Inhibition of ebselen on aflatoxin B$_1$-induced hepatocarcinogenesis in Fischer 344 rats

Cheng-Feng Yang, Jin Liu, Shanthi Wasser$^1$, Han-Ming Shen, Carolyn Eng-Looi Tan$^1$ and Choon-Nam Ong$^2$

Department of Community, Occupational and Family Medicine, National University of Singapore and $^1$Department of Pediatric Surgery, KK Women’s and Children’s Hospital, Singapore
$^2$To whom correspondence should be addressed
Email: cofongcn@nus.edu.sg

Aflatoxin B$_1$ (AFB$_1$), a potent hepatocarcinogen, enhances ROS formation and causes oxidative DNA damage, which may play a role in its carcinogenicity. We have demonstrated recently that ebselen, an organic selenium compound, protects against the cytotoxicity of AFB$_1$ through its antioxidant capability. The present study was designed to investigate the effect of ebselen on AFB$_1$-induced hepatocarcinogenesis in an animal model. Fischer 344 rats were first treated with either deionized water or ebselen (5 mg/kg, 5 days/week) via gavage for 4 weeks, then given AFB$_1$ (0.4 mg/kg, gavage, once a week) or AFB$_1$ plus ebselen (5 mg/kg, 5 days/week) for another 24 weeks. The results showed that the hepatocarcinogenicity of AFB$_1$ in rats was significantly reduced by ebselen treatment as indicated by a decrease in: (i) serum γ-glutamyl transpeptidase activity; (ii) expression of mRNAs of liver α-fetoprotein and the placental form of glutathione S-transferase (GST-P); and (iii) the area and mean density of staining of liver GST-P foci. Ebselen treatment significantly reduced the formation of hepatic AFB$_1$–DNA adducts and 8-hydroxydeoxyguanosine caused by AFB$_1$ exposure. These findings suggest that ebselen can inhibit the carcinogenicity of AFB$_1$. In addition to the reduction of AFB$_1$–DNA adduct formation, the protective effect of ebselen against AFB$_1$-induced oxidative DNA damage may also, at least in part, contribute to its anticarcinogenic property.

Introduction

Aflatoxin B$_1$ (AFB$_1$) is a potent hepatocarcinogen in experimental animals and an etiological factor in human liver cancer (1,2). It is generally believed that AFB$_1$ is activated mainly by cellular cytochrome P450 (CYP450) to form the reactive intermediate, AFB$_1$-8,9-epoxide (AFBO). The subsequent covalent binding of this epoxide to DNA to generate AFB$_1$–DNA adducts is considered to be a critical step in the carcinogenicity of AFB$_1$ (3,4). AFB$_1$ also induces formation of reactive oxygen species (ROS) (5), lipid peroxidation (6,7) and formation of 8-hydroxydeoxyguanosine (8-OHdG) in vivo and in vitro (8,9). Given the importance of oxidative stress in chemically induced carcinogenesis (10,11), it is reasonable to assume that AFB$_1$-induced ROS formation and oxidative DNA damage may, in addition to the formation of AFB$_1$–DNA adducts, also have an important role in AFB$_1$ carcinogenicity.

Ebselen [2-phenyl-1,2-benziselenazol-3(2H)-one], a synthetic seleno-organic compound, has strong antioxidant and anti-inflammatory properties with very low toxicity (12). It is now under active investigation as a neuroprotective agent in clinical trials (12). Although its antioxidant capabilities may play a role in its carcinogenicity, we have demonstrated that ebselen inhibited 12-O-tetradecanoylphorbol-13-acetate (TPA)-induced down-regulation in gap-junctional intercellular communication (GJIC) (13). Reduction in GJIC has been proposed to be involved in the development of a number of pathogeneses, especially carcinogenesis (14). We recently demonstrated that ebselen is potent in protecting against ROS-induced cytotoxicity and DNA damage in HepG$_2$ cells (15). Ebselen also protects against the cytotoxicity of AFB$_1$ via its strong ability to scavenge intracellular ROS and preventing oxidative damage (16). The present study was designed to study the effect of ebselen on AFB$_1$-induced liver carcinogenesis in Fischer 344 rats.

Materials and methods

Chemicals

AFB$_1$, DMSO, 8-OHdG, deoxyguanosine (dG), DNsA I, RNAsA, nuleasA P$_1$ and alkaline phosphatase were all from Sigma (St Louis, MO). The 2,3-dihydro-2-(N$^5$-formyl-2,5,6-triamino-4-oxopyrimidin-5-yl)-3-hydroxy-AFB$_1$ (AFB$_1$–FAPY) standard was kindly given by Dr D.Hsieh (University of California at Davis, USA). Ebselen was generously given by Rhône–Poulenc Rorer (France). Trizol reagent, Superscript RNase H–reverse transcriptase and reverse transcriptase–polymerase chain reaction.

Animals and treatments

Male Fisher 344 rats (140–180 g body wt) were obtained from the animal center at the National University of Singapore. All animals received humane care, in compliance with the university’s and NIH’s guidelines. They were housed in plastic cages with a 12 h light–12 h dark cycle at room temperature and allowed free access to tap water and animal feed (Glen Forrest, Stockfeeders, Glen Forrest, Australia). After a 5 day acclimatization period, 40 rats were randomly assigned into four groups according to their body weight.

Figure 1 illustrates the design of the experiment, which lasted for 28 weeks. In the control group (group I) and the group given ebselen only (group II), rats were gavaged with deionized water or with ebselen (5 mg/kg body wt/day; 5 days/week), respectively, throughout the experiment. In the groups given AFB$_1$ alone (group III) or together with ebselen (group IV), animals were first given deionized water or ebselen (5 mg/kg body wt/day; 5 days/week), respectively, via gavage for 4 weeks followed by administration of AFB$_1$ (0.4 mg/kg body wt, once a week by gavage) (group III) or ebselen plus AFB$_1$ (group IV) for another 24 weeks. The general condition of each

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Blood collection and tissue preparation

The concentration of DMSO given to ebselen- and/or AFB 1-treated rats. Staining of the foci. While the GST-P staining was localized in altered foci in the rat was monitored daily and the body weight was recorded weekly throughout the experiment. Ebselen and AFB 1 were dissolved in DMSO and diluted with deionized water to the required concentrations just before gavage. An equal amount of DMSO was given to control animals during the gavage to balance the percentage of section area occupied by the foci and the mean density of the GST-P foci were assayed by an immunohistochemical method using a344 rats. Group I (control): rats were treated with deionized water via gavage; group II (ebselen control), rats were treated with ebselen (5 mg/kg body wt/day; 5 days/week) via gavage; group III (AFB 1 treatment only), rats were first gavaged with deionized water for 4 weeks, then treated with AFB 1 (0.4 mg/kg body wt, once a week) via gavage for another 24 weeks; and group IV (ebselen plus AFB 1 treatment), rats were first treated with ebselen (5 mg/kg body wt/day; 5 days/week by gavage) for 4 weeks, followed by administering AFB 1 (0.4 mg/kg body weight, once a week by gavage) and ebselen for another 24 weeks.

The system was able to detect the number of GST-P foci per cm² section, the number of discrete foci. Instead, the majority of remaining AFB 1 DNA adducts in rat liver 24 h after AFB 1 and cut

Liver tissue was homogenized in 0.25 M sucrose, 50 mM Tris buffer (pH 7.4) and midazolan (F. Hoffmann-La Roche, Basel, Switzerland). Midline laparotomy of each rat was carried out, a blood sample was collected for isolation of serum and the liver was quickly removed, washed and weighed. Liver tissue was homogenized in 0.25 M sucrose, 50 mM Tris buffer (pH 7.4) for DNA extraction. For RNA analysis, liver sections were harvested into Trizol solution, frozen in liquid nitrogen and stored at –80°C until analysis. Liver tissues were also fixed in buffered 4% paraformaldehyde for paraffin sectioning.

Measurement of serum alanine aminotransferase and γ-glutamyl transpeptidase activity

Increased serum alanine aminotransferase (ALT) activity is a general index reflecting liver cell damage (6), while increased serum γ-glutamyl transpeptidase (GGT) activity is an indicator of the carcinogenicity of AFB 1 (17). Activities of ALT and GGT in rat serum were measured using an Abbott VP Biochemical Analyzer with the appropriate test kits (Abbott Laboratory, Chicago, IL).

Analysis of AFP and GST-P transcripts in rat liver

AFP and GST-P are widely used as biomarkers of hepatocellular carcinoma (HCC) and have been used to evaluate various carcinogens including AFB 1-induced hepatocarcinogenesis (18,19). In the present study, the expression of AFP and GST-P mRNA in rat liver was investigated by reverse transcription–polymerase chain reaction (RT–PCR). Briefly, total RNA was extracted using Trizol solution and treated with DNase I (MessageClean kit) to remove contaminating genomic DNA from the RNA samples. Reverse transcription reactions were carried out using Superscript RNase H reverse transcriptase according to the manufacturer’s instructions. AFP-specific fragments were amplified by 38 cycles of PCR, each cycle comprising 30 s at 95°C, 30 s at 57.6°C and 30 s at 72°C. GST-P-specific fragments were amplified by 27 cycles of 30 s at 95°C, 30 s at 58°C and 30 s at 72°C. An excess of dNTPs (200 μM each dNTP, PCR primers (100 pmol) and HotStar Taq polymerase (2.0 units per 20 μl) was used to ensure that these were not limiting factors in the PCR analysis. All PCR reactions were carried out in the linear, quantitative phase of the amplification process, which was determined in preliminary experiments. The levels of mRNA expression of AFP and GST-P transcripts were normalized to that of the constitutively expressed housekeeping gene, glyceraldehyde-3-phosphate dehydrogenase (G3PDH), mRNA in the same tissue sample (20). The sequences of the PCR primers were as follows: AFP upper primer: 5′-CAAAAGCCGGTTGGAAAGCACA-3′; AFP lower primer: 5′-GCCGAGCTGCTGTTGAGTGATG-3′; GST-P upper primer: 5′-GCCAGCTTCTGA- GTCCAAGCAGC-3′; GST-P lower primer: 5′-GCAGCGTGGTTTCTCTGGATGCT- ATCC-3′ (this paper; based on accession no. X02904); G3PDH upper primer: 5′-CCTCTAATGATCCCTCAACTACAGG-3′; G3PDH lower primer: 5′-CATGTTGGTGAAGAGCCCGAC-3′ (20).

The PCR products were size fractionated on 1.2% agarose gels, visualized by ethidium bromide staining and photographed. The photographs of the gels were scanned with a GS-700 imaging densitometer (Bio-Rad, Hercules, CA). The densities of the bands produced by the PCR products were quantified using Molecular Analyst software (Bio-Rad). In order to compare mRNA levels between different groups, the transcripts were normalized against the corresponding control G3PDH transcript with matched PCR conditions. The identities of the PCR products were confirmed by sequence analysis.

Immunohistochemical analysis of GST-P foci in rat liver tissue

Histochemical detection of GST-P foci in rat liver is commonly used as a marker for AFB 1-induced hepatocarcinogenesis (21). In the present study, GST-P foci were assayed by an immunohistochemical method using a streptavidin–biotin–peroxidase complex (ABC) as described by Hsu et al. (22). Briefly, liver slices (3–5 mm thick) were embedded in paraffin and cut into 5 μm thick sections. After being deparaffinized with xylene, quenched with hydrogen peroxide and blocked with normal serum, the liver tissue sections were incubated with primary antibody (mouse anti-GST-P; Transduction Laboratories, Lexington, KY) and then with secondary antibody (anti-mouse Ig-G; Vector Laboratories, Burlingame, CA). The tissue sections were then incubated with ABC complex (ABC peroxidase staining kit; Vector Laboratories), stained with DAB substrate (DAB substrate kit; Vector Laboratories) and counterstained with hematoxylin.

Staining of GST-P foci in liver tissue section was analyzed and quantified using an image analyzer (KS 300; Carl Zeiss, Hamberg, Germany). The system was able to detect the number of GST-P foci per cm² section, the percentage of section area occupied by the foci and the mean density of staining of the foci. While the GST-P staining was localized in altered foci in the rat treated with ebselen and AFB 1 together (group IV), much of the liver was stained positive for GST-P in the rat treated with AFB 1 only (group III), making it inappropriate to count the number of discrete foci. Instead, the percentage of section area occupied by the foci and the mean density of the foci staining were analyzed.

Measurement of AFB 1–DNA adducts in rat liver

Early studies have demonstrated that the initial major form of AFB 1–DNA adduct is AFB 1–N ²-Gua (1,23). However, AFB 1–N ²-Gua is not stable and the majority of remaining AFB 1–DNA adducts in rat liver 24 h after AFB 1 treatment is an imidazole ring-opened formamidopyrimidine (AFB 1–FAPY) derivative (1,24). In the present study, AFB 1–FAPY was liberated from hepatic DNA using acidic conditions and determined using an HPLC method as previously described (25). Briefly, DNA was extracted using chloroform without phenol as described earlier (8). Five hundred micrograms of DNA in 10 mM Tris–HCl was hydrolyzed in 0.3 N HCl at 100°C for 2 h and the hydrolysate was applied to a C 18 Sep-Pak cartridge. The cartridge was washed with 0.05 N acetic acid and AFB 1–DNA adducts were eluted with methanol. The eluent was analyzed by HPLC with a Whatman Partisphere C 18 column, monitored using a Shimadzu fluorescence detector (excitation, 360 nm; emission, 425 nm). The standard AFB 1–FAPY was treated in exactly the same manner as the DNA samples; results are expressed as peak heights.

Determination of 8-OHdG formation in rat liver

Formation of 8-OHdG in rat liver DNA was determined using HPLC linked to an electrochemical detector (ECD) as previously described (8). Briefly, 200 μg DNA was denatured and then digested by incubation with DNase I, nuclease P1 or alkaline phosphatase. The digested DNA was dissolved in deionized water and 8-OHdG was determined by HPLC, which consisted of a Waters 600E pump, a Whatman Partisphere C 18 column and a UV detector (Hewlett-Packard 1050, λ 254 nm) connected to an ECD (Hewlett-Packard 1049A) in series for monitoring dG and 8-OHdG, respectively. The results are expressed as 8-OHdG/10² dG.
The body weight of rats in all four groups increased throughout the experiment; no obvious changes were detected in any group. The general condition of the rats was monitored daily through the period of the experiment.

### Results

#### General effects of ebselen and AFB<sub>1</sub> treatment

The general condition of the rats was monitored daily throughout the experiment; no obvious changes were detected in any group. The body weight of rats in all four groups increased significantly during the experimental period. Neither ebselen nor AFB<sub>1</sub> had any obvious effect on rat body weight gain. There was no significant difference in mean body weight between the four groups throughout the experiment (data not shown).

#### Effect of ebselen on AFB<sub>1</sub>-induced enzyme activity in rat serum

Serum ALT activity in rats treated with ebselen (5 mg/kg body wt) (group II) for 28 weeks was not significantly different from that in control animals (group I) (Table I). GGT activity was not detected in control or ebselen-treated rats (Table I). Serum ALT and GGT activity in rats treated with AFB<sub>1</sub> (0.4 mg/kg body wt) (group III) for 24 weeks was significantly higher than that of control animals (group I) (Table I). Serum ALT and GGT activities in rats treated with ebselen and AFB<sub>1</sub> together (group IV), however, were significantly lower than those in animals treated with AFB<sub>1</sub> alone (group III) (Table I), suggesting that ebselen treatment could reduce AFB<sub>1</sub>-induced rat liver damage and carcinogenesis.

#### Effect of ebselen on AFB<sub>1</sub>-induced expression of AFP mRNA

Expression of the AFP transcript in rat liver was investigated by RT–PCR; the results are shown in Figure 2. PCR amplification of fragments specific for AFP yielded a fragment of 588 bp (Figure 2a). The values from densitometric analysis, after normalization against the corresponding G3PDH transcript (209 bp), were expressed as the AFP:G3PDH ratio (Figure 2b). It was found that while both control (group I) and ebselen-treated (group II) rat livers expressed AFP transcripts at low levels, treatment with AFB<sub>1</sub> (group III) led to a 93.2% increase in AFP mRNA level (Figure 2). AFP mRNA expression in rats treated with ebselen and AFB<sub>1</sub> together (group IV) was 47.1% lower than that in rats treated with AFB<sub>1</sub> alone (group III) (Figure 2). These results indicate that treatment with ebselen significantly reduced the level of expression of AFP transcript in AFB<sub>1</sub>-treated rat livers.

#### Effect of ebselen on AFB<sub>1</sub>-induced expression of GST-P mRNA in rat liver and formation of GST-P foci

The effect of ebselen treatment on AFB<sub>1</sub>-induced GST-P in rat liver was investigated at both the mRNA level (by RT–PCR; Figure 3) and the protein level (by immunohistochemical analysis; Figure 4). PCR amplification of fragments specific to GST-P yielded a product of 409 bp (Figure 3a). No significant difference in GST-P mRNA expression was observed in control (group I) and ebselen-treated (group II) animal livers (Figure 3b). The expression of GST-P mRNA in AFB<sub>1</sub>-treated rat livers (group III), however, was 50.5% higher than that in control animals (group I) (Figure 3). Treatment with ebselen and AFB<sub>1</sub> together (group IV), on the other hand, resulted in a marked reduction (44.7%) in the amount of AFB<sub>1</sub>-induced GST-P transcript.

### Table I. Effect of treatment with ebselen and AFB<sub>1</sub> on the activities (mean ± SD) of rat serum ALT and GGT

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Number of rats</th>
<th>Serum ALT (IU/l)</th>
<th>Serum GGT (IU/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>control</td>
<td>8</td>
<td>73.5 ± 13.9</td>
<td>0</td>
</tr>
<tr>
<td>II</td>
<td>ebselen</td>
<td>10</td>
<td>67.4 ± 10.9</td>
<td>0</td>
</tr>
<tr>
<td>III</td>
<td>AFB&lt;sub&gt;1&lt;/sub&gt;</td>
<td>10</td>
<td>91.3 ± 10.7*</td>
<td>3.5 ± 1.4</td>
</tr>
<tr>
<td>IV</td>
<td>ebselen + AFB&lt;sub&gt;1&lt;/sub&gt;</td>
<td>10</td>
<td>80.9 ± 3.1†</td>
<td>1.3 ± 0.5†</td>
</tr>
</tbody>
</table>

*Significantly different from control (group I) (P < 0.05; one-way ANOVA with Scheffe’s test).
†Significantly different from treatment with AFB<sub>1</sub> alone (group III) (P < 0.05; Student’s t-test).

Statistical analysis

Data are presented as means ± SD. The differences between groups were analyzed using Student’s t-test or one-way analysis of variance (ANOVA) with Scheffe’s test. A P value of < 0.05 was considered statistically significant.
and liver immunohistochemical detection of GST-P foci (Figure 2); (iii) expression GST-P mRNA in liver (by 44.7%) (Table I); and (iv) the area and mean density of staining of GST-P foci in the liver (by 51.6% and 24.0%, respectively) (Figure 4 and Table II).

It is well known that AFB1 is an indirectly acting carcinogen and that it is activated primarily by CYP450. The binding of its reactive metabolite, AFBO, to DNA to form AFB1–DNA adducts accounts for its carcinogenicity, whereas conjugation of AFBO with glutathione results in detoxification of this potent carcinogen (3,4). As AFB1 poses a great health risk in many parts of the world, a great deal of effort has been made to investigate the possible effects of various chemical agents against AFB1 carcinogenicity (26). Many chemopreventive agents have been demonstrated to reduce AFB1–DNA adduct formation via inhibition on CYP450-mediated AFB1 activation and/or enhancing its detoxification through the conjugation with GSH catalyzed by cytosolic α-class GSTs (26,27). For instance, high concentrations of phenolic antioxidants [butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT)] can prevent AFB1 carcinogenicity via induction of GST activity (28). Oltipraz, a substituted dithiolethione, efficiently reduces the level of AFB1–DNA adducts by inhibiting AFB1 activation and strongly inducing GST enzymes (29).

In order to elucidate the possible mechanisms involved in the inhibition of ebselen on AFB1-induced hepatic preneoplastic alterations, we first examined the effect of ebselen treatment on the formation of AFB1–DNA adducts in rat liver. Ebselen treatment significantly inhibited hepatic formation of AFB1–DNA adducts (Table III). It has been shown previously that ebselen inhibits mouse liver microsomal NADPH-dependent CYP450 reductase and converts rat liver microsomal CYP450 to CYP420 in cell-free systems via interaction with sulphhydryl groups of proteins (30,31). In vivo, however, ebselen is unlikely to inhibit microsomal CYP450, because most of the administered ebselen would combine with serum proteins (albumin) and intracellular thiols (glutathione) before reaching CYP450 located in the endoplasmic reticulum (30,32). Indeed, in mice fed a diet containing ebselen (200 mg/kg) for 1 week, there was no effect on liver microsomal NADPH-dependent CYP450 reductase activity, ethoxyccumarin-O-deethylation rate or the amounts of CYP450 and cytochrome b5 (30). Also, a much higher oral dose of ebselen (500 mg/kg body wt) does not inhibit liver metabolism of the xenobiotics in mice (33). Although changes in AFB1 metabolism by CYP450 were not studied in the present study, it is believed that oral administration of ebselen (5 mg/kg body wt) is not likely to affect the liver microsomal metabolism of AFB1 significantly. Therefore, it seems that the inhibition by ebselen of hepatic AFB1–DNA adduct formation may not be due to modulation by ebselen of AFB1 activation by CYP450. Although no direct evidence is available at present, it is believed that ebselen treatment might affect the AFB1 detoxification pathway catalyzed by GSTs, thus reducing the level of hepatic AFB1–DNA adducts. Further study is needed to clarify the possible mechanisms responsible.
Table II. Inhibition by ebselen of AFB1-induced liver GST-P foci in Fischer 344 rats

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Number of rats</th>
<th>Liver GST-P foci (mean ± SD)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Section area occupied by the foci (%)</td>
<td>Mean density of foci staining (relative units)</td>
</tr>
<tr>
<td>I</td>
<td>control</td>
<td>10</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>II</td>
<td>ebselen</td>
<td>10</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>III</td>
<td>AFB1</td>
<td>10</td>
<td>13 ± 3.5</td>
<td>64 ± 19.2</td>
</tr>
<tr>
<td>IV</td>
<td>ebselen + AFB1</td>
<td>10</td>
<td>6 ± 1.9*</td>
<td>49 ± 8.2*</td>
</tr>
</tbody>
</table>

*Significantly different from treatment with AFB1 alone (group III) (P < 0.05; Student’s t-test).

Fig. 5. Effect of treatment with ebselen and AFB1 on the level of 8-OHdG in rat liver DNA. Data are presented as mean ± SD (n = 7 or 8). *Significantly different from control (group I) (P < 0.05, one-way ANOVA with Scheffe’s test); #significantly different from treatment with AFB1 alone (group III) (P < 0.05; one-way ANOVA with Scheffe’s test).

for the inhibition of ebselen on hepatic AFB1–DNA adduct formation.

There are some discrepancies in studies of the relationship between the level of hepatic AFB1–DNA adducts and ability to protect against AFB1 carcinogenicity. For example, a recent study indicated that dietary administration of β-carotene had no obvious effect on the formation of AFB1–DNA adducts in rat liver, but reduced AFB1-induced liver GST-P foci as efficiently as other carotenoids that dramatically inhibited the formation of hepatic AFB1–DNA adducts (34). In addition, as mentioned above, the protection by high concentrations of BHA and BHT against AFB1 carcinogenicity results from the
inhibition of AFB1–DNA adduct formation through their strong induction of GST activity. Williams and Iatropoulos (35), however, showed that low concentrations of BHA and BHT, which are not able to induce GST activity, still significantly inhibit hepatocarcinogenesis of AFB1 in rats. This evidence thus suggests that there may be other mechanisms responsible for AFB1 carcinogenicity besides formation of AFB1–DNA adducts. It will be of interest to explore further other mechanisms that might be involved in the protective effect of ebselen against AFB1 carcinogenicity.

The role of carcinogen-induced ROS and oxidative DNA damage in carcinogenesis has attracted much attention. It is now widely believed that oxidative DNA damage is implicated in the development of cancer (10,11,36). There is a large body of experimental evidence suggesting that 8-OHdG, a key biomarker of oxidative DNA damage, plays an important role in carcinogenesis: (i) many carcinogens induce 8-OHdG formation in the target organs of experimental animals (37); (ii) 8-OHdG is mutagenic (38); (iii) in some human cancer tissues the 8-OHdG concentration is significantly higher than that in the corresponding non-tumorous tissues (39); and (iv) some chemopreventive agents inhibit 8-OHdG formation caused by carcinogen exposure (40). It was recently shown that AFB1 induces 8-OHdG formation in rat liver in a dose- and time-dependent manner (8,9). However, the significance of AFB1-induced oxidative DNA damage in the carcinogenicity of AFB1 has not been well investigated. In the present study, it was found that AFB1 treatment also significantly increased the level of 8-OHdG in rat liver (Figure 5). Ebselen treatment, however, significantly inhibited AFB1-induced 8-OHdG formation (Figure 5). Given the importance of 8-OHdG formation and its implication in carcinogenesis, the inhibitory effect of ebselen on 8-OHdG formation may also contribute to its protective effect against the carcinogenicity of AFB1.

The formation of 8-OHdG is a result of direct interaction between ROS and DNA bases (41), so any factors interfering with the formation of ROS will also affect 8-OHdG formation. It has been shown that AFB1 enhances ROS formation in hepatocytes in a dose- and time-dependent manner, leading to oxidative damage (5). Moreover, the metabolic processing of AFB1 by CYP450, which accounts for the activation of AFB1 to the reactive intermediate, AFBO, is postulated to be the source of the increased levels of ROS in AFB1-treated hepatocytes (5). Therefore, either inhibition of the CYP450 metabolism of AFB1 or scavenging of ROS will reduce the level of ROS produced by AFB1 exposure and, subsequently, attenuate AFB1-induced oxidative DNA damage. As discussed earlier, it seems unlikely that ebselen administered orally (5 mg/kg body wt) would be able to affect the liver microsomal CYP450 metabolism of AFB1. Therefore, the observed protective effect of ebselen on AFB1-induced oxidative DNA damage is likely to be the result of its antioxidant property as a ROS scavenger.

Earlier animal studies showed that the induction of 8-OHdG by AFB1 exposure seems to occur at higher doses (several hundred micrograms per kilogram) (8), whereas the formation of AFB1–DNA adducts could be detected with exposure to a few nanograms per kilogram (1). In the present study, using AFB1 0.4 mg/kg, increased levels of both AFB1–DNA adduct and 8-OHdG were observed in AFB1-treated rats. Furthermore, ebselen treatment significantly reduced the levels of both AFB1–DNA adduct (Table III) and 8-OHdG (Figure 5) in rat liver. It is, therefore, believed that ebselen prevents AFB1-induced preneoplastic changes by inhibiting the formation of AFB1–DNA adducts and the ROS-scavenging ability of ebselen. On the other hand, ebselen could act by inhibiting AFB1–DNA adduct formation when AFB1 concentrations are low, conditions where AFB1–DNA adduct formation is known to play a more important role in its carcinogenicity.

In summary, the overall results of the present study suggest that ebselen can inhibit the preneoplastic changes caused by AFB1. Considering the importance of oxidative DNA damage in carcinogenesis, it is believed that, in addition to the inhibition of AFB1–DNA adduct formation, the protective effect of ebselen against AFB1-induced oxidative DNA damage may also contribute, at least in part, to its anticarcinogenic property.

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