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Characterization of pancreatic islet cell tumors and renal tumors induced by a combined treatment of streptozotocin and nicotinamide in male SD rats

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ABSTRACT

We herein investigated the histopathological features, including proliferative activity and immunoexpression, of pancreatic islet cell tumors (ICTs) in male SD rats induced by streptozotocin (STZ) and nicotinamide (NA), and discussed their relevance to biological behaviors and prognoses. A total of 70 and 43% of rats developed ICTs 37–45 weeks after the treatment with STZ (50 or 75 mg/kg, i.v.) and NA (350 mg/kg, twice, p.o.), respectively. Among the islet tumors observed in the STZ/NA-treated groups, 75% were adenomas, while 25% were carcinomas. Most STZ/NA-induced carcinomas were characterized by well-differentiated tumor cells with/without local invasion into the surrounding tissues, and weak proliferative activity. No outcome such as distance metastasis and death was noted. All of the ICTs strongly expressed insulin, part of which had hormone productivity; however there were no hypoglycemia-related clinical signs such as convulsion in these rats 36 weeks after the treatment. These results suggested that rat ICTs induced STZ/NA have small impact on biological activity or prognosis. STZ/NA treatment significantly increased of focal proliferative lesions in the kidney, liver and adrenal glands other than pancreatic islets. Of the STZ/NA-induced kidney tumors, more than 60% were renal cell adenomas, and many of them were basophilic type. The incidence of eosinophilic or clear cell type of tumors was less than 10%, respectively. Immunohistochemical analyses revealed that many of the STZ/NA-induced basophilic type of renal tumors were derived from proximal tubules, whereas the clear cell and eosinophilic types were derived from collecting tubules.

1. Introduction

Islet cell tumors (ICTs) are common age-related lesions in rats, with the incidences of spontaneously arising ICTs in males and female Crl:CD (SD) rats being reported as 8.6% and 3.3%, respectively (Giknis and Clifford, 2013; Greaves, 2012). ICTs are also induced by the administration of chemicals that selectively exert toxic effects on islet cells, such as streptozotocin (STZ) or alloxan (Kazumi et al., 1980, 1978; Masiello et al., 1984; Yamagami et al., 1985). Most ICTs induced by a combined treatment of STZ and nicotinamide (NA) are hormone-producing benign adenomas (insulinoma) delimited by a thin capsule from the surrounding acinar tissue, and their morphological features are similar to spontaneous ICTs (Masiello et al., 1984; Spencer et al., 1986). ICTs are regarded as a continuum of islet cell hyperplasia with progression to adenoma and carcinoma (Rosol et al., 2013). ICTs are classified on the basis of tumor size, cellular pleomorphism, invasion, and metastasis; however, there are no definitive morphological criteria that accurately predict their exact biological nature (Riley et al., 1990).

In humans, ICTs (called pancreatic neuroendocrine tumors [PNETs]) constitute approximately 2% of all pancreatic neoplasms; its annual prevalence has been reported as < 1 per 100,000 worldwide, but is gradually increasing (Yao et al., 2008). Patients with clinical symptoms and elevated plasma hormone levels have been diagnosed with functioning ICTs (Gumbs et al., 2002). In the 2010 WHO classification of digestive neuroendocrine neoplasms, ICTs uniformly composed of cancer cells with a neuroendocrine phenotype are classified as PNETs (Grades 1 and 2) and pancreatic neuroendocrine carcinomas (PNECs, Grade 3) (Klimstra et al., 2010). The grading of PNETs is based on two calculations: (1) the mitotic count and (2) ki-67 labeling index (Klimstra et al., 2010). Ordinary PNETs (G1 and G2) are regarded as low-grade malignancies, with an overall 10-year survival rate of 60–70%; however, PNECs (G3) are clearly more aggressive

Abbreviations: ICTs, islet cell tumors; STZ, streptozotocin; NA, nicotinamide; PNETs, pancreatic neuroendocrine tumors; PNECs, pancreatic neuroendocrine carcinomas; PCNA, proliferating cell nuclear antigen

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Table 1
Design for the carcinogenicity study of STZ and NA in male Sprague-Dawley rats.

Group[a]	No. of animals	STZ (i.v.)		NA (i.p.)	
		Dose level[b] (mg/kg)	Dose concentration (mg/mL)	Dose level[b] (mg/kg)	Dose concentration (mg/mL)
Vehicle controls	20[c]	0	0	0	0
Low dose	30[c]	50	10	350 × 2	70 × 2
High dose	30[c]	75	15	350 × 2	70 × 2

[a] Controls received the vehicle control article [0.1 mol/L-citrate buffer solution (i.v.) and saline (i.p.) twice] only.
[b] Animals were dosed at a volume of 5 mL/kg of both STZ and NA.
[c] Animals were serially sacrificed 37–45 weeks after the treatment.

(median survival of 32 months, 5-year survival rate of 22%) (Reid et al., 2014).

As previously reported, it currently remains unclear whether the histopathological criteria for the malignancies of ICTs in rats reflect their true biological nature, in contrast to those in humans. In the present study, we investigated the histopathological features, including proliferative activity and immunoexpression, of ICTs in male SD rats induced by STZ/NA, and discussed their relevance to biological behaviors and prognoses. The principal aims were to consider the similarity between ICTs in rats and those in humans. We also examined the frequency of these tumors in major organs/tissues other than the pancreas.

2. Materials and methods

2.1. Chemicals, animals, and experimental design

STZ and NA were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Five-week-old male Crl:CD(SD) rats were obtained from Charles River Laboratories Japan, Inc. (Kanagawa, Japan) and were acclimated for 1 week. All animals were housed with a 12-h lighting period and were allowed free access to food (CRF-1, ORIENTAL YEAST CO., LTD.) and water, except when urine was being collected.

The experimental design is shown in Table 1 (Yamagami et al., 1985). At 6 weeks of age, rats were intravenously injected once with STZ (50 or 75 mg/kg) in 0.1 mol/L-citrate buffer solution and intraperitoneally co-administrated NA (350 mg/kg) twice in saline 10 min before and 3 h after the STZ treatment. The day of administration was designated as Day 1. Rats were serially sacrificed between 37 and 45 weeks after the treatment. All experimental procedures conducted in the present study were approved by the Institutional Animal Care and Use Committee.

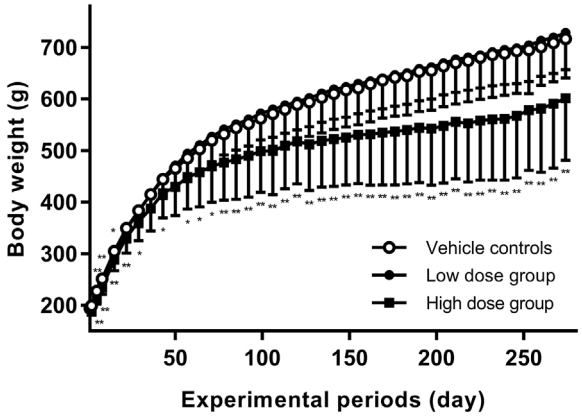

Fig. 1. Effects of STZ/NA on body weight changes in rats administered 0 mg/kg (white circles), 50 mg/kg (black circles), or 75 mg/kg of STZ. Significantly different from vehicle controls (** $p < 0.01$, * $p < 0.05$; Dunnett's 2 Sided).

2.2. Clinical examination

Assessments included clinical observations, body weight measurements, and a urinalysis. Assessments were performed at least once daily during the study period, while body weights were measured on Days 1, 2, and 5 (in Week 1) and then once a week (from Week 2). A urinalysis was conducted on Weeks 4, 12, and 36 with fresh urine to determine general assessment items in the general toxicity study (Tonomura et al., 2011).

2.3. Serum glucose and insulin measurements

Serum glucose and insulin measurements were conducted twice in Week 36 and on each necropsy day under non-fasting conditions. Blood was sampled using a syringe or tube containing heparin sodium from the tail vein under no anesthesia in Week 36 and from the posterior vena cava under isoflurane anesthesia. Plasma was obtained by centrifugation (3000 rpm, 4 °C, 15 min) with a refrigerated centrifuge and then subjected to a glucose analysis using an automatic analyzer (Hitachi 7180; Hitachi High-Technologies Co., Tokyo, Japan) and insulin analysis using an ELISA method with ARVO X3 (PerkinElmer Life and Analytical Sciences, Waltham, MA).

2.4. Histopathology and immunohistochemistry

At necropsy, a comprehensive set of organs/tissues, with the

Table 2
Immunohistochemical methods for the analysis of STZ/NA-induced pancreatic ICTs and kidney tumors.

Antigen	Type of antibody	Manufacturer	Dilution	Antigen retrieval
Insulin	Mouse monoclonal (ab6995)	Abcam[a]	1/1000	Non-treatment (NT)
Somatostatin	Rabbit polyclonal (ab103790)	Abcam[a]	1/4000	Boiled with citrate buffer (pH 6.0)
Glucagon	Mouse monoclonal (ab10988)	Abcam[a]	1/4000	NT
PCNA	Mouse monoclonal (M0879)	Dako[b]	1/200	Boiled with citrate buffer (pH 6.0)
E-Cadherin	Mouse monoclonal (N1620)	Dako[b]	Ready-to-use	Boiled with citrate buffer (pH 6.0)
N-Cadherin	Mouse monoclonal (3B9)	Invitrogen[c]	1/100	Boiled with Tris-EDTA buffer (pH 9.0)
Aquaporin 1	Mouse monoclonal (1/A5F6)	GeneTex[d]	1/1000	Boiled with citrate buffer (pH 6.0)
Aquaporin 2	Rabbit polyclonal (ab85876)	Abcam[a]	1/5000	NT
Dolichos biflorus lectin	HRP-conjugated (H-1201-1)	EY-laboratories[e]	1/20	NT

[a] Abcam Inc., Cambridge, MA.
[b] DAKO, Glostrup, Denmark.
[c] Invitrogen, Carlsbad, CA.
[d] GeneTex, Irvine, CA.
[e] EY-laboratories, San Mateo, CA, USA.