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# Streptozotocin Treatment of Streptozotocin-Induced Islet Cell Adenomas in Rats[1] (39275)

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(Introduced by F. G. STANDAERT)

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We have previously reported that the combination of streptozotocin and nicotinamide administered to Holtzman rats can result in the development of pancreatic islet cell adenomas that have the capacity to produce and secrete insulin (1). Streptozotocin has now been identified as an effective chemotherapeutic agent for the treatment of islet cell carcinoma in man (2-6). The present study was undertaken to determine whether streptozotocin could be used to treat rats whose insulinomas had been induced by this agent and to test the feasibility of using this animal model as a predictive system for selecting further therapies for malignant insulinomas in man.

*Methods.* Streptozotocin, Upjohn (glucopyranose, 2-deoxy-2-(3-methyl-3-nitrosoureido)-D-) was dissolved in citric acid buffer (CAS), pH 4.0, and nicotinamide (Matheson, Coleman, and Bell) was dissolved in normal saline. Twenty-two Holtzman rats, which for 1 1/2 yr previously had been treated with a combination of streptozotocin, 50 mg/kg iv × 1 and nicotinamide, 350 mg/kg ip × 2, 10 min before and 180 min after streptozotocin, were used in this study. Blood glucose (7), which had been serially measured over a period of 15-17 months, and the presence of hypoglycemia, defined as a blood glucose of less than 50 mg/ml, were used as the principal indicators of the development of a functioning insulin-producing islet-cell tumor. Pancreatic islet-cell tumor-bearing animals were divided into two groups: Group 1 rats were treated with streptozotocin 50 mg/kg iv every week for 1-8 weeks, total dose 50-400 mg/kg; Group 2 rats, treated with citric acid buffer every week for 2-4 weeks, served as controls. Seventy-two hours following each dose of streptozotocin or CAS buffer, blood glucose was measured in caudal vein blood from fed animals, and prior to sacrifice both immunoreactive insulin, IRI (8), and blood glucose were determined. After sacrifice with sodium nembutal, the islet-cell tumors were resected and rapidly weighed on a Roller-Smith balance. For the measurement of IRI content, 20-50 mg of the tumor were homogenized in 10 ml of 10% NaCl solution and brought to 100 ml with 95% ethanol. The remainder of the tumor was fixed in 10% neutral formalin and later stained for beta granules by the aldehyde-fuchsin method of Gomori (9), and with hematoxylin and eosin. An equivalent sample of uninvolved normal pancreas was similarly fixed and stained, as were the pancreata of control animals.

*Results.* The initial evidence of hypoglycemia appeared between Days 308 and 428 after treatment with the combination of streptozotocin and nicotinamide, whereas control animals maintained a blood glucose within a normal range. Streptozotocin treatment (Table I) resulted in an elevation of blood glucose ($P < 0.01$), reduction in plasma IRI in all tumor-bearing animals so treated, when compared with tumors from CAS-treated controls ($P < 0.1$). The uninvolved normal pancreas was similarly reduced in IRI content after streptozotocin ($P < 0.05$-$0.1$). These hormonal responses

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were correlated with objective histologic changes in the tumors of streptozotocin-treated animals, which showed cellular necrosis, degeneration, and hemorrhage (Figs. 1 and 2).

*Discussion.* Streptozotocin can produce selective necrosis of the pancreatic islet cells and diabetes in rodents, dogs, and monkeys (10–12). Nicotinamide administered in a pharmacologic dose has been demonstrated to protect against the diabetogenic action of streptozotocin (12–13) by preventing the rapid reduction of islet pyridine nucleotide concentrations (14). Beta cells which have

TABLE I

| Treatment | Blood glucose (mg/100 ml) | | | Immunoreactive insulin post-treatment | | |
|---|---|---|---|---|---|---|
| | No. of treatments | Prior | Post-treatment | Plasma ($\mu$V/ml) | Tumor ($\mu$/g) | Uninvolved pancreas ($\mu$/g) |
| Streptozotocin | | | | | | |
| 50 mg/kg/wk, iv | 1 | 41 | 94 | | | |
| | 3 | 21 | 173 | 63 | 14.8 | 0.1 |
| | 4 | 37 | 232 | 23 | | 0.1 |
| | 4 | 17 | 169 | 14 | 1.8 | 0.1 |
| | 8 | 19 | 50 | 26 | 3.2 | 0.1 |
| Mean ± SE | | | 144 ± 32 | 32 ± 11 | 7 ± 4 | 0.1 |
| Citrate buffer | | | | | | |
| 0.025 *M*, iv | 2 | 47 | 22 | 26 | 143 | 0.6 |
| | 2 | 42 | 37 | 144 | 60 | 0.1 |
| | 2 | 39 | 49 | 14 | | 0.2 |
| | 2 | 37 | 41 | 18 | 32 | 2.0 |
| | 4 | 42 | 35 | 28 | 750 | 1.2 |
| Mean ± SE | | | 37 ± 4 | 46 ± 24 | 246 ± 170 | 0.8 ± 0.4 |

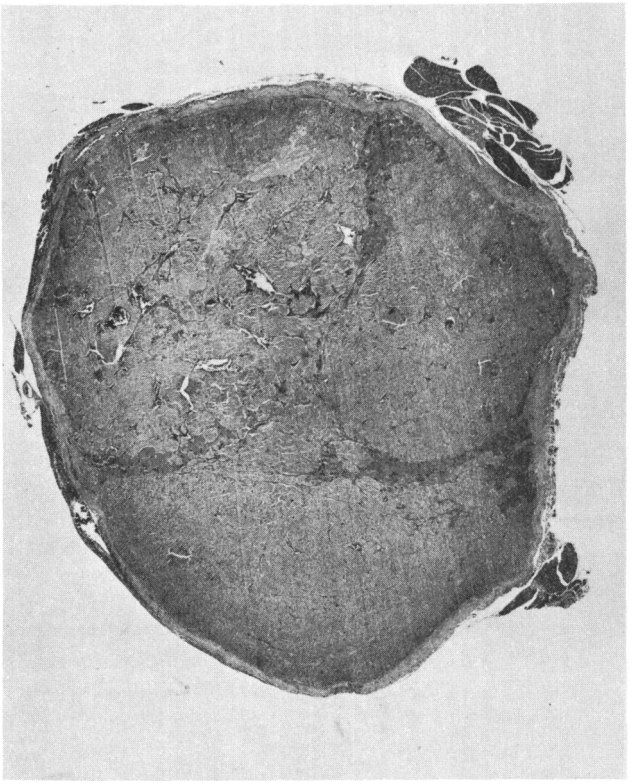

FIG. 1. Untreated pancreatic islet cell tumor in a male Holtzman rat. Hemotoxylin and eosin 16 ×.

FIG. 2. Marked necrosis and hemmorrhage in a pancreatic islet-cell tumor present in a male Holtzman rat treated with streptozotocin. Hemotoxylin and eosin 28 ×.

taken up the drug, but have been protected against destruction by nicotinamide, remain subject to methylation of DNA by the 1-methyl-1-nitrosourea cytotoxic moiety of the streptozotocin molecule (15). Such alkylation, as has been demonstrated for nitrosamines and methyl-1-nitrosourea, has been correlated with neoplastic transformation of the affected tissue (16–17). It is probable that this series of biochemical actions takes place within the beta cells of rats treated with the nicotinamide-streptozotocin combination, and that the chemical transformation is expressed after a long latent period, 1–1$^1/_2$ yr, with the proliferation of neoplastic beta cells. It is of interest that the agent that originally induced this neoplastic transformation retains its ability

to destroy the tumor it has created. This is demonstrated in these studies by the increase in blood glucose, reduction in plasma and tumor IRI, and histopathologic changes in the neoplasms of animals treated with streptozotocin.

This model may lend itself to the investigation of antitumor activity and mechanism of action of potential agents to be used in the treatment of malignant insulinoma in man. Of particular interest will be a future study to determine whether nicotinamide can prevent the antitumor activity of streptozotocin against the rat islet-cell tumor.

*Summary.* Hypoglycemic rats bearing insulin-secreting islet-cell adenomas produced by the combined action of streptozotocin and nicotinamide were treated with streptozotocin. Antitumor response was demonstrated by elevation of blood glucose, reduction in plasma and tumor IRI, and histopathologic changes in the beta-cell neoplasm. The rodent tumor model may serve as a predictive system for selection and investigation of mechanisms of action of future antitumor agents to be used in the treatment of malignant insulinoma in man.

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