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Is human insulin without effect in the diabetic rat?

Dear Sir,

It was with great interest that I read the paper by Soulis-Liparota et al. [1], not only because it contains fine experimental work but also because I wonder if they had the same experience as I had recently. Blood glucose levels in their rats were above 30 mmol/l despite treatment with 2 IU ultralente insulin. In a paper by Rasch [2] a similar insulin dose kept the blood glucose levels below 20 mmol/l. I would like the authors' opinion as to whether they think the type of insulin given had any effect on blood glucose level. I have recently found it impossible to purchase non-human insulin. In the work by Rasch [2] the most efficient insulin was bovine ultralente (Novo Nordisk, Copenhagen, Denmark). I used this insulin for maintaining a blood glucose level of around 20 mmol/l (Fig. 1). Diabetes mellitus was induced with an intraperitoneal injection of streptozotocin (90 mg/kg) in 24 male Sprague-Dawley rats (weight 140 g). Up to a diabetes duration of 33 days their blood glucose level was kept at 19 mmol/l with a mean dose of one IU of bovine insulin given once a day. Thereafter, 18 animals were changed from bovine to human insulin (Ultratard HM 40 IU/

ml; Novo Nordisk). Despite successively increasing the insulin dose to 15 IU per day it was not possible to lower the blood glucose levels below 22 mmol/l. In contrast the blood glucose level in the six bovine-insulin-treated rats was maintained with a dose of 2 IU per day.

The experiment leads to the following questions: is it possible to purchase non-human insulin? Is the human insulin without effect in the rat?

Yours sincerely,
O. Torffvit

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Fig. 1. Insulin doses (IU/day) in insulin-treated diabetic rats. Bovine = Bovine ultralente treated rats ($n = 6$) and bovine + human = initially for 33 days bovine-insulin-treated rats, thereafter switched to human insulin (Ultratard)

Response from the authors

Dear Sir,

We thank Dr. Torffvit for his query regarding the efficacy of different forms of insulin in the rat. Our own study has not specifically addressed this issue. However, over the last 10 years, we have used different doses of both bovine and human ultralente insulins in rats with both short- and long-term experimental diabetes. We were unable to locate a direct comparison of bovine and human insulin in rats although this issue has been addressed in a range of human studies. These studies have suggested that human ultralente insulin, although similar in potency to bovine insulin, has a shorter duration of action in man [1, 2].

In our previous animal studies, we used heat-treated bovine Ultralente insulin and as noted by Dr. Torffvit, we achieved blood glucose levels of less than 22 mmol/l with a dose of 2 IU/day [3]. However, as indicated previously, there was a heterogeneous response among the diabetic rats. In our more recent studies, using human ultralente insulin, the dose of 2 IU/day was not associated with blood glucose levels greater than 20 mmol/l [4]. However, the possibility that human insulin is ineffective in rats is unlikely since in a recent study using human insulin administered via silastic implants, we were able to achieve euglycaemia [5].

Another factor which needs to be considered relates to the severity of the diabetes that is induced by streptozotocin. This relates not only to the susceptibility of the individual rat strain to streptozotocin but also to the dose and mode of administration of streptozotocin [6]. In our studies, streptozotocin was given at a dose of about 45–60 mg/kg whereas Dr. Torffvit gave a

much higher dose of 90 mg/kg. Our own experience involving serial sampling of diabetic animals for blood glucose levels has indicated marked temporal variability of glucose control within individual animals. This emphasises the importance of using glycated haemoglobin in long-term studies in diabetic rats. Finally, it is possible to obtain both bovine insulin (Ultralente MC beef) and human insulin (Ultratard Human Mono-component) from Novo Nordisk (Copenhagen, Denmark).

Yours sincerely,

T. Soulis-Liparota, M. Cooper, M. Dunlop, G. Jerums

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Class I HLA is associated with age-at-onset of IDDM, while class II HLA confers susceptibility to IDDM

Dear Sir,

Among patients with insulin-dependent diabetes mellitus (IDDM), heterogeneity is observed for age of the disease onset. Previous reports in different ethnic groups suggested that the heterogeneity in age-at-onset of IDDM may be determined by gene(s) in the HLA region [1–5]. To investigate the relative contribution of HLA loci to genetic susceptibility to IDDM and to heterogeneity in IDDM, we typed 10 loci in the HLA region, i.e. HLA-A, B, C, DR, DQA1, DQB1, TAP1, TAP2, LMP2 and HSP70-2 genes.

Eighty-one unrelated patients with IDDM and 71 healthy control subjects were studied. Mean $\pm$ SD age-at-onset of the disease was 20.8 ± 13.8 years (range: 0–59 years). The patients were divided into tertiles according to their age-at-onset of IDDM, such that each group contained an equal number of pa-

tients: early-onset (0–12 years), intermediate-onset (13–24 years), and late-onset (> 25 years). HLA-A, HLA-B, HLA-C and HLA-DR antigens were serologically typed. HLA-DQA1 and DQB1 alleles and polymorphism of TAP1, TAP2 and LMP2 genes were determined by PCR-RFLP methods as reported previously [1, 6, 7]. A Pst I polymorphism of heat shock protein (HSP) 70-2 gene was examined by the Southern blot hybridization method with probe (American Type Culture Collection, Rockville, Md., USA) [8]. The significance of the difference in allele frequencies was determined by the chi-square test or Fisher's exact test, with corrected p values (p_c).

As reported previously [1], susceptibility to IDDM was strongly associated with class II HLA loci. HLA-DR9, DQA1*0301, DQB1*0303 and DQB1*0401 were positively associated with IDDM, whereas HLA-DR2, DQA1*01, DQA1*0103, DQB1*0501 and DQB1*0601 were negatively associated. In contrast to the strong association of class II alleles with disease susceptibility, class II alleles showed little effect on the age of disease onset. Instead, class I loci were strongly associated with the age-at-onset of IDDM. Significant heterogeneity was observed in the distribution of B7 and Cw7 alleles. The frequency of B7 was highest in early-onset patients (35%), was 4.4% in the intermediate age-at-onset group, and none of the late-onset patients had this allele ($\chi^2 = 15.0$, 2 degrees of freedom [df], uncorrected $p = 0.0006$,

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