Keywords: Prieurianin; Obesity; Appetite; Adipogenesis; Weight loss; Tolerance; Preadipocytes; Adipocytes

Introduction

The alarming rise in the incidence of obesity worldwide has emerged as one of the most serious public health risks in recent years. Despite the enormity of the obesity pandemic, there are currently only two FDA-approved therapies for its treatment and these drugs exhibit modest efficacy and have limiting side effects. Prieurianin is a plant limonoid product that deters feeding in insect larvae. We investigated in this study the effects of prieurianin on weight loss and adipogenesis. Our results showed that prieurianin causes weight loss by reducing energy intake in obese mice on high-calorie diet. We also found that prieurianin is anti-adipogenic in cultured preadipocytes and adipocytes by inhibiting proliferation and differentiation of preadipocytes into adipocytes, and induces either dedifferentiation or delipidation of mature adipocytes. Whether prieurianin can potentially be used for obesity treatment in human warrants further investigation.

Abstract

The global increase in the incidence of obesity has emerged as one of the most pressing public health issues in recent years. Despite the enormity of the obesity pandemic, there are currently only two FDA-approved therapies for its treatment and these drugs exhibit modest efficacy and have limiting side effects. Prieurianin is a plant limonoid product that deters feeding in insect larvae. We investigated in this study the effects of prieurianin on weight loss and adipogenesis. Our results showed that prieurianin causes weight loss by reducing energy intake in obese mice on high-calorie diet. We also found that prieurianin is anti-adipogenic in cultured preadipocytes and adipocytes by inhibiting proliferation and differentiation of preadipocytes into adipocytes, and induces either dedifferentiation or delipidation of mature adipocytes. Whether prieurianin can potentially be used for obesity treatment in human warrants further investigation.

Materials and Methods

Animals, diets, drugs, and reagents

All animal procedures were approved by the Institutional Animal Care and Use Committee. For all in vivo efficacy studies, prieurianin was dissolved in DMSO and then given intraperitoneally as a mixture in 10% Cremophor EL (Sigma-Aldrich, St. Louis, MO) [16].

The completion of some recent late stage anti-obesity clinical trials using combinations of various repositioned drugs including anticonvulsant, antidepressant and a controlled substance of the amphetamine class, seemed to meet FDA criteria for clinical efficacy [12]. Other than the novel monotherapeutic lorcaserin, these repositioned drugs, however, all have a history of safety issues. The potential side effects associated with the long-term use of these agents remain to be evaluated. Despite these progresses, it is imperative to identify new drugs to address the unmet needs for the treatment of obesity.

Some compounds in plants have been developed for their use in insect pest management without harming the environment [13]. Prieurianin, a limonoid isolated from several plant species, exhibits specific anti-feedant activity in insect using a diet choice bioassay [14]. The drug has no apparent effects on the efficiency of conversion of ingested food in larvae and seems to antagonize 20-hydroxyecdysone activity in Drosophila cells [14, 15].

In light of its ability to deter feeding in insects, we hypothesized that prieurianin would interfere with feeding behaviour in mice and cause weight loss. In this report, we showed that prieurianin suppresses appetite and causes weight loss in diet-induced obese mice and inhibits adipogenesis in cell culture model of preadipocytes. Our results suggest that prieurianin may be an effective anti-obesity drug candidate and also serve as a pharmacological tool for probing the biology of adipose tissue.
to the animals, and vehicle-treated controls received equivolume injections of 0.5% of DMSO in Cremophor. For diet-induced obesity studies, 12-14 week-old normal C57BL/6 (B6) mice (The Jackson Laboratory, Bar Harbor, ME) were acclimatized for at least one week on a 12-hour light/dark cycle at 68-72°F and then fed a high-fat diet containing 45 kcal% fat (Research Diets, Inc., New Brunswick, NJ), until their mean body weight reached approximately 30 g, and remained on the high-fat diet for the duration of the study. Mice had access to the high fat diet ad libitum during treatment in all studies. Blood glucose and insulin levels were measured and adipose tissues were harvested for weighing at the end of the experiments. Food intake and body weight were measured every two days for the duration of the study. Mice were euthanized by CO2 asphyxiation according to the Association for Assessment and Accreditation of Laboratory Animal Care International guidelines. All studies were performed with at least 10 to 20 animals per group. 

Prieurianin was obtained from MicroSource Discovery Systems (Gaylordsville, CT), and sibutramine from Sigma-Aldrich (St. Louis, MO). Tumor necrosis factor (Gaylordsville, CT), and sibutramine from Sigma-Aldrich (St. Louis, MO) were given either 1 or 3 mg kg-1 of prieurianin for three weeks. Prieurianin causes weight loss and reduced food consumption (C) in DIO B6 mice compared to untreated and vehicle-treated controls. All studies consisted of ten mice per group. Statistics were conducted as an ANOVA; asterisk, P<0.05 versus untreated and vehicle-treated controls. Error bars indicate s.e.m.

Cell culture and adipocyte differentiation

NIH-3T3/L1 (L1) (American Type Culture Collection, Manassas, VA) and OP9 stromal (gift of Dr. Perry Bickel, University of Texas Health Science Center, Houston, TX) cells were cultured at 37°C with 10% CO2 in Dulbecco’s modified Eagle’s medium (DMEM) (Invitrogen, Carlsbad, CA) supplemented with 10% (v/v) fetal calf serum (Invitrogen), 1 mM sodium pyruvate, 0.1 mM non-essential amino acids, 2 mM L-glutamine, 100 μg/ml streptomycin sulfate, and 100 U ml-1 penicillin. To assess preadipocytes proliferation, cells were plated in 12 well dishes and then treated with various concentrations of prieurianin. Cell growth was measured daily on a Coulter cell counter. To differentiate L1 cells into adipocytes, cells were incubated with 250 nM dexamethasone, 450 μM 3-isobutyl-1-methylxanthine, and 167 nM insulin for 2 days, followed by 167 nM insulin for an additional 3 days as described (17). For OP9 cells (18), differentiation was initiated with a serum replacement medium composed of MEM-a with 15% KnockOut SR (Invitrogen, Carlsbad, CA) or the data analysis add-in for Microsoft Excel. Either single factor ANOVA or Student’s-t tests were used for all analysis as indicated. P values of less than 0.05 were considered statistically significant.

Results

Anorexigenicity. The observation that prieurianin (Figure1A) is a feeding deterrent in insect larvae that presumably acts by

Figure 1: Prieurianin in mouse model of diet-induced obesity (DIO). A, Structure of prieurianin. B, C, Body weight and amount of food consumed by DIO B6 mice that were given either 1 or 3 mg kg-1 of prieurianin for three weeks. Prieurianin causes weight loss (B) and reduced food consumption (C) in DIO B6 mice compared to untreated and vehicle-treated controls. All studies consisted of ten mice per group. Statistics were conducted as an ANOVA; asterisk, P<0.05 versus untreated and vehicle-treated controls. Error bars indicate s.e.m.
antagonizing 20-hydroxyecdysone in Drosophila cells prompted us to ask whether the anti-feedant effects of prieurianin can be exploited for the treatment of obesity. To test this hypothesis, we examined the effects of prieurianin on weight loss in the diet-induced obese (DIO) C57BL/6 (B6) mice, since this model has been widely used to investigate the underlying mechanisms of obesity in human [20, 21]. DIO B6 mice were given high fat diet for 15 weeks and then prieurianin was administered intraperitoneally (i.p.) daily with either 1 or 3 mg kg⁻¹ prieurianin for three weeks. Prieurianin caused a dose-dependent weight loss (Figure 1B) that was accompanied by 70-80% decrease in food consumption, which eventually returned a dose-dependent weight loss (Figure 1B) that was accompanied by “drug holidays”, mice were treated with either 3 or 5 mg kg⁻¹ prieurianin for 5 or 3 days, respectively, followed by 5 days of drug withdrawal, and repeating this regimen for 4 cycles (Figure 2A and B). DIO B6 mice from the experiments in (Figure 1) were maintained with intermittent drug holiday to overcome drug-induced tolerance. Notably, a gradual regain in body weight was observed following withdrawal, and repeating this regimen for 4 cycles (Figure 2A and B). DIO B6 mice from the experiments in (Figure 1) were maintained on the high calorie diet for four additional weeks without treatment and then subjected to the drug holiday treatment cycle. This treatment protocol, at either 3 or 5 mg kg⁻¹ prieurianin, produced a greater response than daily treatment, with up to 20% weight loss at either dosage, and no observable weight regain at the end of the treatment cycle (Figure 2C). Food consumption decreased precipitously initially, but gradually returned to approximately 60-70% of normal feeding and maintained at that level for the duration of the treatment cycle (Figure 2D). Fed glucose and insulin levels also showed a trend of decrease at the end of treatment period (Figure 3A and B).

Anti-adipogenesis

Post-mortem analysis showed >50% decrease in subcutaneous and visceral fat depots in prieurianin-treated DIO B6 mice (Figure 3C and D), compared to untreated and vehicle-treated controls. The ability of prieurianin to reduce fat mass led us to determine the effects of prieurianin on the differentiation of cultured preadipocytes into mature adipocytes using either the L1 preadipocytes [17], or the OP9 mouse stromal cells [18] that are capable of differentiating into adipocytes. Prieurianin inhibited the proliferation of L1 preadipocytes in a dose-dependent manner (Figure 4A), as well as their post-confluent mitosis and clonal expansion, evident from the reduced ³²P]thymidine uptake following differentiation induction (Figure 4B). Prieurianin also inhibited the differentiation of OP9 stromal cells into adipocytes as evident from the reduced Oil Red O stained lipid-accumulating adipocytes relative to the untreated/undifferentiated and the differentiated controls (Figure 5A). The positive control, TNF α [22,23], also inhibited OP9 cells differentiation (Figure 5A). These effects of prieurianin were recapitulated in the L1 preadipocytes (data not shown). The decrease in adipocytes was not a result of prieurianin-induced apoptosis, as indicated by the lack of annexin V binding to phosphatidylserine, in contrast to stromal cells treated with doxorubicin, a cytotoxic drug that causes apoptosis (Figure 4C). Similarly, apoptosis was not observed in prieurianin-treated L1 preadipocytes (data not shown).
To assess the effects of prieurianin in mature adipocytes, L1 preadipocytes were differentiated into adipocytes followed by treatment with either TNFα or prieurianin. Consistent with previous report [22], TNFα-induced rapid lipolysis, resulting in significant loss of Nile Red stained adipocytes (Figure 5B). Though less potent, prieurianin also caused a loss of lipid in the adipocytes, marked by reduced Nile Red positive cells compared to differentiated control and vehicle-treated cells (Figure 5B).

**Discussion**

Despite a growing obese population worldwide, pharmacotherapy for obesity is limited as there are currently only two FDA-approved drugs for its treatment in U.S. Recent FDA issued heart risk warning in U.S. for sibutramine and its withdrawal in Europe, as well as new concerns for potential liver toxicity with orlistat, further reduced the armamentarium for the combat of obesity. Moreover, the use of repositioned drugs comprising of combinatorial mixture of anticonvulsant, antidepressant, or psychoactive stimulant, also poses questions of unknown longterm safety concerns, as these drugs all have various known risks and side effects [24]. These issues all point to an urgent need for the development of more efficacious and safe pharmacotherapeutics for obesity.

Our studies here showed that prieurianin, a plant-derived limonoid product, which exhibits feeding deterrent activity in insect larvae, has biological activity in high-fat diet induced obese mice that results in appetite suppression, leading to decreased energy intake in mice and weight loss. Further, prieurianin also inhibits the proliferation and differentiation of preadipocytes, as well as causing mature adipocytes to lose their lipid content. These findings suggest a novel anti-obesity drug candidate characterized by a dual anorexigenic and anti-adipogenic property that is distinct from currently available pharmaceutical therapies and pipeline products in development that focused on either suppressing satiety or inhibiting energy homeostasis [11]. The anorexigenic effect of prieurianin could not have been due to taste aversion because the drug was delivered by IP injection into the mice. Moreover, the diet choice bioassay in insect larvae also suggests a specific effect of prieurianin on feeding stimulus [14]. Although it was shown to antagonize 20-hydroxyecdysone action in Drosophila cells [15], the precise mechanism of action of prieurianin, however, is unknown with respect to its effects on appetite suppression in mice and its inhibition of adipogenesis in cultured preadipocytes. It is noteworthy that prieurianin was recently shown to inhibit endocytosis and also block brassinosteroid-induced gene expression in germinating seedlings of Arabidopsis thaliana [25]. It is unclear how prieurianin-inhibited endocytosis translates into its anti-obesity effects. Furthermore, the inhibition of hormone-induced transcription in germinating seedlings by prieurianin suggests that the drug may have similar effects on the transcriptional regulation of adipogenesis in preadipocytes by disrupting gene expression program during differentiation. Hence, identifying the molecular target(s) of prieurianin might yield insights into its anorexigenic activity in suppressing energy intake and its anti-adipogenic potential in the development of adipose tissue.

Long-term efficacy and effectiveness of obesity treatments are known to have poor outcome [26]. The potential of drug-induced tolerance associated with anti-obesity therapeutics is long recognized, but circumventing its development has not been fully addressed [6]. Our studies here demonstrated that drug-induced tolerance resulting from chronic pharmacotherapy for obesity could be overcome by a cyclical drug holiday treatment strategy, which produces a greater and more durable therapeutic response as evident by the sustained weight loss and reduced energy intake. Hence, the on-off treatment protocol may potentially restore the efficacy of currently available anti-obesity drugs as well as other experimental pharmacotherapeutics that are prone to drug-induced tolerance in the long-term treatment of obesity. Besides, decreasing the frequency of drug exposure using the drug holiday treatment protocol may reduce the incidence and disposition to the toxicities of these anti-obesity drugs.
Taken together, the findings in this study showed that prieurianin is a novel anti-obesity therapeutic candidate that exhibits a dual anorexigenic and anti-adipogenic property. Therefore, investigating the precise molecular target of prieurianin, and characterizing its long-term efficacy and toxicology profile will determine its suitability for further development as a pharmacotherapeutic for obesity.

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References

1. Swinburn B, Sacks G, Ravussin E (2009) Increased food energy supply is more than sufficient to explain the US epidemic of obesity. Am J Clin Nutr 90: 1453-1456.
2. Bray GA, Bellanger T (2006) Epidemiology, trends, and morbidities of obesity in the United States. JAMA 295: 761-775.
3. Guerciolini R (1997) Mode of action of orlistat. Int J Obes Relat Metab Disord 21: S12-23.
4. Ryan DH, Kaiser P, Bray GA (1995) Sibutramine: a novel new agent for obesity treatment. Obes Res 3: 553S-559S.
5. Bray GA (2008) Lifestyle and pharmacological approaches to weight loss: efficacy and safety. J Clin Endocrinol Metab 93: S81-88.
6. Fernstrom JD, Choi S (2007) The development of tolerance to drugs that suppress food intake. Pharmacol Ther 117: 105-122.
7. Li Z, Maglione M, Tu W, Mojica W, Shugarman LR, Arteburn D, et al. (2005) Meta-analysis: pharmacologic treatment of obesity. Ann Intern Med 142: 532-546.
8. Pi-Sunyer FX, Aronne LJ, Heshmati HM, Devin J, Rosenstock J (2006) Current and emerging weight-loss medications: current and emerging weight-loss medications? Curr Diab Rep 9: 368-375.
9. Sarker SD, Savchenko T, Whiting P, Sik V, Dinan L (1997) Two limonoids from Turraea obtusifolia (Meliaceae), prieurianin and rohitukin, antagonize 20-hydroxyecdysone action in a Drosophila cell line. Arch Insect Biochem Physiol 35: 211-217.
10. Dorr RT (1994) Pharmacology and toxicology of Cremophor EL diluent. Ann Pharmacother 28: S11-S14.
11. Green H, Meuth M (1974) An established pre-adipose cell line and its differentiation in culture. Cell 3: 127-133.
12. Wolins NE, Quaynor BR, Skinner JR, Tzvetk A, Park C et al. (2006) OP9 mouse stromal cells rapidly differentiate into adipocytes: characterization of a useful new model of adipogenesis. J LipidRes 47: 450-460.
13. Gonzales AM, Orlando RA (2007) Role of adipocyte-derived lipoprotein lipase in adipocyte hypertrophy. Nutr Metab 4: 22.
14. Tschop M, Heiman ML (2001) Rodent obesity models: an overview. Exp Clin Endocrinol Diabetes 109: 307-319.
15. Collins S, Martin TL, Surwil RS, Robidoux J (2004) Genetic vulnerability to diet-induced obesity in the C57BL/6J mouse: physiological and molecular characteristics. Physiol Behav 83: 243-248.
16. Simons PJ, van den Pangaart PS, van Roomen CP, Aerts JM, Boon L (2005) Cytokine-mediated modulation of leptin and adiponectin secretion during in vitro adipogenesis: evidence that tumor necrosis factor-alpha and interleukin-1beta-treated human preadipocytes are potent leptin producers. Cytokine 32: 94-103.
17. Spiegelman BM, Hotamisligil GS (1993) Through thick and thin: wasting, obesity and TNF alpha. Cell 73: 625-627.
18. Robinson JR, Niwender KD (2009) What are the risks and the benefits of current and emerging weight-loss medications? Curr Diab Rep 9: 368-375.
19. Robert S, Chary SN, Drakaki G, Li S, Yang Z, et al. (2008) Endosidin1 defines a compartment involved in endocytosis of the brassinosteroid receptor BR11 and the auxin transporters PIN2 and AUX1. Proc Natl Acad Sci U S A 105: 8464-8469.
20. Mauro M, Taylor V, Wharton S, Sharma AM (2008) Barriers to obesity treatment. Eur J Intern Med 19: 173-180.