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Protective effects of xanthohumol against the genotoxicity of benzo(a)pyrene (BaP), 2-amino-3-methylimidazo[4,5-f]quinoline (IQ) and *tert*-butyl hydroperoxide (*t*-BOOH) in HepG2 human hepatoma cells

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Abstract

Xanthohumol is the major prenylated flavonoid present in the hop plant *Humulus lupulus* L. (Cannabinaceae) and a common ingredient of beer. Recently, xanthohumol has gained considerable interest due to its potential cancer chemo-preventive effect. The aim of this study was to reveal the possible anti-genotoxic activity of xanthohumol in metabolically competent human hepatoma HepG2 cells, by use of the comet assay. Xanthohumol by itself was neither cytotoxic nor genotoxic to the cells at concentrations below 10 μM. However, a significant protective effect against the pro-carcinogens benzo(a)pyrene (BaP) and 2-amino-3-methylimidazo[4,5-f]quinoline (IQ) was observed at concentrations as low as 0.01 μM. In cells treated with xanthohumol in combination with *tert*-butyl hydroperoxide (*t*-BOOH) – an inducer of reactive oxygen species (ROS) – no protective effect was observed and xanthohumol also showed no significant scavenging activity against 1,1-diphenyl-2-picrylhydrazyl (DPPH) radicals. On the other hand, HepG2 cells pre-treated with xanthohumol showed significantly reduced levels of *t*-BOOH-induced DNA strand breaks, indicating that its protective effect is mediated by induction of cellular defence mechanisms against oxidative stress. As xanthohumol is known to be an effective inhibitor of cytochrome P450 enzymes and an inducer of NAD(P)H: quinone reductase (QR), our findings can be explained by an inhibition of metabolic activation of pro-carcinogens and/or by induction of carcinogen-detoxifying and anti-oxidative enzymes by xanthohumol. These results provide evidence that xanthohumol displays anti-genotoxic activity in metabolically competent human cells.

Keywords: Anti-genotoxic; Comet assay; HepG2 cells; Reactive oxygen species; Xanthohumol

1. Introduction

Many epidemiological studies have shown that nutritional factors play an important role in the aetiology of human cancer. Human diet often contains ingredients that cause DNA damage. On the other hand, numerous food constituents of plant origin have been identified that show promising results in preventing cancer and other mutation-related diseases [1,2].

The female inflorescences (hops) of the hop plant *Humulus lupulus* L. are used in the brewing industry to add bitterness and flavour to beer. Hops are known not only in beer brewing processes but also as a tranquilizer in folk medicines. Prenylated flavonoids from hops have

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recently gained considerable attention due to various biological effects [3–6]. Several recent studies showed that xanthohumol (3′-[3,3-dimethyl allyl]-2,4,4′-trihydroxy-6′-methoxychalcone), the major prenylated flavonoid of hops, has chemo-preventive properties relevant to suppression of cancer development at the initiation, promotion and progression phases. It was postulated that xanthohumol may modulate the activities of enzymes involved in carcinogen metabolism and it has been shown to have antioxidant and free-radical scavenging activity [5,7]. Furthermore, xanthohumol acts as an anti-inflammatory agent by inhibiting cyclooxygenases −1 and −2, and it has been reported to inhibit DNA synthesis and induce cell-cycle arrest, apoptosis and cell differentiation [5,7].

Genetic damage, which can lead to mutations, is a crucial event for the initiation of carcinogenesis, so that its prevention can be the key step in cancer prevention [8]. The anti-mutagenic effect of xanthohumol at the cellular level has been shown in a bacterial test system with *Salmonella typhimurium,* where the bacteria were exposed to the heterocyclic aromatic amine 2-amino-3-methylimidazo[4,5-f]quinoline (IQ) in the presence of cDNA-expressed human CYP1A2 [9]. Recently, Dietz et al. [10] showed that xanthohumol prevented formation of menadione-induced DNA strand breaks in murine hepatoma Hepa1c1c7 cells.

The first aim of the present study was to ascertain whether xanthohumol protects against DNA damage induced by two pro-carcinogens that require metabolic activation by cytochrome P450 enzymes – the heterocyclic aromatic amine 2-amino-3-methylimidazo[4,5-f]quinoline (IQ) and the polyaromatic hydrocarbon benzo(a)pyrene (BaP) – in human HepG2 liver cells, which have retained the inducible activities of xenobiotic-metabolizing enzymes [11,12]. The second aim was to determine whether xanthohumol protects against the formation of reactive oxygen species (ROS) by *tert*-butyl hydroperoxide (*t*-BOOH) in HepG2 cells. With the single-cell gel electrophoresis (comet) assay, which is a rapid and sensitive method for analyzing DNA damage at the level of the individual cell [13,14], we investigated the potential genotoxicity of xanthohumol and its protective effects against DNA damage induced by IQ, BaP and *t*-BOOH.

2. Materials and methods

2.1. Chemicals

Williams' medium E, penicillin/streptomycin, foetal bovine serum (FBS), l-glutamine, phosphate-buffered saline (PBS), trypsin, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT [CAS 298-93-1]), *tert*-butyl hydroperoxide (*t*-BOOH [CAS 75-91-2]), benzo(a)pyrene (BaP [CAS 50-32-8]), ethidium-bromide solution (CAS 1239-45-8), dimethyl sulfoxide (DMSO [CAS 67-68-5]) and EDTA [CAS 6381-92-6] were obtained from Sigma-Aldrich (St. Louis, USA); Xanthohumol was obtained from the Nookandeh-Institut für Naturstoffchemie GmbH (Homburg/Saar, Germany) and IQ (CAS 093-02571) from Wako Chemical Industries (Osaka, Japan). Normal melting-point (NMP) agarose and low melting-point agarose (LMP [CAS 9012-36-6]) were from Gibco BRL (Paisley, Scotland).

A 100 μM stock solution of *t*-BOOH was prepared in sterile PBS (pH 7.0). Further dilutions were prepared in complete growth medium just before treatment of the cells. Xanthohumol, IQ and BaP were dissolved in dimethyl sulfoxide (DMSO); the final concentration of DMSO in incubation mixtures was not higher than 1%.

2.2. Human HepG2 cells

HepG2 cells were provided by Prof. Firouz Darroudi, Department of Radiation Genetics and Chemical Mutagenesis, University of Leiden, The Netherlands. The cells were grown in Williams' medium E containing 15% foetal bovine serum, 2 mM L-glutamine and 100 U/ml penicillin/streptomycin at 37 °C in 5% CO_2. Cells were used at passages between 4 and 9.

2.3. Cytotoxicity assay

Cytotoxicity of xanthohumol was determined with the MTT assay, as described by Mossman [15] with minor modifications. This assay measures the conversion of MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) to insoluble formazan by dehydrogenase enzymes of intact mitochondria of living cells. HepG2 cells were seeded onto a 96-well microplate at a density of 7500 cells/well. The next day, the medium was replaced by complete growth medium, containing 0.1, 1, 10, 50 and 100 μM xanthohumol and incubated for 24 h. At the end of the exposure, the cells were washed twice with PBS and the medium was replaced by fresh complete growth medium. MTT was then added to a final concentration of 0.5 mg/ml. After 3 h, the medium was removed and the formazan crystals dissolved in DMSO. Optical density (OD) of the solution in each well was measured against a blank (a well with DMSO) at 570 nm (the formazan absorption peak) and at 690 nm (measurement of the medium turbidity caused by cell debris) with a microplate spectrofluorimeter (Tecan Genios). The OD difference OD_{570}–OD_{690} is proportional to the concentration of formazan, which is proportional to the number of viable cells. Viability of cells was determined by comparing the relative formazan concentration (OD_{570}–OD_{690}) of the treated cells with that of untreated cells. Five individual wells were measured per treatment point.