Increased susceptibility of poly(ADP-ribose) polymerase-1 knockout mice to nitrosamine carcinogenicity

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The involvement of poly(ADP-ribose) polymerase-1 (Parp-1), one of the poly(ADP-ribose) polymerase family proteins, in genomic stability, DNA repair and cell death triggered by DNA damage has been well documented. However, the potential role of Parp-1 in carcinogenesis has not been well evaluated. In this study the carcinogenic activity of N-nitrosobis(2-hydroxypropyl)amine (BHP) was studied in Parp-1–/– mice, generated by disrupting Parp-1 gene exon 1. Parp-1–/– and Parp-1+/+ male mice received 0, 250 and 500 p.p.m. BHP in their drinking water for 20 weeks and were then killed. The percentage of animals bearing hemangiomomas and hemangiosarcomas in the liver and numbers of tumors per mouse were markedly higher in the Parp-1–/– groups given 250 or 500 p.p.m. BHP than in their Parp-1+/+ counterparts. Hemangiosarcomas developed only in Parp-1–/– mice. In the lung the numbers of adenomas per mouse were increased in Parp-1–/– mice given BHP at 250 and 500 p.p.m. (P < 0.01) compared with the Parp-1+/+ case. The results show that susceptibility to BHP is significantly elevated in Parp-1–/– mice, thus providing direct evidence that Parp-1 is relevant to carcinogenesis.

Poly(ADP-ribose) polymerase (Parp) catalyzes the transfer of ADP-ribose units to various acceptor proteins, including the enzyme itself, resulting in the synthesis of poly(ADP-ribose) from nicotinamide adenine dinucleotide (1,2). Parp-1 is a nuclear enzyme comprising N-terminal DNA-binding, automodification and C-terminal catalytic domains and is activated by DNA strand breaks. The existence of other Parp-1 homologues developed in animals given 0, 250 and 500 p.p.m. BHP at a low incidence and number and in Parp-1–/– mice that received 250 and 500 p.p.m. BHP. Data for incidences and numbers of tumors in the liver after BHP administration are summarized in Table I. Neither Parp-1–/– nor Parp-1+/+ mice which received 0 p.p.m. BHP showed any spontaneous tumor development in this experimental period (groups 1 and 4, respectively). Heman- giomas developed in Parp-1–/– mice that received 500 p.p.m. BHP at a low incidence and number and in Parp-1–/– mice which received 250 and 500 p.p.m. BHP at a markedly higher incidence and number. In addition, hemangiosarcomas were only observed in Parp-1–/– mice that received 250 and 500 p.p.m. BHP. Metastases from one such lesion were found in the lung of a Parp-1–/– mouse that received 500 p.p.m. BHP.

In the lungs of Parp-1–/– and Parp-1+/+ mice given BHP alveolar hyperplasias, adenomas and adenocarcinomas were observed. The incidences of mice bearing lung tumors in Parp-1–/– and Parp-1+/+ mice at 250 p.p.m. were 5/9 and 5/8, respectively, with no significant difference. However, the incidence in Parp-1–/– mice was higher than that in Parp-1+/+ mice with 500 p.p.m. BHP.

Abbreviations: BHP, N-nitrosobis(2-hydroxypropyl)amine; Parp, poly(ADP-ribose) polymerase.

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<table>
<thead>
<tr>
<th>Mouse</th>
<th>Group</th>
<th>BHP (p.p.m.)</th>
<th>Effective no. of mice</th>
<th>No. of mice bearing tumors</th>
<th>No. of tumors per mouse</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>H</td>
<td>HS</td>
<td>Total</td>
</tr>
<tr>
<td>Parp-1+/–</td>
<td>1</td>
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<td>10</td>
<td>0</td>
<td>0</td>
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<td></td>
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<td>9</td>
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<td>3</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>500</td>
<td>9</td>
<td>7d</td>
<td>3</td>
</tr>
<tr>
<td>Parp-1+/+</td>
<td>4</td>
<td>0</td>
<td>10</td>
<td>0</td>
<td>0</td>
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<tr>
<td></td>
<td>6</td>
<td>500</td>
<td>8</td>
<td>1</td>
<td>0</td>
</tr>
</tbody>
</table>

H, hemangioma; HS, hemangiosarcoma.

Significant difference compared with group 5 (P < 0.02).

Significant difference compared with group 5 (P < 0.01).

Significant difference compared with group 5 (P < 0.001).

Significant difference compared with group 6 (P < 0.02).

Significant difference compared with group 6 (P < 0.01).

p.p.m., at 8/9 and 4/8, respectively. Furthermore, compared with the Parp-1+/+ case, the numbers of lung adenomas per mouse in Parp-1+/– mice were higher at both 250 (3.2 ± 3.8 and 1.4 ± 1.7, respectively) and 500 p.p.m. (3.0 ± 2.5 and 0.9 ± 1.4, respectively; P < 0.01). These results for liver and lung lesions demonstrated elevated susceptibility of Parp-1+/– mice to BHP carcinogenicity.

BHP is reported to be an environmental contaminant of commercial samples of disopropanolamine and trisopropanolamine (22) which is metabolized through oxidation by cytochrome P450 enzymes primarily in the liver, finally generating methylating or hydroxypropylating species (23,24). Methylated bases produced by BHP, including O6- and N2- methylguanine, can be repaired by alkylguanine alkyltransferases or base excision repair. Hydroxypropylated guanines such as O6- and N2-hydroxypropylguanine might be repaired through nucleotide excision repair. The elevated susceptibility to carcinogenicity induced by BHP in Parp-1+/– mice compared with Parp-1+/+ mice may be due to a deficiency in base excision repair (25) or other DNA repair pathways. The resistance of Parp-1+/– cells to cell death after extensive DNA damage (13) could also be related to a higher sensitivity to the carcinogen. Not only initiation of carcinogenesis through gene mutation but also tumor progression may be enhanced in the Parp-1-deficient state, as hemangiosarcomas only developed from hemangiomas in Parp-1+/– animals. This malignant progression could have been caused by dysfunction of Parp-1 in transcriptional regulation of genes involved in cell differentiation or maintenance of genomic integrity.

Increased incidences of spontaneous tumors have not been found in any organs of Parp-1+/– mice (8,10,21) other than a reported higher level of spontaneous T cell lymphomas in Parp-1+/– SCID compared with Parp-1+/+ SCID mice (26). Furthermore, although alterations in Parp-1 expression in cancer cells have been described (27), inactivation or genetic alteration of the Parp-1 gene in neoplasia has not been reported so far. The present study clearly indicates that dysfunction of Parp-1 could be considered as one of the risk factors for carcinogenesis. Thus Parp-1+/– mice should provide a useful animal model for the sensitive detection of carcinogenicity of genotoxic agents.

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**References**


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