Effect of Insulin on Testicular Alterations in the Nonobese Diabetic Mouse*

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ABSTRACT

Severe spermatogenic alterations occur in association with diabetic manifestations in the nonobese diabetic (NOD) mouse. A study was undertaken to determine whether or not administration of insulin during initial appearance of diabetic changes could inhibit the interference with spermatogenesis. Male NOD mice injected with cyclophosphamide to promote onset of overt diabetes were divided into insulin-treated and nontreated groups. Testicular specimens were then examined by light and electron microscopy. Insulin-treated animals showed variable changes ranging from normal spermatogenesis to moderate to severe alterations. Animals with diabetes that did not receive insulin exhibited extensive spermatogenic disruption. The findings indicate a blunting of testicular damage when insulin is administered early in the development of diabetic manifestations. Although spermatogenic abnormalities could not be prevented entirely by insulin treatment, the results provide evidence for a direct metabolic effect on the pathogenesis of the testicular alterations.

Introduction

Effects on the male reproductive system in diabetes mellitus include impotence resulting from neurovascular complications and testicular alterations associated with defective spermatogenesis. There are few critical studies of the significance of the testicular damage. Early clinical investigations reported reduced testicular volume, sperm output, and semen volume in male diabetics, but this has not been a consistent finding. More recently, observations from testicular biopsies in oligospermic men with diabetes indicated a variety of abnormalities affecting germ cells, Sertoli cells, the tubular boundary tissue, and vascular structures in the interstitial compartment.

Studies on testicular alterations in diabetic animal models have been performed in the alloxan-diabetic rat, the streptozotocin-diabetic rat, the spontaneously diabetic BB Wistar rat, the spontaneously diabetic Chinese hamster and in mice with various forms of hereditary diabetes and obesity. The changes are varied, with effects on germ cells, peritubular tissue and Leydig cells.

A model of special interest is the nonobese diabetic (NOD) mouse, first introduced in Japan in 1980. The NOD mouse develops diabetes spontaneously

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on an apparently autoimmune basis and thus resembles human type I insulin-dependent diabetes mellitus. Although not all NOD mice become overtly diabetic, more than 95 percent of male and female mice display lymphocytic infiltration within their pancreatic islets after puberty. Diabetic manifestations are first observed at 12 weeks, and thereafter the number of mice with insulin-dependent diabetes increases to reach a peak at 28 weeks.\textsuperscript{15}

Previously a report was made of the presence of severe testicular alterations in the NOD mouse.\textsuperscript{16} The principal changes were in the seminiferous tubules, including extensive germ cell degeneration, disruption of spermatogenesis, tubular fibrosis, and calcification. Leydig cells had a normal appearance, and no vascular abnormalities were present. Although it was initially suspected that the testicular damage would be on an autoimmune basis, there was no evidence of any mononuclear cell infiltration. Consequently, the factors responsible for the spermatogenic disruption were unclear.

With a view to investigating the possible role of hyperglycemia in the development of testicular alterations in the NOD mouse, the present study was carried out to determine whether or not insulin could prevent the testicular damage. The objective was to evaluate the effects of early administration of insulin following onset of diabetic manifestations on changes in spermatogenesis. Cyclophosphamide was utilized to promote the development of diabetes as a convenient method for monitoring the onset of diabetic changes. Previous reports have shown cyclophosphamide to be effective in promoting the onset of overt diabetes in NOD mice.\textsuperscript{17}

**Materials and Methods**

**ANIMALS**

Male NOD mice from the Sansum Medical Research Foundation colony were used in this study. The colony was established in 1984 with five pairs of mice from Clea Japan, Inc. The breeding protocol at Sansum has been designed to keep approximately 60 breeding pairs of mice. Brothers are mated with sisters if the mother became diabetic. Female NOD mice start developing overt diabetes at 12 weeks of age, and the cumulative incidence of diabetes in the colony is 70 percent by 35 weeks of age. About 8 percent of male mice become diabetic by 30 weeks. An NOD mouse is overtly diabetic when the blood glucose level is above 12 mM (200 mg/dl).

Animals started the experiment at 12 weeks of age. They were randomly assigned to groups receiving cyclophosphamide and untreated age-matched controls. The treated group was further subdivided into animals developing diabetes and not receiving insulin (Group 1); animals developing diabetes and receiving insulin (Group 2); and animals that did not develop diabetes (Group 3). The experiments were approved by the Institutional Animal Use and Care Committee of the Sansum Medical Research Foundation.

**TREATMENT PROTOCOL**

All mice were normoglycemic with blood glucose levels of 5 to 7 mM (90–126 mg/dl) when cyclophosphamide was first given (day 0). Cyclophosphamide\textsuperscript{*} was injected intraperitoneally, 250 mg/Kg in a volume of 300 µl of normal saline. Weight and blood glucose measurements were made on days 7 and 14 to determine which animals were diabetic. Diabetic animals were randomly divided into groups receiving and not receiving insulin.

Animals receiving insulin were given 1 U ultralente (Ultralente Iletin I\textsuperscript{†}) daily in

\textsuperscript{*} Sigma Chemical Company, St. Louis, MO.

\textsuperscript{†} Eli Lilly & Company, Indianapolis, IN.
the hind leg using the Tender Touch injection device.† Treatment with insulin lasted 3 to 4 weeks.

BLOOD GLUCOSE MEASUREMENTS

Blood glucose determinations were made on whole blood obtained from the retroorbital sinus under light anesthesia. Blood was drawn into heparinized tubes and analyzed using the glucose oxidase method.§

TESTICULAR SPECIMEN PREPARATION

Animals were anesthetized with ether and killed by cervical dislocation. One testis was fixed in Bouin’s solution for processing for light microscopy. Slides were examined and photographed with a Reichert Diastar microscope. The other testis was diced and placed in Karnovsky’s fixative. Following postfixation in osmium tetroxide and embedding in Spurr’s resin, sections stained with uranyl acetate and lead citrate were examined with an ISI LEM-2000 electron microscope.

SPECIMEN ANALYSIS

Slides from the testicular specimens were analyzed in a blind fashion by one of the authors (B.G.). The overall extent of seminiferous tubule damage was assessed by examining sections prepared from different areas of the specimens. Effects on spermatogenesis were expressed semiquantitatively as follows: 0 (normal, active spermatogenesis); + (mild disruption, patchy hypospermatogenesis); ++ (moderate to severe disruption, diffuse hypospermatogenesis); + + + (total disruption, germ cell depletion). In specimens with variable findings, the predominant pattern was indicated.

STATISTICAL ANALYSIS

Evaluation of the effects of treatment was analyzed by the chi-square method with Statview.*

Results

In the untreated control group, two animals developed diabetes as indicated by the presence of severe hyperglycemia (567 and 488 mg/dl). Both showed extensive germ cell degeneration, depletion of the spermatogenic cell population, and tubular atrophy. Leydig cells appeared normal by both light and electron microscopy, and vascular structures were thin-walled without endothelial abnormalities. No mononuclear cell infiltration was present. Of the 17 untreated animals which did not exhibit diabetic manifestations, three showed minimal and the remainder no effects on spermatogenesis.

Effects on spermatogenic maturation in the different experimental groups are indicated in table I. Variable degrees of damage were evident in the animals with hyperglycemia. Those that did not develop diabetic changes had normal appearing tubules or minimal alterations, consisting of mild hypospermatogenesis and irregular maturation.

Animals in Group 1, those with diabetic changes (blood glucose 238 to 543 mg/dl) not receiving insulin, all showed advanced stages of damage with the exception of one animal. This animal, which initially had developed hyperglycemia (389 mg/dl), was found to have become normoglycemic (105 mg/dl). The three animals with the most severe alterations had an appearance comparable to that of the untreated control mice which had developed diabetes.

In Group 2, animals with diabetic changes (blood glucose 238 to 559 mg/dl) receiving insulin, there were variable

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† Derata Corporation, Minneapolis, MN.
§ Yellow Springs Instrument Company, Yellow Springs, OH.
* Abacus Concepts, Berkeley, CA.
TABLE I

Effects of Insulin on Spermatogenesis in Diabetic Mice

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of Animals</th>
<th>Normal</th>
<th>Mild Disruption</th>
<th>Mod - Sev Disruption</th>
<th>Germ Cell Depletion</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (CP, DM+)</td>
<td>5</td>
<td>1*</td>
<td>1</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>2 (CP, DM+, I)</td>
<td>5</td>
<td>1</td>
<td>2</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>3 (CP, DM-)</td>
<td>7</td>
<td>5</td>
<td>2</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Animal became non-diabetic.
CP = cyclophosphamide.
DM = diabetic manifestations.
I = insulin.

findings in the tubules reflecting a limited degree of damage. One animal had essentially normal spermatogenesis. Mild hypospermatogenesis was present in two of the animals. Two animals had more extensive damage, although without complete germ cell depletion. The findings indicated a significant reduction in testicular damage in diabetic animals with insulin treatment (p < 0.05).

All of the mice in Group 3 had either no or minimal abnormalities. The findings were comparable to those in the untreated control animals that did not develop diabetes.

Evaluation of Leydig cells, including light and electron microscopic studies, showed no morphologic abnormalities in any of the experimental groups. No evident vascular alterations were present. Lymphocytic infiltration was absent in all of the specimens.

Discussion

Previous studies of testicular alterations associated with diabetes in animal models have shown profound changes in Leydig cell structure and function. In drug-induced forms, such as following streptozotocin treatment, the severe testicular dysfunction can be attributed to hormonal factors involving the pituitary and resulting in Leydig cell abnormalities. Such changes do not occur in the insulin-dependent form of diabetes found in the NOD mouse.

Evidence for autoimmune damage in the testis is also lacking in this model. This was an early consideration in view of the extensive autoimmune insulitis responsible for the diabetic state in NOD mice. The characteristic pattern of autoimmune orchitis, as observed in a variety of animal models and clinical conditions, includes lymphocytic infiltration of the testicular interstitium with extension of the mononuclear infiltrate into seminiferous tubules in advanced stages. No such changes were seen in NOD mice. It was therefore necessary to seek other explanations for the testicular alterations.

Investigation of the role of hyperglycemia as a causative factor for the spermatogenic disruption was the basis for the present study. Hyperglycemia as a cause of testicular dysfunction has been demonstrated in the spontaneously diabetic BB rat. The resulting abnormality in spermatogenic maturation is associated with reduced fertility. Gonadal dysfunction in the BB rat includes both endocrine and spermatogenic abnormalities.

Carbohydrate metabolism is known to be important in the regulation of spermatogenesis by Sertoli cells. This is particularly evident during early devel-
development. Sertoli cells isolated from immature rats convert glucose to lactate under defined incubation conditions.\textsuperscript{26} Lactate from Sertoli cells influences the survival of pachytene spermatocytes during the initial spermatogenic cycle.\textsuperscript{28} Glucose metabolism therefore appears to be critical for the establishment and maintenance of spermatogenesis.\textsuperscript{29}

The findings of the present study also raise the possibility that insulin deficiency per se could be the critical factor related to testicular damage in the NOD mouse. Several experiments have attempted to clarify the relative effects of follicle-stimulating hormone (FSH), insulin and insulin-like growth factor (IGF-1) on glucose utilization and lactate production by cultured Sertoli cells.\textsuperscript{30,31} In general, these studies conclude that FSH and insulin both can rapidly stimulate lactate production, although by different mechanisms.\textsuperscript{31,32} Insulin by itself has been shown to directly influence glucose uptake by cultured Sertoli cells.\textsuperscript{30} The mechanism appears to be via stimulation of hexose transport.\textsuperscript{33}

The role of cyclophosphamide in this study needs to be considered, since CP treatment by itself is known to produce testicular damage.\textsuperscript{34} However, the spermatogenic alterations produced by CP in rats\textsuperscript{35} and mice\textsuperscript{36} under conditions comparable to the present study are not as severe as those associated with diabetes. As shown here, extensive damage following CP treatment occurred only when diabetic manifestations were present.

In summary, while it is not possible to separate out the roles of hyperglycemia and insulin deficiency in regard to the testicular damage in NOD mice, the present findings suggest that metabolic effects related to carbohydrate metabolism are critical. The blunting effect of insulin treatment on the testicular alterations provides support for a direct metabolic role in the pathogenesis of the spermatogenic abnormalities. The findings indicate that the NOD mouse is a useful model for studying the mechanisms involved in diabetes-induced effects on spermatogenesis.

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References


