Regulatory Toxicology and Pharmacology 73 (2015) 442–451

Contents lists available at ScienceDirect

Regulatory Toxicology and Pharmacology

journal homepage: www.elsevier.com/locate/yrtph

Broccoli seed extract: Genotoxicity and subchronic toxicity studies

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ARTICLE INFO

Article history:
Received 24 June 2015
Received in revised form
6 August 2015
Accepted 7 August 2015
Available online 11 August 2015

Keywords:
Broccoli seed extract
Rat
Subchronic toxicity
Genotoxicity
Mouse sperm abnormality assay

ABSTRACT

Potential health benefits have been attributed to broccoli consumption. Hence, there is potential for use of broccoli seed extract (BSE) in food or for use as a dietary supplement. To assess the potential safety of a BSE product, three genotoxicity experiments, including an Ames, *in vivo* mouse micronucleus, and *in vivo* mouse sperm abnormality assay, were carried out. BSE was subject to an acute oral toxicity test and was evaluated in a 30-day feeding study in rats. BSE showed no mutagenic activity in the Ames assay and no evidence of genotoxic potential in the *in vivo* assays at doses up to 10 g/kg body weight (bw). The LD_{50} of BSE in rats was >10 g/kg bw/d. In the 30-day feeding study, in which BSE was administered in the diet to provide doses of 0, 0.3, 1.0, or 3.0 g/kg bw/d, no toxicological significant effects were noted on body weight, body weight gain, organ weights, or on the results of hematological, clinical chemistry and histopathological evaluations. The no-observed-adverse-effect level was considered to be 3.0 g/kg bw/d, the highest dose tested. Collectively, these results support the safe use of BSE as a food ingredient or product.

1. Introduction

The genus *Brassica* (family Brassicaceae or Cruciferae) includes many commonly eaten vegetables (in the form of top sprouts, stems, roots or oils) such as broccoli, cauliflower, Brussels sprout, cabbage, and others (Latté et al., 2011).

Consumption of broccoli provides an excellent natural source of dietary fiber, folate, minerals (calcium and potassium) and vitamins (A, C, E, and K). Other important components which have specifically been linked to the beneficial effects of broccoli consumption include the glucosinolates (Traka and Mithen, 2009; Latté et al., 2011; Dinkova-Kostova and Kostov, 2012; Veeranki et al., 2013). These are secondary plant products, the aglycones of which can be grouped into a number of different structural classes depending on the nature of the side chain present (Fahey et al., 2001). At least 16 glucosinolates are present in common broccoli cultivar samples (Vang et al., 2001; Vallejo et al., 2003; Meyer and Adam, 2008; Latté et al., 2011). The most common of the glucosinolates present are glucoraphanin and glucoiberin. Of the indole glucosinolates, glucobrassicin and neoglucobrassicin predominate. The exact nature of the glucosinolates present though is highly dependent upon the cultivar used, the habitat/environment present, and the stage of plant development (Kurilich et al., 1999; Vallejo et al., 2003; Verkerk et al., 2009; Latté et al., 2011). The basic structure of the glucosinolate molecule, and associated potential side chains, is shown in Fig. 1.

Glucoraphanin is hydrolyzed by myrosinase intrinsic to gut microflora (Shapiro et al., 1998; Conaway et al., 2000; Shapiro et al., 2006) to the respective isothiocyanate sulforaphane (Fig. 2). Some of the glucoraphanin in broccoli may also be digested by myrosinase present within the plant cells, whereby the enzyme is released upon chewing or mechanical processing (Fahey et al., 2001). Also, glucoraphanin is absorbed to some extent intact and undergoes enterohepatic circulation with subsequent hydrolysis to sulforaphane in the lower gut (Bheemreddy and Jeffery, 2007). Isothiocyanates, including sulforaphane, are absorbed from the small intestine and colon (Shapiro et al., 1998; Conaway et al., 2000; Fahey et al., 2001; Petri et al., 2003; Holst and Williamson, 2004). Sulforaphane itself is metabolized by the mercapturic acid pathway (Kolm et al., 1995; Kassahun et al., 1997; Conaway et al., 2000;

Abbreviations: 2-AA, 2-Aminoanthracene; 2-NF, 2-nitrofluorene; 9-AA, 9-aminoacridine; BSE, broccoli seed extract; bw, body weight; FDA, United States Food and Drug Administration; GSH, glutathione; GST, glutathione transferase; LD_{50}, median lethal dose; NOAEL, no-observable-adverse-effect level; PCE, polychromatic erythrocytes; RBC, red blood cells; WBC, white blood cells.

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http://dx.doi.org/10.1016/j.yrtph.2015.08.003
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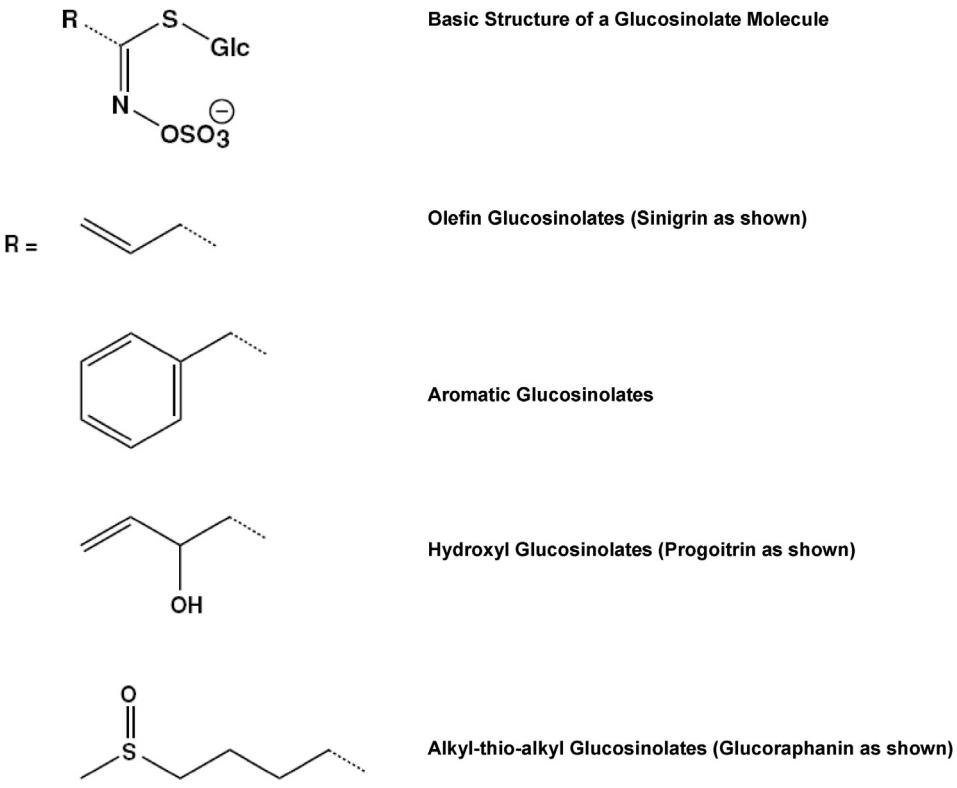

Fig. 1. Basic structures of broccoli-derived glucosinolate molecules.
Adapted from Halkier and Gershenzon (2006).

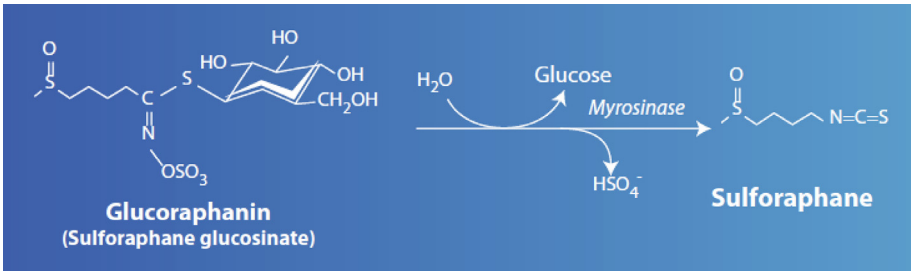

Fig. 2. Hydrolysis of glucoraphanin to sulforaphane.
Anonymous (2010).

Shapiro et al., 2006). Bioavailability and peak plasma concentrations of sulforaphane following oral consumption of broccoli are higher for fresh broccoli compared to cooked or steamed broccoli (Vermeulen et al., 2008).

Cruciferous vegetable consumption, specifically broccoli, has been associated with decreases in the risk of developing several chronic conditions, including various types of cancer (Kristal and Lampe, 2002; Kensler et al., 2005; Juge et al., 2007; Traka et al., 2008; Verkerk et al., 2009; Latté et al., 2011; Wu et al., 2013a,b). Broccoli also has been shown to possess anti-oxidant activity (Latté et al., 2011).

The apparent chemopreventive effect of broccoli and its extracts has been attributed in large part to the action of sulforaphane on both phase I and phase II metabolizing enzymes (Mahéo et al., 1997; Talalay and Fahey, 2001; Latté et al., 2011; Boddupalli et al., 2012; Dinkova-Kostova and Kostov, 2012; James et al., 2012; Veeranki et al., 2013). Phase I enzymes, such as the CYP family, are responsible for the oxidation of many compounds to more polar, easily excreted metabolites (Dauterman, 1994). However, for many chemicals shown to be either carcinogenic in animals or humans (Williams et al., 2007; Macherey and Dansette, 2008), this oxidative metabolism is associated with the production of reactive intermediates which are capable of damaging DNA and which act as the ultimate carcinogen (Levi, 1994; Delclos and Kadlubar, 1997; Dragan, 1997; Caldwell and Mills, 1999; Clayson and Kitchin, 1999; Williams et al., 2007; Macherey and Dansette, 2008). Sulforaphane has been shown to competitively inhibit, or reduce, the activity of several isoforms of CYP enzymes (*e.g.*, CYP1A1, CYP1A2, CYP2E1) (Clarke et al., 2008; Latté et al., 2011). Inhibition of these CYP enzymes can be an important mechanism, specifically for sulforaphane (Clarke et al., 2008), in the prevention of the formation of carcinogenic reactive intermediary metabolites (Higdon et al., 2007; Williams et al., 2007; Macherey and Dansette, 2008).

In addition to modulating the activity of CYP enzymes, sulforaphane has also been demonstrated in *in vitro* and *in vivo* to upregulate the activity of phase II detoxification enzymes, including NAD(P)H-quinone reductase and glutathione transferase (GST) (Talalay and Fahey, 2001; Cornblatt et al., 2007; Clarke et al., 2008;