Induction of Rat Pancreatic B-Cell Tumors by the Combined Administration of Streptozotocin or Alloxan and Poly(Adenosine Diphosphate Ribose) Synthetase Inhibitors

Takashi Yamagami, Atsuo Miwa, Shin Takasawa, Hiroshi Yamamoto, and Hiroshi Okamoto

Departments of Biochemistry [T. Y., S. T., H. Y., H. O.] and Pathology [A. M.], Toyama Medical and Pharmaceutical University School of Medicine, Toyama 930-01, Japan

ABSTRACT

Streptozotocin and alloxan were administered to Wistar rats in combination with poly(adenosine diphosphate ribose) synthetase inhibitors. Ten to 16 months after the injection of streptozotocin (50 mg/kg body weight i.v.) and 3-aminobenzamide (345 mg/kg i.v.), streptozotocin (50 mg/kg) and nicotinamide (350 mg/kg i.p.), streptozotocin (50 mg/kg) and picolinamide (250 mg/kg i.p.), alloxan (40 mg/kg i.v.) and nicotinamide (350 mg/kg), alloxan (40 mg/kg) and 3-aminobenzamide (345 mg/kg), and alloxan (40 mg/kg) and picolinamide (250 mg/kg) pancreatic islet cell tumors developed in 100, 98, 60, 26, 22, and 20% of surviving rats, respectively. However, after the single injection of streptozotocin and alloxan, islet cell tumors developed in 42 and 11% of surviving rats, respectively. The tumors were rich in B-granules on electron micrographs and contained as large amounts of proinsulin messenger RNA as normal pancreatic islets. The results indicate that poly(adenosine diphosphate ribose) synthetase inhibitors enhance the tumorigenic effect of streptozotocin and alloxan on islet B-cells.

INTRODUCTION

The induction of pancreatic islet cell tumors in rats by a combined administration of streptozotocin and nicotinamide was first reported by Rakieten et al. (22) in 1971. Histochemical and biochemical properties of the tumors have been described (12, 14, 23). Our recent study revealed that the streptozotocin- and nicotinamide-induced tumor cells contain as much proinsulin mRNA as do normal pancreatic islets (9, 11, 19). Islet cell tumors were also found in rats given streptozotocin and picolinamide, an isomer of nicotinamide, and in rats given alloxan and nicotinamide (13, 14). However, how the combined administrations cause the islet tumors is not yet understood.

Recently, we found that both streptozotocin and alloxan cause DNA strand breaks in islet B-cells to activate poly(ADP-ribose) synthetase (31) and that nicotinamide and picolinamide are potent inhibitors of islet poly(ADP-ribose) synthetase (26, 29). The present study was designed to investigate whether the combined administration of streptozotocin or alloxan with various poly(ADP-ribose) synthetase inhibitors to rats induces islet B-cell tumors and to determine the proinsulin mRNA content of the tumors induced. As a result, we found that, in any combination examined, islet B-cell tumors containing significant amounts of proinsulin mRNA sequences as well as B-granules were induced with a high incidence. A possible mechanism of the B-cell tumorigenesis is discussed.

MATERIALS AND METHODS

Animals and Chemicals. Male Wistar rats were purchased from Shizuoka Laboratory Animal Center, Hamamatsu, Japan; streptozotocin was from The Upjohn Co., Kalamazoo, MI; alloxan and nicotinamide were from Wako Pure Chemical Industries, Osaka, Japan; picolinamide was from Tokyo Kasei Kogyo Co., Tokyo, Japan; [α-32P]dCTP (3000 Ci/mmoll from Amersham International, Amersham, Buckinghamshire, England; aminophenyl thioether papers from Schleicher and Schuell, Inc., Keene, NH. 3-Aminobenzamide-HCl was donated from Research Laboratories, Chugai Pharmaceutical Co., Tokyo, Japan.

Induction and Morphological Examination of Tumors. Rats weighing 150 g were divided into 8 groups; streptozotocin group; streptozotocin-nicotinamide group; streptozotocin-picolinamide group; streptozotocin-3-aminobenzamide group; alloxan group; alloxan-nicotinamide group; alloxan-picolinamide group; and alloxan-3-aminobenzamide group. Each group consisted of 17 to 99 rats (see Table 1). All chemicals were dissolved in 0.9% NaCl solution. Streptozotocin and alloxan were dissolved just before injection and were administered i.v. via the tail vein into ether-anesthetized rats at doses of 50 and 40 mg/kg body weight, respectively (13, 16, 22, 32). Nicotinamide (350 mg/kg) was injected i.p. into rats 10 min before and 3 hr after the administration of streptozotocin or alloxan (22). Picolinamide (250 mg/kg) was injected i.p. 10 min before streptozotocin or alloxan injection (14). 3-Aminobenzamide-HCl (345 mg/kg) was injected i.v. via the tail vein 30 min before streptozotocin or alloxan injection. Rats were allowed access to standard rat chow and water. After 10 to 16 months, rats were anesthetized with pentobarbital sodium (45 mg/kg body weight i.p.), and laparotomy was carried out under sterile conditions. Grossly visible tumors were excised and fixed; one half was immediately fixed in 2.5% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.4) for histological evaluations, and the other half was stored at −80°C until RNA isolation. For an ultrastructural study, fixed segments of tumors were postfixed in 2% osmium tetroxide in 0.1 M cacodylate buffer (pH 7.4), dehydrated in graded ethanol solutions, embedded in Epon 812, and sectioned on a LKB Ultratome 8000. The sections were stained with uranyl acetate and lead citrate and examined in a Hitachi H-300 electron microscope.

Northern Blot Hybridization. RNA was isolated as described previously (9) from tumors induced by streptozotocin and alloxan with or without poly(ADP-ribose) synthetase inhibitors and from various tissues of untreated rats including pancreatic islets, liver, kidney, and spleen. Islets were isolated by the collagenase method as described previously (20). RNA was denatured by glyoxalation, electrophoresed on a 1% agarose gel at 90 V for 100 min, and blotted to a diazophenyl thioether paper (an active form of aminophenyl thioether paper) by the method of Alwine et al. (1). Proinsulin cDNA insert, the hybridization probe, was cut off from a recombinant plasmid and nick-translated in the presence of...
Table 1

Incidence of islet cell tumors induced with streptozotocin or alloxan and poly(ADP-ribose) synthetase inhibitors

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Surviving 10 mo or longer</th>
<th>Single</th>
<th>Multiple</th>
<th>Total</th>
<th>Mean no. of tumors/bearing rat</th>
<th>Tumor size (mean diameter, mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Streptozotocin (50 mg/kg i.v.)-nicotinamide (350 mg/kg i.p. in 2 injections)</td>
<td>99</td>
<td>82</td>
<td>28</td>
<td>52</td>
<td>80$^d$ (98)$^b$</td>
<td>2.3</td>
</tr>
<tr>
<td>Streptozotocin (50 mg/kg i.v.)-picolinamide (250 mg/kg i.p.)</td>
<td>23</td>
<td>15</td>
<td>8</td>
<td>1</td>
<td>9 (60)</td>
<td>1.1</td>
</tr>
<tr>
<td>Streptozotocin (50 mg/kg i.v.)-aminobenzamide (345 mg/kg i.v.)</td>
<td>35</td>
<td>24</td>
<td>14</td>
<td>10</td>
<td>24$^b$ (100)</td>
<td>1.8</td>
</tr>
<tr>
<td>Streptozotocin (50 mg/kg i.v.)</td>
<td>26</td>
<td>19</td>
<td>6</td>
<td>2</td>
<td>8 (42)</td>
<td>1.3</td>
</tr>
<tr>
<td>Alloxan (40 mg/kg i.v.)-nicotinamide (350 mg/kg i.p. in 2 injections)</td>
<td>60</td>
<td>38</td>
<td>9</td>
<td>1</td>
<td>10 (26)</td>
<td>1.2</td>
</tr>
<tr>
<td>Alloxan (40 mg/kg i.v.)-picolinamide (250 mg/kg i.p.)</td>
<td>36</td>
<td>20</td>
<td>4</td>
<td>0</td>
<td>4 (20)</td>
<td>1.0</td>
</tr>
<tr>
<td>Alloxan (40 mg/kg i.v.)-aminobenzamide (345 mg/kg i.v.)</td>
<td>17</td>
<td>9</td>
<td>1</td>
<td>1</td>
<td>2 (22)</td>
<td>1.5</td>
</tr>
<tr>
<td>Alloxan (40 mg/kg i.v.)</td>
<td>25</td>
<td>18</td>
<td>2</td>
<td>0</td>
<td>2 (11)</td>
<td>1.0</td>
</tr>
</tbody>
</table>

$^a$ Occurrence of tumors was determined after 10 to 13 months for streptozotocin groups and after 13 to 16 months for alloxan groups because islet cell tumors tend to develop more slowly in the latter than in the former.

$^b$ Statistically significant at $p < 0.01$ by the $x^2$ test as compared with the group treated with streptozotocin alone.

$^c$ Numbers in parentheses, rats with tumors as a percentage of surviving rats.

$^d$ Mean ± S.D.

RESULTS

Table 1 shows the incidence, number, and size of tumors which were histopathologically confirmed to be islet cell tumors. Islet cell tumors developed in all the surviving rats of the streptozotocin-3-aminobenzamide group, 98% of rats in the streptozotocin-nicotinamide group, and 60% of rats in the streptozotocin-picolinamide group. Islet cell tumors were also found in rats treated with combined administrations of alloxan and poly(ADP-ribose) synthetase inhibitors as frequently as 20 to 26%. Tumors also developed in rats treated with streptozotocin or alloxan alone, but the incidence was lower (42 or 11%, respectively) than in rats treated with the combined administrations. The tumors induced with streptozotocin-nicotinamide and alloxan-nicotinamide developed at almost the same time periods as the tumors induced with the single administration of streptozotocin and alloxan. One to 6 tumors were found per rat. The tumors were well delineated, round or polyhedral, and brownish red or white and ranged from 1 to 10 mm in greatest diameter. Light microscopic examination showed that proliferated tumor cells were in a cord-like or tubular arrangement and stainable by aldehyde-fuchsin which specifically stains B-cells (5). The morphological findings were in agreement with those reported previously for streptozotocin- and nicotinamide-induced tumors (12, 22) and alloxan- and nicotinamide-induced tumors (13). In electron micrographs, as shown in Fig. 1, tumor cells were rich in B-granules in all tumors examined. Cells containing A-granules or D-granules were not encountered.

Next, the amount of proinsulin mRNA in the tumors was estimated by Northern blot hybridization using rat proinsulin cDNA as a probe. As shown in Fig. 2, distinct bands of hybridization were obtained at the same mobilities as those of authentic rat proinsulin mRNA were detected on the lanes of RNAs from tumors and islets, but no visible band was detected on the lanes of RNAs from liver, kidney, and spleen, non-insulin-producing tissues. Liquid scintillation counting of the hybridization bands cut from the gels showed that $32^P$ radioactivities in the bands on lanes of tumor RNAs were 85 to 126% of that of islet RNA, except alloxan- and nicotinamide-induced tumor RNA in which $32^P$ radioactivity was 24% of that of islet RNA. The result indicates that the tumors contained as large amounts of proinsulin mRNA sequences as did normal islets.

DISCUSSION

The present study demonstrated that the combined administration of streptozotocin or alloxan and poly(ADP-ribose) synthetase inhibitors causes a high incidence of islet B-cell tumors. The tumors were confirmed to be B-cell tumors by the finding that they were rich in B-granules and contained large amounts of proinsulin mRNA.

The incidences of islet B-cell tumor development in rats receiving streptozotocin and nicotinamide (98%), streptozotocin and picolinamide (60), and alloxan and nicotinamide (26%) are comparable with those reported by others; 65 to 79% (14, 22,
28S RNA—
18S RNA—
4S RNA—

Fig. 2. Proinsulin mRNA content of tumors induced with streptozotocin or alloxan and poly(ADP-ribose) synthetase inhibitors. One ng of rat proinsulin mRNA and 5 μg (b to k) or 2 μg (l to n) of LiCl-precipitated RNA from tumors and normal tissues were denatured, electrophoresed, transferred to a diazophenyl thioether paper and hybridized with nick-translated rat proinsulin cDNA as described in "Materials and Methods." Exposure time was 2 hr for Lane a, 1 hr for Lanes b to k, and 22 hr for Lanes l to n. a, rat proinsulin mRNA; b, streptozotocin- and nicotinamide-induced tumor RNA; c, streptozotocin- and picolinamide-induced tumor RNA; d, streptozotocin- and 3-aminobenzamide-induced tumor RNA; e, alloxan- and nicotinamide-induced tumor RNA; f, alloxan- and picolinamide-induced tumor RNA; g, alloxan- and 3-aminobenzamide-induced tumor RNA; h, normal islet RNA; i, normal liver RNA; j, normal kidney RNA; k, normal spleen RNA; l, streptozotocin-induced tumor RNA; m, alloxan-induced tumor RNA; n, normal islet RNA. Bars, relative migration of molecular weight markers run on the same gel.

23), 63% (14), and 14% (13), respectively. On the other hand, after a single administration of streptozotocin and alloxan, the B-cell tumors developed at a much lower incidence (42 and 11%, respectively) than after the combined administrations (Table 1).

Poly(ADP-ribose) synthetase, an enzyme which catalyzes chromatin-bound polymerization of an ADP-ribose moiety of NAD+, has been correlated to DNA synthesis, DNA repair, and cell differentiation (7, 8, 17, 24). The enzyme is inhibited by aromatic acid amides such as nicotinamide, picolinamide, and 3-aminobenzamide, and these agents have been widely used to investigate the functions of poly(ADP-ribose) synthetase, although multiple metabolic effects of the agents were recently suggested (18). We have already shown that when islet B-cell DNA is fragmented by streptozotocin and alloxan (31, 32), poly(ADP-ribose) synthetase is activated to cause NAD+ depletion, which leads to impairment of B-cell functions including proinsulin synthesis (19, 26, 31). It has been considered that NAD+ depletion is incompatible with survival of islet B-cells (6, 19, 21). When poly(ADP-ribose) synthetase inhibitors coexist with streptozotocin or alloxan, cellular NAD+ level is maintained, and the B-cells may be allowed to survive (21, 31). In this case, however, B-cell DNA was found to remain fragmented (26). Therefore, in the coexistence of poly(ADP-ribose) synthetase inhibitors, the relative abundance of surviving B-cells in which the DNA had been fragmented with streptozotocin and alloxan is considered to be exceedingly larger than in the absence of the inhibitors. This may be one explanation for the increase in B-cell tumor incidence in animals treated with streptozotocin or alloxan and poly(ADP-ribose) synthetase inhibitors. Furthermore, poly(ADP-ribose) synthetase inhibitors retard the rejoining of DNA strand breaks, as we and others have shown previously (4, 30). This repair retardation may increase the frequency of alteration in gene structure, which may result in abnormal expression of certain genes that are crucially involved in tumorigenesis (21). We are now searching for the genes which are specifically expressed in the B-cell tumor through a comparative analysis between normal islet and B-cell tumor cDNA libraries. Recently, Konishi et al. (15) observed that poly(ADP-ribose) synthetase inhibitors such as 3-aminobenzamide and 5-methyl nicotinamide enhanced the diethylnitrosamine-initiated induction of γ-glutamyl transpeptidase-positive focal, a precancerous state, in rat liver. Diethylnitrosamine causes DNA fragmentation (25) as well as streptozotocin and alloxan (31). Therefore, it is supposed that poly(ADP-ribose) synthetase inhibitors and DNA-damaging agents synergistically induce tumors or pretumorous lesions.

The incidences of development of B-cell tumors were lower in alloxan groups than in streptozotocin groups (Table 1). This may reflect some differences in the mode of action between these 2 agents. Alloxan has been shown to cause DNA strand breaks through generation of radical oxygens, especially the hydroxyl radical (26). On the other hand, streptozotocin-induced DNA breaks seem to be mediated by alkylation of DNA bases (2). In addition, it seems possible that alloxan not only promotes DNA strand breaks but also affects B-cell plasma membranes and other cell components (3).

ACKNOWLEDGMENTS

We thank K. Takahashi and T. Sawa for their assistance and N. Tsuneda for typing.

REFERENCES

2. Bennett, R. A., and Pegg, A. E. Alkylation of DNA in rat tissues following...

CANCER RESEARCH VOL. 45 APRIL 1985
1848

Downloaded from cancerres.aacrjournals.org on April 14, 2017. © 1985 American Association for Cancer Research.
Fig. 1. Electron micrographs of tumors induced with streptozotocin or alloxan and poly(ADP-ribose) synthetase inhibitors. Tumor induced with streptozotocin and nicotinamide (a), streptozotocin and picolinamide (b), streptozotocin and 3-aminobenzamide (c), streptozotocin (d), alloxan and nicotinamide (e), alloxan and picolinamide (f), alloxan and 3-aminobenzamide (g) and alloxan (h). Arrows, typical B-granules which have the crystalline core and halo. × 20,000.
Induction of Rat Pancreatic B-Cell Tumors by the Combined Administration of Streptozotocin or Alloxan and Poly(Adenosine Diphosphate Ribose) Synthetase Inhibitors

Takashi Yamagami, Atsuo Miwa, Shin Takasawa, et al.


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/45/4/1845

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.