FEBS 29961 FEBS Letters 579 (2005) 5343–5346

Quercetin appears in the lymph of unanesthetized rats as its phase II metabolites after administered into the stomach

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Received 1 August 2005; revised 22 August 2005; accepted 23 August 2005

Available online 16 September 2005

Edited by Barry Halliwell

Abstract **Quercetin is a major flavonoid in plant foods and potentially has beneficial effects on disease prevention. The present work demonstrated that quercetin was transported into the lymph after being metabolized in the gastrointestinal mucosa of rats. Glucuronide/sulfate and methylated conjugates of quercetin appeared in the lymph, but not quercetin aglycone. The highest lymphatic concentration was found at as rapid as 30 min after administration, suggesting gastric absorption, whereas the mucosal glucuronidation activity was significantly higher in the duodenum and jejunum than in the stomach. This is the first report to show the lymphatic flavonoid transport pathway from the gastrointestinal tract.**

Keywords: Quercetin; Lymph; Gastrointestinal absorption; Conjugation; UGT activity; Rat

1. Introduction

Dietary flavonoids have attracted much attention because of the potentially beneficial effects on various disease prevention [1–5]. Not only the function but also the bioavailability of flavonoids have been widely investigated in cell culture [6,7], animal models [8,9] and in human volunteers [10–12]. It is well known that the small intestine is one of the major tissues as well as the liver to metabolize flavonoids. Quercetin is a major dietary flavonoid found in high amounts in onions, apples, and broccoli. Its metabolic pathway and plasma metabolites have been well investigated among various flavonoids.

Flavonoids are widely distributed in plant foods as glycosidic derivatives in which one or more phenolic hydrogens are substituted with sugar moieties. The type of sugar moiety is a major determinant of flavonoid absorption in the small intestine [13,14]. While there are several glycosides that are taken up into the enterocyte via sodium glucose co-transporter 1 (SGLT1) [7,15,16], many glycosides need de-glycosylation to be absorbed by intestinal enzymes or microflora to produce the corresponding aglycone [14,17], which is more lipophilic than glycosides [18,19]. Generally, when a lipophilic nutrient is absorbed in the small intestine, its intestinal cellular metabolite is secreted to the interstitium as a component of chylomicrons. Thus, whereas hydrophilic nutrients like glucose are mainly transported to the plasma, hydrophobic lipids are transported into the lymph. Although analyses of plasma flavonoid metabolites of rodents [20] and human [21] have been performed, it has never been investigated whether flavonoids could enter the lymph, like tocopherols, another major dietary antioxidant [22,23]. The initial lymphatic capillaries in the gastrointestinal tract, into which absorbed nutrients are transported, are gathered to form a larger collecting lymph duct and reach the thoracic lymph duct. In this study, whether quercetin could be transported into the lymph was investigated using unanesthetized thoracic lymph-cannulated rats.

2. Materials and methods

2.1. Animals

Male Wistar rats (8–9-week old) were purchased from Japan SLC Co. (Hamamatsu, Japan). The rats were allowed free access to drinking water and commercial laboratory diet (Lab MR Breeder, Nosan Co., Yokohama, Japan). The animal care and experimental procedures followed were approved by the Committee for Animal Experimentation of the Faculty of Medicine, The University of Tokushima.

2.2. Chemicals

Authentic quercetin, sodium taurocholate and β-glucuronidase/sulfatase from *Helix pomatia* (type H-5) were obtained from Sigma Chemical Co. (St. Louis, MO). Authentic isorhamnetin (4′-methylated quercetin) was purchased from Extrasynthese (Genay, France). Methanol (HPLC grade) and other chemical reagents were obtained from Kanto Chemical Co., Inc. (Tokyo, Japan).

2.3. Administration and recovery of quercetin in rats

Under anesthesia, the surgical operation of the thoracic lymph duct cannulation for rats, along with the gastric tubing introduction for sample administration, was performed according to the procedure previously described [24]. The lymph was collected from the rats 30 min prior to quercetin administration after an overnight recovery in restraining cages with free access to glucose–NaCl solution (25 g and 4.97 g/L, respectively) . Then, rats were infused with 1 ml of quercetin solution in propylene glycol (2 mg/ml, 5.9 mM) at a dose of 10 mg/kg via gastric tubing, followed by the continuous infusion of the glucose–NaCl solution. The lymph was fractionated at 10, 20, 30, 45, 60 min and other desired time points for 7 h after the quercetin infusion in glass tubes with 30 μl of 10 mM Na-EDTA solution to avoid coagulation.

2.4. Analysis of quercetin metabolites in lymph

An aliquot of the collected lymph was mixed with the same volume of methanol/acetic acid (100:5, v/v) followed by the centrifugation at 12000 rpm for 10 min at 4 °C, and the supernatant was applied to

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Abbreviations: SGLT1, sodium glucose co-transporter 1; UDP, uridine 5′-diphosphoglucuronic acid; UGT, uridine 5′-diphosphate glucuronosyltransferase

0014-5793/$30.00
doi:10.1016/j.febslet.2005.08.060

HPLC analysis. Another aliquot was treated with β-glucuronidase/sulfatase to hydrolyze metabolized quercetin conjugates to aglycone and isorhamnetin, as previously described [6]. Quercetin and isorhamnetin were determined by HPLC, which were monitored with a UV detector at 365 nm, using a C18 column, detailed elsewhere [6]. The retention time served as a method of identification, based on the previous confirmation by NMR data [20].

2.5. Determination of uridine 5′-diphosphate glucuronosyltransferase activity in the gastrointestinal mucosa

Uridine 5′-diphosphate glucuronosyltransferase (UGT; EC 2.4.1.17) activity in the gastrointestinal mucosa was measured as previously described [25]. The rat mucosa of stomach, duodenum and jejunum were homogenized with 0.15 M Tris–HCl buffer, pH 7.6, and the supernatant was used as the enzyme solution. Tissue enzyme preparation was diluted 1:1 (v/v) with the Tris–HCl buffer containing 21 mM of castanospermine, 20 mM $MgCl_2$ and 2 mM DL-dithiothreitol. Then 10 μM quercetin and/or 1.25 mM uridine 5′-diphosphoglucuronic acid (UDP) were added to the enzyme solution and incubated at 37 °C in a shaking water bath for 30 min. The difference in quercetin content between the control without UDP and the sample incubated with UDP was assumed to be the amount of the glucuronide conjugates. The apparent UGT activity was expressed as pmol quercetin conjugated/min/mg protein in the mucosa homogenate.

2.6. Statistics

Results are expressed as means ± S.E.M. ($n = 4$). A two-way ANOVA was used when the difference of the data among three groups was analyzed, followed by a Bonferroni correction post hoc test. Statistical calculation was carried out using ystat2002, an Excel Statistical Program File (Igaku Tosho Shuppan Co., Tokyo, Japan). Data are considered statistically significant at $P < 0.05$.

3. Results

3.1. Lymphatic output of the gastrointestinal metabolites of quercetin

There was no quercetin or its related compounds detected in the lymph before administration. The lymphatic concentration of quercetin metabolites after administration is shown in Fig. 1. During only the first 30 min after the quercetin injection, the intact aglycone was detected in the lymph at the range of 0.04–0.11 μM and then became under the detection limit (0.03 μM). The enzymatic treatment with β-glucuronidase/sulfatase was performed to determine the total quercetin glucuronides/sulfates, which are the metabolized forms present in the plasma, resulted in increasing quercetin aglycone released and the simultaneous detection of methylated quercetin aglycone, isorhamnetin. Thus, in the lymph, quercetin was found mostly to be conjugated with glucuronide and/or sulfate, and partly methylated. The concentration of quercetin conjugates is significantly higher than isorhamnetin conjugates during 180 min after the administration ($P < 0.05$). The highest lymphatic concentration (C_{max}) of quercetin conjugates at 2.54 ± 0.43 μM was found at 30 min (T_{max}) after the administration. The concentration of isorhamnetin conjugates was increased for 30 min and almost constant at the range of 0.55–0.75 μM during 7 h.

The cumulative amounts of lymphatic quercetin aglycone, its conjugates and isorhamnetin conjugates for 7 h after the gastric administration were shown in Fig. 2. Quercetin conjugates were accumulated significantly more than the aglycone and isorhamnetin conjugates 45 min after quercetin administration and later. The total recovery of all quercetin derivatives was 0.44% of the administered quercetin.

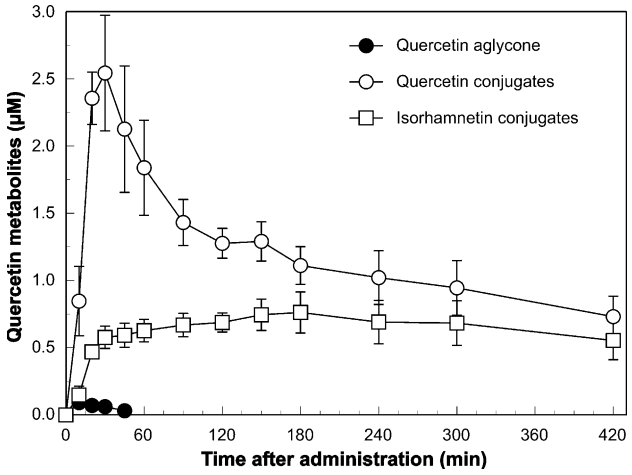

Fig. 1. Lymphatic output of quercetin metabolites in unanesthetized lymph-cannulated rats. Rats were infused intragastrically with 2 mg quercetin in propylene glycol/200 g body weight ($n = 4$). The lymph was fractionated at 10, 20, 30, 45, 60, 90, 120, 150, 180, 240, 300, 420 min after the administration and the concentration of each quercetin metabolites was determined. Values are expressed as means ± S.E.M. ($n = 4$).

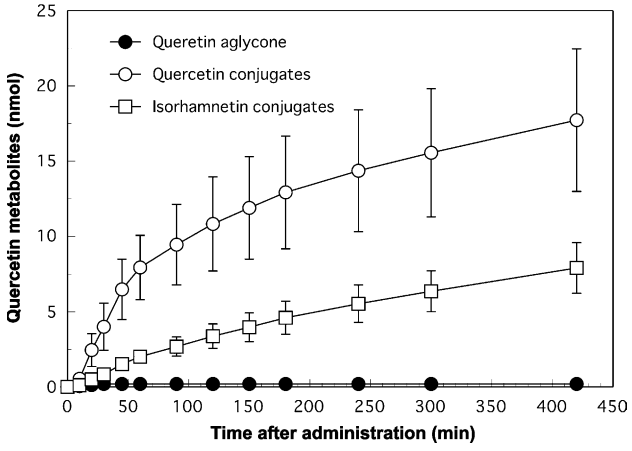

Fig. 2. Cumulative amount of the quercetin metabolites output in the lymph. Rats were infused intragastrically with 2 mg quercetin in propylene glycol/200 g body weight ($n = 4$). The lymph was fractionated at 10, 20, 30, 45, 60, 90, 120, 150, 180, 240, 300, 420 min after the administration and the amount of each quercetin metabolites in each fraction was determined. Values are expressed as means ± S.E.M. ($n = 4$).

The initial lymph flow rate for 1 h after the administration was 3.80 ± 0.82 ml/h and became slower to 1.97 ± 0.72 ml/h for the next 2 h. The flow was stable at approximately1.6 ml/h after that until the end of the lymph collection.

3.2. UGT activity in the upper gastrointestinal tract

The metabolic activity of the gastric mucosa was compared with that of the intestinal mucosa since the rapid increase of quercetin metabolites in the lymph suggested the gastric absorption of quercetin. UGT activity was determined in this study as the representative enzyme to produce flavonoid phase II conjugates. The apparent UGT activity was expressed as the amount of quercetin conjugated with glucuronic acid. The duodenal mucosa homogenate showed the highest activity and the UGT activity in the gastric mucosa homogenate was

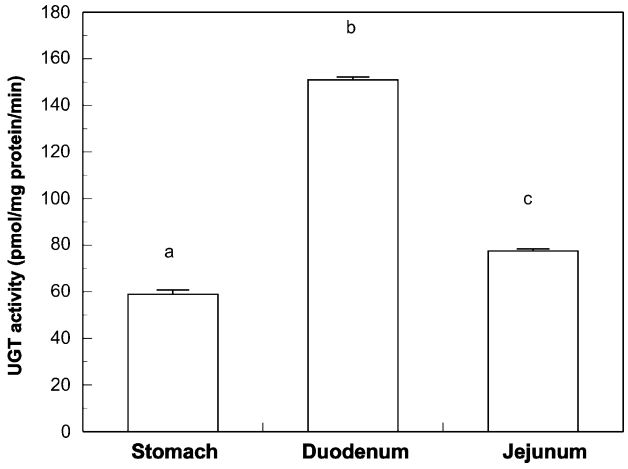

Fig. 3. Apparent enzymatic activity of UGT in the mucosa homogenates of rats. The mucosa of the stomach, duodenum and jejunum were collected and homogenized, and the apparent UGT activity for quercetin as the substrate was determined in those tissues. Values are the means ± S.E.M. ($n = 3$). Means with different letters are significantly different, $P < 0.05$.

significantly lowest among three tissue homogenates (Fig. 3). The non-specific decrease of quercetin was also determined because quercetin is unstable in aqueous solution. The loss of quercetin incubated without UDP was 17–24% and did not significantly differ among three homogenates.

4. Discussion

This is the first report that quercetin is transported to the lymph in the form of its metabolites after the administration in the gastrointestinal tract. Generally, the gastrointestinal lymph plays an important role in the absorption of dietary lipid and other lipophilic compounds. However, little is known about whether the lymphatic pathway has any significance in the absorption of amphiphilic dietary compounds. The lymphatic profile of quercetin in this study was very similar to the plasma profile in rats shown previously [26]. Thus, when quercetin was orally administered with propylene glycol, the C_{max} of its conjugates in the blood was shown at 30 min (T_{max}), and the concentration of isorhamnetin conjugates were increased for 1 h and became constant during 8 h. This suggests that quercetin metabolites transport to both portal vein and the lacteal. Although the mechanism about how the transport pathway is chosen may be unknown, it is likely that the composition of meal affects the pathway. When α-tocopherol, a lipophilic dietary antioxidant, is absorbed, its micellar condition in the intestinal lumen is important in enhancing the absorption in the intestine [22]. Because flavonoids are amphiphilic, the aqueous solubility of quercetin also affects its bioavailability. Azuma et al. [27,28] reported that the co-administration of lipids and emulsifiers enhances the intestinal absorption of quercetin in rats. Furthermore, Lesser et al. [29] showed that high-fat diet enhances the absorption of quercetin in pigs. Thus, lipids have been considered to increase the solubility of quercetin in the intestinal fluid and enhance the uptake into the enterocyte. The result of this study showing that quercetin metabolites appeared in the lymph suggests that quercetin administered with lipids could be easily transferred to the lacteal in the small intestine. When the same dose of quercetin as in this study was administered to rats, the maximum plasma concentration at 1 h was reported to be 9.6 μM of quercetin metabolites and 4.2 μM of isorhamnetin metabolites [30], which were higher than the lymphatic concentration in this study (2.5 and 0.6 μM, respectively). In both cases, quercetin was administered with propylene glycol. These results imply the preferential distribution of quercetin metabolites to the blood on this condition. Alternatively, the lymphatic concentration may be increased when quercetin is administered with dietary lipids. Recently, de Boer et al. [9] reported the tissue distribution of quercetin metabolites in rats fed quercetin for 11 weeks. Interestingly, quercetin was accumulated at the highest amount in the lung. Our findings showing the presence of quercetin in the thoracic lymph may provide a potential mechanism by which quercetin metabolites can be found in the lung at these concentrations.

The maximum concentration of quercetin metabolites appearing in the lymph was as rapid as 30 min after the administration. Crespy et al. [8] previously showed the possibility of quercetin absorption via the stomach when it was administered as its aglycone form. The phase II conjugation activity of the upper gastrointestinal mucosa was investigated in this study since quercetin was mainly converted to its glucuronide/sulfate conjugates. It has been previously reported that the main conjugation enzyme in rat intestinal mucosa was UGT, and sulfation only takes place in the liver [25]. We found that the gastric mucosa does possess UGT activity. However, the highest activity was obtained from the duodenal mucosa, followed by the jejunal mucosa. Shelby et al. [31] reported the mRNA expression of rat UGT family in the gastrointestinal tract. The similar expression levels of UGT1A1, a major UGT isoform in multiple tissues, were shown in stomach, duodenum, and jejunum, whereas several minor isoforms were expressed at higher level in duodenum and jejunum than in stomach. Quercetin has five hydroxyl groups, and the different regioselectivity of glucuronidation is shown by the various UGT isoforms [32]. The difference of the activity among these tissues may depend on the distinctive expression of UGT isoforms. In practice, dietary quercetin is present as its glycosides in plant food, which cannot be absorbed in the stomach [8]. Thus, with dietary exposure, even though a part of quercetin could be absorbed from the stomach like shown in this study, the main organ for the quercetin uptake is probably the upper small intestine.

In conclusion, this result indicates the presence of a novel flavonoid transport pathway, at least in part, from the gastrointestinal mucosa to the circulation, that is, not direct influx into the portal vein but via the lymphatic pathway. Although the significance of the alternative pathways is currently unknown, this finding will give new ideas for the bioavailability and key target tissues of dietary flavonoids.

Acknowledgements: The authors thank Dr. K. Fukuzawa, Dr. A. Tokumura and Dr. K. Kogure in the Department of Pharmaceutical Sciences, The University of Tokushima for their assistance. This work was supported partly by the Ministry of Agriculture, Forestry and Fishery Food Project, Japan.

References

[1] Sun, A.Y., Simonyi, A. and Sun, G.Y. (2002) The French Paradox and beyond: neuroprotective effects of polyphenols. Free Radic. Biol. Med. 32, 314–318.

[2] Ross, J.A. and Kasum, C.M. (2002) Dietary flavonoids: bioavailability, metabolic effects, and safety. Annu. Rev. Nutr. 22, 19–34.
[3] Arts, I.C. and Hollman, P.C. (2005) Polyphenols and disease risk in epidemiologic studies. Am. J. Clin. Nutr. 81, 317S–325S.
[4] Lambert, J.D., Hong, J., Yang, G.Y., Liao, J. and Yang, C.S. (2005) Inhibition of carcinogenesis by polyphenols: evidence from laboratory investigations. Am. J. Clin. Nutr. 81, 284S–291S.
[5] Manach, C., Mazur, A. and Scalbert, A. (2005) Polyphenols and prevention of cardiovascular diseases. Curr. Opin. Lipidol. 16, 77–84.
[6] Murota, K., Shimizu, S., Chujo, H., Moon, J.H. and Terao, J. (2000) Efficiency of absorption and metabolic conversion of quercetin and its glucosides in human intestinal cell line Caco-2. Arch. Biochem. Biophys. 384, 391–397.
[7] Walgren, R.A., Lin, J.T., Kinne, R.K. and Walle, T. (2000) Cellular uptake of dietary flavonoid quercetin 4′-beta-glucoside by sodium-dependent glucose transporter SGLT1. J. Pharmacol. Exp. Ther. 294, 837–843.
[8] Crespy, V., Morand, C., Besson, C., Manach, C., Demigne, C. and Remesy, C. (2002) Quercetin, but not its glycosides, is absorbed from the rat stomach. J. Agric. Food Chem. 50, 618–621.
[9] de Boer, V.C., Dihal, A.A., van der Woude, H., Arts, I.C., Wolffram, S., Alink, G.M., Rietjens, I.M., Keijer, J. and Hollman, P.C. (2005) Tissue distribution of quercetin in rats and pigs. J. Nutr. 135, 1718–1725.
[10] Moon, J.H., Nakata, R., Oshima, S., Inakuma, T. and Terao, J. (2000) Accumulation of quercetin conjugates in blood plasma after the short-term ingestion of onion by women. Am. J. Physiol. Regul. Integr. Comp. Physiol. 279, R461–R467.
[11] Manach, C. and Donovan, J.L. (2004) Pharmacokinetics and metabolism of dietary flavonoids in humans. Free Radic. Res. 38, 771–785.
[12] Manach, C., Williamson, G., Morand, C., Scalbert, A. and Remesy, C. (2005) Bioavailability and bioefficacy of polyphenols in humans. I. Review of 97 bioavailability studies. Am. J. Clin. Nutr. 81, 230S–242S.
[13] Hollman, P.C., Bijsman, M.N., van Gameren, Y., Cnossen, E.P., de Vries, J.H. and Katan, M.B. (1999) The sugar moiety is a major determinant of the absorption of dietary flavonoid glycosides in man. Free Radic. Res. 31, 569–573.
[14] Arts, I.C., Sesink, A.L., Faassen-Peters, M. and Hollman, P.C. (2004) The type of sugar moiety is a major determinant of the small intestinal uptake and subsequent biliary excretion of dietary quercetin glycosides. Br. J. Nutr. 91, 841–847.
[15] Day, A.J., Gee, J.M., DuPont, M.S., Johnson, I.T. and Williamson, G. (2003) Absorption of quercetin-3-glucoside and quercetin-4′-glucoside in the rat small intestine: the role of lactase phlorizin hydrolase and the sodium-dependent glucose transporter. Biochem. Pharmacol. 65, 1199–1206.
[16] Wolffram, S., Block, M. and Ader, P. (2002) Quercetin-3-glucoside is transported by the glucose carrier SGLT1 across the brush border membrane of rat small intestine. J. Nutr. 132, 630–635.
[17] Manach, C., Morand, C., Demigne, C., Texier, O., Regerat, F. and Remesy, C. (1997) Bioavailability of rutin and quercetin in rats. FEBS Lett. 409, 12–16.
[18] Yamamoto, N., Moon, J.H., Tsushida, T., Nagao, A. and Terao, J. (1999) Inhibitory effect of quercetin metabolites and their related derivatives on copper ion-induced lipid peroxidation in human low-density lipoprotein. Arch. Biochem. Biophys. 372, 347–354.
[19] Rothwell, J.A., Day, A.J. and Morgan, M.R. (2005) Experimental determination of octanol-water partition coefficients of quercetin and related flavonoids. J. Agric. Food Chem. 53, 4355–4360.
[20] Moon, J.H., Tsushida, T., Nakahara, K. and Terao, J. (2001) Identification of quercetin 3-*O*-beta-D-glucuronide as an antioxidative metabolite in rat plasma after oral administration of quercetin. Free Radic. Biol. Med. 30, 1274–1285.
[21] Day, A.J., Mellon, F., Barron, D., Sarrazin, G., Morgan, M.R. and Williamson, G. (2001) Human metabolism of dietary flavonoids: identification of plasma metabolites of quercetin. Free Radic. Res. 35, 941–952.
[22] Koo, S.I. and Noh, S.K. (2001) Phosphatidylcholine inhibits and lysophosphatidylcholine enhances the lymphatic absorption of alpha-tocopherol in adult rats. J. Nutr. 131, 717–722.
[23] Tso, P., Lee, T. and DeMichele, S.J. (2001) Randomized structured triglycerides increase lymphatic absorption of tocopherol and retinol compared with the equivalent physical mixture in a rat model of fat malabsorption. J. Nutr. 131, 2157–2163.
[24] Murota, K., Kawada, T., Matsui, N., Sakakibara, M., Takahashi, N. and Fushiki, T. (2000) Oleyl alcohol inhibits intestinal long-chain fatty acid absorption in rats. J. Nutr. Sci. Vitaminol. (Tokyo) 46, 302–308.
[25] Piskula, M.K. and Terao, J. (1998) Accumulation of (−)-epicatechin metabolites in rat plasma after oral administration and distribution of conjugation enzymes in rat tissues. J. Nutr. 128, 1172–1178.
[26] Piskula, M.K. and Terao, J. (1998) Quercetin's solubility affects its accumulation in rat plasma after oral administration. J. Agric. Food Chem. 46, 4313–4317.
[27] Azuma, K., Ippoushi, K., Ito, H., Higashio, H. and Terao, J. (2002) Combination of lipids and emulsifiers enhances the absorption of orally administered quercetin in rats. J. Agric. Food Chem. 50, 1706–1712.
[28] Azuma, K., Ippoushi, K., Ito, H., Horie, H. and Terao, J. (2003) Enhancing effect of lipids and emulsifiers on the accumulation of quercetin metabolites in blood plasma after the short-term ingestion of onion by rats. Biosci. Biotechnol. Biochem. 67, 2548–2555.
[29] Lesser, S., Cermak, R. and Wolffram, S. (2004) Bioavailability of quercetin in pigs is influenced by the dietary fat content. J. Nutr. 134, 1508–1511.
[30] da Silva, E.L., Piskula, M.K., Yamamoto, N., Moon, J.H. and Terao, J. (1998) Quercetin metabolites inhibit copper ion-induced lipid peroxidation in rat plasma. FEBS Lett. 430, 405–408.
[31] Shelby, M.K., Cherrington, N.J., Vansell, N.R. and Klaassen, C.D. (2003) Tissue mRNA expression of the rat UDP-glucuronosyltransferase gene family. Drug Metab. Dispos. 31, 326–333.
[32] Boersma, M.G., van der Woude, H., Bogaards, J., Boeren, S., Vervoort, J., Cnubben, N.H., van Iersel, M.L., van Bladeren, P.J. and Rietjens, I.M. (2002) Regioselectivity of phase II metabolism of luteolin and quercetin by UDP-glucuronosyl transferases. Chem. Res. Toxicol. 15, 662–670.