RESEARCH ARTICLE

Absorption of anthocyanins through intestinal epithelial cells – Putative involvement of GLUT2

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Anthocyanins bioavailability is a major issue regarding their biological effects and remains unclear due to few data available on this matter. This work aimed to evaluate the absorption of anthocyanins at the intestine using Caco-2 cells. Anthocyanin extract, rich in malvidin-3-glucoside, was obtained from red grape skins and tested on Caco-2 cells. The absorption of anthocyanins, in absence or presence of 1% ethanol, was detected by HPLC/DAD/LC-MS. Our results showed that this transport was significantly increased in the presence of ethanol especially after 60 min of incubation. In addition, cells that were pretreated for 96 h with anthocyanins (200 μg/mL) showed an increase of their own transport (about 50% increase). Expression of glucose transporters sodium-dependent glucose transporter 1, facilitative glucose transporters 5, and facilitative glucose transporters 2 was assessed by RT-PCR. It was found that facilitative glucose transporters 2 expression was increased (60%) in Caco-2 cells pretreated with anthocyanins, by comparison with controls. When the effect of anthocyanin extract on ^{3}H-2-deoxy-D-glucose uptake was tested, an inhibitory effect was observed (about 60% decrease). However, the malvidin aglycone was tested and had no effect. In conclusion, anthocyanins could be absorbed through Caco-2 cells, and can interfere with their own transport and also with glucose intestinal uptake.

Keywords:
Anthocyanin *in vitro* bioavailability / Caco-2 cells / Glucose transporters / Glucose uptake / Malvidin-3-glucoside

1 Introduction

Anthocyanins are water soluble naturally occurring secondary metabolites responsible for the blue, purple, and red color of many plant tissues. They occur primarily as glycosides of their respective anthocyanidin chromophores, with the sugar moiety attached at the position 3 on the C ring or the position 5 on the A ring. The common anthocyanidins (aglycones) are cyanidin, delphinidin, petunidin, peonidin, pelargonidin, and malvidin. Aglycones are rarely found in fresh plant material. Several hundred anthocyanins are known, and they vary as follows: (i) in the number and position of hydroxyl and methoxyl groups on the basic anthocyanidin skeleton to the flavylium cation; (ii) in the identity, number, and positions at which sugars are attached; and (iii) in the extent of sugar acylation and the identity of acylating agent. Glucose, galactose, arabinose, rhamnose, and xylose are the most common sugars that are bonded to anthocyanins [1]. Like other flavonoids, anthocyanins and anthocyanidins (the aglycone form) have antioxidant properties.

Anthocyanin levels (mg/fresh weight of fruits) range from 0.25 mg/100 g to 500 mg/100 g in pear and in blue-

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Abbreviations: GAPDH, glyceraldehyde-3-phosphate dehydrogenase; **GLUT2,** facilitative glucose transporters2; **GLUT5,** facilitative glucose transporters 5; **^{3}H-DG,** ^{3}H-2-deoxy-D-glucose; **MTT,** 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium-bromide; **SGLT1,** sodium-dependent glucose transporter 1

berries, respectively, and the fruits that are the richest in anthocyanins (> 20 mg/100 g fresh weight) are very strongly colored (deep purple or black) berries. Intake of these compounds was estimated to be 200 mg/day/person in the Unites Sates [2], but in other countries with other dietary habits anthocyanins intake could vary. The consumption of anthocyanins is among the greatest within the various classes of flavonoids, with the main dietary sources being red fruit, vegetables, and red wine [3, 4].

Anthocyanins have presented a wide range of biological effects such as antioxidant capacity, anti-inflammatory properties, and anti-carcinogenic activity [1, 5]. However, the role of anthocyanins on health beneficial properties is still unclear due to the lack of knowledge about the rate and extent of their absorption, metabolism, and tissue or cell distribution. There are considerable literature regarding anthocyanins absorption and bioavailability [6–11], but the results and conclusions are controversy and divergent. This issue still remains unclear despite the major role it plays regarding the biological effects of these compounds *in vivo*.

This work aimed to evaluate the absorption of anthocyanins on the intestinal epithelia. The influence of ethanol, as an important food matrix element, and of the chronic exposure of anthocyanins were also evaluated. The hypothesis of the interference of anthocyanins with glucose transporters was also investigated. For this purpose, an extract of anthocyanins from red grape skins was tested on Caco-2 cells, an enterocyte-like cell line derived from a human colonic adenocarcinoma.

2 Material and methods

2.1 Materials

^{3}H-2-deoxy-D-glucose (^{3}H-DG) (2-[1,2-^{3}H]; specific activity 40–50 Ci/mmol) from Amersham Pharmacia Biotech, Buckinghamshire, UK; Toyopearl gel was purchased from Tosoh® (Tokyo, Japan); Hepes, NaCl, Tris.HCl, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium-bromide (MTT) and cytochalasin B (from *Diechslera dematioidea*) were purchased from Sigma-Aldrich® (Madrid, Spain); TFA, 2-deoxi-D-glucose and malvidin were purchased from Fluka® (Madrid, Spain); Triton X-100 from Merck® (Darmstadt, Germany); malvidin-3-glucoside was purchased from Extrasynthèse (Genay, France).

2.2 Anthocyanins extract preparation

Skins of red grapes (*Vitis vinifera*) were subjected to extraction with 40% aqueous ethanol. The extract was filtrated in a 50 μm nylon membrane and then purified by Toyopearl gel HW-40(S) column chromatography according to the procedure previously described [12]. The fraction corresponding to the anthocyanidin monoglucosides was collected, the solvent was partially evaporated in a rotary evaporator at 30°C and the sample was freeze dried and stored at −20°C until use.

2.3 Anthocyanin extract analysis

The extract was analyzed by HPLC (Knauer K-1001) on a 250 × 4.6 mm id reversed-phase C18 column (Merck, Darmstadt); detection was carried out at 520 nm using a diode array detector (Knauer K-2800). The solvents were A: H2O/HCOOH (9:1) and B: H2O/CH3CN/HCOOH (6:3:1). The gradient consisted of 20–55% B for 50 min at a flow rate of 1.0 mL/min. The column was washed with 100% B for 20 min and then stabilized at the initial conditions for another 20 min. For LC-MS analyses, a liquid chromatograph (Hewlett-Packard 1100 series) equipped with an AQUATM (Phenomenex, Torance, CA, USA) reversed-phase column (150 × 4.6 mm, 5 μm, C18) thermostatted at 35°C was used. Solvents were (A) aqueous 0.1% TFA and (B) acetonitrile, establishing a gradient as reported elsewhere [12]. Double online detection was made in a photodiode spectrophotometer and by mass spectrometry. The mass detector was a Finnigan LCQ (Finnigan, San Jose, USA) equipped with an API source, using an ESI interface. Both the auxiliary and the sheath gas were a mixture of nitrogen and helium. The capillary voltage was 3 V and the capillary temperature 190°C. Spectra were recorded in positive ion mode between m/z 120 and 1500. The mass spectrometer was programmed to do a series of three scans: a full mass, a zoom scan of the most intense ion in the first scan, and an MS-MS of the most intense ion using relative collision energy of 30 and 60 V.

2.4 Cell culture conditions

The Caco-2 cell line was obtained from the American-Type Culture Collection (ATCC37-HTB, Rockville, MD, USA) and was used between passage number 41–58. Caco-2 cells were maintained according to the procedure described elsewhere [13]. They were grown in a humidified atmosphere of 5% CO_2-95% air, in minimum essential medium (Sigma, St. Louis, MO, USA) supplemented with 15% fetal bovine serum, 25 mM HEPES, 100 units/mL penicillin, 100 μg/mL streptomycin, and 0.25 μg/mL amphotericin B (all from Sigma). Culture medium was changed every 2–3 days and the culture was split every 7 days. For subculturing, the cells were removed enzymatically (0.25% trypsin-EDTA, 1 min, 37°C), split 1:3, and subcultured in plastic culture dishes (21 cm^2; ∅ 60 mm; Corning Costar, Corning, NY). For transport experiments, Caco-2 cells were seeded on transwell inserts (polycarbonate membrane, 0.4 μm pore size, 24 mm diameter, Corning Costar). Inserts were placed in a six well plates. For uptake studies, cells were seeded on 24-well plastic cell culture clusters (2 cm^2; ∅ 16 mm; Corning Costar).

Cells were allowed to grow and differentiate to confluent monolayers for 25 days after the initial seeding. The cell medium was free of fetal calf serum for 24 h before the experiments. Pretreatment of cells was achieved by the incubation of cells with 200 μg/mL anthocyanin extract during 4 days.

2.5 Transport studies

Transport studies experiments were performed using a modified procedure from Yi *et al.* [14]. Transepithelial electrical resistance of cells grown in the transwell was measured using an epithelial voltohmmeter fitted with planar electrodes (EVOM; World Precision Instruments, Stevenage, UK). Experiments were conducted only in cell monolayers that showed a transepithelial electrical resistance > 230 Ω. Medium was removed and cells were washed with Hanks' medium with the following composition (in mM): 137 NaCl, 5 KCl, 0.8 $MgSO_4$, 1.0 $MgCl_2$, 0.33 Na_2HPO_4, 0.44 KH_2PO_4, 0.25 $CaCl_2$, 0.15 Tris.HCl, and 1.0 sodium butyrate, pH 7.4. Anthocyanin solution in Hanks (200 μg/mL) was added to the apical side of the cells and Hanks containing 2% fetal bovine serum was added to the basolateral compartment. Transepithelial transport was followed as a function of time. Samples (150 μL) were taken from the basolateral side and replaced by fresh medium. The samples were acidified with HCl and frozen until HPLC analyses (according with the procedure described in Section 2.3. The cellular uptake of anthocyanins was also measured following the procedure below. The cell membranes were washed with Hanks' medium and then removed/tore off from the insert. Anthocyanins were extracted using methanol with 5% formic acid. Cells were sonicated for 5 min and centrifuged at 2000 × *g*. The supernatant was collected, and cells were rinsed two more times with acidified methanol and centrifuged again at 2000 × *g*. The supernatant solutions were evaporated under nitrogen and reconstituted in 150 μL of methanol with 5% formic acid. The samples were injected into HPLC and anthocyanins were quantified using a malvidin-3-glucoside calibration curve.

2.6 Glucose uptake

Caco-2 cells were incubated in glucose-free HEPES buffered saline (containing, in mM: 140 NaCl, 5 KCl, 2.5 $MgSO_4$, 1 $CaCl_2$, and 20 HEPES, pH 7.4). The culture medium was aspirated and cells were washed with 0.3 mL buffer at 37°C. Uptake was initiated by the addition of 0.3 mL medium at 37°C containing 1 μM ^{3}H-DG, according to procedure described elsewhere [15]. At the end of the incubation period, incubation was stopped by placing the cells on ice and rinsing them with 0.3 mL ice-cold buffer. The cells were then solubilized with 0.3 mL 0.1% v/v Triton X-100 (in 5 mM Tris.HCl, pH 7.4), and placed at 37°C overnight. Radioactivity in the cells was measured by liquid scintillation counting. When anthocyanins and cytochalasin B were tested, they were present during both preincubation and incubation periods.

2.7 RT-PCR

RNA was extracted from the Caco-2 cells using Tripure Isolation Reagent (Roche, Indianapolis, USA), according to the producer's instructions. RNA was dissolved in water (diethylpyrocarbonate treated) and stored at −80°C.

Reverse transcription was performed with SuperScript First Strand System for RT-PCR (Invitrogen, Carlsbad, CA), using 3 μg/μL random hexamers as primers at 55°C, according to manufacturer's instructions. cDNA was synthesized from 5 μg of total RNA in a total volume of 20 μL.

PCR amplification was performed in the presence of 1.5 mM $MgCl_2$ for facilitative glucose transporters 2 (GLUT2) and facilitative glucose transporters 5 (GLUT5) and 2 mM of $MgCl_2$ for sodium-dependent glucose transporter 1 (SGLT1). Five hundred micromolar of primer, 0.2 mM dNTPs, 2 U of Taq DNA polymerase (DFS-Taq DNA polymerase, Bioron GmbH) and 3.5 μL of RT product were added in a final volume of 50 μL. Simultaneous amplification of the invariant housekeeping gene glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was performed.

For GLUT2 the following primer was used: 5′-CAACCATTGGAGTTGGCGCTGTAA-3′ (forward primer), 5′-AGGTCCACAGAAGTCCGCAATGTA-3′ (reverse primer). Thermocycling consisted of 35 cycles of 30 s at 95°C, 30 s at 57°C, and 30 s at 72°C. The predicted size of the PCR products was 368 bp [16]. The primer for GLUT5 was the following: 5′-TGGAGCAACAGGATCAGAGCATGA-3′ (forward primer), 5′ACATGGACACGGTTACAGACCACA-3′ (reverse primer). Thermocycling consisted of 30 cycles of 30 seg at 95°C, 30 seg at 57°C, and 30 seg at 72°C. The predicted size of the PCR products was 231 bp [16]. For SGLT1 the following primer was used: 5′-TCTTCGATTACATCCAGTCAA-3′ (forward primer), 5′-TCTCCTCTTCC TCAGTCATC-3′ (reverse primer). Thermocycling consisted of 35 cycles of 45 seg at 94°C, 1 min at 56°C, and 1 min at 72°C [17]. The predicted size of the PCR products was 521 bp. GAPDH primer was the following: 5′-ACT GGC GTC TTC ACC ACC AT-3′ (forward primer), 5′-TCC ACC ACC CTG TTG CTG TA-3′ (reverse primer) (Primers from Metabion International – Martinsried, Deutschland).The predicted size of the PCR product was 682 bp. PCR products were visualized on a 1.6% agarose gel with ethidium bromide staining. The expression of all tested enzymes was normalized to the expression of GAPDH of each sample and compared using Gel Pro Analyser software.

2.8 Viability studies

Cell viability was assessed by the MTT assay for the treatment with ethanol [18]. Briefly, cells were pretreated with 1% ethanol in medium at 37°C. This treatment was removed after 4 days and the cells were incubated for 3 h with 500 μL of Hanks' medium and 50 μL of MTT (5 mg/mL in PBS). This solution was carefully aspirated and the formazan produced by mitochondrial dehydrogenase activity dissolved in DMSO. Absorbance at 660 nm corresponds to unspecific reduction of MTT. The difference in absorbance between 540 and 660 nm was calculated and registered. Results were expressed in % of control.

Owing to the interference of anthocyanins with MTT, viability of cells pretreated with these compounds for 4 days was tested by trypan blue staining [19]. After the treatment, cells were incubated with 250 μL trypsin 0.05% for 7 min and resuspended in medium. An equal volume of trypan blue (0.4% in NaCl 0.9%) and cell suspension was added to a microtube. The number of dead cells, which retained the dye, was compared with the total number to calculate the viability percentage.

2.9 Protein determination

The protein content of cell monolayers was determined by Bradford's method [20], using human serum albumin as standard.

2.10 Statistical analyses

Values are expressed as the arithmetic mean ± SEM. All the assays were performed in $n \geq 3$. Statistical significance of the difference between various groups was evaluated by one-way analysis of variance followed by the Bonferroni test, using GraphPad Prism 5 software, version 5.01, GraphPad Software. For comparison between two groups, Student's *t*-test was used. Differences were considered significant when $p < 0.05$.

3 Results

The anthocyanin extract obtained from red grape skin extraction was analyzed by HPLC and LC-MS and is comprised by anthocyanins, mainly by malvidin-3-glucose (approximately 90% of the anthocyanins is malvidin-3-glucose) (Fig. 1).

The pigment profile of the extract, which is shown in the legend of Fig. 1, was determined through LC/DAD-MS.

This extract (200 μg/mL) was applied to the apical side of Caco-2 cells cultured on inserts and the basolateral media was collected, analyzed, and quantified by HPLC. The results are presented in Table 1. Anthocyanins stability was measured on the apical side, and no significant degradation was observed (data not shown). The transport of anthocyanins across Caco-2 was time dependent. The same experiments were performed in the presence of ethanol (1%), and anthocyanin absorption was significantly increased.

Caco-2 cells were treated during 4 days with 200 μg/mL of anthocyanin extract. Anthocyanin transport across treated cells was notably increased when compared with control cells (Table 1). In order to assess if cells stored anthocyanins during this (pre)treatment, treated-cells during 4 days with this extract were incubated for a period of 120 min in the absence of anthocyanins. No anthocyanins were detected on the basolateral side and in the cellular compartment (data not shown).

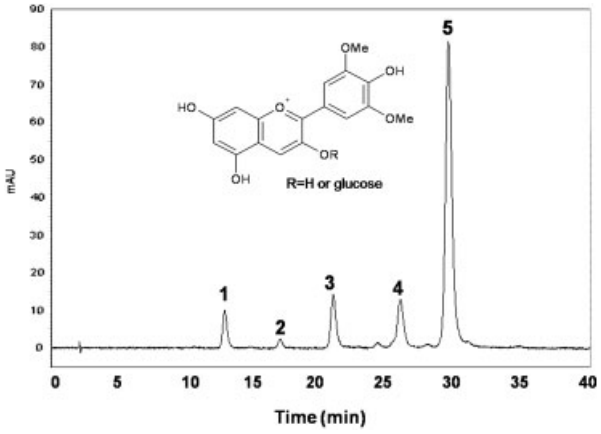

Figure 1. Representative structure of malvidin and malvidin-3-glucose. HPLC chromatogram of the anthocyanins extract recorded at 520 nm: (1) delphinidin-3-glucose; (2) cyanidin-3-glucose; (3) petunidin-3-glucose; (4) peonidin-3-glucose; (5) malvidin-3-glucose.

Table 1. Transport efficiency of anthocyanins (200 μg/mL) through Caco-2 cells (Apical → basolateral), in the presence or absence of ethanol (1%)

Time (min)	Anthocyanin	Anthocyanin with 1% ethanol	Pre-treatment with anthocyanin
15	0.14 ± 0.04	0.17 ± 0.05	0.83 ± 0.40*
30	0.26 ± 0.09	0.23 ± 0.05	1.21 ± 0.92*
45	0.32 ± 0.07	0.53 ± 0.07*	1.64 ± 1.15*
60	0.42 ± 0.09	0.80 ± 0.09*	1.92 ± 1.14*
75	0.80 ± 0.19	1.27 ± 0.17*	2.71 ± 1.23*
90	0.79 ± 0.07	1.41 ± 0.16*	2.59 ± 1.24*
120	1.77 ± 0.36	2.68 ± 0.21*	3.53 ± 1.08*

Effect of four days pretreatment with anthocyanins (200 μg/mL) on this transport. Results are presented as transport efficiency (%) (mean ± SEM, $n = 3$–14). Transport efficiency percentages were calculated based on: (anthocyanin concentrations at the basolateral side overtime)/(anthocyanin concentrations at the apical side at the zero hours) × 100%. *Significantly different from anthocyanin transport ($p < 0.05$).

The expression of glucose transporters of Caco-2 cells was analyzed by semi-quantitave RT-PCR. Remarkably, neither GLUT5 nor SGLT1 had their expression significantly altered, whereas GLUT2 revealed a significant increase in its expression compared with control cells (no treatment) (Fig. 2).

The activity of this transporter was evaluated using ^{3}H-DG. In pretreated cells, glucose uptake was decreased (Fig. 3A). Cytochalasin B, an inhibitor of GLUT2, was used and glucose transport was inhibited in control cells, as expected, and was even more decreased on pretreated cells (Fig. 3B).

Table 2 shows the kinetic parameters obtained after the exposure of cells to 200 μg/mL of anthocyanins during 60 min (acute incubation). These data suggest that the affinity of glucose transporter for ^{3}H-DG was significantly reduced, whereas the activity increased after preincubation with anthocyanins.

In Fig. 4, it can be seen that acute incubation with anthocyanins decreased glucose uptake. Similar assay was performed using not only malvidin-3-glucose, but also malvidin – the aglycone molecule of malvidin-3-glucoside – which does not bear any glucose moiety. Malvidin-3-glucose mimics the effect observed with anthocyanins, validating the results obtained with this extract. In contrast, the aglycone did not have any effect on glucose uptake.

4 Discussion

Since anthocyanins are widely consumed, and there are several beneficial properties described for these compounds, it was of great importance to investigate their absorption. Anthocyanins are unique compared with other flavonoids in that they are absorbed intact as glycosides. The mechanism of absorption is not known. However, Passamonti *et al.* [21] found that anthocyanins can serve as ligands for bilitranslocase, an organic anion transporter present in the epithelial cells of the gastric mucosa, and according to these authors bilitranslocase could play a role in the bioavailability of anthocyanins. The results described by Mulleder *et al.* [22] were interesting; they found that the addition of sucrose to elderberry juice led to reduced and delayed excretion of the anthocyanins. This means that sugars may interfere with anthocyanin transport mechanism. Different studies

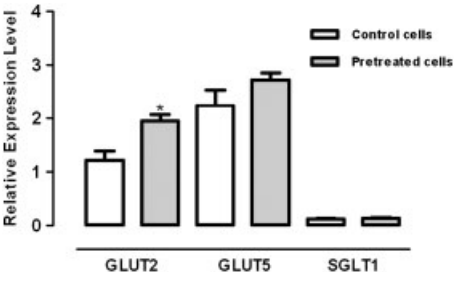

Figure 2. Effect of the treatment with anthocyanins (200 μg/mL) for 4 days on glucose transporters (GLUT2, GLUT5, and SGLT1) expression in Caco-2 cells, in three independent cell culture plates. The expression of transporters was normalized by comparison with the expression of GAPDH of each sample. $*p<0.05$ *versus* control Caco-2 cells (without treatment).

Table 2. Kinetic characterization of ^{3}H-DG apical uptake on Caco-2 cells in presence or absence (control) of anthocyanins (200 μg/mL)

	K_m (μM)	V_{max} (nmol/mg protein/6 min)
Control	6368 ± 776.8	53.9 ± 4.7
Anthocyanins (200 μg/mL)	93059 ± 3010*	405.7 ± 12.7*

Cells were pre-incubated for 60 min with buffer (control) or anthocyanins and incubated for 6 min with 3H-DG. Each value represents the mean ± SEM ($n = 4$). The value followed by (*) in each column is significantly different from control, $p<0.05$.

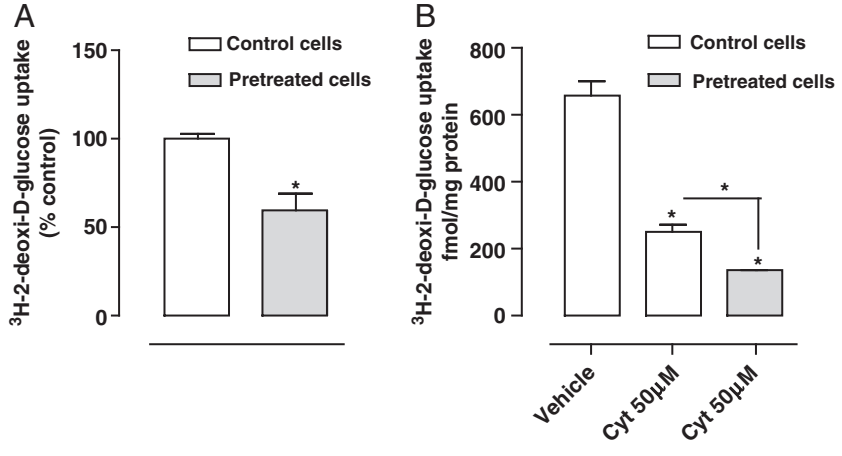

Figure 3. (A) Effect of the pretreatment with anthocyanins (200 μg/mL) for 4 days on the uptake of ^{3}H-DG 1 μM by Caco-2 cells. Caco-2 cells were incubated at 37°C with ^{3}H-DG, for 6 min. (B) Effect of cytochalasin B 50 μM (Cyt) on the uptake of ^{3}H-DG 1 μM by Caco-2 cells (control *versus* pretreated cells). Caco-2 cells were preincubated for 20 min with Cyt and incubated with ^{3}H-DG, for 6 min. Each value represents the mean ± SEM ($n = 6$). *Significantly different from the respective control ($p<0.05$).

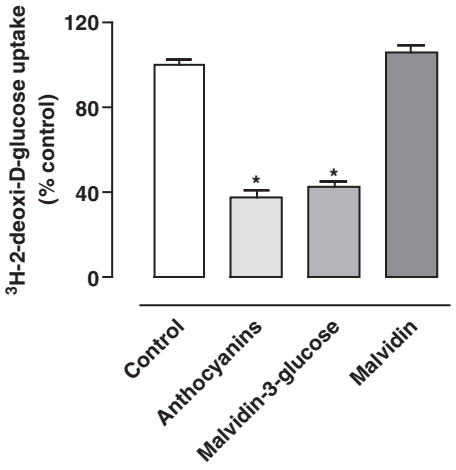

Figure 4. Effect of preincubation with 200 μg/mL of anthocyanins, malvidin-3-glucose, and malvidin aglicone on the uptake of ^{3}H-DG 1 μM. Caco-2 cells were preincubated for 60 min with the compounds and incubated with ^{3}H-DG, for 6 min. Each value represents the mean ± SEM. *Significantly different from the respective control ($p < 0.05$).

demonstrated that anthocyanins from several food sources are absorbed intact and presented in plasma or urine [11, 23–27]. Although no data are available on the exact amount of anthocyanins that are absorbed, together the plasma kinetic profile and the recovery of anthocyanins in the urine suggest that relatively small portions are absorbed. However, urinary excretion does not provide an accurate measure of bioavailability because biotransformation, by gut microflora originating phenolic acids [28], and bile excretion may alter amounts excreted in the urine.

The concentration used in this work (200 μg/mL) was chosen based on previous results (not published) in which there was a saturation of transport above this concentration. On the other hand, this value is easily attained in the gut with a normal diet by consuming two glasses of red wine or 100 mg of blueberries [29].

Anthocyanins were able to cross Caco-2, but only about 1% of the anthocyanins passed through these cells and reached the basolateral side. This was somehow expected since some studies in the literature reported that these compounds are not absorbed in great extension presenting low percentages of transport, as the ones found in this study [14]. In fact, maximum plasma levels of total anthocyanins are reported in the range of 1–590 nmol/L with doses of 60–1300 mg in human studies [27, 30–32].

As red wine is one of the main sources of anthocyanins and ethanol is one of its constituents, some assays were carried out in the presence and absence of ethanol. The concentration of ethanol used (1%) did not appear to be cytotoxic for Caco-2 cells according to the MTT assay. This concentration was chosen based on previous studies [33] where it was demonstrated that above this concentration cell viability was compromised. Our results showed that ethanol improved anthocyanins absorption sustaining the theory that ethanol facilitates flavonoid absorption [34]. Still, the information regarding this issue is controversy since there are some reports referring to anthocyanins that claim no influence of ethanol on their absorption [32, 35]. However, according to these authors, ethanol may modify the time course of malvidin-3-glucoside, enhancing the early absorption of this compound [32]. It should be taken into consideration that the impact of ethanol on anthocyanin absorption and bioavailability may depend on the models used for these studies (cell monolayers, animals models, or humans trials).

The increase of anthocyanin absorption observed with anthocyanins pretreatment was very unexpected and intriguing. Indeed, the chronic exposure with anthocyanins seems to be one way to increase these compounds absorption, even more than ethanol presence. Therefore, it can be assumed that anthocyanins must be interfering in some system.

The hypothesis raised was that anthocyanins could interfere with the transporters responsible for their own transport. The candidates for anthocyanin transporters were the glucose transporters, since anthocyanins possess a sugar moiety, in particular, a glucose residue. It has been suggested in the literature that glucoside flavonoids, such as quercetin-3-glucose, could enter the cell anchored by the glucose residue, using hexose transporters [36].

Hexose transporters described in Caco-2 cells are SGLT-1, GLUT5, and GLUT2. SGLT-1 is an energy-dependent sodium-dependent cotransporter, GLUT5 and GLUT2 are facilitated transporters, whereas GLUT5 only transports fructose. Both SGLT-1 and GLUT5 are present only on the apical membrane, and until a few years ago, GLUT2 was described to be present only in the basolateral membrane and in some pathologies on the apical membrane [37]. Recently, it has been described and accepted that GLUT2 is present on the apical side, and in the presence of great amount of glucose it is recruited to the membrane, becoming the main responsible for glucose uptake [38]. Unexpectedly, SGLT1, a transporter previously described in the literature as a flavonoid transporter [36], did not have its expression altered. GLUT5 expression did not change, but GLUT2 had its expression enhanced. Pretreatment with anthocyanins induced this transporter expression, altering not only anthocyanin absorption but certainly glucose transport as well.

This was confirmed by studying ^{3}H-DG uptake. Despite GLUT2 augmented its expression, glucose uptake was decreased, similar to what was observed with other flavonoids [39–41]. This could possibly be due to competition between glucose and anthocyanins for the same transporter, GLUT2. In view of the fact that this substrate (^{3}H-DG) enters the cell by facilitated transporters and that GLUT5 only transports fructose, GLUT2 was likely to be the target of this modulation.

The result obtained with cytochalasin B is in agreement with the observed overexpression of GLUT2 on anthocyanins treated-cells, since this compound is a good inhibitor of this transport system. Once chronic exposure to anthocyanins interfered with glucose transporter GLUT2 expression and with its activity, their effect after acute exposition was also an issue to investigate.

The changes on kinetic parameters of ^{3}H-DG uptake after acute treatment with anthocyanins also indicate that they largely interfere with glucose transport, and again, corroborate the involvement of GLUT2. The glucose residue was the essential part so that the rest of the molecule could be dragged into the cell with glucose. This was ultimately confirmed when malvidin aglycone, without any glucose residue, was tested and no interference with glucose transport was observed.

In conclusion, anthocyanins could be absorbed through Caco-2 cells, and chronic exposure interferes with their own transport. Ethanol seems to be a very important *solvent*, when present in the same food matrix, which increases anthocyanins *in vitro* bioavailability. Additionally, anthocyanins interfered with intestinal glucose uptake. Thus, this study allow us to speculate that: (i) intestinal glucose uptake could be inhibited by anthocyanins consumption, with a special impact in a diabetic population and (ii) the increase of GLUT2 expression by chronic consumption of anthocyanins could be favorable for their own bioavailability, which is crucial to their bioactivity. However, these results were obtained based on *in vitro* studies, and therefore the extrapolation to the complex *in vivo* procedures, which take place in the human gut, should be done with some caution. Thus, further additional approaches, especially *in vivo* studies using animal models followed by using studies with human samples (from human gut byopsis), should be performed in order to confirm our results.

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The authors have declared no conflict of interest.

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