Impaired GFP-vinculin adhesions in human lens epithelial cells due to the T-type calcium channel blocker mibefradil

DGZ 2007

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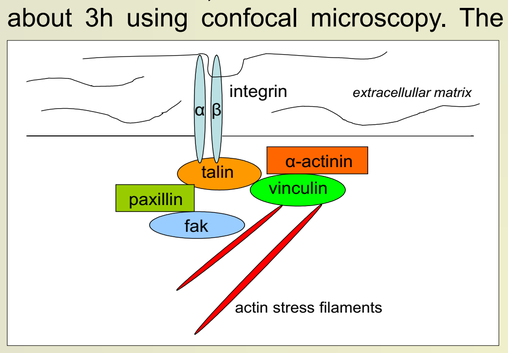
Abstract:

Previous findings demonstrated that the T-type calcium channel blocker mibefradil reduced integrin expression and interfered signaling pathways in human lens epithelial cells which could be an important therapeutic aspect in posterior capsule opacification after cataract surgery. Beside integrins and adapter proteins the adhesion complex is also characterized by vinculin which is spatially associated with transmembrane integrin receptors. In our present experiments GFP-vinculin was observed to investigate the time dependent influence of mibefradil. Primary human lens epithelial cells and immortalized HLE-B3 cells were transfected in suspension with a GFP-vinculin construct, cultivated for 24h and then observed in the presence of mibefradil (10, 20µM) about 3h using confocal microscopy. The length and the number of vinculin contacts were significantly decreased, which was accompanied by a decrease in vinculin protein expression and by a significantly reduced cell area. Moreover mibefradil seems to induce a shift of direction of vinculin mobility from peripheral direction in normal cells to centripetal direction in mibefradil treated cells. We conclude that this early contact loss of vinculin adhesions in lens epithelial cells due to this calcium channel inhibitor is one of the key events for the already observed apoptosis and suggest a detachment-induced cell death (anoikis).



Material & Methods:

Calcium channel antagonist

The T-type calcium channel antagonist mibefradil dihydro-chloride was used. The substance was solved in distilled water (stock solution 50 mg/10 ml).

Cell culture

Primary human lens epithelial cells (hLEC) from cataract surgery and the human lens epithelial cell line HLE-B3 (ATCC No: CRL-11421) were used. The cells were cultured in DMEM with 10 % FCS and 1 % gentamicin at 37°C with 5 % CO_2 atmosphere.

Transfection with GFP-vinculin

Live cells were transfected in suspension with a GFP-vinculin construct using Effectene transfection reagent. Tranfected cells were seeded in a subconfluent density in Lab-Tek 4-well chambered cover glass and cultured for 24 h at 37°C with 5 % CO_2 atmosphere.

Immunoblotting by Western blot

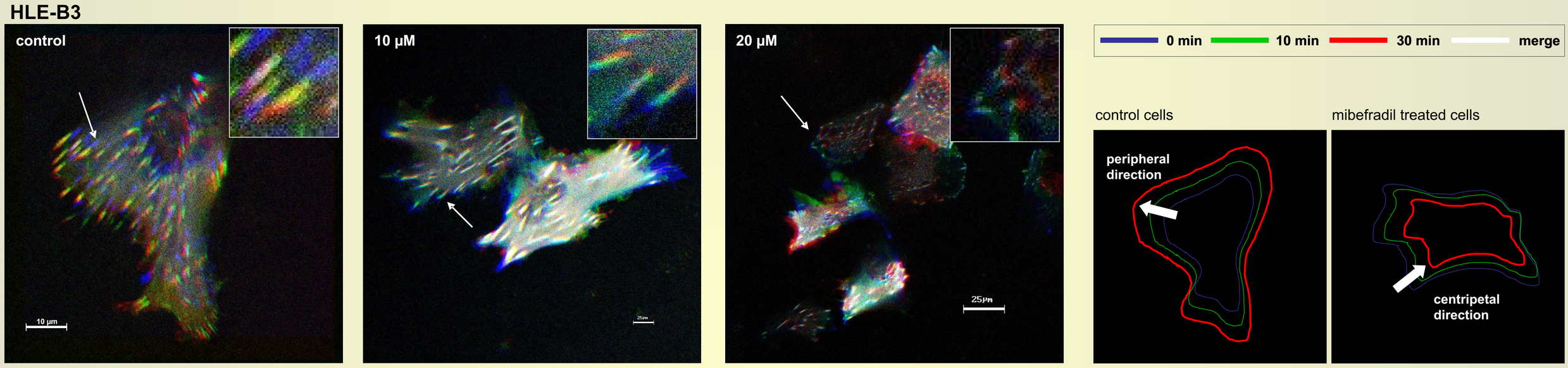
The cells were run on 4-20 % Tris-glycine gels and transferred onto PVDF membrane. The membrane was blocked for 1 h followed by an incubation with a monoclonal mouse anti-human vinculin antibody (1:1000 dilution). After incubation with the secondary AP-conjugated rabbit anti-mouse antibody the membrane was detected by CDP-Star luminescence solution.

Microscopical analysis of GFP-vinculin

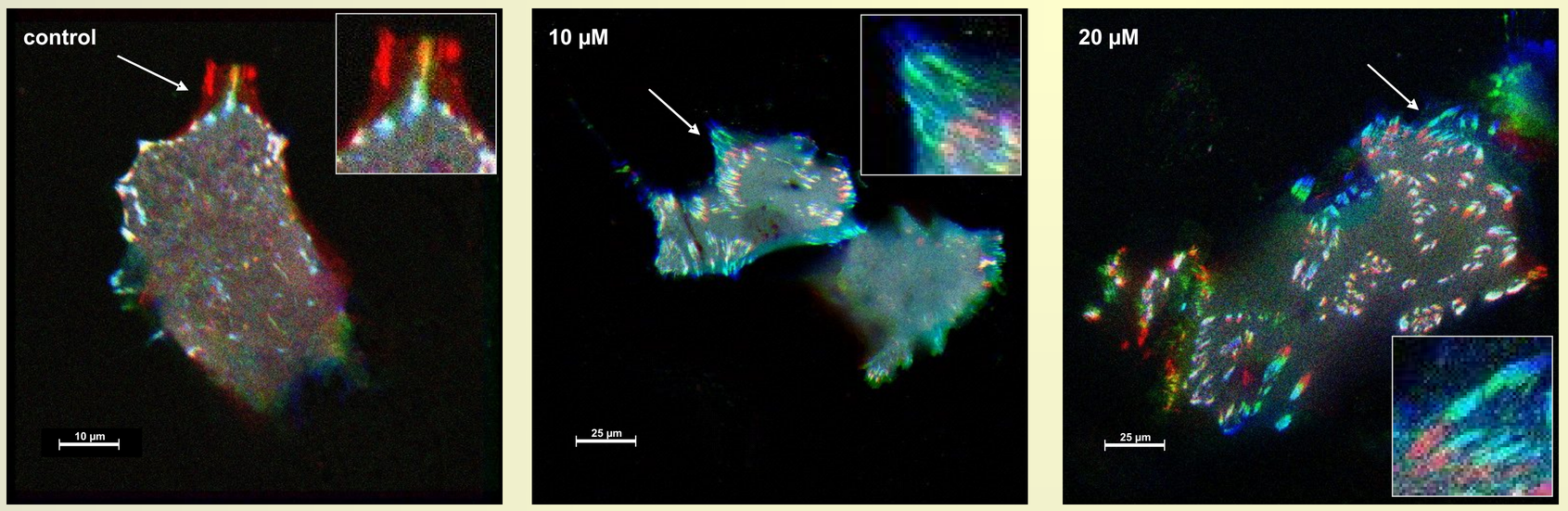
The transfected living cells were observed using then confocal laser scanning microscope LSM Leica TCS SP2 AOBS (Leica Microsysteme GmbH, Bensheim) with an incubator at 37°C and 5 % CO_2. We used a 63 x oil immersion objective and an excitation wavelength of 488 nm with a long-pass 515 nm filter. Number, length and cell area of GFP-vinculin containing focal contacts in transfected cells were analyzed with the Leica LCS Lite software.

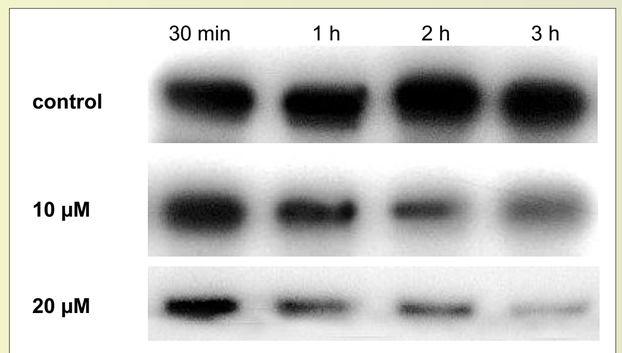
Results:

HLE-B3



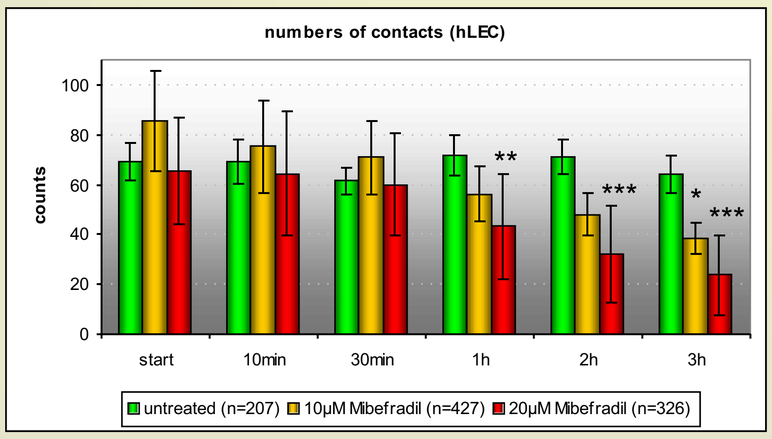
hLEC



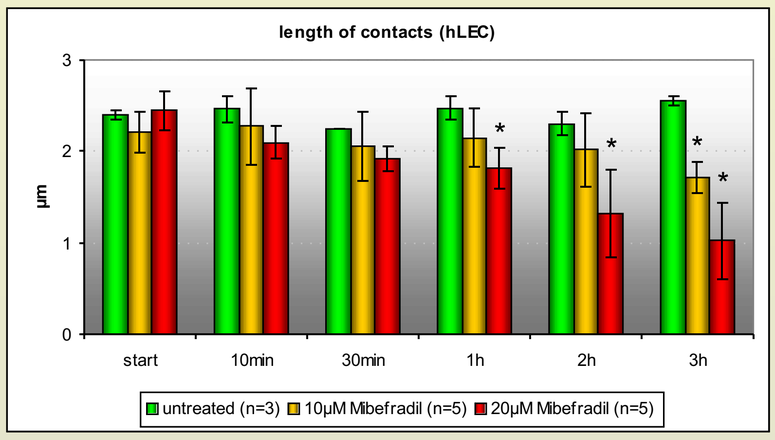


Western blot: Vinculin expression in HLE-B3 cells. A decrease of vinculin protein expression occurs due to mibefradil.

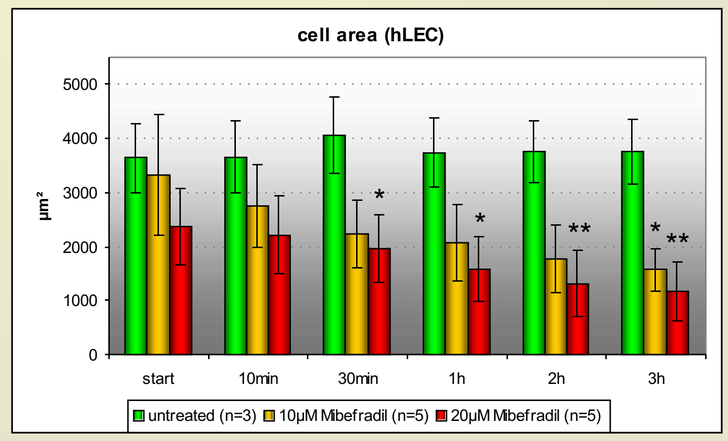
Vinculin mobility in primary human lens epithelial cells and human lens HLE-B3 cells. Note that the calcium channel blocker induces a shift of direction of vinculin mobility from peripheral direction in control cells to centripetal direction in mibefradil treated cells. Insets are magnified views of indicated areas (arrow). Confocal microscopy of living GFP transfected cells. LSM Leica.



Number of GFP-vinculin contacts in transfected human lens epithelial cells (hLEC) at different time points after treatment with mibefradil (mean ± SEM). An incubation with the higher concentration of mibefradil (20 µM) results in a significantly loss of focal adhesions compared to the starting point already after 1 h (t-test, paired, ** p<0.01, *** p<0,001). Treatment of cells with the lower concentration of mibefradil (10 µM) results in a significantly decrease of the focal contact's number after 3 h (U-test, * p<0.05).



Length of GFP-vinculin contacts in transfected human lens epithelial cells (hLEC) at different time points after treatment with mibefradil (mean ± SEM). Statistical analyses reveal that vinculin contacts in hLEC were significantly shorter already after 1 h with 20 µM mibefradil compared to the contact length of the starting point (t-test, paired, * p<0.05). With the lower concentration of mibefradil (10 µM) the vinculin containing contacts are significantly decreased after 3 h (t-test, paired, * p<0.05).



The cell area of hLEC cells is significantly decreased already after 30 min with 20 µM mibefradil (t-test, paired, * p<0.05, ** p<0.01, mean ± SEM). With the lower concentration of mibefradil (10 µM) the cell area is significantly decreased after 3 h (t-test, paired, * p<0.05).

Acknowledgements
Arne Weidmann was gratefully supported by the Deutsche Forschungsgemeinschaft DFG (NE 560/5-5).
We thank Dr. B. Geiger (The Weizmann Institute, Rehovot, Israel) for providing the GFP-vinculin-construct.

References

Beck R, Nebe B, Rychly J, Guthoff R: Inhibition der Adhäsion von Linsenepithelzellen durch den Kalzium-Kanalblocker Mibefradil: In: DGII, 14. Kongress der Deutschsprachigen Gesellschaft für Intraokularlinsen-Implantation und refraktive Chirurgie. (Hrg.: Wenzel M, Kohnen T, Blumer B) Biermann Verlag GmbH Köln (2000) 473-479.

Beck R, Nebe B, Guthoff R, Rychly J: Inhibition of lens epithelial cell adhesion by the calcium antagonist Mibefradil correlates with impaired integrin distribution and organization of the cytoskeleton. Graefes Arch Clin Exp Ophtalmol 239 (2001) 452-458.

Diener A, Nebe B, Lüthen F, Becker P, Beck U, Neumann HG, Rychly J: Control of focal adhesion dynamics by material surface characteristics. Biomaterials 26 (2005) 383-392.

Kunz F, Beck R, Rychly J, Guthoff R, Nebe B: Induction of apoptosis in lens epithelial cells by a calcium antagonist correlates with impaired integrin-mediated adhesion. Biol Cell 93 (2001) 222.

Nebe B, Kunz F, Peters A, Rychly J, Noack T, Beck R: Induction of apoptosis by the calcium antagonist mibefradil correlates with depolarization of the membrane potential and decreased integrin expression in human lens epithelial cells. Graefe's Arch Clin Exp Ophtalmol 242 (2004) 597-604.

Nebe B, Beck R, Peters A, Guthoff R, Rychly J: The calcium channel antagonist Mibefradil inhibits integrin mediated cell growth on intraocular lenses. Eur J Cell Biol (2000) 94.

Weidmann A, Müller PD, Beck R, Nebe B: Influence of the T-type calcium channel blocker mibefradil into signaling pathways in human lens epithelial cells. Eur J Cell Biol 85S1, Suppl. 56 (2006) 51.

Conclusion:

We suggest that the specific influence of the T-type calcium channel blocker mibefradil on vinculin containing focal contacts in living lens epithelial cells - beside earlier findings like inhibition of integrin expression, depolarization of membrane potential and destruction of actin cytoskeleton - is the reason for the time dependent loss of cell adhesion. Thus, mibefradil seems to be a suitable drug to inhibit proliferation and migration of outgrowing lens cells of the equatorial zone of the eye after cataract surgery. Our current experiments reveal a possible practicable approach - the immobilized mibefradil in microparticles which can be affixed at the haptic of artificial lenses.