NOTE
Pancreatic Islet Cell Tumors Found in Rats Given
Alloxan and Nicotinamide¹⁾

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Abstract

Morphological and biochemical studies were performed in pancreatic islet cell tumors found in rats given alloxan (40 mg/kg) and nicotinamide (305 mg/kg). Complete serial sections of the whole pancreas, combined with planimetric analysis, uncovered islet cell tumors in 5 of 7 rats which were killed 10 to 14 months after treatment. Hypoglycemia associated with hyperinsulinemia was found in a rat which developed a tumor which consisted of cells which reacted lightly with aldehyde fuchsin. Another rat developed a gross tumor which was composed of cells stained deeply with aldehyde fuchsin. However, flat insulin response to glucose associated with glucose intolerance was found in this rat. In addition to B cells, a few A and D cells were found within the two tumors.

The present study suggests that pancreatic islet cell tumors found in rats given alloxan and nicotinamide are composed of at least three endocrine cell populations, although the majority of tumor cells are insulin-producing B cells.

We found that pancreatic islet cell tumors can be induced by streptozotocin with and without nicotinamide or picolinamide (Kazumi *et al.*, 1978a; Yoshino *et al.*, 1977). Induced tumors were classified into two groups on the basis of the insulin responses to glucose in their hosts: "responders" characterized by markedly elevated insulin responses to glucose associated with low blood glucose levels and "non-responders" characterized by flat responses associated with glucose intolerance. Rats given combined treatment developed "responders" whereas "non-responders" were found in rats treated with streptozotocin alone (Kazumi *et al.*, 1978b; Kazumi *et al.*,

1979). Alloxan as well as streptozotocin produces diabetes in many species (Rerup, 1970). The present study deals with morphological and biochemical findings of islet cell tumors of the pancreas found in rats given alloxan and nicotinamide.

Materials and Methods

After overnight fasting, 10 male Wistar rats, weighing 178 ± 3 g, were given a single i.v. injection of alloxan monohydrate, 40 mg/kg of body weight, 10 to 15 min after a single i.p. injection of nicotinamide, 305 mg/kg of body weight. The alloxan (Lot M5T8188, Nakarai Chemicals, Kyoto, Japan) and nicotinamide (Lot WTE1323, Nakarai Chemicals) were dissolved in distilled water. Rats were kept in metal cages and were given laboratory chow pellets (Oriental Yeast Co., Tokyo, Japan) and water *ad libitum*. They were monitored for urine glucose by Testape® one week after treatment.

Ten or fourteen months after treatment, their pancreases, kidneys and portions of their hepatic

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Rat	Glycosuria ^{a)}	Survival (months)	Death ^{b)}	Mean area of islets ($\times 10^{-4}$ mm ²)	Pancreatic islet cell tumors
1	+	8	D	not examined	
2	+	8	D	not examined	
3	+	8	D	not examined	
4	++	10	K	52 ± 5 (74) ^{c)}	+
5	+	10	K	33 ± 3 (67)	+
6	++	14	K	27 ± 3 (87)	+
7	—	10	K	29 ± 3 (73)	—
8	—	10	K	39 ± 3 (62)	+
9	—	14	K	60 ± 6 (65)	+
10	—	14	K	27 ± 3 (102)	—

a) examined by Testape 1 week after treatment.

b) D: dead, K: killed.

c) The number in parentheses indicates the number of islets examined.

Table 1. Survival, mean areas of pancreatic islets ($\pm$ SEM) and pancreatic lesions found in rats given alloxan monohydrate (40 mg/kg body weight, iv) with nicotinamide (305 mg/kg body weight, ip).

tissues were dissected and were fixed in Bouin's solution. Paraffin-embedded sections were cut into 5 to 6 μ m thickness and stained routinely with hematoxylin and eosin. Complete serial sections were cut in all of the whole pancreas of 5 untreated, age-adjusted male Wistar rats and experimental animals. Some of the experimental rats were subjected to identical morphological studies after oral glucose tolerance tests (Kazumi *et al.*, 1978b). Blood glucose was measured by the method of Hoffman (1937) using a Technicon Auto-Analyzer. Plasma insulin was assayed by a radioimmunoassay (Morgan and Lazarow, 1963) using rat insulin as a standard. In the two tumors, serial sections (2-3 sections each) were stained with aldehyde fuchsin of Gomori (1964) or with phosphotungstic acid hematoxylin (Levene and Feng, 1964), or were impregnated with the silver method of Hellerström and Hellman (1960) for the demonstration of B, A and D cells, respectively.

Planimetric analysis was performed on at least 5 sections which were selected randomly in different regions from each pancreas. At least 60 islets were photographed and prints were made at a magnification of $\times 800$. The surface area of sectioned islets was measured with a planimeter. Values were expressed not in terms of volume but of area, since islets of experimental animals varied in shape. Because the mean area plus 5 SD ranged from 194 to 226×10^{-4} mm² and none of the 434 islets exceeded 200×10^{-4} mm² in control rats, we judged those above 226×10^{-4} mm² as microtumors.

Statistical analysis was done by Student's *t* test.

Results

Among 10 rats treated, 4 rats were aglycosuric while the other 6 were glycosuric one week after treatment. Survivals, pancreatic lesions and the mean areas ($\pm$ SEM) of islets are summarized in Table 1.

Of the three rats which had been glycosuric and survived for 10 months or longer, one rat (Rat 6) killed at 14 months developed a grossly visible islet cell tumor measuring $2 \times 2 \times 1$ mm in the pancreas. Serial sections uncovered small tumors (one tumor each) in all 3 of them. None of the aglycosuric rats developed grossly visible tumors. However, serial sections uncovered small islet cell tumors in 2 of 4 rats. Rats 8 and 9 had 2 and 5 tumors, respectively. Thus pancreatic islet cell tumors were found in 5 of 7 rats which survived for 10 to 14 months after treatment with alloxan and nicotinamide. The smallest tumor measured 312×10^{-4} mm² and the largest one measured 1.2 mm in diameter. Complete serial sections, however, did not uncover such

Fig. 1. Oral glucose tolerance tests in rats 10 (lines) and 14 months (dashes) after treatment with alloxan and nicotinamide. Mean data ($\pm$ SEM) obtained from 10 untreated rats of the same sex and body weight are indicated by open circles.

tumors in the pancreas of 5 untreated controls.

Planimetric study revealed that mean areas of sectioned islets of control animals ranged from 41 ± 4 to 44 ± 3 (SEM) $\times 10^{-4}$ mm². The majority (98–100%) of islet areas were below 140×10^{-4} mm² and none exceeded 200×10^{-4} mm². Tumor-free rats, Rat 7 and 10, had significantly reduced mean areas compared with controls ($p < 0.025$, and 0.05 , respectively). They showed glucose intolerance associated with flat insulin response to oral glucose loading (Fig. 1). Islets in the pancreases surrounding tumors varied in mean area. The mean area of islets in Rat 6 bearing a grossly visible tumor was significantly less than those of controls ($p < 0.01$), whereas Rat 9 had a significantly increased one ($p > 0.01$).

The tumors varied from round to polyhedral in shape. They were highly vascular and were encapsulated. The small tumors detected by light microscopic examination resembled the normal islet histologic structure. The gross tumor found in Rat 6 was circumscribed by a thin fibrous

capsule. The tumor cells stained deeply with aldehyde-fuchsin (Fig. 2, a) and b)). In addition to B cells, there were a few A and D cells (Fig. 2, c) and d), respectively). Despite intense aldehyde-fuchsin-positive granulation in the tumor, flat and depressed insulin response to glucose was associated with glucose intolerance. The tumor found in Rat 4 contained numerous vascular spaces with frank hemorrhage (Fig. 3). The tumor cells stained lightly with aldehyde fuchsin. This animal showed hypoglycemia (22 mg/100 ml) and hyperinsulinemia (14.12 ng/ml). This neoplasm also contained a few A and D cells.

Neither were ductural structures seen nor mitoses observed within the tumors. Neither capsular nor vascular infiltration by tumor cells was observed.

No grossly visible or microscopic tumoral lesion was found in kidneys, livers or portions of hepatic tissues in any of the rats used in this study.

Fig. 2. Pancreatic islet cell adenoma found in a rat killed 14 months after treatment with alloxan monohydrate (40 mg/kg body weight, iv) and nicotinamide (305 mg/kg body weight, ip).

Fig. 2a. The tumor consisted of aldehyde-fuchsin-positive cells. $\times 87$

Fig. 2b. Higher magnification of the adenoma. $\times 550$ Aldehyde fuchsin

Fig. 2c. Section stained with phosphotungstic acid hematoxylin. $\times 550$

Fig. 2d. Section impregnated with the silver method of Hellerström and Hellman. $\times 550$

Fig. 3. Islet adenoma of the pancreas found in a rat killed 10 months after treatment with alloxan and nicotinamide. Tumor cells stained lightly with aldehyde fuchsin. $\times 87$

Discussion

Alloxan produces diabetes through the destruction of pancreatic B cells in many species (Rerup, 1970). Spontaneous recovery from alloxan diabetes has also been observed in many species. In the course of a long term study on alloxan diabetes in the rat (Lazarow, 1952), spontaneous recovery occurred during the second year of the disease. A beta cell adenoma was found in the pancreas of two of the rats. Because the number of animals treated and survived was not cited and complete serial sections were not employed, the details are obscure. The present study using complete serial sections combined with planimetric analysis reveals that islet cell tumors of the pancreas were found in 5 of 7 rats which survived for 10 to 14 months after treatment with alloxan and nicotinamide. Such tumors were not found in the pancreas of

untreated controls examined in the same manner. Although these results suggest the oncogenicity of alloxan and nicotinamide, further studies are required, because the number of animals used in this study is too small.

The two tumors examined were B cell tumors and appeared to be producing insulin as evidenced by basophilic cytoplasmic granulation and hyperinsulinemia associated with hypoglycemia. In addition to B cells, histochemical examination demonstrated the presence of A and D cells in these tumors, indicating that the neoplasms are multicellular as are human pancreatic endocrine tumors (Creutzfeldt, 1975) and streptozotocin-induced pancreatic islet cell tumors in the rat pancreas (Kazumi *et al.*, 1979). The circumscription of the tumors, the absence of mitotic activity and failure to find vascular invasion of metastasis strongly point toward a benign tumor. The tumor examined, however, failed to display any

morphologically obvious histogenetic relationship to either pancreatic ducts or non-neoplastic islets.

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