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Validation and implementation of a liquid chromatography/tandem mass spectrometry assay to quantitate dimethyl benzoylphenylurea (BPU) and its five metabolites in human plasma and urine for clinical pharmacology studies

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Abstract

A method has been developed for the quantitation of *N*-[4-(5-bromo-2-pyrimidinyloxy)-3-methylphenyl]-*N*′-(2-dimethylamino-benzoyl)urea (BPU) and its metabolites in human plasma and urine. BPU and metabolites were separated on a C18 column with acetonitrile–water mobile phase containing 0.1% formic acid using isocratic flow for 5 min. The analytes were monitored by tandem mass spectrometry. Calibration curves were generated over the range of 2.5–500 ng/mL for BPU, mmBPU, and aminoBPU in plasma; and 0.1–20, 0.1–20, 0.5–100, 10–2000, 1–200, and 3–600 ng/mL for BPU, mmBPU, aminoBPU, G280, G308, and G322 in urine, respectively. The method has been successfully applied to study the pharmacokinetics of BPU.

Keywords: Dimethyl benzoylphenylurea (BPU); LC/MS/MS; Pharmacokinetics

1. Introduction

Benzoylphenylureas were initially developed as insecticides [1,2]. *N*-[4-(5-Bromo-2-pyrimidinyloxy)-3-chlorophenyl]-*N*′-(2-nitrobenzoyl)urea (HO-221) was the lead benzoylphenylurea compound with noted antitumor activity but poor physico-chemical characteristics therefore limiting its potential clinical utility [2–4]. *N*-[4-(5-Bromo-2-pyrimidinyloxy)-3-methylphenyl]-*N*′-(2-dimethylamino-benzoyl)urea (BPU, NSC 639829, Fig. 1), an HO-221 analogue and poorly water-soluble benzoylphenylurea derivative, has reported cytotoxic activities [5]. The mechanism of action for benzoylphenylurea derivatives includes tubulin polymerization inhibition and microtubule depolymerization in vitro [4,6].

In murine pharmacokinetic studies, BPU was metabolized to monomethyl-BPU (mmBPU, Fig. 1) and didesmethyl-BPU (aminoBPU, NSC 647884, Fig. 1), which were shown to have in vitro cytotoxic activity similar to the parent compound with activity against murine P388 leukemia, human AIDS-related lymphoma, breast, and prostate carcinoma [4,6,7]. Bioavailability was low and variable for both a 5 mg (12–29%) and a 25 mg (4.4–26%) capsule in dogs [8]. The mechanism of action, oral formulation, and favorable anti-tumor activity in preclinical models lead to the clinical development of BPU as an anticancer agent.

BPU is currently being evaluated in phase I clinical trials in patients with refractory metastatic cancers with the drug being administered orally once weekly on a continuous schedule or for 6 out of 8 weeks [9,10]. Initially, BPU was quantitated using LC/MS/MS over the range of 0.05–10 ng/mL [11]. As dose escalation continued in the phase I clinical trial, a LC/UV method was developed to quantitate BPU in the range of 10 ng/mL–10 μg/mL [12]. Using the LC/UV and the LC/MS/MS methods, five BPU metabolites were identified in vivo in either urine or plasma from patient receiving oral BPU [11–13]. In order to comprehensively characterize the clinical

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pharmacology of BPU, a method for the quantitation of BPU and its metabolites in plasma and urine was necessary. BPU, mmBPU, and aminoBPU were quantitated in a clinically relevant range in plasma and urine, while BPU's three non-cytotoxic metabolites (G280, G308, and G322) were quantitated in urine. G280, G308, and G322 were not assessed in plasma since initial identification of these metabolites was limited to urine samples. The assay reported in this paper utilizes LC/MS/MS to achieve a rapid, sensitive, and specific method in plasma and urine of patients receiving BPU.

2. Experimental

2.1. Chemical and reagents

BPU (NSC 639829) and aminoBPU (NSC 647884) were a gift from the Developmental Therapeutics Program, Cancer Therapy Evaluation Program, National Institute of Health (Bethesda, MD, USA). mmBPU, G280, G308, and G322 were synthesized in the Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins Medicinal Chemistry Core (Baltimore, MD, USA). The internal standard, temazepam, was obtained from Sigma Chemical Co. (St. Louis, MO, USA). Formic acid (88%, v/v in water) was purchased from J.T. Baker (Phillipsburg, NJ, USA) and *n*-butyl chloride from Honeywell, Burdick & Jackson (Muskegon, MI, USA). Acetonitrile and methanol were HPLC Grade and were obtained from EM Science (Gibbstown, NJ, USA). Deionized water was obtained from a Milli-Q-UF system (Millipore, Milford, MA, USA) and used in all aqueous solutions. Drug-free (blank) human plasma originated from Pittsburgh Blood Plasma Inc. (Pittsburgh, PA, USA). Human urine was obtained from healthy volunteers that were willing to donate urine.

2.2. Preparation of stock solutions

Stock solutions of BPU, mmBPU, G280, G308, and G322 were prepared in duplicate at 0.1 mg/mL by dissolving 2 mg, accurately weighed, in 20 mL of methanol. The stock solution of aminoBPU was prepared in duplicate at 0.01 mg/mL by dissolving 2 mg, accurately weighed, in 200 mL of methanol. The area counts for each of the duplicated aliquots were checked in quintuplicate, and if the mean value for area counts was within 5%, the stock solutions were then stored in a glass vial at −20 °C. Stock solutions of BPU, mmBPU, aminoBPU, and G280 were stable for 3, 4, 4, and 4 months, respectively.

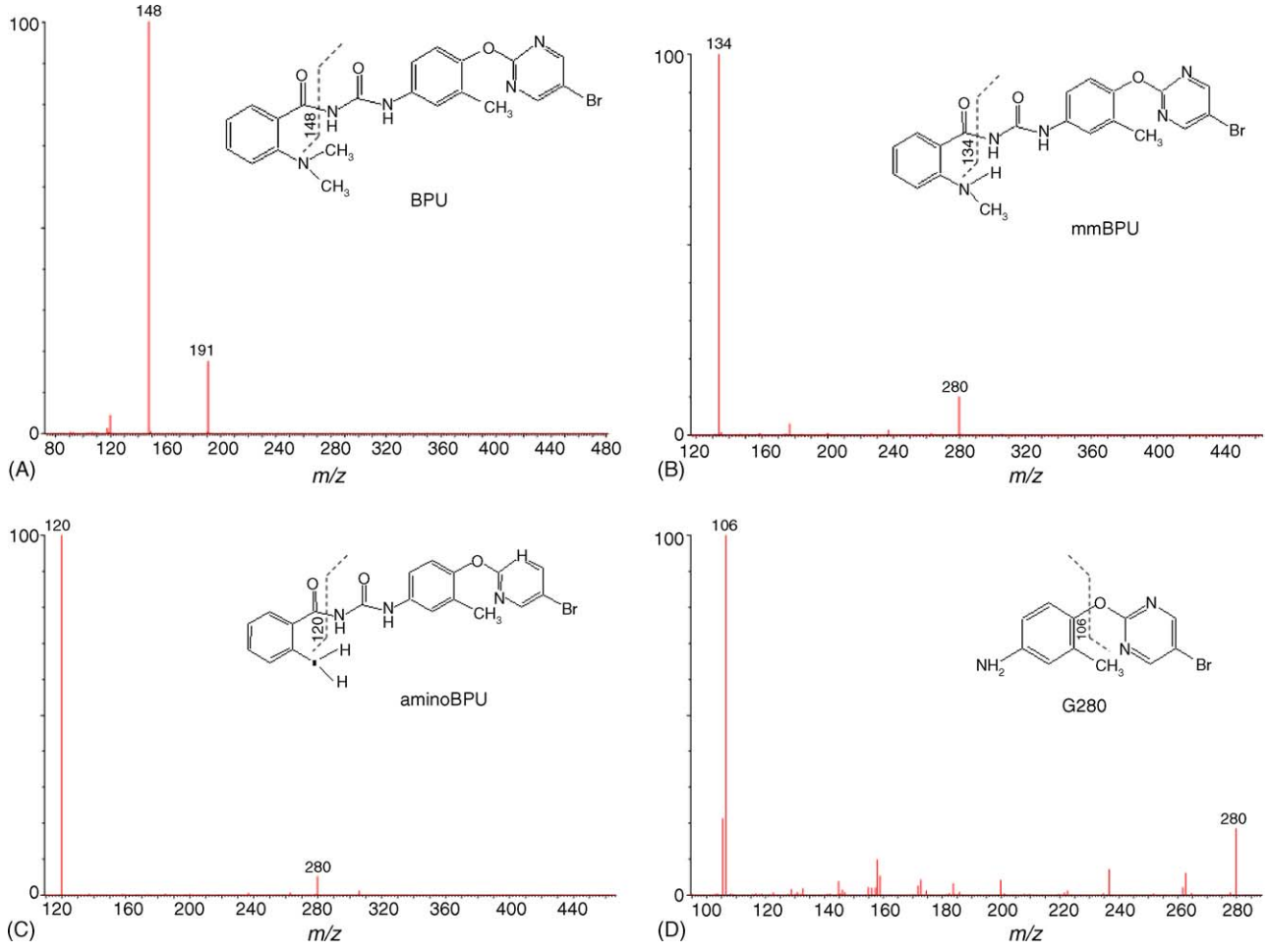

Fig. 1. Full-scan product ion spectrum and chemical structure for BPU (A), mmBPU (B), aminoBPU (C), G280 (D), G308 (E), G322 (F), and temazepam (G).