Effects of Naringenin on Lipogenesis, Lipolysis and Glucose Uptake in Rat Adipocyte Primary Culture: A natural antidiabetic agent

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Abstract

The flavonoid naringenin is commonly found in citrus fruits and tomato. The main aim of this study was to assess the in vitro insulin-like effects of naringenin using rat adipocyte primary culture. The adipogenic, lipolytic and glucose uptake activities of naringenin in primary rat adipocytes were assessed using Oil Red O, glycerol, and 2-deoxy-D-[2,6-^{3}H]glucose uptake quantitative assays respectively. Naringenin exhibited significant increase of lipogenesis in the presence and absence of insulin in primary rat preadipocytes. At the concentration range of 0.01 – 100 µM, naringenin inhibited 50 % of epinephrine induced lipolysis in rat adipocytes and enhanced insulin's antilipolytic activity. Naringenin (100 µM) stimulated 163 % glucose uptake in rat adipocytes (compared to untreated cells) and this was significantly higher than the insulin mediated glucose uptake at similar concentration. Thus, naringenin may play an important role as an adjuvant and/or alternative to insulin therapy for the management of diabetes mellitus.

INTRODUCTION

Diabetes mellitus (DM) is a life-long metabolic disorder characterized by inability of glucose transportation from bloodstream into adipose tissue due to defects in insulin action, insulin secretion or both [1]. In the year 2000, the total number of people diagnosed with DM was 171 million but this number is estimated to increase to 366 million in the year 2030 [2]. Type 2 DM is the most common form of diabetes which contributes to more than 85 % of all cases of diabetes worldwide [3]. This phenomenon is worrying because Type 2 DM usually occurs in conjunction with other metabolic syndrome including obesity, hypertension and dyslipidemia [4]. Obesity has a strong correlation with Type 2 DM and high prevalence of obesity had proven to cause epidemic condition of diabetes mellitus [5]. The adipocytes in obese individuals which are enlarged with high concentration of lipid develop resistance to insulin [6].

Insulin plays an important role in regulating glucose and lipid metabolism. Insulin inhibits lipolysis elicited by epinephrine as well as promotes triglyceride storage in preadipocytes and glucose uptake in adipocytes [7]. The effects of insulin are counteracted by multiple hormones, such as glucagon, epinephrine, and isoproterenol which convert glycogen and triglyceride to glycerol [8]. Lipid metabolism in adipocytes has great influence on the occurrence of obesity and Type 2 DM. Hence, identification of compounds that are able to mimic insulin in lipid metabolism will add credence to the development of new anti-diabetic drugs or nutraceuticals that can be adjuvants to insulin therapy.

Flavonoids are plant metabolites with benzo-gamma-pyrone structure, which are found ubiquitously in all parts of plants [9]. Flavonoids are known to exert beneficial effects such as decrease total cholesterol, improve control of blood glucose concentration, and help in weight control in diabetic patients [10]. The flavonoid naringenin (4',5,7-trihydroxyflavanone) is commonly found in citrus fruits and tomato. Many experiments have been carried out with animal models to find a cure for obesity and DM. However the present study was conducted in vitro using primary rat preadipocytes as a cellular model, to investigate the insulin-like effects of naringenin.

MATERIALS AND METHODS
MATERIALS

All cell culture media and culture flasks were purchased from Sigma-Aldrich (St. Louis, USA) and Nunc (Naperville, IL, USA) respectively. Penicillin-Streptomycin-Glutamine

and insulin were obtained from Invitrogen (Carlsbad, California) whereas 1-methyl-3-isobuylxanthine (IBMX), glycerol standard, dexamethasone, adenosine-5-triphosphate (ATP) and liquid scintillation counting cocktail were purchased from ICN (Aurora, Ohio). Phosphate-buffered saline tablets (PBS), Type-II collagenase and 2-deoxy-D-[2,6-3H]glucose were obtained from Flowlab (Sydney, Australia), Worthington Biochemical (Lakewood, New Jersey) and Amersham Biosciences (Piscataway, New Jersey) respectively. Epinephrine, isoproterenol, propanolol, 4-aminoantipyrine, N-ethyl-N-(3-sulfopropyl)-m-anisidine, sodium azide, naringenin and 2-deoxyglucose were obtained from Sigma-Aldrich (St. Louis, USA). Peroxidase, glycerokinase, and glycerol phosphate oxidase were purchased from Roche (Mannheim, Germany).

METHODS
TISSUE CULTURE

The intra-abdominal and epididymal fat pads were obtained from normal male Sprague-Dawley rats aged 6-8 weeks according to published articles with minor modifications [11] . Preadipocytes obtained were cultured in RPMI 1640 media supplemented with 2 mM penicillin-streptomycin-glutamine and 10 % foetal bovine serum (FBS). Upon confluency, the preadipocytes were differentiated with DMEM supplemented with penicillin-streptomycin-glutamine, 10 % FBS, 17 μM pantothenic acid, 0.5 mM IBMX, 1 μM dexamethasone, 10 μg/ml insulin and 33 μM biotin. After two days, IBMX and dexamethasone were removed from the differentiation media. The preadipocytes were allowed to differentiate in this media for up to 15 days.

PREPARATION OF NARINGENIN

Naringenin was dissolved in 100% DMSO and subsequently diluted to a concentration range of 0.01μM, 0.1μM, 1.0μM, 10μM and 100μM using 1% DMSO.

LIPOGENESIS ASSAY

Preadipocytes in 12-well tissue culture plate were incubated with naringenin in humidified air jacketed incubator containing 5 % CO_2 at 37 ?C. On Day-12 after the initiation of differentiation, the cells were fixed using 10 % formalin and the lipid accumulated during lipogenesis was stained with Oil Red O dye before quantified using spectrophotometer at 510nm.

LIPOLYSIS ASSAY

The glycerol released in lipolysis was measured according to the method by Trinder [12] . The glycerol reagent was prepared by mixing ATP, magnesium salt, 4-aminoantipyrine, sodium-N-ethyl-N0-(3-sulfopropyl)-m-anisidine, glycerol kinase, glycerol phosphate oxidase, peroxidase and TRIS buffer. Naringenin was incubated with the fully differentiated adipocytes at 37 ºC for 5 hours. Then, the glycerol reagent was added to the incubation mixture and the resulting colored complex was measured at 510nm.Quantitation was carried out using glycerol standard run concurrently with the incubations in the presence of the test compounds..

GLUCOSE UPTAKE ASSAY

The glucose label was prepared by mixing 2-deoxyglucose, 2-deoxy-D-[2,6-^{3}H]glucose and phosphate buffered saline (PBS). Fully differentiated adipocytes were serum-starved for 2 hours prior to the glucose uptake assay. Naringenin was pre-incubated with the adipocytes for 30 minutes, followed by the incubation with [^{3}H]2-deoxyglucose label for another10 minutes. The reaction was terminated by washing the cells thrice with ice-cold PBS. Finally, scintillation cocktail was added into each well and the amount of [^{3}H]2-deoxyglucose label taken up by adipocytes was quantified through scintillation counting.

RESULTS AND DISCUSSION

Adipose tissues are insulin-responsive; therefore they are widely used as the cellular model to investigate the biochemical pathways and physiological mechanisms related to diabetes and obesity. The adipocyte primary culture model can serve as a good platform for early assessment of potential hypoglycaemic agents and their properties against lipid metabolism. This could minimize the usage of large number of animals for such preliminary assessments.

In this study, confluent preadipocytes were induced to differentiate in DMEM media. Confluent state is an indicator of preadipocyte growth arrest stage, which is a prerequisite for the cells to become committed to adipocyte cell lineage [13] . Fully differentiated adipocytes accumulate lipid droplets in their cytoplasm. Insulin plays a vital role in preadipocyte differentiation/lipogenesis by activating insulin-signaling cascade (ISC) which leads to lipid metabolism as well as preadipocyte differentiation. Therefore, compounds that are able to induce lipogenesis could potentially have insulin-like property. The extent of lipogenesis was reflected by the amount of lipid accumulation in mature adipocytes. Naringenin showed very mild stimulation of lipogenesis in

primry rat adipocytes at the concentration range tested with only 40 % stimulation at the maximum dose of 100 μM. When co-incubated with insulin, naringenin synergistically increased at least 50 % of insulin induced lipogenesis (Figure 1).

Figure 1

Figure 1: Effects of Naringenin and Insulin (1 ÂμM) on Primary Rat Preadipocyte Differentiation/Lipogenesis. (* indicates P< 0.05, ** indicates P< 0.01 and *** indicates P<0.001 compared to the positive control as determined by Student's t-test)
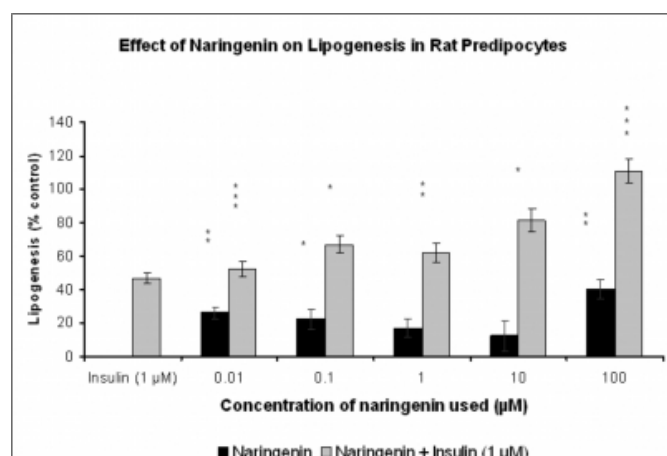
Lipolytic activity in adipocytes is highly dependant on hormone sensitive lipase (HSL), which is regulated by insulin and catecholamines [14]. Epinephrine is well known to stimulate lipolysis by promoting adenylate cyclase activity to increase cAMP level leading to the activation of HSL [15]. However, the activation of HSL could be blocked by insulin through the activation of cAMP phosphodiesterase (PDE) and stimulation of protein phosphatise-1, which in turn will lead to HSL dephosphorylation. Hence, epinephrine induced lipolysis in adipocytes was used as our model to investigate naringenin's antilipolitic activity. Epinephrine (1 μM) exerted 125 % of lipolysis whereas insulin (1 μM), an antilipolytic agent showed a baseline lipolytic level of 17 % lipolysis in primary rat adipocytes compared to untreated cells. At the concentration range of 0.01 – 100 μM, naringenin inhibited almost 50 % of epinephrine induced lipolysis in rat adipocytes as well as enhanced insulin's antilipolytic activity (Figure 2).

Figure 2

Figure 2: Effects of Epinephrine (1 ÂμM), Insulin (1 ÂμM) and Naringenin on Primary Rat Adipocyte Lipolysis. (* indicates P< 0.05, ** indicates P< 0.01 and *** indicates P<0.001 compared to the positive control as determined by Student's t-test.)
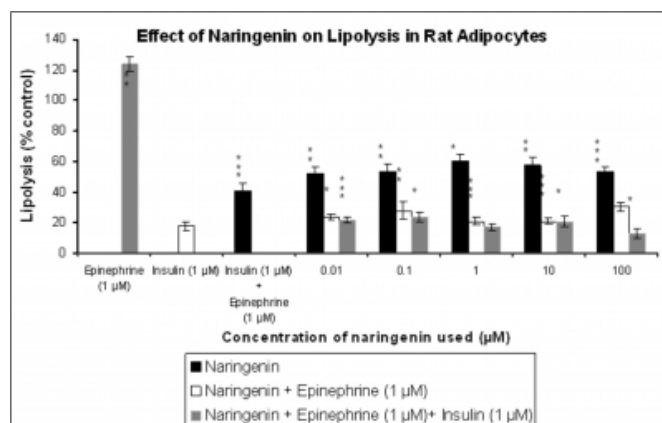
Insulin is also known to stimulate glucose uptake in adipocytes by activating several insulin-signalling proteins, namely insulin receptor substrates (IRS), phosphatidylinositol 3-kinase (PI3-K), and protein kinase B (PKB) [16]. The insulin-signalling proteins will eventually lead to the translocation of insulin-sensitive glucose transporter 4 (GLUT4) vesicles to the plasma membrane and facilitates glucose uptake into adipose tissue [16]. The present study showed that naringenin stimulated a significantly higher glucose uptake activity in primary rat adipocytes when compared to insulin. At a concentration of 100 μM, insulin stimulated 130 % glucose uptake whereas naringenin (100 μM) stimulated 163 % glucose uptake in rat adipocytes (Figure 3).

Figure 3

Figure 3: Effects of Insulin (100 μM) and Naringenin on Glucose Uptake in Rat Adipocytes. (** indicates P< 0.01 and *** indicates P
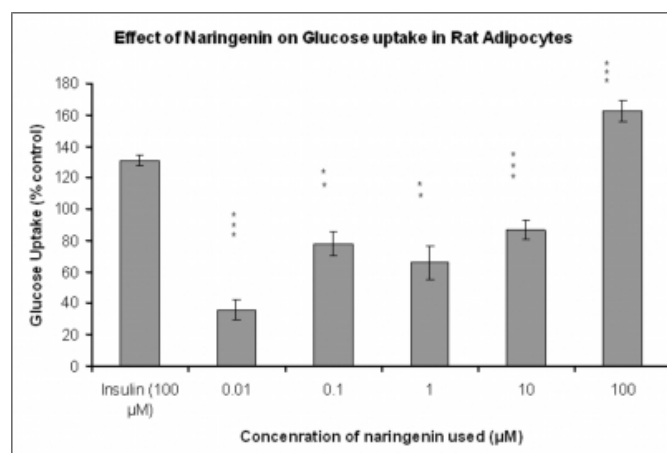
Insulin and some common antidiabetic drugs (e.g. Rosiglitazone) are known to have lipogenic, glucose uptake and epinephrine induced antilipolytic activities. Similarly, flavanoids which have been shown to have these activities were considered to be potential antidiabetic agents. Naringenin stimulated lipogenesis and glucose uptake but inhibited epinephrine induced lipolysis in adipocytes both in the presence and absence of insulin. These findings correlated with other studies using in vivo rat models which showed that naringenin helps to reduce blood glucose in streptozotocin-induced diabetic rats [17,18] . Hence, the foods rich in naringenin may be able to help attenuate the post-prandial hyperglycemic state in diabetic patients. Naringenin could serve as a therapeutic agent or an adjuvant to insulin therapy in the management of diabetes mellitus.

CONCLUSION

Naringenin is an 'insulin-like' agent since it significantly induced adipogenesis in primary rat adipocytes but inhibited epinephrine induced lipolytic activity apart from exerting a higher efficacy in stimulating glucose uptake activity when compared to insulin. The exact pathways involved in the activation of insulin-like activities require further investigations and

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