The alpha-glucosidase inhibitor miglitol increases hepatic CYP7A1 activity in association with altered short-chain fatty acid production in the gut of obese diabetic mice

Yoji Hamada a, *, Moritaka Goto b, Go Nishimura b, Hiroshi Nagasaki c, Yusuke Seino d, Hideki Kamiya a, Jiro Nakamura a

a Division of Diabetes, Department of Internal Medicine, Aichi Medical University School of Medicine, 1-1 Yazakokarimata, Nagakute, 480-1195, Japan
b Pharmaceutical Research Laboratories, Sanwa Kagaku Kenkyusho Co, Ltd, 363 Shiozaki, Hokusei-cho, Inabe, 511-0406, Japan
c Department of Physiology I, Fujita University School of Medicine, 1-98 Kutsukake-cho, Toyoake, Aichi, 470-1192, Japan
d Department of Endocrinology and Diabetes, Fujita University School of Medicine, 1-98 Kutsukake-cho, Toyoake, Aichi, 470-1192, Japan

ABSTRACT

Purpose: Bile acids (BAs) have been shown to contribute to glucose and energy homeostasis. We have recently reported that miglitol, an alpha-glucosidase inhibitor, increases fecal BA excretion and ameliorate insulin resistance and obesity in mice. The aim of this study was to clarify the mechanisms by which miglitol affects BA metabolism. The expression of genes regulating BA metabolism, gut microbiome and short-chain fatty acids (SCFA) were examined.

Procedures: NSY mice, representing an obese type 2 diabetic model, were fed with a high-fat diet with or without miglitol for 4 weeks. The expression of BA-related genes in the liver and the lower intestine were measured. Alterations in fecal microbiome, fecal SCFA along with plasma lipid levels were also evaluated.

Major findings: Miglitol significantly increased fecal BA secretion and markedly upregulated the mRNA expression, protein levels and enzyme activity of hepatic cholesterol 7α-hydroxylase, a rate-limiting enzyme of BA synthesis. In the intestine, miglitol treatment significantly suppressed the mRNA expression of apical sodium-dependent bile acid transporter and ATP-binding cassette transporter G5 and G8. In fecal microbiome, the prevalence of prevotella was remarkably reduced and that of clostridium subcluster XIVa was increased by miglitol. Miglitol elevated formic and n-butyric acids along with total SCFA concentration in feces, while succinic acid was decreased. There was no change in plasma total cholesterol levels.

Conclusions: Collectively, miglitol may affect BA metabolism via enhanced CYP7A1 activity resulting from at least in part the alterations in gut microbiome and SCFA production in obese diabetic mice.

© 2020 Published by Elsevier Inc. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

1. Introduction

Bile acids (BAs) have been shown to contribute to energy homeostasis and glycolysis [1,2]. We have recently reported that miglitol, an alpha-glucosidase inhibitor (αGI), influences fecal and blood BA concentrations, and ameliorates insulin resistance along with obesity in mice [3]. The mechanisms by which miglitol affects BA metabolism, however, remained to be clarified.

BA concentrations are strictly regulated by the feed-back mechanisms mainly via farnesoid X receptor (FXR)-small heterodimer partner (SHP) pathway along with numerous receptors, enzymes and transporters in the liver and gut [4,5]. On the other hand, various factors other than BAs may influence these molecules. For example, cholesterol 7α-hydroxylase (CYP7A1), a rate-limiting enzyme of BA synthesis, is known to be regulated by FXR-independent mechanisms [6,7].

Present study aimed to clarify the mechanism(s) for miglitol-induced BA hypersecretion. We conclude that miglitol upregulates hepatic CYP7A1 possibly via increased short-chain fatty acid production resulting from alterations in gut microbiome.
2. Materials and methods

2.1. Animals

All procedures were conducted according to the Guidelines for the Care and Use of Laboratory Animals of Sanwa Kagaku Kenkyusho (SKK). Five-week-old male Nagoya-Shibata-Yasuda (NSY) mice (Hoshino Laboratory Animals, Ibaragi, Japan), known as a spontaneous-onset obese type 2 diabetes model [8], were fed with normal chow (n = 10) or a high-fat diet (HFD) (Casein, 23.6%; Sucrose, 20.4%; Iard, 20.93% in composition; Oriental Yeast, Tokyo, Japan) (n = 10) that were free from or mixed with 0.08% miglitol for 4 weeks.

2.2. Measurement of total bile acid concentrations in feces

Feces were collected over a period of 24 h two days prior to the sacrifice and were frozen-dried and powdered followed by extraction twice with 90% ethanol at 65 °C for 1 h. After centrifugation, the supernatants were dried under a nitrogen stream and re-dissolved in 90% ethanol. Total bile acids were determined using the Total bile acid-test kit (Wako, Osaka, Japan).

2.3. Gene expression analysis

Mice were sacrificed at 4 weeks after treatment, and the liver and the distal ileum were immediately removed and frozen. Total RNA was extracted from the frozen tissues and cDNA was synthesized using High Capacity cDNA Reverse Transcription® kit (Applied Biosystems, Tokyo, Japan). The mRNA of BA-related genes were purchased from Applied Biosystems. (Applied Biosystems, Tokyo, Japan). The mRNA expression of hepatic CYP7A1 was clearly increased by miglitol (Fig. 1A) and tended to suppress body weight (Fig. 1B). The mRNA expression of Na(+)-taurocholate co-transporting polypeptide (NTCP) was mildly reduced whereas other hepatic BA transporters were unchanged. Fibroblast growth factor receptor (FGFR) 4 and β-klotho (KLB) expression was not altered by the agent (Fig. 1C).

In accordance with mRNA expression, miglitol significantly enhanced the protein expression as well as the activity of hepatic CYP7A1 (Fig. 1D and E).

In the intestine, miglitol increased the mRNA expression of SHP, FXR and other genes relating to hepatic BA production. The expression of Na(+)-taurocholate co-transporting polypeptide (NTCP) was mildly reduced whereas other hepatic BA transporters were unchanged. Fibroblast growth factor receptor (FGFR) 4 and β-klotho (KLB) expression was not altered by the agent (Fig. 1C).

In accordance with mRNA expression, miglitol significantly enhanced the protein expression as well as the activity of hepatic CYP7A1 (Fig. 1D and E).

In the intestine, miglitol increased the mRNA expression of SHP and suppressed that of FXR (Fig. 1F). The mRNA expression of apical sodium-dependent BA transporter (ASBT), a primary BA transporter in enterocytes, was significantly decreased by miglitol and the mean expression of other BA transporters was also lowered. FGF15 mRNA was not altered by the agent. Miglitol significantly suppressed ATP-binding cassette transporter G5 and G8 (ABCG5/8) expression in the intestine, but not in the liver.

2.4. Determination of CYP7A1 activity

The livers were homogenized in 100 mM Na3PO4, pH 7.2, containing 100 mM sucrose, 50 mM KCl, 50 mM NaF, 5 mM EGTA, 3 mM DTT, 1 mM EDTA, 1 mM PMSE and 100 μM leupeptin, and then centrifuged twice at 10,000 g for 10 min. The supernatant was centrifuged at 38,000 rpm for 60 min and the pellets were mixed with 0.1 M NaH2P2O7, 50 mM NaF, and 1 mM EDTA. The specific activity of CYP7A1 was determined using an HPLC assay procedure as described previously [9].

2.5. Western blot analysis

Proteins extracted from the liver microsomal fraction were loaded on SDS-PAGE and then transferred onto PVDF membranes. The membranes were blocked, incubated with primary antibodies against CYP7A1 and calnexin, and washed. The membranes were then incubated with HRP-conjugated secondary antibodies and washed, followed by detection with ECL Plus® (GE Healthcare, Tokyo, Japan).

2.6. Determination of gut microbiome and SCFA concentrations

Cecal samples were collected at the sacrifice and immediately frozen. Microbiome was measured using terminal restriction fragment-length polymorphism (T-RFLP) analysis as previously described [10]. SCFA concentrations were quantified by HPLC using a post column reaction with a detector, tandemly arranged two columns and a guard column as mentioned previously [11].

2.7. Statistical analyses

Data were analysed using analysis of variance (ANOVA) with post-hoc comparisons.

3. Results

3.1. Effects of miglitol on genes involved in BA metabolism

Miglitol enhanced BA excretion to feces in HFD-fed mice (Fig. 1A) and tended to suppress body weight (Fig. 1B). The mRNA expression of hepatic CYP7A1 was clearly increased by miglitol (Fig. 1C), whereas miglitol did not alter the mRNA expression of SHP, FXR and other genes relating to hepatic BA production. The expression of Na(+)-taurocholate co-transporting polypeptide (NTCP) was mildly reduced whereas other hepatic BA transporters were unchanged. Fibroblast growth factor receptor (FGFR) 4 and β-klotho (KLB) expression was not altered by the agent (Fig. 1C).

In accordance with mRNA expression, miglitol significantly enhanced the protein expression as well as the activity of hepatic CYP7A1 (Fig. 1D and E).

In the intestine, miglitol increased the mRNA expression of SHP and suppressed that of FXR (Fig. 1F). The mRNA expression of apical sodium-dependent BA transporter (ASBT), a primary BA transporter in enterocytes, was significantly decreased by miglitol and the mean expression of other BA transporters was also lowered. FGF15 mRNA was not altered by the agent. Miglitol significantly suppressed ATP-binding cassette transporter G5 and G8 (ABCG5/8) expression in the intestine, but not in the liver.

3.2. Alteration of gut microbiome by miglitol

Miglitol markedly reduced the prevalence of prevotella and suppressed clostridium cluster XVIII (Fig. 2A). In contrast, the prevalence of clostridium cluster XIV and bifidobacterium was significantly increased by miglitol and lactobacillales was prone to increase. Miglitol did not affect the total cecal bacterial counts (10.44 ± 0.05 vs. 10.46 ± 0.05 log/g).
3.3. Fecal SCFA

Miglitol significantly increased formic and n-butyric acids in feces of HFD-fed mice (Fig. 2B). While saccinate was decreased by miglitol, the total amount of fecal SCFA was significantly increased by the agent.

Between hepatic CYP7A1 mRNA levels and fecal SCFA concentrations, there was a significant positive correlation in total mice and a tendency for correlation in control mice (Fig. 2C).

3.4. Serum lipids

Miglitol did not show significant effect on serum cholesterol or triglyceride levels (Fig. 2D).

4. Discussion

In present study, miglitol clearly enhanced the expression and activity of hepatic CYP7A1 and suppressed ileal ASBT expression. These results suggest that the agent elevates hepatic BA production and its secretion to gut, which in turn suppress BA absorption by transporters in the ileum. The data seems to account for our present and previous [12] data that the agent enhances fecal BA secretion.

It is intriguing that the miglitol effects appear inconsistent in some points with known feedback regulation of BAs. BAs are well demonstrated to stimulate FXR and subsequently to induce SHP, which results in the suppression of hepatic CYP7A1 [4] and ileal BA transporters. The BA activation of ileal FXR-SHP pathway augments humoral factors FGF15/19 that suppress hepatic CYP7A1 via FGFR1/4 with β-klotho [12]. Our present data showed that FXR mRNA was not altered in the liver and lowered in the intestine, and ileal FGF15 and hepatic FGFR4 along with KLB were not upregulated. Because the transcriptional activity of FXR is independent of its mRNA expression [13], this may give explanation for the discrepancy between the FXR expression and its downstream molecules. Nevertheless, it is puzzling that FGF15-FGFR/KLB axis was not upregulated under the condition where ASBT expression was clearly suppressed.

Several factors other than BAs have been reported to regulate hepatic CYP7A1.

SCFAs such as acetate, propionate and butyrate have been observed to upregulate liver CYP7A1 expression in vivo [14] and in vitro [15]. In present study, miglitol altered gut microbiome and SCFA concentrations, in accordance with previous papers where an αGI acarbose was reported to affect gut bacterial flora and SCFA [16,17] as well as plasma SCFA [18,19]. This is probably because the agent delays saccharide absorption in the upper intestine and subsequently increase saccharide concentrations in the lower intestine, which alters fermentation and intestinal environment. These findings may indicate that the miglitol effect on CYP7A1 is attributable in part to SCFA elevation accompanying gut microbial
changes, and the observed correlation between fecal SCFA concentrations and hepatic CYP7A1 expression possibly supports the hypothesis.

Cholesterol levels may also affect CYP7A1 via LXR \[4,5\]. Although SCFA have been reported to lower plasma cholesterol \[15\], we failed to observe reduction in cholesterol levels by miglitol. Decreased intestine ABCG5/8 expression and resultant reduction in sterol excretion possibly intertwines the cholesterol metabolism.

In addition to above mechanisms, our previous study \[3\] has demonstrated that miglitol induces deiodenase 2, an enzyme converting thyroid hormone T4 to T3, in brown adipose tissues. Because T3 is known to increase CYP7A1 activity \[6,7,20\], this effect possibly mediates to some degree the miglitol enhancement of CYP7A1.

Limitation of our study is the lack of direct evidence for the cause-effect relationship between SCFAs and CYP7A1. The mechanism for non-activation of FGF15-FGFR/KLB pathway under treatment with miglitol also remains to be clarified.

In conclusion, an αGI miglitol affects BA metabolism via upregulation of hepatic CYP7A1, and the alterations in gut microbiome along with SCFA production may underlie the effect. Given the fact that BA metabolism is involved in the energy homeostasis and nutrient metabolism, the effects of αGI appear worth further investigation.

**Contribution statement**

YH was responsible for the conception and design of the study, the interpretation of data and writing the manuscript. MG and GN conducted experiments and contributed to discussions. HN, YS, ST and HK contributed to discussions. JN supervised the study and reviewed the manuscript.

**Declaration of competing interest**

YH, HN and YS have received grant support from SKK. MG and GN are employees of SKK.

**CRediT authorship contribution statement**

Yoji Hamada: Conceptualization, Methodology, Writing - original draft. Moritaka Goto: Methodology, Investigation, Validation. Go Nishimura: Investigation, Resources. Hiroshi Nagasaki: Writing - review & editing. Yusuke Seino: Methodology. Hideki Kamiya: Writing - review & editing. Jiro Nakamura: Supervision.

**Acknowledgements**

This study was supported by research grants from SKK.
References

[1] Houten SM, Watanabe M, Auwerx J. Endocrine functions of bile acids. EMBO J 2006;25:1419–25. https://doi.org/10.1038/sj.emboj.7601049.
[2] Cao R, Cronik ZK, Zha W, Sun L, Wang X, Fang Y, et al. Bile acids regulate hepatic gluconeogenic genes and farnesoid X receptor via G(α/β)-protein-coupled receptors and the AKT pathway. J Lipid Res 2010;51:2234–44. https://doi.org/10.1194/jlr.M004929.
[3] Hamada Y, Nagasaki H, Fuchigami M, Furuta S, Seino Y, Nakamura J, et al. The alpha-glucosidase inhibitor miglitol affects bile acid metabolism and ameliorates obesity and insulin resistance in diabetic mice. Metabolism 2013;62:734–42.
[4] Chiang JY. Bile acids: regulation of synthesis. J Lipid Res 2009;50:1955–66.
[5] Dawson PA, Lan T, Rao A. Bile acid transporters. J Lipid Res 2009;50:2340–57.
[6] Davis RA, Miyake JH, Hui TY, Spann NJ. Regulation of cholesterol-7alpha-hydroxylase: BAREly missing a SHP. J Lipid Res 2002;43:533–43.
[7] Lammel-Lindemann JA, Angajala A, Engler DA, Webb P, Ayers SD. Thyroid hormone induction of human cholesterol 7 alpha-hydroxylase (Cyp7a1) in vitro. Mol Cell Endocrinol 2014;388:32–40. https://doi.org/10.1016/j.mce.2014.02.003.
[8] Ueda H, Ilegami H, Yamato E, Fu J, Fukuda M, Shen G, et al. The NSY mouse: a new animal model of spontaneous NIDDM with moderate obesity. Diabetologia 1995;38:503–8.
[9] Hylemon PB, Studer EJ, Pandak WM, Heuman DM, Vlahcevic ZR, Chiang JY. Simultaneous measurement of cholesterol 7 alpha-hydroxylation activity by reverse-phase high-performance liquid chromatography using both endogenous and exogenous [4-14C]cholesterol as substrate. Anal Biochem 1989;182:212–6.
[10] Nagashima K, Hisada T, Sato M, Mochizuki J. Application of new primer-enzyme combinations to terminal restriction fragment length polymorphism profiling of bacterial populations in human feces. Appl Environ Microbiol 2003;69:1251–62. https://doi.org/10.1128/aem.02.1251-1262.2003.
[11] Higashimura Y, Naito Y, Takagi T, Uchiyama K, Mizushima K, Ushiroda C, et al. Protective effect of agaro-oligosaccharides on gut dysbiosis and colon tumorigenesis in high-fat diet-fed mice. Am J Physiol Gastrointest Liver Physiol 2016;310:C367–75. https://doi.org/10.1152/ajpgi.00324.2015.
[12] Stroeve JH, Brufau G, Stellard F, Gonzalez FJ, Staels B, Kuipers F. Intestinal FXR-mediated FGF15 production contributes to diurnal control of hepatic bile acid synthesis in mice. Lab Invest 2010;90:1457–67. https://doi.org/10.1038/labinvest.2010.107.
[13] Xu G, Pan LX, Li H, Forman BM, Erickson SK, Shefer S, et al. Regulation of the farnesoid X receptor (FXR) by bile acid flux in rabbits. J Biol Chem 2002;277:50491–6.
[14] Imazumi K, Hirata K, Yasun Sugano M. Propionate enhances synthesis and secretion of bile acids in primary cultured rat hepatocytes via succinyl CoA. Biochem Biotechnol 1992;56:1894–6. https://doi.org/10.1271/bbb.56.1894.
[15] Zhao Y, Liu J, Hao W, Zhu H, Liang N, He Z, et al. Structure-specific effects of short-chain fatty acids on plasma cholesterol concentration in male syrian hamsters. J Agric Food Chem 2017;65:10984–92. https://doi.org/10.1021/acs.jafc.7b04666.
[16] Smith BJ, Miller RA, Ericsson AC, Harrison DC, Strong R, Schmidt TM. Changes in the gut microbiome and fermentation products concurrent with enhanced longevity in acarbose-treated mice. BMC Microbiol 2019;19:130. https://doi.org/10.1186/s12866-019-1494-7.
[17] Holt PR, Atillasoy E, Lindenbaum J, Ho SB, Lupton JR, McMahon D, et al. Effects of acarbose on fecal nutrients, colonic pH, and short-chain fatty acids and rectal proliferative indices. Metabolism 1996;45:1179–87. https://doi.org/10.1016/0026-0495(96)90020-7.
[18] Wolfer TM, Chaisson JL. Acarbose raises serum butyrate in human subjects with impaired glucose tolerance. Br J Nutr 2000;84:57–61.
[19] Lee EY, Zhang X, Miyamoto J, Kimura I, Taknaka T, Furusawa K, et al. Gut carbohydrate inhibits GIP secretion via a microbiota/SCFA/FFAR3 pathway. J Endocrinol 2018;239:267–76. https://doi.org/10.1530/JOE-18-0241.
[20] Drover VA, Agellon LB. Regulation of the human cholesterol 7alpha-hydroxylase gene (CYP7A1) by thyroid hormone in transgenic mice. Endocrinology 2004;145:574–81. https://doi.org/10.1210/en.2003-0993.