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EX VIVO RECEPTOR OCCUPANCY ASSAY

Animal dosing and tissue collection

Animals are administered the test article (oral, i.p., s.c or i.v) or vehicle alone. After allowing for tissue uptake and distribution, the animals are sacrificed and the brain or other organs removed. A blood sample is collected at the time of sacrifice for subsequent quantification of drug levels in the plasma.

Sectioning

Frozen brains (or organ) from the drug-treated animals are trimmed with a razor blade and mounted in a cryostat chuck. Tissue sections are cut at a thickness of 20 µm using a cryostat (Bright OFT5000) and thaw mounted onto Superfrost® slides. Three consecutive sections are placed on each slide, with a total of two slides (6 sections) per brain.

Incubation in radiotracer

Slides are placed horizontally in a humidified box and radioligand in assay buffer (1 ml) layered over each slide. Sections are incubated in the radioligand solution for 5 - 10 minutes at room temperature. The radioligand solution is then rapidly aspirated off and the slides immediately placed in ice-cold wash solution (e.g. three washes, 5 minutes each). Following the final wash, the slides are dipped briefly in distilled water and dried under a stream of warm air.

Phosphorimaging

Sections are placed over a multipurpose (^{125}I) or tritium-sensitive (^{3}H) phosphor screen together with autoradiographic standards. The screen is exposed for 1 - 7 days and then scanned on a phosphorimager (Cylone Storage Phosphor System).

Data analysis

Regions-of-interest (ROIs) are drawn over each section and radioactivity levels measured in digital light units / mm^2 (DLU/mm^2). The DLU/mm^2 values are converted to CPM/mm^2 by reference to the autoradiographic standards. A value for specific binding is generated by the subtraction of mean nonspecific binding from mean total binding for each brain or organ. Percent inhibition of specific binding is plotted against the drug dose or plasma or tissue drug concentration to determine receptor occupancy.

IN VIVO RECEPTOR OCCUPANCY ASSAY

Animal dosing and tissue collection

Animals are administered the test article (oral, i.p., s.c or i.v.) or vehicle alone. After allowing for tissue uptake and distribution, the animals are placed in a restraint device and administered a radiolabeled tracer for the target receptor (in 0.2 ml saline) via a tail vein and returned to the home cage. 30 minutes later the animals are sacrificed and the brain or other organs removed. A blood sample is collected at the time of sacrifice for subsequent quantification of drug levels in the plasma. Individual brain or tissue regions are dissected out and placed into pre-weighed vials and reweighed. Once all the tissue regions have been harvested the tissues are digested overnight in Solvable® (1 ml). Following digestion, scintillation cocktail (10 ml) is added to each vial and the vials counted in a liquid scintillation counter (Wallac® TriLux 1450 MicroBeta).

Data analysis

DPM values for each tissue region are converted to DPM/mg tissue and thence to percentage injected dose per gram. In vivo receptor binding of the radiotracer is quantified by determining the tissue radioactivity (DPM/mg) in a receptor-rich tissue area relative to that in a receptor-deficient, reference region. The latter comprises non-specific binding whereas the former comprises both receptor binding and non-specific binding. Receptor occupancy for the drug-treated animals is determined as a percentage of that in the vehicle-treated animals and the percentage receptor occupancy values plotted against plasma or tissue drug levels.