THE EXACERBATING EFFECT OF INSULIN-INDUCED HYPOGLYCEMIA ON SPONTANEOUS PERIPHERAL NEUROPATHY IN AGED B6C3F1 MICE

Hisashi IKEGAMI, Hajime TABATA, Toshiaki MATSUZAWA and Hiroshi SUZUKI

Safety Research Laboratories,
Yamanouchi Pharmaceutical Co., Ltd.,
1-1-8 Azusawa, Itabashi-ku, Tokyo 174-8511, Japan

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ABSTRACT — The effect of insulin-induced hypoglycemia on spontaneous peripheral neuropathy in aged mice was examined. Ninety-five-week-old female B6C3F1 mice were infused subcutaneously for 2 weeks with 40 or 80 IU/kg/day of insulin with a micro osmotic pump. Blood glucose level was decreased during the infusion (4.3-6.8 mmol/L in mice receiving 40 IU/kg/day of insulin or 2.4-5.4 mmol/L in mice receiving 80 IU/kg/day of insulin versus 6.5-7.6 mmol/L in control mice). In histopathological examination, axonal degeneration and/or remyelination were observed in a small number of nerve fibers of control mice. Similar nerve fiber lesions were observed in mice receiving 40 IU/kg/day of insulin, whereas severer lesions with an increase in segmental axonal degeneration of nerve fibers were observed in 47 mice receiving 80 IU/kg/day of insulin. These findings suggest that spontaneous peripheral neuropathy in aged mice is exacerbated by sustained hypoglycemia induced by insulin treatment.

KEY WORDS: Peripheral neuropathy, Hypoglycemia, Insulin, Mice

INTRODUCTION

Hypoglycemic peripheral neuropathy in humans has been shown to be associated with hypoglycemia due to insulinoma (Jaspau et al., 1982; Jayasinghe et al., 1983). The reports on insulin-induced hypoglycemic neuropathy in rats have shown that a morphological feature of hypoglycemic neuropathy is axonal degeneration with ovoid formation, and that sustained and severe hypoglycemia is necessary to induce the neuropathy (Sidenius and Jakobsen, 1983; Potter et al., 1988; Yasaki and Dyck, 1990; Yasaki and Dyck, 1991). Hypoglycemic neuropathy in mice has also been reported in cases of insulinoma (Dyer and Messing, 1989), but there are very few experimental studies to evaluate the correlation between the neuropathy and the severity of hypoglycemia.

Spontaneous peripheral neuropathy is observed in aged rodents, and the incidence and severity of the neuropathy increase with age (Thomas et al., 1980; Krinke et al., 1981; Mitsumori et al., 1981; Mitsumori et al., 1986; Majeed, 1992a; Majeed, 1992b). The most characteristic morphological feature of the peripheral neuropathy is axonal atrophy with dilatation of the myelin sheath in the proximal part of the nerve fiber and axonal degeneration with ovoid formation in the distal part. There are interstrain differences in the incidence, and B6C3F1 mice, a hybrid strain between the C57BL and C3H strains, show a high incidence of the neuropathy (71% in the sciatic nerve of male mice aged 80 to 104 weeks and 89% in female) (Majeed, 1992b).

The present study was carried out to study the effect of insulin-induced hypoglycemia on spontaneous peripheral neuropathy using aged female mice of 95 weeks.

MATERIALS AND METHODS

Animals

Nine-week-old female B6C3F1 mice were purchased from Charles River Japan Inc. (Kanagawa, Japan) and acclimated until 95 weeks of age. During the
acclimation and the experiment, animals were maintained individually in a hanging stainless steel wire-bottomed cage (16W×23L×13Hcm) in an animal room under controlled conditions (temperature: 23±3°C, humidity: 55±10%, lighting: 13hr (8:00-21:00), and ventilation: 20 times an hr) and fed pelleted diet (CRF-1, Oriental Yeast Co., Ltd., Tokyo, Japan) and tap water ad libitum.

Animals were observed daily for clinical signs. Body weight and food consumption were measured weekly to assess the general health of the animals. The rectal temperature was also measured weekly using a Termo-Finer CTM-303 electronic thermometer (Termo Corporation; Tokyo, Japan).

**Insulin treatment**

Bovine insulin was obtained from Wako Pure Chemical Industries Co., Ltd. (Osaka, Japan). It was diluted in distilled water with a small amount of HCl (0.1 N), and stuffed in a micro osmotic pump (Alza Corp., California, U.S.A), which continuously delivers the solution at the rate of 0.5μL. The vehicle pumps, stuffed with distilled water with the same amount of HCl (0.1 N), were prepared for the control mice. The pumps were implanted subcutaneously on the backs of mice. The mice were infused with 40 or 80 IU/kg/day of insulin or vehicle for two weeks. The pumps were replaced on Day 5 and 10 to maintain the appropriate blood glucose levels.

During the experiment, two control mice and one mouse receiving 40 IU/kg/day of insulin died due to abdominal tumor masses and three mice receiving 80 IU/kg/day of insulin died due to exhaustion associated with hypoglycemia; they were excluded from the study.

**Blood glucose**

Blood samples were collected daily from incisions (1 mm) at the tip of the tail at 9:00 during the insulin-treatment. Blood glucose levels were measured by the immobilized enzyme membrane/H2O2 method with a compact glucose analyzer “Antsense (Miles-Sankyo Co., Ltd., Tokyo, Japan)”.

**Histopathology**

At necropsy, the animals were perfused under ether anesthesia through the left ventricle with saline followed by 0.1 M phosphate-buffered 4% paraformaldehyde. The femoral part of the sciatic nerve and the brachial plexus were post-fixed in the same fixative, and embedded longitudinally in paraffin or water-soluble plastic resin (historesin plus, Leica instruments GmbH, Baden-Wüttemberg, Germany), sectioned at a thickness of 4 or 2 μm, respectively. The sections were stained with hematoxylin and eosin (H&E) or hematoxylin, eosin and Luxol fast blue (H&E-LFB) for light microscopic observation.

The numbers of nerve fiber lesions (segmental axonal degeneration) per 1 mm² of the longitudinal paraffin sections were counted. We considered this method reliable enough to quantitatively evaluate the lesions, since the lesions were segmental and the numbers of lesions were relatively few (0-38.1 per 1 mm² of tissue). The areas of the nerve tissues were measured with a morphometrical analyzer (LUZEX3U, Nikon Corp., Tokyo, Japan).

Additional samples of the sciatic nerve were fixed in 0.1 M phosphate-buffered 4% paraformaldehyde, post-fixed in 0.1 M phosphate-buffered 1% osmium tetroxide, and immersed in glycerol for teasing.

**Statistical analysis**

Body weight, food consumption, rectal temperature, blood glucose and numbers of segmental axonal degeneration per 1 mm² per group are expressed as the mean with the standard errors. The one-way analysis of variance (ANOVA) technique was used with Turkey-Kramer’s procedure (Turkey, 1949; Kramer, 1956).

**RESULT AND DISCUSSION**

**Clinical signs**

Insulin-treated mice intermittently exhibited decreased locomotor activities and a hunched posture. Although the mice receiving 80 IU/kg/day additionally exhibited hypothermia and a decrease in food consumption, no significant change was seen in body weights (Table 1). Concerning the observations, it has been reported that insulin causes hypothermia with sedation (Kawasaki et al., 1983a; Kawasaki et al., 1983b).

**Blood glucose**

The mean blood glucose levels in mice treated with insulin were significantly lower than those in control mice (4.3-6.8 mmol/L in mice with 40 IU/kg/day of insulin or 2.4-5.4 mmol/L in mice with 80 IU/kg/day of insulin versus 6.5-7.6 mmol/L in control mice, Fig.1). Further, hypoglycemia (less than 2.0 mmol/L) was frequently observed in all the mice receiving 80 IU/kg/day of insulin, although rarely observed in mice receiving 40 IU/kg/day of insulin. However, transient increases in blood glucose (more than 8 mmol/L) were occasionally
observed in mice receiving 80 IU/kg/day of insulin, which might be a rebound such as a Somogyi phenomenon (Gerich, 1988). The phenomena were not observed in mice receiving low doses of insulin.

**Histopathology and nerve teasing**

In control mice, as shown in Photo 1, peripheral nerve lesions were observed in the sciatic nerve and brachial plexus. The lesions consisted of segmental axonal degeneration with ovoid formation, which was eosinophilic and positive for Luxol fast blue, and segmental remyelination characterized by thinning of the myelin sheath and Schwann cell proliferation. The segmental axonal degeneration was observed in a small number of nerve fibers, 1.6 ± 0.4 / mm² in the sciatic nerve and 1.6 ± 0.3 / mm² in the brachial plexus (Fig 2).

Similar peripheral nerve lesions were observed in the sciatic nerve and brachial plexus in mice receiving 40 IU/kg/day of insulin. The frequency of segmental axonal degeneration was similar to that in control mice, 2.2 ± 0.4 / mm² in the sciatic nerve and 0.9 ± 0.2 / mm² in the brachial plexus.

When the mice were given a high dose of insulin (80 IU/kg/day), an increase in segmental axonal degeneration was observed in the sciatic nerve of mice with a mean of 17.3 ± 6.2 / mm² (Photo. 2&3). In fact, 4 mice out of 7 showed severer lesions compared to those in control mice. In the brachial plexus, the frequency of the lesions was slightly increased (2.4 ± 0.9 / mm²), but one mouse showed severe lesions.

Examination of the teased sciatic nerve fibers showed segmental demyelination and myelin degradation with ovoid formation, which was positive for osmium (Photo.3, insert). There were no morphologic differences in the lesions between the insulin-treated and control mice.

The present study demonstrates that insulin-induced hypoglycemia exacerbates the spontaneous peripheral neuropathy in aged B6C3F1 mice. In the affected peripheral nerve, segmental axonal degeneration was increased in number in comparison to the aged control mice, although there were no qualitative morphologic differences in the axonal degeneration between the hypoglycemic mice and the aged control. The ovoid

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<th>Table 1. Body weight, food consumption and rectal temperature of 97-week-old mice treated subcutaneously with insulin 2 weeks.</th>
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<td><strong>Vehicle</strong></td>
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<td>Body weight (g)</td>
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<td>Rectal temperature (℃)</td>
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**p<0.01, significantly different from vehicle control. Mean±S.E.**

**Fig. 1.** Daily blood glucose levels in aged female B6C3F1 mice treated subcutaneously with insulin for 2 weeks. *p<0.05, **p<0.01 (Mean±S.E.).

C: Vehicle control; △: Insulin infusion 40 IU/kg/day; ▼: Insulin infusion 80 IU/kg/day.

**Fig. 2.** Frequency of segmental axonal degeneration in the peripheral nerve of aged female B6C3F1 mice treated subcutaneously with insulin for 2 weeks.

○: Sciatic nerve; ◊: Brachial plexus.
Photo 1. Sciatic nerve in the control mouse. Segmental axonal degeneration of nerve fibers (a) and Schwann cell proliferation (b). LFB & H&E stain. Bar: 100 μm.

Peripheral Neuropathy in hypoglycemic aged mice.


formation was observed in axonal degeneration in both paraffin-embedded tissue and teased nerve of control and insulin-treated mice. It is observed in many cases of axonal degeneration, and is thought to emerge as a result of collapse of the myelin sheath (Dyck et al., 1993) Remyelination is known to be an event in axonal damage repair. In this study, it was observed in a few nerve fibers of control and insulin-treated mice, and is thought to have occurred as a result of spontaneous peripheral neuropathy rather than hypoglycemic neuropathy.

A certain degree of hypoglycemia is necessary to aggravate the peripheral nerve lesions. The peripheral nerve lesions were aggravated in some mice receiving 80 IU/kg/day of insulin which were frequently accompanied by severe hypoglycemia (less than 2.0 mmol/L). In contrast, the lesions were not exacerbated in mice receiving 40 IU/kg/day of insulin which rarely showed severe hypoglycemia. Therefore, it is conceivable that hypoglycemia (less than 2.0 mmol/L) is necessary to aggravate the peripheral neuropathy in aged mice. Similar results were reported with a significant correlation between episodes of hypoglycemia (less than 2 mmol/L) and prevalence of the nerve lesions in young adult rats (Potter et al., 1988).

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