Development and Application of an MRM Method for Simultaneous Quantification of Sodium Channels Na v 1.1, Na v 1.2 and Na v 1.6 in Stable HEK293 cell lines, and Solubilized Membrane Proteins from Rodents and Human Brain Tissues.

Luis Sojo[1], Raibow Kwan[1], Prerna Das[1], Neelan Gerrebos[1], Jenny Li[1], Xin Yin Wang[1], Gina DeBoer[1], Vanessa Emnacen-Pankhurst[1], Sophia Lin[1], Raymond Feng[1], and Sam Goodchild[1]

[1]Xenon Pharmaceuticals Inc

June 30, 2023

Abstract

Rationale Na $_v$1.1, 1.2 and 1.6 are transmembrane proteins acting as voltage gated sodium channels implicated in various forms of epilepsy. There is a need for knowing their actual concentration in target tissues during drug development. **Methods** Unique peptides for Na $_v$1.1, Na $_v$1.2 and Na $_v$1.6 were selected as quantotropic peptides for each protein and used for their quantification in plasma membranes from stably transfected HEK293 cells and rodent and human brain samples using ultra-high-performance liquid chromatography/electrospray ionization tandem mass spectrometry. **Results** Na $_v$ 1.1, 1.2 and 1.6 protein expressions in three stably individually transfected HEK293 cell lines were found to be 2.1±0.2, 6.4±1.2 and 4.0±0.6 fmole/µg membrane protein respectively. Na $_v$1.2 showed the highest expression, with approximately 3 times higher ($p<0.003$) in rodents than in human at 3.05±0.57, 3.35±0.56 in mouse and rat brains and 1.09±0.27 fmole/µg in human, respectively. Both Na $_v$1.1 and 1.6 expressions were much lower than Na $_v$1.2, with approximately 40% less expression in human Na $_v$1.1 when compared with rodents Na $_v$1.1 at 0.49±0.1 (mouse), 0.43±0.3 (rat), and 0.28±0.04 (human); while Na $_v$1.6 was approximately 60% less expression in human when compared to rodents at 0.27±0.09 (mouse), 0.26±0.06 (rat) and 0.11±0.02 (human) fmole/µg membrane proteins. **Conclusions** MRM was used to quantify sodium channels Na $_v$1.1, 1.2 and 1.6 expressed in stably transfected HEK293 cells and brain tissues from mouse, rat, and human. We found significant differences in the expression of these channels in mouse, rat, and human brains. Na $_v$ expression ranking among the three species was Na $_v$1.2 >> Na $_v$1.1> Na $_v$1.6, with the human brain expressing much lower concentrations overall in comparison to rodents.

Hosted file

`Nav_quantification_Kwan et al_2023_RCMS_Submission_1.docx` available at https://authorea.com/users/634613/articles/652414-development-and-application-of-an-mrm-method-for-simultaneous-quantification-of-sodium-channels-na-v-1-1-na-v-1-2-and-na-v-1-6-in-stable-hek293-cell-lines-and-solubilized-membrane-proteins-from-rodents-and-human-brain-tissues