Sulodexide increases mRNA expression of glutathione-related genes in human umbilical endothelial cells exposed to oxygen-glucose deprivation

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Submitted: 19 March 2019
Accepted: 7 July 2019

Arch Med Sci
DOI: https://doi.org/10.5114/aoms.2019.87504
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Abstract

Introduction: Sulodexide (SDX), a heparinoid used to treat vascular diseases, exerts anti-ischemic properties. However, the underlying molecular mechanisms remain unclear. Induction of glutathione (GSH)-dependent genes protects against ischemia. Here, we investigated the effect of SDX on GSH-associated gene expression in human umbilical endothelial cells (HUVECs) using an in vitro ischemia model.

Material and methods: The transcriptional expression of GSH-related genes (GCLc, xCT, GS, GPx1 and GR) in HUVECs treated without/with SDX (0.5 LRU/ml) under oxygen-glucose deprivation (OGD) condition for 1–6 h was analyzed by real-time polymerase chain reaction.

Results: GCLc and xCT were strongly up-regulated by SDX in HUVECs in the first 2 h of OGD. GS and GPx1 mRNA expression levels were significantly increased during any time interval in ischemic HUVECs treated with SDX. Furthermore, incubation of HUVECs with SDX in OGD for 1–4 h resulted in enhanced expression of GR.

Conclusions: Our studies provide the first evidence that SDX activates GSH-related genes in OGD-injured HUVECs.

Key words: sulodexide, endothelium, glutathione, gene expression, ischemia.

Introduction

Sulodexide (SDX) is a heparinoid used in chronic venous and arterial diseases [1]. It is also proved that SDX protects against ischemia/reperfusion injury [2]. SDX possesses endothelial protective effects due to its antioxidant, anti-inflammatory and antiproteolytic properties [1]. In endothelium, SDX interacts with nuclear factor-erythroid-2-related factor (Nrf2)-antioxidant response element (ARE) and nuclear factor-κB (NF-κB) signaling pathways [1, 3]. Nrf2 regulates the expression of enzymes involved in glutathione (GSH) synthesis and utilization such as γ-glutamylcysteine ligase (GCL), cysteine-glutamate exchanger (xCT), glutathione synthase (GS), glutathione peroxidase (GPx) and glutathione reductase (GR). ARE was found in the promoters of all these GSH-associated genes [4]. GSH, a key antioxidant, is synthesized in two ATP-dependent steps.
The first step is rate limiting and determined by GCL activity and the intracellular level of cysteine. GCL catalyzes a coupling reaction between glutamate and cysteine, whose product is γ-glutamylcysteine (γGC). GCL consists of a catalytic subunit (GCLc) and modifier subunit (GCLm) which are regulated independently at transcriptional and post-transcriptional levels. However, the transcriptional control of GCLc gene expression is the most important for GCL activity [5]. System xCT, a cysteine/glutamate antiporter with xCT and 4F2hc subunits, facilitates the uptake of cystine, which is quickly reduced to cysteine for GSH synthesis. Transcriptional regulation of the xCT gene is controlled by the Nr2f-ARE pathway and xCT mRNA expression correlates with system xCT activity [6]. The second step of GSH synthesis is catalyzed by GS, which adds a glycine to γGC. GS expression is regulated only at the transcription level [4]. During oxidative stress, GPx catalyzes the reaction of hydro and lipid peroxides with GSH resulting in the formation of oxidized glutathione (GSSG). GPx1 is the most abundant intracellular isoform of GPx in human endothelium. GR then converts GSSG to GSH using NADPH [5]. GPx1 and GR are regulated at the mRNA level [7].

In the present study, the variations in the transcript levels of GSH-related genes (GCLc, xCT, GS, GPx1 and GR) were evaluated in HUVECs subjected to oxygen-glucose deprivation (OGD) and treated with SDX.

Material and methods

Cell culture and treatment

HUVECs (Lonza, USA) were cultured in EGM-2 with EGM-2 BulletKit as previously described [3]. SDX (0.5 LRU/ml) was added to glucose-free DMEM and cells were harvested in a hypoxic chamber (3% O2, 5% CO2, 92% N2, at 37°C) after different OGD times (1–6 h). External control groups (normoxia) in EGM-2 with EGM-2 BulletKit were exposed to OGD (Figures 1 C, E). There is evidence by SDX was observed in HUVECs exposed to OGD (Figures 1 A, B). Since both xCT and 4F2hc were up-regulated in response to SDX in HUVECs exposed to OGD [3]. Here we found that SDX induces very early and transient transcription of de novo GSH synthesis genes, including GCLc and xCT (Figures 1 A, B). Since both GCLc and xCT are linked to GSH production, these alterations could have an initial effect in increasing the GSH levels by SDX, resulting in cellular resistance to oxidative stress [9]. In our study, also transcriptional activation of GS and GR by SDX was observed in HUVECs exposed to OGD (Figures 1 C, E). There is evidence
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that in endothelial cells with a requirement for a large capacity to synthesize GSH it is necessary for the expression of GS and GR genes to be adequately elevated [10]. GSSG accumulation in cells upon ischemic stress is highly toxic and therefore GR is one of the most relevant factors involved in protection against oxidative damage. Furthermore, the time-course analysis shows that SDX induced a prolonged antioxidant response by a stronger GPx1 expression in later hours of OGD (Figure 1 D).

Hence, this study suggests that SDX acts as a strong protector of endothelial cells under ischemic injury. For clinical application to patients with ischemic vascular disease, this study has many limitations because of confirmation only by in vitro study. However, these findings may provide the basis for further experimental and clinical studies on the endothelial protective effects of SDX in ischemic events.

In conclusion, we report the novel finding that SDX induces GSH-related genes in human endothelial cells exposed to simulated in vitro ischemia. Our results provide further insights into antioxidant properties of SDX.

Acknowledgments
This work was supported by Grant No. KNW-1-116/ N/5/0 (B.G.) from Medical University of Silesia.
Conflict of interest

The authors declare no conflict of interest.

References

1. Carroll BJ, Piazza G, Goldhaber SZ. Sulodexide in venous disease. J Thromb Haemost 2019; 17: 31-8.
2. Jiang QJ, Bai J, Jin J, Shi J, Qu L. Sulodexide for secondary prevention of recurrent venous thromboembolism: a systematic review and meta-analysis. Front Pharmacol 2018; 9: 876.
3. Gabryel B, Bontor K, Jarzabek K, et al. Sulodexide up-regulates glutathione S-transferase P1 by enhancing Nrf2 expression and translocation in human umbilical vein endothelial cells injured by oxygen glucose deprivation. Arch Med Sci DOI: https://doi.org/10.5114/aoms.2019.82818
4. Tonelli C, Chio IIC, Tuveson DA. Transcriptional regulation by Nrf2. Antioxid Redox Signal 2018; 29: 1727-45.
5. Espinosa-Diez C, Miguel V, Vallejo S, et al. Role of glutathione biosynthesis in endothelial dysfunction and fibrosis. Redox Biol 2018; 14: 88-99.
6. Habib E, Linher-Melville K, Lin HX, Singh G. Expression of xCT and activity of system xc(-) are regulated by NRF2 in human breast cancer cells in response to oxidative stress. Redox Biol 2015; 5: 33-42.
7. Iskusnykh IV, Popova TN, Agarkov AA, Pinheiro de Carvalho MA, Rjevskiy SG. Expression of glutathione peroxidase and glutathione reductase and level of free radical processes under toxic hepatitis in rats. J Toxicol 2013; 1013: 870628.
8. Duan Q, Sun W, Yuan H, Mu X. MicroRNA-135b-5p prevents oxygen-glucose deprivation and reoxygenation-induced neuronal injury through regulation of the GSK-3beta/Nrf2/ARE signaling pathway. Arch Med Sci 2018; 14: 735-44.
9. Gabryel B, Jarząbek K, Machnik G, et al. Superoxide dismutase 1 and glutathione peroxidase 1 are involved in the protective effect of sulodexide on vascular endothelial cells exposed to oxygen-glucose deprivation. Microvasc Res 2016; 103: 26-35.
10. Espinosa-Diez C, Miguel V, Mennerich D, et al. Antioxidant responses and cellular adjustments to oxidative stress. Redox Biol 2015; 6: 183-97.