Disposition and Metabolism of Bruceantin in the Mouse*

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Summary. A microbiologic assay of the agar diffusion type, employing a strain of yeast tentatively identified as Candida macedoniensis, was developed to study the disposition, excretion, and metabolism of the antitumor agent bruceantin (BN; NSC 165563) in the mouse. Bioautographic studies showed the assay to be specific for BN. Normal and tumor-bearing male BDF1 mice were studied. Tumor-bearing mice were implanted sc with 10^6 L1210 ascites cells and held for 7 days prior to dosing. Average tumor weight was 307 mg and advanced generalized disease was evident. Three groups of 5 or 10 mice were injected iv with BN (1.5 mg/kg; approx. LD_{50}). At various times after injection, blood, tissues, urine, and feces were obtained and extracted with chloroform to recover BN. Recovery of BN was in the range of 91 to 121%. Disposition and excretion of BN were similar for normal and tumor-bearing mice. Decay of BN in blood was biphasic with \( \alpha \)-phase half-lives of 5 to 6 min. Estimated half-lives for the \( \beta \)-phase were possibly > 0.5 day. Average zero time intercepts for \( \alpha \)- and \( \beta \)-phases were 550 and 51 ng/ml respectively. Higher levels of BN were found in lung, pancreas, intestine, and spleen (1–6 \mu g/g) than in liver, kidney, and tumor (0.3–0.5 \mu g/g) after 15 min. Concentrations of BN in brain and peritoneal fat were below detectable limits (< 0.1 \mu g/g tissue). Urine and fecal excretion of BN accounted for < 2% of the dose after 24 h. In vitro metabolism studies using a postmitochondrial microsome fraction of liver, lung, and kidney suggest that bruceantin is inactivated by an NADPH-dependent enzyme present in liver but not in lung or kidney.

Introduction

Bruceantin (NSC 165563; Fig. 1) is a member of the quassinoid or simaroubolide group of compounds and was originally isolated from the stem bark of Brucea antidysenterica (J. F. Mill) (Kupchan et al., 1973, 1975). The drug has shown good activity against KB cells in vitro as well as against B16 melanoma, L1210 leukemia, and P388 leukemia in the mouse (Hartwell, 1976; Douros and Suffness, 1978). Bruceantin does not appear to have antibacterial activity and shows only weak activity (10–1000 \mu g/ml) against some strains of yeast (Suling, unpublished results). Studies of the mechanism of action of bruceantin suggest that the compound primarily inhibits initiation of protein synthesis in susceptible cells (Liao et al., 1976).

Preclinical toxicological studies of bruceantin in mice, dogs, and monkeys have been completed (Castles et al., 1976) and the drug is now in clinical trials.

The current study involved the disposition and excretion of bruceantin in both normal and solid L1210 tumor-bearing mice as well as the in vitro metabolism of the drug. Both normal and tumor-bearing mice were studied to compare levels of drug in the tumor relative to non-malignant tissues, and to determine possible effects of the malignancy on drug disposition. A sensitive and specific microbiological assay was developed and used to quantify levels of bruceantin in urine and tissues.

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Fig. 1. Structure of bruceantin
**Materials and Methods**

**Chemicals.** Bruceantin was obtained from the National Cancer Institute, Bethesda, Maryland. Thin-layer chromatography of the drug preparation, with three solvent systems, yielded a single migrating component after charring with 50% sulfuric acid. Glucose-6-phosphate (G-6-P) and nicotinamide adenine dinucleotide phosphate (NADP) were purchased from the Sigma Chemical Co., St. Louis, Missouri.

**Experimental Protocol.** Male BDF1 mice (20 g) were used throughout this study. Tumor-bearing mice were obtained by subcutaneous implantation of 10^6 L1210 ascites cells per mouse. Animals were held for seven days at which time the average tumor weight was 307 mg and advanced generalized disease was evident.

Normal and tumor-bearing mice were given single iv doses of bruceantin (1.5 mg/kg; approx. LD<sub>50</sub>) via the tail vein. The delivery vehicle was 3% ethanol and 0.05% Tween 80 in 0.9% saline. Control mice were injected with the vehicle alone. At various time intervals up to and including 60 min, three groups of 5 or 10 mice were anesthetized and blood was taken by cardiac puncture with heparinized syringes. The blood was pooled into tubes held in ice. Two groups of 10 mice were used for later time intervals. Tissues were excised, blotted on saline-saturated gauze pads, weighed, and homogenized syringes. The blood was pooled into tubes held in ice. Two groups of 10 mice were used for later time intervals. Tissues were excised, blotted on saline-saturated gauze pads, weighed, and homogenized in nine parts of cold 0.9% saline. Urine and feces were collected over a period of 24 h from mice that were housed in metabolism cages. The urine was collected into chilled tubes and all samples were stored at -20°C until they were extracted and assayed. Fecal material was homogenized in nine parts of saline prior to extraction.

Samples were extracted three times with equal volumes of chloroform. Emulsions were centrifuged in order to hasten the separation of layers. The lower chloroform layers from the three extractions were pooled, concentrated to about 0.5 ml using a Buchler Evaporator, quantitatively transferred to one dram glass vials, and evaporated to dryness under a stream of nitrogen. The dried extracts were then dissolved in 0.5 or 1 ml of chloroform and evaporated to dryness under a stream of nitrogen. The dried extracts were then dissolved in 0.5 or 1 ml of chloroform and assayed for bruceantin using the above standard curves.

**Microsomal Enzyme Preparation.** Liver, kidney, and lung were obtained from anesthetized male BDF1 mice (20–25 gm) that were first bled by cardiac puncture. The 9000 x g supernatant (S-9) of homogenates of the above organs was prepared as described by Aman et al., 1975.

**Microsomal Enzyme Assay Conditions.** The assay mix contained (per ml) 8 μmol MgCl<sub>2</sub>, 33 μmol KCl, 5 μmol G-6-P, 4 μmol NADP, 100 μmol phosphate buffer (pH 7.4), 10 μg bruceantin, and S-9 (0.1–0.3 ml). All components, except the S-9 fraction, were preincubated at 37°C for 10 min. The reaction was initiated by the addition of S-9 and incubation was continued for various time periods. Reactions were stopped by the addition of 2 ml of chloroform to 0.5 or 1 ml of mix. Each mixture was vortexed, centrifuged, and the chloroform layer transferred to a two-dram vial. A second chloroform extraction was done on the aqueous phase and the two extracts were pooled and evaporated to dryness under a stream of nitrogen. The dried extracts were then dissolved in 0.5 or 1 ml of chloroform and assayed for bruceantin.

**Bioautography.** Thin-layer chromatography of the chloroform extracts of homogenized tissues, urine, and feces was done on glass fiber sheets impregnated with polyacrylic acid gel (Gelman type SA) using chloroform/acetone (C:A; 9:1), chloroform/methanol (C:M; 95:5), benzene/methanol (B/M; 95:5), and hexane/ether (H/E; 4:1) solvent systems. After development, the chromatograms were air-dried and placed onto the surface of agar assay medium that had been seeded with CM1021 and solidified (1.5% agar) in Pyrex baking dishes. After 60 min, the chromatograms were removed from the agar plates and the plates were incubated at 37°C for 16 to 18 h. The position of bruceantin on the chromatograms was visualized as a distinct zone of growth inhibition on the assay plates. Also, chloroform solutions of bruceantin and chloroform extracts from the microsomal enzyme reaction studies were chromatographed as described and the components visualized by spraying with 50% sulfuric acid followed by charring at 130°C. Chromatograms were also sprayed with 5% ferric chloride in 95% ethanol (Kupchan et al., 1975) for the detection of bruceantin and possible metabolites.