Fluorescence-based Neurotransmitter Uptake Assay in Primary Neuronal Cultures. Parsa Safa, MDS Analytical Technologies, Sunnyvale, CA 94089 & Lesley Radov, AstraZeneca Pharmaceuticals, CNSP Discovery, Wilmington, DE 19803

Abstract

Depression is a common mental disorder. It is estimated that the lifetime prevalence of major depressive disorder (MDD) in the US has increased 3-fold since the 1960s to a current prevalence of 8.3%. Depression treatment was the development of dual uptake inhibitors (SSRI/SSNI). These agents have gained acceptance in the clinic, but are not totally satisfactory due to delayed onset, low response rates and their side effect profile. The latest advance in depression therapy has been the development of the triple uptake inhibitors (SSRI/SSNI/selective dopamine uptake inhibitors).

The most accurate technique used to estimate ligand affinity has been monitoring the accumulation of radiolabeled substrates or inhibitors. One major drawback in the use of these radiolabeled methods is the inherent monitoring multiple changes. A second consideration is the increased pressure in animal use of radiolabeled material due to public safety concern and increasing disposal costs. An alternative, non-radioactive approach for determining ligand affinity is available. The use of a fluorescence-based assay for the determination of ligand affinity is in cell lines expressing the human DAT, NET and SERT genes has been previously reported. A question that constantly arises is the relevance of the use of cell lines over-expressing the recombinant neuronal culture model.

Materials and Methods

Nucleus accumbens cultures of the same age are used. The confidence limits overlap between all 6 neuronal cultures with reported K_i values. Excellent correlation exists between the published K_i values and those generated with the MDS Analytical Technologies Neurotransmitter Transporter Uptake Assay Kit®.

Conclusion

Dye uptake in primary neuronal cultures is time, temperature, sodium, chloride and temperature dependent.

Assay is reproducible if culture age is kept constant.

Tissue source is critical to determine valid NET vs DAT vs SERT inhibitor selectivity & potency.

Excellent correlation exists between the published K_i values for the 14 drugs evaluated and the K_i values generated in the primary neuronal culture system. The correlation for each transporter is as follows: NET: 0.9801; SERT: 0.9948 & DAT: 0.9895.

Table 3. Comparison of K_i results from primary neuronal cultures with reported K_i values.

Table 2. K_i values are reproducible in primary neuronal cultures of the same age.

Table 1. The effect of culture age on compound potency.

References