ION CHANNEL MODULATION SYMPOSIUM
2022 - THE UNITED KINGDOM

Voltage-gated sodium channels: Multi-faceted involvement in cancer

Serife Yerlikaya[1*], Scott P. Fraser[2] and Mustafa B. A. Djamgoz[2,3]

[1]Istanbul Medipol University, Research Institute for Health Sciences and Technologies (SABITA), Istanbul, Turkey

[2]Department of Life Sciences, Imperial College London, London, UK

[3] Biotechnology Research Centre, Cyprus International University, Haspolat, Nicosia, TRNC, Mersin 10, Turkey

* Correspondence:

Dr Serife Yerlikaya

E-mail: serife.yerlikaya@medipol.edu.tr

Tel: +90 543 365 55 12

Background

A substantial body of in vitro and in vivo evidence suggests that de novo expression of voltage-gated sodium channels (VGSCs) occurs in several carcinomas and drives the metastatic process (Djamgoz et al., 2019). It is well known that chemotherapy can lead to peripheral neuropathy and this could be associated with an alteration in VGSC expression/activity. We questioned whether blocking VGSC activity would affect the cytotoxic impact of paclitaxel (PTX) upon human strongly metastatic MDA-MB-231 cells.

Aim

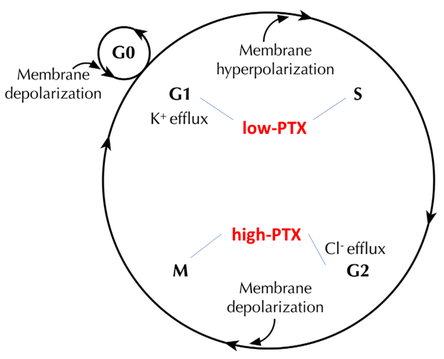
The overall aim was to present further evidence for functional involvement of VGSCs in some novel aspects of cancer, including in relation to chemotherapy.

PACLITAXEL - VGSC Hypothesis

Paclitaxel targets microtubules:

At relatively low concentration (assumed 50pM) - apoptosis is induced at G0 and G1/S phase (either via Raf-1 kinase activation or p53/p21).

At relatively high concentration (assumed 50nM) - PTX would cause mitotic arrest at G2/M phase.



Hypothesis - General

PTX → ΔV_m → $\Delta[Ca^{2+}]i$ → ΔCV (cell viability)

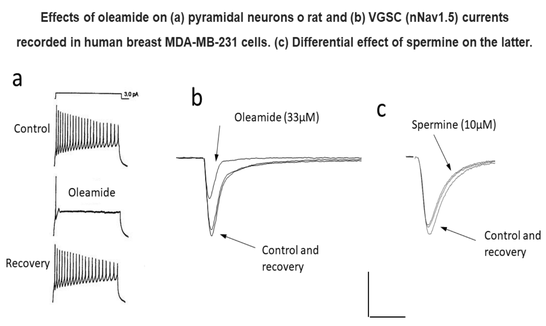
Tetracaine → ΔTE (treatment efficiency)

METHODS

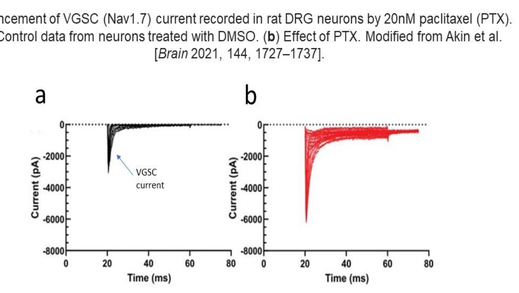
Human breast cancer MDA-MB-231 cells were used as a 'model'. Cell viability (CV) was measured by the trypan blue exclusion (TBE) assay and defined as CV (%) = (No. live cells / Total no.) x 100. Proliferation assay was performed by the MTT method. Paclitaxel (PTX) - treated and tetracaine-(co)treated MDA-MB-231 cells were incubated under normoxic conditions for 24 - 48 h. We first tested the cytotoxicity of a series of PTX concentrations: 500 nM, 50 nM, 5 nM, 500 pM and 50 pM for monotherapy. Two test concentration of PTX (50pM and 50nM) were used in combination with tetracaine, a blocker of VGSCs. The effectiveness of tetracaine in the combination was defined as "Treatment Efficiency", as follows: **TE (%) = {1 - [CV(drug) / CV(control)]} x 100**

RESULTS

VGSC – Oleamide



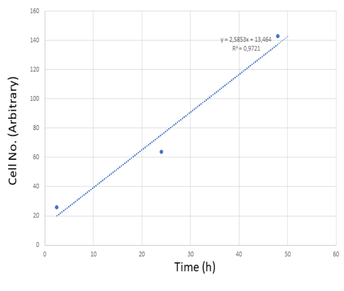
VGSC – Chemotherapy



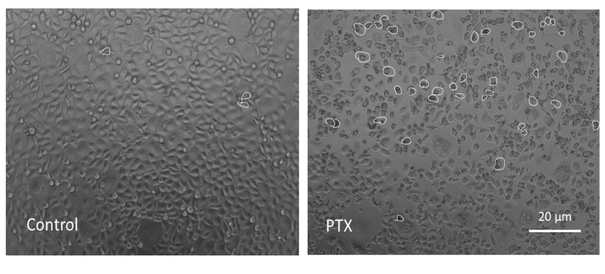
Controls

The TBE assay revealed:
i) that the cells proliferated normally, cell number increasing linearly; and
ii) that CV could readily be quantified.

Proliferation of MDA-MB-231 cells (TBE Assay)

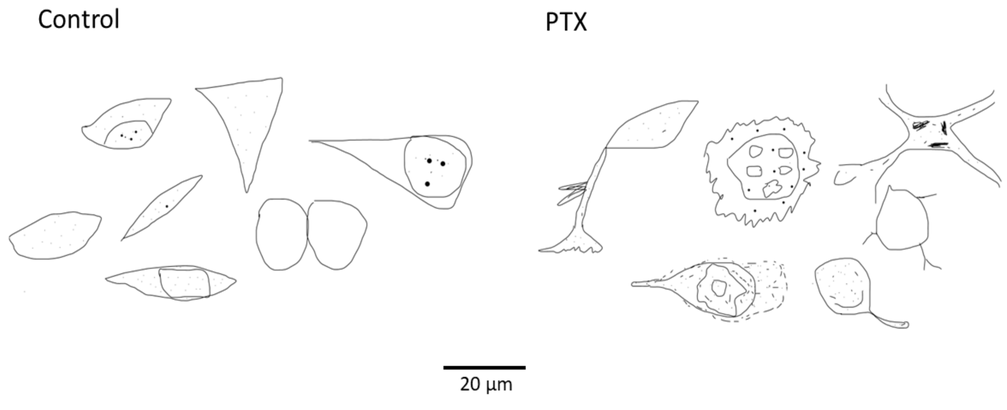


Effect of 50pM paclitaxel (PTX) on viability of MDA-MB-231 cells (TBE Assay)



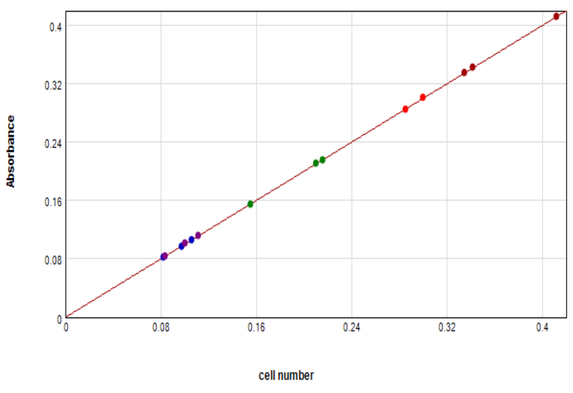
Change of cell morphology towards a more delineated phenotype

Effect of 50pM paclitaxel (PTX) on morphological appearance of MDA-MB-231 cells

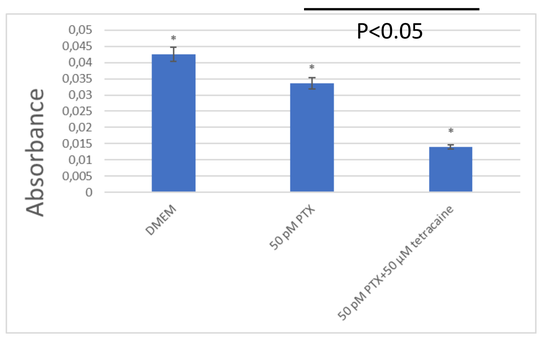


Data from the MTT assay

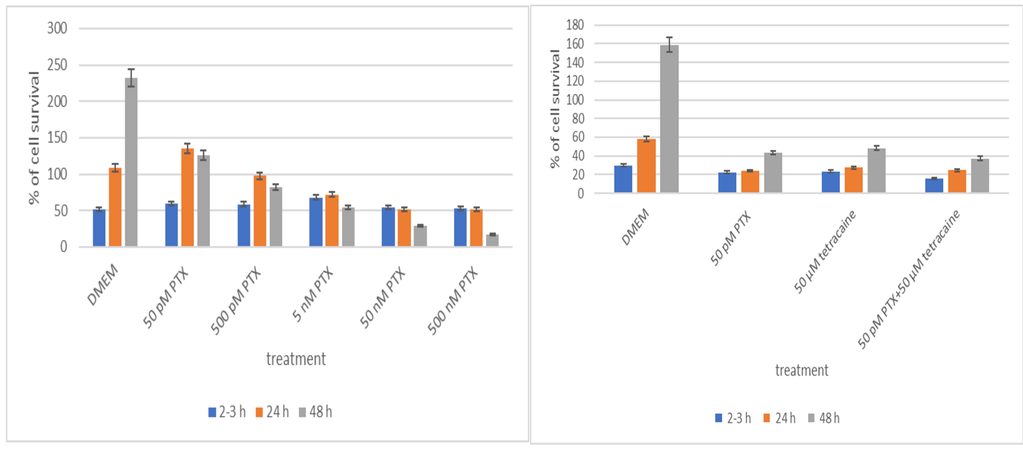
Standard curve for the MTT 'growth' assay on MDA-MB-231 cells



Tatracaine enhanced the inhibition of 'growth' induced by PTX



Effect of 50pM paclitaxel (PTX) on cell viability: Dose dependence and combination with tetracaine

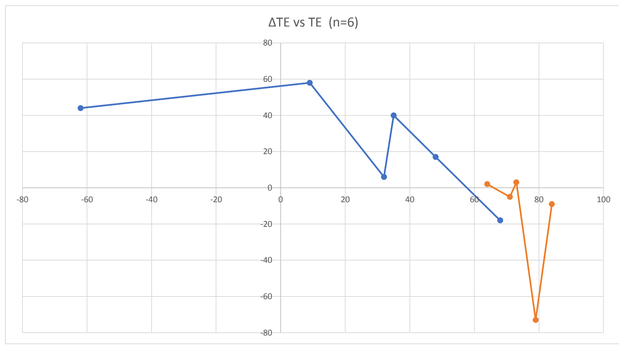


Conclusion

Specific: PTX suppressed the viability of MDA-MB-231 cells; the effect was concentration dependent. Tetracaine potentiated effectiveness of 50pM PTX (increased TE) but tended to inhibit the effectiveness of high-dose (50nM) PTX.

General: Extended to the clinic, the results imply that low-dose PTX combined with tetracaine (or another VGSC blocker) could be equally effective in killing tumour cells whilst reducing undesirable side effects.

Treatment efficiency (TE) of 50 pM PTX (blue) vs 50 nM PTX (orange) combined with tetracaine



References

Akin, E.J.; Alsaloum, M.; Higerd, G.P.; Liu, S.; Zhao, P.; Dib-Hajj, F.B.; Waxman, S.G.; Dib-Hajj, S.D. Paclitaxel increases axonal localization and vesicular trafficking of Nav1. 7. *Brain* **2021**, *144*, 1727–1737

Djamgoz, M., Fraser, S. P., & Brackenbury, W. J. (2019). In vivo evidence for voltage-gated sodium channel expression in carcinomas and potentiation of metastasis. *Cancers, 11*(11), 1675.