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Correlation between the cellular metabolism of quercetin and its glucuronide metabolite and oxidative stress in hypertrophied 3T3-L1 adipocytes

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ABSTRACT

Background: Quercetin (Q) is one of the most abundant flavonoids in human dietary sources and has been related to the capacity to ameliorate obesity-related pathologies. Quercetin-3-O-β-D-glucuronide (Q3GA) is supposed to be the main metabolite in blood circulation, but the intracellular final effectors for its activity are still unknown.
Hypothesis/purpose: To identify and quantitate the intracellular metabolites in hypertrophied adipocytes incubated with Q or Q3GA and to correlate them with the intracellular generation of oxygen radical species (ROS).
Methods: Cytoplasmic fractions were obtained and quercetin metabolites were determined by liquid chromatography coupled to a time-of-flight mass detector with electrospray ionization (HPLC-DAD-ESI-TOF). Intracellular ROS generation was measured by a ROS-sensitive fluorescent probe.
Results: Both Q and Q3GA were absorbed by hypertrophied adipocytes and metabolized to some extent to Q3GA and Q, respectively, but Q absorption was more efficient (1.92 ± 0.03 µg/µg protein) and faster than that of Q3GA (0.12 ± 0.0015 µg/µg protein), leading to a higher intracellular concentration of the aglycone. Intracellular decrease of ROS correlated with the presence of the most abundant quercetin metabolite.
Conclusion: Q and Q3GA are efficiently absorbed by hypertrophied adipocytes and metabolized to some extent to Q3GA and Q, respectively. The intracellular decrease of ROS in a hypertrophied adipocyte model treated with Q or Q3GA is correlated with the most abundant intracellular metabolite for the first time. Both compounds might be able to reach other intracellular targets, thus contributing to their bioactivity.

Introduction

Plant-derived polyphenols have demonstrated the potential to improve some disease states by a multitargeted mode of action (Barrajon-Catalan et al., 2014; Joven et al., 2012; Joven et al., 2014). Quercetin (3,3′,4′,5,7-pentahydroxyflavone) (Q) derivatives are the most abundant flavonoids in human dietary sources; ubiquitously present in fruits and vegetables (Ahn et al., 2008), they are converted into aglycone on the cell surface of intestinal epithelial cells and bacteria. Upon absorption, Q is subjected to different types of metabolism, with quercetin-3-O-β-D-glucuronide (Q3GA) as the major metabolite circulating in the bloodstream. The localization of Q3GA in macrophages, atherosclerotic lesions, brain and immune cells and lipid droplets of the liver by using specific antibodies has been reported (Joven et al., 2012; Kawai et al., 2008).

Q3GA is proposed to be deconjugated by β-glucuronidase into hydrophobic Q aglycone in cells, such as macrophages or vascular tissue, which in turn may improve intracellular pathological conditions (Ishisaka et al., 2013; Kawai et al., 2008; Menendez et al., 2011). Nevertheless, no deconjugation has been demonstrated in other cell models.

Abbreviations: Q, quercetin; Q3GA, quercetin-3-O-β-D-glucuronide; ROS, radical oxygen species; UGT, UDP-glucuronosyltransferase; PPAR, peroxisome proliferator-activated receptor; H2DCF-DA, 2′,7′-dichlorodihydrofluorescein diacetate.

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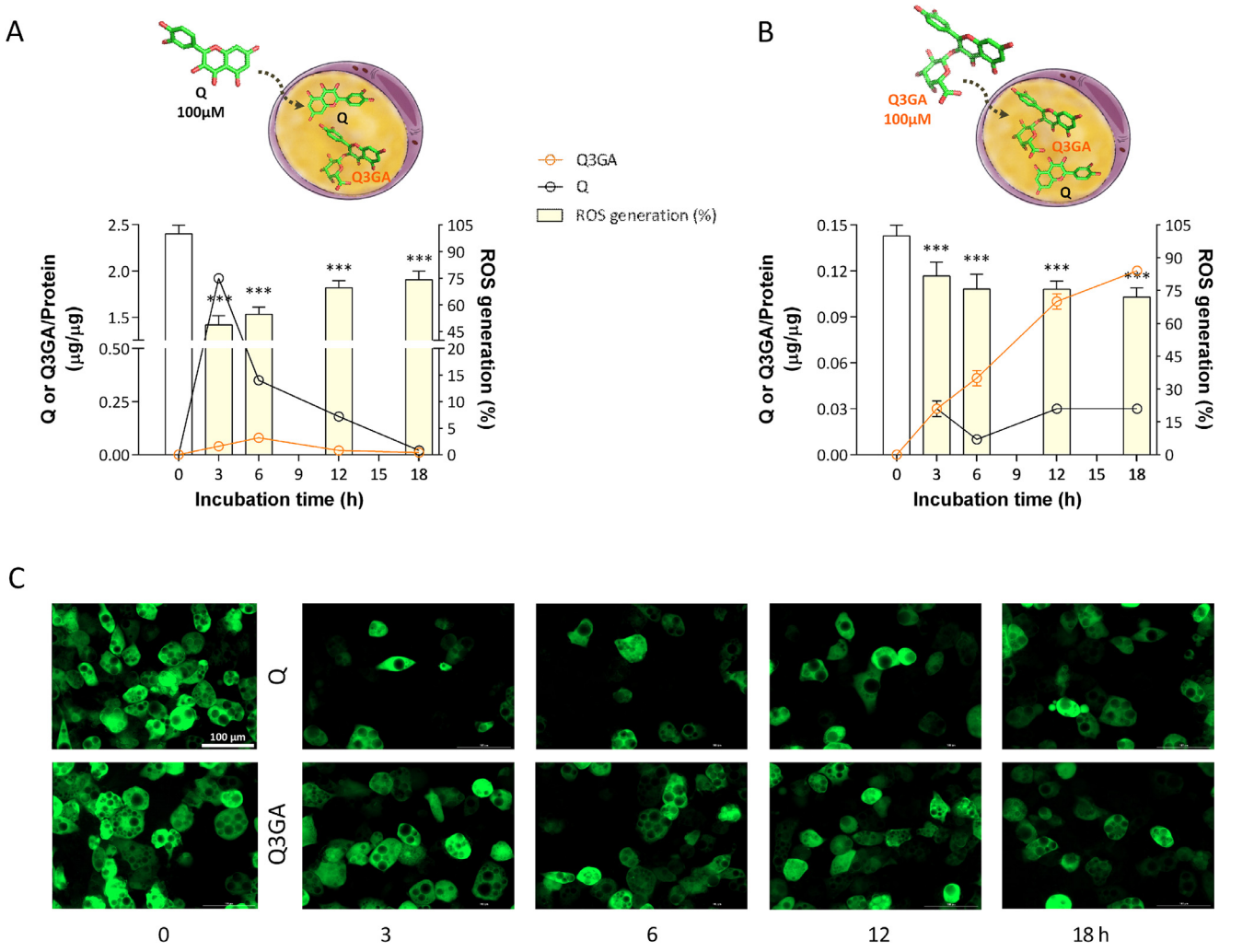

Fig. 1. Cellular metabolism of Q and Q3GA in hypertrophied adipocytes and the concomitant intracellular ROS decrease.
Hypertrophic adipocytes were treated with 100 µM Q (A) or 100 µM Q3GA (B) for 0, 3, 6, 12 and 18 h, and the cellular levels of both the aglycone and glucuronide metabolites were determined by HPLC-ESI-TOF-MS (black and orange circles, respectively). An inverse correlation was observed between intracellular ROS generation, determined by using the ROS-sensitive fluorescent probe H2DCF-DA (yellow bars) and the cytoplasmic levels of Q (A) and Q3GA (B). Representative photomicrographs of intracellular ROS after incubation of adipocytes with Q or Q3GA for 3, 6, 12 and 18 h (C). The intensity of green fluorescence represents the level of intracellular ROS compared to the control (0 h). All data were analyzed by using one-factor ANOVA and Tukey test for multiple comparisons. The results were expressed as the mean ± standard deviation. Differences showing $p < 0.05$ were considered statistically significant (n = 6).

Adipocyte hypertrophy compromises cell function, initiating an oxidative stress-related inflammatory process leading to metabolic disorders associated with obesity (Furukawa et al., 2004). Our findings strongly support that polyphenols from *Hibiscus sabdariffa* L. (Malvaceae) may become an alternative way to alleviate the metabolic disturbances associated with obesity through the modulation of energy management and inflammation pathways (Barrajon-Catalan et al., 2014; Beltran-Debon et al., 2010; Herranz-Lopez et al., 2012). Evidence in animal models leads us to propose that Q and Q3GA, among other flavonols, are the major blood metabolites accounting for these effects (Fig. 1, Supplementary information) (Fernandez-Arroyo et al., 2012; Joven et al., 2012). Nevertheless, the main quercetin metabolites reaching adipocyte intracellular targets are yet to be discovered. In the present work, a comparative study of the cellular metabolism of Q and its glucuronide metabolite was carried out by high performance liquid chromatography coupled to a time-of-flight mass detector with electrospray ionization (HPLC-DAD-ESI-TOF) in hypertrophied adipocytes, and the intracellular metabolites were correlated with the generation of ROS in the cytosol.

Material and methods

Chemicals

LC-MS grade formic acid for mobile phase preparation and the standards Q, Q3GA and naringenin (used as internal standard) were purchased from Fluka, Sigma-Aldrich (Steinheim, Germany). LC-MS grade acetonitrile and analytical reagent grade methanol and ethanol were obtained from Fisher Scientific (Madrid, Spain). Stock solutions containing these analytes were prepared in methanol and stored at −80 °C until use. 3T3-L1 cells and all tissue culture reagents were purchased as reported (Herranz-Lopez et al., 2012). Briefly, 3T3-L1 cells were purchased from the American Type Culture Collection (Manassas, VA, USA). Dexamethasone, 3-isobutyl-1- methylxanthine and insulin were obtained from Sigma–Aldrich (Madrid, Spain). Dulbecco's modified Eagle's medium, calf serum, fetal bovine serum, and an antibiotic mixture (penicillin–streptomycin) were purchased from PAA Laboratories (Linz, Austria). Sodium pyruvate and trypsin–EDTA were obtained from Invitrogen (Carlsbad, CA). Polyvinyldifluoride (PVD) filters, 0.22 µm, were obtained from Millipore (Bedford, MA).