Microsomeal Triglyceride Transfer Protein Gene Expression and ApoB Secretion Are Inhibited by Bitter Melon in HepG2 Cells1,2

Pratibha V. Nerurkar,*3 Laurel Pearson,† Jimmy T. Efird,** Khosrow Adeli,†† Andre G. Theriault,‡ and Vivek R. Nerurkar†‡

*Laboratory of Metabolic Disorders and Alternative Medicine, Department of Molecular Biosciences and Bioengineering, College of Tropical Agriculture and Human Resources; †Retrovirology Research Laboratory, **Department of Tropical Medicine and Medical Microbiology and ‡Department of Medical Biotechnology, John A. Burns School of Medicine, University of Hawaii, Honolulu, Hawaii; and ††Hospital for Sick Children, University of Toronto, Toronto, Canada

ABSTRACT Momordica charantia or bitter melon is traditionally used as an antidiabetic agent in Asia, Africa, and South America. Recent studies indicate that bitter melon can also lower plasma lipids and VLDL in diabetic animal models as well as animals fed a high-fat diet, suggesting an effect on lipoprotein metabolism. The aim of this study was to delineate the cellular and molecular mechanisms involved in the lipid-lowering properties of bitter melon and regulation of apolipoprotein B (apoB). Human hepatoma cells, HepG2, treated with bitter melon juice (BMJ) for 24 h reduced apoB secretion with and without the addition of lipids (P < 0.05). However, BMJ did not increase apoB secretion in cells treated with N-acetyl-leucyl-leucyl-norleucinal, indicating a lack of effect on the proteasomal degradation pathway. BMJ reduced the secretion of new triglycerides (P < 0.05) and decreased microsomal triglyceride transfer protein (MTP) mRNA expression, suggesting that lipid bioavailability and lipidation of lipoprotein assembly are likely involved in decreased apoB secretion. Interestingly, BMJ increased the nuclear translocation of the mature form of sterol regulatory element-binding protein-1c (SREBP-1c, P < 0.05), involved in MTP secretion. Our data suggest that BMJ is a potent inhibitor of apoB secretion and TG synthesis and secretion that may be involved in the plasma lipid- and VLDL-lowering effects observed in animal studies. J. Nutr. 135: 702–706, 2005.

KEY WORDS: • bitter melon • hyperlipidemia • apolipoprotein B • transcription factors • triglycerides

The incidence and prevalence of type 2 diabetes is rapidly increasing globally. Current pharmacologic agents used for treating type 2 diabetes improve glycemic control but have varying effects on dyslipidemia, which is commonly associated with this disease (1). Therefore, therapeutic approaches that would ameliorate diabetic dyslipidemia would form an important cornerstone in treating these patients. Type 2 diabetic patients are at high risk for developing cardiovascular diseases (CVD)4 due to associated hyperlipidemia and increased levels of plasma apolipoprotein B (apoB)-containing VLDL and LDL (2). Among conventional treatment strategies, a combination of 2 or more drugs is usually necessary to achieve the target glucose and lipid levels; this likely affects compliance and the quality of life due to possible drug-drug interactions (3). Therefore, regardless of enormous advances in medical care, alternative therapies have become increasingly popular over the past several years, including medicinal herbs and functional foods (4).

Momordica charantia, also known as bitter melon, balsam pear, or karela, is widely cultivated in Asia, Africa, and South America and extensively used in folk medicines as a remedy for diabetes, specifically in India, China, and Central America [(5) and references therein]. Freeze-dried bitter melon capsules are widely available and marketed in health food stores across North America and Western European countries. All parts of the plant (fruit, seed, and leaves) were shown to possess hypoglycemic properties in normal and diabetic animal models (6–11). To date, only a few, nonrandomized, clinical studies conducted using bitter melon juice (BMJ) or fried fruit have demonstrated a significant decrease in blood glucose levels among type 2 diabetic patients (12–15).

Although empirically used to lower blood glucose, studies in animal models now indicate that bitter melon may also

lower hepatic and serum lipids (7,9,10,16,17). Feeding of freeze-dried BMJ to rats fed a high-cholesterol or a normal diet, significantly decreased hepatic triglyceride (TG) and cholesterol and increased serum HDL cholesterol (7,10). Rats fed BMJ along with a high-fat diet demonstrated not only reduced adiposity, but also lower serum insulin and leptin levels and normalized glucose tolerance (9). In streptozotocin-induced diabetic rats, BMJ decreased both hepatic and serum TG and cholesterol (16). In alloxan-induced diabetic rats, as well as untreated control rats, aqueous extracts of bitter melon fruit and seeds significantly reduced VLDL levels, suggesting an effect on lipoprotein metabolism (17). Overall, the effects of bitter melon on various lipid variables in animals are novel findings, but the underlying mechanisms are unknown. To date, only one study demonstrated an upregulation of peroxisome proliferator-activated receptors (PPARα and γ) and acyl CoA oxidase in rat hepatoma cells, H4IIEC3, which could be involved in the lipid-lowering effects of BMJ (8). However, no clinical studies have yet investigated the effects of bitter melon on serum lipids in humans.

Among the various lipoproteins, apoB is the major protein component of VLDL, intermediate density lipoproteins, and LDL. These particles are linked in a delipidation cascade in which TG-rich VLDL, released from the liver, is converted to cholesterol-rich LDL (18). Abnormalities in the metabolism of apoB-containing lipoproteins are responsible for the generation of hyperlipidemia and the associated increased risk of developing coronary heart disease (19). Because bitter melon lowers plasma lipids and VLDL levels in diabetic rats (17), the objective of this study was to investigate the effects of bitter melon on apoB secretion in vitro, using the human hepatoma cell line, HepG2. Our results indicate that BMJ significantly inhibits the synthesis and secretion of cellular TG as well as apoB secretion in HepG2 cells. Understanding the mechanisms of traditional functional foods such as bitter melon may help to identify new molecular targets in the treatment of type 2 diabetes and hyperlipidemia.

MATERIALS AND METHODS

Materials. HepG2 cells and cell culture medium were obtained from the American Type Culture Collections, and fetal bovine serum (FBS), fatty acid-free bovine serum albumin (BSA), oleic acid and N-acetyl-Leucyl-Leucyl-Valinal (ALLN) were obtained from Sigma Chemical.

Preparation of BMJ. The Chinese variety of young bitter melons (raw and green) was obtained from a local farmer’s market, washed, and deseeded. BMJ was extracted according to the published protocol (raw and green) was obtained from a local farmer’s market, washed, and deseeded. BMJ was extracted according to the published protocol (raw and green) was obtained from a local farmer’s market, washed, and deseeded. BMJ was extracted according to the published protocol (raw and green) was obtained from a local farmer’s market, washed, and deseeded. BMJ was extracted according to the published protocol (raw and green) was obtained from a local farmer’s market, washed, and deseeded. BMJ was extracted according to the published protocol (raw and green) was obtained from a local farmer’s market, washed, and deseeded. BMJ was extracted according to the published protocol (raw and green) was obtained from a local farmer’s market, washed, and deseeded. BMJ was extracted according to the published protocol (raw and green) was obtained from a local farmer’s market, washed, and deseeded. BMJ was extracted according to the published protocol (raw and green) was obtained from a local farmer’s market, washed, and deseeded. BMJ was extracted according to the published protocol (raw and green) was obtained from a local farmer’s market, washed, and deseeded. BMJ was extracted according to the published protocol.
inhibited apoB secretion by 17 ± 4% at 0.5% and by 32 ± 4% at 1% BMJ. However, only 1% BMJ concentration was significantly different from control (Fig. 1A). ALLN increased apoB secretion in control cells (148 ± 13%, P < 0.05) indicating an inhibition of apoB degradation (Fig. 1B). However, in the presence of BMJ, ALLN did not significantly increase apoB secretion (95 ± 9 and 89 ± 10 at 0.5 and 1% BMJ, respectively) compared with BMJ-treated with only 1% BSA (Fig. 1A, B). These results suggest that BMJ has no effect on the proteasomal degradation pathway in HepG2 cells, and other factors such as lipid bioavailability could be responsible for the BMJ-associated decrease in apoB secretion.

**BMJ inhibits apoB secretion in presence of increased cellular lipids.** Increased cellular lipids, specifically triglycerides, can limit apoB secretion in HepG2 cells by preventing its proteasomal degradation (26). Oleate increased the net apoB secretion in untreated control cells (147 ± 4.5%, P < 0.05; Fig. 1C). In BMJ-treated cells, oleic acid normalized apoB secretion to untreated control levels without oleate (Fig. 1C; P < 0.05). Interestingly, a lipid-rich environment facilitated the inhibition of proteasomal degradation in cells treated with BMJ, oleate, and ALLN. The apoB levels in BMJ-treated cells (172 ± 6 at 0.5% and 159 ± 9 at 1% BMJ; Fig. 1D) were higher than untreated controls in Figure 1A (P < 0.05), suggesting that BMJ likely affects nonproteasomal degradation of apoB. However, apoB secretion in BMJ-treated cells was significantly lower than in the respective control cells (218 ± 5; Fig. 1D). BMJ did not affect the secretion of the most abundant cellular protein, albumin, suggesting that its effects on apoB secretion are specific (data not shown).

**BMJ inhibits synthesis and secretion of cellular TG.** Because lipid bioavailability was one of the factors affecting the BMJ-associated decrease in apoB (Fig. 1), the rate of incorporation of [3H]glycerol into cellular TG was measured in HepG2 cells treated with BMJ for 24 h. BMJ tended to decrease (P = 0.109) cellular TG synthesis by 17–21%, but both 0.5 and 1% BMJ inhibited TG synthesis (38 ± 5 at 0.5% and 44 ± 6 at 1% BMJ) compared with untreated control cells (P < 0.05, Fig. 2). However, no dose-dependent effects of BMJ were observed. The data suggest that a reduction in TG secretion could be one of the factors in the apoB-reducing effects of BMJ.

**Inhibition of MTP gene expression and induction of nuclear transcription factor, SREBP-1.** Figure 3 depicts a representative gel of MTP and GAPDH gene expression, and the bar graph represents the intensity of 699-bp MTP amplicon expressed as the ratio to GAPDH (Fig. 3). HepG2 cells treated with 0.5 and 1% BMJ decreased MTP gene expression in the presence or absence of oleate, by 30 ± 7% (P < 0.05) and 50 ± 8% (P < 0.05), respectively, compared with untreated controls (Fig. 3).

Nuclear transcription factors such as SREBP are involved in regulating apolipoprotein secretion by affecting MTP levels. Figure 4 is a representative Western blot analysis of the mature SREBP-1 protein in the nuclear fractions of HepG2 cells treated with varying concentrations of BMJ for 24 h. The bar graph represents arbitrary units of the densitometry scan, and data are expressed as a percentage of the control, which indicates increased expression of nSREBP-1c in BMJ-treated cells (517 ± 20 at 0.5%, 485 ± 15 at 1%, 904 ± 22 at 2% and 1100 ± 28 at 5% BMJ; P < 0.05).

**DISCUSSION**

After 24 h of treatment, BMJ decreased apoB secretion in HepG2 cells (P < 0.05). ApoB100, a 556-kDa hydrophobic protein, is synthesized in the endoplasmic reticulum (ER), stabilized through binding with lipids, and then secreted as apoB100-containing lipoproteins such as VLDL. It is therefore possible that the decrease in serum VLDL of diabetic rats fed bitter melon may be due to its ability to inhibit apoB secretion (17). Whether bitter melon can inhibit the actual synthesis of apoB is currently under investigation. Although detailed intracellular processes that lead to the assembly of apoB-containing lipoproteins in the ER are not completely understood, the published literature suggests that proteases located in the endoplasmic reticulum (ER) play a crucial role in apoB degradation. Treatment of HepG2 cells with calpain inhibitor I, ALLN, an inhibitor of proteasomal degradation, did not increase apoB secretion in BMJ-treated cells (Fig. 1B), suggesting that other factors such as lipid bioavailability and MTP activity may be involved. Increased TG synthesis can prevent apoB degrada-

**FIGURE 1** ApoB secretion from HepG2 cells treated with 0.5 and 1.0% BMJ for 24 h in the presence or absence of oleate and the proteasomal inhibitor, ALLN. Data are represented as a percentage of the control (set as 100%). Values are means ± SD from 3 independent experiments performed in duplicate (n = 6). Means with a common letter do not differ, P > 0.05.

**FIGURE 2** Synthesis and secretion of cellular triglycerides in HepG2 cells treated with BMJ for 24 h. Data are represented as a percentage of the control (set as 100%). Values are means ± SD from 3 independent experiments performed in duplicate (n = 6). Means with a common letter do not differ, P > 0.05.
and TG synthesis, our preliminary data indicate that BMJ increases the nuclear translocation of mature SREBP-1 (68-kDa protein) as analyzed by Western immunoblot (Fig. 4, \( P < 0.05 \)). It is further interesting to note that although SREBP-1 is involved in fatty acid and TG synthesis, our preliminary data indicate that BMJ decreases cellular TG synthesis and secretion, suggesting differential effects and alternative mechanisms, such as upregulation of LDL receptor or inhibition of diacylglycerol acyltransferase, an enzyme involved in TG synthesis. Recently, Chao and Huang (8) demonstrated that H4IIEC3 rat liver cells treated with 100 and 150 mg/L of BMJ extract for 72 h activated transcriptional factors such as PPARα and PPARγ, probably contributing to its hypolipidemic and hypoglycemic effects. However, they did not measure the effects of BMJ on cellular lipid levels (8). In our study, HepG2 cells were treated with crude undiluted BMJ at 0.5 and 1% for 24 h (corresponding to 20 and 40 mg/L BMJ protein), which demonstrated a decrease in cellular TG synthesis and secretion independent of SREBP increase. Although a direct comparison between our study and that of Chao and Hung (8) is difficult, due to differences in bitter melon preparations and exposure time, it is highly possible that BMJ exerts its hypoglycemic and hypolipidemic effects through differential regulation of nuclear transcriptional factors, because not only PPARα but also SREBP-1 was shown to regulate insulin effects (28). BMJ concentrations of 0.5 and 1% used in our study were within the range of freeze dried BMJ used in animal studies (7,9). However, our results may reflect acute effects due to differences in absorption and metabolism of BMJ in HepG2 cells compared with in vivo animal models and humans.

Most of the studies conducted to date have used whole fruit juices or crude preparations of bitter melon, and the chemical profile of bitter melon is not well characterized. Therefore, the exact nature of the active ingredients that are responsible for various health-promoting effects is not known. Nevertheless, most preparations from various laboratories were able to demonstrate the beneficial effects of bitter melon in numerous animal studies. In contrast, only a few clinical studies have investigated the effects of bitter melon in humans. Welihinda and co-workers (12) demonstrated an improved glucose tolerance in 73% of type 2 diabetic patients consuming 57 g BMJ/d. In another study, consumption of 15 g/d of the aqueous extract of bitter melon led to a 54% decrease in postprandial glucose levels and a 17% reduction in glycosylated hemoglobin among patients with diabetes.

**FIGURE 3** MTP mRNA expression in HepG2 cells treated with BMJ for 24 h in the presence or absence of oleate. Bar graph represents the densitometry scans of 699-bp MTP amplicon and are expressed as the ratio to GAPDH. Data are expressed as a percentage of the control (set at 100%). Values are means ± SD from 3 independent experiments performed in duplicate (n = 6). Means with a common letter do not differ, \( P > 0.05 \).

**FIGURE 4** Mature form of SREBP in the cytosolic and nuclear fraction (nSREBP) of HepG2 cells treated with BMJ for 24 h. The bar graph represents arbitrary units of the densitometry scan, and data are expressed as a percentage of the control (set at 100%). Values are means ± SD from 3 independent experiments performed in duplicate (n = 6). Means with a common letter do not differ, \( P > 0.05 \).
type 2 diabetes patients (29). Only one study demonstrated that feeding fried bitter melon (rather than raw juice) as a dietary supplement significantly improved glucose tolerance in humans (13). However, these clinical studies were small and they were not randomized. Therefore, adequately powered, randomized, placebo-controlled clinical trials are essential before bitter melon can be recommended as an effective complementary therapy. However, due to its hypoglycemic properties, use of bitter melon with other hypoglycemic agents must occur with medical supervision and monitoring. Some negative side effects such as diarrhea and hepatotoxicity in humans were noted (30); these could be due to excessive consumption. Further studies are warranted to characterize and identify the active ingredients of bitter melon and understand the pharmacokinetics in humans.

ACKNOWLEDGMENTS

We thank Raymond Liu and Hezhi Gan for their technical assistance.

LITERATURE CITED