Study on the analytical application of matrix-assisted laser desorption/ionization mass spectrometry-imaging technique for visualization of polyphenols

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Abbreviations

- 1,5-DAN, 1,5-diaminonaphthalene
- 9-AA, 9-aminoacridine
- ABC, ATP-binding cassette
- ADME, absorption, distribution, metabolism, and excretion
- AMPK, adenosine monophosphate activated-protein kinase
- ANOVA, analysis of variance
- BCRP, breast cancer resistance protein
- CHCA, α-cyano-4-hydroxycinnamic acid
- DHB, 2,5-dihydroxybenzoic acid
- DMAN, 1,8-bis(dimethyl-amino)naphthalene
- DMSO, dimethyl sulfoxide
- EC, epicatechin
- ECG, epicatechin-3-*O*-gallate
- EGC, epigallocatechin
- EGCG, epigallocatechin-3-*O*-gallate
- ESI, electrospray ionization
- FA, formic acid
- IAA, *trans*-3-indoleacrylic acid
- ITO, indium-tin oxide
- KBR, Krebs-Bicarbonate Ringer's
- LC, liquid chromatography
- *m/z*, mass-to-charge ratio
- MALDI-MS, matrix-assisted laser desorption/ionization mass spectrometry
- MCT, monocarboxylic transporter
- MeOH, methanol
- MRP2, multidrug resistance protein 2
- Nd:YAG, neodymium-doped yttrium aluminum garnet
- OATP, organic anion transporting polypeptides
- PA, proton affinity
- PepT1, peptide transporter 1
- P-gp, P-glycoprotein
- *S/N*, signal-to-noise ratio
- SA, sinapinic acid
- SD rat, Sprague-Dawley rat
- SD, standard deviation
- TF, theaflavin
- TF3'G, theaflavin-3'-*O*-gallate
- TF-33'diG, theaflavin-3-3'-di-*O*-gallate
- TF3G, theaflavin-3-*O*-gallate
- THAP, 2',4',6'-trihydroxyacetophenone
- TJ, tight-junction
- TOF, time-of-flight
- UV, ultraviolet

Chapter I

Introduction

A popular beverage of tea, derived from the leaves of the *Camellia sinensis* plant, has been consumed worldwide, and to date, it is considered that the tea intake would be of health-benefit owing to dietary flavonoids (polyphenols). In green or non-fermented tea, major components are monomeric catechins, e.g., epicatechin (EC), epicatechin-3-*O*-gallate (ECG), epigallocatechin (EGC), and epigallocatechin-3-*O*-gallate (EGCG). On the other hands, by fermentation of tea leaves to produce black tea, oxidation and polymerization reactions occur in leaves to form oligomeric catechins, such as theasinensins and theaflavins (TFs) including theaflavin (TF), theaflavin-3-*O*-gallate (TF3G), theaflavin-3'-*O*-gallate (TF3'G), and theaflavin-3-3'-di-*O*-gallate (TF-33'diG) [1]. To date, extensive studies have been performed on health-benefits of tea polyphenols, and showed their potential in preventing cardiovascular diseases [2], diabetes [3], and cancers [4].

Irrespective to the evidences on their preventive effects, it must be essential to know absorption, distribution, metabolism, and excretion (ADME) behavior, since the understanding of ADME is indispensable for elucidating the bioactive mechanism(s) and effective dosage of polyphenols in our body. In general, polyphenols are thought to be absorbed into the circulation system, following distribution at organs, and/or excretion into urine and fecal via metabolism [1]. Among catechins, EC and EGC have been reported to be highly bioavailable, compared to gallate catechins such as ECG and EGCG [5]. In human study, EC, EGC, ECG, and EGCG were detected in plasma to be 174, 145, 50.6, and 20.1 pmol/mL, respectively, after the consumption of tea catechins (EC, 36.54 mg; EGC, 15.48 mg; ECG, 31.14 mg; EGCG, 16.74 mg) [6]. Another human study also revealed the absorption of not only catechins, but also their conjugates in plasma at >50 ng/mL [7]. They also clarified that ECG and EGCG were absorbed in their intact form, while EC and EGC were susceptible to metabolism to produce conjugated forms [7]. Another research group reported high stability of EGCG during absorption process in human [8]. In cell-line experiments using Caco-2 cell monolayers, non-gallate catechin, EC, was found to show lower cellular accumulation than gallate ECG, due to high efflux back of EC to apical side [9]. After 50-μmol/L, 60-min, Caco-2 transport experiments of EC, ECG, and EGCG, only gallate catechins (ECG and EGCG) were predominantly accumulated in cells at 3037 ± 311 and 2335 ± 446 pmol/mg protein, respectively [10].

There were few researches on absorption of black tea TFs. In human study, even at high dose intake of 700 mg TFs, plasma and urine levels of TFs were as low as 1 and 2 ng/mL, respectively [11]. In urine, TFs were not detected after consumption of 1000 mg of TF extract [12]. Non-absorbable property of TFs was also confirmed by Caco-2 cell transport study, in which TF3'G was not detected in basolateral side after 60-min transport [13]. Irrespective to poor absorption or low bioavailability of TFs, it was reported that they have potential in the regulation of intestinal absorption route(s); in turn, TFs may exert physiological function at the small intestine [14]. However, the absorption behavior of TFs still remains unclear whether they could be incorporated into intestinal membrane or not.

Once being absorbed into the circulation system or organs, polyphenols undergo phase II metabolism, namely, methylation, sulfation, and glucuronidation [15][16]. Phase II enzymes catalyzing the methylation, sulfation, and glucuronidation are catechol-*O*-methyltransferase, sulfotransferase, and uridine diphosphate-glucuronosyltransferase, respectively [17]. These metabolic enzymes were found not only in the intestine, but also in the liver and the kidneys [18][19][20]. It has been reported that higher absorbable catechins such as EC and EGC were more susceptible to such metabolic reactions, compared to gallate catechins (ECG and EGCG) [7]. For EC absorption, a predominant sulfate conjugate of EC were effluxed from the enterocytes back to the intestinal perfusate, while glucuronide conjugate was absorbed into blood, bile and urine [21]. When 500 mL of green tea was given to 10 volunteers, only intact ECG and

EGCG were found in human plasma, whereas glucuronide, methyl-glucuronide, and methyl-sulfate conjugates of EC and EGC were detected [5]. In absorption studies of EGCG in mice [15] or ECG in Wistar rats [22], their sulfate and glucuronide conjugates were found in blood, liver, and kidney, suggesting that overall absorption study is still required for further understanding of polyphenol bioavailability.

The low bioavailability of polyphenols is in part due to their pumping out (or efflux) to the apical compartment and/or metabolic degradation. *In vitro* studies suggested that the routes involved in efflux of polyphenols are ATP-binding cassette (ABC) transporters such as multidrug resistance protein 2 (MRP2) and P-glycoprotein (P-gp), which are located in the apical side [23]. In Caco-2 cell transport experiments of monomeric catechin (EC), inhibition of MRP2 route by MK-571, an inhibitor of MRP2, significantly reduced the effluxes of EC and its sulfate conjugates to the apical compartment [24]. In MRP2 transfected and P-gp transfected cells, it was demonstrated that the cellular accumulation of ECG was significantly increased by both MRP2 and P-gp efflux inhibitors, suggesting the involvement of ECG in both ABC transporters [10].

In order to get inside into the absorption and metabolism behaviors of tea polyphenols, some analytical evaluations have been reported. In *in vivo* evaluation, transport routes of polyphenols may not be fully explored [25][26]. Thus, to elucidate intestinal absorption and metabolism of polyphenols, cell-based *in vitro* model, commonly Caco-2 cell, has been widely used. Caco-2 cells, which

are derived from human colon carcinoma, resemble the enterocytes and express transport systems as in small intestine [27]. By using Caco-2 transport system in combination with transporter inhibitors, investigations on transport routes of polyphenols have been widely performed [10][28]. Irrespective to easy set of cell-line experiments, Caco-2 cell model remains some disadvantages such as different protease expression from actual intestinal membranes. An alternative strategy for absorption study has been proposed by using *ex vivo* Ussing Chamber system, which is mounted with animal intestinal membranes [29][30]. Miyake *et al*. [29] evaluated intestinal absorption of drugs with different levels of membrane permeability using rat and human intestine mounted onto the Ussing Chamber system [29]. The *ex vivo* system is considered to be a good tool for investigating transport mechanism as *in vivo* intestinal absorption events, and is used for transport of drugs [29][30], and peptides [31].

It should be noted that analytical assays to monitor target analytes must be needed for absorption study, even though appropriate absorption systems are available. To date, liquid chromatography-mass spectrometry (LC-MS) in electrospray ionization (ESI) mode is commonly used for absorption study of polyphenols [32], since LC-MS system could detect not only target polyphenols, but also metabolites simultaneously or one-in-run assay. Irrespective to its high sensitivity and throughput characteristics, LC-based method remains some drawbacks; it requires tedious pre-treatments such as preparation and extraction steps, and could not obtain the localization of analytes in biological tissues [33]. On the other hand, matrix-assisted laser desorption/ionization MS (MALDI-MS),