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ORIGINAL CONTRIBUTION

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Evaluation of polyphenol bioavailability in rat small intestine

Summary *Background* Dietary polyphenols, which are contained in several foods of plant origin, have been reported to be effective protective agents against cardiovascular diseases and cancer. However, data on their absorption from the gastrointestinal tract are still scarce and, often, contradictory. *Aim of the study* In this report, evaluation of polyphenol bioavailability was carried out by using segments of the small intestine from rat. The extent of absorption throughout the small intestine of rat was evaluated with two model compounds, tannic acid and catechin, as representatives of high and low molecular weight polyphenols, respectively. The consequence of the binding of tannic acid to BSA on both tannic acid absorption and *in vivo* protein digestibility was also examined. *Methods* Polyphenol solutions of different concentrations were injected into the lumen of ligated segments (6 cm) of the small intestine and the segments incubated in buffer for 5 min. The residual amount of polyphenol in the lumen of each segment was assayed by maximum absorption in the UV/VIS optical spectrum as was the amount of compound that had crossed the gut wall into the incubation buffer. Digestibility of BSA and of a BSA-tannic acid complex was assayed with rats. *Results* The results indicated a significant, concentration-dependent, disappearance of both polyphenols from the small intestine of the rat, with higher uptake levels being evident for tannic acid (50 %) than catechin (30 %). However, complete transfer through the gut wall was not observed with tannic acid whilst low but significant amounts (10 %) were detected in incubation buffers with catechin. Partial binding of polyphenols by endogenous proteins in the intestinal lumen was also demonstrated. Complexing tannic acid with BSA (1:10 mol/mol) was not found to affect either the extent of interaction of tannic acid with the small intestine or the *in vivo* digestibility of the protein. *Conclusions* Our experiments show that tannic acid and catechin both interact with the gut but only catechin appears able to traverse the gut. In addition, they provide evidence for binding of tannic acid and catechin by endogenous proteins in the intestinal lumen. This may limit their absorption from the small intestine. BSA complexed with tannic acid was as readily digested as BSA alone. This may suggest that tannic acid exerts anti-nutritional effects by binding to proteins of the gut wall and interfering with gut function rather than by inhibition of dietary protein digestion.

Key words Tannic acid – Catechin – Bioavailability – Rat intestine – Polyphenol/protein interaction

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Introduction

Polyphenols – flavonoids, phenolic acids and tannins – are contained in several foods of plant origin (fruit and vegetables) (1). Much evidence has been provided in recent years supporting a role for these compounds as protective agents against cardiovascular disease and certain forms of cancer, including breast, esophageal, gastrointestinal, lung and skin cancer (2–4). On the other hand, claims about mutagenic activity of some polyphenols, such as rutin from red wine, need also to be carefully considered (5). The biological activity of polyphenolic compounds is related, at least in part, to their antioxidant properties, due to both free-radical scavenging activity and metal chelating properties (6–8). However, other mechanisms of actions, such as regulation of hormone and enzymatic activity, have also been described (9–11).

Increasing evidence for the possible effects of plant polyphenols on human health have been obtained in *in vitro* and *in vivo* systems. However, data on their absorption from the gastrointestinal tract are still scarce and, often, contradictory (12–16), although it is likely that the different biological effects of polyphenols are related to their absorption and bioavailability for target organs and tissues.

Absorption of simple phenolic compounds, such as gallic acid (17), caffeic acid (18) and catechin (19–21) by animals and man has been demonstrated. However, most of the polyphenols are present in food plants as esters of organic acids or as glycosides. The glycosylated forms have long been considered not to be absorbed from the gut unless hydrolysed by the microflora in the large intestine or in the distal ileum (22). Because microorganisms extensively degrade dietary polyphenols, only limited absorption would occur. This claim was later questioned (23–27) because absorption of intact glycosylated form of quercetin, possibly through glucose transport pathway of the small intestine (26), was in fact observed.

The molecular weight and structure of polyphenolic compounds are likely to have a major effect on their metabolic fate. Soluble compounds such as catechin and tannic acid, appeared to be absorbed or partially degraded in the rat intestine, whereas high molecular weight condensed tannins or bound-polyphenols were not (28). Previous studies of Butler et al. (29) indicated absorption of tannins when rats were fed with ^{125}I-labelled tannins, radioactivity being recovered in urine, serum, liver and kidney. However, although increased hepatic UDP glucuronyltransferase activity (an enzyme involved in the detoxification of phenolics) in chickens fed high-sorghum diets suggested absorption of tannins from the gastrointestinal tract (30), this hypothesis was not confirmed later when ^{14}C-condensed tannins were given to chickens (31). Whether the structure of tannins was changed by the iodination process or the result reflected differences in polyphenol absorption in different species could not be ascertained.

The capacity of polyphenols to complex proteins is well documented (1, 7). Nonetheless, only a few studies have been performed so far on the consequence of binding to proteins on both activity and bioavailability of the phenolic compounds (32, 33). In fact, protection of tannic acid hydrolysis in the gut after complexation with proteins has been reported (34).

Most of the studies on polyphenol absorption have been carried out using man or experimental animals in long-term studies. Other reliable methods for the estimation of intestinal uptake of food components, such as *in vitro* or *in vivo* perfusion techniques, are also available. However, these model systems are quite complex, time-consuming and expensive and often require sophisticated equipment.

In the present study polyphenol bioavailability was evaluated using segments of the small intestine of rats. Two polyphenolic compounds, tannic acid and catechin, were tested in our model system as representatives of high and low molecular weight polyphenols with gallic acid and flavonoid structures, respectively. After a short incubation time, both the amount of polyphenol in the lumen of each segment and that in the incubation buffer were measured by absorption maxima in the optical spectrum. The consequence of formation of a complex of tannic acid with proteins on both tannic acid absorption and *in vivo* protein digestibility was also examined.

Materials and methods

In vitro experiments with small intestinal segments of rat

All management and experimental procedures were carried out in strict accordance with the requirements of UK Animals (Scientific Procedures) Act 1986 by staff licensed under this Act to carry out such procedures.

Three rats were used for each determination. For *in vitro* experiments, rats of the Hooded Lister (Rowett) strain, weaned at 19 days, were maintained on stock diet until they reached approximately 140 g. They were then given a semisynthetic based lactalbumin-control diet for 7 days. Rats were fasted overnight before each experiment and killed by anaesthetic (halothane) overdose. The small intestine was removed and 15 consecutive sections of 6 cm length were taken from the pylorus along the duodenum, jejunum and ileum. The segment was washed with phosphate buffered saline pH 7.5, tied at one end and loosely ligated at the other end. 0.2 ml of solutions containing increasing concentrations of tannic acid (0.059–0.29 μM) or (+)catechin (0.17–3.44 μM) (Sigma Chemical Co. (Poole, Dorset)) in 0.05 M

$NaHCO_3$-0.08 M taurocholate buffer, pH 7.4, were inserted into each segment via the loosely ligated end by syringe. This end of the segment was then tied tightly and the ligated segment was incubated at 37 °C for 5 minutes in vials containing 2 ml of buffer. At the end of the incubation period, the segment was opened and the contents were gently washed out with 2 ml of the same buffer and centrifuged (8000*g*, 15 min). Incubation buffers were also centrifuged. Optical density values (OD) were measured at 313 nm and at 281 nm for tannic acid and catechin, respectively. The concentration of polyphenols in each gut piece and the corresponding incubation buffer was calculated from tannic acid (5.9×10^{-4}–0.029 μM) or catechin (6.89×10^{-3}–0.34 μM) standard curves, made in $NaHCO_3$-taurocholate buffer and run in triplicate. The percentage of residual polyphenol in the intestinal washing was calculated from a comparison of OD values of the tannic acid or catechin solutions inserted into the segments. The amount of polyphenol taken up by the gut was obtained by difference between the amount inserted and that left in the segment. The extent of interaction of polyphenols with duodenal, jejunal and ileal tissue was tested by inserting the same amount of tannic acid (100 μg) into 6 cm segments taken sequentially along the whole small intestine of rat (90 cm). The percentage of residual polyphenol in the intestinal washing was calculated as described above. The amount of protein (mg/cm of gut) present in each segment was calculated after precipitation of proteins in the intestinal washing with trichloroacetic acid (TCA), at a final concentration of 5 % (w/v). The suspension was centrifuged and the precipitate redissolved in 0.1 M NaOH. Protein in the precipitate was then assayed by the Lowry method (35). To correct for the contribution of the protein background, the OD at 313 nm (for tannic acid) and at 281 nm (for catechin) of appropriate blanks (in which only buffer was inserted into the intestinal segment) was determined and subtracted from the OD values measured in presence of tannic acid or catechin solutions.

Estimation of the amount of polyphenol that was protein-bound in the intestinal segments was performed after precipitation of proteins in the intestinal washings with trichloroacetic acid (TCA), at a final concentration of 5 % (w/v). The suspension was centrifuged and the supernatant was brought to neutral pH by addition of concentrated NaOH. Free polyphenol content (tannic acid and catechin) in the supernatant was quantified as described above and protein-bound polyphenol was calculated by difference.

The effect of the binding of tannic acid to bovine serum albumin (BSA) on both tannic acid absorption and *in vivo* protein digestibility was examined. In the former case, tannic acid in taurocholate-$NaHCO_3$ buffer (pH 7.4) at a concentration from 0.06 to 0.29 μM was complexed with a 10-fold molar excess of BSA in the same buffer and the solution was incubated for 30 minutes at room temperature. Of these tannic acid-BSA solutions 0.2 ml was inserted into rat segments and the experiment carried out as described above. The concentration of tannic acid measured in the presence of BSA in each gut segment was compared with that of tannic acid solutions at the same concentration but in absence of BSA.

In vivo digestibility experiments

The effect of the binding of tannic acid to proteins on the *in vivo* digestibility was tested in rats by measuring the digestibility of BSA, either free or complexed with tannic acid. Digestibility *in vivo* was determined as described by Carbonaro *et al.* (36) in acute (1 h) experiments with growing rats. Male rats of the Hooded Lister (Rowett) strain (40 days of age) were adapted to experimental conditions by giving them a lactalbumin-diet for 7 days. Rats (140±1 g) were then housed individually in polypropylene and stainless-steel cages and fasted overnight before the experiment. Rats were intubated with 3 ml of a 0.5 μM BSA solution in taurocholate-$NaHCO_3$ buffer (pH 7.4) or with a BSA solution at the same concentration but in presence of tannic acid (BSA-tannic acid 10:1 on a molar basis). Control values were obtained by giving rats 3 ml taurocholate-$NaHCO_3$ buffer. One hour after gavage the rats were killed by halothane overdose. The time of 1 h was chosen because the digesta would not yet have reached the large intestine by this time (37). A longitudinal incision along the midline of the abdomen was made and the stomach and small intestine were taken out separately. The stomach and intestinal contents were washed out with ice-cold water containing 0.1 mg/ml Aprotinin (Sigma Chemical Co., St. Louis, MO, USA) and centrifuged at $4000\times g$ for 45 min. The protein content of the supernatants was determined by the method of Lowry *et al.* (35). Protein digestibility (%) in the small intestine was calculated, after correction of the amount of protein in stomach and small intestinal content for non-dietary protein, by the following expression:

$$\text{P.D. (\%)} = \frac{P_{ing}-P_{st}-P_{int}}{P_{ing}-P_{st}} \times 100$$

where P.D. = protein digestibility; P_{ing} = mg of protein ingested; P_{st} = mg of protein in the stomach; P_{int} = mg of protein in the intestine.

Statistical analysis

Data from parallel determinations were subjected to analysis of variance. The significance of the differences

between means was obtained by Student's *t*-test ($p < 0.05$).

Results and discussion

Interaction of tannic acid and catechin with the small intestine

Nutrients may be absorbed with differing efficiencies at various sites along the small intestine. As shown in Fig. 1, the extent of interaction of tannic acid with duodenum (up to 6 cm gut length), jejunum (up to 48 cm) and ileum (up to 90 cm) was similar ($p > 0.05$). The percentage of residual tannic acid in the gut contents ranged from 40 to 60 % of the original.

A major point likely to affect polyphenol bioavailability is their ability to interact with dietary or endogenous proteins in the gut and the effects this potentially has on the efficacy of absorption of polyphenols from the gut. Quantification of proteins in the intestinal segments after precipitation with TCA indicated that as much as 0.4–1.0 mg of protein/cm of gut could be detected. These proteins may have been released from the gut wall possibly due to the sloughing off cells during the incubation period. In fact, about half of the tannic acid that was present in the intestinal washing after 5 minutes incubation was found to be protein-bound (Fig. 1). Tannic acid is a very powerful protein-binding compound: indeed, complexation of proteins by high MW tannins (either hydrolysable or condensed tannins) during gastrointestinal digestion has long been reported to account for limited digestion of proteins in tannin-containing foods, such as legumes and cereals (7, 38).

The amount of polyphenol that was apparently taken up by the gut from the lumen appeared to increase in a dose-dependent manner – when increasing concentrations of tannic acid or catechin were inserted into segments of small intestine from rat (Figs. 2 and 3, respectively). The percentage of polyphenol taken up by the gut was higher for tannic acid (40–60 %, Fig. 2) than for catechin (25–35 %, Fig. 3). However, the UV/VIS spectra for the outer incubation buffers at the various polyphenol concentrations tested showed no absorption peak that could be ascribed to tannic acid. Therefore, it appeared that no tannic acid was transported completely through the gut wall. In the case of catechin, the amount that could be detected in the external buffer was around 10 % of total catechin inserted into each segment.

The amount of tannic acid that was bound to proteins in the intestinal washings was 20–50 % (Fig. 1). Estimation of the amount of catechin that was protein-bound in the intestinal contents indicated that it ranged from 40 to 70 % of the total catechin that was not taken up by the gut (results not shown). Therefore, not only high MW polyphenols such as tannic acid can bind proteins in the small intestine, but also low MW flavonoids, i. e. catechin. The interaction between low molecular weight phenolics, including catechin, and BSA has been recently studied *in vitro* (32) and changes in the activity of phenolics after binding to proteins have been reported (33). However, the interaction between phenolic compounds and endogenous proteins in the small intestine has not been thoroughly investigated, although bioavailability of the compounds may be changed after binding to the protein matrix.

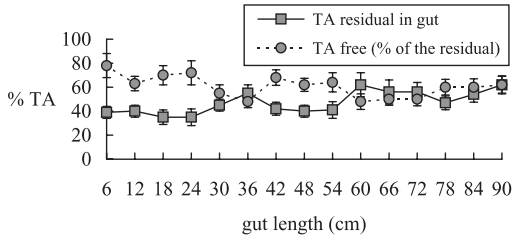

Fig. 1 Interaction of tannic acid (TA) with different parts of the whole small intestine. An identical amount of tannic acid (100 µg) was inserted into each of the 6 cm segment taken along the 90 cm-small intestine and the residual amount of tannic acid was obtained by the optical density values of luminal contents at 313 nm, as described under Materials and Methods. cm 0–6: duodenum; cm 6–48: jejunum; cm 48–90: ileum. The values are the means ±SD (n = 3).

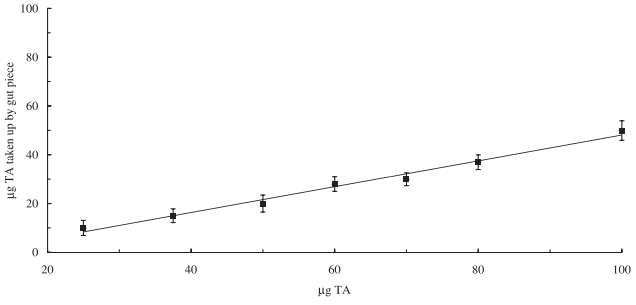

Fig. 2 Dose-dependence of tannic acid (TA) disappearance after 5 min incubation in the jejunum. The amount of tannic acid that was taken up by the gut was obtained as described under Materials and Methods. The values are the means ±SD (n = 3).

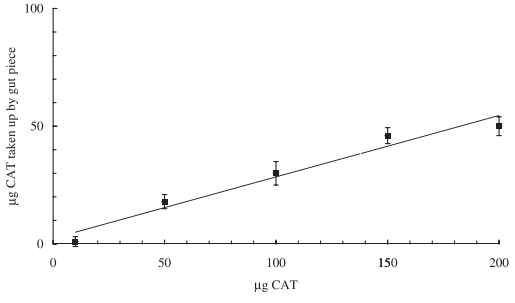

Fig. 3 Dose-dependence of catechin (CAT) disappearance after 5 min incubation in the jejunum. The amount of catechin that was taken up by the gut was obtained as described under Materials and Methods. The values are the means ±SD (n = 3).

Data on polyphenol absorption are still insufficient and often controversial. *In vivo* studies with rat or man have indicated that bioavailability of polyphenols can vary widely, likely depending on both the experimental system and the chemical structure of the polyphenolic compound (23, 24). Catechin appears to be absorbed in man, but only 10 % of the total amount was detected in serum after oral administration (20, 39), a value that appears consistent with that determined in our system. Apparent digestibility of the flavonoid quercetin appeared to be higher than that of catechin, being 15–20 % in rat (27, 40) and 24 % in human ileostomy subjects (23). Although only the aglycone forms of quercetin (and of other flavonoids) were assumed to be absorbed in the small intestine, absorption of quercetin-glucosides in man was recently found much higher (52 %) than that of the corresponding aglycone (20 %) (24).

As concerns polymeric phenolic compounds such as tannins, only a few studies concerning their absorption in the small intestine are available. However, most of the evidence is consistent with our finding that transfer of these compounds from the small intestine is slight. Despite results of studies with ^{125}I-labelled polyphenolics from Quebracho (*Schinopsis* spp.) (29) suggesting the absorption of tannins in rats, the opposite was found by the same authors when ^{14}C-condensed tannins were given to chickens (31). As only small amounts of tannic acid and catechin were recovered in the faeces, Bravo et al. (28) suggested their degradation and absorption in the intestinal tract of rats. However, no indication about the fate of these compounds in the small intestine was provided by the authors. Degradation of tannic acid to gallate during gastrointestinal digestion with recovery of gallate in the urine has also been observed: on the contrary, when complexed with proteins, tannic acid was protected against hydrolysis in the gut (34).

To test whether complexation of tannic acid to proteins prevented its interaction with the gut wall, tannic acid was complexed with an excess of BSA and increasing concentrations of the tannic acid-BSA complex were inserted into the small intestinal segments. The residual concentration of tannic acid was then measured after 5 minutes incubation (Fig. 4). Unexpectedly, the percentage of tannic acid that was apparently taken up by the gut in the presence and absence of BSA was the same. Therefore, the binding of tannic acid to BSA does not seem to impair its interaction with the small intestine of rat. This may suggest that the sites of interaction of tannic acid with proteins and with the gut wall are different.

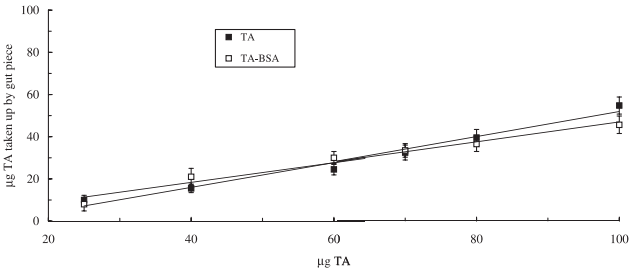

Fig. 4 Effect of BSA binding on the interaction of tannic acid (TA) with the small intestine. The amount of tannic acid that was taken up by the gut in presence and in absence of BSA was obtained as described under Materials and Methods. The values are the means ±SD (n = 3).

In vivo digestibility of the BSA/tannic acid complex

Table 1 gives the *in vivo* digestibility values for BSA alone or when complexed to tannic acid. The binding of tannic acid to BSA appeared to have little or no effect on either the digestibility of the protein or on the amount of protein that was recovered in stomach and small intestine after 1 hour. Indeed, digestibility of BSA in the small intestine was practically complete, being about 97 % in both cases. This result was in line with the previous observation of the lack of any effect of binding of BSA to tannic acid on its interaction with the small intestine (Fig. 4).

Anti-nutritional effects of tannins, such as reduced growth rate or decreased protein and amino acid digestibility, are well established. Nonetheless, the present findings do not appear to support the generally accepted view of a major negative effect of polyphenols on protein digestion through complexation to dietary proteins in the gastrointestinal tract. This may be because the amount of tannin used was relatively low. Alternatively, it has recently been suggested that most of the antinutritional effects of tannins in monogastric animals can be attributed to systemic effects, such as inhibition of post-digestive metabolism or impaired utilisation of absorbed amino acids, by low MW polyphenol components readily absorbed in the small intestine (31, 34). Thus, the consequences of direct binding of polyphenol to the gut wall may be relevant and also affect the overall absorption. Inhibition of sucrase activity and of the Na^+-dependent glucose transporter by tannic acid and low MW phenolic compounds (epicatechin, catechol) in rat brush border membrane vesicles have indeed been assessed (41, 42).

Table 1 Effect of tannic acid (TA) on the amount of total protein (mg) in the gastrointestinal tract of rats and on the in vivo digestibility (%) of BSA. Rats were killed after 1 h intubation with either 100 mg of BSA or 100 mg of BSA complexed with 300 µg of TA (a)

Sample	BSA	BSA-TA
Residual protein in:		
Stomach	0.8±0.5	0.9±0.7
Small intestine	2.8±0.6	3.6±2.1
Intestinal digestibility	97.2±0.6	96.4±2.3

(a) Values are means and standard deviations of three determinations.

Conclusions

The results of this present study indicate a concentration-dependent disappearance of polyphenols from the lumen of segments of the small intestine of rat, which was higher with tannic acid than with catechin. However, passage of polyphenols through the gut wall was only observed with catechin but even with this it was quite low (10 %). A consistent part of the polyphenols was found to be bound by endogenous proteins in the intestinal lumen. However, binding of tannic acid to BSA appeared to have little or no effect on either the extent of interaction of tannic acid with the small intestine or the *in vivo* digestibility of BSA. This might suggest that the antinutritional effect of high MW polyphenols, such as tannic acid, is more likely to be due to their direct binding to proteins of the gut wall rather than by their complexation with dietary proteins during gastrointestinal digestion.

The model system used in this study appears to be suitable for a rapid screening of polyphenol bioavailability, as well as of the potential interactions among dietary or endogenous components that may affect their binding to the gut wall. Further application of the model with other dietary polyphenolic compounds is currently in progress.

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