Endocrine, *vol. 23, no. 1, 39–43, March 2004* 0969–711X/04/23:39–43/$25.00

Comparison of Voglibose and Nateglinide for Their Acute Effects on Insulin Secretion and Free Fatty Acid Levels in OLETF Rat Portal Blood after Sucrose Loading

Yutaka Mori,[1] Yoshirou Kitahara,[2] Kyouko Miura,[2] and Naoko Tajima[3]

[1]Department of Internal Medicine, National Higashi Utsunomiya Hospital; [2]Central Research Laboratories, Ajinomoto Co., Inc.; and [3]Division of Diabetes and Endocrinology, Department of Internal Medicine, The Jikei University School of Medicine, Japan

Objective: Short-term hypoglycemic effects of single-dose voglibose and nateglinide were compared after sucrose loading in spontaneously diabetic Otsuka Long–Evans Tokushima fatty (OLETF) rats.

Materials and Methods: After a 17-h fasting period, the animals received 0.06 mg/kg of voglibose (VOG group, *n* = 6), 50 mg/kg of nateglinide (NAT group, *n* = 6), or 0.5% methyl cellulose (control group, *n* = 6), immediately followed by 2.5 g/kg of sucrose.

Results: Compared to control group values, glucose levels after sucrose loading were significantly decreased in the portal blood in the VOG group and in the peripheral blood in the NAT and VOG groups. The portal glucose $AUC_{0–120\ min}$ was significantly lower in the VOG group than in the control and NAT groups, whereas the peripheral glucose $AUC_{0–120\ min}$ was significantly lower in the VOG and NAT groups than in the control group. Portal insulin levels in the VOG group were significantly decreased compared to the control group. However, portal insulin levels in the NAT group were acutely increased, peaking 15 min after sucrose loading. Portal FFA levels were decreased in the NAT group 15, 30, and 60 min after sucrose loading; no FFA reductions were seen in the VOG group.

Conclusions: Although both drugs produced similar hypoglycemic effects after sucrose loading in the peripheral blood, these drugs generated vastly different results in portal blood. Reduced FFA in the portal blood, observed after single administration of nateglinide, may have a favorable impact not only on glucose metabolism but also on lipid metabolism.

Key Words: Nateglinide; voglibose; portal FFA; OLETF rats.

Introduction

α-Glucosidase inhibitors and fast-acting, short-duration insulin secretagogues are commonly used today in the treatment of type 2 diabetes. Although both these drugs have proven efficacy in suppressing postprandial hyperglycemia, they have completely different mechanisms of action. Voglibose, a α-glucosidase inhibitor, inhibits the breakdown of disaccharides into monosaccharides by acting competitively on the activities of disaccharidase (a α-glucosidase), in the final stage of carbohydrate digestion. This delays the digestion and absorption of carbohydrates, controlling postprandial hyperglycemia *(1)*. In contrast, the fast-acting, short-duration insulin secretagogue nateglinide acts to reduce postprandial plasma glucose levels by promoting early short-term insulin secretion. The nateglinide-induced promotion of early insulin secretion, peaking 15 min after glucose loading in normal rats, has been reported previously *(2,3)*.

The Otsuka Long–Evans Tokushima Fatty (OLETF) rats originated from the outbred strain of Long–Evans rats that were purchased from Charles River Canada in 1982 and maintained at Tokushima Research Institute, Otsuka Pharmaceutical Co., Ltd. (Tokushima, Japan). In 1983, a spontaneously diabetic rat with polyuria, polydipsia, and slight obesity was first discovered in this strain and after the 20th generation of selective breeding, the diabetic strain (OLETF) was established in 1990 *(4)*.

As an animal model of spontaneous type 2 diabetes accompanied by obesity *(5)*, the OLETF rat is characterized by insulin resistance *(6)* and accumulated intra-abdominal fat *(7)*, and as its early insulin response to glucose loading is first reduced and then eliminated with aging, the animal progresses from impaired glucose tolerance (IGT) to type 2 diabetes *(7)*. Hyperglycemia, hyperinsulinemia, and hyperlipidemia, as they characterize this rat, are more noticeable under nonfasting than fasting conditions. The OLETF rat can be used as a model for various forms of human postprandial disease including postprandial hyperglycemia, hyperinsulinemia, and hyperlipidemia, providing an optimal animal model that helps elucidate the pathological conditions involved.

Received October 13, 2003; Revised December 16, 2003; Accepted January 5, 2004.

Author to whom all correspondence and reprint requests should be addressed: Yutaka Mori, MD, Department of Internal Medicine, National Higashi-Utunomiya Hospital, 2160 Shimookamoto, Kawachi-machi, Kawachi-gun, Tochigi 329-1193, Japan. E-mail: moriyutakajp@yahoo.co.jp

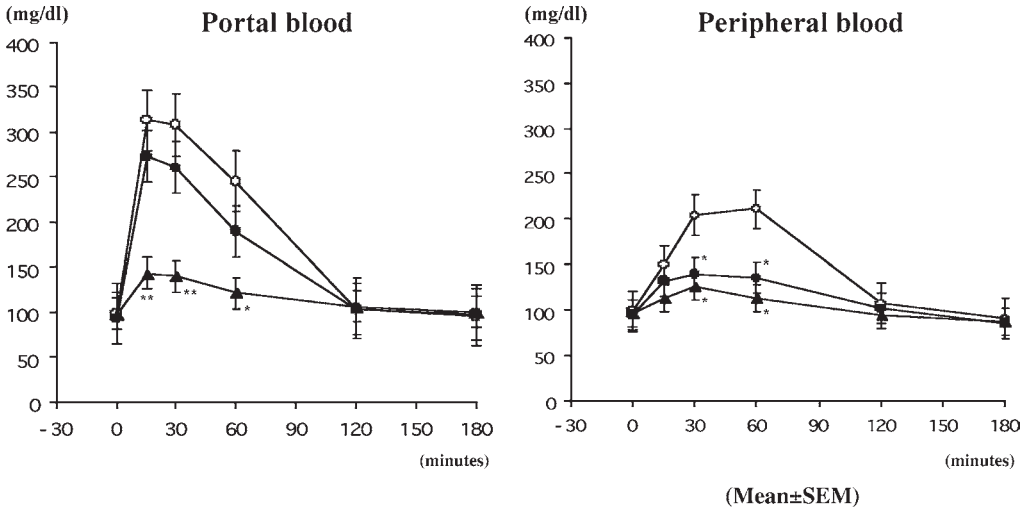

Fig. 1. Changes in post-sucrose loading glucose levels in the portal and peripheral blood after single administration of voglibose and nateglinide. -○- control group, -▲- VOG group, -●- NAT group. *$p < 0.05$, **$p < 0.01$ vs control.

To elucidate differences in the mechanisms of action of these drugs, we compared single-dose voglibose and single-dose nateglinide for their short-term effects on insulin secretion and free fatty acid (FFA) levels in the portal blood in OLETF rats after sucrose loading.

Results

The weights of the animals were 398.8 ± 10.1 g in the VOG group, 402.0 ± 11.9 g in the NAT group, and 398.3 ± 11.1 g in the control group. There was no significant difference in body weight among the groups.

Glucose levels in the portal blood in the VOG group were not increased after sucrose loading, and were significantly decreased compared to those in the control group. However, glucose levels in the portal blood in the NAT group after sucrose loading merely showed a tendency toward a decrease compared to those in the control group (Fig. 1, left). Glucose levels in the peripheral blood in the VOG and NAT groups after sucrose loading were decreased significantly compared to those in the control group (Fig. 1, right). The portal glucose $AUC_{0–120\,min}$ was significantly ($p < 0.01$, $p < 0.05$) lower in the VOG group than in the control and NAT groups, while the peripheral glucose $AUC_{0–120\,min}$ was significantly lower in both the VOG and NAT groups ($p < 0.01$, $p < 0.05$) than in the control group (Fig. 2).

Insulin levels in the portal blood in the VOG group after sucrose loading were not increased, and were significantly lower than in the control group. However, portal insulin levels in the NAT group showed a sharp increase, peaking 15 min after sucrose loading (Fig. 3, left). Although similar tendencies were observed in the peripheral blood, the differences were not significant (Fig. 3, right). The portal blood insulin $AUC_{0–120\,min}$ (ng·h/mL) was 14.7 ± 4.7, 10.0 ± 3.1, and 20.2 ± 4.0 for the control, VOG, and NAT groups, respectively. While no significant differences were seen compared with the control group, there was a trend toward decrease in the VOG group, as well as an increasing trend in the NAT group.

Moreover, while portal FFA levels had fallen significantly in the NAT group 30 and 60 min after sucrose loading, no reductions were seen in the VOG group (Fig. 4, left). The portal FFA $AUC_{0–120\,min}$ value was significantly lower in the control and NAT groups (both $p < 0.05$) than in the VOG group (Fig. 4, right).

Discussion

The character of diabetes mellitus in OLETF rats that were used in this experiment involves postprandial hyperglycemia, and a slight rise in the fasting blood glucose level compared to the rise in blood glucose level following glucose loading, even as age progresses *(7)*. Because the purpose of this experiment was to compare the acute effects of VOG and NAT on blood glucose levels following sugar loading, only young, 12-wk-old rats whose blood glucose level rose following glucose loading were used. Even in an experiment on the acute effects of nateglinide, in which 24-week old OLETF rats whose fasting blood glucose level rose with an advance in age were used, the same results were obtained. The experiments in this study revealed that single-dose VOG given to OLETF rats led to delayed carbohydrate absorption due to suppressed decomposition of sucrose to glucose in the small intestine, so that the glucose levels in the portal vein were not increased. Accordingly, glucose levels were not increased in the peripheral blood. Furthermore, single administration of VOG resulted in no increase in insulin levels in the portal blood. Therefore, it was assumed that hormone-sensitive lipase had not been suppressed in the adipose cells, causing no suppression of lipolysis in visceral fat and no reduction of FFA levels in the portal blood. In contrast, single-dose NAT led to an acute

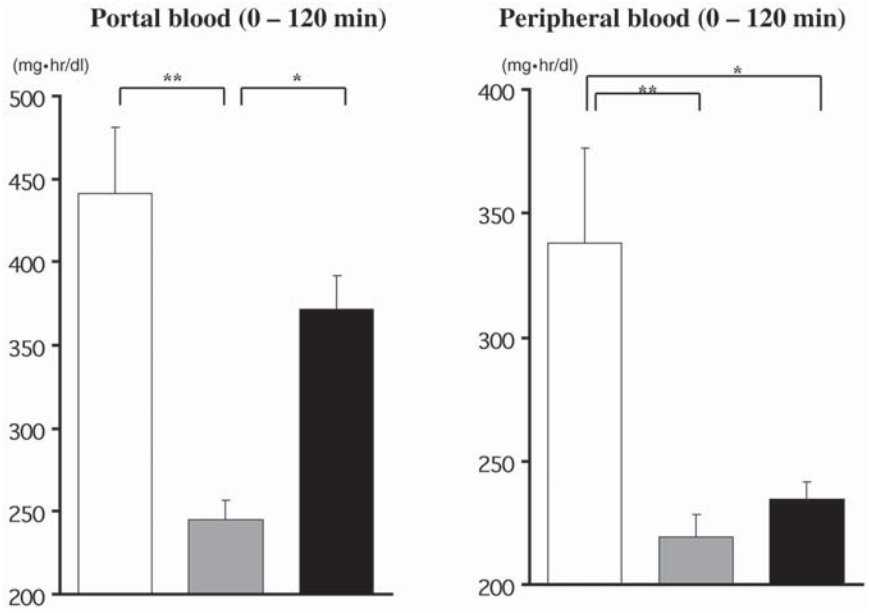

Fig. 2. Post-sucrose loading glucose AUC in the portal and peripheral blood after single administration of voglibose and nateglinide. -○- control group, -▲- VOG group, -●- NAT group. *$p < 0.05$, **$p < 0.01$ vs control.

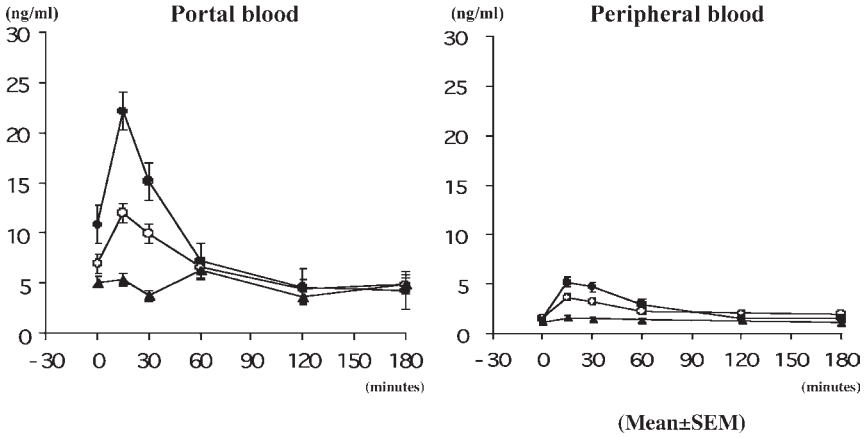

Fig. 3. Post-sucrose loading changes in insulin values in the portal and peripheral blood after single administration of voglibose and nateglinide. -○- control group, -▲- VOG group, -●- NAT group.

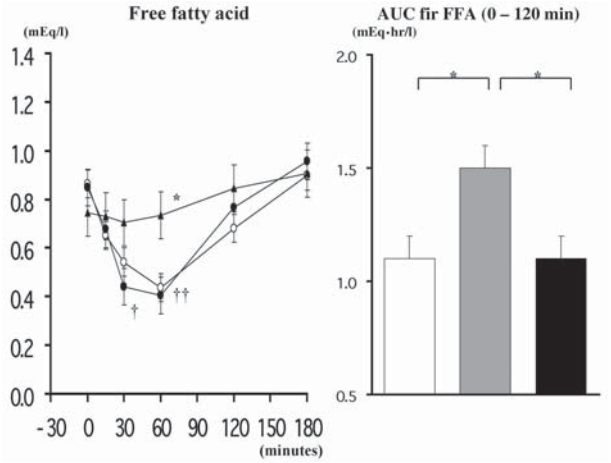

Fig. 4. Post-sucrose loading changes in free fatty acid values and free fatty acid area under the curve (AUC) in the portal blood after single administration of voglibose and nateglinide. -○- control group, -▲- VOG group, -●- NAT group. *$p < 0.05$ vs control, **$p < 0.01$ versus VOG group.

increase in insulin levels in the portal blood. It was assumed that the increased insulin levels led to suppression of hormone-sensitive lipase in the adipose cells and lipolysis in visceral fat, which in turn resulted in decreased FFA levels in the portal blood *(8,9)*. The acute increase in insulin levels in the portal vein was also thought to have resulted in decreased hepatic glucose production, as well as increased hepatic glucose uptake. This resulted in a decrease in glucose levels in the peripheral blood after sucrose loading. Thus, although single administration of the α-glucosidase inhibitor VOG and the fast-acting, short-duration insulin secretagogue NAT produced similar hypoglycemic effects in the peripheral blood after sucrose loading, these drugs generated vastly different results with regard to insulin secretion and FFA levels in the portal blood.

FFAs are reported to interfere with insulin-induced inhibition of hepatic glucose production *(10)* and insulin-induced acceleration of hepatic glucose uptake. When glucose was

administered directly into the portal vein in OLETF rats, which have impaired capability for inhibiting lipolysis of viceral fat by secreting endogenous insulin, there was no decrease in free fatty acid levels. As a result, hepatic glucose production was higher and hepatic glucose uptake was lower than in normal rats *(11)*. Consequently, the reduction of FFA in portal vein blood brought about by a single-dose of NAT is assumed to provide an important effect on glucose metabolism in the liver.

In a study using Zucker fatty rats, Mine et al. *(12)* reported that acute increases in insulin induced by single-dose NAT resulted in a reduction in plasma triglyceride levels after fat loading. They also showed through analysis of the lipoprotein fraction that single-dose NAT led to a reduction in both very low density lipoprotein (an endogenous triglyceride-rich lipoprotein) and chylomicron (an extrinsic triglyceride-rich lipoprotein), suggesting that the acute increase in insulin secretion in the portal vein induced by NAT may have impacted not only the lipoprotein synthesis and secretion process in the liver by reducing portal FFA levels but also the metabolism of triglyseride-rich lipoprotein through accelerating lipoprotein lipase.

We therefore conclude that reduced FFA levels associated with an acute increase in insulin levels in the portal blood—a finding in our experiment with single administration of NAT—is likely to have had an impact on not only the insulin-mediated glucose metabolism in the liver, which suppressed hepatic glucose production and promoted hepatic glucose uptake, but also affected the lipid metabolism in the liver.

When considered from this viewpoint, nateglinide may be more appropriate than voglibose for patients with type 2 diabetes who have marked postprandial hyperglycemia combined with postprandial hypertriglyceridemia.

Materials and Methods

Animals

Male OLETF rats *(5)* were obtained at 4 wk of age from the Tokushima Research Institute, Otsuka Pharmaceutical Co. Inc. (Tokushima, Japan). The animals were housed in plastic cages (320 × 270 × 175 mm) in an animal room with controlled temperature (23 ± 2°C) and relative humidity (55 ± 15%), and a 12-h light/12-h dark cycle (lights on at 0700 h). They were supplied with rat chow (CE-2; Clea Japan, Inc.) and tap water *ad libitum* until 12 wk of age. The guidelines for Laboratory Animal Facilities of the Jikei University School of Medicine were followed for the care and use of the animals in this study.

Drugs

Nateglinide [(-)-*N*-(*trans*-4-isopropylcyclohexanecarbonyl)-D-phenylalanine] and voglibose were synthesized at Central Research Laboratories of Ajinomoto, Co. Inc. (Kanagawa, Japan). These were suspended in 0.5% methylcellulose and administered at doses of 50 and 0.2 mg/kg to rats via a stomach tube in volume of 10 mL/kg. These doses showed a similar suppressive effect on the peak blood glucose levels after oral glucose loading of fasted normal rats in our previous study *(3)*. Control rats were treated with 0.5% methylcellulose alone (the vehicle).

Experimental Design

An in-dwelling catheter was surgically implanted in the portal vein in 12-wk-old OLETF rats (n = 18), with the exposed end of the catheter positioned behind the head. Rats were fasted for 17 h, and were then given 2.5 g/kg of sucrose orally (oral sucrose tolerance test). The rats were not anesthetized, and their movement was not restricted. Immediately prior to sucrose loading, the rats were given oral voglibose (VOG group, n = 6), nateglinide (NAT group, n = 6), or a control solution of 0.5% methylcellulose (control group, n = 6). Blood samples were drawn from the in-dwelling catheter (portal blood) and the caudal vein (peripheral blood) at baseline and at 15, 30, 60, 120, and 180 min after sucrose loading. Blood glucose and plasma insulin levels were measured in both portal and peripheral blood samples, and free fatty acids (FFA) in portal blood were assessed. The area under the curve from 0 to 120 min ($AUC_{0–120\ min}$) was measured for portal glucose, insulin, and FFAs, as well as for peripheral glucose.

Biochemical Analysis

Blood glucose levels were determined by the glucose oxidase methods using a Fuji Dri-Chem 5500 auto analyzer (Fuji Medical Sysytems, Tokyo, Japan). Plasma FFA levels were measured by an enzyme technique, where the samples were incubated with enzyme reagent (NEFA-SS, Eiken, Japan) and the optical density determined. Plasma insulin levels were determined with a commercial enzyme immunoassay kit (Ultra Sensitive Rat Insulin Kit, Seikagaku Co., Tokyo, Japan) using rat insulin as the standard.

Statistical Analysis

All numerical values were expressed as mean ± SEM. Statistical analysis was performed by using one-way analysis of variance (ANOVA), followed by Scheffe's method, as a post-hoc test to detect any significant differences among the groups ($p < 0.05$). The level of $p < 0.05$ was regarded as statistically significant.

References

1 Goto, Y., Yamada, Y., Ohyama, K., Matsuo, T., Odaka, H., and Ikeda, H. (1995). *Diabetes Res. Clin. Pract.* **28,** 81–87.
2 Ikenoue, T., Akiyoshi, M., Fujitani, S., Okazaki, K., Kondo, N., and Maki, T. (1997). *Br. J. Pharmacol.* **120,** 137–145.
3 Ikenoue, T., Okazaki, K., Fujitani, S., et al. (1997). *Biol. Pharm. Bull.* **20,** 354–359.
4 Kawano, K., Hirashima, T., Mori, S., Kurosumi, M., and Saitoh, Y. (1991). *Rat News Lett.* **25,** 24–26.
5. Kawano, K., Hirashima, T., Mori, S., Saitoh, Y., Kurosumi, M., and Natori, T. (1992). *Diabetes* **41,** 1422–1428.

6. Nakayama, N., Sato, T., Asahi, Y., Toide, K., and Yabuuchi, Y. (1993). In: *Insulin resistance in human disease.* Huh, K. B., Shinn, S. H., and Kaneko, T. (eds.), Elsevier Science: Amsterdam, pp. 389–392.
7. Mori, Y. and Ikeda, Y. (1999). In: *Obesity and NIDDM. Lessons from the OLETF rat.* Shima, K. (eds.), Elsevier Science: Amsterdam, pp. 237–244.
8. Brasaemle, D. L., Levin, D. M., Adler-Wailes, D. C., and Londos, C. (2000). *Biochem. Biophysi. Acta* **1483,** 251–262.
9 Elks, M. L. and Manganiello, V. C. (1985). *Endocrinology* **116,** 2119– 2121.
10 Groop, L. C., Saloranta, C., Shank, M., Bonadonna, R. C., Ferrannini, E., and DeFronzo, R. A. (1991). *J. Clin. Endocrinol. Metab.* **72,** 96–107.
11 Shiba, Y., Yamasaki, Y., Kubota, M., et al. (1986). *Metabolism* **35,** 323–327.
12. Mine, T., Miura, K., Kitahara, Y., Okano, A., and Kawamori, R. (2002). *Biol. Pharm. Bull.* **25,** 1414–1416.