Catechin Is Metabolized by Both the Small Intestine and Liver of Rats

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ABSTRACT Flavan-3-ols are the most abundant flavonoids in the human diet, but little is known about their absorption and metabolism. In this study, the absorption and metabolism of the monomeric flavan-3-ol, catechin, was investigated after the in situ perfusion of the jejunum + ileum in rats. Five concentrations of catechin were studied, ranging from 1 to 100 µmol/L. The absorption of catechin was directly proportional to the concentration, and 35 ± 2% of the perfused catechin was absorbed during the 30-min period. Effluent samples contained only native catechin, indicating that intestinal excretion of metabolites is not a mechanism of catechin elimination. Catechin was absorbed into intestinal cells and metabolized extensively because no native catechin could be detected in plasma from the mesenteric vein. Mesenteric plasma contained glucuronide conjugates of catechin and 3′-O-methyl catechin (3′OMC), indicating the intestinal origin of these conjugates. Additional methylation and sulfation occurred in the liver, and glucuronide + sulfate conjugates of 3′OMC were excreted extensively in bile. Circulating forms were mainly glucuronide conjugates of catechin and 3′OMC. The data further demonstrate the role of the rat small intestine in the glucuronidation and methylation of flavonoids as well as the role of the liver in sulfation, methylation and biliary excretion. J. Nutr. 131: 1753–1757, 2001.

KEY WORDS: • catechin • metabolism • absorption • rats • methylation

Flavan-3-ols are a class of flavonoids that are widely distributed in fruits and beverages including green tea, red wine, apples and chocolate (1–4). In foods, they are present as monomers, oligomers and polymers; they may be esterified with gallic acid but are generally not present as glycosides (5). Although their intake levels are not precisely known, they are likely the most abundant flavonoids in the human diet with estimates of consumption ranging from 0.1–0.5 g/d (6,7). In addition to the widely reported in vitro biological activities of flavan-3-ols (8,9), consumption of purified monomers and foods containing predominantly flavan-3-ols has been shown to reduce platelet activity, fatty streak development and certain cancers (6,10–17). The particular mechanism(s) of action are impossible to establish without a complete understanding of their uptake as well as their metabolism and distribution among tissues and cells. The monomers catechin and epicatechin are absorbed and are present as glucuronidated, sulfated and methylated conjugates in human plasma (16,18–21). However, it is unclear which tissues are responsible for their metabolism.

Until recently, the liver has been presumed to be responsible for most flavonoid metabolism due to its high concentrations of UDP-glucuronosyltransferase (22,23) sulfotransferase (24,25) and catechol-O-methyltransferase (COMT)2 (26). However, conjugation enzyme activities are widely distributed among tissues (27). In vitro studies indicate that the metabolism of epicatechin and other flavonoids can occur in the small intestine (28–30). Studies with isolated rat intestine (31–33) also indicate that metabolism of several other flavonoids occurs with efficiency in the small intestine. The flavonol, quercetin, is extensively absorbed, metabolized and then reexcreted by the small intestine as was shown in an in situ perfusion model (34).

In this study, the absorption and metabolism of catechin was investigated after in situ perfusion in the jejunum and ileum in rats. Like many intestinal perfusion models, the model allows the direct calculation of the amount absorbed as well as the characterization of metabolites formed by the intestine by their appearance in mesenteric blood. In contrast to models using isolated intestines, the in situ model can be used to study the metabolism and excretion at other sites such as the liver and can give insight into how the different organs function together in the living animal. Thus, the aim of the present work was to investigate the absorption and metabolism of catechin by the small intestine as well as the subsequent contribution of the liver in metabolism and excretion.

MATERIALS AND METHODS

Chemicals and reagents. (+)-Catechin, and (+)-taxifolin were purchased from Extrasynthese (Genay, France). The 3′- and 4′-O-methylated conjugates of catechin were synthesized using a mixture of 250 mg (+)-catechin, 500 mg K2CO3 and 1 mL methyl iodide in 20 mL acetone, which was placed in an ultrasonic bath for 2.5 h. The 3′- and 4′-O-methylated conjugates were purified by semipreparative HPLC and the positions of the methyl groups were confirmed by one-dimensional (1D)-difference nuclear Overhauser effect spectroscopy as previously described (35). β-Glucuronidase (G-0376; EC 3.2.1.31) was purchased from Wako Chemicals USA Inc. (Richmond, VA). Albumin (fraction V), bovine serum albumin (BSA) (Bio-Rad Laboratories, Richmond, CA), bovine serum albumin (BSA) (Sigma-Aldrich, St. Louis, MO), and bovine serum albumin (BSA) (Sigma-Aldrich, St. Louis, MO) were purchased from Sigma-Aldrich (St. Louis, MO). Phenol was purchased from Sigma-Aldrich (St. Louis, MO). Water used in all experiments was deionized and passed through a Milli-Q system (Millipore, Saint-Laurent, Quebec, Canada). Glucuronic acid was purchased from Sigma-Aldrich (St. Louis, MO). All other chemicals and reagents were of the highest grade available.
Absorption of catechin. Five different concentrations of catechin, ranging from 1 to 100 μmol/L, were perfused through the small intestine in situ. The absorption of catechin was directly proportional to the amount perfused (Fig. 1). The percentage absorbed (35 ± 2%) was not significantly different at any of the concentrations. Catechin in the effluent was present exclusively in its native form.

Mesenteric plasma. Plasma was taken from the mesenteric vein at the end of the 30-min perfusion period to determine the presence of metabolites that were formed in the small intestine. Levels of specific forms of catechin metabolites in mesenteric plasma, abdominal aortic plasma and bile after perfusion with 30 and 100 μmol/L are shown in Table 1. Native catechin was not detected in untreated plasma taken from the mesenteric vein. Plasma taken from the mesenteric vein contained glucuronide conjugates of catechin and glucuronide conjugates of 3′OMC. No 4′-O-methylcatechin (4′OMC) or conjugates were detected. A chromatogram of plasma taken from the mesenteric vein after hydrolysis by β-glucuronidase is shown in Figure 2. The sum of the conjugate forms of catechin and 3′OMC in mesenteric plasma was proportional to the amount perfused and averaged 1.2 μmol/L after perfusion with 30 μmol/L catechin and 3.8 μmol/L after perfusion with 100 μmol/L catechin. The proportion of methylated metabolites tended to be higher (0.05 < P < 0.1) when perfusion was performed at 30 μmol/L than at 100 μmol/L.

Excretion by bile. Metabolites of catechin were eliminated extensively by bile. Bile flow was 238 ± 97 μL during the first 20 min of perfusion and 116 ± 28 μL during the last 10 min of perfusion. Specific conjugate forms were collected during the last 10 min of perfusion with 30 and 100 μmol/L in bile. The concentration of metabolites excreted in bile were 26.8 and 58.2 μmol/L after perfusion with 30 and 100 μmol/L, respectively (Table 1). The major metabolite present in bile was a glucuronidated + sulfated form of 3′OMC, but a glucuronide conjugate of 3′OMC was also present. No conjugates of 4′OMC were detected. Unmethylated forms of catechin were also excreted in bile, but at much lower concentrations (<0.1 μmol/L) (Table 1).

Abdominal aortic plasma. Plasma taken from the abdominal aortic artery contains the circulating metabolites that reach peripheral tissues. Aortic plasma contained total concentrations of catechin metabolites of 0.5 and 1.5 μmol/L after perfusion with 30 and 100 μmol/L catechin, respectively (Table 1). Catechin and 3′OMC were both in glucuronidated form.

The results are presented as means ± SEM. Differences with P < 0.05 were considered significant.
form, although in some rats, very small but detectable quantities of the glucuronidated and sulfated forms of both catechin and 3’OMC were present. No 4’OMC or its conjugates could be detected. The proportion of methylated metabolites tended to be higher (0.05 < P < 0.1) when perfusion was performed at 30 μmol/L.

DISCUSSION

We used an in situ model of intestinal perfusion to study the absorption and metabolism of catechin. The model allows the direct calculation of absorption by the small intestine. The animals are intact and alive throughout the experiment; thus, metabolism can occur in several different tissues. The model can distinguish between metabolic conjugation in the intestine as well as the liver and peripheral tissues by identifying metabolites in mesenteric plasma, bile and abdominal aortic plasma. The circulating metabolites after in situ perfusion are in agreement with those observed in rat feeding studies, namely, glucuronide conjugates of catechin and 3’OMC (37) although some sulfate conjugates have been reported (38).

Catechin in liver samples was extensively methylated (37), which is consistent with our finding that the biliary metabolites were methylated. Shaw and Griffiths (39) reported that the major metabolite in bile was a glucuronidated form of 3’OMC; we report this metabolite as well as a form of 3’OMC that is both glucuronidated and sulfated. The agreement in metabolite composition with in vivo studies indicates that the metabolic processes that occur after in situ perfusion reflect the processes that occur after consumption of catechin by rats.

The effect of the catechin dose on absorption was investigated by perfusion at five different concentrations. A maximum concentration of 100 μmol/L catechin was chosen because the concentration could rarely be higher in the small intestine even after a concentrated food source of catechin (40). The absorption of catechin was directly proportional to the concentration perfused, and this was reflected in metabolite levels in both mesenteric and aortic plasma. This finding is consistent with two studies that showed that plasma levels of epicatechin metabolites were directly proportional to the dose from chocolate (21,41). The mechanism by which catechin is absorbed by the small intestine is not known, but catechin probably enters the enterocytes by passive diffusion, and absorption by this mechanism should be proportional to the concentration (42). If catechin enters the cell by another mechanism such as facilitated transport, it appears not to be saturated even at concentrations of 100 μmol/L. The paracellular transport of catechin by Caco-2 cells, a well-established model of human intestinal absorption, was recently described (43). However, paracellular transport was clearly not the mechanism in the rat small intestine, given the extensive metabolism that must occur within the enterocytes.

Intestinal excretion of conjugated metabolites may constitute an important excretion mechanism for some flavonoids. Using the same perfusion model, 67% of quercetin aglycone was absorbed and conjugated by the small intestine, but the majority of absorbed quercetin was then excreted as conjugates back into the intestinal lumen (34) presumably by the multidrug resistance protein pump (30,44). Our results suggest that the efflux of conjugated forms does not occur because only
native catechin was present in the perfusion effluent. Therefore, it appears that when catechin is absorbed into the enterocytes, it is converted to metabolites that are then excreted or transported exclusively on the serosal side to the mesenteric blood where they are delivered to the liver.

The composition of metabolites in mesenteric and aortic plasma as well as bile reveals differences in metabolism in different tissues. Glucuronidation and methylation clearly occurred in the small intestine in this study. Sulfated metabolites were present almost exclusively in bile and thus were formed in the liver. Excretion of high amounts of methylated conjugates in bile, as well as their increased proportion in aortic compared with mesenteric plasma, indicates that methylation also occurs in the liver. Piskula and Terao (29) proposed, on the basis of the enzyme activities present in isolated rat tissues, that epicatechin is glucuronidated in the intestine and then sulfated in the liver, and methylated in the liver and possibly the kidney. We also conclude that glucuronidation is the major conjugation process that occurs in the small intestine and that sulfation and methylation occur in the liver. In addition, methylation occurred in the small intestine, as has been previously described for flavonoids (33,34,45).

A schematic representation of the possible mechanisms of absorption and metabolism of catechin in rats is shown in Figure 3. Absorbed catechin enters intestinal epithelial cells where it is always glucuronidated and sometimes methylated. Apparently, some of the glucuronides are able to enter hepatocytes as has been described for other glucuronides (46,47). Manach et al. (37) reported that after feeding rats catechin, only half of the catechin was glucuronidated in liver samples, suggesting that some glucuronides are de-glucuronidated and then re-glucuronidated in the liver, but this may occur only if pre-existing glucuronides have access to β-glucuronidases in the endoplasmic reticulum (37,47). Nevertheless, it appears that in the cytosol of hepatocytes, catechin glucuronides are sulfated and/or methylated and are eliminated extensively by bile. The circulating forms are then exclusively glucuronide conjugates of catechin and 3′OMC. If metabolism also occurs in the kidneys as suggested by Piskula and Terao (29), the metabolites are likely excreted by urine immediately after. Metabolism by other tissues cannot be excluded, although this was not observed in this study.

Recently, it was reported that after isolated intestinal perfusion of 50 μmol/L catechin or epicatechin, large amounts of the native compounds were transported across the small intestine and O-methylation occurred at both the 3′ and 4′ positions (33). In contrast, we observed that all of the absorbed catechin was metabolized by the small intestine with concentrations up to 100 μmol/L and that methylation occurred exclusively on the 3′ position (Fig. 2). In vivo studies in rats, marmosets and humans have also demonstrated that catechin is methylated at the 3′ position (18,20,37–39,48). Methylation at this position may be due to specificity of COMT or a cytochrome P450 that later catalyzes 4′-O-demethylation. However, if pre-existing glucuronides from the intestine are subsequently methylated in the liver as our results suggest, the methylated glucuronides would require sufficient access to cytochrome P450 to become 4′-O-demethylated (42,47,49).

The dose of catechin could affect the composition of individual metabolites. However, no significant differences were observed between the proportions of the conjugate forms at 30 and 100 μmol/L. Complete glucuronidation in the small intestine occurred even at 100 μmol/L. Slightly higher proportions of methylated metabolites existed in both mesenteric and aortic plasma after perfusion with 30 vs. 100 μmol/L, but these differences were not significant (0.05 < P < 0.1). Only a very small amount of sulfate conjugation occurred in the small intestine at both 30 and 100 μmol/L. However, sulfotransferases are generally saturated at much lower concentrations than are glucuronyl transferases (50,51); whether increased proportions of catechin are conjugated with sulfate in the small intestine after lower doses remains to be determined. Similar proportions of sulfate conjugates in bile were observed after both concentrations, indicating that sulfate conjugation in the liver was not affected by increasing the dose.

From this study, it is clear that the small intestine is the most important organ of glucuronidation and that it also plays a role in the methylation of catechin. The liver was certainly the major site of sulfation as well as additional methylation. We conclude that native catechin enters intestinal epithelial cells, glucuronides enter hepatocytes and that extensive metabolism occurs in both organs in rats. It is essential to further characterize the specific metabolites formed, their subsequent distribution in tissues and cells, as well as their specific biological activities.

LITERATURE CITED

ABSORPTION AND METABOLISM OF CATECHIN IN RATS


