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Pre-clinical pharmacokinetics of the cyclooxygenase-inhibiting nitric oxide donor (CINOD) AZD3582

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Abstract

The pre-clinical pharmacokinetics of AZD3582 (4-(nitrooxy)butyl-(2S)-2-(6-methoxy-2-naphthyl) propanoate) and its primary metabolites (naproxen and nitrate) were evaluated. AZD3582 had intermediate and passive intestinal permeability (40 times lower than for naproxen), high systemic plasma clearance (CL), substantial gastrointestinal hydrolysis, intermediate volume of distribution (V_{ss}; ≥3.4 L kg^{-1}) and half-life (t½; 7 h), negligible plasma protein binding (~0.1%), low/intermediate oral uptake (≥13% as intact substance) and low and varying oral bioavailability (mean 1.4% in minipigs and 3.9% in dogs). Following administration of therapeutically relevant oral doses, plasma concentrations of AZD3582 were very low (≤13 nM in minipigs and ≤442 nM in dogs; rat data not available) and varying, and accumulation was not apparent. The pharmacokinetics of AZD3582 did not show apparent dose-, time- or gender-related dependency. In blood and intestine, AZD3582 was hydrolysed to naproxen, nitrate and other metabolites. The rate of this conversion was higher in rats than in non-rodents, including man. Despite near-complete to complete uptake of the oral dose, AZD3582 administration resulted in a lower bioavailability (F) of total naproxen than naproxen administration: 55% and 85% relative bioavailability (F_{rel}) in rats and minipigs, respectively. An increased distribution to metabolizing tissues of naproxen (as AZD3582), and thereby enhanced naproxen CL, is believed to be responsible. Following dosing of AZD3582 or naproxen, the t½ of naproxen was 5, 9–10 and >40 h in rats, minipigs and dogs, respectively. The V_{ss} and CL for naproxen were small. Plasma protein binding was extensive, and saturation was observed within the therapeutic dose and concentration range. Intake of food prolonged the systemic absorption of naproxen in the minipig. The pharmacokinetics of naproxen did not show apparent time- or gender-related dependency. Following oral dosing of [^{3}H]-, [^{14}C]- and [^{15}N]-AZD3582, most [^{14}C]- and [^{3}H]-activity was excreted in urine and expired air, respectively. Seventeen per cent of [^{15}N] was recovered in minipig urine as [^{15}N]-nitrate. About 30% of [^{3}H]-activity (naproxen and/or naproxen-related metabolites) was excreted in bile and re-absorbed. Concentrations of [^{14}C]-activity (nitrooxy-butyl group and/or its metabolites) in milk were higher than in plasma and [^{3}H]-activity in milk. [^{3}H]- and [^{14}C]-excretion data indicated that intact AZD3582 was not excreted in urine, bile or milk to a significant extent. There was no apparent consistency between tissue distribution of [^{14}C]- and [^{3}H]-activity in the rat, which suggests rapid and extensive metabolism of extravascularly distributed AZD3582. A substantial increase of plasma nitrate levels was found after single and repeated oral doses of AZD3582 in the minipig. No inhibition or induction of CYP450 was found.

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Introduction

Non-selective non-steroidal anti-inflammatory drugs (NSAIDs) inhibit two isoforms of cyclooxygenase (COX): COX-1 and COX-2. These NSAIDs are frequently associated with gastrointestinal damage, which is attributed to a combination of the COX-1 and -2 inhibitory activity (local and systemic) and a direct local toxic effect associated with the carboxylic moiety of these compounds (Somasundaram et al 1997; Wallace et al 2000; Buttergereit et al 2001). Attempts to overcome the gastrointestinal side effects include the development of non-acidic (including ester pro-drugs of conventional NSAIDs) and COX-2-selective NSAIDs, combination therapies with proton pump inhibitors and prostaglandin analogues and minimization of gastrointestinal mucosal NSAID exposure (e.g. enteric coating, extended release and dose and

permeability reduction) (Jacobs & Bijlsma 1997; Buttergereit et al 2001). With these approaches, a reduction, but not elimination, of gastrointestinal side effects has been reached (Buttergereit et al 2001). Also associated with NSAID use is an increased incidence of cardiovascular and cardiorenal side effects, including hypertension and oedema (Whelton 2001; Perazella 2002). Such problems have not been overcome with the 'gastrointestinal safer' NSAID therapies (Mukherjee et al 2001; Whelton 2001; Perazella 2002). Thus, there is still a need to improve on the overall safety profile of NSAIDs.

Nitric oxide stimulates many of the protective factors that are negatively affected by COX inhibition, such as mucus production, secretion of bicarbonate and blood-flow in the gastric mucosa (Del Soldato et al 1999; Muscara & Wallace 1999). It has also been shown to exhibit biological activity, such as reduction of neutrophil adherence, T-cell activation and reduction of tumour necrosis factor release (Del Soldato et al 1999; Muscara & Wallace 1999). An improved gastrointestinal safety profile has been observed with nitric oxide donor and NSAID co-therapy vs NSAID therapy in man (Lanas et al 2000), and the blood-pressure-lowering effect of nitric oxide donors indicates a potential to counterbalance the hypertensive effect of NSAIDs.

COX-inhibiting nitric oxide donors (CINODs) are a new class of agents being pre-clinically and clinically tested for the potential to have similar anti-inflammatory and analgesic efficacy to other NSAIDs, but with a clinically significant reduction in overall side effects. Besides their nitric-oxide-donating property, CINODs have other beneficial (gastrointestinal-sparing) characteristics, such as non-acidity (masked carboxylic acid group) and slower gastrointestinal absorption than for NSAIDs in general.

The CINOD AZD3582 (4-(nitrooxy)butyl-(2S)-2-(6-methoxy-2-naphthyl) propanoate) was chosen by AstraZeneca (under a license agreement with its inventor, NicOx, Sophia Antipolis, France) as a drug candidate for oral treatment of acute and chronic pain. The molecule AZD3582 (Figure 1A) consists of a naproxen moiety (Figure 1B), a commonly used, effective and non-COX-selective NSAID and a nitric oxide-donating part, which are linked together by a butyl moiety. Naproxen, in contrast to COX-2-selective NSAIDs, may have a cardioprotective effect (Mukherjee et al 2001; Schlienger et al 2002). Besides this advantage AZD3582 also has a favourable pharmacokinetic (PK) profile (including higher gastrointestinal permeability, better gastrointestinal stability and longer elimination half-life) compared with other CINOD candidates.

AZD3582 is a lipophilic, viscous oil with low aqueous solubility and a molecular weight of 347.4 g mol^{-1}. It is unionized at all pHs, and the log P and log D (octanol/water) were estimated to be 4. To reach the desired drug profile (rapid onset of effect, sufficient analgesic and anti-inflammatory effects and sufficient donation of nitric oxide to the upper gastrointestinal tract and blood circulation), the aim was to find and develop a formulation that provided as rapid and extensive an absorption as possible. In formulation testing studies in rats and minipigs, an emulsion was found to give the desired in-vivo absorption profile in animals. This emulsion was very similar to the Self Emulsifying Drug Delivery System (SEDDS) used in human studies (Fagerholm & Björnsson 2005). For several reasons (eating behaviour, gastrointestinal anatomy and physiology similar to man), the minipig was chosen as the main non-rodent animal species for toxicology and pharmacokinetic studies (Davis et al 2001). Dogs are known to have a potential for higher gastrointestinal absorption capacity than man and other animals (Lennernäs 1997; Chiou et al 2000), to be sensitive to NSAID treatment and to have a much longer half-life (t½) of naproxen than other animals and man (Runkel et al 1972). Furthermore, the gastrointestinal characteristics (motility patterns, pHs, transit times) in the minipig are more similar to those in man than those in dogs (Kararli 1995).

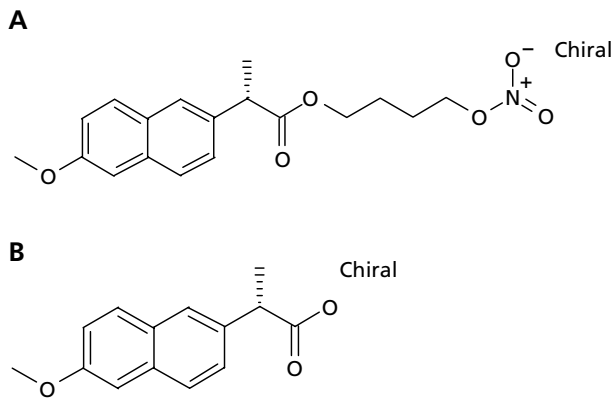

Figure 1 Chemical structure of AZD3582 (MW 347 g mol^{-1}, log D and log P (octanol/water) 4, pK_a —) (A) and naproxen (MW 230 g mol^{-1}, log D (octanol/water) at pH 7.4 0.2, weak acid with pK_a 4.15) (B).

In a biological environment including intestine and blood, AZD3582 is hydrolysed to naproxen, nitrate and other metabolites. The absorption, distribution, metabolism, excretion and pharmacokinetics (ADME/PK) of naproxen (MW 230 g mol^{-1}; weak acid with dissociation constant (pK_a) 4.15, log D 0.2 at physiological pH and 0.1 at pH 6.5) in animals and man are well described in the literature (Runkel et al 1972; Davies & Anderson 1997; FDA 1997). The intestinal permeability (P_e) of naproxen is very high (8×10^{-4} cm s^{-1} in man; about five times higher than the minimum value for complete gastrointestinal uptake) (Fagerholm et al 1996), in-vivo gastrointestinal solubility is high, and in-vitro solubility is high except at gastric pH (naproxen therefore belongs to Class II of the Biopharmaceutical Classification System; BCS). Oral uptake of naproxen is complete and rapid, oral bioavailability (F) is high (50% in rat and near complete in minipig, dog and man), volume of distribution (V_{ss}) is very low (0.1–0.3 L kg^{-1} in various species, including man) and unbound fraction in plasma (f_u) is very low (at low concentrations 1–2% in rats and <0.1% in man) (Runkel et al 1972; Davies & Anderson 1997). The metabolism of naproxen, which is the major route of elimination, varies among species and is largely determined by conjugation

(Runkel et al 1972; Davies & Anderson 1997). The t½ obtained in rats, guinea-pigs, dogs, minipigs, monkeys and man is reported to be approximately 5, 9, 35, 5, 2 and 12–17 h, respectively (Runkel et al 1972; Davies & Anderson 1997). Thus, the clearance (CL) and t½ do not appear to follow the general allometric rule that species with higher body weight have a lower CL per kg body weight and longer t½. The binding to plasma proteins (mainly albumin) is concentration dependent within the therapeutic concentration range, and a less than dose-proportional increase of plasma exposure is observed at increasing doses (Davies & Anderson 1997). The human cytochrome P450 (CYP450) enzymes responsible for the demethylation of naproxen have been identified as CYP1A2 and CYP2C9 (Davies & Anderson 1997). Only a small fraction of naproxen is excreted in human breast milk (Davies & Anderson 1997). Excretion of naproxen in bile has not been studied in man, but is reported to be about 7% in the rat (Iwakawa et al 1991). It has not yet been shown (at least to our knowledge) whether naproxen utilizes drug transporters, such as tubular uptake by renal transporters reported for other NSAIDs (Mulato et al 2000). However, naproxen does have the ability to interact with drug transporters; it inhibits organic anion uptake by hOAT1–4, organic cation uptake by hOCT1–2 (Khamdang et al 2002) and active uptake of D-glucose and P-glycoprotein efflux of verapamil in the rat intestine in-vitro (AstraZeneca, data on file).

In-vitro ADME data of AZD3582 and naproxen were obtained in gastrointestinal fluids (stability and dissolution), liver microsomes (CYP450 inhibition), plasma (binding) and intestine (permeability) from rat, minipig and man. In-vivo ADME/PK/exposure data of AZD3582, naproxen and nitrate (metabolite, and also endogenous substance) were obtained in PK and toxicology studies in mice, rats, rabbits, dogs and minipigs. In mice and rabbits, only toxicokinetic data were obtained. We have focused on presenting data obtained at therapeutically relevant doses ($< 30\ \mu mol\ kg^{-1}$) in rats, dogs and minipigs. Due to instability (hydrolysis by esterases) in rat blood and plasma, it was not possible to fully evaluate the ADME/PK characteristics of AZD3582 in the rat. Investigation of binding to plasma proteins and blood components was not possible due to affinity of AZD3582 to glass and plastics. Therefore, an in-silico approach was used for prediction of the binding of AZD3582 to plasma proteins. In distribution, excretion and mass-balance in-vivo studies, [^{14}C]-, [^{3}H]- and/or [^{15}N]-labelled AZD3582 was administered. AZD3582 and naproxen were dosed both orally and intravenously. AZD3582 was administered as a Phospholipon 80–Lutrol F127–coconut oil–water emulsion or as a Lutrol F127–coconut oil–water emulsion, whereas naproxen was dosed as a sodium naproxen solution.

A prediction of the ADME/PK in man was done using the in-vitro and in-vivo data. The methodology and predicted estimates are reported separately, together with the clinical ADME/PK data (Fagerholm & Björnsson 2005).

Materials and Methods

In-vivo studies and in-vitro studies using animal materials were approved by the animal research ethical committees in the countries where the studies were performed: Sweden, UK, Germany and Denmark.

In-vitro studies

The main human and animal in-vitro/in-silico ADME studies are presented in Table 1. Stability in rat, minipig and human plasma or blood, and in liver microsomes (various species including man) and rat hepatocytes, was also studied in-vitro (Swedmark et al 2002).

Gastrointestinal stability in-vitro

The objective of this study was to determine the gastrointestinal stability of orally administered AZD3582, and whether, and how much of, the dose was hydrolysed into naproxen in this environment. Rat material (duodenum, jejunum, ileum and colon) was obtained from the intestine of rats. Human gastrointestinal fluids (from stomach and jejunum) were taken from subjects participating in perfusion experiments. The incubation mixture consisted of human or rat gastrointestinal fluid or rat mucosal scrapings and was performed at 37°C in a shaking water bath for 2 h. A concentration of 1 mM was assumed to be a relevant maximum gastrointestinal concentration after dosing in man. Samples were withdrawn at different time points and analysed.

Dissolution in simulated gastric fluid in-vitro

The dissolution rate of the AZD3582 self-emulsifying drug delivery system (SEDDS) was obtained in phosphate

Table 1 In-vitro/in-silico absorption, distribution, metabolism, excretion (ADME) studies with AZD3582 and naproxen

Study	Short title	Species	Drug	Concn (mM)
A	Stability in gastrointestinal fluids or tissues in-vitro	Rat and man	AZD3582	1
B	Dissolution rate in simulated gastric fluid in-vitro		AZD3582	1
C	Intestinal permeability (P_e) in-vitro (Ussing chambers)	Rat	AZD3582 and naproxen	0.2–1.1
D	Plasma protein binding in-vitro	Rat and minipig	Naproxen	0.01–1
E	Plasma protein binding in-silico	—	AZD3582	—
F	Inhibition of microsomal CYP450 in-vitro	Man	Naproxen	0–1

buffer (paddle method 75 rev min^{-1} at pH 6.8) + 8 g L^{-1} cetyltrimethylammonium bromide (CTAB) in-vitro.

Intestinal permeability in-vitro

The objective was to determine the P_e of AZD3582 and to predict its absorption following oral administration. P_e values, both from the lumen towards the bloodstream and vice versa, were studied for 180 min at 37°C in Ussing chambers with rat jejunal intestinal mucosa in-vitro. Samples from donor and receiver were analysed.

The cumulative amount of compound that had diffused across the mucosal tissue was calculated from the analysed concentrations for each of the Ussing chambers. When the cumulative amounts were plotted vs time, steady state appeared as a linear part of the curve. The flux of molecules (dQ/dt) across the area (A) exposed in the chamber was driven by the concentration gradient across the membrane (ΔC). The resulting equation for the P_e was: $P_e = dQ/dt \times 1/(A \times \Delta C)$. During the experiments, some of the AZD3582 was hydrolysed into naproxen, which has higher membrane permeability than AZD3582. Similar Ussing chamber experiments were performed with only sodium naproxen in the donor emulsion solution. The P_e and flux values were used to distinguish the contribution of naproxen to the total P_e and to calculate the P_e of AZD3582 only. The ratio of P_e in the mucosal-to-serosal direction (absorption) and serosal-to-mucosal direction was calculated to indicate whether or not AZD3582 was subject to active transport.

Plasma protein binding in-vitro and in-silico

The unbound fraction (f_u) vs total concentration relationship of naproxen was determined in spiked plasma samples from rat and minipig. The unbound naproxen concentrations were determined after pre-treatment based on ultrafiltration. Data for binding of naproxen in human plasma were obtained from the literature (Runkel et al 1972; Davies & Anderson 1997; FDA 1997). Plasma protein binding with AZD3582 was not performed because of its high affinity to plastics and glass, and its instability in plasma and blood (t½ < 2 min). Plasma protein binding for AZD3582 was calculated using an in-house computational chemistry model based on molecular descriptors and protein binding data from more than 5000 compounds, and multivariate (partial least squares; PLS) statistics.

Inhibition of microsomal CYP450 in-vitro

The study was undertaken to investigate whether the in-vitro metabolism of substrates in human liver microsomes dependent on the specific CYP450 enzymes CYP1A2, CYP2C9, CYP2C19, CYP2D6, CYP2E1 or CYP3A4 is influenced by the presence of naproxen. Methoxyresorufin was used as a probe substrate for CYP1A2, diclofenac for CYP2C9, R-omeprazole for CYP2C19, dextromethorphan for CYP2D6, chlorzoxazone for CYP2E1 and testosterone for CYP3A4. Pooled human liver microsomes were incubated with the probe substrates after pre-incubation with naproxen. Samples were taken at various time points and analysed.

In-vivo studies

In-vivo data were obtained in pharmacokinetic and toxicology studies in mice, rats, rabbits, dogs and minipigs. In mice and rabbits, only toxicokinetic data were obtained. In this article ADME/PK data obtained at therapeutically relevant doses (< 30 μmol kg^{-1}) in rats, dogs and minipigs are presented (Table 2). Most data were obtained following single-dose administration, except for the toxicology studies. AZD3582 and naproxen were dosed orally (by gavage; 4 or 5 mL kg^{-1}) and intravenously (0.5 or 1 mL kg^{-1}), and generally to fasted animals. AZD3582 was dosed as a Phospholipon 80–Lutrol F127–coconut oil–water emulsion or as a Lutrol F127–coconut oil–water emulsion (chosen in formulation testing studies in rats and minipigs), whereas naproxen was dosed as a sodium naproxen solution. In mass-balance, distribution and excretion studies, AZD3582 was administered as [^{14}C]-AZD3582, [^{3}H]-AZD3582 or [^{15}N]-AZD3582. A dose of 10 μmol kg^{-1} equals 3.5 mg kg^{-1} AZD3582 and 2.3 mg kg^{-1} naproxen. A therapeutic naproxen dose in Caucasians is 250–500 mg twice daily (7–13 mg kg^{-1}).

Pharmacokinetic studies

Pharmacokinetic data for AZD3582 and naproxen were obtained at clinically relevant, single-bolus doses (< 30 μmol kg^{-1}) of AZD3582 or naproxen in rats (Sprague-Dawley), dogs (Beagle) and minipigs (Göttingen) (Table 2). Due to instability (hydrolysis by esterases) in rat blood and plasma, it was not possible to fully evaluate the pharmacokinetic characteristics of AZD3582 in the rat. Except for a food interaction study in the minipig, doses were administered in fasted animals.

Mass-balance studies

Mass-balance and excretion studies were conducted after single oral administration of combined [^{3}H]- and [^{14}C]-AZD3582 (15 μmol kg^{-1}, 4 mL kg^{-1}, 2.8 MBq mL^{-1} for [^{3}H] and 0.56 MBq mL^{-1} for [^{14}C]) in male and female Sprague-Dawley rats, and a mixture of [^{3}H]-, [^{14}C]- and [^{15}N]-AZD3582 (15 μmol kg^{-1}, 4 mL kg^{-1}, 1.3 MBq mL^{-1} for [^{3}H] and 0.67 MBq mL^{-1} for [^{14}C]) in male and female Göttingen minipigs. Urine, faeces and expired air were sampled. All carcasses were examined for residual radioactivity. The [^{3}H]-, [^{14}C]-activity and [^{15}N]-levels represented the fates of the naproxen, butyl linker and nitrogen oxide moieties, respectively. Comparison of [^{3}H]-, [^{14}C]- and [^{15}N]-patterns/relative levels was used for evaluation of excretion of intact AZD3582.

Bile excretion studies

Bile excretion was investigated after single oral administration of a mixture of [^{3}H]- and [^{14}C]-AZD3582 (15 μmol kg^{-1}, 4 mL kg^{-1}, 2.8 MBq mL^{-1} for [^{3}H] and 0.56 MBq mL^{-1} for [^{14}C]) to bile-duct-cannulated male and female Sprague-Dawley rats. Urine, faeces and bile were collected and the collected bile was pooled. A second group of male and female rats was infused in the duodenum with the pooled bile from the first group. Samples

Table 2 In-vivo ADME/PK studies with AZD3582 and naproxen

Study	Short title	Compound	Dosing, dose	Species (number/gender)
1	Single-dose pharmacokinetics	AZD3582	Intravenous, single (10 μmol kg^{-1})	Rat (3M)
		AZD3582	Oral, single (10 μmol kg^{-1})	Rat (3M)
		AZD3582	Oral, single (15 and 30 μmol kg^{-1})	Rat (3M + 3F)
		Naproxen	Intravenous, single (15 μmol kg^{-1})	Rat (3M)
		Naproxen	Oral, single (15 μmol kg^{-1})	Rat (3M)
2	Mass-balance and excretion	[^{14}C]-, [^{3}H]-AZD3582	Oral, single (15 μmol kg^{-1})	Rat (6M + 6F)
3	Bile excretion	[^{14}C]-, [^{3}H]-AZD3582	Oral, single (15 μmol kg^{-1})	Rat (3M + 3F)
4	Milk secretion	[^{14}C]-, [^{3}H]-AZD3582	Oral, single (15 μmol kg^{-1})	Rat (12F)
5	CYP450 induction	AZD3582	Oral, multiple (14, 43 and 101 μmol kg^{-1} daily for 3 months)	Rat (10M + 10F per dose level)
6	Distribution	[^{14}C]-AZD3582	Oral, single (14 μmol kg^{-1})	Rat[a] (7M + 4F)
7	Distribution	[^{3}H]-AZD3582	Oral, single (18 μmol kg^{-1})	Rat[b] (6M + 4F)
		[^{3}H]-AZD3582	Intravenous, single (10 μmol kg^{-1})	Rat[b] (8M)
8	Distribution	[^{3}H]-naproxen	Oral (20 μmol kg^{-1})	Rat[a] (12M)
9	Single-dose pharmacokinetics	AZD3582	Intravenous, single (1, 3 and 10 μmol kg^{-1})	Dog (3M)
		AZD3582	Oral, single (10 and 20 μmol kg^{-1})	Dog (3M)
10	Single-dose pharmacokinetics	AZD3582	Intravenous, single (0.75 μmol kg^{-1})	Minipig (4M)
		AZD3582	Oral, single (15 μmol kg^{-1})	Minipig (4M + 4F)
		AZD3582	Oral, single (15 μmol kg^{-1}) with food	Minipig (4M)
		Naproxen	Oral, single (15 μmol kg^{-1})	Minipig (4M)
11	Mass-balance and excretion	[^{14}C]-, [^{3}H]-, [^{15}N]-AZD3582	Oral, single (15 μmol kg^{-1})	Minipig (4M + 4F)
12	Nitrate exposure	AZD3582	Oral, repeated (46 μmol kg^{-1} daily for 14 days)	Minipig (4M + 4F)

M, male; F, female. [a]Long Evans black hooded rats. [b]Lister hooded rats.

of urine, faeces and bile were collected. Samples from the carcasses were investigated for residual radioactivity.

Milk excretion study

Secretion in milk was studied after single oral administration of a mixture of [^{3}H]- and [^{14}C]-AZD3582 (15 μmol kg^{-1}, 2.8 MBq mL^{-1} for [^{3}H] and 0.56 MBq mL^{-1} for [^{14}C]) to lactating Sprague-Dawley rats. Samples of milk and plasma were collected, and ratios of both [^{3}H]- and [^{14}C]-activity in milk and plasma were calculated.

CYP450 induction study

This study was part of a 3-month, oral toxicity study in male and female Sprague-Dawley rats. Rats were given either water, vehicle or AZD3582 14.4, 43.2 or 101 μmol kg^{-1} daily. Microsomes were prepared from liver samples obtained from the rats, and the concentrations of CYP450 isoforms 1A, 2B, 3A and 4A were determined.

Distribution studies

Two whole-body autoradiography studies were performed with AZD3582: one involving single oral administration of [^{14}C]-AZD3582 (14 μmol kg^{-1}; 12 MBq mL^{-1}) in male and pregnant female Long Evans black hooded rats, the other involving oral (18 μmol kg^{-1}; 18 MBq mL^{-1}) and intravenous (10 μmol kg^{-1}; 38 MBq mL^{-1}) administration of [^{3}H]-AZD3582 in male and pregnant female Lister hooded rats. For comparison, a whole-body autoradiography study with oral administration of [^{3}H]-naproxen (20 μmol kg^{-1}; 108 MBq mL^{-1}) was performed in male Long Evans black hooded rats. The [^{3}H]- and [^{14}C]-activity represented the fates of the naproxen and butyl linker moieties, respectively. Comparison of [^{3}H]- and [^{14}C]-patterns/relative levels was used to evaluate distribution of AZD3582 metabolites rather than of intact AZD3582.

Nitrate exposure study

The systemic exposure to nitrate, which is an endogenous compound and metabolite of AZD3582, was measured on the first and last days of a 2-week toxicology/toxicokinetic study with 46 μmol kg^{-1} AZD3582 daily in male and female Göttingen minipigs.

Calculation of ADME/PK parameters

ADME/PK parameters were analysed non-compartmentally using WinNonlin (Pharsight Corporation, Mountain View, CA) and/or Microsoft Excel (Microsoft Corporation, CA). In many cases, low (in relation to the limit of quantification; LOQ) and irregular plasma concentrations did not allow evaluation of the full PK profile for the parent compound AZD3582. Individual concentration data from each subject and the actual time points for sampling were used throughout the PK analysis. Samples with concentrations below the LOQ in early time points were treated as zero. Levels below the LOQ appearing in terminal samples were omitted from the analysis. The AUC was calculated using the linear trapezoidal rule on the ascending and logarithmic trapezoidal rule on the descending

part of the plasma concentration vs time curves, with extrapolation to infinity by adding C_{pred}/λ_z.

Bioanalysis
Naproxen was analysed by liquid chromatography with ultraviolet (UV) or fluorescence detection (LOQ 0.5 μM) and AZD3582 was analysed by coupled column liquid chromatography and electrospray mass spectrometry (LOQ 4 nM). Nitrate levels were determined following ultrafiltration sample pretreatment by anion exchange liquid chromatography (LOQ 1 μM). The [^{15}N]-to-[^{14}N] isotope ratio was determined by a method based on conversion of nitrate to nitrobenzene; the isotopic ratio was established by gas chromatography-mass spectroscopy (Jungersten et al 1996). The degree of radioactivity excreted was determined by liquid scintillation.

Statistical analysis

In-vitro gastrointestinal stability data was examined using analysis of variance. $P < 0.05$ denoted significance. Naproxen exposure (C_{max} and AUC) after oral AZD3582 and naproxen dosing was also examined statistically and in a similar way. The significance values for these exposures should, however, be taken with caution. This is because naproxen data were obtained at therapeutic dose levels where plasma protein binding shows saturation (probable underestimation of exposure difference between AZD3582 and naproxen dosing), and the number of animals in each dosing group was limited to 3 or 4.

Results

In-vitro and in-silico data

Gastrointestinal stability in-vitro
AZD3582 was less stable in rat gastrointestinal material than in human gastrointestinal material. Most of the hydrolysed AZD3582 was found as naproxen, but some small and unidentified chromatographic peaks could also be observed. The t½ for AZD3582 in rat material was 0.6–6 h; the t½ was shortest in duodenal fluid and mucosa (0.6 and 0.7 h, respectively) and significantly different ($P < 0.05$) from the other intestinal regions from the rat, and longest in ileal fluid (> 6 h). AZD3582 was very stable in human stomach fluid (t½ 11 ± 3 h) and significantly less stable ($P < 0.05$) in the human jejunal fluid (t½ 3 ± 2 h).

Dissolution in simulated gastric fluid in-vitro
The dissolution t½ of AZD3582 SEDDS in phosphate buffer + CTAB in-vitro was approximated to 0.5 h.

Intestinal permeability in-vitro
When AZD3582 was given in the donor compartment of an Ussing chamber, only naproxen could be measured in the receiver compartment. Moreover, naproxen was detected in the donor compartment, indicating that hydrolysis of AZD3582 also occurred before uptake. Between 0% and 6% of the donor AZD3582 was hydrolysed during these 3-h experiments. The flux across the jejunal intestinal mucosa was divided into the contribution of naproxen (formed before uptake) and the contribution of intact AZD3582. Hydrolysis into naproxen contributed 28–56% to the overall permeability. The P_e of AZD3582 in the absorptive direction (mucosa to serosa) was $7.0 \pm 3.2 \times 10^{-6}$ cm s^{-1}, and was not different from the P_e in the opposite direction (i.e. 4.8×10^{-6} cm s^{-1} (n = 2: 3.9 and 5.7). The highly permeable naproxen had a P_e ($2.6 \pm 0.82 \times 10^{-4}$ cm s^{-1}) that was about 40 times higher than the intermediately permeable AZD3582.

Plasma protein binding in-vitro
In male rats, the f_u of naproxen was about 3% at 10 μM total plasma concentration; f_u increased linearly with increasing concentrations, reaching 19% at 995 μM. A similar trend was also found in female rats, where the f_u at 10 μM and 995 μM was 1% and 14%, respectively. In male and female minipigs, the f_u was approximately 0.1% at 40–113 μM, and increased thereafter to 9.2% at 993 μM. The increase in minipigs appeared to be exponential.

Plasma protein binding in-silico
The f_u for AZD3582 was predicted to be 99.9% in-silico.

Inhibition of microsomal CYP450 in-vitro
No inhibition of microsomal CYP450 enzymes occurred at therapeutically relevant naproxen concentrations. At high doses CYP1A2 and CYP2C9 were inhibited; this can be attributed to competitive inhibition, since it is well established that naproxen is metabolized to desmethyl naproxen by these two CYP450 enzymes.

In-vivo data

Pharmacokinetic studies

AZD3582. Due to instability in rat plasma and blood, ADME/PK data for AZD3582 in the rat are lacking. Mass-balance and naproxen F_{rel} data indicated, however, that ≥ 35–43% of AZD3582 absorbed in rats was intact.

The V_{ss} and plasma CL of AZD3582 in minipigs, obtained following dosing of a very low intravenous bolus dose (0.75 μmol kg^{-1}), were ≥ 3.4 L kg^{-1} and 175 mL min^{-1} kg^{-1}, respectively. The initial (distribution) t½ was approximately 5 min. The terminal t½ could not be well determined in that study, which also caused uncertainties of the V_{ss} and CL estimates. The mean apparent t½ was, however, 0.3 h. At high oral doses (576–1150 μmol kg^{-1} in toxicology studies) where plasma concentrations of AZD3582 could be quantified for a longer time, the terminal t½ was about 7 h. AZD3582 accumulated to some extent in plasma after these high once-daily doses. However, there was a large difference between the minimum and maximum steady-state concentrations ($C_{ss,min}$ and $C_{ss,max}$) in these studies. At therapeutically relevant oral doses (15 and 30 μmol kg^{-1}) given to fasted male minipigs, AZD3582 levels in plasma were very low. At 15 μmol kg^{-1} there were no quantifiable levels (including data obtained for investigation of food effect), whereas the

maximum individual concentration found after 30 μmol kg^{-1} was 13 nM. Plasma concentrations and plasma concentration vs time profiles showed large variability. The apparent oral F in the minipig averaged 1.4%, and ranged between 0% and 3.7%. Assuming that naproxen was stable within gastrointestinal fluids, the fraction of intact AZD3582 absorbed was ≥13% in minipigs. The maximum individual concentration measured in the minipig (after a single dose of 576 μmol kg^{-1}) was 777 nM. There was no apparent gender-, dose- and time-related dependency for the PK of AZD3582 in minipigs.

In the dog, where higher intravenous doses were given (1, 3 and 10 μmol kg^{-1}), the V_{ss}, plasma CL and t½ were 6.8 L kg^{-1}, 40 mL min^{-1} kg^{-1} and 7.3 h, respectively (Figure 2). The mean (maximum individual) oral F after 10 and 20 μmol kg^{-1} AZD3582 was 3.9 (14)%. The mean (maximum individual) C_{max} obtained after administration of these oral doses were 33 (45) nM and 211 (442) nM. As in the minipig, plasma concentrations and plasma concentration vs time profiles following oral dosing showed large variability.

Naproxen. Following single intravenous bolus dosing of AZD3582, the t_{max} of naproxen in minipigs and dogs was, on average, 0.4 and 1 h, respectively.

Compared with intravenous and oral naproxen dosing, AZD3582 gave a lower systemic exposure to naproxen, in both rats and minipigs. The mean F_{rel} of naproxen after an intravenous AZD3582 dose (vs intravenous naproxen administration) in the rat was 60%. In the minipig, the mean F_{rel} of naproxen after an intravenous AZD3582 dose (vs oral naproxen dosing) was 65%. At an oral dose level of 15 μmol kg^{-1} in male rats and male and female minipigs, the mean F_{rel} vs oral naproxen dosing was 55% and 85%, respectively (Figure 3). The naproxen exposure difference between oral AZD3582 and naproxen dosing in rats was significant ($P < 0.05$), whereas that in the minipig was not. Instead of a distinct peak shortly after oral naproxen dosing in the rat, plasma concentrations reached a plateau within 15 min and remained at this level for several hours. The t½ of naproxen in the rats and minipigs was, on average, 5 and 9–10 h, respectively, and did not differ between AZD3582 and naproxen dosing.

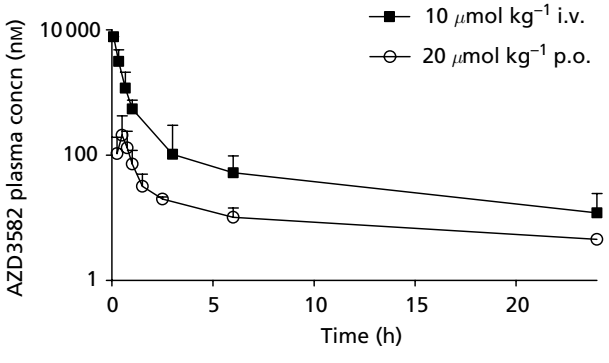

Figure 2 Mean ± s.d. plasma concentration of AZD3582 vs time following single intravenous (10 μmol kg^{-1}) or oral (20 μmol kg^{-1}) administration of AZD3582 to fasted male dogs.

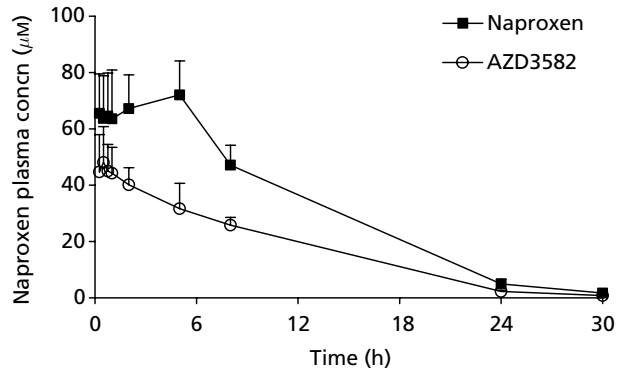

Figure 3 Mean ± s.d. plasma concentration of naproxen vs time following single oral administration of 15 μmol kg^{-1} AZD3582 or naproxen to fasted male rats.

The t½ of naproxen in the dog was >40 h. The F of naproxen (oral vs intravenous dosing) in the rat was, on average, 97%. The V_{ss} and plasma CL obtained after intravenous dosing of naproxen in rats were, on average, 0.12 L kg^{-1} and 0.29 mL min^{-1} kg^{-1}, respectively. The plasma exposure and t½ were approximately 10–20% higher/longer in female than in male rats.

The systemic exposure (C_{max} and area under the curve (AUC)) of naproxen increased less than proportionally in relation to dose, in both rats and minipigs. A reduced C_{max} and AUC were observed after 14 days' oral administration with 216 μmol kg^{-1} AZD3582 in rats and after 5 days' dosing with 921 μmol kg^{-1} AZD3582 in minipigs. In parallel with this, significant treatment-related decreases in plasma albumin (hypoalbuminaemia) and total protein levels were observed at these high doses. No other time- or gender-related dependency was observed.

A slight decrease of the C_{max} and a prolongation of t_{max} (0.5–7 vs 4–24 h) were observed when AZD3582 was administered together with food in minipigs. The AUC was unchanged.

Mass-balance studies

The [^{14}C]-activity in AZD3582 represented the fate of the butyl linker moiety and the [^{3}H]-activity the fate of the naproxen moiety. In the rat, 84–91%, 10%, <10% and 4–6% of orally dosed [^{14}C]-activity was excreted in expired air, faeces, urine and carcass, respectively. The corresponding values for [^{3}H]-activity were very low: 2–4%, 13–16%, >65% and 1–2%, respectively. Assuming that faeces recoveries (10 and 13–16% recovery of [^{14}C]- and [^{3}H]-activity, respectively) reflected the unabsorbed extent, then 84–90% of the dose had been absorbed.

In the minipig, 47–49%, 2%, 9% and 8% of orally dosed [^{14}C]-activity was excreted in expired air, faeces, urine and carcass, respectively. The values for [^{3}H]-activity in faeces, urine and carcass were 2%, >77% and 2–3%, respectively. Two per cent recovery of [^{14}C]- and [^{3}H]-activity in faeces indicates 98% absorption of the dose. Seventeen per cent of the [^{15}N] was recovered in minipig urine as [^{15}N]-nitrate. Most of the [^{15}N]-nitrate excretion

occurred during the first 24 h. No major differences between males and females were observed.

Bile excretion studies
Recovery of orally administered [^{14}C]-activity at 48 h post dose in rat bile, urine, faeces and carcass accounted for 8–12%, 4–6%, < 3% and 10–16%, respectively, of the amount administered. The total recovery of [^{14}C]-activity was 32–35%, the remainder possibly being lost in the expired air. The corresponding recoveries of orally administered [^{3}H]-activity in bile, urine, faeces and carcass accounted for 29–30%, 50–52%, 3% and 2%, respectively. The total recovery of [^{3}H]-activity was 87–90%. Less than 3% recovery of [^{14}C]-activity and 3% recovery of [^{3}H]-activity in faeces indicates 97% absorption of the dose.

In rats infused intraduodenally with pooled samples of bile collected in the first phase, recovery of orally administered [^{14}C]-activity at 54 h post dose in bile, urine, faeces and carcass accounted for 20–27%, 3–10%, 14–16% and 7–11% of the amount in administered bile, respectively. The average total recovery of [^{14}C]-activity was 52%. The corresponding recoveries of orally administered [^{3}H]-activity in bile, urine, faeces and carcass accounted for 23–29%, 30–43%, 8–12% and 4–6%, respectively. The total recovery of [^{3}H]-activity was about 85%. The ratio between [^{3}H]-activity in bile from intraduodenally and from orally administered rats averaged 0.88.

Milk excretion study
Radioactivity associated with [^{14}C] was found at higher concentrations in rat plasma than milk at first and last post-dose stages, with mean milk–plasma ratios of 0.56 and 0.75 at 0.5 and 75 h post dose, respectively. At 5 and 24 h post dose, higher concentrations of [^{14}C]-activity were observed in milk than in plasma, the ratios being 11 and 1.9, respectively. Radioactivity associated with [^{3}H] was found at higher concentrations in plasma than in milk at all time points, ratios being 0.13, 0.15, 0.58 and 0.81 at 0.5, 5, 24 and 72 h post dose, respectively.

CYP450 induction study
There was no induction of CYP1A, 2B, 3A or 4A in response to 3 months of oral dosing with AZD3582 in the rat.

Distribution studies
At 15 min post dose in male and female Long Evans black hooded rats, high levels of [^{14}C]-activity were found in all tissues except for the lens and periosteum. The highest [^{14}C]-activity was found in the bile, followed by urine, liver, gastric mucosa and kidney. The [^{14}C]-activity declined markedly after 2 days in all tissues except for adrenal cortex, bile and skin. After 28 days, low levels of [^{14}C]-activity were still present in several tissues; the most prominent levels were found in skin, eye, skeletal muscle, brain and pituitary gland. Other tissue levels were close to, or below, the limit of detection.

In male and female Lister hooded rats the [^{3}H]-activity was widely distributed throughout the tissues within 15 min of oral dosing. Peak tissue concentrations were generally attained at 1 h post dose, with highest concentrations observed in the kidney (medulla), lung and blood. The tissue distribution and elimination of total [^{3}H]-activity following intravenous and oral AZD3582 administration was similar. Concentrations of total [^{3}H]-activity declined, and by 48 h were below the limit of reliable measurement.

In the pregnant Long Evans black hooded rat, [^{14}C]-activity was extensively and rapidly distributed in all maternal and foetal tissues, crossing the placental barrier. The highest [^{14}C]-activity was found in maternal liver. After 1 h, all tissue levels had increased except for the maternal liver. The highest foetal tissue concentration was observed in liver and in the lens. After 16 h, the [^{14}C]-activity markedly declined in maternal tissues, although the foetal radioactivity was fairly constant. The concentration of [^{3}H]-activity in the foetal tissues was highest at 5 h in the placenta. Lower concentrations were measured in foetal blood, liver, foetus, eye and brain. [^{3}H]-activity concentrations in all tissues declined, and by 16 h they had decreased significantly, although radioactivity was still measurable in most maternal and foetal tissues.

For comparison, an autoradiography study was made with [^{3}H]-naproxen in male Long Evans black hooded rats. Oral administration of AZD3582 led to higher tissue concentrations (based on the [^{3}H]-activity) in the renal medulla (1, 5 and 16 h), small intestinal wall (1 and 16 h), thyroid gland and prostate gland (5 and 16 h) and epididymis (16 h), even though AZD3582 was given at a slightly lower dose (18 vs 20 μmol kg^{-1}). At time points not mentioned, and in other organs, there were no apparent differences. It should be noted that [^{3}H]-AZD3582 experiments were performed in male and female Lister hooded rats, and [^{3}H]-naproxen in male Long Evans black hooded rats. These strain and gender differences may have influenced the comparison. For example, a different naproxen t½ was observed in the rat strains (5.2 vs 3.4 h), and we cannot exclude the possibility that this could have been due to strain/gender differences in plasma protein binding and/or metabolism, etc.

Nitrate exposure study
In the 14-day oral toxicity study with 46 μmol kg^{-1} AZD3582 daily in minipigs, basal (pre-dose/trough) nitrate levels in plasma increased from 6 ± 2 to 17 ± 9 μM. The C_{max} on day 1 was 80 ± 17 μM.

Discussion

AZD3582 had an intermediate intestinal P_e that was approximately 40 times lower than that of the highly permeable naproxen. The similar bi-directional permeabilities predict that AZD3582 was not a substrate for intestinal transport proteins. The stability in human gastric juice was good; hydrolysis was observed in human jejunal fluid and in rat intestinal scrapings and

fluids. The hydrolysis rate in upper small-intestinal fluids in the rat was, on average, five times more rapid than in man, corresponding well with the generally higher enzymatic activity in rodents. It should be noted that incubations in stored material might lead to slower hydrolysis than in the in-vivo situation. However, this method has been tested for other drugs (Lehr et al 1992; Krondahl et al 1997), and good comparison with in-vivo results has been achieved. The stability in rat intestinal fluids increased from proximal to distal parts. Upper small-intestinal stability and P_e data predicted AZD3582 to be degraded five and three times more rapidly than it was absorbed in rat and man, respectively. The stability in rat ileal fluid was 10 times better than in the upper small intestine, suggesting that each AZD3582 molecule is approximately twice as likely to be absorbed than to be degraded in the rat ileum. The predicted human P_e for AZD3582 corresponds to a fraction absorbed (f_a) of 70–90% and, by taking the gastrointestinal hydrolysis into account, the f_a of intact AZD3582 was predicted to be 23–24%. Near-complete uptake of naproxen in man could be expected. Assuming that the recovery of [^{14}C]- and [^{3}H]-activity in faeces was unabsorbed dose, 84–97% and 98% of oral AZD3582 doses was absorbed in rats and minipigs, respectively. The [^{14}C]- and [^{3}H]-activity in faeces was consistent in each study, which indicates that intact AZD3582 had been excreted. Despite good absorption, the F_{rel} of total naproxen vs naproxen dosing was only 55% and 85% in rats and minipigs, respectively. This indicates a higher CL of naproxen after AZD3582 dosing (see below). A similarly lower naproxen exposure was found after intravenous AZD3582 (vs naproxen) dosing. Assuming that naproxen was not metabolized in gastrointestinal fluids, the fraction of AZD3582 absorbed as intact AZD3582 was estimated to be ≥35–43 and ≥13% in rats and minipigs, respectively. The apparently higher value for the lower range limit in the rat seems contradictory to the expected lower gastrointestinal stability and absorptive capacity in this species. However, this could possibly be explained by a higher relative volume of the rat intestine occupied with dosing solution. The dosing volumes in rats (approximate body weight 250 g) and minipigs (approximate body weight 10 kg) were 4–5 mL kg^{-1}. Dimensions (diameter and length) of the duodenum and jejunum in rats, minipigs and man suggest a storage capacity (volume) of approximately 6 mL, 2.5 L and 2 L fluid, respectively (Kararli 1995). Thus, the dosing volumes in rats corresponded to approximately 20% of the capacity of the duodenum and jejunum. The value in minipigs was much lower at 2%. Therefore, it cannot be ruled out that the relatively high dosing volume in rats and inferior mixing may have resulted in a better protection from hydrolysis by esterases. Duodeno-jejunal transit times in rats, minipigs and man are approximately 1.0, 1.0 and 1.5 h, respectively (Kararli 1995). As a result of hydrolysis in the gastrointestinal tract, intermediate intestinal P_e and high systemic plasma CL (higher than liver plasma flows), AZD3582 had a very low and varying oral F. The oral F in dogs was, on average, three times higher (3.9% vs 1.4%) than in minipigs. It is not clear whether this difference was due to higher absorptive capacity or better stability in the dog, or both. Dogs are known to have a potential for higher gastrointestinal absorption capacity than man and other animals (Lennernäs 1997; Chiou et al 2000). Because of instability in rat blood and plasma, no AZD3582 oral bioavailability (and other ADME/PK) data are available.

In a rat study by Davies et al (1997), the ulcerogenic, analgesic and anti-inflammatory effects and naproxen plasma exposures of AZD3582 were compared with those of naproxen. The extent of absorption in that study was lower than in the current studies (F_{rel} of 36% compared with 55%). One plausible reason could be the different formulations and dosing volumes. In the studies reported here, AZD3582 was given as an emulsion (4 or 5 mL min^{-1}), compared with a 5% dimethyl sulfoxide (DMSO) solution (1 mL kg^{-1}) in the study by Davies and colleagues.

The t½ of naproxen in rats (5 h) and minipigs (9–10 h) was not different after AZD3582 administration. The intestinal P_e of AZD3582 in rats (approximately 6×10^{-6} cm s^{-1}) corresponds to an absorption rate constant of approximately 0.25 h^{-1} and an absorption t½ of approximately 3 h. This agrees with the absence of absorption-rate-controlled elimination for naproxen after AZD3582 dosing in the rat. The t½ of AZD3582 in minipigs and dogs (7 h) was shorter than for naproxen, which also indicates that the elimination of naproxen was not limited by the AZD3582 uptake in these species.

With intermediate P_e and negligible plasma protein binding, AZD3582 has the potential to permeate from the blood circulation and into capillary walls and tissues at quite a high rate, and also to act as a Trojan Horse by transporting naproxen from the blood circulation more rapidly and extensively than circulating naproxen. The low F vs naproxen dosing is an indication of increased volume of distribution and CL of naproxen during the time period when AZD3582 was present in the body. Since both AZD3582 and naproxen have to traverse the gastrointestinal mucosal cells, and a similar naproxen loss was found after intravenous and oral dosing, it seems likely that the increased CL occurred in central metabolizing organs (such as the liver and capillary walls) rather than in the gastrointestinal mucosa. The metabolic loss of naproxen after oral dosing (vs naproxen administration) was greater in the rat than in the minipig. Based on in-vitro stability, dissolution and permeability data, the F vs naproxen dosing in man was predicted to be a minimum of 70%, which is similar to the F_{rel} values found in rats and minipigs.

The binding sites of AZD3582 outside plasma and within the body are not known. Its intermediate V_{ss} is consistent with its high lipophilicity, which could mean that AZD3582 is extensively distributed to tissues. However, this does not exclude the possibility that AZD3582 is highly bound to blood cells and blood capillary walls, without being extensively distributed to, or bound in, other body tissues. AZD3582 is metabolized within intestinal and capillary wall cells (Berndt et al 2005) and in media containing esterases. This indicates that a

substantial loss of AZD3582 might occur outside the blood circulation, and that the apparent V_{ss} is dependent on the extravascular stability. Since the t½ of naproxen was similar following dosing of AZD3582 or naproxen, a deep compartment for AZD3582 is not likely to play a major role. There was no apparent consistency between tissue distribution of [^{14}C]- and [^{3}H]-activity in the rat, which suggests rapid and extensive metabolism of extravascularly distributed AZD3582.

As a consequence of saturable plasma protein binding, the plasma exposure to naproxen increased less than proportionally in relation to dose. The plateau found for total naproxen concentrations after oral dosing of naproxen 15 μmol kg^{-1} could be an indication of saturation at this dose level. After oral dosing of AZD3582 15 μmol kg^{-1} to rats, plasma levels of naproxen were lower and no plateau was found. Thus, the F_{rel} of 55% (significant difference between AZD3582 and naproxen dosing) in rats and 85% in minipigs (significant difference between AZD3582 and naproxen administration not found) may be overestimates. The oral F of naproxen after naproxen dosing in rats (97%) was higher than values obtained by others (50%) (Runkel et al 1972). Different dose levels and saturation degree of plasma protein binding are probable explanations for this difference. The higher plasma exposure and longer t½ observed in female rats (vs male rats) are consistent with their higher degree of plasma protein binding. In agreement with the loss of albumin and total protein (increased f_u) after repeated high doses in rats and minipigs, the plasma exposure to total naproxen decreased.

The total recovery of [^{14}C]-activity in urine and bile was very low, whereas some [^{14}C] was detected in milk. The [^{14}C]-activity (nitrooxy-butyl group and/or its metabolites) was almost exclusively excreted via expired air, and was expected to be metabolized into endogenous compounds and distributed as small carbon fragments. Following oral administration, [^{3}H]-activity (naproxen and/or naproxen-related metabolites) was excreted mainly in urine, while very little was excreted in faeces, air and milk, and retained in carcass. This difference between [^{3}H]- and [^{14}C]-excretion data indicates that intact AZD3582 was not excreted in urine, bile or milk to a significant extent. Intermediate bile excretion (29–30% of oral dose) and enterohepatic recirculation (88% of amount excreted in bile was reabsorbed) of [^{3}H]-activity (naproxen part of the molecule) were found in the rat. The lower gastrointestinal uptake compared with an oral naproxen dose and higher [^{3}H]-activity excreted in faeces in rats given bile from orally dosed rats suggests bile secretion of non-absorbable naproxen-related metabolites after enterohepatic re-circulation. The fraction of [^{3}H]-activity that was excreted in bile (29–30%) was higher than the 7% that Iwakawa et al (1991) found for naproxen. This indicates that two-thirds of the excreted [^{3}H]-activity consisted of naproxen-related metabolites. The excretion of [^{15}N]-nitrate in minipig urine was quite low (17% of dose). This result indicates that [^{15}N] might not only be excreted as nitrate, but perhaps also as urea or expired nitric oxide gas, or may be retained in proteins, for example. The baseline plasma nitrate level in minipigs increased 3 fold after repeated oral administration of a therapeutically relevant dose. Intake of AZD3582 with food in the minipig resulted in a prolonged t_{max} for naproxen, which is in agreement with the markedly prolonged and varying gastric emptying that has been observed in the fed state in the minipig (Davis et al 2001). AZD3582 could not be quantified in the food interaction study, and information on food effects on AZD3582 pharmacokinetics and exposure is therefore lacking.

Conclusions

AZD3582 had intermediate and passive P_e (40 times lower than for the highly permeable naproxen), high systemic plasma CL (higher than liver plasma flows), substantial gastrointestinal hydrolysis, intermediate V_{ss} (≥3.4 L kg^{-1}) and terminal t½ (7 h), negligible plasma protein binding (approximately 0.1%), low/intermediate oral uptake (≥13% as intact substance) and low and varying oral F (mean 1.4% in minipigs and 3.9% in dogs; rat data not available). Following administration of therapeutically relevant oral doses, plasma concentrations of AZD3582 were very low (≤ 13 nM in minipigs and ≤ 442 nM in dogs; rat data not available) and varying, and accumulation was not apparent. The PK of AZD3582 did not show apparent dose-, time- or gender-related dependency. In a biological environment including blood and the intestine, AZD3582 was hydrolysed to form naproxen, nitrate and other metabolites. The rate of this conversion in blood and the gastrointestinal material in-vitro was higher in rats than in non-rodents, including man.

Despite near-complete to complete uptake of the oral dose, administration of AZD3582 resulted in a lower F of total naproxen than naproxen administration: 55% and 85% in rats and minipigs, respectively. An increased distribution to metabolizing tissues of naproxen (as AZD3582), and thereby enhanced naproxen CL, is believed to be the reason. Following dosing of AZD3582 or naproxen, the t½ of naproxen was 5, 9–10 and > 40 h in rats, minipigs and dogs, respectively. The V_{ss} and plasma CL for naproxen were small. The binding of naproxen to plasma proteins was extensive, and saturation was observed within the therapeutic dose and concentration range. Intake of food prolonged the systemic absorption rate of naproxen in the minipig. The PK of naproxen did not show apparent time- or gender-related dependency.

Following oral dosing of [^{3}H]- and [^{14}C]-AZD3582, most [^{14}C]- and [^{3}H]-activity was excreted in urine and expired air, respectively. About 30% of the [^{3}H]-activity (naproxen and/or naproxen-related metabolites) was excreted in bile and re-absorbed. Concentrations of [^{14}C]-activity (nitrooxy-butyl group and/or its metabolites) in milk were higher than in plasma and [^{3}H]-activity in milk. A difference between [^{3}H]- and [^{14}C]-excretion data indicated that intact AZD3582 was not excreted in urine, bile or milk to a significant extent. The binding sites of AZD3582 outside plasma and within the body are not known. There was no apparent consistency between tissue distributions of [^{14}C]- and [^{3}H]-activity in the rat, which

suggests rapid and extensive metabolism of extravascularly distributed AZD3582. This was further indicated by a similar naproxen t½ after AZD3582 and naproxen dosing.

A substantial increase in plasma nitrate levels was found after single and repeated oral doses of AZD3582 in the minipig.

No inhibition or induction of CYP450 was found.

Crucial properties for anti-inflammatory and analgesic activity together with gastrointestinal tolerability of a CINOD are assumed to be sufficiently high intestinal P_e and good gastrointestinal stability, sufficiently rapid and extensive systemic uptake of its NSAID, and acceptable dose size and dosing strategy. Pre-clinical ADME/PK studies show that AZD3582 appears to have these properties, and the compound was therefore further evaluated in man.

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