

Kv11.1 (hERG1) channels are aberrantly expressed in several human cancers where they control different aspects of the neoplastic cell biology such as proliferation and apoptosis, invasiveness and angiogenesis, the latter through the regulation of VEGF secretion (reviewed in¹). The hERG1-dependent effects were shown *in vitro* and, more recently, *in vivo*. In preclinical models of both leukemia¹ and colorectal cancer³, hERG1 overexpression confers a higher malignancy to neoplastic cells. Moreover, hERG1 blockers have therapeutic potential, since preclinical tests showed that treatment with specific hERG1 blockers overcame chemoresistance in acute leukemias⁴ as well as reduced GI cancer growth, angiogenesis and metastatic spread³. The overall message emerging from our data is that the hERG1 protein represents a novel biomarker and drug target in oncology. Up to now, hERG1 was considered an "antitarget" due to the cardiac side effects that many (but not all) hERG1 blockers produce and that result in lengthening the electrocardiographic QT interval. We report here recent studies on known and newly developed hERG1 blockers that exhibit no cardiotoxicity and are more specific for the hERG1 channels expressed in cancer cells.

1. Arcangeli et al., *Curr Med Chem* 16: 66-93 (2009);
2. Wulff et al., *Nature Rev Drug Discovery* 8: 982-1001 (2009);
3. Crociani et al., submitted; Arcangeli et al., 30th Anniversary Conference AICR, 7-9 April 2010, abstract;
4. Pilozzi et al., *Blood*, 117: 902-914, 2011.

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O-559

Analysis of the K⁺ current in human T cells in hypercholesterinaemic state

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We reported previously that the increase of the cholesterol content of the cell membrane (*in vitro*) modified the biophysical parameters of the gating of Kv1.3 K⁺ ion channels in human T-lymphocytes. In our present study we aimed to determine the effect of hypercholesterolemia on the biophysical parameters of Kv1.3gating and the proliferation of T cells.

T-lymphocytes were isolated from the peripheral blood of patients with cholesterol level considered normal (<5.2 mmol/l, control group) and patients with hypercholesterinaemia (hc). Whole-cell K⁺ currents were measured in patch-clamped T cells and the kinetic (activation and inactivation kinetics) and equilibrium parameters (voltage-dependence of steady-state activation) of Kv1.3 gating were determined. Lymphocyte proliferation was measured using CFSE staining with and without anti-CD3 and anti-CD28 stimulation.

Our results indicate that the biophysical parameters of Kv1.3 gating are similar in the control group and in the 'hc' samples. The CFSE-based assay showed that hypercholesterolemic T cells had higher spontaneous activation rate compared to control group. However, T cells from high cholesterol level patients challenged by anti-CD3 and anti-CD28 exhibited lower proliferation rate than control cells.

P-560

Endothelial cells involved in chronic thromboembolic pulmonary hypertension have altered calcium homeostasis

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Chronic thromboembolic pulmonary hypertension (CTEPH) is a rare and late possible consequence of venous thromboembolism. In this study, cellular properties of endothelial cells isolated from surgical material of CTEPH patients (CTEPH-hECs) were compared with control human pulmonary artery endothelial cells (hPAEC).

The purified endothelial cells from CTEPH surgical tissue and the control hPAECs were cultured under standard conditions. CTEPH-hECs and hPAECs were stained for von Willebrand factor and vascular-endothelial cadherin. To address the intracellular calcium changes, Fura-2/am loaded cells were challenged with 10 μM histamine, intracellular calcium stores were depleted with 15 μM BHQ and the activation of transient receptor potential canonical channels (TRPCs) were followed.

CTEPH-hECs and hPAECs both express similar pattern of von Willebrand factor and vascular-endothelial cadherin. CTEPH-hEC show no significant differences in basal calcium level (140.89±51.40 mM Ca²⁺ for CTEPH-hEC, n=221; 128.61±49.16 for hPAEC, n=245) as well as in the histamine-induced calcium peak (278.78±21.97 for CTEPH-hEC, n=110; 242.40±7.65 for hPAEC, n=133) in comparison to control hPAECs. Interestingly, the challenge with BHQ leads to a significant increase in calcium store depletion signal (505.27±37.51 for CTEPH-hEC, n=111; 200.67±25.77 for hPAEC, n=112). By adding back the calcium to the cells a significant difference in the calcium influx occurs (652.39±46.88 for CTEPH-hEC, n=111; 322.84±19.33 for hPAEC, n=112).

The isolated and purified endothelial cells from surgical material of CTEPH patients present the characteristic endothelial phenotype, but they show altered calcium homeostasis at the stored calcium and store operated calcium influx level.

Hiperkoleszterinámiás T-sejtek proliferációjának és Kv1.3 ioncsatornáinak vizsgálata

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Korábbi kísérleteink során kimutattuk, hogy a sejtmembrán koleszterintartalmának módosítása (*in vitro*) megváltoztatta a humán T-sejtek Kv1.3 kálium ioncsatornáinak kapuzási kinetikáját. Jelen munkánk során arra kerestük a választ, hogy a hiperkoleszterinémia milyen hatással van a T-sejtek proliferációjára és a csatorna kapuzási paramétereire.

Ennek érdekében először is egészséges (kontroll) és hiperkoleszterinémiás személyek perifériás véréből izoláltunk T-limfocitákat. A Kv1.3 csatorna kapuzását jellemző aktivációs, inaktivációs és egyensúlyi aktivációs időállandókat patch-clamp technika segítségével, feszültség-zár üzemmódban határoztuk meg. A limfociták proliferációját CFSE hígítási esszé felhasználásával anti-CD3 és anti CD28 stimulációt követően, illetve annak hiányában határoztuk meg.

Ereményeink alapján kijelenthetjük, hogy a Kv1.3 kapuzási paraméterei nem változtak meg hypercholesterinaemia hatására. A proliferációs esszé során azonban azt találtuk, hogy a hiperkoleszterinémiás T-sejtek spontán osztódási rátája emelkedettebb volt a kontroll sejtekhez képest. Ezzel szemben a stimuláció hatására a kontroll sejtek osztódtak jobban.