Abstract An improved method of inducing diabetes in dogs was developed. This method included 90% pancreatectomy, 2 mg/kg streptozotocin (STZ) perfused into pancreaticoduodenal artery, and the fixation suture of the duodenum to the costo-abdominal wall. Vasopressin injection administered to the animals before surgery reduced bleeding. All dogs used in this procedure survived and became diabetic. One month after the procedure the pancreatic islets were reduced in volume and the number compared with pancreas tissue obtained during the surgery. Acinar tissue remained with a normal histology, and exocrine function maintained the physiological parameters, except for a soft faecal consistency. We conclude that this procedure is effective in inducing experimental diabetes in dogs.

Key words Pancreatectomy • Streptozotocin • Diabetes • Dogs

Introduction

The experimental diabetic dog model has been used to validate various insulin delivery systems, to test several insulin preparations [1] and to apply pancreatic implant testing models [2]. Some disadvantages of using the dog as a diabetic model may be the cost of the surgery and postoperative care.

Tschoepe et al. [3] reported that the systemic administration of streptozotocin (STZ) (28–38.5 mg/kg body weight, b.w.) into a foreleg vein was not effective in inducing diabetes in beagle dogs, leading to a variety of toxic secondary effects, even when low doses (28 mg/kg b.w.) are used. The authors proposed a combined subtotal pancreatectomy consisting of pancreatic corpus and cauda resection, with a selective 25-mg/kg STZ infusion into the superior pancreaticoduodenal artery. Using this technique, the three studied dogs resulted in a diabetic state, maintaining their initial weight for 4 weeks without any toxic effects. When a selective low STZ dose (2 mg/kg b.w.) infusion into the superior pancreaticoduodenal artery was used, none of the studied dogs became diabetic. Previously, Freyse et al. [4] had reported diabetes induction in 16/55 dogs with 77% pancreatic resection, associated with selective 2 mg/kg of STZ infusion into the superior pancreaticoduodenal artery.

STZ, an antibiotic extracted from Streptomyces achromogenes, is highly toxic and side effects may be shown in the kidney and liver [5]. STZ has a glucose molecule with a highly reactive nitrosourea side chain, which is responsible for the initial cytolysis action to β cells [6]. Recent information described the host DNA alkylation, and the absence of nitric oxide affects radicals in the pathogenesis of β-cell damage after STZ administration [7].

In the present study, we describe a selective application of a low dose of STZ directly to the remnant 10% pancreas tissue, followed by fixation suture of the duodenum to the costo-abdominal wall.
Subjects and methods

Vasopressin (Ferring AB, Malmo, Sweden) (0.3 μg) was administered subcutaneously 3 h before the procedures. Twenty minutes prior to surgery, 2 mg/kg propionil promacin IM (Combelen, Bayer, Mexico DF, Mexico) and 0.1 mg/kg atropine IV were administered to five fox terrier dogs weighing 5–6 kg. Their abdominal skins were shaved and disinfected. Brachial vein was then cannulated with a #23 paediatric catheter and perfused with 25 mg/kg sodium pentobarbital (Anestephorte, Salud y Bienestar Animal, Mexico DF, Mexico) until deep anaesthesia was obtained. A medial laparotomy was performed with an upper incision from the middle of the abdominal rectum muscles to the peritoneum. The tail and the head of the pancreas were surgically excised, and approximately 10% of the corpus of the pancreas with the duct of Wirsung was preserved. The cranial pancreaticoduodenal artery was dissected and canalised with a #23 needle coupled to a plastic catheter and perfused with 2 mg/kg of STZ in 3 ml of saline solution (HPLC 97%; Calbiochem Biosciences Inc., La Jolla, CA, USA). Blood supply was temporarily stopped by an intermediate ligature immediately before venopuncture. A fixation suture of the duodenum to the costo-abdominal wall was made 10 cm from the remnant pancreas tissue. Dog care was maintained with analgesics and antibiotics until complete recuperation. Blood sugar was measured every day for 8 days before and for 1 month after the surgical procedure, using a One-Touch System (Lifescan, Milpitas, CA, USA). When blood sugar values reached 200 dl/ml or more, the dogs were considered clinically diabetic. To keep dog glucose values <200 dl/ml, 20–30 units of human insulin was injected i.m. every 2 or 3 days. After a month of diabetes, a blood sample was obtained from the studied dogs without insulin supply for 5 days (Fig. 1).

Exocrine function maintained the physiological parameters, except for a soft faecal consistency.

The pancreas tissue obtained during the partial pancreatectomy showed a normal pattern of acinar cell groups and islets. Maldonado stain showed pale blue β cells and the β-cell granules deep blue to red. Immunofluorescent stain for anti-pig insulin showed numerous fluorescent cells at the islets (Fig. 2a). Pancreas tissue obtained 1 month after the partial pancreatectomy and studied for histology showed fibroplasia at the periphery and in the septal tissue. The islets were small in volume and few in number, without signs of inflammation. Few islet cells showed red cytoplasmic granules after Maldonado’s stain and anti-insulin fluorescent stain (Fig. 2b). Acinar tissue remained normal in appearance.

Discussion

In this study using our modified technique, all studied dogs survived and developed a hyperglycaemic stage, mimicking diabetes. Tschoepe et al. [3] also obtained 100% (n=3) diabetes-like stage in the dogs when 25 mg of STZ was used but failed to induce hyperglycaemia when 2 mg STZ was employed. On the other hand, Freyse et al. [4] showed that a combination of 77% pancreatectomy and 2 mg STZ selectively applied into the pancreaticoduodenal artery produced hyperglycaemia in 16/55 dogs. Differences