Ultrastructural Changes in Mouse Leydig Cells after Streptozocin Administration

Roberto E. SANGUINETTI\(^{1(2)},\) Kenji OGAWA\(^1\),
Masamichi KUROHMARU\(^1\), and Yoshihiro HAYASHI\(^1\)

\(^{1}\)Department of Veterinary Anatomy, Faculty of Agriculture, the University of Tokyo, Bunkyo-ku, Tokyo 113, Japan and \(^{2}\)Present address: Institute of Anatomy, Faculty of Veterinary Science, La Plata University, 60 y 118 La Plata (1900), Buenos Aires, Argentina

Abstract: Male ICR mice were intraperitoneally injected with a single dose (100 mg/kg BW) of streptozocin (STZ). The pancreas and testis were excised at 1, 2 or 4 weeks after STZ administration and observed by light and electron microscopy. At 2 weeks after injection (p.i.), the islets of Langerhans in the pancreas showed severe atrophy, indicating a diabetic state. In the testis, although no conspicuous morphological changes were detected at 2 weeks p.i., noticeable changes had occurred in the Leydig cells at 4 weeks p.i. In the cells, lipid droplets increased in number, whereas smooth endoplasmic reticulum (sER) decreased. Giant whorl-like sER appeared frequently at this time. These findings indicate the declined secretory activity of Leydig cells in STZ diabetic mice.

Key words: Leydig cell, mouse, streptozocin, ultrastructural changes

Administration of streptozocin (STZ) induces diabetes by selective destruction of islet B cells. It is well known that experimental diabetes caused by STZ results in impaired reproductive function [2, 4–7]. The testicular dysfunction in STZ-diabetic rats is thought to be caused by the disturbance of pituitary LH secretion and a subsequent notable decrease in testosterone secretion from Leydig cells [1, 4, 5]. In fact, ultrastructural changes, such as the accumulation of lipid droplets, reduced smooth endoplasmic reticulum (sER) and appearance of myelin-like structures, were observed in the Leydig cells of STZ-diabetic rats [3, 8, 9]. To the best of our knowledge, however, no morphological studies have been carried out on the Leydig cells of STZ-diabetic mice. In the present study, ultrastructural changes in mouse Leydig cells were investigated at 1, 2 and 4 weeks after STZ injection (p.i.) and compared with those in rat Leydig cells.

Twenty-two male ICR mice (8 weeks old) were used in this study. Thirteen animals were intraperitoneally injected with a single dose of streptozocin (STZ: 100 mg/kg BW, diluted in 0.1 M citrate buffer, pH 4.5). The animals were sacrificed at 1 (4 mice), 2 (4 mice) and 4 (5 mice) weeks p.i. As controls, six animals were intraperitoneally injected with the buffer solution only and sacrificed 4 weeks later. For light microscopy, the animals were perfused with Bouin’s fixative after brief washing with 0.9% saline. The excised organs (testis and pancreas) were sliced, dehydrated in an ascending series of ethanol and embedded in paraffin. Six μm sections were stained with PAS-hematoxylin and observed with a light microscope. For transmission elec-
Fig. 1. A transmission electron micrograph of a mouse Leydig cell at 2 weeks after streptozocin injection. A number of lipid droplets (asterisks) are observed within the Leydig cell cytoplasm. arrows: mitochondria, G: Golgi complex, N: nucleus (× 17,000).

Throughout the period, exocrine cells of STZ-mice were normal. No apparent morphological changes were observed in the pancreas of the control mice.

The size of the testis decreased to approximately 75% of that of normal mice at 2 weeks and to almost 60% at 4 weeks p.i. In control mice, Leydig cells contained small amounts of lipid droplets, a quantity of sER, some mitochondria with tubular cristae and a well-developed Golgi complex. At 1 week p.i., no morphological changes were detected in Leydig cells of STZ mice. At 2 weeks p.i., the Leydig cells appeared similar to those in the control mice although the number of Leydig cell was slightly increased. Active spermatogenesis was still recognized, and no conspicuous changes were observed in the seminiferous epithelium. At 4 weeks p.i., lipid droplets in the Leydig cells showed a tendency to increase in number. Although normal sER decreased in amount to a degree, a giant whorl-like sER was frequently found within the Leydig cell cytoplasm. Mitochondria showed no obvious changes. At this time, spermatogenesis was still active, although degenerating germ cells were frequently encountered.

Previous studies [3, 9] showed marked ultrastruc-
nural changes in the Leydig cells of STZ-treated rats at 2 weeks after injection. In this study, however, only small changes were detected in the cells of STZ-treated mice at 2 weeks p.i. Thereafter, some of the changes became apparent at 4 weeks p.i. The mice used in this study were given a higher dose (100 mg/kg) of STZ than rats (65 mg/kg) [3, 9]. Mouse Leydig cells would therefore be less sensitive to STZ than those of rats.

Lipid droplets that were absent from the Leydig cells of normal rats appeared after STZ administration [3, 8, 9]. But lipid droplets are seen in normal mouse Leydig cells. At 4 weeks p.i., the number of lipid droplets increased in STZ mice, indicating the accumulation of cholesterol esters for testosterone synthesis; the amount of sER decreased and giant whorl-like sER which may be an inactive form of sER, appeared frequently within the Leydig cell cytoplasm. These findings indicate declined testosterone secretion in Leydig cells. Although it remains unclear how insulin insufficiency causes the changes in the hypophysial-testis axis in the diabetic animal, the declined secretion of testosterone is thought to result from a partial blockage of the secretion of LH [5]. The present study demonstrated that the same phenomenon also occurs in STZ-treated diabetic mice.

It was conclusively revealed that the mouse Leydig cell is, at least morphologically, inactive 4 weeks after STZ injection.

References