Enhanced thyroid carcinogenicity of N-nitrosobis(2-oxopropyl)amine in Otsuka Long-Evans Tokushima Fatty rats, a model of type II diabetes mellitus

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Abstract

Diabetes mellitus is a group of common metabolic diseases that feature hyperglycemia in common and are classified into the two broad categories, type I diabetes mellitus and type II diabetes mellitus. Type I diabetes mellitus is characterized by absolute deficiency of insulin caused mainly by genetic processes. Type I diabetes accounts for about 5–10% of cases. Type II diabetes mellitus is microangiopathy, diabetes mellitus type II is known as diabetes mellitus/hyperlipidemia onset of hyperglycemia (after 20 weeks of age), mild course of diabetes, and conversion to insulin-dependent diabetes after 40 weeks of age (8–10). It has been reported that both males and females develop atypical hyperplasia of the cholelithocarcinoma duct by 15 weeks of age (11), frequently accompanied by papillary growth with cellular atypia. However, no progression of the lesion to malignant tumors has been observed in older OLETF rats.

N-Nitrosobis-(2-oxopropyl) amine (BOP) is a potent pancreatic carcinogen, being able to induce a high incidence of pancreatic ductal neoplasms, both adenomas and adenocarcinomas, in Syrian hamsters (12,13). In contrast, in rats, BOP induces tumors mainly in the thyroid, liver and lungs (14–16). In the present study, we examined the carcinogenic potency of BOP in the OLETF and Long-Evans Tokushima Otsuka (LETO) rats, to determine whether diabetic and/or hyperlipidemic conditions influence carcinogenesis by BOP.

Materials and methods

Introduction

Diabetes mellitus is a group of common metabolic diseases that feature hyperglycemia in common and are classified into the two broad categories, type I and type II, on the basis of the underlying pathogenic processes. Type I diabetes, which accounts for about 5–10% of patients, is a state of absolute deficiency of insulin caused mainly by autoimmune destruction of pancreatic β cells. Type II diabetes accounting for >90% of cases is characterized by high insulin resistance in fat and muscle tissue and leads to an inadequate, compensatory increased production of insulin. Decompensation of β cells and low absolute insulin concentrations eventually occur, but only in later stages of the disease. Type II diabetes is a major health problem in developed countries, where it affects ~7% of adults and ~15% of people older than 60 years. Although the major complication of diabetes mellitus is microangiopathy, diabetes mellitus type II is known to be associated with various neoplasms and is recognized as a risk factor for several types of cancer, including pancreatic, liver, colon and thyroid cancers (1–3). In fact, the risk of colon cancer appears to be elevated by a high-fat diet, and epidemiological studies have shown a clear association with hypertriglyceremia and hypercholesterolemia (4,5). Epidemiological findings have also shown that fat intake and obesity might increase pancreatic cancer risk and that diabetes mellitus might be related with thyroid cancer (3,6,7).

The Otsuka Long-Evans Tokushima Otsuka (OLETF) strain of rats spontaneously develops hyperglycemia, hyperinsulinemia, insulin resistance and mild obesity and has been well studied as an animal model for type II diabetes mellitus (8–10). OLETF rats show late onset of hyperglycemia (after 20 weeks of age), mild course of diabetes, and conversion to insulin-dependent diabetes after 40 weeks of age (8–10). It has been reported that both males and females develop atypical hyperplasia of the cholelithocarcinoma duct by 15 weeks of age (11), frequently accompanied by papillary growth with cellular atypia. However, no progression of the lesion to malignant tumors has been observed in older OLETF rats.

N-Nitrosobis-(2-oxopropyl) amine (BOP) is a potent pancreatic carcinogen, being able to induce a high incidence of pancreatic ductal neoplasms, both adenomas and adenocarcinomas, in Syrian hamsters (12,13). In contrast, in rats, BOP induces tumors mainly in the thyroid, liver and lungs (14–16). In the present study, we examined the carcinogenic potency of BOP in the OLETF and Long-Evans Tokushima Otsuka (LETO) rats, to determine whether diabetic and/or hyperlipidemic conditions influence carcinogenesis by BOP.

Carcinogenicity study

Groups of 25 OLETF and LETO rats at 5 weeks of age were each given four subcutaneous injections of BOP on days 1, 3, 5 and 7 at a dose of 10 mg/kg body wt, and additional groups of 25 animals each received saline alone as vehicle controls. At the final sacrifice time point at weeks 62–64 (67–69 weeks of age), all surviving animals were anesthetized with diethyl ether, and blood samples were collected from the abdominal aorta for measurement of various serum parameters, including insulin, glucose, triglyceride, total cholesterol, phospholipids, free fatty acids, tetraiodothyronine (T4), triiodothyronine (T3) and thyroid-stimulating hormone (TSH), conducted by SRL, Inc. (Tokyo, Japan). At autopsy, the pancreas, thyroid, heart, lung, kidneys, liver, spleen, testis, stomach, intestine (small intestine, cecum and colon) and bile duct were carefully examined macroscopically. Each pancreas and colon were carefully dissected from surrounding tissue and fixed in 10% phosphate-buffered formalin (pH 7.4) after spreading on filter paper. The other organs were also fixed in 10% phosphate-buffered formalin (pH 7.4) and routinely processed for embedding in paraffin. Sections were stained with hematoxylin and cosin for assessment of histopathological features.

Histopathological examination of thyroid tumors and scoring of fibrosis

Thyroid neoplasms were histopathologically diagnosed according to the criteria described by Kawaoi et al. (17). Fibrosis in the thyroid glands was assessed as percent areas of thyroid sections. The image analysis software National Institute of Radiological Sciences Institute, 1-1 Tsukiji 5-chome, Chuo-ku, Tokyo 104-0045, Japan,1Japan Food Cancer Prevention Basic Research Project, National Cancer Center Research Institute, 1-1 Tsukiji 5-chome, Chuo-ku, Tokyo 104-0045, Japan,1 Japan Food Research Laboratories, Bunkyo-ku 2-3, Chitose-shi, Hokkaido 066-052, Japan and 3Department of Oncologic Pathology, Kanazawa Medical University, 1-1 Daigaku, Uchida, Ishikawa 920-0293, Japan

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Epidemiologic data suggest that diabetes mellitus type II is a risk factor for several types of cancer, including pancreatic, liver, colon and thyroid cancers. In the present study, effects of diabetes/hyperlipidemia on N-nitrosobis(2-oxopropyl)amine (BOP)-induced cancer development were examined in Otsuka Long-Evans Tokushima Fatty (OLETF) rats, model animals for non-insulin-dependent diabetes mellitus and Long-Evans Tokushima Otsuka (LETO) rats, appropriate controls. Male of both strains were given four subcutaneous injections of BOP (10 mg/kg body wt) or saline on alternative days, starting at 5 weeks of age. BOP induced tumors in a variety of tissues, including the thyroid gland, colon, kidney, liver and lung. The highest yields were noted for thyroid tumors, the incidence (P = 0.0182) and multiplicity (P < 0.001) of BOP-induced thyroid cancers with marked fibrosis being significantly higher in OLETF than in LETO rats. Interestingly, anaplastic thyroid carcinomas were observed limited to the BOP-treated OLETF rats. Additionally, a greater incidence and frequency of aberrant crypt foci, putative precursor lesions for colon tumors, was observed in the BOP-treated OLETF group. However, BOP was ineffectual at inducing pancreatic ductal tumors. No thyroid, liver, lung or colon tumors were found in the OLETF and LETO rats receiving the vehicle. Significant increases in serum levels of insulin, glucose, phospholipids, triglycerides and total cholesterol were detected in the OLETF rats compared with the LETO rats, regardless of the treatment. Our results indicate that diabetic/hyperlipidemic state can enhance BOP-induced carcinogenesis of the thyroid gland and to a lesser extent the colon in OLETF rats.
Institutes of Health Image v.1.63 was used for calculation and the following grading system was applied to quantify the degree of fibrosis: grade 0 (<5% fibrosis), grade 1 (<6–20% fibrosis), grade 2 (21–35% fibrosis), grade 3 (36–50% fibrosis), grade 4 (51–65%) and grade 5 (>66%).

**Determination of aberrant crypt foci**

All colons were carefully removed, flushed with saline, slit open longitudinally from the cecum to the anus, placed between filter papers and fixed in 10% neutral buffered formalin for 24 h. They were then stained with 0.2% methylene blue in saline and placed, mucosal side up, on a microscope slide and examined under a microscope. Aberrant crypt foci (ACF) were recorded according to standard procedures used routinely in our laboratory (18).

**Statistical analysis**

The significance of differences in the incidences of tumors and other lesions was analyzed by the Fisher’s exact probability test. Variation in other data was evaluated by the Student’s unpaired t-test, one-way analysis of variance and post hoc (Tukey) multiple comparison test or one-way analysis of variance with a Bonferroni correction for multiple comparisons. A P value of <0.05 was regarded as significant.

**Results**

**General observation**

In the BOP-treated OLETF group, 15 rats were killed or died between weeks 42 and 58. At week 42, one rat was killed because of a large tumor mass developing in the lower abdomen. The mass was histopathologically diagnosed as granulomatous prostatitis and seminal vesiculitis. The first tumor that developed in the thyroid of an OLETF rat treated with BOP was observed at week 44, and therefore animals that survived beyond this time point were counted in the effective numbers. In the saline-treated OLETF group, six rats were killed or died at weeks 37–58. One rat of this group developed an inflammatory granuloma in the forestomach. In the BOP-treated LETO group, one rat was killed at week 32 because of dyspnea, but had not developed any tumors. In the saline-treated LETO group, one rat that had an inflammatory granuloma in the forestomach was killed at week 48. The final survival rates at weeks 62–64 were 76% (19/25 rats) in the saline-treated OLETF group, 96% (24/25 rats) in the saline-treated LETO group, 40% (10/25 rats) in the BOP-treated OLETF group and 96% (24/25 rats) in the BOP-treated LETO group.

Figure 1 shows body weight curves during the study. The mean weight of OLETF rats treated with or without BOP increased until week 30 and was significantly heavier than that of LETO groups from weeks 5 to 30. The average food consumption (g/day per rat) of OLETF rats treated with BOP (32.6 ± 3.3, P < 0.001) or saline (32.9 ± 3.2, P < 0.001) was significantly greater than that of their LETO counterparts given BOP (20 ± 1.2) or saline (20.7 ± 1.1). BOP treatment did not affect final body weights and food consumption in either OLETF or LETO animals.

Data on serum levels of phospholipid, triglyceride, total cholesterol, insulin and glucose are summarized in Table I. Those values for the OLETF rats were significantly higher than those of the LETO rats. The data suggested the OLETF rats to be hyperlipidemic and hyperglycemic, in a type II diabetic state, as reported previously (8,9). The serum glucose levels of the BOP-treated OLETF rats (208–608 mg/dl) were higher than those of the saline-treated OLETF rats (109–384 mg/dl), though the difference was not statistically significant (P = 0.06). Any influences of BOP treatment on the other values were not observed.

**Carcinogeticity of BOP in OLETF and LETO rats**

As summarized in Table II, neoplasms developed in a variety of tissues of the BOP-treated OLETF and LETO rats, including the thyroid, colon, liver, lungs, kidney, pancreas and subcutis. The highest yield was for thyroid tumors in the BOP-treated OLETF rats. The incidence was significantly higher than in the BOP-treated LETO group, being 96 and 71%, respectively. Pancreatic neoplasms developed in the BOP- and saline-treated OLETF rats at similar incidences. In each case, four pancreatic tumors were found, histopathologically diagnosed as one ductal adenocarcinoma and three acinar cell adenocarcinomas. In the LETO rats treated with BOP or saline, no pancreatic tumors were observed. In the tissues other than thyroid and pancreas, only few tumors developed in both strains (Table II). They included one colonic adenocarcinoma, one liver cell adenoma, one liver cell carcinoma, four lung adenomas and two renal fibrosarcomas in the BOP-treated OLETF rats, and two liver cell adenomas, eight lung adenomas and two renal cell adenomas, four renal cell adenocarcinomas, one renal mesenchymal tumor and one renal rhabdomyosarcoma of the BOP-treated LETO rats. However, there are no differences of the incidences in any of these neoplasms between the OLETF and LETO groups.

**Enhanced development of BOP-induced thyroid cancer in OLETF rats**

Thyroid neoplasms developing in the BOP-treated OLETF and LETO rats were histopathologically diagnosed as follicular cell adenomas (Figure 2A), follicular cell adenocarcinomas (Figure 2B and C) and anaplastic carcinomas (Figure 2D). In addition to spindle cell type of anaplastic carcinoma (Figure 2D), the coexistence of a type that consisted of spindle neoplastic cells and foci of follicular cell carcinoma with zones of transition was observed. As summarized in Table III, the incidences of BOP-induced thyroid follicular cell adenomas, poorly differentiated/anaplastic thyroid carcinoma, total carcinomas and total tumors (adenomas plus carcinomas) were significantly greater in the OLETF than the LETO rats. The incidence of well-/moderately differentiated thyroid carcinoma was also greater in the OLETF rats than the LETO rats, but the difference was not statistically significant. The multiplicities of follicular cell adenoma, well-/moderately differentiated follicular carcinomas, poorly differentiated/anaplastic follicular adenocarcinomas, total carcinomas and total tumors (adenomas plus carcinomas) in the thyroid were all significantly higher in the OLETF than the LETO rats. While the highest incidences of malignant epithelial neoplasms were for well-/moderately differentiated follicular cell carcinoma in both OLETF and LETO rats, large anaplastic carcinomas developed only in the OLETF rats and invaded into the surrounding tissues, including muscle, esophagus, trachea and lung.

The weights of thyroid glands were 1.5 times heavier in the salinetreated OLETF rats than in the saline-treated LETO rats, and BOP treatment significantly increased the weights for each strain (Table IV). The thyroid glands of BOP-treated OLETF rats were four times heavier than those of BOP-treated LETO rats. In addition, fibrosis accompanied by inflammation was prominent in the BOP-treated OLETF rats with thyroid carcinomas (Figure 2C) when compared with the BOP-treated LETO rats (Table IV).

Values for T3, T4 and TSH are shown in Table V. The T3 level in the OLETF rats was significantly higher than in their LETO counterparts, with or without BOP. The T4 level was increased by BOP.

![Fig. 1. Growth curves for OLETF (circles) and LETO (triangles) rats receiving BOP (closed symbols) or saline (open symbols).](https://academic.oup.com/carcin/article-abstract/28/10/2193/2476227/20/24/276277)
treatment in both strains, especially in the LETO rats and was consistently lower in OLETF than LETO rats. There were no differences in the TSH levels between the OLETF and LETO rats treated with or without BOP.

Colon carcinogenesis in OLETF rats

As mentioned, only one adenocarcinoma developed in the colon of a rat from the BOP-treated OLETF group. We further analyzed the number of ACF, putative precursor lesions (19), in order to determine

Table I. Serum lipid, insulin and glucose levels in OLETF and LETO rats given BOP or saline

<table>
<thead>
<tr>
<th>Strain</th>
<th>Treatment</th>
<th>No. of rats</th>
<th>Triglyceride (mg/dl)</th>
<th>Total cholesterol (mg/dl)</th>
<th>HDL-C (mg/dl)</th>
<th>LDL-C (mg/dl)</th>
<th>Phospholipids (mg/dl)</th>
<th>FFA (mEq/l)</th>
<th>Insulin (ng/ml)</th>
<th>Glucose (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>OLETF</td>
<td>BOP</td>
<td>23</td>
<td>433 ± 323&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>309 ± 124&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>71 ± 30&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>62 ± 36&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>399 ± 149&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>1041 ± 859&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>1.24 ± 1.28&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>391 ± 160&lt;sup&gt;bc&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Saline</td>
<td>22</td>
<td>535 ± 268&lt;sup&gt;b&lt;/sup&gt;</td>
<td>331 ± 77&lt;sup&gt;b&lt;/sup&gt;</td>
<td>70 ± 13&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>63 ± 24&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>430 ± 91&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1029 ± 432&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>0.83 ± 0.94&lt;sup&gt;b&lt;/sup&gt;</td>
<td>203 ± 106&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>LETO</td>
<td>BOP</td>
<td>24</td>
<td>93 ± 30</td>
<td>135 ± 13</td>
<td>29 ± 3</td>
<td>23 ± 5</td>
<td>182 ± 25</td>
<td>533 ± 167</td>
<td>0.2 ± 0.20</td>
<td>130 ± 55</td>
</tr>
<tr>
<td></td>
<td>Saline</td>
<td>24</td>
<td>95 ± 33</td>
<td>132 ± 20</td>
<td>29 ± 5</td>
<td>23 ± 4</td>
<td>171 ± 23</td>
<td>510 ± 132</td>
<td>0.26 ± 0.33</td>
<td>90 ± 9</td>
</tr>
</tbody>
</table>

HDL-C, high-density lipoprotein-cholesterol; LDL-C, low-density lipoprotein-cholesterol; FFA, free fatty acids.
<sup>a</sup>Means ± SDs (n = 22–24).
<sup>b</sup>Significantly different from the LETO group with each treatment at P < 0.001.
<sup>c</sup>Significantly different from the LETO group with each treatment at P < 0.01.
<sup>d</sup>Means ± SDs (n = 5).
<sup>e</sup>Significantly different from the LETO group with each treatment at P < 0.05.

Enhancement of thyroid cancer in OLETF rats

Fig. 2. Histopathology of thyroid proliferative lesions (A–D) developing in male OLETF rats receiving BOP. (A) A follicular cell adenoma. Tumor cells with slight nuclear atypia show a papillary to follicular cell pattern. The tumor is well demarcated by a fibrous capsule and compresses adjacent follicles. (B) A follicular cell carcinoma. The neoplastic cells with cellular pleomorphism form irregular follicles. Minimal invasion (arrow) is seen along one margin. (C) A follicular cell carcinoma with a scirrhus response. The tumor cells with nuclear atypia show irregular and follicular (tubular) growth patterns with extensive fibrosis. (D) An anaplastic carcinoma of spindle cell type. The tumor consists of pleomorphic spindle cells that show striform patterns, closely simulating the appearance of malignant fibrous histiocytoma. Hematoxylin and eosin stain. Original magnification: ×4 for A; ×10 for (B–D).

Table II. Tumor incidences in OLETF and LETO rats treated with BOP or saline

<table>
<thead>
<tr>
<th>Strain</th>
<th>Treatment</th>
<th>Effective number of rats&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Number (%) of rats with tumors</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Thyroid</td>
</tr>
<tr>
<td>OLETF</td>
<td>BOP</td>
<td>24</td>
<td>23&lt;sup&gt;b&lt;/sup&gt; (96)</td>
</tr>
<tr>
<td></td>
<td>Saline</td>
<td>25</td>
<td>0</td>
</tr>
<tr>
<td>LETO</td>
<td>BOP</td>
<td>24</td>
<td>17 (71)</td>
</tr>
<tr>
<td></td>
<td>Saline</td>
<td>25</td>
<td>0</td>
</tr>
</tbody>
</table>

<sup>a</sup>Number of animals that survived beyond week 44 (49 weeks of age).
<sup>b</sup>Significantly different from the LETO-BOP group at P = 0.0240.

Colon carcinogenesis in OLETF rats

As mentioned, only one adenocarcinoma developed in the colon of a rat from the BOP-treated OLETF group. We further analyzed the number of ACF, putative precursor lesions (19), in order to determine
Table III. Histopathological diagnosis of thyroid tumors in the OLETF and LETO rats receiving BOP

Strain | Incidencea (%) | Multic和平ilityb |
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Follicular cell adenoma</td>
<td>Well- or moderately differentiated carcinoma</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Total carcinoma</td>
</tr>
<tr>
<td>OLETF</td>
<td>19/24 (79)</td>
<td>10/24 (42)</td>
</tr>
<tr>
<td>LETO</td>
<td>12/24 (50)</td>
<td>15/24 (63)</td>
</tr>
</tbody>
</table>

aNumber of rats with thyroid tumors/effective number of rats
bSignificantly different from the LETO-BOP group at P = 0.0344.
Significantly different from the LETO-BOP group at P = 0.0022.
Significantly different from the LETO-BOP group at P = 0.0182.
Significantly different from the LETO-BOP group at P = 0.0240.
Significantly different from the LETO-BOP group at P < 0.001.
Significantly different from the LETO-BOP group at P < 0.005.

Table IV. Mean weights of thyroid glands and scores for fibrosis in the OLETF and LETO rats

<table>
<thead>
<tr>
<th>Strain</th>
<th>Treatment</th>
<th>Weight of thyroid glands (g)</th>
<th>Score of fibrosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>OLETF</td>
<td>BOP</td>
<td>1.026 ± 0.871ab (n = 18)</td>
<td>2.50 ± 1.32b (n = 24)</td>
</tr>
<tr>
<td></td>
<td>Saline</td>
<td>0.045 ± 0.016c (n = 22)</td>
<td>0.05 ± 0.22a (n = 25)</td>
</tr>
<tr>
<td>LETO</td>
<td>BOP</td>
<td>0.251 ± 0.114d (n = 24)</td>
<td>1.25 ± 0.90df (n = 24)</td>
</tr>
<tr>
<td></td>
<td>Saline</td>
<td>0.031 ± 0.005c (n = 24)</td>
<td>0.08 ± 0.28c (n = 25)</td>
</tr>
</tbody>
</table>

Means ± SDs.
Significantly different from the OLETF-saline, LETO-BOP and LETO-saline groups at P < 0.01 for each comparison.
Significantly different from the LETO-BOP group at P < 0.001.
Significantly different from the LETO-saline group at P < 0.001.
Significantly different from the LETO-saline group at P < 0.001.

Table V. Serum thyroid hormone and TSH levels in OLETF and LETO rats

<table>
<thead>
<tr>
<th>Strain</th>
<th>Treatment</th>
<th>No. of rats</th>
<th>T3 (ng/ml)</th>
<th>T4 (µg/dl)</th>
<th>TSH (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>OLETF</td>
<td>BOP</td>
<td>23</td>
<td>1.2 ± 0.3abc</td>
<td>3.3 ± 0.5b</td>
<td>5.9 ± 1.7</td>
</tr>
<tr>
<td></td>
<td>Saline</td>
<td>22</td>
<td>1.1 ± 0.2de</td>
<td>3.0 ± 0.7bc</td>
<td>7.0 ± 2.1</td>
</tr>
<tr>
<td>LETO</td>
<td>BOP</td>
<td>24</td>
<td>0.9 ± 0.1</td>
<td>4.2 ± 0.5a</td>
<td>5.9 ± 3.4</td>
</tr>
<tr>
<td></td>
<td>Saline</td>
<td>24</td>
<td>0.9 ± 0.1</td>
<td>3.3 ± 0.4</td>
<td>6.0 ± 2.6</td>
</tr>
</tbody>
</table>

Means ± SDs.
Significantly different from the LETO-BOP group at P < 0.001.
Significantly different from the LETO-saline group at P < 0.001.
Significantly different from the LETO-BOP group at P < 0.001.
Significantly different from the LETO-saline group at P < 0.001.

Discussion

In the present experiment, BOP induced tumors in a variety of organs, such as thyroid, colon, liver, lungs and kidneys of OLETF and LETO rats, but did not significantly increase pancreatic ductal carcinoma development. The incidence and multiplicity of thyroid cancer in the BOP-treated OLETF group were the highest among the tumors developing and significantly greater than in the BOP-treated LETO rats.

BOP-treated OLETF group were the highest among the tumors developing and significantly greater than in the BOP-treated LETO rats. Of particular interest, anaplastic thyroid carcinomas were developed in BOP-treated OLETF rats with hyperglycemia and hyperlipidemia, but not in any of the LETO rats. Our results thus suggest that diabetes mellitus and/or hyperlipidemic status affect carcinogenicity and promote cancer development in the thyroid. Since a history of diabetes mellitus is reported to be a risk factor for anaplastic thyroid cancer in humans (3), it is probable that the biochemical status in the body accelerates transformation from differentiated to anaplastic tumors.

Thyroid cancer is a relatively rare cancer, representing ~1% of all malignancies and accounting for ~0.5% of all deaths caused by malignant tumors (20). The well-differentiated thyroid carcinoma (21) is generally characterized by slow growth and a low mortality rate (8–15%) (22–26) and is only infrequently a cause of death. In contrast, anaplastic thyroid carcinomas, 5–15% of all thyroid cancers, are responsible for most thyroid cancer deaths (27,28), with only short survival periods (29,30). Such a histologic type of thyroid cancer usually occurs in elderly patients as a rapidly growing mass associated with dyspnea, dysphagia and hoarseness. In most of the cases, extra-thyroidal extension is observed at the time of initial presentation; growth of anaplastic thyroid carcinoma is very rapid with infiltration of surrounding muscle, esophagus, trachea, skin and even bone. The fact that poorly differentiated/anaplastic thyroid carcinomas more frequently developed in the BOP-treated OLETF rats is therefore of major interest. The pathogenesis of anaplastic thyroid carcinoma is not completely understood— it could arise de novo or from a preexisting well-differentiated thyroid carcinoma. Most aggressive tumors, however, arise as a result of anaplastic transformation of a preexisting well-differentiated tumor, papillary or follicular carcinoma, as found in this study, although they histologically mimic sarcoma or carcinosarcoma (31). Immunohistochemistry of anaplastic thyroid carcinomas found in this study showed negative to weak positivity against an
antibody of thyroid transcription factor-1 in tumor nuclei (data not shown), as in human cases (32). In the present study, fibrosis was also remarkable in follicular adenocarcinomas of the BOP-treated OLETF rats, suggesting the presence of factors promoting stromal cell proliferation.

As indicated in an N-bis(2-hydroxypropyl)nitrosamine-induced thyroid cancer model using F344 rats, where decreased T3 and T4 levels and an increased TSH level in the serum were described (33), thyroid hormones participate in thyroid carcinogenesis. Studies on tumor-promoting effects of p-aminobenzoic acid, xylazine and β-estradiol 3-benzoate on thyroid follicular cells suggest an involvement of inhibition of thyroid iodine uptake and organification, resulting in serum TSH stimulation dependent on continuous reduction of serum T4 levels through the feedback system in the pituitary–thyroid axis (34–36). In the present study, the incidence of thyroid carcinoma in the BOP-treated OLETF group that exhibited mild hyperthyroidism with hyperlipidemia was significantly higher than in the BOP-treated LETO group. These results suggest that disrupted thyroid hormone levels and diabetes mellitus/hyperlipidemic status in concert affect carcinogenicity and promote thyroid cancer development in the OLETF rats. In fact, clinically, association between Hashimoto thyroiditis and thyroid cancer (37), and that between subclinical hyperthyroidism and hyperlipidemia (38) is reported. Epidemiological data suggest a clear association between colon cancer development and hypertriglyceridemia/hypercholesterolemia (4,5) and our previous study revealed that APC106 and Min mice, known to feature a hyperlipidemic state, develops more numerous polyps in their intestinal tracts than do wild-type mice (39,40). In the current study, the colons of OLETF rats that were hypertriglyceridemic and hyperlipidemic appeared more sensitive to carcinogen than those of LETO rats based on the results for ACF that are putative precursor lesion of adenocarcinomas (19). The findings are consistent with a recent report that type II diabetes mellitus can enhance the generation and growth of colon carcinoma in OLETF rats treated with 1,2-dimethylhydrazine (41).

In conclusion, our findings described here indicate that a diabetic and/or hyperlipidemic state could enhance BOP-induced carcinogenicity of the thyroid gland and colon, in particular the thyroid, in OLETF rats. This strain can thus be considered as good model animals for investigating the relation between diabetes/hyperlipidemia and development of cancer.

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References

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