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Absorption, metabolism, distribution and excretion of (−)-epicatechin: A review of recent findings

Gina Borges [a], Javier I. Ottaviani [b], Justin J.J. van der Hooft [c], Hagen Schroeter [b], Alan Crozier [a, *]

[a] Department of Nutrition, University of California, Davis, CA 95616, United States
[b] Mars Inc., McLean, VA 22101, United States
[c] Bioinformatics Group, Department of Plant Sciences, Wageningen University, 6708 PB, Wageningen, The Netherlands

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ABSTRACT

This paper reviews pioneering human studies, their limitations and recent investigations on the absorption, metabolism, distribution and excretion (aka bioavailability) of (−)-epicatechin. Progress has been made possible by improvements in mass spectrometric detection when coupled to high performance liquid chromatography and through the increasing availability of authentic reference compounds of in vivo metabolites of (−)-epicatechin. Studies have shown that [2-^{14}C](−)-epicatechin is absorbed in the small intestine with the 12 structural-related (−)-epicatechin metabolites (SREMs), mainly in the form of (−)-epicatechin-3′-*O*-glucuronide, 3′-*O*-methyl-(−)-epicatechin-5-sulfate and (−)-epicatechin-3′-sulfate, attaining sub-μmol/L peak plasma concentrations (C_{max}) ~1 h after ingestion. SREMs were excreted in urine over a 24 h period in amounts corresponding to 20% of (−)-epicatechin intake. On reaching the colon the flavan-3-ol undergoes microbiota-mediated conversions yielding the 5C-ring fission metabolites (5C-RFMs) 5-(hydroxyphenyl)-γ-valerolactones and 5-(hydroxyphenyl)−γ-hydroxyvaleric acids which appear in plasma as phase II metabolites with a C_{max} of 5.8 h after intake and are excreted in quantities equivalent to 42% of the ingested (−)-epicatechin. Other catabolites excreted in 0−24 h urine in amounts equivalent to 28% of intake included 3-(3′-hydroxyphenyl)hydracrylic acid, hippuric acid and 3′-hydroxyhippuric acid. Overall (−)-epicatechin is highly bioavailable with urinary excretion indicating that 95% is absorbed and passes through the circulatory systems as a diversity of phase II metabolites. Rats produce a very different profile of SREMs than that of humans. These findings demonstrate that ex vivo studies investigating the mechanisms underlying the protective effects of (−)-epicatechin on human health should make use of physiological concentrations human of SREMs and 5C-RFMs, and not the parent (−)-epicatechin, with model systems derived from human cells. In epidemiological studies 5-(4′-hydroxyphenyl)-γ-valerolactone-3′-sulfate and 5-(4′-hydroxyphenyl)-γ-valerolactone-3′-*O*-glucuronide, the principal 5C-RFMs in both plasma and urine, could serve as key biomarkers of (−)-epicatechin intake.

1. Introduction

Cocoa beans are a rich source of flavan-3-ols, in the form of the monomers (−)-epicatechin and (+)-catechin (Fig. 1) (Crozier et al., 2006; Rothwell et al., 2013). As well as simple monomers, flavan-3-ols also exist as proanthocyanidins which are found in fruits, bark, leaves and seeds of many plants, and in foods such as fruits and berries, nuts, beans, some cereals (barley and sorghum), spices such as curry and cinnamon, and in cocoa and some dark chocolates (Gu et al., 2004). This review will focus principally on the absorption, metabolism, distribution and excretion (ADME) of (−)-epicatechin, the principle flavan-3-ol monomer in cocoa beans. Processing of cocoa beans can, however, result in some epimerisation of (−)-epicatechin to form (−)-catechin and as a result the predominant form of catechin in some chocolate products can be the (−)-isomer rather than the naturally occurring (+)-form (see structures in Fig. 1) (Gotti et al., 2006; Cooper et al., 2007).

There is a wealth of data derived from human dietary

* Corresponding author.
E-mail address: alan.crozier44@gmail.com (A. Crozier).

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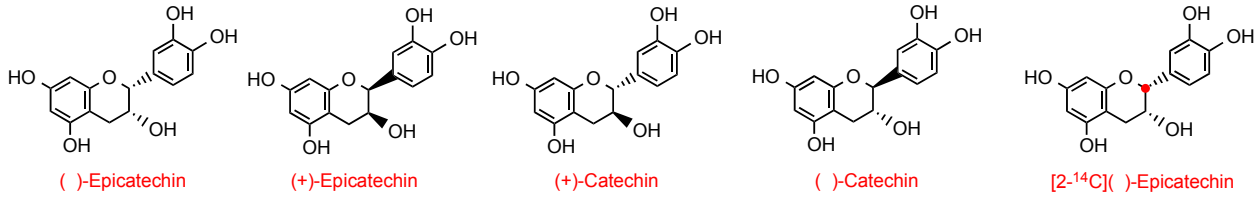

Fig. 1. Structures of epicatechin and catechin stereoisomers and [2-^{14}C](−)-epicatechin in which the red circle indicates the position of the ^{14}C-label. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

intervention studies linking the consumption of flavan-3-ols derived from cocoa to improved cardiovascular health and cognitive function (Heiss et al., 2010; Del Rio et al., 2013; Brickman et al., 2014; Rodriguez-Mateos et al., 2014). In this context, (−)-epicatechin can, at least partially, be causally linked with these beneficial effects (Schroeter et al., 2006; Loke et al., 2008). Knowledge of the ADME of (−)-epicatechin, that is its metabolism within the proximal and distal gastrointestinal (GI) tract, absorption of metabolites into the circulatory and their transport through the body prior to urinary excretion is key to:

- the development of objective biomarkers of intake and, thus, the interpretation of epidemiological data on associations between intake and health.
- the assessment of safety and risks associated with intake.
- the design and execution of dietary intervention studies.
- the use of cell cultures in vitro and organ/tissue preparations ex vivo that are aimed at elucidating the mechanisms of action that causally underlie observations in vivo.

2. Pioneering studies and their limitations

Upon intake, (−)-epicatechin enters the circulatory system in nmol/L concentrations as phase II glucuronide, sulfate and methyl metabolites (Crozier et al., 2010). At the time of the initial bioavailability investigations nothing was known about the identity of the in vivo (−)-epicatechin metabolites so human studies on the ADME of flavan-3-ols following the intake of cocoa products, treated plasma and urine samples with β-glucuronidase/sulfatase prior to the analysis of the released (−)-epicatechin by reverse phase HPLC, typically with fluorescence (Richelle et al., 1999) or electrochemical detection (Rein et al., 2000; Wang et al., 2000). With this methodology Richelle et al. (1999) showed that following the consumption of 40 g of dark chocolate containing 282 μmol of (−)-epicatechin, the epicatechin levels rose rapidly and reached a peak plasma concentration (C_{max}) of 355 nmoL/L after 2.0 h (T_{max}). Wang et al. (2000) carried out a study in which varying amounts of chocolate were consumed with 40 g of bread which revealed a positive relationship between intake and (−)-epicatechin plasma concentrations.

β-Glucuronidase/sulfatase treatment of samples in such studies was convenient as metabolites, were converted to a single product, (−)-epicatechin, thus simplifying the subsequent quantitative analysis. However, relatively little was known about the individual metabolites present prior to enzyme treatment. A further limitation that has become apparent is that β-glucuronidase/sulfatase preparations fail to fully hydrolyse all (−)-epicatechin-sulfates and methyl-(−)-epicatechin sulfates, and as a consequence (−)-epicatechin bioavailability was underestimated (Saha et al., 2012). Use began to be made of HPLC coupled with ion trap mass spectrometric detection in the early 2000s, which represented a significant step forward towards unravelling the metabolism of (−)-epicatechin. This analytical approach enabled direct information to be obtained about individual metabolites without the use β-glucuronidase/sulfatase enzymes. In this way, human (−)-epicatechin metabolites that were partially identified included an (−)-epicatechin-*O*-glucuronide, an (−)-epicatechin sulfate and various *O*-methyl-(−)-epicatechin sulfates (Mullen et al., 2009; Stalmach et al., 2009). However, this approach did not permit the full structural elucidation of the metabolites and a further limitation was that quantification of metabolites was by reference to the parent compound, unmetabolized (−)-epicatechin, posing questions about the accuracy of the levels reported.

These problems have been now been overcome with the development of methods for the synthesis of a range structurally-related (−)-epicatechin metabolites (SREMs) (Sharma et al., 2010; Mull et al., 2012; Zhang et al., 2013a,b) as well as 5C-ring fission metabolites (5C-RFMs) such a 5-(hydroxyphenyl)-γ-valerolactones and their phase II metabolites (Sánchez-Patán et al., 2011; Curti et al., 2015; Brindani et al., 2017). The availability of standards enabled the development and validation of sample preparation methods which facilitated the accurate identification and quantification of SREMs and 5C-RFMs for the assessment of (−)-epicatechin bioavailability in humans and other species. Alongside these developments, substantially enhanced selectivity and sensitivity of analysis has been achieved with HPLC-MS through the use of high resolution triple quadrupole and orbitrap mass spectrometers. This has been of particular value in the detection of microbiota-derived catabolites, not just of flavan-3-ols, but also other dietary (poly)phenols (Pereira-Caro et al., 2016, 2017a,b).

3. Initial investigations in human volunteers analysed using authentic (−)-epicatechin metabolites

The first cocoa flavan-3-ol bioavailability study in which the identification and quantification of SREMs was aided by the availability of authentic (−)-epicatechin metabolites was that of Ottaviani et al. (2012). Ten volunteers ingested a cocoa-based test drink, which when consumed by a 75 kg subject, contained 476 μmol of (−)-epicatechin and 66 μmol of (±)-catechin. SREMs identified in plasma all attained C_{max} 2 h after cocoa intake which is indicative of absorption in the small intestine. The main metabolite was (−)-epicatechin-3′-*O*-glucuronide, which had a C_{max} of 589 nmoL/L. *O*-Methylated glucuronides, such as 4′-*O*-methyl-epicatechin-7-*O*-glucuronide, were also detected, but in much smaller concentrations. The main sulfated SREM was (−)-epicatechin-3′-sulfate (C_{max} 331 nmoL/L) together with lower amounts of (−)-epicatechin-5-sulfate (C_{max} 37 nmoL/L) and (−)-epicatechin-7-sulfate (C_{max} 12 nmoL/L). Other SREMs that were detected in low nmol/L concentrations were 4′-*O*-methyl-(−)-epicatechin-7-*O*-glucuronide, and 3′- and 4′-*O*-methyl-(−)-epicatechin-5/7-sulfates.

Subsequently Actis-Goretta et al. (2012) also identified and quantified an array of (−)-epicatechin metabolites in plasma after the ingestion of 100 g of dark chocolate containing 241 μmol of (−)-epicatechin and 90 μmol of (±)-catechin. Again, the main

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