Inhibition of Streptozotocin-induced Islet Cell Tumors and N-Nitrosobis(2-oxopropyl)amine-induced Pancreatic Exocrine Tumors in Syrian Hamsters by Exogenous Insulin

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The Eppley Institute and Department of Pathology and Microbiology, University of Nebraska Medical Center, Omaha, Nebraska 68105-1065

ABSTRACT

The effects of streptozotocin (SZ) and N-nitrosobis(2-oxopropyl)amine (BOP), separately or in combination, on the pancreas, common duct, and gallbladder, all target tissues of BOP, were studied in Syrian golden hamsters. Groups of hamsters were treated with either a single dose (20 mg/kg body weight) of BOP (BOP group), or a single i.p. dose (50 mg/kg body weight) of SZ and 14 days later with a single s.c. injection of the same dose of BOP (SZ + BOP group). Another group of animals was treated similarly with BOP and SZ except that they received twice daily injections of insulin, beginning 1 day after SZ administration and for the duration of the experiment (52 weeks) (SZ + insulin + BOP group). The control group consisted of hamsters treated with a single dose of BOP and daily doses of insulin (insulin + BOP group). Hamsters treated with SZ recovered spontaneously from their diabetes, although the mortality was high (86%). BOP treatment induced the diabetogenic effects of SZ in both SZ + BOP and SZ + insulin + BOP groups and reduced the mortality to 43 and 74%, respectively. SZ pretreatment inhibited the incidence of BOP-induced pancreatic ductal/ductular cell carcinomas in the SZ + BOP group (P < 0.01); this protective effect of SZ on carcinoma development was potentiated by additional treatment with insulin (SZ + insulin + BOP group, P < 0.001). Although the frequency of BOP-induced tumors in the gallbladder (all polyps) was not altered by either SZ or insulin, the frequency of the common duct polyps was significantly lower in the SZ + insulin + BOP group than in the BOP group (P < 0.005). Hamsters in the SZ, SZ + BOP, and SZ + insulin + BOP groups developed islet cell adenomas (insulomas). However, the SZ + insulin + BOP group had significantly fewer insulomas than in the SZ + BOP group (P < 0.0005). The overall data confirm the inhibitory effect of SZ on BOP-induced pancreatic cancer and suggest that this effect is related to the diabetic condition of hamsters rather than insulin deficiency and that intact islets appear to be prerequisite for exocrine pancreatic cancer induction by BOP. On the other hand, the inhibitory action of insulin on insuloma induction by SZ and on ductal/ductular cancer induction by BOP seems to be related to the suppressive effect of this hormone on β-cell and ductal/ductular cell replication, respectively.

INTRODUCTION

Epidemiological studies suggest that diabetes presents a predisposing factor for pancreatic cancer (1-6) and that the risk of pancreatic cancer development remains for years after the diagnosis of diabetes (1). Moreover, histopathological studies have revealed hyperplastic changes in the pancreatic ducts of deceased diabetics more often than in those of nondiabetics (7). Reasons for the particular predisposition of diabetics toward pancreatic cancer could include perpetual proliferation of ductular cells in diabetic patients (8, 9), a condition known to increase the risk for carcinogenesis in many tissues, including the pancreas (10).

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2 To whom requests for reprints should be addressed, at The Eppley Institute for Research in Cancer, University of Nebraska Medical Center, 42nd and Dewey Avenue, Omaha, NE 68105-1065.
3 The abbreviations used are: SZ, streptozotocin; BOP, N-nitrosobis(2-oxopropyl)amine.
4 BOP was synthesized as reported (13) and was given once s.c. SZ (Sigma Chemical Co., St. Louis, MO), was prepared immediately before use as a 10-mg/ml solution in 0.1 M citrate buffer (pH 4.5) and was given i.p. as described.
5 Insulin (Eli Lilly and Co., Indianapolis, IN) was prepared in normal saline and injected s.c. twice a day during the experiment. Hamsters received 4 units Lente and 1 unit regular insulin/kg body weight 2 h before the dark cycle began (at 4 p.m.) and then received 4 units Lente and 4 units Ultra Lente/kg body weight 16 h later (at 8 a.m. the following day). Injection of each insulin type was given by separate syringe. This treatment schedule was found to be optimal in controlling...
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Table 1 Effective number of animals (ENA), survivals and final body weights (bw), type and incidence of tumors induced in Syrian hamsters with BOP and/or SZ with or without insulin treatment

<table>
<thead>
<tr>
<th>Group</th>
<th>ENA</th>
<th>Survival (wk ± SD)</th>
<th>Initial bw (g)</th>
<th>Final bw (g)</th>
<th>Insuloma no. (%)</th>
<th>Adenoma no. (%)</th>
<th>Carcinoma no. (%)</th>
<th>Gallbladder polyps no. (%)</th>
<th>Common duct polyps no. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BOP</td>
<td>33</td>
<td>48 ± 7</td>
<td>130 ± 11</td>
<td>127 ± 11</td>
<td>0a</td>
<td>14 (42)a</td>
<td>11 (33)a</td>
<td>18 (54)b</td>
<td>14 (42)b</td>
</tr>
<tr>
<td>Insulin + BOP</td>
<td>34</td>
<td>45 ± 10</td>
<td>132 ± 13</td>
<td>136 ± 32</td>
<td>1 (3)</td>
<td>14 (41)c</td>
<td>7 (20)d</td>
<td>16 (47)f</td>
<td>13 (38)f</td>
</tr>
<tr>
<td>SZ + BOP</td>
<td>42</td>
<td>48 ± 6</td>
<td>124 ± 13</td>
<td>135 ± 21</td>
<td>22 (52)c,d</td>
<td>9 (21)</td>
<td>3 (7)f</td>
<td>19 (54)b</td>
<td>9 (32)</td>
</tr>
<tr>
<td>SZ + insulin + BOP</td>
<td>43</td>
<td>42 ± 9</td>
<td>120 ± 10</td>
<td>126 ± 22</td>
<td>4 (9)c</td>
<td>4 (9)c</td>
<td>1 (2)d</td>
<td>14 (33)c</td>
<td>3 (8)c</td>
</tr>
<tr>
<td>SZ</td>
<td>15</td>
<td>45 ± 10</td>
<td>118 ± 21</td>
<td>138 ± 17</td>
<td>7 (21)f</td>
<td>0^e</td>
<td>0^e</td>
<td>0^e</td>
<td>0^e</td>
</tr>
</tbody>
</table>

*P < 0.0005 * *P < 0.005 * *P < 0.001 * >P < 0.0005

The average survival, the initial and final body weights, and the type and incidence of tumors are listed in Table 1; blood glucose levels are shown in Fig. 1.

Blood Glucose

The blood glucose levels of hamsters in the SZ group decreased gradually during the observation period and reached the normal value at 48 weeks. Most animals in this group died between weeks 16 and 48, and only 14% were still alive at the experiment end.

Although initially the blood glucose levels were equally high in all three groups of hamsters treated with SZ (SZ, SZ + BOP, SZ + insulin + BOP), the degree of hyperglycemia became significantly less after BOP treatment in the SZ + BOP group, compared with the SZ group at weeks 8-30 (P < 0.0001) and at week 36 (P < 0.03). The blood sugar level reached the normal value at week 30. The rate of survival in hamsters from the SZ + BOP group was significantly higher (57%) than in the SZ group.

In hamsters treated with SZ + insulin + BOP, the blood glucose levels were high and remained high until week 16, at which time they were higher than levels in SZ-treated hamsters (P < 0.04). At weeks 8 and 16, the hyperglycemia in the SZ + insulin + BOP group was also significantly higher than in the SZ + BOP group (P < 0.0001 and P < 0.0002, respectively). Thereafter, the levels dropped sharply to values lower than in the SZ group at weeks 30 (P < 0.02) and 36 (P < 0.002). The survival rate in the SZ + insulin + BOP group was 26%.

Histology

In BOP and insulin + BOP groups most islets showed normal configuration and distribution. A few or several islets in both groups showed intrainsular ductular proliferation, some of cystic type (Fig. 2), causing fragmentation and atrophy of endocrine tissue. The number of these intrainsular ductules increased with the experimental time. Also, in these altered islets, the normal ratio of β-, α-, and δ-cells was retained.

In SZ + BOP-treated hamsters, islets varied in size from atrophie (20-50 μm) to hyperplastic (>500 μm; Fig. 3) and contained either uniformly shaped cells, as in control hamsters, or a few or several hyperplastic cells with hyperchromatic nuclei. In addition, the number of endocrine cells, and their distribution within the islets, varied between the normal appearing, atrophic, and hyperplastic islets and from area to area in the same pancreas or in different animals. In 6 of 42 hamsters

Fig. 1. Blood glucose levels of hamsters treated with BOP, BOP and streptozotocin (BOP + SZ) or BOP + SZ plus insulin (SZ+BOP). BOP was injected once s.c. 14 days after SZ treatment. Insulin was given daily for life. The numbers refer to the number of animals at the experimental intervals. * P < 0.05; ** P < 0.01; *** P < 0.005; **** P < 0.0005 compared with SZ-treated hamsters.

Fig. 2. Intrainsular ductular proliferation in a hamster treated with insulin + BOP. The pseudopapillary structures within the cystic ductules represent ruptured ductular walls. Note the presence of small and large groups of cells immunoreactive with anti-insulin. Avidin-biotin peroxidase complex method, × 200.
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Fig. 3. Islets in a hamster treated with SZ + BOP are of different size but with the regular distribution of the endocrine cells. As in the normal hamster, α-cells occupy the outer zone of islets (black in photo). There are several extrainsular α-cells (arrows). Avidin-biotin-peroxidase complex method, × 96.

Fig. 4. A degenerated islet in a SZ + BOP-treated hamster composed of a large number of “light, ballooned” cells intermingled with a few acinar cells (long arrow) and unstained islet cells. There is a single cell immunoreactive with anti-insulin (arrowhead). A small islet in the vicinity of this degenerated islet shows no staining (short arrow). Avidin-biotin-peroxidase complex, × 200.

Fig. 5. A degenerated islet in a hamster of the SZ + BOP group. As in Fig. 4, there are several “ballooned cells” surrounded by cells immunoreactive with antisomatostatin. Note the irregular arrangement of the δ-cells. Avidin-biotin-peroxidase complex method, × 200.

Fig. 6. Extrainsular cells immunoreactive with anti-insulin showing long and slender cytoplasmic processes, which extend between the acinar cells. Avidin-biotin-peroxidase complex method, × 200.

of this group, varying numbers of “ballooned cells” were observed in islets (Fig. 4). All of these hamsters had normal blood glucose levels (129 and 128 mg/dl) at the time of death, whereas they all were hyperglycemic when the experiment began. In some of these islets with ballooned cells, none or only a few cells immunoreactive with any of the three antihormones could be found, whereas in others, primarily α- and δ-cells were present (Figs. 4 and 5). Generally, the atrophic islets were composed almost entirely of α-cells with normal or increased numbers of δ-cells, which showed derangement of their usual peripheral position in the islets (Fig. 5). However, in some islets the normal distribution of β-, α-, and δ-cells were retained. On the other hand, all but one hyperplastic islet found in these hamsters were composed of β-cells with only a few or none α- and/or δ-cells. The number of extrainsular endocrine cell foci increased remarkably, a phenomenon found consistently during pancreatic carcinogenesis with BOP (11, 14). Some of these extrainsular endocrine cells showed tiny and long cytoplasmic processes extending between and around the neighboring acinar cells (Fig. 6).

In the SZ + insulin + BOP group, most hamsters showed small islets with irregular borders. Ballooned cells were found in the islets of 5 of 43 hamsters, 4 of which were still diabetic at the time they were killed (blood glucose between 318 and 386 mg/dl). The remaining hamster was normoglycemic throughout the experiment. In most hamsters of this group, islets were depleted of β-cells and contained primarily α-cells (Fig. 7), with a normal number of δ-cells randomly distributed within the islets. In a few hamsters groups of δ-cells could be identified in the islets, but the number of β-cells was low, ranging from 10 to 50%/islet. In this group of hamsters, many
extrainsular cells were immunoreactive with $\alpha$-, $\beta$-, or $\delta$-cells. Histological findings in SZ-treated hamsters were generally similar to those in the SZ + BOP group with regard to the size and the cell composition of islets. In 8 of 15 hamsters, balloon cells were identified in a number of islets. Of these 8 hamsters, 7 were diabetic at the time they were euthanized and the remaining one was diabetic at the beginning of SZ treatment. In hamsters of this group, many islets of regular or small size contained none or only a few cells immunoreactive with anti-insulin, whereas most of these cells were of the $\alpha$-cell type, between and around which $\delta$-cells were demonstrable. However, almost all hyperplastic islets (>500 $\mu$m in diameter) were composed of $\beta$-cells with none or a few $\alpha$- and/or $\delta$-cells, except in one case, in which 50% of cells were $\alpha$-cells and the remaining half were $\beta$-cells.

**Patterns of Tumors**

The incidence and type of tumors found in each group are listed in Table 1.

**Exocrine Tumors.** Ductular proliferation (pseudoductules, tubular complex) was seen in all groups. Higher incidences occurred in the BOP (100%), insulin + BOP (79%), SZ + BOP (60%), and SZ + insulin + BOP (44%) groups than in the SZ (9%) group. As stated above, in many cases the ductules were found, often in cystic patterns, within the islets.

Significantly, more ductular adenomas were found in hamsters treated with BOP or insulin + BOP than in the other two groups. Ductal/ductular carcinomas occurred in all groups. Higher incidences occurred in the BOP (100%), insulin + BOP (79%), SZ + BOP (60%), and SZ + insulin + BOP (44%) groups than in the BOP and insulin + BOP groups. Adenocarcinomas in the BOP and insulin + BOP groups were large (about 10 mm in diameter) and invasive, whereas in the SZ + BOP group all 3 cancers were of microscopic size. There were also many more in situ carcinomas in hamsters treated with BOP (27%) or with insulin + BOP (32%) than with SZ + BOP (12%) or with SZ + insulin + BOP (7%, $P < 0.001$).

Although the incidence of gallbladder tumors (all polyps) did not differ among the BOP-treated groups, significantly fewer common duct tumors (all polyps) were found in SZ + insulin + BOP-treated hamsters.

**Endocrine Tumors.** Islet cell tumors were seen in hamsters treated with SZ, SZ + BOP, and insulin + BOP. (The one tumor seen in a hamster of the insulin + BOP group was most probably a spontaneously developed lesion). The tumor incidence was significantly lower in the SZ + insulin + BOP and SZ + BOP groups than in the BOP and insulin + BOP groups. Adenocarcinomas in the BOP and insulin + BOP groups were large (about 10 mm in diameter) and invasive, whereas in the SZ + BOP group all 3 cancers were of microscopic size. There were also many more in situ carcinomas in hamsters treated with BOP (27%) or with insulin + BOP (32%) than with SZ + BOP (12%) or with SZ + insulin + BOP (7%, $P < 0.001$).

The first insuloma was found at week 28 in the SZ and SZ + insulin + BOP groups and at week 27 in SZ + BOP group. The neoplasms were generally small, ranging from 2 to 4 mm in diameter; only a few of them were encapsulated. Because of the small size of insulomas and because only a few sections from each pancreas were examined histologically, the insuloma incidence could have been higher than that listed in Table 1. In fact, in some cases tumors were found only in a single section and not in the subsequent slide. For this reason, not all of these tumors could be examined by all three antibodies.

Morphologically, all of the endocrine neoplasms were adenomas (insulomas) with no differences in their patterns and sizes among the groups. The neoplasms were, in most cases, ill defined and in some regions extended into the surrounding tissue. Immunohistochemical examination did not reveal differences in the cellular composition of adenomas among the groups. About 60% of the tumors, regardless of their size, were composed of $\beta$-cells with none or a few (1-20%) $\alpha$-cells, fewer numbers of $\delta$-cells (0-10%), and various number of unstained cells. In the remaining 40% of the neoplasms, the $\beta$-cells contributed to 15-50% of the cell population with only a few $\alpha$- or $\delta$-cells and many cells that did not react with any of the three antibodies used (Figs. 8-10). In these adenomas, the immunoreactive cells were arranged either in groups within or individually in random fashion between the unstained cells (Figs. 8 and 9). In some cases the apparently newly formed $\beta$-
the hyperplastic (preneoplastic) islets did not correlate with that in neoplastic islets. For example, in SZ + insulin + BOP-treated hamsters in whom hyperplastic islets were almost entirely composed of α-cells, tumors contained a few or none α-cells, various numbers of β-cells, and none or a few b-cells (Fig. 10). There was also no correlation between the severity of hyperglycemia, either at the beginning or the end of the experiment, and the pattern and incidence of tumors. Table 2 summarizes the initial and final blood glucose levels of hamsters in each group.

**DISCUSSION**

In the present study, hamsters treated with SZ only developed severe hyperglycemia and surviving hamsters (14%) recovered gradually from the SZ-induced diabetes spontaneously, although 86% of hamsters died from the consequences of diabetes between weeks 16 and 48. In our previous study, recovery from diabetes occurred in 50% of hamsters at 70 days post-SZ. BOP treatment (SZ + BOP) clearly reduced the diabetogenic effects of SZ in this species and prolonged the survival significantly (57% versus 14%). Although SZ + BOP-treated hamsters had blood glucose levels initially as high as in the SZ group, the glucose levels dropped rapidly after BOP treatment and normalized much earlier than did in the SZ group. In the SZ + BOP group, none of the hamsters were hyperglycemic at the end of the experiment.

A similar situation was seen in the SZ + insulin + BOP group. In the previous study, administration of insulin at the same dose and frequency as given to SZ-treated hamsters (SZ + insulin group) adversely affected diabetes and caused persistent severe hyperglycemia during the 70-day observation period. In the present experiment, BOP given to SZ + insulin-treated hamsters inhibited the dramatic increase of blood sugar and its persistency. Moreover, in the SZ + insulin + BOP group, blood glucose levels dropped drastically after week 16 and reached levels lower than in the SZ group thereafter, a situation that could have as well increased survival of animals in this group as compared with those in the SZ group.

The reasons for the beneficiary effects of BOP on the course of SZ-induced diabetes is obscure. The pronounced formation in the SZ + BOP and SZ + insulin + BOP groups of extrainsular endocrine cells (nesidioblastosis), a phenomenon known to occur consistently during BOP pancreatic carcinogenesis (11, 14, 15), could be one explanation. Whether the number or the functional activity of these extrainsular β cells was sufficient to compensate for the β-cells destroyed by SZ or in these animals extrapancreatic sources of insulin may have existed (16) is presently unknown.

With regard to the carcinogenicity, our results confirm the study of Bell and Strayer (17) and show that pretreatment of hamsters with SZ inhibits the pancreatic carcinogenicity of BOP. Because simultaneous administration of SZ and BOP paradoxically resulted in an enhancement of exocrine pancreatic carcinogenesis (18), the timing between SZ and BOP treatment appears to be crucial in modifying tumor induction. The extent of islet cell necrosis seems to be a key factor in the neoplastic process. In the study of Bell and Strayer (17), islet cells were destroyed completely by daily injections of SZ for 3 consecutive days. Although BOP was given weekly for 24 weeks beginning 7 days after SZ treatment, no pancreatic tumors were induced. In the present experiment, insulin given to normoglycemic hamsters (insulin + BOP group) did not influence the tumor yield. Therefore, it appears that intact islet cells rather than the availability of insulin are prerequisite for triggering

<table>
<thead>
<tr>
<th><strong>Table 2 Initial and final blood glucose levels (in mg/dl) of hamsters with induced tumors</strong></th>
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<tr>
<td><strong>Group</strong></td>
</tr>
<tr>
<td>BOP</td>
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<td>Insulin + BOP</td>
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<td>SZ</td>
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* Numbers in parentheses, percentage.

Fig. 10. Two consecutive sections of a pancreas in a hamster treated with SZ + insulin + BOP. All nonneoplastic islet cells in this case are composed of α-cells (a), whereas only a few α-cells are present in islet cell adenoma (center). On the other hand most tumor cells are of β-cell type showing different staining intensity (a), whereas only a few «-cells are present in islet cell adenoma (center). On the other hand most tumor cells are of β-cell type showing different staining intensity with antiinsulin (ft) and no insulin-containing cells are in surrounding nonneo...
the neoplastic effects of BOP. In fact, intrinsurals ductular proliferation, an early event in pancreatic carcinogenesis (11, 14, 15), was found predominantly in hamsters whose islets contained many \( \beta \)-cells. Whether \( \beta \)-cells exert their effects by factors other than insulin or \( \alpha \)-cells (the numbers of which were increased significantly in these animals) act as inhibitors for ductular proliferation remains to be seen.

The possibility that tumor inhibition by SZ could be related to toxic effects of this compound on the liver, the suggested major site of BOP metabolism to a proximate pancreatic carcinogen (19), seems unlikely because pancreatic cells can metabolize BOP directly (20). In the present study, tumor induction in other target tissues of BOP, such as gallbladder, was not altered in SZ pretreated hamsters. Alloxan, another \( \beta \)-cell cytotoxic substance, in a nonhepatotoxic dose also inhibited toxic effects of this compound on the liver, the suggested ductular proliferation remains to be seen.

factors other than insulin or \( \beta \)-cells (the numbers of which were metabolism to a proximate pancreatic carcinogenicity (20). In the present study, tumor induction by BOP could be related to the suppressive effects of insulin on ductular cell regeneration.4 The inhibitory action of SZ given 7 days after BOP has failed to inhibit tumorigenesis.5 The mechanisms of this action are not yet known.

Inhibition by insulin of insuloma induction by SZ could well have the same cause, for example suppressive action of the hormone on islet cell regeneration.4 Remarkably, most insulomas induced by SZ contained more cells immunoreactive with antinsulin than those reactive with antigliucagon and antisomatostatin, although \( \alpha \)-cells and, to a lesser degree, \( \delta \)-cells were the cell types that initially proliferated and replaced the damaged \( \beta \)-cells. This observation indicates that \( \alpha \)- and \( \delta \)-cells are not the target of SZ toxicity. The malignant endocrine cells seem to arise either from the \( \beta \)-cells that may have survived the

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8 R. H. Bell, personal communication.

REFERENCES

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