

³H-Penciclovir (³H-PCV) Uptake Assay

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[Abstract] Thymidine Kinase from human Herpes simplex virus type 1 (HSV1-TK) in combination with specific substrate prodrug nucleotide analogue ganciclovir (GCV) has been widely used as suicidal therapeutic gene for cancer gene therapy. *HSV1*, and its mutant (*HSV1-sr39TK*) with improved substrate specificity, were used as reporter genes for PET-imaging of various biological functions in small animals, by combining with radiolabeled substrates such as ¹⁸F-FHBG and ¹²⁴I-FIAU. ³H-Penciclovir (PCV) uptake assay is a method of choice used to determine the expression level of HSV1-TK in mammalian cells and tissues. HSV1-TK phosphorylate PCV and result in the formation of penciclovir monophosphate, and its subsequent phosphorylation by cellular TK lead to the formation of penciclovir triphosphate, which is trapped selectively in cells expressing HSV-TK. ³H-Penciclovir enables the detection of penciclovir uptake of mammalian cells and tissues by radioactive procedures such as scintillation counting. Here we describe the protocol to carry out ³H-Penciclovir uptakes in mammalian cells.

Materials and Reagents

1. Control and experimental cells (HEK293T and HEK293T-sr39TK)
2. Dulbecco's Modified Eagle Medium (DMEM) (Life technologies, catalog number: 11965-084)
3. ³H-Penciclovir (specific activity 14.9 ci mmol⁻¹) (Moravek Biochemicals, La Brea, CA, USA)
4. Sodium hydroxide (NaOH) (0.1 N)
5. Phosphate buffered saline (PBS) (pH 7.4) (Life technologies, catalog number:10010-056)
6. Glass Scintillation vials
7. Cytoscint Scintillation fluid (Acros Organics, Geel, Belgium)
8. Cell culture plates (Greiner Bio-One, USA)
9. Bradford protein assay reagent (Bio-Rad Laboratories, Hercules, CA)
10. BSA (Sigma-Aldrich, catalog number: A9418)
11. Wash solution (Ice cold PBS) (pH 7.4)

Equipment

1. 37 °C 5% CO₂ Cell culture incubator (Thermo Fisher Scientific, model: Napco 8000)
2. Scintillation counter (Beckman Coulter)
3. Shaker (Boekel scientific, Model: 260350)
4. Radioactive Chemical hood (Hamilton, Two Rivers, WI)
5. Pipettes (Gilson, Middleton, WI)
6. Disposal (As per Environment and Health safety regulation)
7. Spectrophotometer (Varian Inc, model: Varian Cary 50)

Procedure

1. Mammalian HEK293T and HEK293T cells stably expressing sr39-HSV1-TK (HEK293T-sr39TK) should be plated at a confluency of 60-80% in 12 well culture plates (150,000 cells/well in 1 ml DMEM). Incubate at 37 °C with 5% CO₂ for 24 h.
2. Twenty-four hours after initial plating remove the medium and add 0.5 µl of ³H-Penciclovir/well (0.5 mCi/ml of 8-³H-Penciclovir; specific activity 14.9 ci mmol⁻¹) in 0.5 ml of respective culture medium.
3. Incubate at 37 °C for 3 h. Maintain the incubation time consistent between samples.
4. Keep the same cells (HEK293T) plated in equal number express no HSV1-TK as control.
5. After 3 hours of incubation remove the medium carefully without disturbing cells, and wash the plates twice with 0.5 ml each of ice cold PBS. Pool the medium and wash solutions from respective well to measure the remaining total activities.
6. Add 0.5 ml of 0.1 N NaOH to each well and leave it for 10 min at room temperature in a shaker to lyse the cells.
7. Transfer 20 µl of homogenous cell lysate to measure total protein concentration by using Bradford protein assay reagent. In brief, to each 20 µl of cell lysate add 1 ml of 1x Bradford protein assay reagent and read the absorbance at 595 nm (A₅₉₅) 5 min after incubation at room temperature in a spectrophotometer. Use standard graph prepared with the standards (BSA) diluted in 0.1 N NaOH to calculate the total protein present in 0.5 ml of cell lysate.
8. Transfer the remaining cell lysate (480 µl) to scintillation vial and 5 ml of biodegradable scintillation fluid (Cytoscint fluid). Cells lacking HSV1-TK expression exposed to ³H-Penciclovir serve as negative control.
9. The total remaining activity from each sample should be measured by using 480 µl of solution taken from the pooled medium and the wash solution, and also measured from 0.5 µl of ³H-Penciclovir diluted in 480 µl of medium.

10. Measure the radioactivity from different samples in a Scintillation counter by taking an average reading/min by reading for 5 min. Normalize the results with protein concentration and relate with total activity.
11. Results should be expressed as the percentage conversion of ^3H -PCV per milligram of protein/total count, or as the conversion of ^3H -PCV in moles per milligram of protein/total activity (Radioactivity within cells/[Radioactivity remained in medium + Radioactivity within cells]).

Acknowledgments

We thank Dr Sanjiv Sam Gambhir, Chairman, Department of Radiology, Stanford University for providing helpful support and facility for conducting this research. We thank the Department of Radiology, Stanford University for funding support (R. Paulmurguan), and thank the Canary Center at Stanford for providing the facilities. We also thank NIH-NCI RO1CA161091 (R. Paulmurugan) for partial funding support.

References

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