Impaired Transient Receptor Potential Melastatin 3 ion channels in Natural Killer cells from Gulf War Illness veterans

Etianne Martini Sasso [1,2,3], Katsuhiko Muraki[2,4], Natalie Eaton-Fitch[1,2], Peter Smith[2,5], James N Baraniuk[6], Sonya Marshall-Gradisnik[1,2].

[1] The National Centre for Neuroimmunology and Emerging Diseases, Griffith University, Gold Coast, QLD, Australia.
[2] Consortium Health International for Myalgic Encephalomyelitis, National Centre for Neuroimmunology and Emerging Diseases, Griffith University, Gold Coast, QLD, Australia.
[3] School of Pharmacy and Medical Sciences, Griffith University, Gold Coast, QLD, Australia.
[4] Laboratory of Cellular Pharmacology, School of Pharmacy, Aichi-Gakuin University, Nagoya, Japan.
[5] Clinical Medicine, Griffith University, Gold Coast, QLD, Australia.
[6] Department of Medicine, Georgetown University, Washington DC, USA.

e.martinisasso@griffith.edu.au
ncned@griffith.edu.au

Background

In 1990 and 1991, almost one million defence personnel from 41 countries engaged in combat in the Gulf War, being exposed to diverse hazardous environmental stressors, biological or chemical agents and prophylactic strategies[1,2,3]. It is estimated that from 25% to 32% of those veterans engaged in the Gulf War continue to suffer from health issues and disabling symptoms, which characterise a chronic, debilitating, and multisystem disorder known as chronic multi-symptom illness or Gulf War Illness (GWI)[4,5]. Although GWI aetiology remains elusive, exposure to multiple toxicant agents, vaccines and medications is the most plausible cause of this condition[3,6]. Interestingly, GWI significantly overlaps another multisystem disorder, Myalgic Encephalomyelitis/Chronic Fatigue Syndrome (ME/CFS), which is associated with impaired Transient Receptor Potential Melastatin 3 (TRPM3) ion channel[7, 8, 9].

Aim

Given the potential for TRP channels to be modulated by various environmental and toxic stimuli, such as those experienced by veterans during the Gulf War and the similarities between GWI and ME/CFS, this study aimed to investigate TRPM3 function in Natural Killer (NK) cells from veterans diagnosed with GWI in comparison with healthy controls (HC).

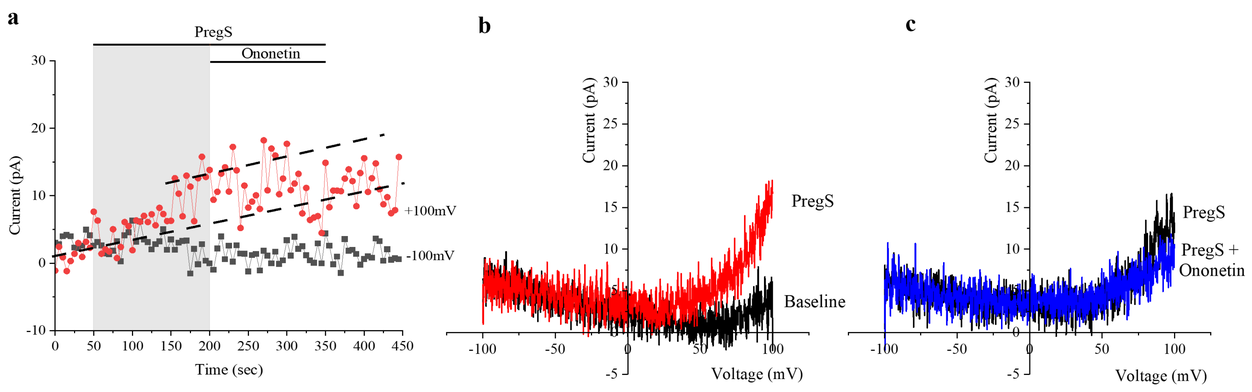
Methods

The gold standard technique, whole-cell patch-clamp, was performed to measure TRPM3 ion channel activity in isolated NK cells of N=7 Australian males veterans who engaged in combat in Gulf War (fulfilling the CDC Case Definition and Kansas criteria for GWI, aged 52.57 ± 1.99), and N=7 male HC aged (45.86 ± 9.30). The TRPM3 agonist, pregnenolone sulfate (PregS) was used to activate TRPM3 function, while ononetin was applied as a TRPM3 antagonist. Statistical comparison was performed by Mann-Whitney and Fisher's exact test.

Results

1- TRPM3 ion channel in HC and GWI participants.

- **Healthy control**



- **GWI**

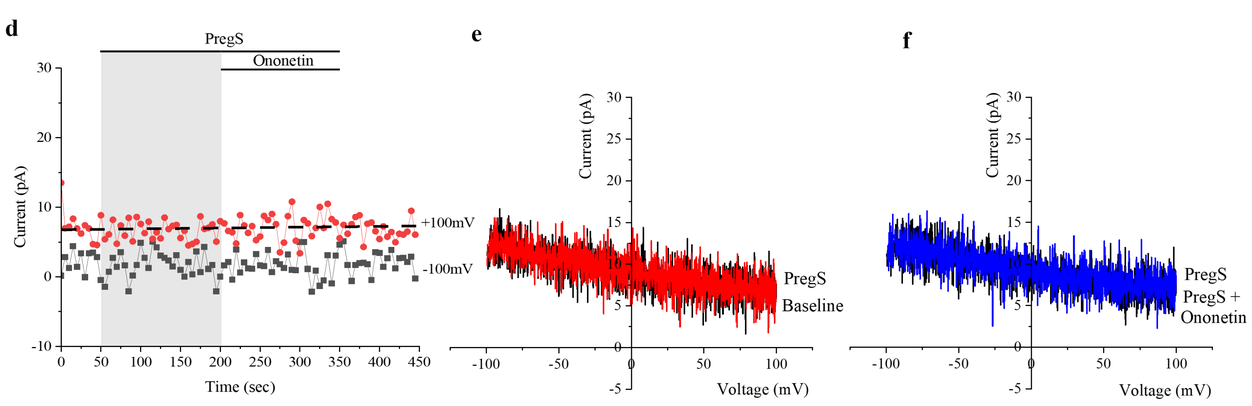


Figure 1.a.d. A representative time-series of current amplitude at +100mV and -100mV showing the effect of 100μM PregS and 10μM ononetin on ionic currents in isolated NK cells from HC (**a**) and GWI participants (**d**). **b.e.** Current-voltage relationship (*I–V)* before and after PregS stimulation in a HC cell (**b**) and GWI participant cell (**e**). **c.f.** *I–V* before and after application of ononetin in a HC cell (**c**) and GWI participant cell (**f**) .

2- Impaired TRPM3 ion channel activity after PregS in NK cells from GWI participants.

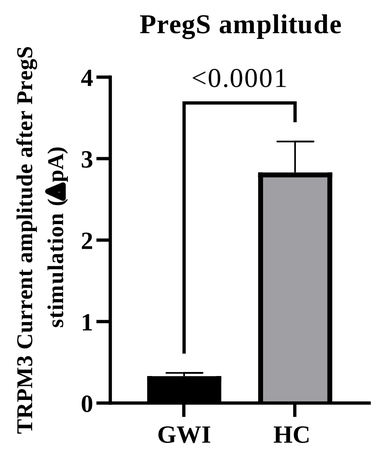


Figure 2. Bar graphs representing TRPM3 current amplitude at +100mV after stimulation with 100μM PregS in GWI (N=7; n=52) compared with HC (N=7; n=56). N refers to number of participants and n to number of records analysed. Data are represented as mean ± SEM, analysed using Mann-Whitney.

3- PregS-evoked currents are resistant to ononetin in NK cells from GWI participants.

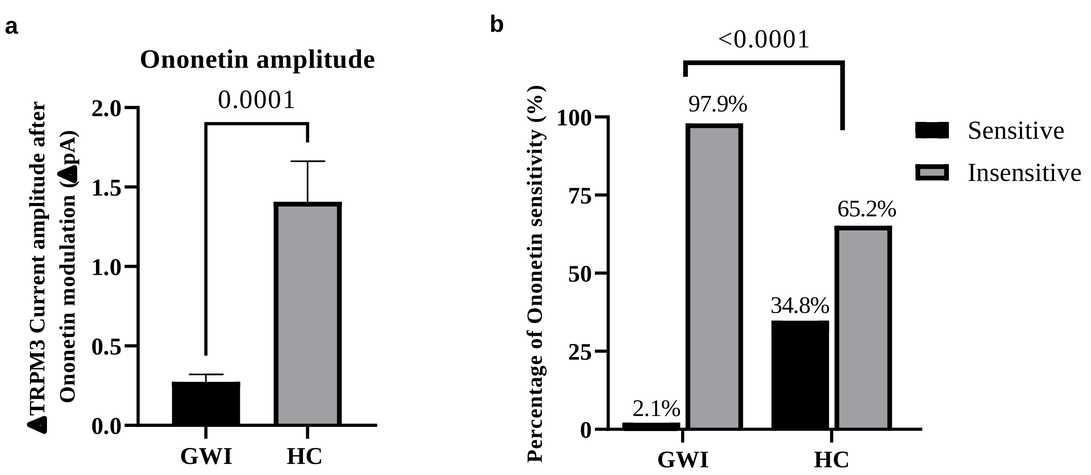


Figure 3. Summary TRPM3 activity after ononetin modulation. **a**. Bar graphs representing TRPM3 current amplitude at +100mV after inhibition with 10μM ononetin in presence of PregS, GWI (N=7; n=48) compared with HC (N=7; n=46). **b**. Bar graphs representing sensitive and insensitive cells to 10 μM ononetin in presence of PregS. Data are analysed using Mann-Whitney and Fisher's exact test, represented as mean ± SEM. N refers to number of participants and n to number of records analysed.

Conclusions

- **A significant impairment in TRPM3 ion channel function in NK cells from GWI veterans**.
- **TRPM3 dysfunction** is potentially involved in the **pathomechanism of GWI.**
- **Pharmacotherapeutic intervention for TRPM3** may provide a possible **treatment for GWI**.

Acknowledgements

Those participants who volunteered for this project. This study was supported by NCNED, Disability and Rehabilitation, Griffith University.

References

1. Gean EG, Ayers CK, Winchell KA, Freeman M, Press AM, Paynter R, et al. Biological measures and diagnostic tools for Gulf War Illness—A systematic review. Life Sci. 2021; 275:119360. Epub 20210316. https://doi.org/10.1016/j.lfs.2021.119360 PMID: 33741418.
2. Institute of Medicine. In: Hernandez LM, Durch JS, Blazer DG II, Hoverman IV, editors. Gulf War Veterans: Measuring Health. Washington (DC): The National Academies Press; 1999.
3. Elhaj R, Reynolds JM. Chemical exposures and suspected impact on Gulf War Veterans. Mil Med Res. 2023; 10(1):11. Epub 20230308. https://doi.org/10.1186/s40779-023-00449-9 PMID: 36882803; PubMed Central PMCID: PMC9993698.
4. National Academies of Sciences, Engineering, and Medicine. In: Cory-Slechta D, Wedge R, editors. Gulf War and Health: Volume 10: Update of Health Effects of Serving in the Gulf War, 2016. Washington (DC) 2016.
5. Moffett K, Crosson B, Spence JS, Case K, Levy I, Gopinath K, et al. Word-finding impairment in veterans of the 1991 Persian Gulf War. Brain Cogn. 2015;98:65-73. doi: 10.1016/j.bandc.2015.05.005.
6. White RF, Steele L, O'Callaghan JP, Sullivan K, Binns JH, Golomb BA, et al. Recent research on Gulf War illness and other health problems in veterans of the 1991 Gulf War: Effects of toxicant exposures during deployment. Cortex. 2016;74:449-75. Epub 20150925. doi: 10.1016/j.cortex.2015.08.022.
7. James LM, Georgopoulos AP. At the Root of 3 "Long" Diseases: Persistent Antigens Inflicting Chronic Damage on the Brain and Other Organs in Gulf War Illness, Long-COVID-19, and Chronic Fatigue Syndrome. Neurosci Insights. 2022;17:26331055221114817.
8. Steele L. Prevalence and patterns of Gulf War illness in Kansas veterans: association of symptoms with characteristics of person, place, and time of military service. Am J Epidemiol. 2000;152(10):992-1002. doi: 10.1093/aje/152.10.992.
9. Cabanas H, Muraki K, Eaton N, Balinas C, Staines D, Marshall-Gradisnik S. Loss of Transient Receptor Potential Melastatin 3 ion channel function in natural killer cells from Chronic Fatigue Syndrome/Myalgic Encephalomyelitis patients. Mol Med. 2018;24(1):44.. doi: 10.1186/s10020-018-0046-1.