Comprehensive Analysis of Neurotransmitters from Regenerating Planarian Extract Using UHPLC-MS/SRM Method

Kannan Rangiah¹, Dasaradhi Palakodeti²

¹Centre for Cellular and Molecular Platforms, ²Institute of Stem Cell Biology and Regenerative Medicine, NCBS-TIFR, GKV, Bellary Road, Bangalore-560065, India.

Abstract

Quantification of neurotransmitters (NTs) from biological systems is imperative to track how changes in concentration of active neurochemicals may affect biological behavior. A stable isotope dilution ultra-high performance liquid chromatography/mass spectrometry/selected reaction monitoring (UHPLC-MS/SRM) assay has been developed for a sensitive and quantitative assessment of NTs in planarians. We used this method for the simultaneous quantification of sixteen NTs from both sexual, asexual and regenerating planarians. Each of the sixteen well resolved analytes showed a linear relationship between concentrations (0.78–50 ng/mL), regression coefficients higher than 0.97, accuracy (91–109%) and low coefficients of variation (CVs). The inter-day CVs for the lowest quality controls (1.56 ng/mL) were in the range between 2–11%. The levels of most of the NTs were similar in both sexual and asexual planarians except for glutamic acid, which was about two-fold higher in asexual compared to sexual planarian. Further, the levels of melatonin and epinephrine were lower than the limit of quantification (LOQ) in both the strains. We also identified high levels of serotonin and failed to detect tryptamine suggesting that the pathway essential for the conversion of tryptophan to tryptamine is absent in planarians. Interestingly, we also found high levels of dopamine and L-DOPA in regenerating planarians suggesting their possible role in regeneration. For the first time, we developed novel methodology based on UHPLC-MS/SRM and quantified sixteen NTs with high sensitivity and specificity from sexual and asexual strain of planarian Schmidtea mediterranea. This method will also have great application in quantifying various neurotransmitters with great precision in different model systems.

Role of Neurotransmitters

Chemical NTs and hormones occupy key positions in the regulation of physiological processes. A diverse array of NTs and neuromodulators control and affect brain function. Several groups of NTs like amino acids, catecholamines and neuropeptides are present in the biological system. Communications of cells in the neural networks completely rely on the maintenance of balance among the concentrations of such neurochemicals. Disturbed balance between two or more such NTs in the brain has already been observed in many diseases like Parkinson’s disease, Alzheimer’s disease and Schizophrenia.

Neurotransmitters in Planaria

Serotonin, dopamine, tyramine, histamine, noresinephrine and octopamine were reported in the flatworms. Major role in the muscle contraction and the spike activity in the CNS. Serotonin and dopamine are known to increase the regeneration in planaria. It has been shown that various enzymes like tyrosophan hydroxylase, tyramine p-hydroxylation in planarian Dugesia japonica.

Nothing much is known about the species Schmidtea mediterranea.

Sample preparation

Three worms of each sexual and asexual were taken in endopod tube (in triplicates) and washed with water thrice.

- Worms were weighed without water in the accurate analytical balance.
- Worms were crushed using plastic pestle in 200 μl of aceticone (0.1 % FA)
- Then Centrifuged (14000 rpm, 5 min) and transferred the supernatant to the fresh endopod and ISTDs (10 ng) were spiked.
- It was then dried in speed vacuum and reconstitute it in 50 μl of 0.5% acetonitrile (0.1% FA) and transferred into the HPLC vial for the injection.
- 10 μl was injected for the UHPLC-MS/SRM analysis.

ISTDs Used

Standard curves and regression line analysis of sixteen NTs

Validation for UHPLC-MS/SRM analysis of NTs on five different days

Quantification of neurotransmitters from planarial extract

Conclusions

Stable isotope dilution UHPLC-MS/MS assay was developed and validated to evaluate the concentrations of sixteen NTs from the planarian extract. The LOQ for each analyte was 1.56 ng/mL and standard curves were linear in the range of 0.78–50 ng/mL. The assay was sensitive, specific and very robust. Various NTs were up regulated in the regenerating planarians suggesting their role in regeneration.

Acknowledgments

We thank the department of biotechnology (DBT), Government of India for financial assistance.