

December 7, 2013

Dear Director Wang, the Editor of World Journal of Gastroenterology

Please find enclosed the edited manuscript in Word format (file name: 5171-review.doc).

Title: ZD 7288, an HCN channel blocker, attenuates chronic visceral pain in rats

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The manuscript was carefully revised and English edited by AJE according to yours and reviewers' suggestions and comments as follows:

1. The format was updated according to the World Journal of Gastroenterology.

2. Revision has been made according to the point-by-point suggestions of the reviewer

(1) A number of statements are not references for instance in the introduction 1, 3rd, 4 and 5th sentences, and those which are referenced do not reflect an over all knowledge of the field. For instance 2nd sentence in the introduction the references selected are not the main stay in the field. Reviews from M. Camilleri Expert Opin Pharmacother. 2013; 14(9):1151-60. Aliment Pharmacol Ther. 2010 Jan;31(1):35-46 are more relevant. Similarly in the discussion there are a number of assertions not referenced that show poor knowledge of the field of visceral pain and IBS.

Response (1): Thank you for your kind suggestion. According to these suggestions of reviewer, some references were added in the introduction and discussion. (See page 3; line:2-6 & page 7-9)

(2) The use of semiquantitative AWR is no longer a standard. It is not clear since EMG regarding was performed why this quantification was used and compared with the EMG, these data are largely descriptive and it would have been better to focus on the underlying mechanism of action of drug, in term of modulation of sensory nociceptive afferents. Therefore half of the figures are not warranty.

Response (2): Thanks. In general, AWR scores are also used to assess visceral hypersensitivity (Xu et al., 2008; Li et al., 2012). AWR scores are semi-measurement data. EMG recording results are pure measurement data without objective factor influence. The authors realized that EMG is more reliable in quantification than AWR scores. However, other signals easily interfere with EMG recordings during the experimental process. Thus, the combined application of EMG and AWR scores improves the credibility of results in this study. (See Page 9; line 5-12 from the bottom up).

(3) The discussion is mostly a repeat of results; insight to the mechanisms of action of the presented data in light of what is known in the somatic field should be discussed and whether there is indication that is more advanced field there is some clinical studies indicating its potential to be translational.

Response (3): The authors sincerely thank you for this suggestion. We deleted some repeated description and improved following contents according to the review's comments in discussion (See: pages 7-9)

The effect of ZD 7288 on spinal central sensitization in chronic visceral pain rats

Emery et al. reported that HCN channels play a central role in neuropathic pain^[8]. ZD 7288 effectively attenuates neuropathic pain^[16]. Most of the drugs that are specifically approved for the treatment of visceral pain syndromes are effective treatments for chronic neuropathic pain states^[23], which suggests chronic visceral pain and chronic neuropathic pain share a common mechanism. Treatment with 50 nM and 100 nM ZD 7288 significantly inhibited EMG amplitudes in IBS-like rats in our study (16%-41% and 40%-62%, 80-20 mmHg CRD, respectively). Intrathecal administration of 100 nM ZD 7288 significantly relieved mechanical allodynia in neuropathic pain rats with spinal nerve ligation, but 50 nM ZD 7288 had no effect^[16]. These results indicated that HCN channels may be the common mechanism in visceral and neuropathic pain, and these channels may play a more important role in visceral hypersensitivity.

Neuropathic pain is characterized by ectopic discharges, which is similar to the discharges observed in IBS-like rats^[19]. The spontaneous activity of lumbosacral afferents and the number of dorsal roots activated by CRD significantly enhanced in IBS-like rats compared to controls^[19, 21]. Spinal HCN channels contribute to the maintenance of neuropathic pain, most likely at the primary afferent terminals^[6]. Its amplitude is augmented in the ventral-lateral periaqueductal gray neurons in neuropathic pain models, and an increase in the frequency of ZD 7288-attenuated action potential firing is observed^[24]. Therefore, we inferred that the hyperexcitability of spinal ascending neurons due to an up-regulation of HCN channels might underlie spinal sensitization in chronic visceral pain. However, more electrophysiological studies, such as whole-cell patch clamp recordings, are required.

Potential of ZD 7288 as a treatment for chronic visceral pain

Several treatments, such as anti-spasm medications, antidepressants, probiotics and acupuncture, are efficacious IBS treatments, but patients and clinicians question their efficacy due to the recurrence of abdominal pain, diarrhea and other symptoms^[1-4]. For example, acupuncture is clinically effective for visceral pain due to bowel obstruction, inflammation or ulcer, but controversies exist due to the high recurrence rate^[25, 26]. Scientific evidence of acupuncture treatment efficacy is lacking, and its mechanisms require to be investigated^[25]. Antagonists to NMDA receptors, such as MK-801^[27] and AP-7^[5], inhibit visceral hypersensitivity. However, the use of these agents in the treatment of chronic pain is restricted due to their serious side effects, including hallucinations, learning and memory impairments, and sensorimotor disturbances^[28, 29].

In this study, 100 nM ZD 7288 exhibited stronger analgesic effects without apparent side effects, which is consistent with the results of Wan in neuropathic pain^[16]. The intrathecal administration of ZD 7288 increased pain thresholds in IBS-like rats in a dose-dependent manner. Visceral pain hypersensitivity includes allodynia and hyperalgesia. Allodynia indicates that an originally non-noxious stimulation induces pain, and hyperalgesia indicates that an originally noxious stimulation induces a supernormal reaction^[21, 30, 31]. Neonatal CRD in the present study may result in allodynia and hyperalgesia, which is consistent with previous studies^[20]. ZD 7288, an HCN channel blocker, attenuated visceral pain at 20-40 (non-noxious stimulation) and 60-80 mmHg CRD (noxious stimulation). Therefore, ZD 7288 attenuated allodynia and hyperalgesia in rats with chronic visceral pain. Our results suggest that ZD 7288, an HCN channel blocker, is a useful drug for the treatment of chronic visceral pain in the future.

Some specific comments

1. Method: additional information how the CRD was applied using anesthesia for inserting the

probe, how the animal was restrained during the stimulus.

6. The parameters of CRD are not clearly described what was the mmHg for each pressure and how it was given? Using a barostat? For each experimental protocol, the intensity in mmHg, its duration for each CRD, time interval between each CRD and time period of repeated CRD should be specified.

Response 1&6: The authors sincerely thank your kind reminding for pointing out this mistakes. We added detailed description of electromyographic recordings in the Method section. (See page 4 line 27 to page 5 line 8)

Rats were anesthetized with ether, and distention balloons (5 cm in length; made of the finger of a latex glove attached to polyethylene tubing) were inserted through the anus into the rectum and descending colon of adult rats. The tubing was taped to the tail to hold the balloon in place. Two silver bipolar electrodes were inserted into the external oblique muscle of the abdomen (EOMA). The rats were maintained in a supine position in a self-made restrainer and allowed at least 30 min to recover from inhalational anesthesia. The tubing was attached through a T-connector to a sphygmomanometer pump and a pressure gauge. Distention was produced by rapid inflation of the balloon to the desired pressure (20, 40, 60 or 80 mmHg) for 10 s followed by a 4-min rest.

2. Information as whether the body weight of rats exposed to CRD differs from sham should be indicated after the period of CRD and thereafter.

Response 2: Thank you for raising this question. No significant differences in body weight were seen over time between control and neonatal colon irritation (CI) rats (see Figure 1).

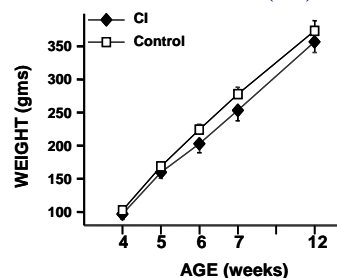


Figure 1 Body weight between control and CI rats

3. Information on the anesthesia used to place the electrodes and the electrodes themselves are not specified.

Response 3: Thank you. In our study, two silver bipolar electrodes were inserted into the external oblique muscle of the abdomen under anesthesia with aether. (see page 5, line 2-3)

4. EOMA, RM6240BD meaning?

Response 4: Thank you again. EOMA is abbreviations of “External Oblique Muscle of the Abdomen” and RM6240BD (made in Chengdu, China) is the instrument number of the multi-channel physiological signal acquisition and processing system used in this study. (see page 5, line 3, 8-9)

5. “Rats were constricted and allowed time for recovery from inhalational anesthesia for at least 30 minutes”. The meaning of this sentence is not clear as well as the exact experimental protocol.

Response 5: Thanks a lot. This paragraph was revised as: “Then, rats were kept supine position in a self-made restrainer and allowed at least 30 minutes for recovery from inhalational anesthesia.” Experimental protocol was also described in detail in Method Section. (See pages 4-5)

7. Protocols of CRD in the experiment with drug administration should be specified and on which base the selection of dose was used.

Response 7: Our protocols of CRD in the experiment were the same as described in “Electromyographic recordings and AWR scores” (Al-chaer, 2000). Measurements of visceral pain were performed 30 min after intrathecal administration. In our study, doses of ZD 7288 were selected according to a previous reference (Wan, 2008). In addition, some preliminary experiments were performed to find the appropriate dose for IBS-like rats (Data not shown in this manuscript).

8. Histology of the colon at the end of CRD from 8 and 15 days will be important to assess the integrity under these conditions of CRD.

Response 8: Thanks. No significant structural damage was found in the colon tissues of the adult rats exposed to neonatal CRD in this study (also reported in Al-chaer, 2000). Damage caused by distension at the end of CRD (8-15 days) surely exists. However, it is unlikely affect the present results, since we detect visceral pain of these rats 8 weeks after the model initiation.

9. It is not clear if the same balloon and CRD procedure in rats aged 8 to 15 and adults at age 8 weeks old.

Response 9: Of course, they are not the same. The balloon used in CRD procedure in rats aged days 8 to 15 was made of angioplasty balloons (length, 20.0 mm; diameter, 2.5 mm), while the balloon (5 cm in length) used in adult rats was made of the finger of a latex glove attached to polyethylene tubing.

10. Most likely the sensitivity of the monitoring is not exact at two decimal numbers as listed in abstract and results (40.62%, 34.63%, 21.63%, and 16.48%).

Response 10: The authors thank you for your advice. These data were done by rounding down decimal places in abstract, results and discussion in the revised manuscript.

11. Discussion the first few sentences of the paragraph “*Potential of ZD 7288 as..*” should be revised as they don’t reflect the state-of knowledge. We encourage the investigators to read a couple of authoritative reviews on pathophysiology and underlying mechanisms of IBS.

Response 11: Thanks for your suggestion. We have revised this paragraph of the discussion section after reading a couple of reviews on mechanisms of IBS. (Camilleri, 2013; 2010. See page: 8; lines 14-16)

Several treatments, such as anti-spasm medications, antidepressants, probiotics and acupuncture, are efficacious IBS treatments, but patients and clinicians question their efficacy due to the recurrence of abdominal pain, diarrhea and other symptoms ^[1-4].

3 References and typesetting were corrected

Thank you again for your kind assistance on our manuscript in the World Journal of Gastroenterology. Please feel free to contact me if there is anything needed.

Sincerely yours,



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