Evaluation of the inhibitory and induction potential of YM758, a novel If channel inhibitor, for human P450-mediated metabolism


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SUMMARY

This study was designed to examine the in vitro metabolism of YM758, a novel cardiovascular agent, and to evaluate its potential to cause drug interactions and induction of CYP isozymes. After incubation with pooled human liver microsomes, YM758 was converted to two major metabolites (AS2036313-00, and YM-394111 or YM-394112). The formation of AS2036313-00, and YM-394111 or YM-394112 were mediated by CYP2D6 and CYP3A4, respectively, which was elucidated by using a bank of human liver microsomes and recombinant CYP enzymes in combination with the utilization of typical substrates and inhibitors. The KI values of YM758 for midazolam, nifedipine, and metoprolol metabolism ranged from 59 to 340 μM, being much higher than the YM758 concentration in human plasma. The formation of AS2036313-00, and YM-394111 or YM-394112 was inhibited by quinidine and ketoconazole with KI values of 140 and 0.24 μM, respectively, which indicates that YM758 metabolism may be affected by co-administration of strong CYP2D6 and 3A4 inhibitors in vivo, given the clinical plasma concentrations of quinidine and ketoconazole. After human hepatocytes were exposed to 10 μM YM758, microsomal activity and mRNA level for CYP1A2 were not induced while those for CYP3A4 were slightly induced. The tested concentration was much higher than that in human plasma, which suggests that the induction potential of YM758 is also negligible.

INTRODUCTION

YM758 monophosphate, (-)-N-{2-[(R)-3-(6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline-2-carbo-nyl)pi­peridino]ethyl}-4-fluorobenzamide monophosphate is a novel funny If current channel (If channel) inhibitor that has a specific bradycardiac effect, and had been under development for the treatment of stable angina and atrial fibrillation by Astellas Pharma Inc. (Fig. 1). The If channel is expressed in the sinus node (1), and If channel inhibitors have been shown to act by reducing the heart's demand for oxygen and by increasing the diastolic period. It has been reported that, in clinical trials, the If channel inhibitors zatebradine and ivabradine reduce the heart rate without concomitant negative inotropic or hypotensive effects (1,2). In fact, ivabradine recently entered the market as a treatment for stable angina (1,2). The pharmacokinetic and excretion profiles of zatebradine in humans have already been investigated in a single administration study of 14C-zatebradine (3).
For YM758, the tissue distribution and excretion of radioactivity have already been investigated after a single oral administration of \(^{14}\text{C}-\text{YM758}\) to rats, with most of the radioactivity found to be distributed to the rat liver. The tissue-to-plasma concentration ratio of the radioactivity was calculated to be 43.5 at 0.5 h after dosing (4). In addition, after a single oral administration of \(^{14}\text{C}-\text{YM758}\) to bile-ducted cannulated rats, the urinary and biliary excretion of radioactivity were 17.8% and 57.3%, respectively (4). These findings imply that YM758 was distributed and eliminated mainly from the rat liver. In terms of the distribution of YM758 to the liver, uptake of the unchanged drug into the liver via organic anion transporting polypeptide (OATP)1B1 and excretion into the bile via P-glycoprotein (P-gp) in humans (5) were also investigated. However, the metabolic pathway of YM758 in the liver has not been investigated yet in rats or humans. Ivabradine, an \(I_{f}\) channel inhibitor like YM758, was mainly metabolized by CYP3A4, and thus was thought to carry a potential of pharmacokinetic interaction (6). Moreover, \(N\)-dealkylated ivabradine, S-18982, is known to be an active metabolite in humans due to the discrepancy in the times taken to reach the maximum plasma concentration of unchanged drug and to achieve the most potent heart rate reduction (7).

The present study was carried out to study the in vitro metabolism of YM758, including elucidation of the in vitro metabolites and characterization of drug-metabolizing enzymes involved in the formation of metabolites. In addition, experiments to evaluate the potential of YM758 to inhibit or induce human microsomal CYP enzymes were undertaken.

**MATERIALS AND METHODS**

**Chemicals**

YM758 monophosphate was synthesized at the Process Chemistry Laboratories of Astellas Pharma Inc. (Ibaraki, Japan). YM-202023 (an internal standard for YM758; Fig. 1) and the elucidated metabolites of YM758 (Fig. 3) were synthesized at Chemistry Laboratories of Astellas Pharma Inc. (Ibaraki, Japan). Pooled human liver microsomes and a bank of human liver microsomes (Reaction Phenotyping Kit, Version 5) were purchased from XenoTech LLC. (Kansas City, KS, USA), and recombinant human CYP/FMO enzymes from insect cells (Supersomes™) were from Gentest Corp. (Woburn, MA, USA). A bank of human liver microsomes was characterized for the following enzymatic activities: 7-ethoxyresorufin O-dealkylation (CYP1A2), coumarine 7-hydroxylation (CYP2A6), \(S\)-mephenytoin \(N\)-demethylation (CYP2B6), paclitaxel 6\(\alpha\)-hydroxylation (CYP2C8), diclofenac 4\(\beta\)-hydroxylation (CYP2C9), \(S\)-mephenytoin 4\(\beta\)-hydroxylation (CYP2C19), dextromethorphan \(O\)-demethylation (CYP2D6), chlorzoxazone 6-hydroxylation (CYP2E1), testosterone 6\(\beta\)-hydroxylation (CYP3A4/5), lauric acid 12-hydroxylation (CYP4A9/11), and benzydamine \(N\)-oxidation (FMO3) (8,9). Ketoconazole, metoprolol, quinidine, omeprazole, rifampin, phenacetin, and testosterone were obtained from Sigma-Aldrich (St Louis, MO, USA), and midazolam and nifedipine from Bufa B.V. (Uitgeest, The Netherlands). NADPH, glucose-6-phosphate, and glucose-6-phosphate dehydrogenase were purchased from Roche Diagnostics GmbH (Mannheim, Germany). All other chemicals and reagents were of analytical grade and purchased from commercial sources.

![Image of chemical structures](image_url)

**Fig. 1.** Chemical structure of YM758 monophosphate (a) and YM-202023 (b).

**In vitro metabolism of YM758**

**Metabolic reactions and analytical procedures**

Unless otherwise noted, individual (0.5 mg/mL; \(n=16\)) and pooled (0.5 mg/mL; mixed gender) liver microsomes or recombinant CYP/FMO enzymes were incubated at 37 °C with YM758 and 0.1 M potassium phosphate buffer containing 0.1 mM EDTA (pH 7.4) in the presence of an NADPH-generating system consisting of NADPH (0.5 mM), glucose-6-phosphate (5 mM), and glucose-6-phosphate dehydrogenase (1 U/mL), at a final incubation volume of 0.5 mL. Each experiment was performed in triplicate. The final concentrations of YM758 were set at 100 \(\mu\)M for reaction phenotyping experiments using indi-