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Involvement of Breast Cancer Resistance Protein (BCRP1/ABCG2) in the Bioavailability and Tissue Distribution of *trans*-Resveratrol in Knockout Mice

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trans-Resveratrol undergoes extensive metabolism in the intestinal cells, which leads to the formation of glucuronide and sulfate conjugates. Given the important role of the breast cancer resistance protein (ABCG2/BCRP) in the efflux of conjugated forms, the present study investigates the bioavailability and tissue distribution of *trans*-resveratrol and its metabolites after the oral administration of 60 mg/kg in Bcrp1$^{-/-}$ mice. *trans*-Resveratrol and its metabolites were measured in intestinal content, plasma and tissues by HPLC. At 30 min after administration, intestinal content showed decreases of 71% and 97% of resveratrol glucuronide and sulfate, respectively, in Bcrp1$^{-/-}$, indicating a lower efflux from the enterocytes. Furthermore, the area under plasma concentration curves (AUC) of these metabolites increased by 34% and 392%, respectively, whereas a decrease in the AUC of *trans*-resveratrol was found. In conclusion, Bcrp1 plays an important role in the efflux of resveratrol conjugates, contributing to their bioavailability, tissue distribution and elimination.

KEYWORDS: Bioavailability; Bcrp1$^{-/-}$ mice; distribution; HPLC-DAD; *trans*-resveratrol

INTRODUCTION

The breast cancer resistance protein (BCRP/ABCG2) and its murine homologue Bcrp1 belong to the ATP binding cassette (ABC) transmembrane drug transporter family. BCRP is constitutively expressed in healthy tissues, including the intestine, liver, blood−brain barrier, breast and placenta, as well as in tumors, where it is one mechanism contributing to multidrug resistance (*1*). Its location on the apical membrane of epithelial cells of the intestine suggests its strategic function as a protective efflux pump that increases the elimination of ingested xenobiotics and drugs (*2*). In the liver, this transporter has been found in the canalicular membrane, hepatocytes, bile duct, reactive bile ductules and blood vessel endothelium (*3*), thus playing a role in the biliary excretion of drugs, xenobiotics and metabolites (*1*). In kidney, BCRP was located in the brush border membrane of proximal tubules (*4*), suggesting its possible involvement in renal drug excretion. Overall, BCRP favors the excretion of endogenous and exogenous compounds into bile, feces and urine, and dramatically influences the pharmacokinetics of many drugs and dietary bioactive compounds (*1*).

trans-Resveratrol (*trans*-3,4′,5-trihydroxystilbene) is a polyphenol present in grapes, red wine, peanuts and various berries (*5*). This naturally occurring molecule, known as a phytoalexin, is synthesized by plants in response to stress, injury, UV radiation and fungal infections. *trans*-Resveratrol holds a wide range of pharmacological properties (*6*) with no harmful effects (*7*). Numerous biochemical and molecular actions seem to contribute to *trans*-resveratrol effects in different human disease models. The health-protecting properties of this phytoalexin include cardioprotective (*8*), neuroprotective (*9*), spermatogenesis-enhancing (*10*), antiaging (*11*) and cancer chemopreventive actions (*12*). However, these beneficial effects may be affected by its low oral bioavailability, as shown in studies on both laboratory animals and humans (*5*).

Recently, we studied the intestinal absorption of this polyphenol, using an *in vivo* perfusion technique in Sprague−Dawley rats (*13*). Our results showed that, although *trans*-resveratrol quickly enters the enterocyte, it is highly metabolized to glucuronide and sulfate, which are secreted back to the intestinal lumen through multidrug resistance protein 2 (MRP2) and BCRP. All these processes contributed to the limited intestinal net absorption of this polyphenol. Given that BCRP is involved in the intestinal secretion of *trans*-resveratrol conjugates, the present study investigates the bioavailability and tissue distribution of *trans*-resveratrol after the oral administration of 60 mg/kg in a knockout mouse model for this transporter. Transgenic rodent models in which specific proteins are deleted provide a powerful tool for examining the complex processes involved in the bioavailability of *trans*-resveratrol.

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MATERIALS AND METHODS

Chemicals. *trans*-Resveratrol was chemically pure and purchased from Second Pharma Co., Ltd. (Shangyu, P. R. China). All laboratory procedures involving the manipulation of *trans*-resveratrol were performed in dim light to avoid its photochemical isomerization to the *cis* form. Acetonitrile and methanol were from J. T. Baker (Deventer, Netherlands) and acetic acid from Scharlau Chemie S.A. (Barcelona, Spain). β-Glucuronidase (type L-II, *Patella vulgata*) and sulfatase (type H-1, *Helix pomatia*) were from Sigma-Aldrich (St. Louis, MO). All these solvents were HPLC grade. Other chemicals used were analytical grade and obtained from Sigma-Aldrich. Water used in all experiments was passed through a Milli-Q water purification system (18 MΩ) (Millipore, Milan, Italy).

Animal Experiments. Mice were housed and handled according to procedures approved by the Research Committee of Animal Use of the University of León (Spain) and performed according to the "Principles of Laboratory Animal Care" and the European guidelines described in EC Directive 86/609. The animals used in the experiments were Bcrp1$^{-/-}$ and wild-type mice (9−14 wk), all of >99% FVB genetic background. The Bcrp1$^{-/-}$ mice were kindly supplied by Dr. A. H. Schinkel from The Netherlands Cancer Institute. Animals were kept in a temperature-controlled environment with a 12 h light/12 h dark cycle. They received a standard rodent diet (Panlab SA, Barcelona, Spain) and water was available *ad libitum*.

trans-Resveratrol was administered intragastrically by oral gavage feeding in overnight fasted mice, as an aqueous solution of 20% hydroxypropyl β-cyclodextrin at the single dose of 60 mg/kg at a constant volume of 10 mL/kg. Blood samples were taken at 5, 10, and 30 min and placed in tubes containing EDTA-K2 as anticoagulant. Plasma was immediately obtained by centrifugation at 1500*g* for 15 min at 4 °C, immediately frozen in liquid nitrogen and kept at −80 °C until analysis.

For the tissue distribution study of *trans*-resveratrol, a group of 4 mice were killed at 10 min and another at 30 min by cervical dislocation. Small intestine was removed, and the intestinal content was collected. Subsequently, brain, heart, liver, kidney and lungs were rapidly excised and washed with NaCl 0.9% to remove residual blood containing *trans*-resveratrol and its metabolites. Tissues were wiped with filter paper, weighed, immediately frozen in liquid nitrogen and kept at −80 °C until analysis.

Determination of *trans*-Resveratrol and Its Metabolites in Intestinal Content. Intestinal content samples were defrosted at room temperature, and 10 mL of 80% methanol, with 2.5% acetic acid and 10 μL of 15% ascorbic acid as antioxidant, was added. The mixture was shaken with constant stirring for 30 min at 60 °C. Then, samples were transferred to a centrifuge tube and the remaining content in the beaker was gathered with an additional 2 mL of acidified methanol. The homogenates were centrifuged at 33000*g* (Centrikon H-401, Kontron Hermle Instruments, Italy) for 30 min at 4 °C. The supernatant was transferred to a clean tube, and the residue was extracted one more time following the same procedure. The organic solvent of the supernatant was evaporated with a Concentrator 5301 (Eppendorf Iberica S.L., San Sebastián de los Reyes, Spain) at 45 °C to a final volume of 400 μL, which was then placed in a sealed amber vial for HPLC analysis.

Determination of *trans*-Resveratrol and Its Metabolites in Tissues. The defrosted small intestine was placed in a conical tube, and 4 mL of methanol (80%, v/v) acidified with acetic acid (2.5%, v/v) and 10 μL of ascorbic acid (15%, v/v), as antioxidant, was added to the same tube. The entire tissue was stirred for 2 min and kept overnight at 4 °C. After this, the intestine was stirred again for 2 min and centrifuged at 1500*g* for 30 min at 4 °C (Megafuge 1.0R, Heraeus, Boadilla, Spain). The supernatant was placed in a clean tube. The organic solvent of the supernatant was evaporated to a final volume of 400 μL, and placed in a sealed amber vial for HPLC analysis.

The other tissues were finely minced with scissors and placed in a homogenizer vessel, following the validated method described elsewhere (*14*). Briefly, methanol acidified with acetic acid and 10 μL of ascorbic acid was added, and tissues were subsequently homogenized. The homogenization process was adjusted to each tissue. Brain and testes were placed in 3 mL of acidified methanol and ground by a manual glass homogenizer with 30 strokes. An additional 1 mL was used to collect the residues in the glass vase and was added to the initial 3 mL. However, liver, lungs and kidney were homogenized with 6 short pulses of a Polytron tissue homogenizer (Kinematica AG, Lucerne, Switzerland) using 2 mL of acidified methanol. The homogenizer was cleaned twice with 1 mL of acidified methanol, which was added to the 2 mL, making a final volume of 4 mL.

Homogenized samples were transferred to a 10 mL conical glass tube and processed in the vortex for 5 min prior to centrifugation at 1500*g* for 30 min at 4 °C. The supernatant was placed in a clean tube. The residue was extracted twice more with 4 mL of acidified methanol by vigorous shaking in the vortex for 5 min, followed by centrifugation at 3000*g* for 30 min at 4 °C. The organic solvent of the supernatants was evaporated to a final volume of 400 μL and subsequently placed in a sealed amber vial for HPLC analysis.

Determination of *trans*-Resveratrol and Its Metabolites in Plasma. Resveratrol was extracted from plasma samples on a reversed-phase C18 Sep-Pak Classic Cartridge for manual operation (WAT051910, Waters, Milford, MA), using a method described previously (*14*). Briefly, plasma was acidified with acetic acid (30 μL/mL of plasma), stirred in the vortex for 2 min, and slowly loaded onto the cartridge, which was rinsed with water (10 mL/mL of plasma). *trans*-Resveratrol and metabolites contained in the cartridge were eluted with 4 mL of methanol. Ten microliters of ascorbic acid at 15% was added to the eluted liquid, which was evaporated to a final volume of 400 μL. Finally, this was placed in a sealed amber vial for HPLC analysis.

HPLC Analyses. An Agilent model 1100 (Agilent Technologies, Palo Alto, CA) gradient liquid chromatograph equipped with an automatic injector, a Synergi Fusion-RP 80A (250 × 4.6 mm; 4 μm) (Phenomenex, Torrance, CA) with a C18 guard column cartridge, and a diode array UV−visible detector coupled to a ChemStation were used. The temperature of the column was kept at 40 °C. The flow rate was 1.5 mL/min, and injection volume was 100 μL. The mobile phase consisted of two phases: phase A was a 3% acetic acid solution, and phase B was a mixture of phase A:acetonitrile (20:80, v/v). The gradient elution differed, depending on the samples. Plasma: min 0 with 22% solvent B to min 2; 2−6 min, linear gradient from 22 to 30% B; 6−14 min, linear from 30 to 50% B; 14−18 min, increasing to 60% B; 18−25 min, linear from 60 to 100% B; followed by washing and reconditioning the column. Tissue samples and intestinal content: 0−5 min, 15% B; 7 min, 20% B; 10 min, 21% B; 20 min, 22% B; 30 min, 30% B; 35 min, 35% B; 40 min, 40% B; 45 min, 50% B; 50 min, 70% B; 55−60 min, 100% B; 62 min, 15% B. There was a 5 min delay prior to the injection of the next sample to ensure re-equilibration of the column.

The chromatograms were obtained according to the retention time, with detection at 306 nm (**Figure 1**), at which the absorbance of *trans*-resveratrol reaches a maximum. Resveratrol was initially identified using comparative retention times of pure standard and photodiode array spectra (from 200 to 400 nm). The identity of all peaks was confirmed by HPLC−MS (**Figure 1**). *trans*-Resveratrol was quantified by using standard curves constructed after spiking relevant concentrations of it in the appropriate sample matrix, either plasma or homogenized tissues. The curves were characterized by regression coefficients of $R^2 = 0.99$ or above. *trans*-Resveratrol glucuronide and sulfate were quantified after hydrolysis by means of enzyme treatment, as already described (*14*).

Statistical Analysis. All data are given as means ± SEM. Tissue concentrations were analyzed by 1-way ANOVA followed by Bonferroni's *post hoc* test. Statistical differences of plasma concentrations were compared by means of 2-way ANOVA. When the effects were significant, differences between means were assessed with Bonferroni's *post hoc* test. Differences were considered statistically significant when $p < 0.05$.

RESULTS

***trans*-Resveratrol and Resveratrol Glucuronide and Sulfate Concentration in Small Intestine.** No differences were observed in the concentrations of *trans*-resveratrol in the small intestine of wild-type or Bcrp1$^{-/-}$ mice (**Table 1**). The glucuronide and sulfate conjugates were detected at 10 min in wild-type and Bcrp1$^{-/-}$ animals, with no differences between groups. However, at 30 min, the concentrations of *trans*-resveratrol glucuronide and sulfate were inhibited by 68% ($p < 0.05$) and 87% ($p < 0.001$), respectively, in Bcrp1$^{-/-}$ and wild-type mice.

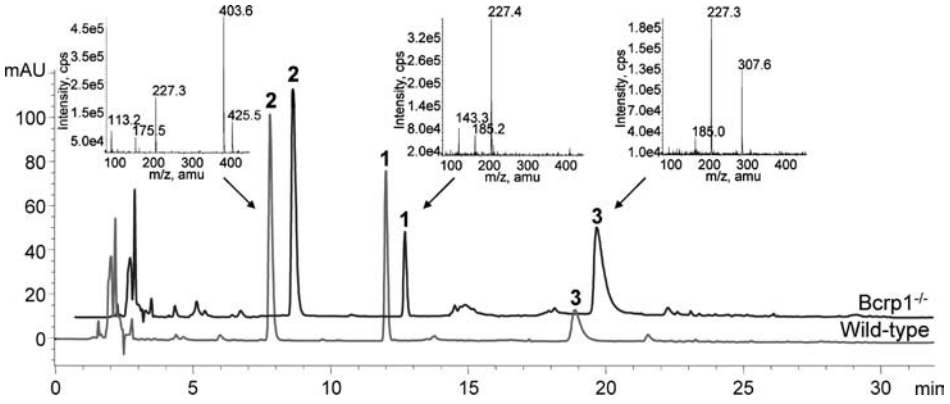

Figure 1. HPLC chromatograms at 306 nm from plasma of wild-type and Bcrp1$^{-/-}$ mice, 30 min after the oral administration of 60 mg/kg *trans*-resveratrol. Peak 1, *trans*-resveratrol; peak 2, *trans*-resveratrol glucuronide; peak 3, *trans*-resveratrol sulfate. The insets depict the full-scan product ion mass spectra of *trans*-resveratrol (1), *trans*-resveratrol glucuronide (2) and *trans*-resveratrol sulfate (3).

Table 1. Concentrations of *trans*-Resveratrol and Glucuronide and Sulfate Conjugates in Tissues and Intestinal Content of Wild-Type and Bcrp1$^{-/-}$ Mice after the Oral Administration of 60 mg/kg of *trans*-Resveratrol[a]

	trans-resveratrol		glucuronide		sulfate	
	wild-type	Bcrp1$^{-/-}$	wild-type	Bcrp1$^{-/-}$	wild-type	Bcrp1$^{-/-}$
			Small Intestine, nmol/g tissue			
10 min	314 ± 38	313 ± 74	198 ± 20	148 ± 66	35.2 ± 9.8	20.4 ± 2.3
30 min	436 ± 66	236 ± 33	345 ± 42	109 ± 25*	119 ± 11$^{\Phi}$	15.9 ± 0.6***
			Intestinal Content, nmol/g content			
10 min	1370 ± 207	1622 ± 563	215 ± 35	142 ± 17	170 ± 34	9.4 ± 4.2*
30 min	5424 ± 806$^{\Phi}$	1394 ± 458**	553 ± 15.1$^{\Phi}$	160 ± 13***	781 ± 49$^{\Phi}$	23.3 ± 7.0***
			Liver, nmol/g tissue			
10 min	5.6 ± 1.4	8.7 ± 0.9	30.2 ± 3.0	31.3 ± 5.8	9.0 ± 1.2	23.1 ± 3.5**
30 min	5.2 ± 1.3	3.6 ± 0.4$^{\varphi}$	32.1 ± 5.4	20.7 ± 3.6	12.6 ± 2.8	13.0 ± 1.3$^{\varphi}$
			Kidney, nmol/g tissue			
10 min	5.7 ± 1.2	16.1 ± 3.6**	99.6 ± 15.5	199.5 ± 13.7**	11.4 ± 2.1	31.0 ± 18.3
30 min	0.8 ± 0.4	3.2 ± 0.8$^{\Phi}$	3.0 ± 0.4$^{\theta}$	98.2 ± 19.1$^{**\theta}$	4.6 ± 1.9	3.2 ± 1.1
			Lung, nmol/g tissue			
10 min	4.8 ± 2.5	6.3 ± 2.4	9.3 ± 1.8	19.4 ± 2.0**	nd[b]	1.0 ± 0.8
30 min	2.1 ± 0.8	12.2 ± 2.2*	10.6 ± 2.9	11.6 ± 1.2$^{\varphi}$	nd	1.7 ± 0.5
			Heart, nmol/g tissue			
10 min	3.5 ± 1.2	3.2 ± 0.5	5.5 ± 1.2	12.1 ± 1.0**	nd	nd
30 min	2.5 ± 0.9	0.8 ± 0.0	12.0 ± 2.0$^{\varphi}$	13.7 ± 1.0	nd	nd
			Brain, nmol/g tissue			
10 min	0.43 ± 0.11	1.25 ± 0.56	0.54 ± 0.14	0.88 ± 0.16	0.01 ± 0.01	0.09 ± 0.03
30 min	0.82 ± 0.10	0.28 ± 0.05	1.28 ± 0.29	1.87 ± 0.75	0.36$^{\varphi}$	0.19 ± 0.12

[a] Results are expressed as the mean ± SEM (n = 3−4). Bcrp1$^{-/-}$ vs wild-type: *p < 0.05, **p < 0.01, ***p < 0.001. Thirty minutes vs 10 min: $^{\varphi}p$ < 0.05, $^{\theta}p$ < 0.01, $^{\Phi}p$ < 0.001. [b] Not detected.

***trans*-Resveratrol and Resveratrol Glucuronide and Sulfate Concentration in Intestinal Content.** After 10 min of the oral administration in wild-type mice, glucuronide and sulfate conjugates were found in intestinal content in concentrations of 215 ± 34 and 170 ± 34 nmol/g, respectively (**Table 1**). At 30 min, the efflux of conjugates increased by 157% (p < 0.001) and 360% (p < 0.001) for the *trans*-resveratrol glucuronide and sulfate, respectively.

trans-Resveratrol glucuronide was 34% and 71% (p < 0.001) lower in Bcrp1$^{-/-}$ mice than in wild-type animals, at 10 and 30 min, respectively. *trans*-Resveratrol sulfate was reduced by 95% (p < 0.05) and 97% (p < 0.001) at 10 and 30 min, respectively.

Plasma Concentrations of *trans*-Resveratrol and Conjugates. After the oral administration of 60 mg/kg of *trans*-resveratrol, blood was withdrawn at different times: 5, 10, and 30 min (**Figure 2**). In wild-type mice, this polyphenol was detected at 5 min at concentrations of 7.3 ± 2.1 μM, which were maintained until 10 min (7.4 ± 1.3 μM). From this time point, the plasma levels decreased to 6.0 ± 1.5 μM at 30 min. *trans*-Resveratrol glucuronide was the most abundant compound in plasma, and was already detected at 5 min, in a concentration 5-fold higher (p < 0.001) than in the parent compound. The concentration of glucuronide increased over time with values of 57.4 ± 5.9 and

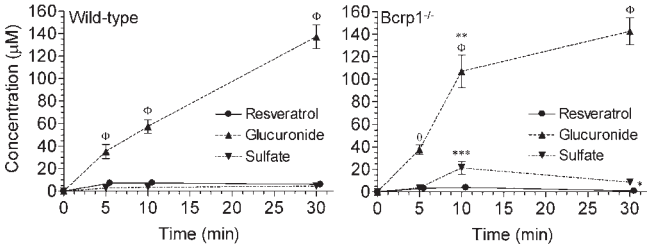

Figure 2. Plasma concentrations after the oral administration of 60 mg/kg of *trans*-resveratrol in wild-type and Bcrp1$^{-/-}$ mice. Values are represented as means ± SEM ($n = 4$). Bcrp1$^{-/-}$ vs wild-type: $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$. Conjugates vs *trans*-resveratrol: $^{\theta}p < 0.01$, $^{\Phi}p < 0.001$. Statistical analysis at different times: In wild-type mice, *trans*-resveratrol and sulfate, 5 = 10 = 30 min; glucuronide, 5 = 10 < 30 min. In Bcrp1$^{-/-}$ mice, *trans*-resveratrol, 5 = 10 = 30 min; glucuronide, 5 < 10 < 30 min; sulfate, 5 = 30 < 10 min; $p < 0.05$.

137 ± 10 μM at 10 and 30 min, which were 8 ($p < 0.001$) and 23 ($p < 0.001$) times the *trans*-resveratrol levels. The sulfate conjugate was also present in plasma of wild-type mice, ranging from 2.8 ± 0.2 μM at 5 min to 4.4 ± 0.8 μM at 30 min.

In Bcrp1$^{-/-}$ mice, the plasma concentrations of *trans*-resveratrol at 5 and 10 min were 3.2 ± 1.0 μM and 4.0 ± 1.8 μM, respectively. At 30 min, the plasma levels were 1.0 ± 0.2 μM, which was 6-fold lower ($p < 0.05$) than the values observed in wild-type animals. *trans*-Resveratrol glucuronide was also the main metabolite found in plasma of mice lacking Bcrp1. At 5 min, the glucuronide was 37.8 ± 4.3 μM; at 10 min, it reached 107 ± 14 μM. The plasma concentration of *trans*-resveratrol glucuronide was 143 ± 12 μM at 30 min, 148 times higher ($p < 0.001$) than that of *trans*-resveratrol. The absence of Bcrp1 influenced the plasma levels of *trans*-resveratrol sulfate. The plasma levels of *trans*-resveratrol sulfate in Bcrp1$^{-/-}$ mice were 3.7 ± 0.7 μM at 5 min, and reached a maximum at 10 min with values of 21.4 ± 5.5 μM, which were 5 times higher ($p < 0.001$) than *trans*-resveratrol alone. At 30 min, the concentration of the sulfate conjugate dropped to 8.6 ± 1.4 μM.

We also determined the area under plasma concentration curves (AUC) for *trans*-resveratrol and its conjugates. The results showed a decrease of 44% in the AUC for *trans*-resveratrol in Bcrp1$^{-/-}$ mice. Conversely, the AUC of glucuronide and sulfate increased by 34% and 392%, respectively.

Tissue Distribution of *trans*-Resveratrol and Resveratrol Glucuronide and Sulfate. The concentrations of *trans*-resveratrol and its metabolites were also examined in the liver, kidney, lung, heart and brain of wild-type and Bcrp1$^{-/-}$ mice. Liver concentrations of *trans*-resveratrol and its glucuronide were similar in wild-type and knockout mice (**Table 1**). The absence of Bcrp1 mainly affected the sulfate content at 10 min, with a concentration 2.6-fold higher in Bcrp1$^{-/-}$ mice ($p < 0.01$).

The organs with the greatest differences between wild-type and Bcrp1$^{-/-}$ mice were kidney and lung (**Table 1**). In kidney, *trans*-resveratrol at 10 min was 3-fold higher ($p < 0.01$) in Bcrp1$^{-/-}$ mice than in wild-type ones. The glucuronide conjugate was 2-fold (10 min) and 32.7-fold (30 min) higher in kidney of Bcrp1$^{-/-}$ mice than in wild-type ones ($p < 0.01$). No differences between animals were observed for the sulfate conjugate, either at 10 or at 30 min.

trans-Resveratrol and its glucuronide and sulfate conjugates were also found in lung (**Table 1**), although the concentrations of these compounds were lower than those in liver or kidney. The concentrations of *trans*-resveratrol were similar in Bcrp1$^{-/-}$ and wild-type mice at 10 min. However, at 30 min the concentration of this compound was 6-fold higher ($p < 0.05$) in Bcrp1$^{-/-}$ than in wild-type mice. At 10 min, glucuronide was 2.1-fold higher in Bcrp1$^{-/-}$ mice ($p < 0.01$). No differences in glucuronide concentrations were observed at 30 min. It is worth mentioning that the sulfate conjugate was only detected in mice lacking Bcrp1.

In heart, no differences between wild-type and Bcrp1$^{-/-}$ mice were observed in the concentration of *trans*-resveratrol (**Table 1**). However, in knockout mice, the glucuronide conjugate was double ($p < 0.01$) that in wild-type mice. The sulfate conjugate was not detected in any group.

The lowest concentrations of *trans*-resveratrol and its glucuronide were measured in the brain (**Table 1**). In wild-type mice the sulfate conjugate was barely detected at 10 min and decreased with time, so that at 30 min it was only present in one mouse brain in a concentration of 0.36 nmol/g.

DISCUSSION

The use of genetically modified mice that lack expression of the transporter Bcrp1 (knockout) has proved a useful tool in studying the *in vivo* function of this efflux pump (*15*). The present study attempts to identify the contribution of this protein to the bioavailability and tissue distribution of *trans*-resveratrol and its conjugates. In a previous study, we established that *trans*-resveratrol was absorbed by simple diffusion and conjugated quickly inside the enterocyte to glucuronide and sulfate. Part of these conjugates was secreted back to the intestinal lumen through Mrp2 and Bcrp1 (*13*). In the present study, the concentrations of *trans*-resveratrol and its metabolites were assessed 10 and 30 min after the oral administration of 60 mg/kg in the intestinal content, small intestine, plasma, liver, kidney, lung, heart and brain in wild-type and Bcrp1$^{-/-}$ mice.

The rapid and extensive metabolism that *trans*-resveratrol undergoes in the enterocytes was clearly seen 10 min after oral administration of 60 mg/kg, since the glucuronide and sulfate conjugates were found in the intestinal tissue as well as in the luminal content in both groups. A significant reduction of the efflux toward the intestinal content was observed in Bcrp1$^{-/-}$ mice, in which secretion of the glucuronide conjugate was inhibited by 70% and the sulfate by 95% more than in wild-type mice. Results here demonstrate that Bcrp1 is predominantly responsible for the intestinal excretion of sulfate conjugates and, to a lesser extent, of glucuronide. Our data corroborate previous findings in Bcrp1$^{-/-}$ mice for 4-methylumbelliferone and its conjugates (*16*) and for minoxidil and its sulfate (*17*). In our experiments, the efflux of both metabolites was not completely halted in Bcrp1$^{-/-}$ mice because the conjugates of *trans*-resveratrol are also substrates of Mrp2, as previously shown in rats (*13*, *18*). The decreased concentrations of glucuronide and sulfate conjugates in the luminal content of Bcrp1$^{-/-}$ mice could not be attributed to changes in the level of conjugating enzymes secondary to the absence of transporters in knockout mice, because no changes in intestinal UDP-glucuronosyltransferase or sulfotransferases were reported in them (*17*).

The analysis of the luminal content revealed that *trans*-resveratrol concentration was higher in wild-type mice than in Bcrp1$^{-/-}$ mice at 30 min. This result cannot be explained by active transport of intact *trans*-resveratrol from the enterocyte, since this compound is not transported by BCRP at physiological pH, as studied in MDCKII cells overexpressing human BCRP (*19*). Moreover, *in vivo* perfusion in rat jejunum using Ko143 as BCRP inhibitor showed that *trans*-resveratrol was not secreted back to the intestinal lumen (*13*). A reasonable explanation could be an intestinal absorption rate of *trans*-resveratrol that is different in wild-type and knockout animals. The lower concentration of *trans*-resveratrol in the luminal content and enterocytes in knockout vs wild-type mice could be due to a major gradient of

concentration of this compound from the lumen to the blood in Bcrp1$^{-/-}$ mice. Moreover, the contribution of enterohepatic circulation cannot be ruled out in wild-type mice, in which the luminal content of *trans*-resveratrol and its conjugates was higher than in knockout ones (*20*).

We also evaluated the plasma levels of *trans*-resveratrol after the oral administration of 60 mg/kg. The higher AUC, observed for *trans*-resveratrol conjugates, is consistent with the fact that these compounds are transported by Bcrp1. The same pattern has been described for other substrates transported by this protein (*17*, *21*). It is worth mentioning that the greater increase of AUC in Bcrp1$^{-/-}$ mice corresponds to resveratrol sulfate, which implies greater affinity of Bcrp1 for this substrate, as pointed out by other authors (*17*, *22*). Moreover, the fact that the AUC of *trans*-resveratrol in knockout mice does not increase indicates that it is not transported by Bcrp1 (*13*). The pattern of plasma concentrations of *trans*-resveratrol and its metabolites is quite similar to that described by van de Wetering et al. (*23*) in wild-type mice. However, important differences were found between our results and van de Wetering's results in plasma from Bcrp1$^{-/-}$ mice. First, van de Wetering et al. (*23*) failed to detect *trans*-resveratrol in plasma. Second, they identified a third metabolite corresponding to *trans*-resveratrol disulfate, which is absent in our samples. These discrepancies may be attributed to the different extraction methods and chromatographic conditions used to determine *trans*-resveratrol from plasma samples.

In the liver, Bcrp1 has been described as mediating the biliary excretion of drugs and its metabolites. No differences between groups for *trans*-resveratrol and its glucuronide were observed. The fact that the glucuronide conjugate did not accumulate in liver could be attributed to the involvement of Mrp2 in its efflux, as previously seen in rats (*18*). In Bcrp1$^{-/-}$ mice, the concentration of *trans*-resveratrol sulfate was higher at 10 min, probably due to the greater affinity of Bcrp1 for sulfates than for glucuronide conjugates (*22*). Moreover, the higher level of *trans*-resveratrol sulfate in Bcrp1$^{-/-}$ mice mirrored the plasma peak observed at this time point.

Kidney was the organ with the highest concentration of *trans*-resveratrol and its conjugates in Bcrp1$^{-/-}$ mice. It is precisely this organ in which the expression of Bcrp1 mRNA has been reported to be the highest (*24*). The absence of Bcrp1 from the apical membrane of the tubular cells leads to the accumulation of the conjugates in the kidney. Our results are consistent with the findings of the Borst group (*23*), who pointed to a decrease in the excretion of resveratrol conjugates in urine of mice with absence of Bcrp1.

The presence of *trans*-resveratrol and its conjugates in lungs has been previously reported in mice and rats (*14*, *26*, *27*). It should be noted that *trans*-resveratrol sulfate was not detected in wild-type mice, whereas it was present in Bcrp1$^{-/-}$ mice, thus confirming the high affinity of this transporter for *trans*-resveratrol sulfate. The concentrations of *trans*-resveratrol in heart have been determined before in rats (*28*) and mice (*26*), but conjugates were not taken into account in those studies. *trans*-Resveratrol glucuronide appeared the only detectable conjugate in this organ, in which it was found in higher amounts than the parent compound was.

The tissue distribution of *trans*-resveratrol was also evaluated in the brain, since Bcrp1 is known to be present in the blood−brain barrier, restricting the penetration of different compounds (*15*). Our results show that this polyphenol is able to cross the blood−brain barrier, although its concentration is lower than that found in other tissues. These data corroborate previous observations in mice and rats (*14*, *26*, *27*). The contribution of Bcrp1 is small, since there is a similar amount of glucuronide and sulfate in knockout and wild-type mice.

In conclusion, the bioavailability and tissue distribution of *trans*-resveratrol is the result of a complex interaction of transporters and enzyme activities in the different tissues. Our results emphasize the role of Bcrp1 in the distribution of *trans*-resveratrol and its conjugates. The most affected organs are the small intestine, kidney and the lungs, with higher amounts in Bcrp1$^{-/-}$ mice. It is worthy of note that Bcrp1 is responsible for the extrusion of the conjugates of *trans*-resveratrol from the enterocytes to the intestinal lumen, which favors high concentrations of these compounds throughout the intestine. Since the conjugates could be converted back to the parent compound (*25*) or even the conjugated compounds could have beneficial effects similar to *trans*-resveratrol's effect (*5*), our results support the suitability of the use of *trans*-resveratrol in therapy for colon cancer (*29*) or intestinal bowel disease (*30*).

ABBREVIATIONS USED

AUC, area under the plasma concentration curve; BCRP/ABCG2, breast cancer resistance protein; ABC, ATP binding cassette; MRP2/ABCC2, multidrug resistance protein 2.

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