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# Assessment of DNA damage and repair in adults consuming allyl isothiocyanate or *Brassica* vegetables☆,☆☆

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**Abstract**

Allyl isothiocyanate (AITC) is a dietary component with possible anticancer effects, though much information about AITC and cancer has been obtained from cell studies. To investigate the effect of AITC on DNA integrity *in vivo*, a crossover study was conducted. Adults ($n$=46) consumed AITC, AITC-rich vegetables [mustard and cabbage (M/C)] or a control treatment with a controlled diet for 10 days each. On day 11, volunteers provided blood and urine before and after consuming treatments. Volunteers were characterized for genotype for GSTM1 and GSTT1 (glutathione *S*-transferases) and XPD (DNA repair). DNA integrity in peripheral blood mononuclear cells was assessed by single-cell gel electrophoresis. Urine was analyzed for 8-oxo-7,8-dihydro-2'-deoxyguanosine (8-oxodG) and creatinine. Ten-day intake of neither AITC nor M/C resulted in statistically significant differences in DNA strand breaks [least squares mean (LSmean) % DNA in tail±S.E.M.: 4.8±0.6 for control, 5.7±0.7 for AITC, 5.3±0.6 for M/C] or urinary 8-oxodG (LSmean μg 8-oxodG/g creatinine±S.E.M.: 2.95±0.09 for control, 2.88±0.09 for AITC, 3.06±0.09 for M/C). Both AITC and M/C increased DNA strand breaks 3 h postconsumption (LSmean % DNA in tail±S.E.M.: 3.2±0.7 for control, 8.3±1.7 for AITC, 8.0±1.7 for M/C), and this difference disappeared at 6 h (4.2±0.9 for control, 5.7±1.2 for AITC, 5.5±1.2 for M/C). Genotypes for GSTM1, GSTT1 and XPD were not associated with treatment effects. In summary, DNA damage appeared to be induced in the short term by AITC and AITC-rich products, but that damage disappeared quickly, and neither AITC nor AITC-rich products affected DNA base excision repair.
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## 1. Introduction

Allyl isothiocyanate (AITC) is a dietary component with potentially important anticancer effects. AITC is derived from the glucosinolate sinigrin, which is found in some *Brassica* vegetables, including cabbage, mustard, brussels sprouts, kale and cauliflower. Isothiocyanates are produced from glucosinolates when plant material is injured, thus releasing the catalyzing enzyme myrosinase to convert glucosinolates to isothiocyanates. Also known as mustard oil, AITC is additionally found in the food supply as a food additive, giving a pungent flavor to mayonnaise products, horseradish spreads, salad dressings, and other spreads and sauces.

There is a substantial body of evidence suggesting that components of *Brassica* vegetables have anticarcinogenic activities. Epidemiological evidence suggests that inclusion of *Brassica* vegetables in the diet leads to lower cancer risk [1–9]. Animal studies have demonstrated that components of *Brassica* vegetables or their metabolites can inhibit tumor growth in transgenic or xenograft models [10–13], and animal and cell studies have suggested that intake of *Brassica* vegetables or components can positively alter physiologic processes associated with cancer, such as induction of phase II enzymes or apoptosis [14–22]. While there is much evidence that AITC has anticarcinogenic activity, much of the evidence is from *in vitro* investigations. Because *in vitro* models do not reflect *in vivo* digestion, metabolism, intracellular and extracellular exposure levels, certain aspects of physiological signaling, etc., it is important to complement *in vitro* studies with *in vivo* studies to gain a fuller understanding of potential for compounds to be anticarcinogenic.

Isothiocyanates are metabolized via the mercapturic acid pathway, being conjugated with glutathione by glutathione *S*-transferase [23]. Of the isoforms of glutathione *S*-transferase, GSTM1 is a

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particularly good catalyst for isothiocyanates [24,25]. It thus follows that individuals with a deletion in the gene for GSTM1 may have higher circulating concentrations of isothiocyanates than individuals with fully functional GSTM1 [26] (though this has not been clearly determined [27,28]), and genotype for GSTM1 may influence response to intake of *Brassica* vegetables or their components. Proteomic analysis has supported that GSTM1 genotype influences serum peptide response to *Brassica* vegetables [29]. In addition, because the gene for GSTT1 can also suffer from a deletion, this gene has also been of interest with respect to isothiocyanate metabolism and cancer risk [30–32]. Supporting the importance of GSTT1 genotype in response to *Brassica* vegetables, GSTT1 genotype has been found to influence the association between *Brassica* intake and risk of myocardial infarction [33].

To investigate the potential of dietary AITC to influence DNA integrity and repair, we have designed a human feeding study in which volunteers consumed AITC or vegetable products containing AITC, then provided blood and urine samples for assessment of DNA damage and repair. Mustard was chosen as a treatment food due to its high concentration of AITC. Cabbage was chosen as an additional treatment food because previous studies have shown that cabbage can contain high amounts of AITC-precursor sinigrin [34], though the actual sinigrin content of cabbage can be highly variable [34,35]. Study volunteers were evaluated for genotype for GSTM1 and GSTT1 since those genotypes have been associated with different responses to *Brassica* vegetables. In addition, because our primary outcome was DNA integrity and repair, volunteers were also evaluated for polymorphisms in the XPD gene, which codes for a DNA repair enzyme.

## 2. Materials and methods

### 2.1. Study design, diet and treatments

The study protocol was approved by the MedStar Health Research Institute (Hyattsville, MD, USA), and written, informed consent was obtained from each study subject. A three-period, three-treatment randomized crossover design was used. Each period lasted 11 days, and there was a 17-day washout between periods. The study was designed to test the effect of daily consumption of AITC or *Brassica* vegetables which provide AITC as well as the effect of an acute bolus dose. To test the effect of daily consumption of AITC or *Brassica* vegetables which provide AITC, volunteers consumed treatments for 10 days and provided fasting blood and urine samples on day 11. To test the acute effect of AITC or *Brassica* vegetables which provide AITC, volunteers consumed a bolus dose of treatment on the 11th morning of the study period and provided blood and urine samples at 3 and 6 h postconsumption. The basal diet consisted of adequate protein, approximately 35% of calories from fat, and three servings of fruits and vegetables per day to be in accord with average intakes in the United States [36,37]. The basal diet excluded foods from the Brassicaceae plant family such as broccoli, brussels sprouts, cabbage, cauliflower, kale, mustard, and wasabi, and prepared foods containing AITC.

The three treatments consisted of a control treatment, a mustard/cabbage (M/C) treatment and an AITC treatment. On days 1 through 10, participants consumed their dietary treatment with dinner. On the morning of day 11, participants consumed their treatment with a slice of bread and a slice of cheese. The control treatment was 20 g of a homemade AITC-free mayonnaise. The M/C treatment was 150 g of homogenized green cabbage (including 50 g of water), 30 g of Grey Poupon Country Dijon Mustard (Kraft Foods, Chicago, IL, USA) and 20 g of AITC-free mayonnaise. Prior to the start of the study, the cabbage was homogenized with a commercial blender at a ratio of 2 g of cabbage to 1 g of water and mixed into a single batch. Aliquots of 150 g were frozen at −20°C until used in the study. The AITC treatment consisted of 114.7 μmol per person of food-grade AITC (Sigma-Aldrich, St. Louis, MO, USA, product no. W203408) incorporated into the AITC-free mayonnaise. The quantity of AITC used in the AITC treatment was chosen to match that measured in the M/C dose prior to the start of the study.

Allyl isothiocyanate content in homogenized green cabbage and Grey Poupon Country Dijon Mustard was measured by a method adapted from Rouzaud et al. [38]. Two grams of homogenized green cabbage was combined with 4 ml of methylene chloride and 25 μl of 100 mM benzyl isothiocyanate as an internal standard in a 9-ml screw-top glass centrifuge tube. The homogenate was vortex-mixed for 5 s and centrifuged at 2500*g* for 5 min, and the methylene chloride extract was removed by Pasteur pipette to a 10-ml glass tube. Homogenates were extracted twice, and the extracts were combined. Extracts were concentrated to a volume of 0.8 ml under nitrogen gas and filtered by centrifugation with 0.2-μm nylon filters (Alltech Associates, Deerfield, IL, USA). Allyl isothiocyanate concentrations in the samples were measured by gas chromatography–mass spectrometry (GC-MS). The column was a DB-5MS capillary column (Agilent Technologies, Santa Clara, CA, USA; length 30 m, diameter 0.25 mm, film thickness 0.25 μm). Helium at 1.0 ml·min$^{-1}$ was used as the carrier gas. One microliter of extract was injected. The oven temperature was held at 55°C for 3 min and then increased by 6°C·min$^{-1}$ to 160°C. Allyl isothiocyanate was identified by comparison of mass spectra of sample peaks to the mass spectrum of an authentic standard (Sigma-Aldrich, product no. 377430). The response factor of AITC was measured under the same analytical conditions as the samples and was determined to be 2.59. Extraction of AITC from mustard was performed by combining 0.5 g of mustard, 4 ml of water, 4 ml of methylene chloride and 100 μl of 5 mM benzyl isothiocyanate. The samples were vortex-mixed for 5 s and centrifuged at 2500*g* for 5 min. The supernatant was transferred to a 10-ml glass tube, and the pellet was extracted with 3 ml of methylene chloride. The supernatants from the two extractions were then pooled, concentrated, filtered and measured by GC-MS in the same way as the cabbage homogenate. The AITC dose per person in the M/C treatment consisted of 114.39 μmol of AITC from the mustard and 0.34 μmol from the cabbage (114.7 μmol/d total). Allyl isothiocyanate concentrations in cabbage and mustard were also measured at the conclusion of the study, and the food-grade AITC was characterized by GC-MS.

Study participants consumed only foods provided by the Beltsville Human Nutrition Research Center (BHNRC). Breakfast and dinner on weekdays were consumed in the BHNRC dining room, and lunches and weekend meals were packed for carry-out. Participants were instructed to eat all foods and only foods provided to them, with the exception of coffee, tea and diet soda. Coffee and tea intake was limited to two cups per day, and intake of diet soda was not limited. Consumption of coffee, tea and soda was recorded by study participants. Study participants were asked to abstain from vitamin and mineral supplements beginning 2 weeks prior to the study and continuing for the duration of the study.

### 2.2. Study participants

Participants were recruited from the Beltsville, MD, area by advertisements in local newspapers. Exclusion criteria were pregnancy; lactation; a history of kidney, liver, gastrointestinal or metabolic disease; a history of cancer; tobacco use during the 6 months prior to the study; or the use of antibiotics or herbal supplements during the month prior to the study, and were determined by self-reporting by potential study participants. A prestudy medical screening assessed the general health of potential participants. Fifty-two participants, ages 40 to 79 years, were selected based on sex and *GSTM1*, *GSTT1* and *XPD* genotypes. Three study participants did not begin the study due to family illness or unknown reasons, two participants dropped from the study during

Table 1
Physical characteristics of study participants[a]

| | Participants who completed the study ($n$=46) | | Participants selected for the comet assay ($n$=20) | |
|---|---|---|---|---|
| | Mean | S.E.M. | Mean | S.E.M. |
| Age | 56.0 | 1.5 | 53.9 | 2.4 |
| BMI | 29.1 | 0.9 | 28.3 | 0.9 |
| Sex | Number | % of Total | Number | % of Total |
| Female | 34 | 73.9 | 12 | 60 |
| Male | 12 | 26.1 | 8 | 40 |
| Ethnicity | | | | |
| African–American | 14 | 30.4 | 5 | 25 |
| Asian | 1 | 2.2 | 1 | 5 |
| Caucasian | 30 | 65.2 | 14 | 70 |
| Hispanic | 1 | 2.2 | 0 | 0 |
| Genotype | | | | |
| *XPD* Codon 312 | | | | |
| Asn/Asn | 4 | 8.7 | 4 | 20 |
| Asp/Asn | 22 | 47.8 | 14 | 70 |
| Asp/Asp | 20 | 43.5 | 2 | 10 |
| *XPD* Codon 751 | | | | |
| Gln/Gln | 9 | 19.6 | 7 | 35 |
| Lys/Gln | 26 | 56.5 | 13 | 65 |
| Lys/Lys | 11 | 23.9 | 0 | 0 |
| *GSTM1* | | | | |
| *GSTM1-null* | 19 | 41.3 | 9 | 45 |
| *GSTM1+* | 27 | 58.7 | 11 | 55 |
| *GSTT1* | | | | |
| *GSTT1-null* | 9 | 19.6 | 1 | 5 |
| *GSTT1+* | 37 | 80.4 | 19 | 95 |

BMI, body mass index.

[a] Forty-six participants completed the study. For 20 of those participants, PBMCs were collected on the final day of each treatment period immediately before treatment consumption and at 3 and 6 h after consumption, and PBMC samples were subsequently assessed for DNA strand breaks by the comet assay.