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Induction of cytotoxic and genotoxic responses by natural and novel quercetin glycosides

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ABSTRACT

The flavonoids quercetin, and its natural glycosides isoquercetin and rutin, are phytochemicals commonly consumed in plant-derived foods. Semi-synthetic water-soluble isoquercetin and rutin glycosides, maltooligosyl isoquercetin, monoglucosyl rutin and maltooligosyl rutin were developed by synthetic glycosylation to overcome solubility challenges for improved incorporation in food and medicinal applications. Quercetin and its natural glycosides are known to induce genetic instability and decrease cell proliferation. Using a system of Chinese hamster ovary (CHO) cells, this study examined the differences in cytotoxic and genotoxic responses induced by natural and synthetic flavonoids. Bioactivity evaluations using poly(ADP-ribose) polymerase (PARP) ELISA showed that the synthetic flavonoids were less effective in inhibiting PARP than the natural flavonoids, where PARP inhibitory effects decreased with glycosylation of flavonoids. In the genotoxic studies, treatments with flavonoids at a concentration range of 0.2 μM–1 mM induced significant frequencies of sister chromatid exchange (SCE) and micronuclei in CHO cells compared to spontaneous occurrences. The synthetic flavonoids monoglucosyl rutin and maltooligosyl rutin induced less genotoxic effects than the natural flavonoids. However, maltooligosyl isoquercetin induced similar responses as isoquercetin and rutin. The growth inhibition studies showed glycosylation dependent cytotoxicity in natural flavonoids. The quercetin aglycone exhibited the highest toxicity out of all the flavonoids studied. Differences in growth inhibition were not observed between the synthetic flavonoids, maltooligosyl isoquercetin and monoglucosyl rutin, and natural isoquercetin and rutin, respectively. Maltooligosyl rutin induced less cytotoxicity than rutin and monoglucosyl rutin. Our in vitro studies demonstrated that the synthetic flavonoids generally induced less genotoxic responses than their natural counterparts.

1. Introduction

Quercetin is a highly abundant flavonoid ubiquitous in plants and distributed in most parts of fruits and vegetables [1]. This polyphenolic compound is found in common dietary sources such as cranberries, apples, blueberries, onions, black tea, red wine, and fruit juices [2]. In the United States, the mean consumption of total flavonoids by adults is estimated at 344 mg per day, where the flavonoid quercetin makes up approximately 4% of flavonoid intake [3]. Quercetin is an aglycone that commonly occurs as glycosides in plants, such as isoquercetin and rutin [4,5]. Isoquercetin and rutin each contains a monosaccharide (glucose) and a disaccharide (rhamnose) residue on the glycosidic linkage, respectively (Fig. 1). These phytochemicals serve a wide range of physiological functions in plants with anti-oxidation being the most notable feature [5,6]. Similar to reported benefits of many flavonoids, studies in cell systems and animals show that quercetin and its natural glycosides are potentially beneficial to human health [7–13]. The health benefits linked to quercetin, isoquercetin and rutin are generally associated with their antioxidant properties [1,12,14].

Quercetin also has pro-oxidant properties that are rooted in the same structural attributes which give rise to its antioxidant properties. It can be metabolically bioactivated to the reactive products,

Abbreviations: CHO, Chinese hamster ovary; SCE, sister chromatid exchange; PARP, poly(ADP ribose) polymerase; ELISA, enzyme-linked immunosorbent assay; IC50, concentration at which 50% of activity is inhibited; ROS, reactive oxygen species; 3-AB, 3-aminobenzamide; SSB, single strand break; PAR, poly(ADP ribose).

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semi-quinone intermediate, and subsequently, *ortho*-quinone [2,15,16]. *Ortho*-quinone can bind DNA causing oxidative damage, which is a potential mechanism of mutagenicity of quercetin [2,28]. The mutagenic property of quercetin has been well established in positive reverse mutation tests in various strains of *Salmonella typhimurium*, though it has not exhibited carcinogenic effects in humans [6,17]. Glycosylation of flavonoid aglycones, on the other hand, tends to diminish pro-oxidant activities, resulting in less mutagenicity [18].

Genetic stability is crucial to cell proliferation and survival, and as a mutagen, quercetin has been shown to induce genomic instability in the form of sister chromatid exchange (SCE) and micronuclei [19]. It has been also demonstrated to impair DNA repair capability by inhibiting poly(ADP-ribose) polymerase (PARP), an enzyme critical to DNA single strand break (SSB) repairs [20]. In addition to reducing genomic stability, quercetin has exhibited cytotoxicity through growth inhibition and cell cycle arrest in various types of cancer cells [21].

As human health stands to potentially benefit from the biological properties of quercetin and its glycosides, there is an increase in interest to incorporate these flavonoids into various food and beverage products. Quercetin, isoquercetin and rutin are already available on the consumer market as dietary supplements, promoting anti-oxidation. However, these compounds are not readily miscible in water which lends difficulty to their incorporation with dietary products [21]. The miscibility issue has led to the development of semi-synthetic quercetin glycosides with improved water solubility: maltooligosyl isoquercetin, monoglucosyl rutin, and maltooligosyl rutin (Fig. 1) [22,23]. Maltooligosyl isoquercetin is currently in the consumer market as dietary supplements. Enzymatic modifications to the quercetin aglycone and glycosides increase the number of carbohydrates that are attached to the glycosidic linkage, resulting in the formation of novel glycosides. The number of glucose molecules distinguishes novel glycosides from one another. Maltooligosyl isoquercetin and maltooligosyl rutin both contain varying numbers of glucose that range from one to seven glucose molecules, where monoglucosyl rutin has only a single glucose. These novel glycosides have been introduced as dietary supplements in beverages, which are currently available to consumers in Japan.

At present, toxicity information for isoquercetin, maltooligosyl isoquercetin, monoglucosyl rutin, and maltooligosyl rutin is scarce [24]. The aim of this study is to examine the cytogenetic responses through comparison of known genotoxic endpoints induced by quercetin with those of the novel glucosyl flavonoids. Using a system of Chinese hamster ovary (CHO) cells, which are commonly used in in vitro toxicity screening studies, cell viability and growth inhibition, SCE, micronuclei and PARP activity were evaluated. We hypothesized that the synthetic flavonoids would induce cytotoxic and genotoxic effects similar to those exhibited by quercetin, but occur in a glucosyl structure-dependent manner.

2. Material and methods

2.1. Cell culture

CHO10B2 (CHO wild type) cells were kindly provided by Dr. Joel Bedford (Colorado State University, Fort Collins, CO) and maintained in culture in minimum essential medium (MEM-α, Gibco, Grand Island, NY), and supplemented with 10% fetal bovine serum (FBS, Sigma, St. Louis, MO) and 1% antibiotics and antimycotics (Gibco) in a humidified incubator at 37 °C and 5% CO_2. The CHO cell doubling time is approximately 12 h.

2.2. Flavonoids

Quercetin (molecular weight 302), isoquercetin (molecular weight 464), maltooligosyl isoquercetin (molecular weight 863.9), rutin (molecular weight 601.5), monoglucosyl rutin (molecular weight 772) and maltooligosyl rutin (molecular weight 1104.4) were developed and provided by the Toyo Sugar Refining Co., Ltd. (Tokyo, Japan). Because maltooligosyl isoquercetin and maltooligosyl rutin have multiple numbers of glycosides, the average

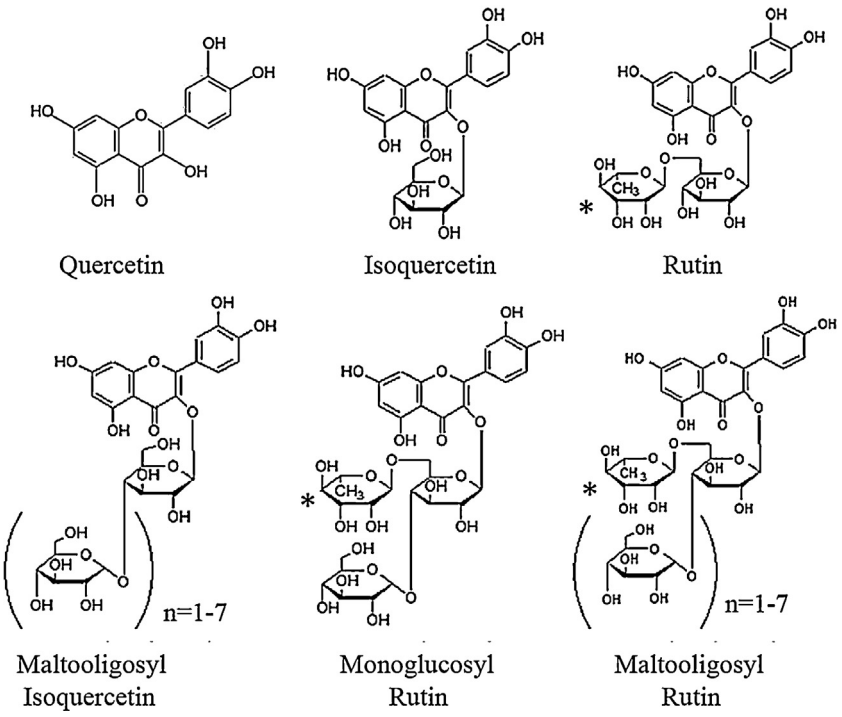

Fig. 1. Chemical structures of natural (top) and synthetic (bottom) flavonoids. A "*" indicates rhamnose structure.