Fundamental Toxicological Sciences (Fundam. Toxicol. Sci.)
Vol.8, No.4, 97-101, 2021

The Japanese Society of Toxicology

Fundamental Toxicological Sciences

URL : http://www.fundtoxicolsci.org/index_e.html

Letter

Antidiabetic agent did not impair spermatogenesis in spontaneously hyperglycemic and diabetic rats

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(Received June 7, 2021; Accepted June 15, 2021)

ABSTRACT — In previous studies, we suggested that hypoglycemia induced by antidiabetic drugs causes testicular toxicity. In this study, we evaluated whether spontaneously hyperglycemic and diabetic rats (Goto-Kakizaki rats) treated with an antidiabetic drug showed testicular histopathological changes. TMG-123, a glucokinase activator, was given to the rats for 4 weeks at 12.5, 25 and 50 mg/kg. The exposures of TMG-123 at 50 mg/kg were similar to those that caused hypoglycemia and testicular toxicity in SD rats, and the decrease in blood glucose levels on day 28 of dosing was similar to that in SD rats, which showed that the pharmacological action of TMG-123 was similar in both SD and Goto-Kakizaki rats. However, blood glucose levels in Goto-Kakizaki rats were originally much higher than those in SD rats, and hypoglycemia was not induced in the Goto-Kakizaki rats. The histopathological evaluation showed no testicular changes. These results corroborate our hypothesis.

Key words: Hypoglycemia, Testicular toxicity, Testis, Sperm, Germ cell, Reproductive organs

INTRODUCTION

In our previous studies (Kobayashi *et al.*, 2015; Kobayashi *et al.*, 2021), the effects on rat testes of a long-lasting hypoglycemic condition caused by antidiabetic agents were evaluated. Insulin and a novel glucokinase activator, TMG-123, were used in those studies. Tsumura and colleagues reported that TMG-123 is assumed to improve glucose tolerance not by stimulating insulin secretion, but mainly by increasing hepatic glucose uptake (Tsumura *et al.*, 2017). These antidiabetic agents with different mechanisms were repeatedly administered to SD rats, and they caused similar long-lasting hypoglycemia and histopathological testicular damages. Therefore, we suggested that hypoglycemia induced by antidiabetic agents causes testicular toxicity.

In this study, spontaneously hyperglycemic and diabetic rats, Goto-Kakizaki (GK) rats, were used. The GK rat is a non-obese model of non-insulin-dependent diabetes mellitus (NIDDM). This rat was developed by repeated selective breeding of normal Wistar rats with a selection index of glucose intolerance (Tourrel *et al.*, 2002; King, 2012). This model shows impaired insulin secretion and glucose intolerance on glucose loading (King, 2012; Koyama *et al.*, 1998). However, this model shows rather high blood insulin levels under the fed condition and insulin resistance (Sugiyama *et al.*, 1989; Berthelier *et al.*, 1997).

If our hypothesis is correct, hyperglycemic GK rats failing to achieve hypoglycemic status by TMG-123

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administration are not likely develop histopathological testicular changes. Therefore, TMG-123 was repeatedly administered to GK rats, the blood exposure of TMG-123 and blood glucose levels were examined, and histopathological examinations of the testes and epididymides were performed.

MATERIALS AND METHODS

Animal

Male Goto-Kakizaki (GK) rats were obtained from Japan SLC, Inc. (Shizuoka, Japan). The rats were provided *ad libitum* access to pellet diet (CLEA Japan, Inc., Tokyo, Japan) and water. The animal room was maintained at 23.5°C to 25.0°C and relative humidity of 48% to 73%, with a 12-hr light/dark cycle. All experimental procedures were approved by the Animal Care and Use Committee of Teijin Institute for Bio-Medical Research. All efforts were made to minimize animal suffering.

Test substances

TMG-123 was synthesized in KYORIN Pharmaceutical Co., Ltd. (Tokyo, Japan)(content: 100.4% by high performance liquid chromatography) and used as the test article. Gelucire 44/14 (abbreviated as Gelucire, Gattefossé Corporation, Saint-Priest, France) and Polyethylene glycol 400 (abbreviated as PEG400, FUJIFILM Wako Pure Chemical Corporation, Osaka, Japan) were weighed at a ratio of 3:2. This mixture, named PEG400/Gelucire (abbreviated as PEG/Gel), was used as a vehicle.

Dosing

For the toxicity groups in this study, TMG-123 (12.5, 25, 50 mg/kg) or vehicle was administered repeatedly for 4 weeks to GK rats (n = 10) at 6 weeks of age. For the satellite groups, the same amounts of TMG-123 were administered to GK rats (n = 5) at the same age in the same manner.

Blood sampling for evaluations of blood exposure to TMG-123 and the blood glucose level

Blood samples were obtained from 5 animals of each satellite group at various time points (0, 0.5, 1, 2, 4, 8, and 24 hr) on days 1 (first day of administration) and 28 (last day of administration). Plasma glucose levels were measured using Glucose C II test Wako (FUJIFILM Wako Pure Chemical Corporation). For blood exposure to TMG-123 on day 28, samples were analyzed by LC-MS/MS, and then parameters such as maximum concentration (C_{max}), time to maximum concentration (T_{max}) and area under the plasma concentration-time curve ($AUC_{0–24hr}$) were calculated.

Histopathology

The rats were sacrificed under *ad libitum* feeding conditions on day 29. After the rats were necropsied, the testes and epididymides of all animals of the toxicity groups were collected and immersion-fixed in Bouin's solution and 10% neutral buffered formalin, respectively. The tissue specimens were prepared by sectioning the paraffin-embedded tissues and staining with hematoxylin and eosin, and they were examined microscopically.

RESULTS

In the evaluation of blood exposure to TMG-123, the mean values of C_{max} and $AUC_{0–24hr}$ increased with doses ranging from 12.5 to 50 mg/kg (Table 1).

Plasma glucose levels on days 1 and 28 decreased with increasing doses over the dose range of 12.5 to 50 mg/kg (Fig. 1). At 25 and 50 mg/kg, blood glucose levels after administration decreased by clearly exceeding 50% compared to that before administration, but no individual showed a blood glucose level of about 50 mg/dL or lower, which is one of the criteria for diagnosing hypoglycemia (Rosenstock *et al*., 2001; Bonds *et al*., 2010; International Hypoglycaemia Study Group, 2017).

In histopathological evaluation (Table 2), there were no treatment-related changes in the testes and epididymides. Some findings were observed in the testes and epididymides, but these findings were considered incidental because they were unilateral changes or were also observed in the vehicle control group.

Table 1. Blood exposures to TMG-123 on day 28.

	12.5 mg/kg	25 mg/kg	50 mg/kg
t_{max} (hr)	1.60 ± 1.34	1.20± 0.45	1.40 ± 0.55
C_{max} (ng/mL)	1095.69 ± 226.40	2363.24± 303.83	4452.95 ± 935.47
$AUC_{0–24hr}$ (ng·hr/mL)	7197.8 ± 434.9	16713.7± 1499.7	29955.8 ± 3828.9

Mean ± S.D. (n = 5).

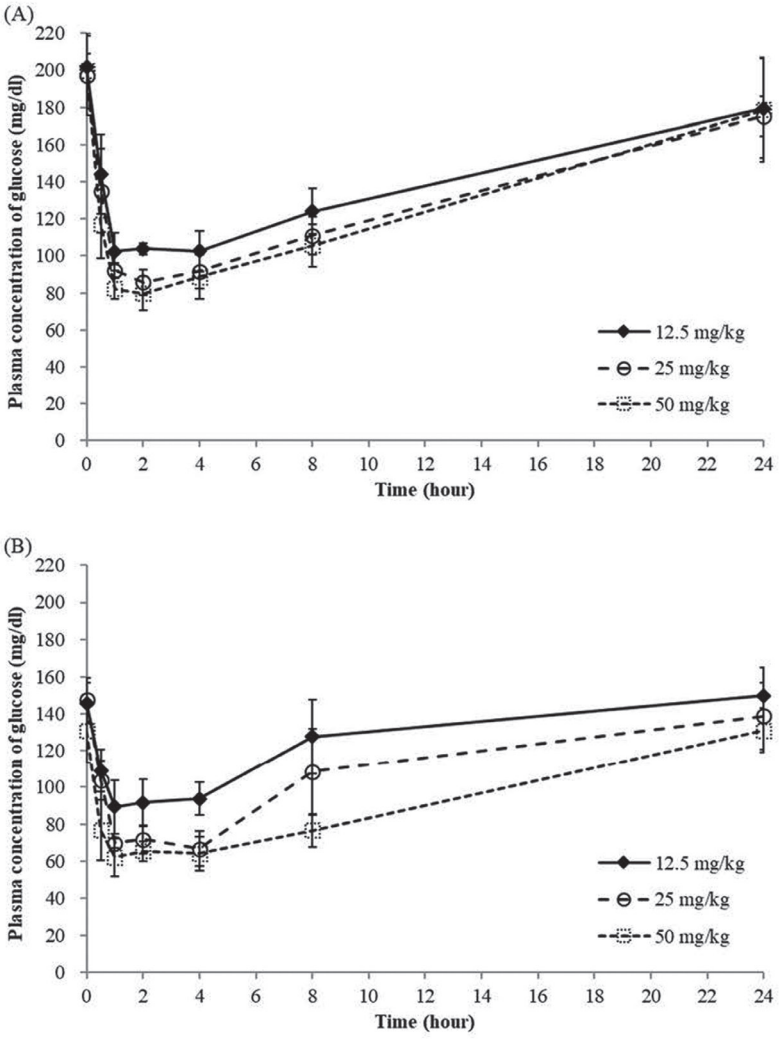

Fig. 1. Plasma glucose levels on days 1 (A) and 28 (B) of dosing. Blood samples were obtained at 0, 0.5, 1, 2, 4, 8, and 24 hr after administration of the test article. The data are presented as means ± S.D. (n = 5).

DISCUSSION

In this study, GK rats were used as spontaneously hyperglycemic and diabetic rats. The GK rat is one of the non-obese models of NIDDM and is often used in efficacy studies of antidiabetic drugs. A novel glucokinase activator, TMG-123, was repeatedly administered to the GK rats for 4 weeks. TMG-123 is a selective activator of glucokinase enzyme. Glucokinase lowers blood glucose concentrations by enhancing glucose uptake and glycogen synthesis in the liver and increasing insulin secretion from pancreatic beta-cells (Matschinsky, 1990; Ferre *et al*., 1996). Tsumura and colleagues reported the unique characteristics of TMG-123 (Tsumura *et al.*, 2017). TMG-123 decreased plasma glucose levels without increasing plasma insulin levels in several animal models of T2DM (Goto-Kakizaki rats, db/db mice, and ZDF rats). Therefore, TMG-123 is assumed to improve glucose tolerance not by stimulating insulin secretion, but mainly by increasing hepatic glucose uptake.

The evaluations of blood exposure to TMG-123 showed that the AUC_{0-24hr} (29955.8 ng·hr/mL at 50 mg/kg) of the male GK rats after 4-week administration was similar to those of the male SD rats after 4-week administration (29200 ng·hr/mL at 100 mg/kg) and after 13-week administration (28500 ng·hr/mL at 100 mg/kg) in a 13-week repeated toxicity study (Kobayashi *et al.*, 2021) and after 4-week administration (25400 ng·hr/mL

Table 2. Histopathological findings.

	Vehicle*	TMG-123		
		12.5 mg/kg	25 mg/kg	50 mg/kg
Testis				
Hypoplasia, seminiferous tubules, unilateral				
no finding	9/9	9/10	9/10	9/10
minimal	0/9	0/10	0/10	0/10
mild	0/9	0/10	0/10	0/10
moderate	0/9	0/10	0/10	0/10
severe	0/9	1/10	1/10	1/10
Dilation, seminiferous tubules, unilateral				
no finding	9/9	10/10	10/10	9/10
minimal	0/9	0/10	0/10	1/10
mild	1/9	0/10	0/10	0/10
moderate	0/9	0/10	0/10	0/10
severe	0/9	0/10	0/10	0/10
Epididymis				
Sperm loss, ductus epididymides, unilateral				
no finding	9/9	9/10	9/10	9/10
minimal	0/9	0/10	0/10	0/10
mild	0/9	0/10	0/10	0/10
moderate	0/9	0/10	0/10	0/10
severe	0/9	1/10	1/10	1/10

*: One animal was euthanized because of misadministration and was excluded from this study.

at 100 mg/kg) in a 4-week repeated toxicity study (data not shown). Regarding blood glucose levels, the amount of reduction on day 28 at 50 mg/kg in the male GK rats was 69 mg/dL, which was similar to that (70 mg/dL) at 100 mg/kg in the male SD rats of the 13-week TMG-123 repeated toxicity study (data not shown). Therefore, the pharmacological action of TMG-123 appears similar in GK rats and in normal rats. However, blood glucose levels in GK rats were originally much higher than those in SD rats, and no individual showed a blood glucose level of about 50 mg/dL or less in the GK rats up to 50 mg/kg.

Administration of TMG-123 to SD rats for 13 weeks caused scattered degeneration of seminiferous tubules in testes and exfoliation of germ cells in the lumen of epididymides in 2 of 8 males in the 100 mg/kg group and in 1 of 8 males in the 20 mg/kg group (Kobayashi *et al.*, 2021), and administration for 4 weeks also caused similar testicular changes in 3 of 10 males in the 100 mg/kg group (data not shown). In addition, administration of insulin to SD rats for 4 weeks caused scattered degeneration of seminiferous tubules in testes and exfoliation of germ cells in the lumen of epididymides in 4 of 15 males in the 400 IU/kg group (Kobayashi *et al.*, 2015). However, in the present study, administration of TMG-123 to the GK rats (10 males in each group) did not result in testicular histopathological changes up to 50 mg/kg.

Germ cells require glucose as an energy source (Rato *et al.*, 2012), and therefore it is considered that the absence of testicular histopathological findings in the GK rats was likely due to the lack of hypoglycemia after administration of the antidiabetic agent. These data support our hypothesis that hypoglycemia induced by antidiabetic drugs causes testicular toxicity. It is necessary to pay attention to secondary changes caused by hypoglycemia when the toxicity of antidiabetic agents is evaluated.

ACKNOWLEDGMENTS

The authors wish to thank all members of the Toxicology Research Department, Teijin Institute for Bio-medical Research, TEIJIN PHARMA LIMITED, for their help and support during this study. The authors are also grateful to Chikae Matsumoto of KYORIN Pharmaceutical Co., Ltd. who allowed us to introduce the data of a 4-week repeated oral dose toxicity study of TMG-123 in this report.

Conflict of interest---- The authors declare that there is no conflict of interest.

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