Lipid raft modulation in rat visceral hypersensitivity: proof of principal study

Sara Botschuijver[1];Sophie van Diest[1]; Anne Strik[1]; Zhumei Yu[1,2]; Olaf Welting[1]; Guy Boeckxstaens[3]; Gary Jennings[4]; Coen Paulusma[1]; Ronald Oude Elferink[1]; Wouter de Jonge[1]; René van den Wijngaard[1].

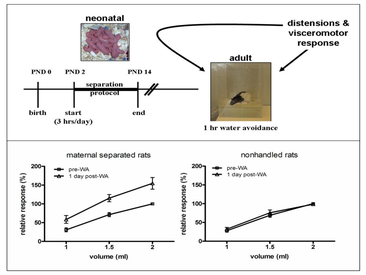
[1]Tytgat Institute for Liver and Intestinal Research, Division of Gastroenterology, Academic Medical Center, Amsterdam, The Netherlands.
[2]Department of Neurobiology, Tongji Medical College, Wuhan, China. [3]Division of Gastroenterology, University Hospital Gasthuisberg, Belgium. [4]Jado Technologies GmbH, Dresden, Germany.

Background

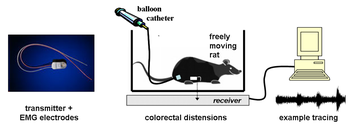
Irritable bowel syndrome (IBS) is one of the most common functional gastrointestinal disorders and abdominal pain is the key contributing factor. Up to 50% of patients have increased perception of gastrointestinal stimuli and this so called visceral hypersensitivity is considered a major pathophysiological mechanism. In our maternal separation model we previously identified the TRPV1 ion channel as a possible target for treating visceral hypersensitivity. Others have shown that TRPV1 is located in so called lipid rafts that function to protect the signaling complex from non-raft enzymes that could otherwise affect the signaling process. In this proof of principle study we used, in two different animal models, the prototype raft modulator Miltefosine to establish whether lipid raft modulation should be considered as a treatment option for visceral hypersensitivity.

General model

Maternal separation



Colonic distension in rat



In the maternal separation model Long-Evans pups are separated from their mothers for 3 hours daily from postnatal days 2 to 14. Upon exposure to water avoidance stress at adult age, maternal separated rats display enhanced sensitivity to colorectal distension whereas non-handled rats remain normo-sensitive. In these experiments, visceral sensitivity is quantified by measuring the visceromotor response (EMG) to colonic distension in freely moving rats.

Conclusion

Showing beneficial effects on capsaicin-induced (i.e. TRPV1-mediated) colonic hypersensitivity in normal rats as well as post-stress visceral hypersensitivity in maternal separation rats, our results provide strong evidence for the concept of lipid raft modulation to reduce TRPV1 dependent abdominal pain in IBS. Although current lipid raft modulators like miltefosine cannot be targeted to specific rafts of interest, our findings do encourage future development of specific small lipid-like molecule drugs with enhanced specificity for evaluation in IBS therapy.

Findings

In vitro TRPV1 activation is modulated by miltefosine

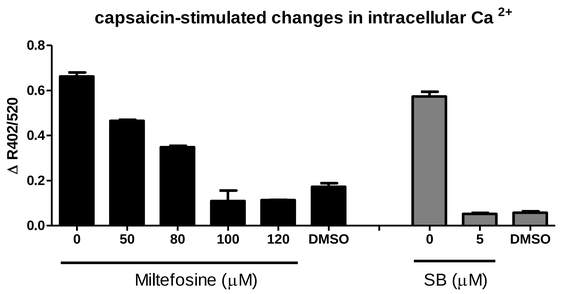


Fig 1. Changes in intracellular free calcium levels in capsaicin-stimulated TRPV-1 recombinant SH-SY5Y human neuroblastoma cells pre-treated with the specific TRPV1 antagonist SB-705498 or miltefosine. **Method**: Dissociated SH-SY5Y- TRPV1 cells were incubated with the indo-1 indicator dye for measuring intracellular calcium. TRPV-1 antagonist or Miltefosine was added 10 minutes before capsaicin activation. Cytosolic free calcium / calcium influx is represented as the change in fluorescence at 405 nm divided by that at 520 nm = Δ405/520

Miltefosine reverses *in vivo* post stress colonic hypersensitivity in maternal separated rats

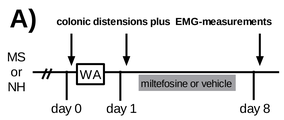
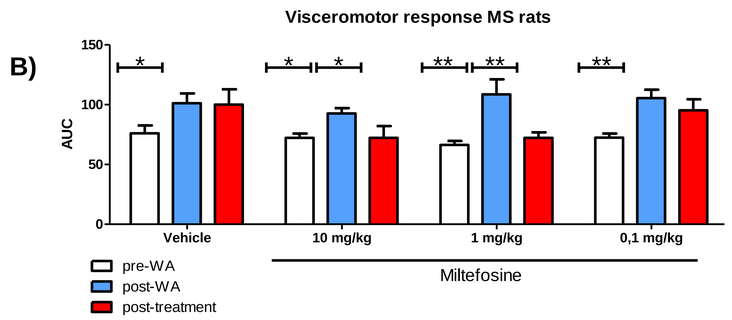
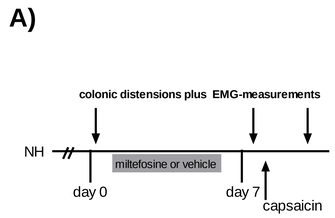


Fig 2A). Experimental protocol. Miltefosine was administered post water avoidance to adult maternal separated rats by daily oral gavage (7 days). **B). Results** Area under the curve (AUC) of the visceromotor response to colorectal distension of rats treated with vehicle or miltefosine.



Miltefosine modulates *in vivo* capsaicin-induced (TRPV1-dependent) hypersensitivity in normal rats



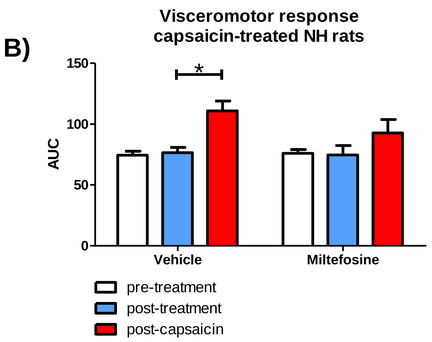


Fig 3A). Experimental protocol. Prior to TRPV1 activation by capsaicin, normal Long Evans rats were treated with Miltefosine (or vehicle) by oral gavage for a 7 day period. Baseline visceromotor response was determined at day 0 and the second distension protocol was performed at day 7. Subsequently the TRPV1 agonist capsaicin was administered rectally via a fine cannula, followed by a third distension protocol. **B)**. **Results** Area under the curve (AUC) of the visceromotor response to colorectal distension in vehicle or miltefosine treated rats.