Extended Abstract

Mass Spectrometry 2017: Quantification of Temozolomide in Nonhuman Primate Fluids by Isocratic Ultra-High Performance Liquid Chromatography Tandem Mass Spectrometry to Study Brain Tissue Penetration Following Intranasal or Intravenous Delivery – Cody j Peer - National Cancer Institute NIH

Abstract

A sensitive and selective ultra-high performance liquid chromatography-Tandem mass spectrometric method was introduced for the quantification of temozolomide (TMZ) in nonhuman primate (NHP) plasma, cerebrospinal fluid (CSF), and brain extracellular fluid (ECF) - microdialysis. Ethyl acetate was to extract the plasma and CSF samples, using theophylline because the internal standard (IS). ECF samples were diluted with acetonitrile prior analysis. TMZ was separated on Waters UPLC® BEH C18 column with an isocratic mobile phase ammonium acetate (10 mM)-0.1% formic acid/ acetonitrile (30:70, v/v) during a positive-ion multiple reaction monitoring mode (m/z 195.5→137.6 for TMZ; m/z 181.5→124.2 for IS). And retention time of TMZ and theophylline has been 0.45 min with a complete time of two .5 min. The method was validated over the various range from 5–2000 ng/mL in NHP plasma, CSF, and ECF with regard to linearity, accuracy, precision, selectivity, and stability. This method was successfully applied towards the measurement of pharmacokinetic samples, various routes of drug administration.

Keywords: ultra-high performance liquid chromatography; tandem mass spectrometry; temozolomide; nonhuman primates

Introduction:

Temozolomide (TMZ) is a prodrug that is ultimately metabolized to an alkylating agent. Upon exposure to physiological pH, TMZ is spontaneously hydrolyzed to the intermediate 5-(3-methyl-triazen-1-yl) imidazole-4-carboxamide (MTIC). MTIC further fragments to 5-amino4-imidazolecarboxamide and the extremely reactive methyldiazonium cation, which methylates DNA at several nucleophilic sites. N7 -guanine, N3 -adenine, and O6 -guanine [1]. Although the formation of O6 -methylguanine accounts for a minority of DNA adducts formed, it is particularly cytotoxic and is correlated with TMZ-mediated cytotoxicity, resulting in mispairing during replication and subsequent strand breakage and tumor cell death. TMZ is FDA approved as a first-line

treatment for patients with newly diagnosed glioblastoma multiforme alongside radiotherapy and as a second-line treatment for refractory anaplastic astrocytoma . Although some drugs have shown modest efficacy, there is continued interest in studying the brain penetration of certain anti-glioma agents to improve therapeutic efficacy and clinical outcome. Intranasal delivery is one route being explored as a means to bypass the blood-brain barrier, as agents can undergo direct transport along the olfactory and trigeminal nerves. Studies are ongoing at the National Cancer Institute (NCI) using rhesus macaques as a nonhuman primate (NHP) model administering several agents in a variety of routes (intranasal, intravenous, oral) to understand the extent of drug penetration into different areas of the brain, and if intranasal administration improves brain penetration vs. systemic delivery. TMZ is being studied as one such agent, since existing research demonstrated relatively high brain penetration (20%–40%) Nonhuman primates are an advantageous model for studying the pharmacokinetics (PK) and brain penetration of neuro-oncology agents, as the pharmacology has been predictive of that in humans. As more researchers wade into this field, there is a lack of robust published LC-MS/MS assays for measuring TMZ in NHP fluids (plasma, CSF) as well as extracellular fluid (ECF) resulting from microdialysis (MD). MD is a technique that allows the opportunity to measure TMZ concentrations directly in the ECF of the tissue or tumor site sampled, as previously described [7]. It has previously been used to study if TMZ brain penetration is enhanced with bevacizumab [8] and cediranib in rats [9], but there is no published study for measuring TMZ from microdialysis in NHP. Several bioanalytical assays measuring TMZ have been published using LC-MS/MS instrumentation; however, these were all based on human or rodent biological matrices. Several additional studies have used less selective and sensitive instrumentation as well to study TMZ pharmacokinetics in rodents and humans. Only one study on TMZ pharmacokinetics used the NHP model, and plasma and CSF levels were measured using a less sensitive HPLC-UV assay (lower limit of quantification (LLOQ):19 ng/mL) [6]. The purpose of this report is to describe a sensitive LC-MS/MS assay that can be used to quantify TMZ concentrations in NHP plasma, CSF, and brain tissue or tumor ECF fluid in situ following MD.

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Cody j Peer
National Cancer Institute NIH
Email id: cody.peer@nih.gov

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