Application Note

on

Morphological Kinetics Analysis

Hungarian Academy of Sciences

Biological Research Centre, Szeged

FEATURED SCIENTIST

"Holographic microscopy analysis enabled us to visualize the kinetics of morphological changes caused by methylglyoxal and the protective effect of edaravone at the cellular level."

Dr. Andrea E. Tóth, Hungarian Academy of Sciences

MAGYAR • TUDOMÁNYOS • AKADÉMIA

1825

Edavarone Protects against Methylglyoxal-induced Barrier Damage in Human Brain Endothelial Cells

Andrea E. Tóth, Fruzsina R. Walter, Alexandra Bocsik, Petra Sántha, Szilvia Veszelka, Lajos Nagy, László G. Puskás, Pierre-Olivier Couraud, Fuyuko Takata, Shinya Dohgu, Yasufumi Kataoka, Mária A. Deli.

ABSTRACT

Methylglyoxal, a reactive carbonyl species present in the blood, can induce inflammation and vascular damage when levels are increased due to certain pathologies. Edavarone, the active substance of a Japanese medicine, used against brain damage after acute brain ischemia and infarction, was studied using human brain endothelial cells. It was found that edavarone protects against methylglyoxal-induced barrier damage in the cells. Co-administration of methylglyoxal and edavarone blocked the effect on cell morphology that methylglyoxal alone caused. These effects include decreased cell area and increased cell thickness due to the contraction of damaged cells.

INTRODUCTION

Methylglyoxal, a highly reactive carbonyl species, is abundant in the blood system in several pathologies. It causes carbonyl- and oxidative stress on the cells, leading to inflammation and damage on the cells of blood vessels among others. Edavarone is the active substance of a Japanese medicine, used against brain damage after acute brain ischemia and infarction. It is a scavenger of reactive molecules and hence protective against the damage.

This study focuses on prevention of methylglyoxal induced damage on the brain blood vessel cells by addition of edavarone. Presented in this application note are the results on cell area and thickness, obtained by the HoloMonitor® M3, of cells treated with either methylglyoxal or combined treatment with methylglyoxal and edavarone. For information about the whole study, please read A.E. Tóth et al. 2014 (http://journals.plos.org/plosone/article?id=10.1371/journal.pone.0100152).

MATERIAL & METHODS

Cell culture

Human hCMEC/D3 brain endothelial cells were plated on collagen-coated culture dishes with borosilicate glass bottom (MatTek, Ashland, MA, USA). All treatments lasted for 4 hours.

Treatments

Human brain endothelial cells were treated with 600 µM methylglyoxal in EBM-2 medium containing 10% fetal bovine serum, HEPES (10 mM) and gentamycin (50 µg/ml). edavarone was used at 3 mM.

Holographic Microscopy

Holograms are interference patterns that are created when a laser beam that has passed through a cell sample is merged with a reference beam. The hologram is captured and used by the software to create a digital holographic 3D cell image (Alm et al. 2013). Digital holographic images were taken with a HoloMonitor M3 instrument (Phase Holographic Imaging AB, Lund, Sweden). Holographic images of the same culture area were captured before and during treatments. Cell morphological changes were analyzed by the HoloStudio 2.4 software provided with the microscope. Optical thickness (optical path length) is based on phase shift, the wavelength of the light and the refractive index of the cell. Optical volume is based on optical thickness and area (Alm et al. 2013).

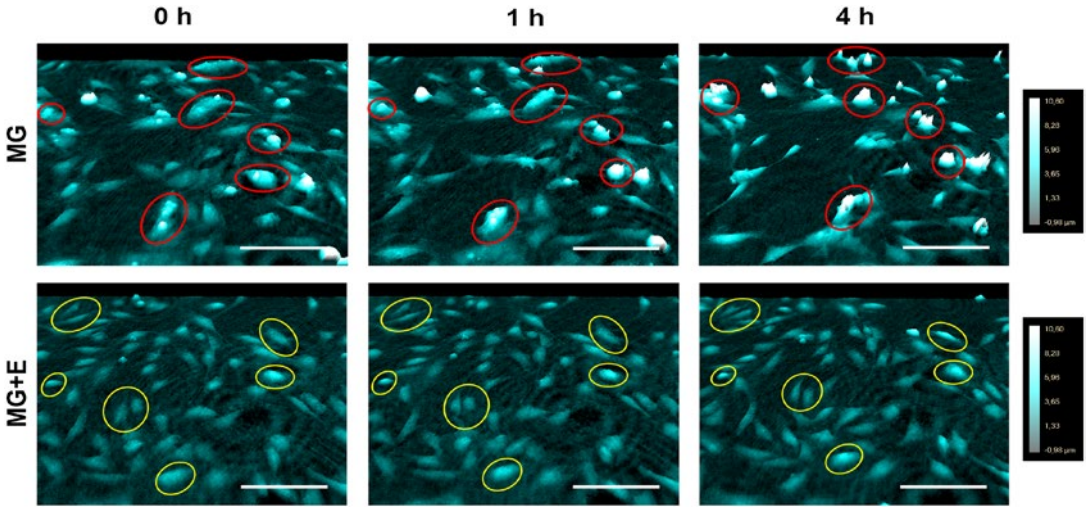

Figure 1. Effect of methylglyoxal and edavarone on cellular morphology. *Holographic images of morphological alterations in human brain endothelial cells by treatment with 600 µM methylglyoxal and co-treatment with 3 mM edavarone for 4 hours. Color scale bar correspond to the height of single cells. Data were analyzed by means of HoloStudio 2.4 software. Red circles indicate cells with drastic changes in cell morphology. Yellow circles indicate cells without any morphological changes. Bar = 100 µm*

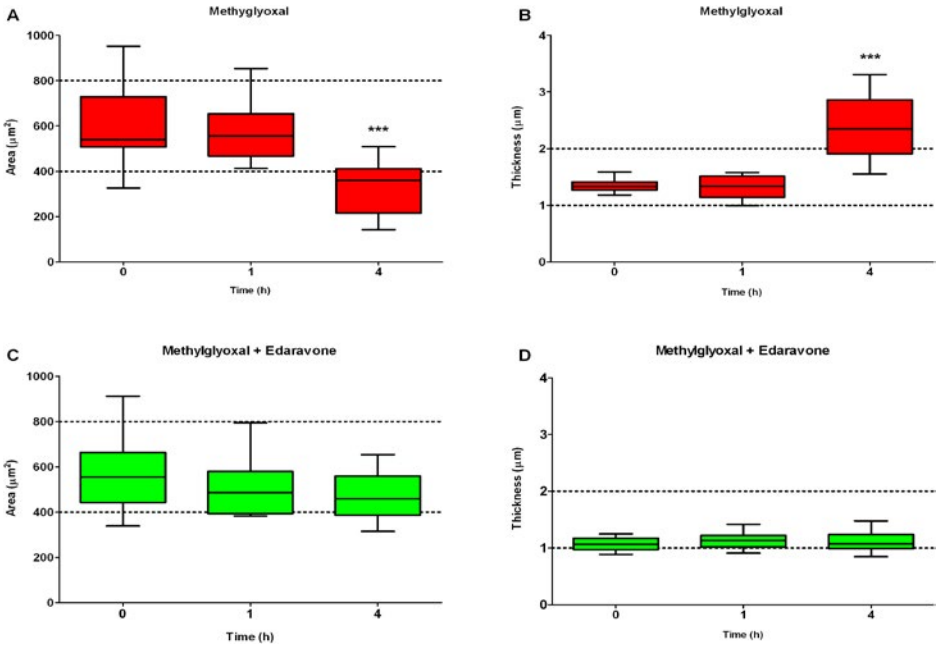

Figure 2. Effect of methylglyoxal and edavarone on area and thickness of adjacent cells. *Morphological alterations induced in human brain endothelial cells by treatment with methylglyoxal at 600 µM (A and B) and co-treatment with edavarone at 3 mM (C and D) for 4 hours. Surface area (A and C) and average optical thickness (B and D) were calculated before and during treatments. Box represents 25 and 75 percentiles. Horizontal line represent the median. Whiskers show minimum and maximum values.*

Statistical analysis: ANOVA followed by Dunnett test, n=12. Statistically significant differences ($p<0.05$) from 0 time-point (*) are indicated.

RESULTS

Holographic microscopy analysis was performed to visualize the morphological changes caused by methylglyoxal and the protective effect of edavarone (Figure 1). This novel technology made it possible to follow living cells in a label-free and noninvasive way. Morphological parameters of treated cells, such as surface area, optical thickness and cell volume could be measured.

Holographic images were taken every 30 minutes before and during the 4-hour treatment of hCMEC/D3 cells. Endothelial cells show a flat, elongated shape and grow next to each other. Treatment with methylglyoxal caused drastic changes in cell morphology: as indicated by the color-scale seen in Figure 1. The increase of cell height was especially prominent (Figure 1). In contrast, in endothelial cells co-treated with edavarone (3 mM) and methylglyoxal (600 µM) there was no change in cell morphology during the treatment period (Figure 1). Two short videos on the cell morphology in both treatment groups at all time-points are shown as supplementary data (Video S1 and S2 http://journals.plos.org/plosone/article?id=10.1371/journal.pone.0100152#pone.0100152.s005).

The analysis of morphological data is shown in Figure 2. During treatment with methylglyoxal the area of the cells significantly decreased (63 ± 34%) and their optical thickness increased by 1.8 fold (176 ± 36%) compared to the values at the beginning of treatment. These data indicate that endothelial cells treated with methylglyoxal contracted and rounded up, which is also visible in Figure 1 and Video S1. The volume of the cells was unchanged. Meanwhile, no changes were observed in cells co-treated with edavarone and methylglyoxal (Figure 2) or in the control group which was treated with medium only. This is the first report on methylglyoxal-induced morphology changes in brain endothelial cells using holographic microscopy imaging.

DISCUSSION

Methylglyoxal, present at elevated levels in vascular systems of patients with e.g diabetes mellitus, exerts toxic effects on brain micro-vessels (Li W. et al. 2013). This study, further supported the fact that methylglyoxal induce damage on cultured brain endothelial cells in a time and dose dependent manner. Methylglyoxal significantly reduced the integrity of the brain barrier, measured by both functional and morphological analyses. Co-treatment with edavarone provided a complete protection against the toxic effect of methylglyoxal. In addition, it was found that edavarone alone tightened the brain endothelial barrier. Two different cell viability assays were in complete agreement on the direct cellular damaging effect of methylglyoxal. Impedance data reflecting changes in cell adhesion, cell shape and number were confirmed by MTT assays measuring the metabolic activity of cells (see article for details).

Using holographic microscopy for label-free analysis of living cells, visualized for the first time the kinetics of morphological changes caused by methylglyoxal. The morphology data confirmed that edavarone completely prevented methylglyoxal-induced changes in morphology and detachment of cells.

CONCLUSIONS

These results show for the first time that edavarone protects cerebral endothelial cells from damage caused by methylglyoxal. These findings could have therapeutic implications for disorders associated with carbonyl stress.

REFERENCES

- Kalapos MP (2008) The tandem of free radicals and methylglyoxal. Chem Biol Interact 171: 251–271. doi: 10.1016/j.cbi.2007.11.009
- Lukic-Panin V, Deguchi K, Yamashita T, Shang J, Zhang X, et al. (2010) Free radical scavenger edaravone administration protects against tissue plasminogen activator induced oxidative stress and blood brain barrier damage. Curr Neurovasc Res 7: 319–329. doi: 10.2174/156720210793180747
- Alm K, El-Schich Z, Miniotis FM, Wingren AG, Janicke B, et al. (2013) Cells and Holograms – Holograms and Digital Holographic Microscopy as a Tool to Study the Morphology of Living Cells. In: Mihaylova, editor. Holography - Basic Principles and Contemporary Applications: ISSB: 978-953-5151117-7, InTech. DOI:10.5772-54405.
- Li W, Maloney RE, Circu ML, Alexander JS, Aw TY (2013) Acute carbonyl stress induces occludin glycation and brain microvascular endothelial barrier dysfunction: role for glutathione-dependent metabolism of methylglyoxal. Free Radic Biol Med 54: 51–61. doi: 10.1016/j.freeradbiomed.2012.10.552