Lifespan Is Prolonged in Autoimmune-Prone (NZB/NZW) F1 Mice Fed a Diet Supplemented with Indole-3-Carbinol

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ABSTRACT Dietary modulation has the potential to prevent or ameliorate systemic lupus erythematosus (SLE). Indole-3-carbinol (I3C), which is abundant in cruciferous vegetables, was evaluated in a murine model of SLE because of its antiestrogenic activities. Female (NZB × NZW) F1 mice, which develop SLE, were fed an AIN76A diet without or with 0.2 g/kg I3C, starting soon after weaning or at 5 mo of age. At 12 mo of age, 80% of mice fed the I3C-supplemented diet soon after weaning were alive compared with only 10% of controls. When experimental diets were initiated at 5 mo of age, 100% of I3C-fed mice and 30% of controls were alive at 12 mo of age. Anti-double-stranded DNA (dsDNA) antibodies developed in all groups, although at several time points, the levels produced in I3C-fed mice were significantly lower. Renal disease (proteinuria, histologic changes, IgG immune complex deposition, subepithelial deposits and diffuse epithelial cell foot process effacement) was more severe in controls. When experimenta diets were initiated at 5 mo of age, whereas males die at 15–20 mo (11). Administration of estradiol to mice expressing a transgene for the heavy chain of an anti-DNA antibody induces a lupus-like phenotype (12). Because indole-3-carbinol (I3C), which is abundant in cruciferous vegetables, is an antiestrogen, we hypothesized that it might prevent, delay or even represent an adjunct treatment for SLE. I3C shifts estrogen metabolism toward less estrogenic metabolites (13) and is a negative regulator of signal that is dependent on the estrogen receptor (14). Many genes activated by estrogen are abrogated by diindolylmethane, a condensation product of I3C (15). We evaluated the effect of a supplement of I3C, at a level obtainable from diet (16), on the outcome of SLE in lupus-prone (NZB × NZW) F1 mice.

MATERIALS AND METHODS

Mice and diets. Female NZB/NZW F1 mice were obtained from the Jackson Laboratory (Bar Harbor, ME). AIN76A diets (17) ± 0.2 g/kg I3C (Sigma, St. Louis, MO) were prepared by Ziegler (Gardner, PA). The AIN76A diet supplies a total of 18.5 MJ/kg with 22% of energy from protein, 11% from fat and 67% from carbohydrates. The nonpurified diet was Harlan Teklad Rodent Chow (Madison, WI). Two feeding protocols were evaluated in parallel with identical sample collections. Early start mice (n = 20) were fed AIN76A diet ± I3C from 1.2 mo of age to determine whether I3C would prevent SLE. Late start mice (n = 20) were fed the nonpurified diet for 5 mo and then fed the AIN76A diet ± I3C, to determine whether I3C ameliorated SLE. Mice consumed their diets ad libitum. Consumption was monitored to ensure comparable intake. Serum, obtained monthly by orbital bleeding, was evaluated for anti-double-stranded DNA (dsDNA) antibodies. Urine samples were collected and evaluated for proteinuria each month early in life and more frequently later. Urine collected at 7 mo was used to measure estrogen metabolites. A subset of mice was killed at 12.5 mo of age, and kidneys removed for evaluation. Lifespan was determined by excessive proteinuria (≥5 g/L); mice were killed by CO2 overdose or unanticipated death. A follow-up study was done with mice (n = 18) fed the AIN76A diet ± I3C from 5 mo and killed at 12–13 mo for kidney evaluation. The study was approved by the Institutional Animal Care and Use Committee at North Shore University Hospital.

Anti-dsDNA antibodies. The ELISA assay for dsDNA antibody was performed as described previously (18). Aliquots (100 µL) of 1:100 diluted serum were incubated overnight at 4°C on high binding.
Dietary indole-3-carbinol (I3C) extends lifespan in NZB × NZW F1 mice (Fig. 1). Control mice began to die at 8 mo. For the early start mice, survival was longer for those fed I3C (*P < 0.0008). At 12 mo, 10% of controls and 80% of I3C-fed mice were alive. Survival for late start I3C-fed mice was also longer (*P < 0.0011). At 12 mo, 30% of controls and 100% of I3C-fed mice were alive. Survival of mice in the early start and late start protocols did not differ. A set of I3C-fed mice lived to 20.5 mo of age.

Effect of indole-3-carbinol (I3C) on anti-double stranded DNA antibody in (NZB × NZW) F1 mice fed an AIN76A diet without or with 0.2 g/kg I3C, starting soon after weaning (left panel) or at 5 mo of age (right panel). Values are means ± SEM of anti-double-stranded DNA antibody titers determined on all surviving mice. Early and late start of diets were done in parallel. Different from I3C at that time, *P < 0.05.

Renal pathology. 13C delayed pathological deterioration of kidneys. Proteinuria was lower in I3C-treated mice, regardless of the time treatment was initiated. An estimated 300 mg/L of protein was detected in urine in almost all mice as early as 7 mo of age (not shown). However, the number of mice with 5 g/L of proteinuria rose more quickly in the controls than with either I3C-fed group (Fig. 3). Morphologically, there was less severe renal damage between 12 and 13 mo of age in mice fed I3C (Fig. 4). Glomeruli of the I3C groups were generally similar to those of normal nonlupus-prone BALB/c mice. In the untreated (NZB × NZW) F1 mice, histological changes included thickening of glomerular capillary loops, nodular expansion of the mesangium and tubular casts. IgG staining was barely evident in normal BALB/c mouse kidneys, weak in I3C-treated mice, but glomerular staining for IgG was weaker in I3C-treated mice, but glomerular staining for IgG was
intense in untreated (NZB × NZW) F1 controls. Semiquantitative results (Table 1) indicated that the difference in severity of kidney disease (glomerulonephritis and interstitial nephritis) was significantly less in I3C-fed mice. In EM studies (Fig. 5, Tables 1 and 2), untreated controls had more extensive glomerular lesions with increased immune complex deposition (granular electron dense deposits) compared with I3C groups. Large subepithelial deposits with spikes and diffuse effacement of visceral epithelial cell foot processes were readily evident in untreated groups but only occasionally seen in the I3C-fed mice.

**Estrogen metabolism.** The ratio of 2-OHE to 16α-OHE in urine at 7 mo of age, was greater than in controls in I3C-treated mice given I3C early (P < 0.002) and later (P < 0.04) in life (Fig. 6), consistent with less estrogenic activity in mice fed I3C.

**DISCUSSION**

Our results indicate that I3C, as a dietary supplement, dramatically increases lifespan of lupus-prone (NZB × NZW) F1 mice. I3C is effective when initiated early, before onset of disease, or later when the disease develops. The findings support the view that I3C may benefit persons at risk for SLE as well as those in the early stages of disease. Therefore, I3C may

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**TABLE 1**

<table>
<thead>
<tr>
<th>Score</th>
<th>Glomerulonephritis</th>
<th>Interstitial nephritis</th>
<th>Foot process effacement</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control I3C</td>
<td>Control I3C*</td>
<td>Control I3C*</td>
</tr>
<tr>
<td>n</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤1+</td>
<td>1</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>2+</td>
<td>0</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>3+</td>
<td>8</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>4+</td>
<td>1</td>
<td>0</td>
<td>3</td>
</tr>
</tbody>
</table>

1 Values show numbers of mice devoid of disease (≤1+) to having severe disease (4+), *P < 0.05 comparing I3C and control between moderate (1–2+) and severe (3–4+) using χ² analysis.

2 Sections were stained for evaluation by hematoxylin and eosin.

3 Sections were evaluated by electron microscopy.

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**TABLE 2**

<table>
<thead>
<tr>
<th>Site of deposits</th>
<th>Control</th>
<th>I3C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mesangium</td>
<td>8</td>
<td>5</td>
</tr>
<tr>
<td>Subepithelial, scattered</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Subepithelial, many, with spikes</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>Intramembranous</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>Subendothelial</td>
<td>1</td>
<td>0</td>
</tr>
</tbody>
</table>

1 Values represent the number of mice out of 8 with electron-dense deposits determined by electron microscopy.
allow a decrease in the dose of immunosuppressive drugs required to treat SLE, reduce toxic side effects and be of value for the prevention of recurrences.

Our findings suggest that I3C suppresses an autoimmune inflammatory response, delaying renal failure due to glomerulonephritis. It is difficult to evaluate precisely the effects of I3C on the development of anti-dsDNA antibodies. The mode by which I3C affects the immune system in SLE may be complex and involve actions other than direct antibody suppression. Tamoxifen, like I3C, often behaves as an antiestrogen, decreasing cytokines and suppressing renal damage in autoimmune (NZB × NZW) F1 and MRL-lpr/lpr mice (21, 22).

Estrogen metabolism was clearly altered in our I3C-treated mice. The ratio of 2-OHE to 16α-OHE was increased by I3C, a result consistent with less estrogenic activity. 16α-OHE binds covalently to the estrogen receptor (23) and has a prolonged estrogenic effect (24), whereas 2-OHE is not estrogenic (25). Lupus patients have decreased 2-hydroxylation (26) and increased 2-OHE/16α-OHE (27) as well as increased levels of urinary estrogen metabolites after oral indole-3-carbinol treatment in humans. J. Natl. Cancer Inst. 89: 718–723.


Figure 6: Indole-3-carbinol (I3C) increases the ratio of 2α:16α-estrone metabolites in urine in (NZB × NZW) F1 mice fed an AIN76A diet without or with 0.2 g/kg I3C, starting soon after weaning (left panel) or at 5 mo of age (right panel). Values show the ratio of 2-hydroxyestrone (2-OHE) to 16α-hydroxyestrone (16α-OHE) ± SEM in urine collected at 7 mo for all mice (n = 10). *Different from I3C at that time, P < 0.05.

ACKNOWLEDGMENTS

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LITERATURE CITED