Metabolism of the New \( \alpha-1_A \)-adrenergic Receptor Antagonist, Phthalimide-phenylpiperazine Analog (RWJ-69442), in Rat, Dog and Human Hepatic S9 Fractions, and in Rats

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SUMMARY

The in vitro and in vivo metabolism of RWJ-69442, an \( \alpha-1_A \)-adrenergic receptor antagonist, was investigated after incubation with rat, dog, and human hepatic S9 fractions in the presence of NADPH-generating system, and a single oral/iv dose administration to rats (oral: 100 mg/kg; iv: 10 mg/kg). Unchanged RWJ-69442 (~30% of the sample in vitro; ~47% of the sample in vivo) plus 14 metabolites were profiled, quantified and tentatively identified on the basis of API-MS and MS/MS data. The metabolic pathways for RWJ-69442 are proposed via the 4 steps: 1. phenyl!piperazinylhydroxylation, 2. N/O-dealkylation, 3. N-dephenylation, and 4. dehydration. Pathway 1 formed OH-phenyl-RWJ-69442 (M1, 4-32% in vitro & in vivo), and diOH-RWJ-69442 (M4, <1-4% in vitro & in vivo). Pathway 2 generated O-desisopropyl-RWJ-69442 (M2, <1-21% in vitro & in vivo), N-desmethyl-RWJ-69442 (M3, 2-3% in vitro & in vivo), N-desmethyl-M2 (M6, 1-8% in vitro & in vivo), and N-dealkylated RWJ-69442 (M9, <1-17% in vitro & in vivo), and in conjunction with pathway 1 produced 6 minor to major oxidized metabolites, OH-M2 (M5, 1-2% in vitro), OH-M9 (M10, <1-34% in vitro & in vivo), O-desisopropyl-M9 (M12, 3-21% in vivo), O-desisopropyl-M10 (M13, 2-12% in vivo), and dehydro-M13 (M14, 25% in vivo). Pathways 3 and 4 formed 2 minor metabolites, N-desphenyl-RWJ-69442 (M7, <1-12% in vitro & in vivo) and dehydrated-RWJ-69442 (M8, <1-2% in vitro), respectively. RWJ-69442 is extensively metabolized in vitro in the rat and human (except dog), and in vivo in the rat.

INTRODUCTION

The \( \alpha_-\)-adrenergic receptors (\( \alpha_-\)-AR), a family of G-protein coupled seven-transmembrane receptors, consist of three native \( \alpha_-\)-AR subtypes, \( \alpha_{1A}^-, \alpha_{1B}^-, \) and \( \alpha_{1D}^+, \) which have been characterized pharmacologically in tissues (1-4). It has been demonstrated that \( \alpha_{1A}^-\)-AR mediates the smooth muscle contraction in human prostate (1-4). Benign prostate hyperplasia (BPH) is the most common benign tumor in men. In a program to select and develop a potent and selective \( \alpha_{1A}^-\)-adrenergic receptor antagonists for the treatment of BPH, two novel series of compounds, pyridine-phenylpiperazines and phthalimide-phenylpiperazines, have been synthesized and evaluated (5-7). Tamsulosin, the first \( \alpha_{1A}^-\)-selective antagonist for the treatment of BPH is currently being marketed in the US and other nations (8-10). RWJ-69442 (Figure 1), a selected analog of phthalimide-phenylpiperazine series, is a new \( \alpha-1_A \)-AR antagonist for the further
pharmacological evaluation (6, 7). The in vitro metabolism of a pyridine-phenylpiperazine analog, RWJ-69597, and three phthalimide-phenylpiperazine analogs, RWJ-69205, RWJ-69442 and RWJ-69471 have been reported previously (11, 12). The objectives of the current study were to investigate the in vitro and in vivo metabolism of RWJ-69442 in rat, dog and human hepatic S9 fractions, and in rats, respectively. This results in profiling, quantifying and identification of unchanged RWJ-69442 and 14 metabolites.

**MATERIALS AND METHODS**

**Chemicals**

RWJ-69442, (S)-2-[4-(dimethylamino)phenyl]-2,3-dihydro-N-[2-hydroxy-3-[4-[2-(1-methylethoxy)-phenyl]-1-piperazinyl]propyl]-1,3-dioxo-1H-isoindole-5-carboxamide (Figure 1), was supplied by Johnson & Johnson Pharmaceutical Research & Development, L.L.C. (Raritan, NJ, USA) with a chemical purity >97% (assessed by HPLC, LC/MS). Diazald® for generating diazomethane was purchased from Sigma-Aldrich (Milwaukee, WI, USA). HPLC grade solvents were obtained from Fisher Scientific (Fair Lawn, NJ, USA), and glass-distilled solvents were purchased from Burdick and Jackson Laboratories (Muskegon, MI, USA). The incubation components for rat, dog, and human hepatic S9 fractions, Tris, potassium chloride, magnesium chloride, NADP+, and glucose-6-phosphate were purchased from Sigma-Aldrich (St. Louis, MO, USA). Glusulase®, a mixture of aryl sulfatase and β-glucuronidase (1:4, v/v) from *Helix pomatia* was purchased from New England Nuclear (Boston, MA, USA).

**Hepatic S9 fractions**

The human hepatic S9 fraction was purchased from Xenotech (Kansas City, KS, USA), and the rat and dog hepatic S9 fractions were obtained from In Vitro Tech.