Endocrinol Diabetes Nutr. 2018;xxx(xx):xxx–xxx

Endocrinología, Diabetes y Nutrición

www.elsevier.es/endo

ORIGINAL

Silymarin prevents diabetes-induced hyperpermeability in human retinal endothelial cells

Marta García-Ramírez[a,b,*], **Mireia Turch**[a], **Olga Simó-Servat**[a,b], **Cristina Hernández**[a,b], **Rafael Simó**[a,b,**]

[a] *Diabetes and Metabolism Research Unit. Vall d'Hebron Research Institute. Universitat Autònoma de Barcelona, Spain*
[b] *Centro de Investigación Biomédica en Red de Diabetes y Enfermedades Metabólicas Asociadas (CIBERDEM), Instituto de Salud Carlos III (ISCIII), Spain*

Received 20 November 2017; accepted 13 December 2017

KEYWORDS
Silymarin;
Diabetic retinopathy;
Diabetic macular edema;
Retinal endothelial cells

Abstract
Introduction: Vascular endothelial growth factor (VEGF) plays an essential role in development of diabetic macular edema (DME). While there is evidence suggesting that silymarin, a flavonoid extracted from *Silybum marianum*, could be useful for prevention and treatment of diabetic nephropathy, no studies have been conducted in diabetic retinopathy (DR). The aim of this study was to assess the effect of silymarin on disruption of inner blood retinal barrier (BRB), the primary cause of DME.
Materials and methods: Human retinal endothelial cells (HRECs) were cultured under standard (5.5 mM D-glucose) and diabetogenic conditions (25 mM D-glucose and 25 mM D-glucose + recombinant vascular endothelial growth factor [rVEGF, 25 mg/mL]). To assess cell viability, three concentrations of silymarin were tested (2, 4 and 10 μg/mL). The effect of silymarin on HREC disruption was determined using a dextran (70 kD) permeability asssay.
Results: No differences were found in the viability of HRECs treated with 2 or 4 μg/mL of silymarin as compared to untreated cells, but viability significantly decreased after using 10 μg/mL. The concentration of 4 μg/mL was therefore selected. Silymarin (4 μg/mL) caused a significant decrease in VEGF-induced permeability in both media with 5.5 nM (422±58 vs. 600±72 ng/mL/cm2; p<0.03) and 25 nM of D-glucose (354 ± 28 vs. 567 ± 102 ng/mL/cm2; p<0.04).
Discussion: Our results show that silymarin is effective for preventing hyperpermeability induced by diabetic conditions in HRECs. Further studies are needed to assess whether silymarin could be useful to treat DME.

* Corresponding author. Tel.: +4 934894172. fax: +34 934894032. Diabetes Research Unit. Vall d'Hebron Research Institute. Pg. Vall d'Hebron 119-129. 08035 Barcelona. Spain.
** Corresponding authors: Diabetes Research Unit. Vall d'Hebron Research Institute. Pg. Vall d'Hebron 119-129. 08035 Barcelona. Spain. Telephone: 34 934894172. FAX: 34 934894032.
E-mail addresses: marta.garcia.ramirez@vhir.org (M. García-Ramírez), rafael.simo@vhir.org (R. Simó).

https://doi.org/10.1016/j.endinu.2017.12.004
2530-0164/

PALABRAS CLAVE
Silimarina;
Retinopatía diabética;
Edema macular diabético;
Células endoteliales de retina

La silimarina previene la hiperpermeabilidad inducida por la diabetes en células endoteliales de retina humana

Resumen
Introducción: El *Vascular endothelial growth factor* (VEGF) juega un papel esencial en el desarrollo del edema macular diabético (EMD). Existe evidencia que indica que el uso de la silimarina, extracto flavonoide del Silybum marianum, podría ser útil en la prevención y el tratamiento de la nefropatía diabética pero no se dispone de datos en retinopatía diabética (RD). El objetivo del estudio es evaluar el efecto de la silimarina sobre la disrupción de la barrera hematorretininana, que es la causa primaria del EMD.
Material y métodos: Células endoteliales de retina humana (HRECs) se cultivaron en condiciones estándar (5.5 mM de D-glucosa) y en condiciones suprafisiológicas de glucosa (25 mM de D-glucosa y 25 mM de D-glucosa + VEGF 25 mg/dl). Para evaluar la viabilidad de las células se probaron 3 concentraciones de silimarina (2, 4 y 10 μg/ml). El efecto de la silimarina sobre la disrupción de las HRECs se determinó mediante análisis de permeabilidad a dextrano (70 kD).
Resultados: No se observaron diferencias en la viabilidad de las HRECs tratadas con 2 o 4 μg/ml de silimarina en comparación con las células no tratadas, pero se observó una reducción de la viabilidad con la concentración de 10 μg/ml. Por consiguiente, se seleccionó la concentración de 4 μg/ml de silimarina. La silimarina (4 μg/ml) produjo un descenso significativo de la permeabilidad inducida por VEGF tanto en medio con 5.5 mM de D-glucosa (422 ±58 vs. 600 ±72 ng/ml/cm2; p<0.03) como en medio con 25 mM de D-glucosa (354±28 vs. 567±102 ng/ml/cm2; p<0.04).
Discusión: Nuestros resultados demuestran que la silimarina es efectiva para prevenir la hiperpermeabilidad inducida por condiciones suprafisiológicas de glucosa en HRECs. Son necesarios más estudios para evaluar si la silimarina podría ser útil para el tratamiento del EMD.

Introduction

Diabetic retinopathy (DR) remains the leading cause for vision loss in developed countries.[1] DR is the most frequent microvascular complication, the prevalence of which increases with the duration of diabetes, with an overall rate of up to 30% and a high risk of severe visual impairment in 10% of subjects.[2,3] Diabetic macular edema (DME) is more frequent in type 2 diabetes, occurs in approximately 7.5% of diabetic patients, and is the main cause of vision loss in working-age adults in industrialized countries.[4,5]

Vascular endothelial growth factor (VEGF) is a well-known pathogenic factor for the disruption of the blood retinal-barrier (BRB) and together with proinflammatory cytokines play a key role in the development of DME.[6,7] For this reason, the intravitreal injection of anti-VEGF agents such as ranibizumab, bevacizumab and aflibercept represents the first-line therapy for DME involving the central macula.[8,9]

Silymarin is a flavonoid extracted from *Silybum marianum* (Milk thistle), which contains seven major components: taxifolin, silychristin, silydianin, silybin A, silybin B, isosilybin A and isolilybin B.[10] Silymarin has mainly been used to treat liver diseases due to its anti-oxidant, anti-fibrotic, and anti-inflammatory properties.[11,12] In addition, recent experimental evidence suggests that silymarin has additional effects, which could be useful for prevention and treatment of diabetic complications.[13–15] In this regard, silymarin is associated with an anti-glycation effect, inhibits aldose reductase, reduces liperperoxidation, and is a partial agonist of peroxixome proliferator-activated receptor γ (PPARγ).[16,17] Notably, silymarin reduces urinary excretion of albumin, TNF-α, and malondialdehyde (MDA) in patients with diabetic nephropathy.[18]

Lin et al.,[19] reported that silybin, a main component of silymarin inhibited VEGF secretion induced by hypoxia in retinal pigment epithelial (RPE) cells, and prevented VEGF- and VEGF plus hypoxia-induced retinal edema. In addition, they also provide evidence that silybin prevented neovascularization in a rat model of age-related macular degeneration (AMD).[19] Zhang et al.,[20] reported that silybin treatment significantly prevented the development of obliterated retinal capillaries in diabetic rats. All these findings points to silymarin as a potential therapeutic approach for VEGF induced hyperpermeability condition such as DR. However, the effect of silymarin on the vascular leakage induced by diabetes has not been previously reported.

On this basis, the main aim of the present study is to evaluate the effect of silymarin on VEGF induced hyperpermeability in human retinal endothelial cells (HREC) under standard and high glucose conditions.

Material and methods

Cell cultures

Human retinal endothelial cells (HRECs) were obtained from a vial of cryopreserved cells purchased from Innoprot

Please cite this article in press as: García-Ramírez M, et al. Silymarin prevents diabetes-induced hyperpermeability in human retinal endothelial cells. Endocrinol Diabetes Nutr. 2018. https://doi.org/10.1016/j.endinu.2017.12.004