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Short Communications

Failure to Find Amyloidosis in Dogs Treated with Long-Term Intravenous Insulin Delivered by a Totally Implantable Pump

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Summary. We examined tissues of seven non-diabetic mongrel dogs and four diabetic beagle dogs treated with constant insulin infusion via totally implantable pumps for from 210 to 880 days. Kidney and skeletal muscle tissue from all dogs were stained with Congo Red and thioflavin-T and appropriately examined. Kidney tissues from the beagle dogs were examined by electron microscopy. No amyloid deposits were found in any of these tissues. Thus, we cannot confirm an earlier report of amyloid occurring in dogs given long-term intravenous insulin. It is concluded that amyloidosis is not a necessary complication of long-term intravenous insulin infusion in dogs.

Key words: Diabetes, insulin, amyloidosis, dogs, kidney.

New technologies including subcutaneous [1], intravenous [2], or intraperitoneal [3] continuous insulin delivery systems have been shown to improve glycaemic control in diabetes, albeit not when compared with the aggressive application of more conventional treatment with multiple daily insulin injections [4]. The value of any approach to improved glycaemic control will ultimately depend upon may variables, including the influence of the improvement on secondary complications of diabetes, patient acceptance, and long-term safety [5]. Thus, the recent report by Williamson et al. [6] demonstrating secondary amyloidosis in beagle dogs receiving intravenous insulin via mechanical pumps is both timely and disturbing. These workers, using portable insulin pumps and indwelling vena caval catheters in 11 normal and four diabetic beagle dogs over 52–250 days, found large amounts of amyloid in liver, kidney, spleen, and/or pancreas in 60% of the animals whose tissues were examined by Congo Red, fluorescence under polarized light and electron microscopy [6]. This report led us to examine tissues of our dogs under study with totally implantable pumps receiving continuous intravenous insulin infusions of 210–880 days duration. We found no amyloid in these dogs.

Materials and Methods

Animals

Seven intact non-diabetic mongrel dogs (five female, two male) received constant insulin infusions using insulin dosages and insulin media and with durations of infusions as outlined in Table 1. Four uninephrectomized male beagle dogs, made diabetic with alloxan [7], received constant insulin infusion at insulin dosages with durations of infusion as outlined in Table 2.

The severity of the diabetic state produced by our alloxan protocol has been documented [8]. All animals received soluble porcine insulin (U100; Lilly, Indianapolis, Indiana).

Pumps

Infusaid TM Model 200 pumps [2, 8] were used in these studies. Of note is the presence of a 0.22-μm filter in the flow path of the pump [8]. The beagles had pumps modified for transcutaneous control of pre-meal insulin boluses by the attachment of a magnetically activated valve. The pumps were implanted in the iliac fossa [8] and cannulae threaded via the deep circumflex iliac vein into the inferior vena cava or via a mesenteric vein into the portal venous system.

Insulin

All animals received regular porcine insulin (U100; Lilly, Indianapolis, Indiana) via implantable pumps (see below). The bioavailability of the insulin prepared in 1% sodium dodecyl sulphate (Table 1) has been demonstrated in diabetic dogs [8] while that of insulin prepared in glycerol (Table 1) has been shown in both dogs [9] and man [2, 10]. The average daily insulin requirement to maintain strict glycaemic control in diabetic dogs treated by insulin pumps tended to be lower than in dogs treated with subcutaneous insulin [11].

Tissue Studies

Kidney biopsies were obtained in all animals as described [12]. Skeletal muscle (abdominal wall) was obtained by open biopsy. Tissues of the mongrel dogs were fixed in 10% buffered formalin for embedding

Table 1. Constant insulin infusions in mongrel dogs

Dog No.	Insulin dose ($U \cdot kg^{-1} \cdot day^{-1}$)	Infusion medium	Duration (days)	Duration (total days)
1	0.5	50% glycerol	84	334
		70% glycerol	40	
		70% glycerol + 0.21% $NaHCO_3$	210	
2	0.5	80% glycerol	80	380
		80% glycerol + 0.21% $NaHCO_3$	300	
3	0.5	40% glycerol	233	404
		40% glycerol + 0.21% $NaHCO_3$	171	
4	0.8	80% glycerol		630
5	0.5	1% sodium dodecyl sulphate		884
6	0.5	1% sodium dodecyl sulphate		750
7	0.5	1% sodium dodecyl sulphate		780

Table 2. Constant insulin infusions

Dog No.	Insulin dose ($U \cdot kg^{-1} \cdot day^{-1}$)	Infusion medium	Duration (days)
B-46	0.32	Glycerol	471
B-50	1.8	Glycerol	352
B-51	0.34	Glycerol	530
B-52	2.1	Glycerol	480

Insulin dose represents sum of constant insulin infusion and bolus delivered by transcutaneous magnetic activation of flow regulator value resulting in increased pump flow rate at meal-times. Animals B-50 and B-52 received an average of 1.5 and 1.3 $U \cdot kg^{-1} \cdot day^{-1}$ of insulin as meal-time boluses each day. Animals B-46 and B-51 did not require bolus insulin to maintain near normoglycaemia

in paraffin and sectioning by standard histological techniques. Sections were stained with Congo Red and with thioflavin-T [13] and examined by polarized light and fluorescence microscopy, respectively. Kidney and skeletal muscle biopsy tissues from the four beagle dogs were snap frozen in isopentane, precooled in liquid nitrogen, sectioned at 4 μ in a Lipshaw cryostat (Lipshaw, Detroit, Michigan) and stained with Congo Red and thioflavin-T and examined as above. Sections of renal tissue from a patient with proven amyloidosis were processed in parallel with the dog tissues and served as positive controls.

In addition, renal biopsies of the four beagle dogs were fixed in 2.5% gluteraldehyde in cacodylate buffer, 0.17 mol/l, post-fixed in 1% osmium and cacodylate buffer, embedded in Polybed 812 (Polysciences, Warrington, Pennsylvania), sectioned at 75 nm, stained, and examined with a JEOL 100 CX electron microscope (JEOL, Medford, Massachusetts) for the presence of amyloid fibrils [14].

Results

The renal tissue from the patient with amyloidosis showed large quantities of amyloid in renal arterial and arteriolar walls by both Congo Red and thioflavin-T. No amyloid was found in any of the dog tissues examined by these techniques.

Electron microscopy of renal tissues from the beagles revealed no glomerular, vascular or other deposition of the characteristic long 100–500 Å fibrils of amyloid [14].

Discussion

Long-term intravenous administration of massive quantities of insulin is presently necessary in a few patients with marked insulin resistance (Brown DM, personal communication). Further, certain developing technologies of insulin delivery are predicated on the hypothesis that long-term intravenous insulin administration in usual therapeutic dose is safe [2]. Thus, the report of Williamson et al. that the majority of beagles receiving intravenous insulin via mechanical pumps develop multisystem amyloid deposition within approximately 2–10 months is of great potential importance and stimulated the present studies [6]. We were unable to confirm this finding in animals treated with continuous intravenous insulin infusions in doses comparable to those used by Williamson et al. and durations of infusion up to 3.5 times those reported by these workers [6]. Koivisto et al. [15] did not find increased levels of serum amyloid A protein in Type 1 diabetic patients treated with continuous subcutaneous insulin infusion for up to 40 months. Nor did they find amyloid in subcutaneous adipose tissue obtained from infusion sites.

It is known that fibrils which have ultrastructural characteristics bearing striking resemblance to native human amyloid fibrils may develop if insulin is treated with hydrochloric acid (pH 2.0) and heated at 80 °C [16]. It is most unlikely that similar conditions could develop in insulin pump systems. However, spontaneous insulin aggregation was noted in the pumps used in the report of Williamson et al. [6] (Albisser AM, personal communication). Perhaps more important, there was no filter in the infusion pathway for insulin delivery in the dogs reported by Williamson et al. [6] (Albisser AM, personal communication).

The Infusaid pump used in the present studies has a 0.22-μm filter in the flow path. Further, nephelometry showed no evidence of aggregation in solutions withdrawn from these pumps after 1 or 2 weeks (unpublished data). One additional and potentially important methodological difference between the present studies and those of the Toronto group is our use of a totally implantable pump compared with that used in the dogs developing amyloidosis, a portable pump requiring a cannula to traverse the skin barrier to infection. This is of particular importance when it is considered that the Toronto group's dogs were found to have massive amyloid deposits after as little as 17 mg of insulin administered over 52 days [17], indicating that the phenomenon described by Williamson et al. [6] could not be due to insulin aggregates alone. The elucidation of the pathogen-

esis of amyloidosis in animals receiving intravenous insulin is of obvious importance. However, it is clear from this report that amyloidosis is not a necessary consequence of long-term intravenous insulin infusion.

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