition. The terminal elimination half-life ($t_{1/2}$) for nadolol did not differ between the two groups, indicating that effects of GTE on the nadolol pharmacokinetics can be attributable to processes that occur in the gut rather than to a modification of its systemic clearance (de Castro et al. 2008). Because EGCG is the most abundant component in the GTE, we focused on EGCG to address whether it contributed to the interaction. EGCG was administered at a dose of 150 mg/kg, which was estimated to be the amount of EGCG contained in the GTE examined above. Pretreatment with EGCG significantly reduced C_{max} and $AUC_{0-\infty}$ of nadolol by 81% and 73%, respectively ($p < 0.001$, Fig. 1A and Table 1). These results suggest that EGCG alone possesses the comparable effect with GTE. Since the $t_{1/2}$ was not altered between EGCG and control, the interaction might be caused in the intestine, probably by inhibiting the absorption of nadolol. The amount of nadolol excreted into the urine was significantly decreased in GTE and EGCG groups compared with control (Fig. 1B), while the urine volumes in control, GTE and EGCG group were 11.7 ± 1.3 ml, 9.4 ± 1.0 ml, and 10.8 ± 0.8 ml, respectively. These findings suggest that the decrease in the plasma concentration of nadolol is not attributed to an increased elimination of nadolol into the urine. On the other hand, the results further support the hypothesis that the intestinal absorption of nadolol may be greatly inhibited by GTE and EGCG treatments. Additionally, it is noted that our results cannot rule out the possibility of the P-gp-mediated interactions by catechins, and further investigations are warranted using other P-gp probe drugs.

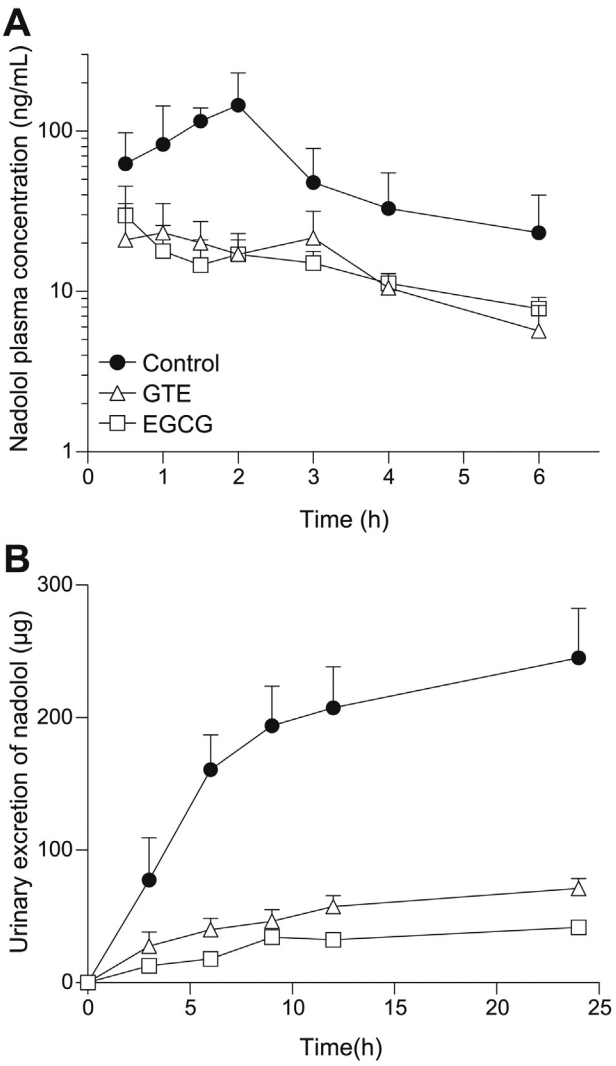

Fig. 1. Plasma concentrations up to 6 h (A) and urinary excretions up to 24 h (B) of nadolol after intragastric administration of 10 mg/kg nadolol in rats pretreated with saline (control, closed circle), green tea extract (400 mg/kg, open triangle) or (−)-epigallocatechin-3-gallate (150 mg/kg, open square) 30 min before nadolol dosing. Data represent mean ± SEM ($n = 5–6$).

Previous *in vitro* studies showed that green tea catechins such as EGCG inhibited the activities of OATP1A2 and OATP2B1 which are expressed in the apical side of the intestinal epithelium in human (Roth et al. 2011). In rats, mRNA expressions of oatp1a5 and oatp2b1 were detected in the intestinal mucosa (MacLean et al. 2010). OATP transporters play important roles in the cellular uptake of variety of endogenic and xenobiotic compounds in the intestine and liver (Klaassen and Aleksunes 2010). The inhibition of the intestinal OATP could lead to a decrease in the absorption of drug from intestinal lumen to the circulation. For instance,