INTRODUCTION

The majority of cancers are considered to be induced by chemicals. Most humans are more or less constantly exposed to a multitude of carcinogens, such as polycyclic aromatic hydrocarbons (PAH), in their environment. Benzo[a]pyrene (B[a]P) is a complete carcinogen, as it initiates and promotes carcinogenesis (Pelkonen and Nebert, 1982). B[a]P is a by-product of incomplete combustion, and is present in tobacco smoke, diesel engine exhaust, urban air, various types of processed foods, coal-tar, creosote, and asphalt, as well as in various occupational settings such as coal gasification plants, coke ovens, and iron foundries (Hattener-Frey and Travis, 1991).

B[a]P is chemically inert and requires metabolic activation by cytochrome P450 enzymes to form more reactive metabolites, such as 7,8-dihydroxy-9,10-epoxy-7,8,9,10-tetrahydrobenzo[a]pyrene (BPDE), in order to exhibit carcinogenicity in animals and humans (Melikian et al., 1990; Wei et al., 1996). BPDE is the ultimate carcinogenic form of B[a]P and binds to the exocyclic nitrogen of deoxyguanosine or deoxyadenosine in DNA (Mehrotra et al., 1994; Chiapperino et al., 2005). In addition, reactive oxygen species (ROS) generated during the metabolic process are capable of directly damaging DNA (Lorentzen and Ts’o, 1977). Oxidative DNA damage has been proven to be involved in cancer (Witz, 1991). Metallothionein (MT) is a cysteine-rich low-molecular-weight protein, and can act as a ROS scavenger (Kang, 1999; Miles et al., 2000; Coyle et al., 2002; Jeong et al., 2004).

MT has a high affinity for metals such as zinc and cadmium, and its expression is induced by these metals and many other factors such as glucocorticoids and cytokines (Miles et al., 2000; Coyle et al., 2002). Among the four

Original Article

Involvement of metallothionein (MT) as a biological protective factor against carcinogenesis induced by benzo[a]pyrene (B[a]P)

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ABSTRACT — The purpose of this study was to examine whether intracellular metallothionein (MT) protects against benzo[a]pyrene (B[a]P)-induced forestomach and lung carcinogenesis. Ten-week-old male MT-I/II null mice and wild-type mice were orally administered B[a]P at a dose of 100 or 250 mg/kg twice a week for 4 weeks. B[a]P-induced mortality was higher in the MT-I/II null mice than in the wild-type mice. The incidence of tumors in the forestomach and lung was 78.6% and 7.1% in the wild-type mice treated with 100 mg/kg B[a]P, respectively. In the MT-I/II null mice treated with B[a]P, tumor incidence in the forestomach and lung was 100% and 33.3%, respectively. The tumor area in the forestomach and lung in the MT-I/II null mice treated with B[a]P was greater than that of wild-type mice. These results suggest that MT acts as a biological protective factor against carcinogenesis induced by B[a]P.

Key words: MT, B[a]P, Carcinogenesis, Survival, Tumor incidence

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isoforms reported thus far, MT-I and MT-II, which are the major isoforms, are ubiquitous in tissues of many animal species (Miles et al., 2000; Coyle et al., 2002). MT is thought to play a role in the homeostasis of essential metals such as zinc and copper, the detoxification of heavy metals and anticancer agents, and protection against the effects of ionizing radiation (Probst et al., 1977; Naganuma et al., 1987; Kägi and Schäffer, 1988; Satoh et al., 1988, 1993a; Lazo and Pitt, 1995; Shibuya et al., 2008a). Pretreatment with MT-inducing metals such as zinc and bismuth was shown to suppress carcinogenesis caused by 7,12-dimethylbenz(a)anthracene (DMBA), 3-methyl cholanthrene, cisplatin, melphalan, and X-irradiation (Poswillo and Cohen, 1971; Duncan and Dreosti, 1975; Kagimoto et al., 1991; Satoh et al., 1993b).

Our recent studies using MT-I and MT-II double knock-out (MT-I/II null) mice have demonstrated that MT plays an important role in defense against B[a]P-induced genotoxicity such as chromosomal aberrations, DNA strand breaks, and oxidative DNA damage (Takaishi et al., 2009). In the present study, to demonstrate the involvement of MT in protecting against the carcinogenicity of B[a]P, we investigated the susceptibility of MT-I/II null mice to carcinogenesis caused by B[a]P.

MATERIALS AND METHODS

Chemicals

B[a]P was purchased from Sigma Chemicals Co. (St. Louis, MO, USA). Other chemicals and reagents used in this study were of the highest quality commercially available.

Animals

MT-I/II null mice with both MT-I and MT-II null mutations were kindly provided by Dr. K.H.A. Choo (Murdoch Institute for Research into Birth Defects, Royal Children’s Hospital, Melbourne, Australia) and were of a mixed genetic background of 129 Ola and C57BL/6 strains (Michalska and Choo, 1993). F1 hybrid mice were mated with C57BL/6J mice and their offspring were back-crossed to C57BL/6J for six generations. Both MT-I/II null mice and wild-type mice were generated by the mating of heterozygous mice.

MT-I/II null mice and wild-type mice were routinely bred in the vivarium of Gifu Pharmaceutical University. Both strains of mice were housed in cages in ventilated animal rooms with a controlled temperature of 24 ± 2°C, a relative humidity of 55 ± 10%, and a 12 hr light/dark cycle. The mice were maintained on standard laboratory chow and tap water ad libitum, and received humane care throughout the experiment in accordance with the guidelines of Gifu Pharmaceutical University.

Treatment

Ten-week-old male MT-I/II null mice and wild-type mice (14-15 mice/group) were orally administered B[a]P at a dose of 100 or 250 mg/kg twice a week for 4 weeks. They were weighed at weekly intervals. At 36 weeks after the first B[a]P administration, the mice were sacrificed by an overdose of diethyl ether anesthetic.

Histological examination

All mice, excluding those that died during the experiment, were examined grossly at necropsy. A histopathological examination was carried out on the forestomach and lung of all mice. Tissue specimens were fixed in neutral-buffered formalin solution and processed for paraffin embedding. Tissue sections (5 μm) were prepared and stained with hematoxylin and eosin for microscopic examination.

In each section, the area of tumor (mm2) was determined.

Statistical analysis

The mortality from all causes as a function of dose was calculated from Kaplan-Meier survival curves (Kaplan and Meier, 1958). The χ2 test was used to analyze differences in tumor frequency between MT-I/II null mice and wild-type control mice. All values were expressed as the mean ± S.D. Differences in the mean values were assessed by ANOVA followed by Newman-Keuls tests for post hoc comparison, or, where applicable, by Student’s t-test. Differences were considered statistically significant at p < 0.05.

RESULTS

The mortality of MT-I/II null mice and wild-type mice after B[a]P treatment was recorded (Fig. 1). In the groups treated with 100 mg/kg B[a]P, 6 of 15 MT-I/II null mice and 1 of 15 wild-type mice had died by the end of the study. Although all of the MT-I/II null mice and wild-type mice died at a B[a]P dose of 250 mg/kg, the MT-I/II null mice died earlier than the wild-type mice. In the control groups, all individuals of both strains of mice were still alive at the end of study.

The occurrence of tumors in MT-I/II null mice and wild-type mice treated with B[a]P is shown in Tables 1 and 2. Forestomach tumors were exhibited by 100% of MT-I/II null mice and 78.6% of wild-type mice upon treatment with B[a]P at 100 mg/kg (Table 1). Lung tumors
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were present in 33.3% of MT-I/II null mice and 7.1% of wild-type mice that underwent B[a]P treatment (Table 2). In the control groups, tumors were not observed in the forestomach or lung of either strain of mice. Tumor area in the forestomach of MT-I/II null mice treated with B[a]P was significantly greater than that of wild-type mice. Histopathological examination revealed that all tumor samples showed neoplastic foci of the epithelial cells in the forestomach showing disarranged structure, cellular pleomorphism and invasive growth beyond the basement membrane (Fig. 2). However, the forestomach tumor stage of B[a]P-administrated mice were not changed between the MT-I/II null mice and wild-type mice.

Fig. 1. Survival of MT-I/II null mice and wild-type mice treated with B[a]P. The treatment groups were as follows: wild-type mice treated with corn oil vehicle (open square); wild-type mice treated with 100 mg/kg B[a]P (open triangle); wild-type mice treated with 250 mg/kg B[a]P (open circle); MT-I/II null mice treated with corn oil vehicle (solid square); MT-I/II null mice treated with 100 mg/kg B[a]P (solid triangle); MT-I/II null mice treated with 250 mg/kg B[a]P (solid circle).

Table 1. Incidence of forestomach tumors in MT-I/II null mice and wild-type mice treated with B[a]P

<table>
<thead>
<tr>
<th>B[a]P (mg/kg)</th>
<th>tumor incidence (%)</th>
<th>tumor/tumor-bearing mouse (mm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>wild-type mice</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>0/14 (0)</td>
<td>-</td>
</tr>
<tr>
<td>100</td>
<td>11/14 (78.6)</td>
<td>13.46 ± 5.05</td>
</tr>
<tr>
<td>MT-I/II null mice</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>0/15 (0)</td>
<td>-</td>
</tr>
<tr>
<td>100</td>
<td>9/9 (100) *</td>
<td>27.61 ± 12.97 *</td>
</tr>
</tbody>
</table>

Values are means ± S.D. *Statistically significant difference from the corresponding wild-type mouse group: p < 0.05.

Table 2. Incidence of lung tumors in MT-I/II null mice and wild-type mice treated with B[a]P

<table>
<thead>
<tr>
<th>B[a]P (mg/kg)</th>
<th>tumor incidence (%)</th>
<th>tumor/tumor-bearing mouse (mm²)</th>
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<tbody>
<tr>
<td>wild-type mice</td>
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<tr>
<td>0</td>
<td>0/14 (0)</td>
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</tr>
<tr>
<td>100</td>
<td>1/14 (7.1)</td>
<td>1.00</td>
</tr>
<tr>
<td>MT-I/II null mice</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>0/15 (0)</td>
<td>-</td>
</tr>
<tr>
<td>100</td>
<td>3/9 (33.3) *</td>
<td>2.00 ± 1.00</td>
</tr>
</tbody>
</table>

Values are means ± S.D. *Statistically significant difference from the corresponding wild-type mouse group: p < 0.05.
DISCUSSION

Several studies have shown that MT-I/II null mice are susceptible to carcinogenesis caused by DMBA (Zhang et al., 1998; Suzuki et al., 2003), N-butyl-N-(4-hydroxybutyl)nitrosamine (Kondo et al., 1999), lead (Waalkes et al., 2004), cisplatin (Waalkes et al., 2006), and X-irradiation (Shibuya et al., 2008b). Our present study shows that MT-I/II null mice are hypersensitive to carcinogenesis induced by B[a]P treatment. In addition, chemical- and radiation-induced carcinogenesis was found to be suppressed by pretreatment with MT-inducing metals such as zinc and bismuth (Poswillo and Cohen, 1971; Duncan and Dreosti, 1975; Waalkes et al., 1989; Kagimoto et al., 1991; Satoh et al., 1993b). These studies indicate that MT plays an important role in protecting against chemical- and radiation-induced carcinogenesis.

The oral administration of B[a]P has been widely used to model chemically induced forestomach and lung carcinogenesis (Wattenberg and Leong, 1970; Ha et al., 1990). In the present study, B[a]P-induced forestomach tumors were observed in all of the MT-I/II null mice and in less than 80% of the wild-type mice. In addition, the area of forestomach tumors per tumor-bearing mouse induced by B[a]P was larger in the MT-I/II null mice than in the wild-type mice. Our present results suggest, therefore, that MT can prevent not only the occurrence of tumors but also their B[a]P-induced growth.

Previous studies have shown that MT-I/II null mice are more susceptible than wild-type mice to acute lethal toxicity of metals (Yoshida et al., 1999; Park et al., 2001). Nevertheless, there are no reports of studies on the lethal toxicity of environmental chemicals using MT-I/II null mice. In the present study, B[a]P-induced mortality was higher among MT-I/II null mice than wild-type mice. The mice died within 36 weeks after the first B[a]P administration, almost all of the mice have tumors in the forestomach, lung or liver. It is through that the cause of death in B[a]P-treated mice may be due to tumor formation and toxic effects caused by B[a]P. Thus, MT appears to also prevent the chronic lethal toxicity caused by B[a]P.

The mechanism by which MT protects against the carcinogenicity of B[a]P is still unclear. However, it has been shown that MT can efficiently eliminate ROS and protect against oxidative stress (Chubatsu and Meneghini, 1993). Our recent studies have demonstrated that MT protects against oxidative DNA damage (Takaishi et al., 2009). Moreover, several studies have shown that antioxidative agents prevent carcinogenesis due to B[a]P (Ha et al., 1990; Wang et al., 1992; Wattenberg and Estensen, 1997; Badary et al., 1999; Yang et al., 2002). Thus, the antioxidative properties of MT are considered as a possible source of the protection against carcinogenesis caused by B[a]P. In addition, the protective effect of MT on B[a]P-induced carcinogenesis may be due to the prevention of genotoxicity, which
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is an initiation stage in carcinogenesis process.

In conclusion, we found that MT-I/II null mice are susceptible to carcinogenesis induced by B[a]P. We previously reported that MT-I/II null mice are susceptible to the genotoxicity of B[a]P (Takaishi et al., 2009). These results suggest that MT acts as a biological protective factor against not only genotoxicity but also carcinogenesis caused by B[a]P. In addition, MT may be one of the factors that determine sensitivity to carcinogenesis caused by B[a]P.

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REFERENCES


