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 (\approx 30\% of the sample in vitro; \leq 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. phenylpiperazinylhydroxylation, 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, \leq 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-M3 (M11, 4-6\% in vivo), 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 \( \alpha_1 \)-adrenergic receptors (\( \alpha_1 \)-AR), a family of G-protein coupled seven-transmembrane receptors, consist of three native \( \alpha_1 \)-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-[(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.