Metabolism and Disposition of Fluticasone Furoate, an Enhanced-Affinity Glucocorticoid, in Humans

Stephen C. Hughes, Peter C. Shardlow, Frank J. Hollis, Rebecca J. Scott, Dimple S. Motivaras, Ann Allen, and Victoria M. Rousell

Division of Drug Metabolism and Pharmacokinetics, GlaxoSmithKline R&D, Ware, Hertfordshire, United Kingdom (S.C.H., P.C.S., F.J.H., R.J.S., D.S.M.); and Clinical Pharmacology, GlaxoSmithKline R&D, Greenford, Middlesex, United Kingdom (A.A., V.M.R.)

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ABSTRACT:
The purpose of this study was to investigate the metabolism and disposition of fluticasone furoate, an enhanced-affinity glucocorticoid receptor agonist, in humans. In a two-part, open-label design study, five healthy male subjects received a p.o. dose of 2 mg of [14C]fluticasone furoate, followed 4 weeks later by an i.v. dose of 0.25 mg of [14C]fluticasone furoate (as a 30-min infusion). Oral absorption was rapid and estimated at approximately 30%, although the oral bioavailability was markedly lower at 1.6%, limited by extensive first-pass metabolism. Plasma clearance was 58.3 l/h, with a volume of distribution of 642 liters, and a terminal elimination half-life of 15.3 h. The major circulating component identified in plasma extracts after i.v. and p.o. dosing was unchanged parent compound, with 17β-carboxylic acid (GW6949301X; M10) also being notable after p.o. administration. Mean recovery of radioactivity was approximately 92 and 102% at 216 and 168 h after i.v. and p.o. administration, respectively, with most (at least 90%) recovered in the feces. Fluticasone furoate was extensively metabolized, with only trace amounts of unchanged parent compound observed in feces following either route of administration. The predominant pathway was removal of the 5-fluoromethyl carboxylic acid group to yield GW694301X (M10). Other pathways included oxidative de-fluorination to yield a hydroxyl at the C6 position. There was no evidence for metabolic loss of the furoate group from fluticasone furoate or any of its metabolites. Evidence presented suggests that enterocytes have a role in the metabolism of unabsorbed fluticasone furoate.

Fluticasone furoate [(6α,11β,16α,17α)-6,9-difluoro-17-[(fluoromethyl)-thio]carbonyl]11-hydroxy-16-methyl-3-oxoandrosta-1,4-dien-17-yl-2-furancarboxylate] is a new enhanced-affinity glucocorticoid receptor agonist. It is a synthetic fluorinated corticosteroid that has been developed as an intranasal treatment for patients with symptoms of rhinitis. Fluticasone furoate, otherwise known as GW685698X, is not a salt or prodrug because the entire molecule is required for pharmacological activity. It has similar or greater potency than other clinically used corticosteroids (including mometasone furoate, budesonide, fluticasone propionate, and the active principle of ciclesonide) for the glucocorticoid receptor and against the proinflammatory transcription factors nuclear factor κB (NF-κB), activation protein-1, and to activate the glucocorticoid response element transactivation pathway (Rhen and Cidlowski, 2005). Inhibition of the NF-κB pathway, in particular, is thought to be intimately involved in the anti-inflammatory activity of glucocorticoids because it is a key pathway in the synthesis of a number of inflammatory cytokines (Karim et al., 2004). It is well documented that topical glucocorticoids interact with many of the inflammatory pathways, and there is a large body of clinical evidence to support their use for the treatment of rhinitis, asthma, and chronic obstructive pulmonary disease (Goodman and Gilman, 2006). The purpose of the present study was to investigate the metabolism and disposition of [14C]fluticasone furoate after p.o. and i.v. administration to healthy male subjects. These dose routes were used as surrogates for the intranasal and inhalation routes, from which the majority of the dose is likely to be swallowed. The p.o. route acted as a surrogate for the portion of an intranasal or inhaled dose that is swallowed, and the i.v. route represented the portion absorbed locally into the systemic circulation.

Materials and Methods

Materials. Fluticasone furoate, [14C]fluticasone furoate (see Fig. 1), and GW694301X, GSK728920A, GSK728921A, and GSK728922A (authentic chemicals of possible metabolites of fluticasone furoate) were supplied by Chemical Development, GlaxoSmithKline R&D (Stevenage, UK). All of the

ABBREVIATIONS: NF-κB, nuclear factor κB; GW694301X, M10, 17β-carboxylic acid; AMS, accelerator mass spectrometry; HPLC, high-performance liquid chromatography; AUC, area under the curve.
other solvents and reagents were of analytical grade and were purchased from commercial suppliers.

**Formulated Drug.** \(^{14}C\)Fluticasone furoate and fluticasone furoate were supplied to the study center by Pharmaceutical Development, GlaxoSmithKline (Ware, UK) as sterile solutions in propylene glycol. The solutions were at a concentration of 0.25 mg/ml and with a specific activity of 100 \(\mu\)Ci/mg. For the p.o. dose, the radiolabeled and nonradiolabeled solutions were mixed in a ratio of 1:1.67 to give a dose of 8-ml volume, equivalent to 2 mg of fluticasone furoate and approximately 75 \(\mu\)Ci.

**Subjects and in Vivo Study Design.** The clinical part of the study was conducted at Charles River Laboratories (formerly known as Inveresk) Edinburgh, UK, in accordance with Good Clinical Practice guidelines and the Declaration of Helsinki. All the subjects provided written informed consent before participation. The protocol was approved by the investigational center ethics committee and by the Administration of Radioactive Substances Advisory Committee, United Kingdom.

Five healthy male Caucasian subjects, aged 50 to 56 years and with a body mass index within the range of 19 to 30 kg/m\(^2\), were enrolled into this study. The study design was an open-label two-period crossover, with a p.o. administration followed by i.v. administration. The subjects were in good health as shown by medical examination, clinical chemistry, hematology, and urinalysis. The subjects were nonsmokers with no history of drug or alcohol abuse, who were taking no other medication at the time of the study, and had taken no prescribed medication within 14 days of the study commencing.

All the subjects received a single p.o. dose of \(^{14}C\)fluticasone furoate at 2 mg (3 MBq, 75 \(\mu\)Ci) and a dose volume of 8 ml. On a separate occasion, each subject received an i.v. infusion of 0.25 mg of \(^{14}C\)fluticasone furoate (1 MBq, 25 \(\mu\)Ci) over 30 min using a dose volume of 1 ml. The two dosing occasions were separated by at least 28 days. Blood samples were collected via an indwelling cannula or by direct venipuncture into lithium heparin-containing polypropylene tubes.

Following p.o. dosing, blood samples (10 ml) were collected at 10, 15, 30, and 45 min and 1, 1.5, 2, 3, 5, 9, 12, 24, 48, 72, 96, and 168 h after dosing, with larger samples (30 ml) for metabolic investigations being collected at 0.5, 2, and 12 h. After the start of the i.v. infusion, samples (10 ml) were collected at 15, 30, 32, 35, and 45 min and 1, 1.5, 2, 3, 4, 6, 9, 12, 24, 48, 72, 96, and 168 h after dosing, with the larger samples being collected at 0.75, 1.5, and 12 h. The samples were processed within 1 h of collection by centrifugation for 10 min at 1500 g at nominally 4°C to yield plasma, which was then stored frozen at nominally ~20°C or processed for the determination of radioactivity levels in plasma. Urine samples were collected at 0 to 6, 6 to 12, and 12 to 24 h and then at 24-h intervals up to at least 168 h. Fecal samples were collected over 24-h periods up to at least 168 h postdose. Both urine and fecal collections continued beyond the 168-h time point following i.v. administration until 90% of the total radioactive dose administered was recovered from all the matrices, or less than 1% of the radioactive dose had been excreted over a 24-h period.

The fecal and urine samples were stored frozen at nominally ~20°C before analysis.

**Radioassay of Samples.** After measurement of the total sample volume or weight (as appropriate) of excreta, the levels of radioactivity in samples were determined by liquid scintillation counting (Beckman LS series, Bucks, UK or PerkinElmer Life and Analytical Sciences, Beaconsfield, Bucks, UK) with quench correction performed by an automatic external standard ratio method, which was established using sealed \(^{14}C\) standards. Aliquots of liquid samples (e.g., urine, dose dilutions) or extracts of samples were mixed with scintillation fluid. Fecal samples were homogenized with an appropriate amount of water. Aliquots of homogenized fecal material were combusted using a model 307 oxidizer (PerkinElmer Life and Analytical Sciences, Waltham, MA) before radioassay by scintillation counting. The plasma had low levels of radioactivity; therefore, these samples were diluted appropriately with water, and the level of radioactivity was determined using accelerator mass spectrometry (AMS).

**Determination of Radiochemical Purity.** The radiochemical purity of \(^{14}C\)fluticasone furoate was confirmed by high-performance liquid chromatography (HPLC). The chromatographic instrument used consisted of an HP1100 series quaternary pump, column oven (50°C), UV detector (A 245 nm), and autosampler using a Zorbas SB-C8 column (150 × 4.6 mm, 3.5-\(\mu\)m particle size). The mobile phase consisted of aqueous 0.1% trifluoroacetic acid (solvent A) and a mixture of acetonitrile/methanol (75:25, solvent B) at a flow rate of 1.5 ml/min. A gradient was used, starting at 60% A with a linear change to 80% B over 30 min. The column was re-equilibrated following each injection. Using on-line radiodetection, the column eluate was combined with scintillant (Ultima-Flu M supplied by Fluorochem, Old Glossop, Derbyshire, UK; 4.5 ml/min) before detection using a Radiomatic Flo-one 150TR radioactivity monitor (PerkinElmer Life and Analytical Sciences). Dose analysis showed that all the p.o. and i.v. doses had a radiochemical purity of 100%.

**Quantification of Fluticasone Furoate and GW694301X in Plasma.** Concentrations of fluticasone furoate and GW694301X (M10) in plasma samples were determined using a validated analytical method using in-line solid-phase extraction, followed by HPLC/tandem mass spectrometry. The validation of this method was performed as detailed for the quantification of fluticasone furoate and GW694301X (Scott et al., 2007) using similar methodology.

\[^{13}C\sub{3}JCC\sub{118781} (fluticasone propionate) and GW819063 (10 \(\mu\)l in 20% acetonitrile in water with both at a concentration of 10 ng/ml) were added to plasma samples (0.15 ml) as internal standards. An aliquot of each sample (0.1 ml) was applied to conditioned Prospect C2 cartridges (10 × 1 mm), which were washed with 5% aqueous methanol containing 0.025% formic acid (2 ml) before being eluted in-line for 30 s onto the analytical column. The cartridge eluant was applied to a HPurity C18 column (100 × 4.6 mm, 5 \(\mu\)m; Thermo Fisher Scientific, Runcorn, Cheshire, UK) eluted at a flow rate of 0.5 ml/min using a linear gradient from 100% solvent A to 100% solvent B over 4.3 min, held until 15 min before returning to 100% solvent A by 15 min. Solvent A was a mixture of methanol and 10 mM ammonium formate (pH 5.7, 75:25, \(\nu/\nu\)), and solvent B was acetonitrile. The column eluant was introduced into a TurboIonSpray source of a Sciex API 4000 mass spectrometer (Applied Biosystems, Warrington, Cheshire, UK) operated in the most sensitive ionization mode initially for 4.4 min before being switched to positive ion mode. The determination of Metabolite Profiles. Representative fecal samples from each subject were obtained by pooling across sampling times on a total sample weight basis to generate sample pools where possible containing 90% or greater of the radioactivity excreted in the feces. Plasma samples from individual subjects were pooled using equal volumes to produce a single representative sample per time point. No further analysis of urine samples was conducted because of the
limited amount of radioactive drug-related material in urine. Radioactive material was extracted from plasma samples using acetonitrile (5 ml/ml sample, rotary mixed at ambient temperature for approximately 1 h), three times with the supernatants being combined. The extracts were then evaporated to near dryness under a stream of nitrogen before being reconstituted in deionized water (0.5 or 1 ml). Radioactive material was extracted from fecal samples using alkaline and acidified methanol as described for plasma, and the reconstituted extracts from each extraction method were combined using equal volumes before analysis by radio-HPLC. Radioisotopic profiles were determined by analysis of appropriate aliquots of plasma and fecal extracts by radio-HPLC using on-line or off-line radiodetection. HPLC method 1 (used for analysis of all the matrices). The chromatographic instrument used consisted of an Agilent (South Queensferry, Scotland, UK) 1100 binary pump, column oven (40°C), UV detector (λ 245 nm), and autosampler (CTC Analytics or LC PAL injector, both Presearch, Basingstoke, Hampshire, UK) using a ThermoHypersil Fluorophase RP column (250 x 4.6 mm, 5-µm particle size). The mobile phase consisted of 50 mM ammonium acetate (BDH; Poole, Dorset, UK) (pH unadjusted) (solvent A) and acetonitrile (solvent B, supplied by Thermo Fisher Scientific, Loughborough, Leicestershire, UK) at a flow rate of 1 ml/min. A gradient was used, starting at 100% A with a linear change to 25% B over 30 min, followed by a linear increase to 40% B by 40 min and then a further increase to 100% by 45 min, with these conditions being held for a further 5 min. The column was re-equilibrated after each injection. Using on-line radiodetection, the column eluate was combined with scintillant (FlowLogic MaxCount supplied by Lablogic, Sheffield, UK; 3 ml/min) before detection using a β-Ram (Lablogic) radioactivity monitor. For off-line radiodetection, fractions were collected using a Gilson 222XL fraction collector (supplied by Gilson, Villiers, France) on 4 x 96 Deepwell LumPlates containing yttrium silicate solid scintillant (PerkinElmer Life and Analytical Sciences) or into standard 96-well plates. Radioactivity determination was performed either by scintillation counting (TopCount MXT counter; PerkinElmer Life and Analytical Sciences) or by AMS analysis of the fractions. HPLC column recoveries were determined on selected samples by collecting the total HPLC column eluate for the appropriate run and assaying the radioactivity to assess recovery of injected radioactive material. Full recoveries of radioactivity were obtained from the HPLC eluant collected.

HPLC method 2 (used for analysis of human plasma samples). The chromatographic instrument is as listed for method 1, with the exception of a different column being used: a ThermoHypersil Gold column (250 x 4.6 mm, 5-µm particle size; Thermo Fisher Scientific, Waltham, MA). The mobile phase consisted of 50 mM ammonium acetate, pH 5, adjusted using acetic acid (supplied by Thermo Fisher Scientific) (solvent A) and acetonitrile (solvent B) at a flow rate of 1 ml/min. A gradient was used, starting at 25% B with a linear increase to 40% B over 8 min, followed by a linear increase to 45% B by 12 min and then a further increase to 100% by 40 min, with these conditions being held for a further 5 min. The column was re-equilibrated after each injection. Using on-line radiodetection, the column eluate was combined with scintillant (FlowLogic MaxCount supplied by Lablogic, Sheffield, UK; 3 ml/min) before detection using a β-Ram (Lablogic) radioactivity monitor. For off-line radiodetection, fractions were collected using a Gilson 222XL fraction collector (supplied by Gilson, Villiers, France) on 4 x 96 Deepwell LumPlates containing yttrium silicate solid scintillant (PerkinElmer Life and Analytical Sciences) or into standard 96-well plates. Radioactivity determination was performed either by scintillation counting (TopCount MXT counter; PerkinElmer Life and Analytical Sciences) or by AMS analysis of the fractions. HPLC column recoveries were determined on selected samples by collecting the total HPLC column eluate for the appropriate run and assaying the radioactivity to assess recovery of injected radioactive material. Full recoveries of radioactivity were obtained from the HPLC eluant collected.

Identification of Metabolites. Structural characterization was performed on selected samples using HPLC/mass spectrometry using Thermo Fisher Scientific and Deca XPplus ion trap mass spectrometers. Electrospray ionization, in positive or negative modes, was used. The HPLC flow was split (1:25–1:50) between the mass spectrometer and on-line or off-line radiodetector. Metabolites of fluticasone furoate were identified based on charged molecular ions and their collision-induced dissociation fragmentation. Authentic standards, where available, were used to compare chromatographic retention time to metabolites that were structurally characterized. Supporting data obtained from metabolites identified from preclinical species or from in vitro incubations with human-derived tissue preparations were also used in the assignment of metabolite structures.

AMS. The 14C content of human plasma, plasma extracts, and HPLC fractions was measured by AMS, which measures the radiocarbon content in a sample through separation of the isotopes of carbon present by their different mass-to-charge ratios. Before AMS analysis, the samples were graphitized via a two-step process of oxidation and reduction (Vogel, 1992). The graphite, containing a cobalt catalyst, was packed into an aluminum cathode and loaded into a sample wheel that was then placed into the ion source of the AMS instrument (NEC 15SDH-2 Pelletron AMS system, Middleton, WI). A generic value of 4.14%, based on historical data, for the carbon content of plasma was used for any neat plasma samples analyzed, and values were adjusted appropriately to allow for any dilution. The carbon content for the HPLC fractions was determined to be insignificant, and only the carbon content of the liquid paraffin carrier (84.17%) was used in calculations to determine the 14C content based on the determined 14C/12C ratio in the samples. The AMS data, which are expressed as percent modern carbon, were used to calculate the disintegration per minute per milliliter of sample, where 100% modern carbon equals 0.01356 dpm/mg carbon.

Pharmacokinetic Calculations. Pharmacokinetic parameters were calculated by noncompartmental methods using WinNonlin Professional version 4.1 (Pharsight, Mountain View, CA). Area under the curve, AUC(0–t), was calculated using the log-linear trapezoidal method and extrapolated to infinity according to AUC(0–t) = C0 t0 + AUC(0–∞), where C0 is the last observed concentration. The t1/2 values were obtained by the ratio of ln 2/λ, where λ is the terminal phase rate constant estimated by linear regression analysis of the log-transformed concentration-time data during the terminal elimination phase. Mean residence time was calculated based on the last measurable concentration. Plasma clearance and volume of distribution at steady state were calculated following i.v. administration only. Oral bioavailability was calculated using the dose-normalized AUC(0–t) p.o./dose-normalized AUC(0–t) i.v.

Results

Demographic, Safety, and Tolerability Data from Humans. Five healthy male Caucasian subjects were enrolled and completed the study. The subjects were of a mean age of 54 years (range 50–56 years), a height of 169 cm (range 163–181 cm), and weight of 79.2 kg (range 71.2–93.0 kg) with a mean body mass index of 27.6 (range 25.1–30.1). Fluticasone furoate was well tolerated, with no drug-related adverse events or clinically significant changes in vital signs observed. No concomitant medication was reported for any subject during the study.

Pharmacokinetics of Fluticasone Furoate and GW694301X. Pharmacokinetic data for fluticasone furoate, its carboxylic acid metabolite (GW694301X, M10), and total radioactivity are listed in Table 1. After p.o. administration of [14C]fluticasone furoate at a dose of 2 mg, maximum mean concentrations (Cmax) of fluticasone furoate occurred within 30 min (tmax) of administration, indicating rapid oral absorption of the dose. Concentrations declined rapidly thereafter, such that they were below the limit of quantification beyond 9 h (Fig. 2). After a 30-min i.v. infusion of [14C]fluticasone furoate at a dose of 0.25 mg, plasma clearance was 58.3 l/h with a mean volume of distribution at steady state of 642 liters. In contrast to post-p.o. administration, concentrations of fluticasone furoate were measurable up to 24 h postinfusion, and mean terminal half-life was estimated at 15.3 h. Mean concentrations of GW694301X peaked at 0.75 h post-p.o. dose of fluticasone furoate and declined rapidly thereafter, with a systemic exposure (based on either AUC(0–t) or AUC(0–t)) approximately 5- to 9-fold lower than for fluticasone furoate. No GW694301X was quantifiable in plasma after i.v. administration of fluticasone furoate. Comparison of the AUC(0–t) values for radioactive activity following p.o. and i.v. administration (normalizing for dose) indicates that oral absorption was approximately 30% (range 18–37%). The mean bioavailability of fluticasone furoate from the oral solution (comparing p.o. and i.v. AUC(0–t) values for fluticasone furoate and normalizing for dose) was considerably lower at 1.6% (range 0.5–2.8%). The AUC(0–t) for total radioactivity in plasma was approximately 36- and 5-fold higher than that for fluticasone furoate following p.o. or i.v. administration, respectively. Furthermore, the mean half-life of total radioactivity (at 35.6 h) was notably longer than that of fluticasone furoate (15.3 h) after i.v. dosing.

Excretion of [14C]Fluticasone Furoate Drug-Related Material. The mean total recovery of radioactive drug-related material in excreta collected after i.v. administration of [14C]fluticasone furoate to

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Pharmacokinetic parameters in humans for fluticasone furoate, GW694301X, and 14C radioactivity in plasma

Mean pharmacokinetic parameters of fluticasone furoate, a carboxylic acid metabolite GW694301X, and 14C radioactivity in plasma of healthy male subjects following a single p.o. (2 mg) or a single i.v. (0.25 mg) administration of 14C-fluticasone furoate are shown. Mean ± S.D., n = 5.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Parameter</th>
<th>Fluticasone Furoate</th>
<th>GW694301X</th>
<th>Radioactivitya</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AUC0-t (pg · h/ml)</td>
<td>511 ± 358</td>
<td>102 ± 61</td>
<td>26,400 ± 14,400</td>
</tr>
<tr>
<td>2 mg p.o.</td>
<td>AUCt-inf (pg · h/ml)</td>
<td>1080b</td>
<td>120 ± 59</td>
<td>39,300 ± 11,700</td>
</tr>
<tr>
<td></td>
<td>Cmax (pg/ml)</td>
<td>213 ± 177</td>
<td>71 ± 42</td>
<td>1380 ± 239</td>
</tr>
<tr>
<td></td>
<td>Tmax (h)</td>
<td>0.50</td>
<td>0.75</td>
<td>0.75</td>
</tr>
<tr>
<td></td>
<td>t1/2 (h)</td>
<td>15.3 ± 2.74</td>
<td>N.D.</td>
<td>35.6 ± 33.3c</td>
</tr>
<tr>
<td></td>
<td>CI (l/h)</td>
<td>58.3 ± 10.9</td>
<td>N.D.</td>
<td>N.A.</td>
</tr>
<tr>
<td></td>
<td>Vss (liter)</td>
<td>642 ± 215</td>
<td>N.D.</td>
<td>N.A.</td>
</tr>
</tbody>
</table>

N.D., not determined, all the concentrations below lower limit of quantification (10 pg/ml); N.A., not applicable.

aRadioactivity concentrations are expressed in terms of picogram equivalents of fluticasone furoate.
bT1/2 of 14C-fluticasone furoate was 8 h in human plasma.

Humans was high (92%) and was complete post-p.o. administration, following collections up to 240 and 168 h, respectively (Table 2). Most of the dose was excreted in the feces for both routes of administration; urinary excretion was a very minor route (<3%). Radioactivity was eliminated rapidly: most was excreted within the first 72 h postdose, with approximately 71 and 91% of the dose recovered over that period following i.v. and p.o. administration, respectively.

Metabolites of 14C-Fluticasone Furoate in Plasma. Proposed structure and spectral data supporting metabolite identification are shown in Table 3. Radioactive drug-related material in plasma is expressed as percentage of sample radioactivity. Radioactive drug-related material in feces is expressed as percentage of administered dose. Where the sample preparation step resulted in some loss of radioactivity, if the recovery was <90%, the chromatogram data have been multiplied by the percentage recovered to calculate percentage of sample radioactivity and percentage of dose.

Metabolites of 14C-Fluticasone Furoate in Plasma. It was established that 14C-fluticasone furoate spiked into plasma was stable when stored under the same conditions as the test samples. Low levels of radioactivity were present in the human plasma extracts; therefore, the profiling of these samples was performed using AMS as the radioactivity detection method. Representative reconstructed HPLC}

{\text{radiochromatograms of plasma extracts following i.v. and p.o. administration, at selected sampling times, are shown in Fig. 3. The extraction efficiency of radioactivity from plasma was in excess of 70% at early time points but notably lower (9–35%) at later time points.}}

The principal radiolabeled component in plasma following a single i.v. infusion of 14C-fluticasone furoate was unchanged fluticasone furoate (P), which accounted for at least 52% of radioactive material in plasma samples up to 1.5 h after dosing. The 17β-carboxylic acid metabolite (GW694301X, M10) of fluticasone furoate was assigned based on retention time and represented up to 4% of plasma radioactivity. Three other minor components were also observed, each representing less than 2% of plasma radioactivity.

After p.o. administration, M10 and fluticasone furoate were the major components in plasma, comprising 28 and 17% of sample radioactivity, respectively, at 0.5 h, and 6 and 7%, respectively, at 2 h post-p.o. administration. Metabolites M21 (6-hydroxyl of parent), M26 (6-hydroxy of M10), and M32 (a hydroxylated product of M10) were observed in human plasma based on their coelution with either authentic standards or identified metabolites from in vitro incubations; all accounted for less than 4% of plasma radioactivity. The proposed structures and supporting spectral data for the metabolites are shown in Table 3.
Metabolites of [14C]Fluticasone Furoate in Feces. It was established that [14C]fluticasone furoate spiked into feces was stable when stored under the same conditions as the test samples. The mean extraction efficiency of radioactivity from the fecal samples was 90% (p.o.) and 84% (i.v.). The extraction efficiency of radioactivity from control human fecal samples spiked with [14C]fluticasone furoate was 95%. Representative HPLC radiochromatograms of human fecal extracts following either i.v. or p.o. administration are shown in Fig. 3.

The predominant identified drug-related component in the fecal extracts from four of five subjects following dosing by either route was M10, representing 32 to 40% of the p.o. dose and 24 to 31% of the i.v. dose. However, in one subject, M10 was a minor component of the total drug-related material excreted in the feces. Other drug-related components assigned by cochromatography with authentic standards were the 6-hydroxy metabolite of M10 (M26) and parent compound, each representing less than 8% of a p.o. or i.v. dose.

Although most of the dose was excreted in feces, the absolute amounts of drug-related material present in the fecal samples were very low. A number of drug-related components remained insensitive to detection by mass spectrometry, and their structures were not elucidated.

Discussion

Fluticasone furoate, administered as an intranasal aqueous suspension via a unique side-actuated device, is a new chemical entity for the treatment of nasal and ocular symptoms of allergic rhinitis (Martin et al., 2007). As such, it is important to gain an understanding of the metabolism and disposition of fluticasone furoate in humans.

The pharmacokinetic profile of fluticasone furoate following i.v. administration to humans is characterized by a high plasma clearance at 58.3 l/h, which is approximately two thirds of human liver blood flow (Davies and Morris, 1993), and a very large volume of distribution of 642 liters, which equates to approximately 15 times total body water (Davies and Morris, 1993), indicating extensive tissue uptake. These properties in combination resulted in a moderate plasma half-life of 15.3 h. In contrast, the plasma half-life observed following p.o. administration was shorter, at approximately 4 h; however, this is likely to be representative of the distribution phase, with plasma concentrations falling below the assay limit of quantification before the true elimination phase was observed. This is consistent with a large first-pass effect resulting in a very low mean oral bioavailability, calculated at 1.6%. The absolute bioavailability of intranasally administered fluticasone furoate in healthy volunteers has been determined to be 0.5% (Allen et al., 2007). The lower bioavailability observed with the clinical intranasal formulation is likely as a result of differences in dose, dose route, and formulation compared with those used in this study.

Systemic exposure to radioactivity was much greater than systemic exposure to fluticasone furoate for both routes of administration, with the observed difference being greater following p.o. administration. In addition, the elimination half-life of radioactivity was greater than that of fluticasone furoate. These observations are consistent with the presence of one or more circulating metabolites with a longer elimination half-life than parent, and of extensive first-pass metabolism after p.o. administration. Oral absorption was estimated to be only moderate (approximately 30%), despite the high tissue permeability suggested by the high volume of distribution. The estimate of oral absorption (obtained by comparison of the AUC_{(o-t)} values for total plasma radioactivity following p.o. and i.v. administration) may be an overestimate because the profiles of drug-related material following the two routes of administration differ; however, it is consistent with the ratios of urinary excretion, although these are based on very low values. A similar extent of absorption was also observed in rats and dogs (S. Hughes, unpublished data), where 29 and 19% (30% of the recovered radioactivity) of a p.o. radioactive dose was recovered in the bile and urine of bile duct–cannulated rats and a dog, respectively.

The principal component in plasma extracts following i.v. administration of [14C]fluticasone furoate (0.25 mg) to healthy male subjects was fluticasone furoate, with low levels of the 17β-carboxylic acid metabolite (M10) of fluticasone furoate also observed. In contrast, following p.o. administration (2 mg) to the same male subjects, M10 and fluticasone furoate were both principal components in the plasma extracts. Three other minor drug-related components (each less than 5% of plasma radioactivity) were observed and were assigned as products of oxidative metabolism. The greater contribution of metabolites to plasma radioactivity after p.o. than after i.v. administration is consistent with high first-pass metabolism. The remainder of the plasma radioactivity following i.v. or p.o. administration was either unextracted or unassigned because of the low levels of drug-related material in the plasma. The unextracted radioactivity represented a very low amount of material, less than 1 ng equivalents/ml, which equates to less than 30 fmol/mg plasma protein.

AMS was used to quantify radiolabeled drug-related components in plasma off-line following separation by HPLC because of the low levels of radioactivity present in human plasma following i.v. or p.o. administration of [14C]fluticasone furoate. AMS is a very sensitive method for the measurement of radiocarbon (Vogel et al., 1995). The assignment of metabolite structures by chromatographic retention time was performed by comparison with retention times of authentic reference standards and a metabolite isolated from in vitro incubations.
followed by definitive identification. The use of AMS resulted in a clearer definition of the circulating components than would have been achieved by conventional off-line radiodetection using a solid yttrium silicate scintillant, which was used to profile plasma samples from nonclinical species. In the nonclinical species, only fluticasone furoate and the metabolite M10 were identified in plasma after i.v. and/or p.o. administration (P. Shardlow and C. Morley, unpublished data). As a result of the difference in sensitivity between AMS and the solid scintillant detection method, it is considered that a number of the minor metabolites (≤5% of radioactive drug-related material) observed in human plasma may not have been observed in nonclinical species because of the difference in limits of detection.

TABLE 3

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>Retention Time</th>
<th>Matrix</th>
<th>Positive (M + H⁺) or Negative (M-H⁻) Ion MS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fluticasone furoate</td>
<td>46.3</td>
<td>Authentic standard</td>
<td>P: 539 (M + H⁺) F1: 519 Loss of HF F2: 473 F3: 333 F4: 313 F5: 293</td>
</tr>
<tr>
<td>M10</td>
<td>37.2</td>
<td>Plasma/Feces</td>
<td>P: 491 (M + H⁺) 471: loss of HF 333: F3 313: F4 293: F5</td>
</tr>
<tr>
<td>M21</td>
<td>44.6</td>
<td>Plasma (based on coelution)</td>
<td>P: 537 (M + H⁺) 311: F4-2 Information on metabolite in hepatocytes</td>
</tr>
<tr>
<td>M26</td>
<td>27.0</td>
<td>Plasma/Feces</td>
<td>P: 489 (M + H⁺) 313: F4 293: F5</td>
</tr>
<tr>
<td>M32</td>
<td>38.1</td>
<td>Human plasma</td>
<td>Negative ion (M-H⁻) P: 505 393</td>
</tr>
</tbody>
</table>

MS, mass spectrometry.
The mean recovery of the radioactive dose following i.v. administration of [14C]fluticasone furoate to male subjects was greater than 92%. Similar recovery was obtained in rats (86%) and dogs (81%) following an i.v. dose (S. Hughes, unpublished data). After p.o. administration to male subjects, the recovery of radioactivity was complete. Most radioactivity was excreted in the feces after both routes of administration. This excretion pattern is similar to that observed for other synthetic corticosteroids, such as ciclesonide (Nave et al., 2004) and mometasone furoate (Affrime et al., 2000).

The major route of fluticasone furoate metabolism in humans is ester hydrolysis, leading to formation of the 17β-carboxylic acid (M10) with loss of the S-fluoromethyl carbothioate moiety. The loss of this moiety is also the significant route of metabolism of fluticasone propionate, with the biotransformation being catalyzed by cytochrome P450 3A4 (Pearce et al., 2006). Oxidative defluorination of fluticasone furoate at the C6 position also occurred and resulted in the formation of M21 and M26; this biotransformation is known to occur in a number of fluorine-containing drugs (Park et al., 2001). Oxidative defluorination is also a metabolic pathway for fluticasone propionate in vitro in human-derived preparations (Sheny et al., 1993). Oxidative defluorination of fluticasone furoate resulted in hydroxylation at the C6 position; C6 hydroxylation is a well precedented route of metabolism for corticosteroids and has been observed in human-derived in vitro systems for budesonide (Jönsson et al., 1995), mometasone furoate (Teng et al., 2003b), and ciclesonide (Peet et al., 2005). A summary of the proposed oxidative pathways for fluticasone furoate in humans is shown in Fig. 4. There was no evidence for metabolic loss of the furoate group from fluticasone furoate or any of its metabolites; this differs to mometasone furoate, where loss of the furoate group is observed with the formation of mometasone in human-derived fluids and tissue preparations (Teng et al., 2003a).

Although limited data were obtained from in vivo excreta because of the low amounts of drug-related material present, it was observed that, although oral absorption of fluticasone furoate in humans was at most approximately 30%, little or no unchanged parent drug was observed in the feces after p.o. administration. A similar pattern was also observed in p.o.-dosed bile duct-cannulated rats, and, furthermore, fluticasone furoate is stable in a preparation of high-density
anaerobic microbiota from either human or rat (N. McArdle, unpublished data). These data, together with evidence that glucocorticoids are substrates for the P-glycoprotein efflux transporter (Yates et al., 2003), suggest that enteroocytes may have a major role in the metabolism of fluticasone furoate in the gastrointestinal tract through a cycle of absorption, metabolism, and efflux, in addition to limiting absorption of this highly permeable moiety.

The major routes of fluticasone furoate metabolism in humans have been defined following a single i.v. and/or p.o. administration of [14C]fluticasone furoate, within the constraints of the very low levels of drug-related material in the plasma and excreta. No notable differences in the routes of metabolism between humans and the nonclinical species were observed.

Most of an intranasal or inhaled dose is likely to be swallowed; therefore, the p.o. and i.v. routes of administration were considered adequate surrogate routes from which to obtain samples to define the major routes of metabolism of fluticasone furoate in humans. The p.o. route acted as a surrogate for the portion of an intranasal or inhaled dose that is swallowed, and the i.v. route represented the drug that is absorbed locally into the systemic circulation.

The routes of fluticasone furoate metabolism identified are consistent with those precedent for other marketed corticosteroids, such as fluticasone propionate, mometasone furoate, ciclesonide, and budesonide. The routes of metabolism for fluticasone furoate and fluticasone propionate are similar, with no evidence that either is metabolized to fluticasone. This shows that they act as different chemical species were observed.

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References


Address correspondence to: Stephen C. Hughes, Division of Drug Metabolism and Pharmacokinetics, GlaxoSmithKline R&D, Park Road, Ware, Hertfordshire, SG12 0DP, UK. E-mail: sch1409@gsk.com