International Journal of Angiology 11: 107–109 (2002)
DOI: 10.1007/s00547-001-0071

Rapid Communication

Suppression of Phosphoenolpyruvate Carboxykinase Gene Expression by Secoisolariciresinol Diglucoside (SDG), a New Antidiabetic Agent

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Abstract. Secoisolariciresinol diglucoside (SDG) from flaxseed has been shown to be effective in preventing/delaying the development of type-1 and type-2 diabetes. The hypoglycemic effect of SDG in type-2 diabetes has been suggested to be due to its antioxidant activity. Hyperglycemia in type-2 diabetes could be due to an increase in the expression of phosphoenolpyruvate carboxykinase (PEPCK), a rate-limiting enzyme in the gluconeogenesis in the liver. It is possible that the hypoglycemic effect of SDG in type-2 diabetes is due to suppression of expression of PEPCK gene. An investigation, therefore, was made on the effect of SDG on the PEPCK gene expression. Primary hepatocyte culture was treated with insulin, a physiological inhibitor of PEPCK gene expression, or with SDG for 12 hours. After RNA extractions, Northern blot analysis was done. SDG in the concentration of 100 μM completely suppressed the expression of PEPCK gene. Insulin in the concentration of 10 nM almost completely suppressed the PEPCK gene expression. The results suggest that SDG suppresses the expression of PEPCK gene and that its known hypoglycemic effect may be due to suppression of PEPCK gene expression.

Introduction

Type-2 diabetes is characterized by hyperglycemia, impaired glucose tolerance, insulin resistance and hyperlipidemia. Its complications are numerous, including coronary artery disease, stroke, polyneuropathy, nephropathy and retinopathy. Besides impaired insulin secretion and insulin resistance [1–3], increased hepatic glucose production is a major cause of hyperglycemia in patients with type-2 diabetes. Increased hepatic glucose production has been reported to be due to increased hepatic gluconeogenesis [4–6]. Phosphoenolpyruvate carboxykinase (PEPCK) is a rate-limiting enzyme for gluconeogenesis in liver [7] and is elevated in all models of diabetes irrespective of the origin (chemical or genetic) [8–11]. Regulation of the activity of PEPCK is primarily controlled through gene expression [12]. Troglitazone, a hypoglycemic drug used in type-2 diabetes [13] inhibits the expression of PEPCK [14] and has antioxidant activity [15].

Recently SDG, an antioxidant [16,17], has been shown to prevent the development of type-1 diabetes (both in streptozotocin-induced and in BBdp rats) [18,19] and type-2 diabetes (zucker diabetes fatty [ZDF] female rats) [20]. The antidiabetic effects were related to a decrease in the oxidative stress. SDG was shown to be hypoglycemic in both the type-1 and type-2 animal model of diabetes. However, the biochemical mechanism by which SDG suppresses serum glucose levels still remains unclear. Hypoglycemic effects of SDG could be due to suppression of PEPCK, a rate-limiting enzyme for gluconeogenesis. The aim of the present study is to assess the ability of SDG to suppress PEPCK. Since the regulation of PEPCK activity is primarily determined at the level of transcription, the effect of SDG on PEPCK gene expression was investigated.

Methods

Male Sprague-Dawley rats weighing between 150–200 g were anesthetized with pentobarbital sodium (40 mg/kg intraperitoneally) and primary hepatocytes were isolated by collagenase method [21]. Viability of the cells that was greater than 90% was determined by Trypan blue exclusion method. Cell cultures were prepared as described by Davies et al. [14]. Cell cultures were treated with 10 nM insulin (physiological inhibitor of PEPCK), 100 μM of SDG, and vehicle control, and incubated for 12 hours in 5% CO_2 in a humidified atmosphere at 37°C. The methods for RNA extraction and Northern blot analysis were as previously described [18]. Total RNA was extracted from the primary cultured hepatocytes using Trizol reagent (Gibco-BRL) according to manufacturer's instructions. For Northern blot analysis, 20 μg of RNA was resolved on 1% agarose-formaldehyde gels and transferred on to Hybond membranes by capillary elution.

Ultraviolet cross-linked membranes were hybridized with the ^{32}P radio-labelled 2 kb cDNA fragment of PEPCK at 65°C overnight. The

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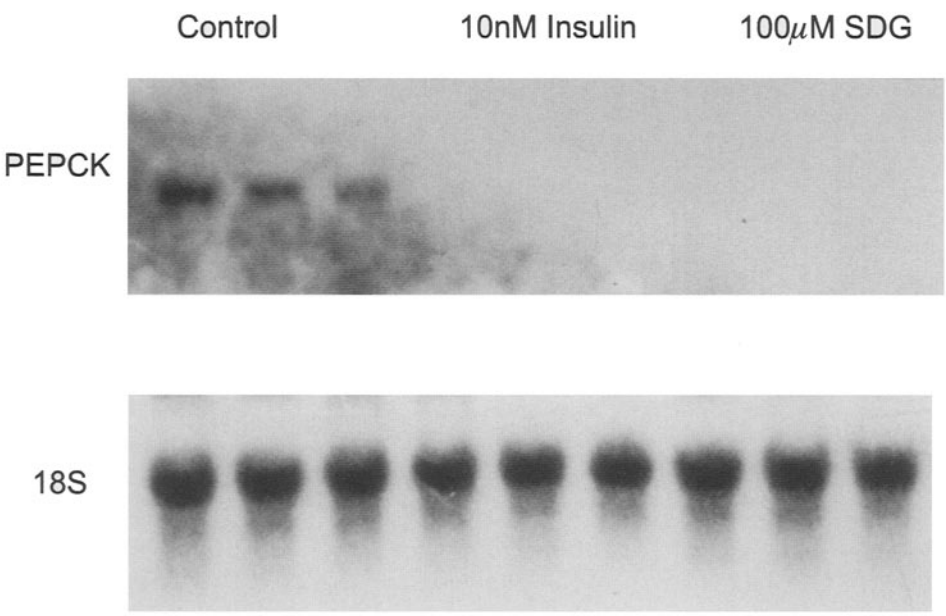

Fig. 1. Effect of SDG and insulin on the levels of PEPCK mRNA in primary rat hepatocytes. Lanes 1-3, basal PEPCK; Lanes 4-6, PEPCK with insulin (10 nM) treatment; Lanes 7-9, PEPCK with SDG (100 μM treatment. 18S ribo-probe for RNA loading equivalent.

membrane was exposed to XAR-5 film (Eastman Kodak) overnight at −80°C. The blot was stripped and re-probed with 32_P labelled cDNA fragment of 18 S ribosomal RNA for verification of equivalent RNA loading.

Results

SDG in the concentration of 100 μM completely suppressed the PEPCK gene expression. Representative blots from three independent experiments are shown in Figure 1. Insulin in the concentration of 10 nM almost completely suppressed the expression of PEPCK gene.

Discussion

PEPCK is the rate-limiting enzyme for hepatic gluconeogenesis and alterations in PEPCK mRNA levels have been reported to accurately reflect changes in the PEPCK enzyme activity and the rate of gluconeogenesis [22]. SDG, a lignan isolated from flaxseed, has been shown to be effective in prevention of both type-1 and type-2 diabetes [18–20]. Prasad [19,20] and Prasad et al. [18] have suggested that the prevention of diabetes may be due to reduction in oxidative stress. The present results suggest that hypoglycemic effect of SDG could be due to inhibition of PEPCK gene expression in addition to reduction in oxidative stress. Troglitazone, a known antidiabetic agent, suppresses PEPCK gene expression [14,15] and is an antioxidant [23]. Vitamin E, an antioxidant [24], also suppresses PEPCK gene expression [15]. Rosiglitazone, which has very little antioxidant activity, is ineffective in inhibition of PEPCK gene expression [15]. It therefore appears that inhibition of PEPCK gene expression by SDG could be due to its antioxidant activity.

Conclusion

The preliminary results suggest that secoisolariciresinol diglucoside isolated from flaxseed suppresses the expression of PEPCK gene, a rate-limiting enzyme in the gluconeogenesis in liver. The antidiabetic effect of SDG could be due to this inhibition of PEPCK gene expression.

Acknowledgements. The technical assistance of Mr. G. F. Davies is highly appreciated.

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