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UPLC–ESI–MS/MS study of the effect of green tea extract on the oral bioavailability of erlotinib and lapatinib in rats: Potential risk of pharmacokinetic interaction

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ABSTRACT

Green tea (GT) is one of the most consumed beverages worldwide. Tyrosine kinase inhibitors (TKIs) belong to the oral targeted therapy that gained much interest in oncology practice, among which are erlotinib (ERL) and lapatinib (LAP). Since green tea polyphenols (GTP) are known to be inhibitors of receptor tyrosine kinases, GTE could likely potentiate the anticancer effect of TKIs, but with a possibility of pharmacokinetic (PK) interaction with co-administered TKIs. In this study, the effect of GTE on the PK of ERL/LAP in rats was studied. UPLC–ESI–MS/MS method has been developed and validated for the quantification of ERL and LAP in rat plasma, using gefitinib (GEF) as the internal standard. Plasma samples were treated extensively by protein precipitation (PPT) followed by solid phase extraction (SPE) using octadecyl C 18/14% cartridges. Chromatographic analysis was carried out on Acquity UPLC BEH^{TM} C18 column with a mobile phase consisting of water: acetonitrile (20: 80, v/v), each with 0.15% formic acid. Quantification was performed in the positive electrospray ionization (ESI+) mode with multiple reaction monitoring (MRM) of the transitions m/z 394.29 → 278.19 (ERL), m/z 581.07 → 365.13 (LAP), and m/z 447.08 → 128.21 (GEF). The method was fully validated as per the FDA guidelines showing linearity over the range of 0.4–1000 (ERL) and 0.6–1000 (LAP) ng/mL with very low lower limit of quantification (LLOQ) of 0.4 and 0.6 ng/mL for ERL and LAP, respectively. The applicability of the method was extended to perform a comparative study of the PK of ERL/LAP following short-term and long-term administration of GTE, compared with their single oral administration. The results revealed that a significant reduction in the oral bioavailability was recorded with both ERL and LAP following the ingestion of GTE particularly for short-term administration. A reduction in C_{max} (AUC) by 67.60% (69.50%) and 70.20% (73.96%), was recorded with short-term administration of GTE, compared with only 16.03% (21.09%) and 13.53% (22.12%) reduction for ERL and LAP, respectively, with long-term administration. Thus patients taking TKIs should preferably avoid drinking GT or ingesting GTE capsules during the period of treatment with TKIs.

1. Introduction

Food-drug interactions have gained much attention in the recent years. The influence of dietary substances on drug exposure constitutes a major challenge during drug development, particularly for oral drugs. Green tea (GT) is among the most consumed beverages worldwide. It is derived from the non-fermented leaves of the *Camellia sinensis* plant. GT has shown promising health beneficial effects and it has been investigated as one of the most important nutraceuticals used as new treatment approaches for oral cancer [1]. GT polyphenols (GTP), particularly catechin (−)-epigallocatechin-3-gallate (EGCG), which constitutes about 50–80% of the total catechins in GT, are reported to have antioxidant [2], and anti-proliferative effect in different types of human malignancies [1–3]. Several interventional studies have explained the anti-carcinogenic effect of tea catechins, with EGCG being the most active, by several mechanisms. Among which are down regulation of the cell cycle, inhibition of receptor tyrosine kinases [3–5], anti-metastatic effect [6], and modulation of the immune system. Multi-targeted anticancer effect of GTP has shown to exhibit promising results against different types of cancer cells including,

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hepatoma cells [7], human placental choriocarcinoma cells [8], and estrogen-receptor positive breast cancer [9]. In addition, GTP are reported to have potent cardio-protective properties besides their protective effect against drug-induced hepatoma [6,10]. Moreover, many studies have shown the synergistic anticancer effect of GT with other chemo-preventive drugs e.g. tamoxifen and 5-FU [11]. Also, GTP could enhance the anti-metastatic effect of TKIs via synergistic inhibition of the epidermal growth factor receptor (EGFR), e.g. gefitinib [12]. Thus combinations of GTE with TKIs could gain prospective action in clinical trials against the progression of oral cancer [12].

Broad evidence of the beneficial effects of GTP in different health aspects starting from enhancing weight loss to their anticancer effect has led to their increased popularity among different age groups whether as beverages or as food supplements. The exposure to high doses of these bioactive compounds daily could be widely encountered. Therefore, many studies have been concerned with investigating the possible effect of GTP on the therapeutic efficacy and toxicity of other co-administered drugs. This is extremely beneficial particularly that GTP could significantly affect the drug-metabolizing enzymes and/or drug transporters on the intestinal and hepatic levels [13–16]. Moreover, it was strongly proposed that GTP (particularly EGCG) could inhibit the activity of p-gp transporter showing reversal of multidrug resistance [14]. Previous studies have reported the effect of GT on the PK of co-administered drugs. Based on different mechanisms, GT could enhance the bioavailability of some drugs (e.g. tamoxifen [17], simvastatin [18], 5-fluorouracil [19]), while decrease the bioavailability of others (e.g. sunitinib [20], bortezomib [21], quetiapine [22], nadolol [23]). Thus ingestion of high doses GTP could significantly affect the PK of concomitantly administered drugs which is considered a matter of public concern [24].

Tyrosine kinase inhibitors (TKIs) are among the oral targeted therapy that gained much interest in oncology practice, among which are erlotinib (ERL) and lapatinib (LAP), Fig. 1. ERL is an orally active selective inhibitor of the ErbB-1 receptor. ERL has been approved for the treatment of $EGFRm^{+}$ NSCLC [25]. Further clinical trials have demonstrated the antitumor activity of ERL in different cancer cases including, head and neck carcinoma, glioma, squamous cell skin carcinoma, and bladder cancer [25–27]. LAP is an orally active dual −kinase inhibitor with specific activity for EGFR, ErbB-1 and ErbB-2 (HER2) [28,29]. LAP has shown significant anticancer effect either as monotherapy or in combination with other chemotherapeutic agents in different cases of malignancies, e.g. HER2-positive metastatic breast cancer [28,29], pancreatic cancer [30], and ovarian carcinoma [31]. Moreover, ERL and LAP are shown to be potent reversal agents of multidrug resistance (MDR) [32]. This suggests the likeliness of their use in combination with other chemotherapeutic agents to overcome the problem of MDR commonly encountered in oncology practice [32]. However, being oral drugs, the PK characteristics of TKIs show large intra and inter-individual variation [33,34]. Different factors could affect their bioavailability including, genetic heterogeneity of drug targets, patient adherence to treatment, in addition to the patient habits. Moreover, drug–drug interactions (DDI) and food-drug interactions (FDI) are widely encountered with this group of oral anticancer drugs. This could be related to the fact that TKIs are mostly substrates of CYP450 metabolizing enzymes as well as drug transporters [33,34].

The effect of co-administered drug/food on the bioavailability of TKIs has attracted much attention. Since tea catechins are known to be inhibitors of receptor tyrosine kinases [3–5], GTE could likely potentiate the anticancer effect of TKIs, but with a possibility of PK interaction with co-administered TKIs (e.g. ERL, LAP). ERL PK interaction studies with corticosteroids, antiemetics [35], and tamoxifen [36] was conducted with our research group using UPLC–MS/MS. Moreover, other PK interactions were reported for ERL with other drugs (e.g. warfarin [37] and aprepitant [38]). Also, PK interaction studies using LC–MS/MS have been previously performed to investigate the possible interaction of LAP with other co-administered drugs, namely tamoxifen [29], carboplatin [31], sorafenib [39], docetaxel [40], and different food types [41]. Different LC–MS/MS methods have been also reported for the determination of either ERL [35,36,42–44] or LAP [42,44–46] in plasma samples.

In spite of the importance of studying the effect of GTE on the PK of co-administered ERL/LAP, there is no study available in the literature so far dealing with this respect. Therefore the present study aims at studying the effect of GTE on the PK of ERL/LAP in rats using a newly developed UPLC–MS/MS method. The applicability of the method was extended to perform a comparative study of the PK of ERL/LAP following short-term and long-term administration of GTE, compared with their single oral administration.

2. Experimental

2.1. Chemicals and reagents

The reference standards of ERL (purity >99%) and gefitinib (GEF), being used as the internal standard (IS), (purity >99%), were purchased from Pfizer Inc. (NY, USA). LAP reference standard (purity >99%) was supplied by Haoyuan Chemexpress Co., Ltd., Shanghai, P.R. China. HPLC grade solvents namely methanol and acetonitrile (Panreac, E.U.) were involved in the study. Formic acid (Sigma Aldrich, Chemie GmbH, Steinheim, Germany) was also used in the analysis. Capsules of GTE 400 mg, Veg capsules EGCg (NOW FOODS, Bloomingdale, IL, USA) were involved in the study. The standardized extract was labelled to contain a minimum of 80% total catechins and 50% EGCG, 200 mg, in addition to up to 4 mg of naturally occurring caffeine.

Ultrapure water used throughout the study was prepared using a Milli-Q Advantage water purification system (Millipore, Molsheim, France) supplied with 0.22 μm filter.

2.2. Instrumentation and analytical conditions

Analysis was performed on Waters Model Xevo TQ-S UPLC–MS/MS separation system (Singapore) equipped with binary solvent manager (Acquity ™ Ultra-performance LC) and sample manager (Acquity ™ Ultra-performance LC). Mass spectrometric detection was carried out using triple-quadrupole mass spectrometric detector (STEP WAVE ™, Ultra-performance LC) with multiple reaction monitoring (MRM)-mode and supplied with different ionization modes (Zspray ™ ESI-APCI-ESCI, Ultra-performance LC). Data acquisition was performed with Masslynx™ Version 4.1 (Micromass) software.

J.T. Bakers vacuum system was used in the solid-phase extraction (SPE) procedure using octadecyl C 18/14% (200 mg, 3 mL) Spe-ed cartridges (Applied Separations, Allentown, Pennsylvania, USA). Nitrogen evaporator N-EVAP 112 with heating system OA-SYS (Organomation Assocciates, Inc, MA, USA) was used in sample preparation. Sample filtration was performed using disposable syringe filters (CHROMAFIL ® Xtra PA-20/25 polyamide filters, pore size: 0.2 μm, filter-Θ: 25 mm), (MACHEREY NAGEL, GmbH & Co. KG, Duren, Germany).

Chromatographic analysis was carried out on Acquity UPLC BEH™ C 18 column (100 × 1.0 mm, i.d., 1.7 μm particle size) (Waters, Ireland). Isocratic elution was carried out using a mobile phase consisting of water: acetonitrile (20: 80, v/v), each with 0.15% formic acid, at a flow rate of 0.2 mL/min. The injection volume was 5 μL with the full loop mode. The auto-sampler and column temperature were maintained at 10° and 45 °C, respectively.