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Original Article

Quercetin uptake and metabolism by murine peritoneal macrophages *in vitro*

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ABSTRACT

Quercetin (Q), a bioflavonoid ubiquitously distributed in vegetables, fruits, leaves, and grains, can be absorbed, transported, and excreted after oral intake. However, little is known about Q uptake and metabolism by macrophages. To clarify the puzzle, Q at its noncytotoxic concentration (44μM) was incubated without or with mouse peritoneal macrophages for different time periods. Medium alone, extracellular, and intracellular fluids of macrophages were collected to detect changes in Q and its possible metabolites using high-performance liquid chromatography. The results showed that Q was unstable and easily oxidized in either the absence or the presence of macrophages. The remaining Q and its metabolites, including isorhamnetin and an unknown Q metabolite [possibly Q^{-} (*O*-semiquinone)], might be absorbed by macrophages. The percentage of maximal Q uptake by macrophages was found to be 2.28% immediately after incubation; however, Q uptake might persist for about 24 hours. Q uptake by macrophages was greater than the uptake of its methylated derivative isorhamnetin. As Q or its metabolites entered macrophages, those compounds were metabolized primarily into isorhamnetin, kaempferol, or unknown endogenous Q metabolites. The present study, which aimed to clarify cellular uptake and metabolism of Q by macrophages, may have great potential for future practical applications for human health and immunopharmacology.

1. Introduction

Quercetin (Q), a typical antioxidative flavonoid ubiquitously distributed in vegetables, fruits, leaves, and grains, can be absorbed, transported, and excreted after oral intake, suggesting that cellular uptake of quercetin aglycone and quinine formation are possible [1]. An aglyconic version of Q can be found in the blood after the digestion of quercetin glycosides via the small intestine, although Q may conjugate with different glycosides to form quercetin glycosides in plants. After Q supplementation, levels of Q and its methylated metabolite isorhamnetin in the plasma and brain increase markedly, further indicating Q uptake and metabolism *in vivo* [2]. Q, in its aglyconic form, can transiently reach any blood

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cells including immune cells *in vivo*. However, little is known about Q uptake and metabolism by macrophages.

Macrophages are the mature form of monocytes resident in almost all tissues and are pivotal cells in innate immunity. Macrophages are relatively long-lived and versatile cells that perform several different functions throughout the innate immune response and subsequent adaptive immune responses [3]. It is clear that monocytes and macrophages are vital to immune response regulation and inflammation development. Q was found to significantly reduce the production of proinflammatory cytokines interleukin-6 and tumor necrosis factor-α in THP1 macrophages [4]. Recently, Q-rich extracts from strawberry and mulberry fruits were found to significantly decrease mouse splenocytes' tumor necrosis factor-α/interleukin-10 (pro-/anti-inflammatory) cytokine secretion ratios in the presence of lipopolysaccharide (LPS) in concentration-dependent manners [5]. Most recently, it was found that Q administered in a prophylactic manner might act as an immunostimulatory agent; however, Q exhibited a therapeutic, but not prophylactic, effect on spontaneous or LPS-induced inflammation *in vivo* [6]. Taken together, Q has been considered a potent bioflavonoid, and widely used in health foods and pharmacology. Undoubtedly, Q may affect immune cells. However, only a few reports regarding research on Q uptake and metabolism by macrophages were found.

Q was found to have great immunomodulation potential *in vitro* and *in vivo*. We hypothesized that macrophages might have a specific mechanism to Q uptake and metabolism. To validate this assumption, Q was incubated with mouse peritoneal macrophages for different time periods. Extra- and intracellular fluids from the cultures were collected. Changes in the levels of Q and its possible metabolites were measured using high-performance liquid chromatography (HPLC) to clarify Q uptake and metabolism. The present study to clarify cellular uptake and metabolism of Q by macrophages may have great potential for its future practical applications for human health and immunopharmacology.

2. Materials and methods

2.1. Chemicals

Quercetin dihydrate ($C_{15}H_{10}O_7 \cdot 2H_2O$; 338.27 g/mol; Sigma-Aldrich Co., Steinheim, Switzerland) was purchased at the highest available purity (>98%, HPLC) and dissolved in dimethyl sulfoxide (Wako, Osaka, Japan) to prepare a stock solution at a concentration of 20mM. The stock solution was sterilized using a filter (Millipore, Carrigtwohill, Cork, Ireland) with 0.22 μm pore size and stored at −80°C for future use. Q, rutin hydrate, ellagic acid, *p*-coumaric acid, morin, kaempferol (Sigma-Aldrich Co., Steinheim, Germany), and isorhamnetin (MP Biomedicals, lllkirch, France) were purchased at the highest available purity (>98%, HPLC) for HPLC standards.

2.2. Source of mouse primary peritoneal macrophages

Female BALB/cByJNarl mice (6-week old) were obtained from the National Laboratory Animal Center, National Applied Research Laboratories, National Science Council in Taipei, Taiwan, R.O.C., and maintained in the Department of Food Science and Biotechnology, National Chung Hsing University, Taichung, Taiwan, R.O.C. The mice were housed and kept on a chow diet (laboratory standard diet, Diet MF 18; Oriental Yeast Co., Ltd., Osaka, Japan). The animal room was kept on a 12-hour-light and 12-hour-dark cycle. Constant temperature (25 ± 2°C) and relative humidity (50–75%) were maintained. After the mice were acclimatized for 4 weeks, they were sacrificed to obtain peritoneal macrophages. BALB/c strain mice weighing 20–25 g were used throughout the experiment. The animal-use protocol for these experiments was reviewed and approved by the Institutional Animal Care and Use Committee, National Chung Hsing University.

The primary peritoneal macrophages from mice were collected according to the method described by Lin and Tang [7] and Liu and Lin [8]. Briefly, the adult female BALB/c mice were anesthetized with diethyl ether, bled using a retro-orbital venous plexus puncture to collect blood, and immediately euthanized by CO_2 inhalation. Peritoneal macrophages were prepared by lavaging the peritoneal cavity with two aliquots of 5 mL sterile Hank's balanced salts solution [50 mL of 10 × Hank's balanced salts solution (Hyclone Laboratories Inc., Logan, UT, USA); 2.5 mL of antibiotic–antimycotic solution (100 × penicillin-streptomycin-amphotericin (PSA)) containing 10,000 units of penicillin, 10 mg streptomycin, and 25 μg amphotericin B per milliliter in 0.85% saline (Atlanta Biologicals Inc., Norcross, GA, USA); 20 mL of 3% bovine serum albumin (Sigma-Aldrich Co., St. Louis, MO, USA) in phosphate-buffered saline (PBS; 137mM NaCl, 2.7mM KCl, 8.1mM Na_2HPO_4, 1.5mM KH_2PO_4, pH 7.4, 0.2 μm filtered); 2.5 mL of 7.5% $NaHCO_3$ (Wako), and 425 mL sterile water], for a total of 10 mL, through peritoneum. The peritoneal lavage fluid was centrifuged at 400 × *g* for 10 minutes at 4°C. The cell pellets were collected and resuspended in a tissue culture medium (TCM, a serum replacement; Celox Laboratories Inc., Lake Zurich, IL, USA), suspended in a medium consisting of 10 mL TCM, 500 mL Roswell Park Memorial Institute 1640 medium (Atlanta Biologicals Inc.), and 2.5 mL antibiotic–antimycotic solution (100 × PSA). The peritoneal adherent cells (>90% of macrophages) from each animal were pooled and adjusted to 2×10^6 cells/mL in TCM medium with a hemocytometer using the trypan blue dye exclusion method.

2.3. Effect of Q treatment on macrophages cell viability

To evaluate the possible cytotoxic effect of Q, the macrophage cell viability was determined using a 3-(4,5-dimethylthiazol-2-diphenyl)-2,5-tetrazolium bromide (MTT) assay. Briefly, peritoneal macrophages (2×10^6 cells/mL in TCM, 50 μL/well) in the absence or presence of Q (88 μM in TCM, 50 μL/well) were cocultured in 96-well plates at 37°C in a humidified incubator with 5% CO_2 and 95% air for 24 hours. Aliquots of 10 μL of 5 mg/mL MTT (Sigma-Aldrich Co., St. Louis, MO, USA) in PBS were added to each well in the 96-well plate. The plates were incubated for another 4 hours. The culture medium was then discarded. The plates were carefully washed with PBS buffer twice. Aliquots of 100 μL of dimethyl sulfoxide were added to each well and oscillated for 30 minutes to extract the formed insoluble formazan. The absorbance was measured at 550 nm