Biochemical Pharmacology 81 (2011) 761–776

Contents lists available at ScienceDirect

Biochemical Pharmacology

journal homepage: www.elsevier.com/locate/biochempharm

In vitro liver metabolism of aclidinium bromide in preclinical animal species and humans: Identification of the human enzymes involved in its oxidative metabolism

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ARTICLE INFO

Article history:
Received 28 October 2010
Accepted 10 December 2010
Available online 22 December 2010

Keywords:
Aclidinium bromide
In vitro metabolism
Interspecies differences
Enzyme identification

ABSTRACT

The metabolism of aclidinium bromide, a novel long-acting antimuscarinic drug for the maintenance treatment of chronic obstructive pulmonary disorder, has been investigated in liver microsomes and hepatocytes of mice, rats, rabbits, dogs, and humans. Due to the rapid hydrolysis of this ester compound, two distinct radiolabeled forms of aclidinium were studied. The main biotransformation route of aclidinium was the hydrolytic cleavage of the ester moiety, resulting in the formation of the alcohol metabolite (M2, LAS34823) and carboxylic acid metabolite (m3, LAS34850), which mainly occurred non-enzymatically. By comparison, the oxidative metabolism was substantially lower and the metabolite profiles were similar across all five species examined. Aclidinium was metabolized oxidatively to four minor primary metabolites that were identified as monohydroxylated derivatives of aclidinium at the phenyl (M4) and glycolyl (m6 and m7) moieties of the molecule. The NADPH-dependent metabolite m4 involved the loss of one of the thiophene rings of aclidinium. Incubations with human recombinant P450 isoforms and inhibition studies with selective chemical inhibitors and antibodies of human P450 enzymes demonstrated that the oxidative metabolism of aclidinium is primarily mediated by CYP3A4 and CYP2D6. Additionally, up to eight secondary metabolites were also characterized, involving further hydrolysis, oxidation, or glucuronidation of the primary metabolites. Also, the liver oxidative metabolism of the alcohol metabolite (LAS34823) resulted in the production of one hydroxylated metabolite (M1) mediated by human CYP2D6, whereas the acid metabolite (LAS34850) was not metabolized enzymatically, although a minor non-enzymatic and NADPH-dependent reduction was observed.

1. Introduction

Aclidinium bromide (AB) (also known as 3R-(2-hydroxy-2,2-dithiophen-2-yl-acetoxy)-1-(3-phenoxy-propyl)1-azonia bicyclo [2.2.2] octane bromide) is a novel, long-acting muscarinic antagonist [1] undergoing Phase III clinical trials for the maintenance treatment of chronic obstructive pulmonary disorder. This ester compound displayed non-enzymatic hydrolysis of its ester bond at neutral and basic pH. Furthermore, AB was rapidly hydrolyzed in plasma of different animal species and humans to yield its alcohol (LAS34823) and carboxylic acid (LAS34850) metabolites [2,3]. The main human esterase involved in the enzymatic hydrolysis of aclidinium was identified as butyrylcholinesterase (BChE), which is found mainly in human plasma. Cytochrome P450-catalyzed ester cleavage was not observed in human liver microsomes [4].

In vitro incubations with liver microsomes and/or hepatocytes can be used to predict potential biotransformations in humans and in those animal species used for preclinical safety studies. Hepatocyte incubations retain Phase I and Phase II enzyme activities and are therefore useful in determining overall metabolism. They also mimic in vivo metabolism more accurately than incubations with subcellular fractions [5]. Previous data using diagnostic substrates have shown that P450 activities in rat, dog, monkey, and human hepatocyte suspensions are not significantly decreased by cryopreservation [6].

The objectives of this study were (a) to compare the in vitro metabolism of aclidinium in liver microsomes and hepatocytes of different preclinical animal species and humans; (b) to identify the oxidative metabolites; and (c) to identify and kinetically characterize the human enzymes responsible for the oxidative

Abbreviations: AB, aclidinium bromide; BChE, butyrylcholinesterase; CID, collision-induced dissociation; P450, cytochrome P450; ESI, electrospray ionization; FMO, flavin-containing monooxigenases; glyc, glycolyl; K_h, hydrolysis rate constant; LC, liquid chromatography; MRM, multiple reaction monitoring; MS, mass spectrometry; *m/z*, mass-to-charge ratio; phe, phenyl; SPE, solid-phase extraction; TEA, triethylamine.

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doi:10.1016/j.bcp.2010.12.013

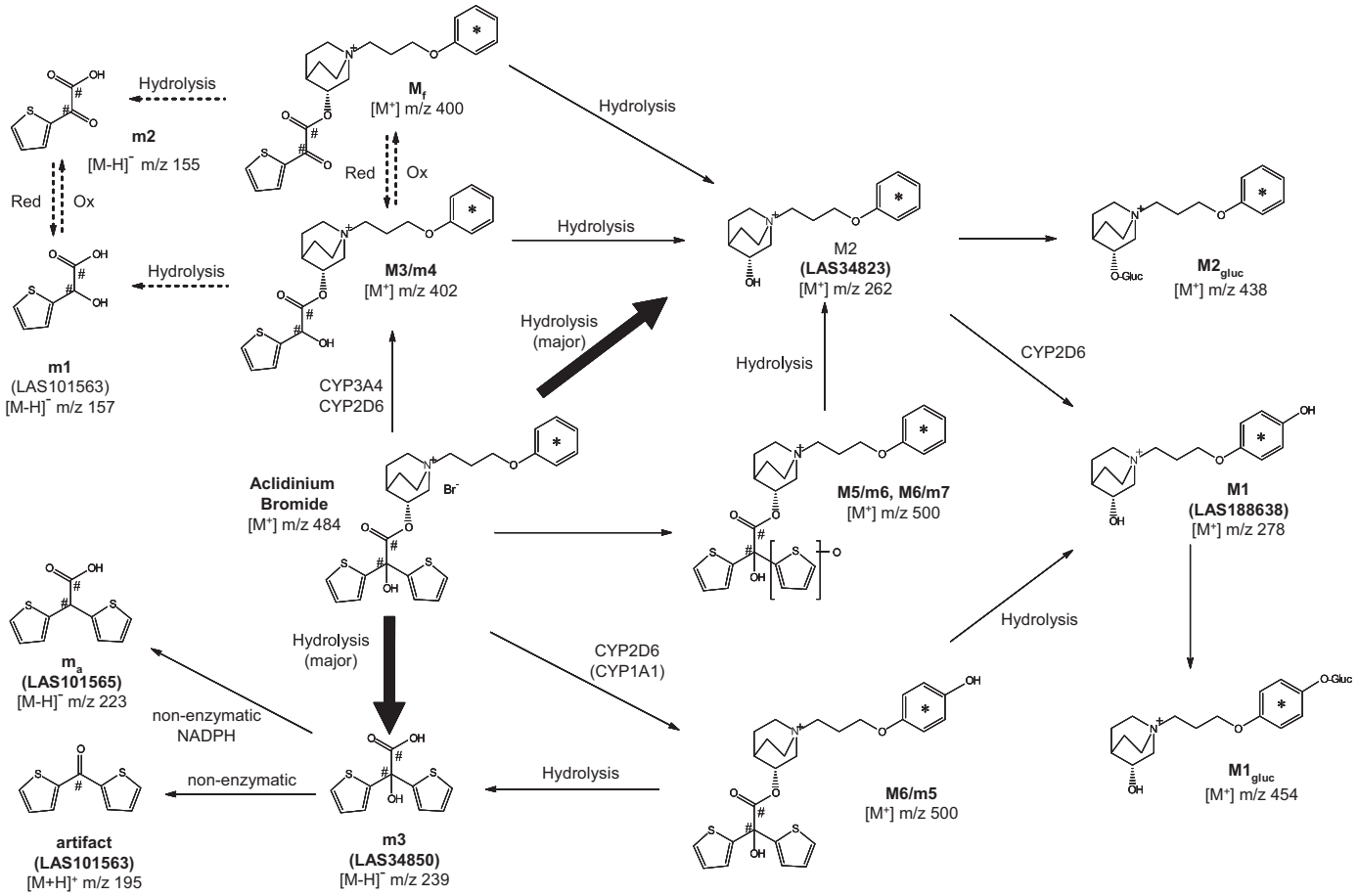

Fig. 1. Proposed in vitro metabolic pathways for aclidinium bromide in animal species and humans. Dashed arrows indicate possible paths of metabolite formation. Symbols * and # denote the positions of ^{14}C-labeled carbon atoms in ^{14}C-phe-AB and ^{14}C-glyc-AB, respectively. Metabolites generated from ^{14}C-phe-AB incubations were coded as "M", whereas metabolites generated from ^{14}C-glyc-AB incubations were coded as "m".

metabolism. Due to the hydrolysis mechanism, two distinct radiolabeled forms of aclidinium were prepared with the radioactive carbon-14 label incorporated into the phenyl or the glycolyl moieties of the molecule (Fig. 1). The phenotyping reaction of aclidinium and its hydrolysis metabolites was performed using human-expressed recombinant P450 isoforms and P450-specific chemical inhibitors and inhibitory antibodies.

2. Materials and methods

2.1. Chemicals

^{14}C-phenyl-AB (^{14}C-phe-AB, 30.2 mCi/mmol) and ^{14}C-glycolyl-AB (^{14}C-glyc-AB, 26.0 mCi/mmol) were synthesized at Quotient BioResearch Ltd. (Northamptonshire, UK). The radiolabeled hydrolysis products ^{14}C-LAS34823 (23.9 mCi/mmol) and ^{14}C-LAS34850 (24.4 mCi/mmol) were prepared by basic hydrolysis from ^{14}C-phe-AB and ^{14}C-glyc-AB, respectively, and further purification (Pharmacokinetics & Drug Metabolism Department, Almirall S.A., Barcelona, Spain). All radiolabeled compounds exhibited purity over 98% (determined by liquid chromatography [LC] with UV and radiometric detection). Stock solutions of 5 mM ^{14}C-phe-AB and 5 mM ^{14}C-glyc-AB were prepared in 0.1N HCl:acetonitrile (10:90, v/v). Stock solutions of 5 mM ^{14}C-LAS34823 and 10 mM ^{14}C-LAS34850 were prepared in aqueous basic solution containing 20% acetonitrile. Non-radiolabeled aclidinium (>99% purity), its alcohol metabolite (LAS34823; [3(R)-hydroxy-1-(3-phenoxy-propyl)-1-azonia-bicyclo[2.2.2]octane, bromide]) and its carboxylic acid metabolite (LAS34850; [dithienyl-glycolic acid, sodium salt]), LAS188638 and LAS101563 standards were synthesized at Ranke Química S.L. (Barcelona, Spain). Working solutions of ^{14}C-phe-AB and ^{14}C-glyc-AB were prepared daily before use in 0.01N HCl:acetonitrile (80:20, v/v) to prevent aclidinium hydrolysis. LC-grade methanol, acetonitrile, dimethylsulphoxide (DMSO), and triethylamine (TEA) were obtained from Scharlab S.L. (Barcelona, Spain). Glucose-6-phosphate, $NADP^+$, glucose-6-phosphate dehydrogenase, 2-thiopheneglyoxylic acid, dithienyl-2-ketone, and Krebs–Henseleit medium with D-glucose were purchased from Sigma–Aldrich (Steinheim, Germany). Williams' E medium and other chemicals used in hepatocyte incubations were purchased from Gibco Invitrogen (Paisley, UK). All other chemicals used were purchased from Sigma–Aldrich (Steinheim, Germany).

2.2. Biological materials

CD-1 mouse (male and female), Sprague–Dawley rat (male and female), New Zealand white rabbit (female), Beagle dog (male), and human (mixed gender, $n = 50$ donors) liver microsomes were purchased from XenoTech (Lenexa, KS, USA). CD-1 mouse (male), Wistar rat (male), New Zealand white rabbit (female) and human (mixed gender, $n = 10$ donors) cryopreserved hepatocytes were purchased from Celsis International (Chicago, USA). Cryopreserved Beagle dog (male) hepatocytes were purchased from XenoTech. Recombinant human P450 (CYP1A1, 1A2, 2C8, 2C9*1, 2C19*1, 2A6, 2B6, 2D6*1, 2E1, 3A4, 3A5, 4A11, 2F2, 4F3A, 4F3B) and flavin-containing monooxigenases (FMO1, FMO3, and FMO5) expressed in microsomes of baculovirus-infected cells (Supersomes™) were