Pharmacological Activation of PIEZO1 and Inhibition by Benzbromarone in Human Liver Sinusoidal Endothelial Cells

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INTRODUCTION

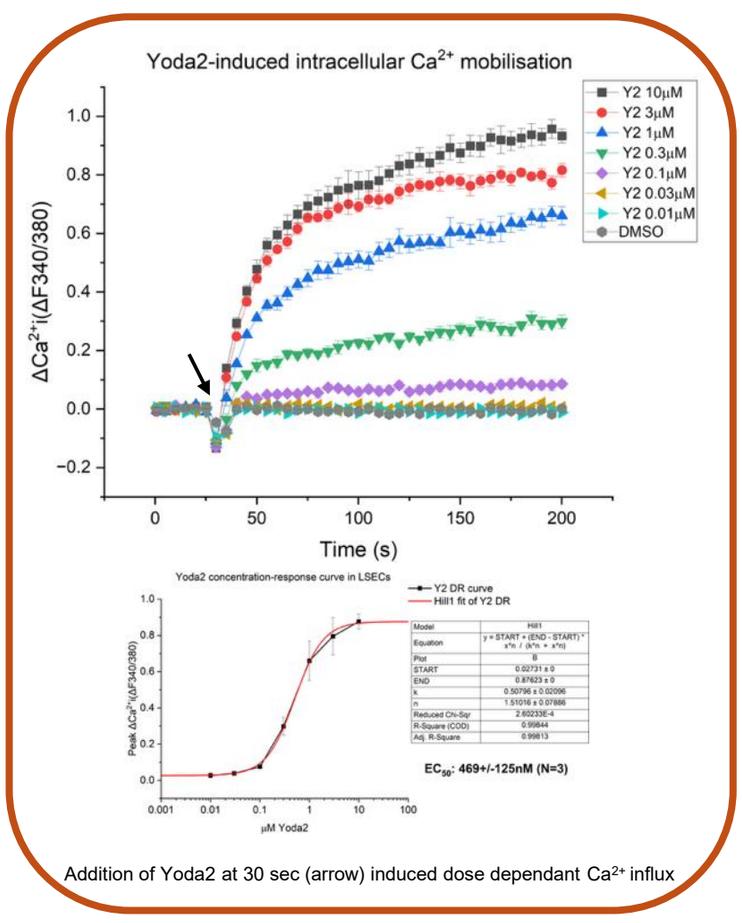
Liver regeneration following resection is critically dependent on haemodynamic changes within the hepatic microcirculation. Liver sinusoidal endothelial cells (LSECs) are among the first cells exposed to altered portal flow and shear stress. PIEZO1, a mechanosensitive Ca^{2+}-permeable ion channel, has been proposed as a key regulator of flow-mediated signaling and endothelial function. Here, we investigated the functional characteristics and pharmacological modulation of PIEZO1 in human LSECs.

METHODS

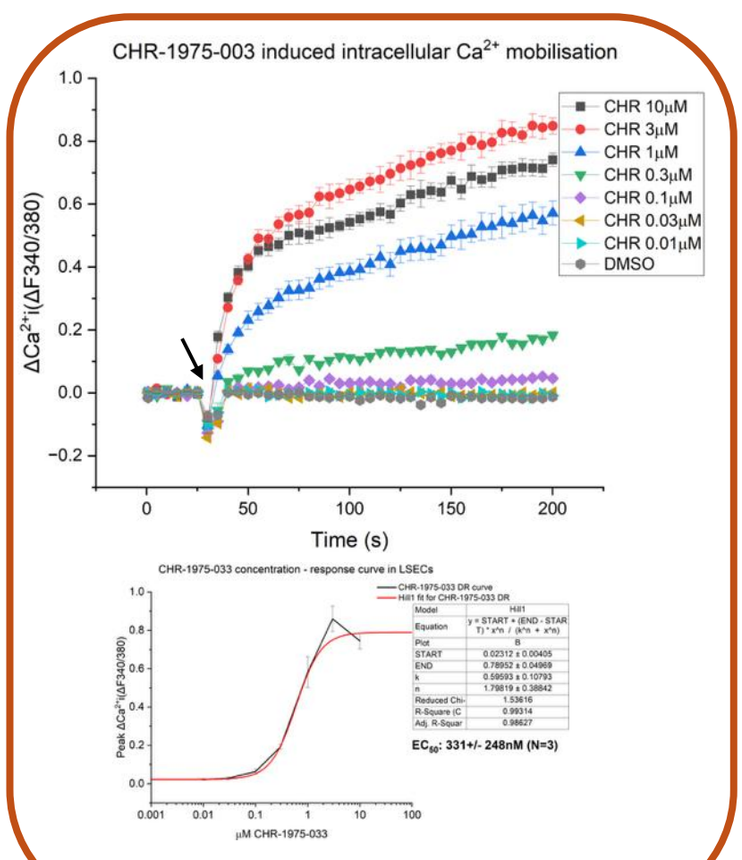
Intracellular Ca^{2+} responses were measured in human LSECs using Fura-2 and ratiometric fluorescence imaging in a FlexStation III. PIEZO1 activation was induced using Yoda2 and a novel small-molecule agonist (CHR), and EC_{50} values were calculated. Specificity was assessed by siRNA-mediated PIEZO1 knockdown. Benzbromarone was investigated as a potential PIEZO1 inhibitor with relevance to liver toxicity. Benchmark modulators ionomycin and ATP were comparators.

RESULTS

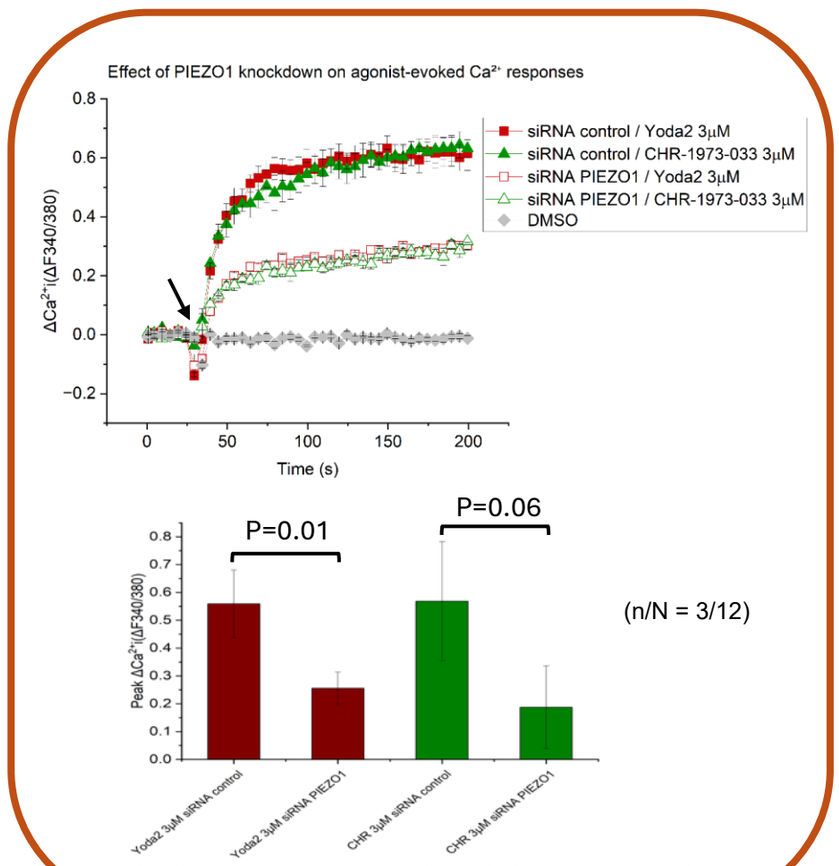
Yoda2 (EC_{50} 469 ± 125 nM) and CHR (EC_{50} 331 ± 248 nM) induced dose-dependent Ca^{2+} influx consistent with PIEZO1 activation. PIEZO1 knockdown similarly attenuated Yoda2 and CHR evoked responses. Benzbromarone inhibited Yoda2-mediated Ca^{2+} influx (IC_{50} 362 ± 164 nM). It also attenuated the ionomycin response but not the ATP response.



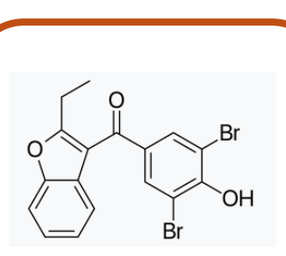
Addition of Yoda2 at 30 sec (arrow) induced dose dependant Ca^{2+} influx



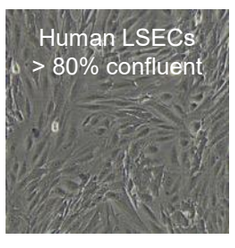
Addition of CHR at 30 sec (arrow) induced dose dependant Ca^{2+} influx

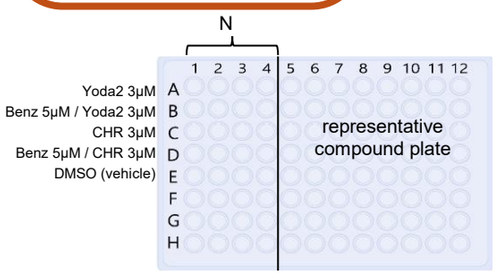


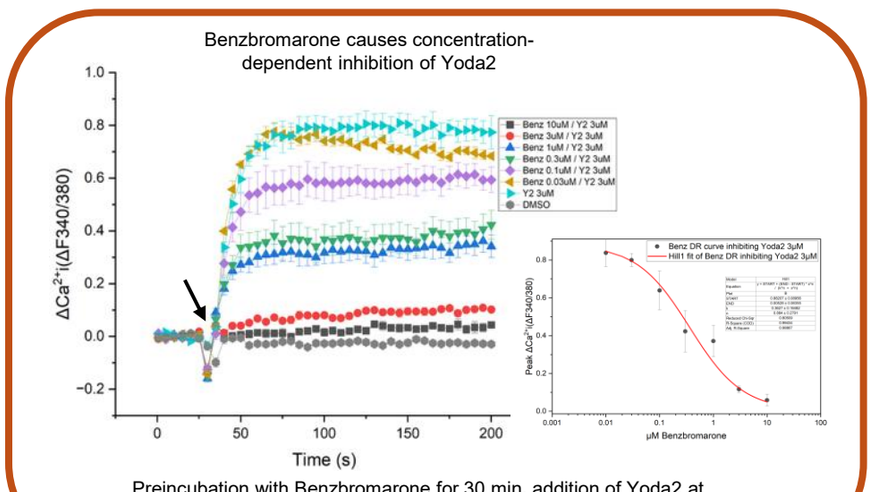
PIEZO1 knockdown reduced Yoda2 and CHR induced Ca^{2+} elevation



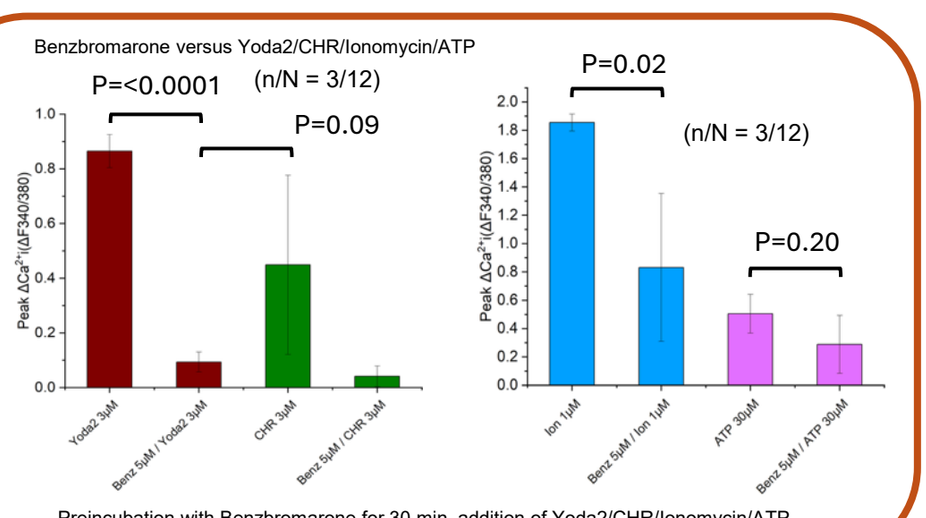
Benzbromarone







Preincubation with Benzbromarone for 30 min, addition of Yoda2 at 30 sec (arrow)



Preincubation with Benzbromarone for 30 min, addition of Yoda2/CHR/Ionomycin/ATP

CONCLUSIONS

Human LSECs exhibit robust PIEZO1-dependent Ca^{2+} signaling, supporting a mechanistic role for PIEZO1 in hepatic haemodynamic sensing. Benzbromarone strongly attenuates PIEZO1-mediated responses and possibly other Ca^{2+} pathways, effects that may contribute to its liver toxicity. These findings support the ideas that PIEZO1 is a determinant of hepatic blood flow and liver toxicity from benzbromarone arises from inhibition of LSEC Ca^{2+} signaling.

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