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Extracts of dietary plants are efficient modulators of nuclear factor kappa B

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Abstract

Convincing evidence suggest that a plant-based diet is associated with a reduced risk of several chronic diseases, but the mechanisms for this association is not fully elucidated. The transcription factor nuclear factor kappa B (NF-κB) plays a critical role in cellular stress-, immune- and inflammatory responses. Also, NF-κB is identified as a promising therapeutic target both in cancer and chronic inflammation. We used monocytes stably transfected with a NF-κB-luciferase reporter construct in a screening of plant extracts for NF-κB modulators. Our aim was to identify dietary components which could induce basal NF-κB activity to produce a preconditioning effect, or inhibit induction of disease related NF-κB activity. When screening 34 dietary plants for their ability to induce basal NF-κB activity or inhibit lipopolysaccharide induced NF-κB activity we observed that 23 dietary plant extracts induced basal NF-κB activity, while 15 extracts attenuate induced NF-κB activation. These results indicate that dietary plants contain compounds that efficiently modulate NF-κB activity. We suggest dietary modulation of NF-κB may contribute to the observed beneficial effects of dietary plants on the risk of chronic diseases.

Keywords: NF-κB; Inhibitor; Diet; Phytochemicals

1. Introduction

A number of epidemiological studies have provided convincing evidence that a diet rich in plant-based foods is correlated with a reduction of the risk of developing several chronic diseases, such as cancer (American Institute for Cancer Research/World Cancer Research Fund, 1997) and cardiovascular disease (Report of a joint FAO/WHO Expert Consultation, 2003). Even with convincing evidence pointing toward a reduction in the risk of several diseases associated with a high intake of fruits and vegetables, mechanisms for this association is not fully elucidated. These risk reductions are generally found only when comparing the intake of whole fruits and vegetables, to disease. Effects of single compounds, on the other hand, are not convincing (Halliwell, 2000) and suggests that several of the components of fruits and vegetables might cooperate (Blomhoff, 2005) to produce health benefits, and thus the benefit will not be evident by supplementation of one or a few compounds.

The nutrigenomics approach holds promise for elucidation of nutrient sensitive signaling pathways, and the characterization of the main dietary components involved. A wide range of dietary components are involved in regulation of gene expression, and these include nutrients like vitamin A, D and E and various fatty acids as well as non-nutrient food components such as phytochemicals (Muller and Kersten, 2003).

Abbreviations: DMSO, dimethyl sulfoxide; EGCG, epigallocatechin gallate; FRAP, ferric reducing ability of plasma; IκB, inhibitor of NF-κB; IKK, IκB kinase; IL, interleukin; IRAK, IL-1-receptor-associated kinase; LPS, lipopolysaccharide; MYD88, myeloid differentiation primary response gene 88; NAC, *N*-acetyl-cysteine; NF-κB, nuclear factor kappa B; TLR4, toll-like receptor 4; TNF, tumor necrosis factor; TNFR1, TNF-receptor 1; TRAF6, TNF-receptor-associated factor 6; TRADD, TNF receptor associated via death domain.

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The transcription factor nuclear factor kappa B (NF-κB) plays a critical role in cellular stress-, immune- and inflammatory responses (Karin et al., 2004). Although NF-κB is essential in normal physiology, a number of human disorders involve inappropriate regulation of NF-κB. Several chronic diseases (Christman et al., 2000; Feldmann et al., 2002; Mattson and Camandola, 2001; Neurath et al., 1998; Valen et al., 2001) and various human cancers (Karin et al., 2002) have been associated with an aberrant up-regulation of NF-κB activity. Also, recent studies have identified NF-κB as a direct link between inflammation and cancer (Greten and Karin, 2004; Luo et al., 2004; Pikarsky et al., 2004). In the pharmacological industry, enormous resources are put into screening for therapeutic targets and compounds that can modulate the activity of these targets.

In vertebrates, the NF-κB family of transcription factors is comprised of five structurally related proteins (p65 (REL A), p50, p52, c-REL, REL B) which form hetero- and homodimers. Normally the NF-κB dimers are mainly located in the cytosol bound to the inhibitors of NF-κB, the IκBs. Numerous stimuli can activate NF-κB through phosphorylation of IκB by the IκB-kinase complex (IKK). Upon phosphorylation, IκB is degraded, and the NF-κB dimer reside mainly in the nucleus, bind to specific target sequences in DNA and promote transcription (Chen and Greene, 2004). NF-κB is activated by a wide variety of stimuli including cytokines such as interleukin (IL)-1, IL-6 and Tumor necrosis factor (TNF)-α , and bacterial- or viral infection, and various forms of stress such as UV-radiation and environmental toxins. NF-κB target genes, more than 150, codes for proteins which are central players in inflammation, activation of the innate immune system and anti-apoptotic signaling (Pahl, 1999).

Even though persistent aberrantly up-regulated NF-κB activity is associated with the pathophysiology of many diseases, small, repetitive episodes of NF-κB activation may also play a role in the prevention of disease. Several investigations have found pre-conditioning mediated through activation of NF-κB to be protective against sepsis (Yang et al., 2000), coronary heart disease (Valen, 2004; Zhang et al., 2003), hepatic ischemia injury (Teoh et al., 2002) and cerebral diseases such as ischemia or epilepsy (Blondeau et al., 2001).

NF-κB is identified as a promising therapeutic target in inflammation, neurodegenerative diseases and cancer. Currently known NF-κB inhibitors include glucocorticoids, ibuprofen, ubiquitin ligase inhibitors, and several structurally unrelated antioxidants such as ascorbic acid, glutathione and *N*-acetyl-cysteine (Gilmore and Herscovitch, 2006). Food stuffs or substances with the ability to modulate NF-κB activity can be of both nutritional and pharmacological interest. In this study, we used a human monocytic cell line stably transfected with a luciferase reporter containing three NF-κB binding sites, to screen a wide variety of dietary plant extracts for their ability to modulate basal or induced NF-κB activity.

2. Materials and methods

2.1. Chemicals

Chemicals used: Curcumin (Cat. No. 28260) Fluka (Buchs, Switzerland). Coumarin (Cat. No. C5782) LKT Laboratories (St. Paul, MN, USA), carnosol (Cat. No. 89800) Cayman Chemical Co. (Ann Arbor, MI, USA) and d-luciferin from Biosynth AG (Staad, Switzerland). Apigenin (Cat. No. A3145), epigallocatechin gallate (EGCG) (Cat. No. E4143), epicatechin (Cat. No. E4018), N-acetyl-cysteine (NAC) (Cat. No. A9165), capsaicin (Cat. No. M2028), thymol (Cat. No. T0501), rutin (Cat. No. R2303), dexamethasone (Cat. No. D8893), genistein (Cat. No. G6776), kaempherol (Cat. No. K0133), sulforaphane (Cat. No. S6317) were all purchased from Sigma–Aldrich (St. Louis, MO, USA). All other chemicals were purchased from Sigma–Aldrich (St. Louis, MO).

2.2. Cell culture

A human monocytic cell line stably transfected with a luciferase reporter containing three NF-κB binding sites (U937-3xκB-LUC cells) were used (Carlsen et al., 2002). Cells were cultured in RPMI-1640 medium supplemented with L-glutamine (2 mM), penicillin (50 U/ml), streptomycin (50 mg/ml), hygromycin (75 μg/ml) and 10% fetal bovine serum at 37 °C and 5% CO_2. For experiments cells were transferred to medium with 2% fetal bovine serum in 24 well plates. For measurements of basal NF-κB activity cells were incubated with extract, phytochemical or vehicle control for 6.5 h. For Lipopolysaccharide (LPS)-induced NF-κB activity, cells were pre-incubated with extract, phytochemical or vehicle control for 30 min, then added LPS (1 μg/ml) and incubated for an additional 6 h. Cell viability was determined by trypan blue exclusion, with a cut-off value of 10% non-viable cells. Results from experiments with extracts of cloudberries and cinnamon were excluded due to cell toxicity.

2.3. Dietary plant extracts

Dietary plants were obtained from local grocery stores or marked places in Oslo, while wild berries were picked in Oslo, or Gol, Norway. All dietary plants were extracted directly or frozen at −20 °C until extraction. Dry samples (spices) were pulverized and to 10 g of the sample was added 20 mL of double distilled water and 20 mL of methanol. Samples of berries, fruits or vegetables were homogenized and to 10 g of the homogenized sample was added 10 ml of methanol. Samples were sonicated in a water bath for 30 min at 0 °C. The sample was centrifuged at 3000g for 15 min, and the liquid phase was concentrated under nitrogen gas to viscid fluid. The concentrated viscid fluid was diluted to 5 ml in PBS or dimetyl sulfoxide (DMSO), and thereafter sterile filtered and stored under argon gas in airtight tubes at −70 °C.

2.4. Phytochemical stock solutions

Phytochemicals were dissolved in DMSO, and stored under argon gas at −70 °C. No more than 0.1% DMSO (v/v) was added to cell cultures.

2.5. Luciferase activity assay

For experiments with dietary plant extracts: Luciferase activity was measured in cell lysates as previously described (Carlsen et al., 2002). For experiments with phytochemicals and spices: Luciferase activity was measured by imaging by use of the IVIS Imaging System 100 from Xenogen Corp., USA. Luminescence was detected for 1 min, 4 min after the addition of 0.2 mg d-luciferin per ml of cell medium. The number of photons emitted from each well was calculated by use of the Living Image Software (Xenogen Corp., USA). Gray scale images were used for reference of position.