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Clinical pharmacokinetics of the cyclooxygenase inhibiting nitric oxide donator (CINOD) AZD3582

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Abstract

The clinical pharmacokinetics of the COX-inhibiting nitric oxide donator (CINOD) AZD3582 and its metabolites, including naproxen, nitric oxide and nitrate, are summarized. AZD3582 has low aqueous solubility, moderate and passive intestinal permeability and is degraded by intestinal esterases. Its oral bioavailability (F) appears to be maximally a few per cent, and increases by several-fold after food intake. Ninety-four per cent or more of an AZD3582 dose is absorbed, of which at least 9–20% appears to be taken up as intact substance. AZD3582 has a predicted plasma protein binding degree of $\sim$ 0.1%, a half-life ($t_{½}$) of 3 to 10 h and does not accumulate after repeated once- and twice-daily dosing. In patients AZD3582 does not provide a significantly better gastrointestinal (GI) side-effect profile than the highly permeable and locally irritating naproxen. Possible reasons for this include considerable GI uptake as naproxen, limited duration and extent of nitric oxide donation in the GI mucosa and the circulation, tolerance development (involving auto-inhibition of nitric oxide catalysing enzymes) and mucosal damage caused by nitric oxide. Blood pressure data suggest that nitric oxide is mainly donated within 3 h. The uptake of naproxen is slightly slower and lower ($\geq$ 94% relative GI uptake and 80–85% relative F) after AZD3582 administration compared with naproxen dosing. The naproxen $t_{½}$ and trough steady-state concentrations after AZD3582 and naproxen dosing are similar. The average systemic nitrate exposure is approximately doubled after dosing of 375 to 750 mg AZD3582 twice daily.

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Introduction

Background

Non-selective, non-steroidal anti-inflammatory drugs (NSAIDs), which inhibit both cyclooxygenase (COX)-1 and COX-2, are effective in the management of pain and inflammation. However, their long-term use is compromised by significant gastrointestinal (GI) toxicity (Haslock 1998; Wolfe et al 1999; Moore 2002), which results in substantial morbidity and mortality. These GI-toxic effects are thought to be due to topical irritation of the epithelium (Somasundaram et al 1997; Buttergereit et al 2001), as well as local and systemic COX inhibition (Wallace 1997). Also associated with NSAID use is an increased incidence of cardio-vascular and cardio-renal side-effects, including hypertension and oedema (Whelton 2001; Perazella 2002).

A therapeutic goal, therefore, has been to maintain the efficacy of NSAIDs, and to achieve an improved safety profile. Development of the COX-inhibiting nitric oxide donating (CINOD) class of drugs, whose mechanism of action involves COX inhibition and nitric oxide donation, was inspired by the known protective effects of nitric oxide, which replicate many of the protective effects of prostaglandins (Wallace 2001). Besides their nitric oxide donating property, CINODs have other potential beneficial (GI sparing) characteristics, such as non-acidity (masked carboxylic acid group) and slower GI absorption compared with NSAIDs in general.

AZD3582 (4-(nitrooxy)butyl-(2S)-2-(6-methoxy-2-naphthyl) propanoate) was the first CINOD to enter extensive clinical trials. Other names for this compound are HCT 3012 and NO-naproxen. The molecule AZD3582 (Figure 1) consists of a naproxen moiety, a commonly used, effective and non-COX-selective NSAID, and a nitric oxide donating part linked together by a butyl moiety. Seven hundred and fifty

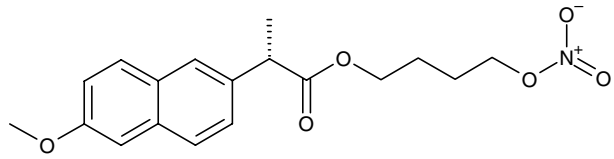

Figure 1 Chemical structure of AZD3582.

milligrams of AZD3582 is equimolar to 500 mg naproxen (approximately 2200 μmol or 30 μmol kg^{-1}). Efficacious doses are 375 and 750 mg AZD3582 twice daily (Lohmander et al 2005; Schnitzer et al 2005). Seven hundred and fifty milligrams AZD3582 once-daily dosing is associated with lower analgesic efficacy, better GI safety profile and lower minimum inhibition of serum thromboxane B_2 (TxB_2) than 375 and 750 mg AZD3582 given twice daily (Wilder-Smith et al 2005). In patient studies, no significant differences in GI safety (30% lower gastroduodenal ulcer incidence with AZD3582, $P = 0.07$) (Lohmander et al 2005) and similar analgesia (Hill et al 2005; Lohmander et al 2005; Schnitzer et al 2005) vs equimolar naproxen doses have been shown. In a volunteer study, the renal effects of AZD3582 were similar to those of naproxen (Huledal et al 2005). The contribution of nitric oxide to the GI safety of AZD3582 has not yet been proven. The absorption, distribution, metabolism, excretion and pharmacokinetic (ADME/PK) characteristics of AZD3582 and its metabolites (including naproxen, nitric oxide and nitrate) are valuable for the explanation and understanding of these pharmacodynamic (PD) findings. Clinical ADME/PK data following intravenous dosing are lacking and the ADME/PK data for the active metabolite nitric oxide are very difficult to obtain. We have therefore used preclinical ADME/PK data, prediction methodology, and preclinical and clinical PD data to enable the interpretation of the ADME/PK data of AZD3582 and its metabolites. The ADME/PK data of AZD3582, naproxen and nitrate in animals are summarized and discussed in Fagerholm et al (2005).

The absorption capability and characteristics of the GI tract are crucial issues for CINODs and therefore an optimization of absorption properties is important in the design of new CINODs. AZD3582 (molecular weight 347.4 g mol^{-1}; log P and log D (octanol/water) 4) has many obstacles for GI absorption: (i) it is a lipophilic, viscous oil with low aqueous solubility; (ii) it is degraded by esterases in the intestines; and (iii) its GI permeability (P_e) is not sufficient for complete absorption. Phospholipon 80:Lutrol F127:coconut oil:water and Lutrol F127:coconut oil: water emulsions were found to give the desired in-vivo absorption profile (rapid and extensive absorption to assure rapid onset of effect, sufficient analgesic and anti-inflammatory effects, and sufficient donation of nitric oxide to the upper GI and blood circulation) in animals (Fagerholm et al 2005). These formulations were the basis for the self-emulsifying drug delivery system (SEDDS) developed for use in humans.

Methods

In-vitro/in-silico studies

Human in-vitro/in-silico ADME data (GI stability and dissolution, plasma protein binding, CYP450 inhibition) for AZD3582 were obtained in preclinical studies. Methods and data are presented in a separate paper (Fagerholm et al 2005).

Predictions of human ADME/PK and exposure

Prediction of fraction absorbed and absorption rate

In-vitro small intestinal P_e (rat Ussing), in-vitro stability in human GI fluids, in-vitro dissolution in phosphate buffer + cethyltriammoniumbromide, GI physiology variables (gastric emptying rate, small intestinal radius and transit time), and established relationships between rat in-vitro P_e, human in-vivo P_e and human fraction absorbed (f_a), enabled a prediction of the intrinsic (P_e-based) and apparent absorption rate constants (k_a) and f_a for intact AZD3582 and naproxen (Fagerholm et al 1996). The predicted k_a represented disappearance from the GI and not appearance in plasma. The calculations were performed using Microsoft Excel (Microsoft Corp., USA) according to the following scheme:

1 Predictions of small intestinal P_e in humans

The human in-vivo small intestinal P_e of AZD3582 was predicted from rat in-vitro P_e values of AZD3582 (6×10^{-6} cm s^{-1}) and naproxen (2.6×10^{-4} cm s^{-1}) inserted into the relationships between small intestinal P_e data in rats in vitro (Ussing) and in situ (perfusion), and humans in vivo (perfusion) (Fagerholm et al 1996; Berggren et al 2004; data and relationships established at AstraZeneca). P_e values for naproxen were already available from previous perfusion studies in rats (2.1×10^{-4} cm s^{-1}) and humans (8.0×10^{-4} cm s^{-1}, about five times higher than the minimum value corresponding to complete GI uptake) (Fagerholm et al 1996). The P_e of nitrate was not measured. It was, however, assumed to be high (at least higher than the P_e for AZD3582) because nitrate is a small compound (MW 62 g mol^{-1}) that is rapidly and completely absorbed from the human GI tract (Schultz et al 1985).

2 Predictions of absorption rate constant (k_a) in humans

The intrinsic k_a for AZD3582 and naproxen from the human small intestine was estimated from the following relationship:

$$k_a = 2 \times P_e / r$$

where P_e is the measured or predicted human P_e (Sinko et al 1991) and r is the human small intestinal radius (1.75 cm). The slowest process of gastric emptying, dissolution, degradation (hydrolysis) and permeation was assumed to determine the apparent k_a. The gastric emptying rate constant (k_{ge}) for solutions, 3.8 h^{-1}, was taken from the literature (Adkin et al 1995). The dissolution and degradation (AZD3582 to naproxen) rate constants (k_{diss}

and k_{degr}) were taken from in-vitro dissolution and stability tests in simulated gastric and human jejunal fluid, respectively. Only a small percentage of AZD3582 was degraded after 30 min (approximated gastric retention time) in human gastric juice in vitro ($t_{½} = 11$ h) and therefore gastric degradation was not considered in the calculations. The dissolution of a hard gelatin capsule is more rapid than any of the other steps and therefore was not considered in the calculations.

3 Predictions of f_a in humans

Predicted or observed human in-vivo small intestinal P_e data were used to predict the f_a in humans:

$$f_a = 1 - e^{-(5.6 \times Pe \times tres/r)}$$

where r and t_{res} are the human small intestinal radius (1.75 cm) and transit time (3 h), respectively (Fagerholm et al 1996). For AZD3582, f_a represents the value it would have had if AZD3582 were not degraded in GI fluids. The intrinsic k_a and k_{degr} values for AZD3582 and the intrinsic k_a value for naproxen were used to calculate the f_a of intact AZD3582 and naproxen, respectively. An average GI transit time of 30 h was used in these calculations (Davis 1986).

The f_a was also estimated using known relationships of absorptive capacity between different animal species and humans, and the general allometric principle that species with higher body weight have a slower metabolism per kilogram of body weight. Naproxen has a very high P_e and therefore species differences in oral uptake are not expected. AZD3582, which has an intermediate P_e, could potentially be absorbed to a greater extent in an animal species with a higher absorptive capacity. Rats generally have similar or slightly lower absorption capacity to humans (Fagerholm et al 1996; Lennernäs 1997; Chiou & Barve 1998), whereas the dog appears to have the potential for an overall higher capacity than rats and humans (Lennernäs 1997; Chiou et al 2000). For several reasons (eating behaviour, GI anatomy, transit times and physiology similar to humans), the minipig is considered to be a (probably the most) suitable animal species for studies of oral drug absorption (Davis et al 2001). Absorption data in the minipig are, however, quite limited. The estimated f_a values of intact AZD3582 in rats, minipigs and dogs were ≥ 35–43, ≥ 13 and ≥ 3.9%, respectively (Fagerholm et al 2005), and the f_a values of the AZD3582 dose (and its naproxen content) in rats and minipigs were 84–97 and 98%, respectively (Fagerholm et al 2005). Naproxen is reported to be completely absorbed in various animal species and humans (Runkel et al 1972; Davies & Anderson 1997; Fagerholm et al 2005). Naproxen belongs to Class II (high P_e and low solubility) in the Biopharmaceutics Classification System (BCS). The in-vivo GI absorption of naproxen is not limited by solubility and dissolution (Class I behaviour), which indicates that the solubility definition in the BCS may be too strict for acidic compounds (Yazdanian et al 2004).

In these predictions, the following assumptions were made: (i) AZD3582 and naproxen fit into the established relationships for P_e and f_a (Fagerholm et al 1996; Berggren et al 2004); (ii) the formulation, AZD3582 and naproxen did not change residence times in various parts of the GI tract; (iii) dissolution and degradation rate constants were similar in vitro and in vivo; (iv) dissolution, degradation and absorption rate constants were stable along the intestine; (v) GI degradation of AZD3582 was most efficient in smaller animals and least efficient in humans; and (vi) the capacity of the GI tissues to absorb AZD3582 and naproxen in the used species agreed with the general species similarities and differences.

Prediction of plasma exposure and PK

Predictions of plasma exposure and PK in humans were made for several reasons: (i) for evaluating the potential of AZD3582 to donate nitric oxide during a whole dosing interval; (ii) for evaluating the potential of AZD3582 to provide sufficiently rapid and high extent of naproxen to the blood circulation; (iii) for selection of doses in the first study in humans; and (iv) for evaluation of increased nitrate exposure.

AZD3582. The metabolism and excretion of AZD3582 were not fully characterized in vitro or in animals and therefore there were insufficient data to make an appropriate prediction of the ADME/PK and plasma concentration vs time profile of AZD3582 in humans. For a rough prediction to be made, general allometric scaling principles were applied: (i) the unbound CL (CL_u) and metabolism increases less than proportionally in relation to body weight; (ii) different species have similar V_{ss} per kilogram of body weight (Mordenti 1986); and (iii) there are similarities/differences in absorptive capacity between species (as described in Prediction of fraction absorbed and absorption rate). The potential for AZD3582 to be degraded in blood capillary walls and body tissues, and thereby not be redistributed back into the blood circulation, was also taken into consideration. The rapid decline of plasma concentrations following peak levels in animals was taken into account when predicting the accumulation potential after repeated dosing. There were no apparent dose- and time-related dependencies in animals and this was also expected in humans. The F, V_{ss}, CL and $t_{½}$ of AZD3582 in minipigs and dogs were 1.4 and 3.9%, ≥ 3.4 and ≤ 7.7 L kg^{-1}, ≤ 175 and 43 mL min^{-1} kg^{-1}, and 7 and 7 h, respectively (Fage-rholm et al 2005).

Naproxen. The same allometric scaling and absorption capacity principles as for AZD3582 were used for prediction of the oral F and exposure of naproxen after AZD3582 vs naproxen dosing. The predicted apparent k_a for naproxen following AZD3582 administration was compared to its $t_{½}$ to evaluate possibilities for 'flip-flop' (absorption-rate-limited elimination). In-vitro dissolution data were available for AZD3582, but not for naproxen tablets or granulates. Differences in absorption rates after AZD3582 and naproxen dosing therefore could not be predicted. The lower P_e, degradation step and the expected longer dissolution for AZD3582 were used to estimate the trend of absorption rate vs naproxen administration. Since no inhibition or induction of metabolism has been observed in vitro, and the

daily exposure of naproxen does not seem to change with time, a similar accumulation of naproxen after AZD3582 and naproxen dosing was assumed; dose- and time-related dependencies similar to naproxen dosing were also expected.

Nitrate. Prediction of the increase in nitrate exposure was based on data obtained after oral dosing of AZD3582 46 μmol kg^{-1} qd to minipigs (corresponds to a daily dose of 1200 mg to humans) and on the maximum possible nitrate content in therapeutically relevant daily AZD3582 doses (375 to 750 mg twice daily) vs the daily endogenous production and exogenous supply by food and drink (FDA/WHO 1995; Jungersten et al 1996). Inorganic nitrate is known to have near-complete bioavailability after oral administration (Schultz et al 1985).

Human in-vivo studies

Human ADME/PK data for AZD3582, naproxen, naproxen-related metabolites and nitrate were obtained in 16 studies in healthy volunteers and patients (Table 1). Naproxen was dosed and used as the reference compound in six of the studies. AZD3582 was administered orally as a SEDDS hard gelatin capsule, with the exception of the first AZD3582 study, in which two of the doses were given as an emulsion. No intravenous AZD3582 formulation has been developed for human use. Thus, no intravenous studies have been performed, and F, V_{ss} and CL estimates are not available. Naproxen was given as encapsulated (hard gelatin) tablets (Naproxen Astra) or granulates. Doses were administered with water, with or without food, to various populations, and under certain restrictions (normal or nitrate- and sodium-restricted diets).

The human studies were performed in accordance with the ethical principles consistent with the Declaration of Helsinki, the International Conference on Harmonization/Good Clinical Practice and applicable regulatory requirements. Study protocols were approved by the Institutional Ethics Committees or Independent Review Boards. All subjects gave written, informed consent.

Bioanalytical methods

The determination of total plasma concentrations of AZD3582 was made by coupled column liquid chromatography–electrospray tandem mass spectrometry. The limit of quantification (LOQ) was 4 nM. The determination of total and unbound concentrations of naproxen in plasma samples was based on reversed-phase liquid chromatography with fluorescence detection, with direct injection of diluted plasma. The LOQs were 0.5 μM and 5 nM, respectively. The concentrations of naproxen-related metabolites were determined by liquid chromatography and on-line radiochemical detection. [^{3}H] activity levels were measured using Packard liquid scintillation counters with the facility for computing quench-corrected disintegrations per minute (dpm). The limit of detection (LOD) was twice background radioactivity levels. Nitrate concentrations were determined by anion-exchange liquid chromatography with UV detection. The LOQs in plasma and urine were 0.5 and 2.3 μM, respectively. The analysis of [^{15}N]-nitrate concentrations was performed using an isotope ratio method based on conversion of nitrate to nitrobenzene followed by gas chromatography–mass spectrometry.

Calculation of PK parameters

PK parameters were calculated non-compartmentally using WinNonlin Professional (Pharsight Corporation, Mountain View, CA), with the exception of F_{rel}, f_u and the blood/plasma ratio, which were calculated in Microsoft Excel (Microsoft Corp., USA) or SAS (SAS Institute Inc., Cary, NC). In many individuals and in all studies, low plasma concentrations (in relation to the

Table 1 Human in-vivo studies with AZD3582 and naproxen containing ADME/PK data

Study	Study description	Dosing	Demographics
1	Single and multiple dose	N: single 250–750 mg, multiple 500 mg bid	Y/H/C/M
2	Single and multiple dose	A: single 50–2250 mg, multiple 1125 mg bid	Y/H/C/M
3	Gastroduodenal endoscopy	A: 750 mg bid; N: 500 mg bid	Y/H/C/M + F
4	Acute analgesia	A: single 375–2250 mg; N: single 500 mg	Y/P/V/M + F
5	Formulation, food interaction	A: single 375 mg	Y/H/C/M + F
6	Single and multiple dose in elderly	A: 750 mg bid	E/H/C/M + F
7	Mass-balance	[^{15}N]-[^{3}H]-A: single 750 mg	Y/H/C/M
8	Chronic analgesia (OA)	A: 125–750 mg bid; N: 500 mg bid	Y+E/P/V/M + F
9	Formulation, food interaction	A: single 375 mg	Y/H/C/M
10	Renal function	A: single 750–1500 mg; N: single 500 mg	Y/H/C/M + F
11	Single dose in Japanese	A: single 50–2250 mg	Y/H/J/M
12	High multiple dose	A: 750–1500 mg bid	Y/H/C/M + F
13	Chronic analgesia (OA)	A: 750–1125 mg bid, 750 mg qd	Y + E/P/V/M + F
14	Multiple dose in Japanese	A: 375–1125 mg bid, 750 mg qd	Y/H/J/M
15	Gastroduodenal endoscopy	A: 375–750 mg bid, 750 mg qd; N: 250–500 mg bid	Y/H/C/M + F
16	Capsule hardening	A: single 375 mg	Y/H/C/M + F

A, AZD3582; C, Caucasian; E, elderly; F, female; H, healthy; J, Japanese; M, male; N, naproxen; OA, osteoarthritis; P, patient; V, various ethnic groups; Y, young.

LOQ) and irregular plasma concentration vs time profiles did not allow evaluation of the full PK profile for 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 LOQs appearing in terminal samples were omitted from the analysis.

In Study 8, plasma was taken over a 12-h period at steady state following AZD3582 and naproxen dosing. NONMEM (version V) (Beal & Sheiner 1992) was used for the population PK analysis of naproxen following AZD3582 and naproxen dosing in this study. Xpose 3.0 (Uppsala University), run in an S-PLUS (MathSoft Inc., version 2000) environment, was used for data checkout, graphics and other diagnostic techniques to assist the model building (Jonsson & Karlsson 1999). The covariate models were built using the stepwise covariate model-building algorithm implemented in PsN, version 1.41 (Division of Pharmacokinetics and Drug Therapy, Department of Pharmacy, Uppsala University) as described by Jonsson and Karlsson (Jonsson & Karlsson 1998). Covariates (gender, race, age, body weight, S-albumin and S-creatinine) were tested on the PK parameters CL_u, $V_{c,u}$ and B_{max} (maximum binding capacity to plasma proteins). Both total and unbound naproxen concentrations were included in the analysis. Examination of goodness-of-fit plots and comparison of the objective function values of competing models determined the form of the structural model. A drop in objective function of 10.83, corresponding to a P value of less than 0.001, was required for the selection of a more complex model.

Statistical methods

Differences in plasma exposures between treatments were examined using analysis of variance (ANOVA) or non-parametric pair-wise comparisons. A significance level of $P < 0.05$ denoted significance. Only differences within a study were analysed. Non-linearity in the dose vs plasma exposure relationships was investigated using a power model, which assumes a linear relationship between log plasma exposure and log dose. It should be noted that AZD3582 demonstrates low, irregular and varying plasma exposure, which is sensitive to food intake at any time, naproxen has saturable PK, and unbound naproxen plasma exposure is affected by food intake at any time. For these reasons, statistical analyses of AZD3582 data often fail to demonstrate differences or have not been included, and differences of total naproxen exposures between treatments are less than for unbound naproxen exposures.

Results

In-vitro/in-silico data and predictions of human ADME/PK and exposure

The preclinical ADME/PK data presented and used in this section are taken from Fagerholm et al (2005).

Prediction of fraction absorbed and absorption rate

The predicted or measured human P_e and f_a values, and rate constants for AZD3582 and naproxen are presented in Table 2. The gastric emptying and intrinsic absorption (permeation) rates appeared to be the most and least rapid, respectively, of the absorption processes for AZD3582. AZD3582 was predicted to have intermediate P_e (corresponding to an f_a of 70–90% if the compound were stable in GI fluids) and f_a (23–24%) values, and to be degraded to naproxen about three times more rapidly as it was absorbed as intact AZD3582. Near-complete uptake of naproxen (94–97%: 23–24% from absorbed AZD3582 and 71–73% from naproxen formed within the GI tract) was estimated. The absorption processes for AZD3582 and naproxen following AZD3582 administration are illustrated in Figure 2.

A higher f_a of the AZD3582 dose than in rats (84–97%) and a similar value to minipigs (98%) was expected. This agrees well with the value predicted from in-vitro data (94–97%; see above). The fraction of dose absorbed intact was also assumed to be higher than in the rat (at least 35–43%) and similar to that in the minipig (≥ 13%). These numbers are also similar to those predicted from in-vitro data (23–24%; see above).

Prediction of plasma exposure and PK

AZD3582. The degree of plasma-protein binding of AZD3582 in silico was predicted to be approximately 0.1%. Thus, plasma-protein binding was assumed to be negligible and not to cause species differences in tissue distribution. In contrast to the minipig, AZD3582 exposure in humans was expected to be favoured by an expected lower CL and first-pass metabolism, and an expected greater potential for distributed AZD3582 to be redistributed back to the blood circulation. Thus, the C_{max} of AZD3582 was assumed to be higher than in minipigs (≤ 13 nM after 10 mg kg^{-1}, which corresponds to a dose of 750 mg in humans). It was not clear whether to expect lower, similar or higher C_{max} values than in dogs

Table 2 Predicted or measured human P_e and f_a values and rate constants for AZD3582 and naproxen following oral administration of AZD3582 and naproxen

Parameter	Unit	Dosed AZD3582		Dosed naproxen
		AZD3582	Naproxen	Naproxen
P_e	($\times 10^{-6}$ cm s^{-1})	20	800	800
Intrinsic k_a	(h^{-1})	0.08	3.2	3.2
k_{diss}	(h^{-1})	1.4	–	n.a.
k_{degr}	(h^{-1})	0.23	–	–
Apparent k_a	(h^{-1})	0.08	0.31*	≤ 3.2**
f_a	(%)	23–24	94–97***	100

n.a., not available. *0.08 h^{-1} for naproxen absorbed as AZD3582 and 0.23 h^{-1} for naproxen formed in the GI tract. **Slower than 3.2 h^{-1} if dissolution is slower than permeation. ***71–73% as naproxen formed from AZD3582 in the GI tract.

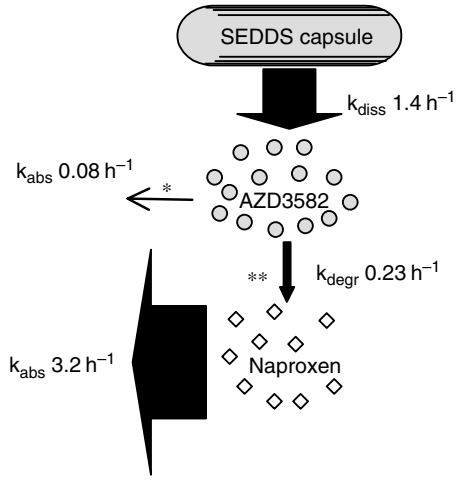

Figure 2 Intrinsic rate constants in the absorption process for AZD3582 and naproxen following AZD3582 administration. The rate constant for gastric emptying (k_{ge}; 3.8 h^{-1}) is not included in the figure. * and ** represent the rate-limiting steps for the GI uptake of AZD3582 and naproxen, respectively.

(≤ 442 nM after 7 mg kg^{-1}). The absorptive capacity (which favours high exposure in the dog), but the smaller body weight of the dog (which favours high exposure in humans) indicated that we could expect a similar C_{max} in dogs and humans. The CL was predicted to be lower and the apparent V_{ss} to be higher in humans compared with animals. Thus, $t_{½}$ was expected to be longer in humans than in animals. Based on the very short $t_{½}$ (few minutes) during the distribution phase in animals, a large difference between peak and trough concentrations was also expected in humans. Consequently, and regardless of the terminal $t_{½}$, negligible accumulation following repeated dosing was anticipated. There were no apparent time-, dose- and gender-related dependencies in animal studies, and this was also expected in humans.

Naproxen. The predicted f_a for intact AZD3582 and naproxen indicated 94–97% uptake of the AZD3582 dose (as AZD3582 and its metabolites). Absorption of intact AZD3582 was expected to be greater in humans than in animals, while metabolism was expected to be slower. Consequently, it was difficult to predict the metabolic loss of naproxen in humans. According to the predictions, a maximum loss of 23–24% naproxen was anticipated. Thus, maximally, about 30% of the naproxen content in AZD3582 (vs naproxen dosing) was expected to be lost. A slower systemic uptake of naproxen following AZD3582 administration was also expected. The elimination rate constant of naproxen in humans, 0.04 to 0.06 h^{-1} (Davies & Anderson 1997), was slower than the predicted apparent k_a of naproxen. Thus, absorption-rate-limited elimination of naproxen following AZD3582 dosing was not anticipated, and a similar naproxen $t_{½}$ after AZD3582 and naproxen administration was expected. As reported for naproxen in humans and observed in animal studies, we assumed a less-than-proportional increase of total naproxen exposure with increasing doses, but a dose-proportional increase of unbound naproxen exposure with dose. A changed PK with time for naproxen was not indicated by our induction and inhibition data or by the literature. Thus, a time-dependent PK of naproxen following AZD3582 dosing was not expected. Food caused a prolonged time to naproxen C_{max} in minipigs, and a similar finding was anticipated in humans.

Nitrate. The maximum amounts of nitrate that can be formed from AZD3582 750 and 1500 mg are 135 mg and 270 mg, respectively. These amounts are comparable to the daily mean dietary (water exclusively) intakes of nitrate in various countries (31 to 409 mg per person per day) (FDA/WHO 1995) and are higher than that formed endogenously (50 mg per day) (Jungersten et al 1996). Thus, it was predicted that AZD3582 (following repeated dosing) would add approximately as much nitrate to the body as was already circulating. Plasma nitrate levels increased from 34 ± 2 to 205 ± 27 μM following intake of nitrate-rich food containing 1000 mg nitrate (Jungersten et al 1996). AZD3582 750 mg contains, maximally, 13.5% of the nitrate content in this portion. Based on the exposure increase and this difference, it was predicted that a single dose of AZD3582 750 mg would give a nitrate C_{max} in plasma of, maximally, approximately 60 μM, which is substantially lower than after intake of nitrate-rich food. A prediction was made from data obtained in minipigs. After administration of 46 μmol kg^{-1} (dose corresponds to 1200 mg daily in humans), a doubling of the basal plasma nitrate levels was observed, and the maximum increase from baseline after a dose was approximately 70 μM.

ADME/PK and exposure

AZD3582

Plasma concentrations of AZD3582 were in the lower nM range and the plasma concentration vs time profile showed large intra- and interindividual variability (Figure 3). AZD3582 did not accumulate after repeated once- and twice-daily dosing. With the exception of a few subjects given AZD3582 1125 to 2250 mg, plasma levels at 12 h post dosing were below the LOQ (4 nM) and these quantifiable 12-h plasma levels were just above the LOQ. In general, plasma levels declined to levels below the LOQ within 6 h after dosing. C_{max} values after dosing of AZD3582 375 and 750 mg in fasted subjects ranged between < 4 (LOQ) to 64 nM and < 4 to 57 nM, respectively. Intake of food, both together with AZD3582 and up to 2.5 h after AZD3582 administration, led to an increase in plasma exposure (Figure 3). In a food interaction study (fasted vs fed; Study 5) the C_{max} and AUC were 4 and 7 times higher, respectively, when AZD3582 was taken with food. C_{max} values after dosing of AZD3582 375 and 750 mg together with food ranged between 6–111 nM and 8–192 nM, respectively. In fasted subjects, the median t_{max} was 1.5 h. When administered with food, the median t_{max} of AZD3582 occurred at 2 h. The highest individual C_{max} observed when food was ingested at 1.5 h after a 750 mg dose was 188 nM. When food was taken at 2 to 2.5 h after AZD3582 administration (Study 2), a second peak was observed at

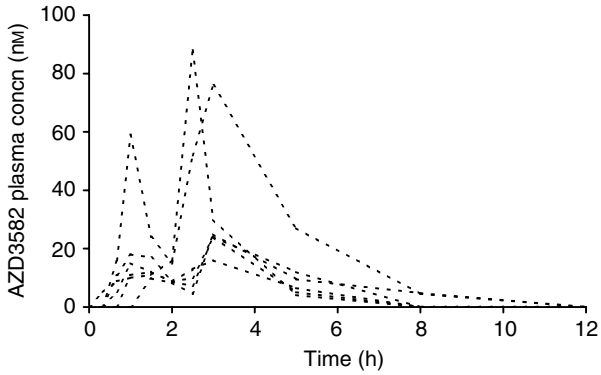

Figure 3 Individual plasma concentrations of AZD3582 1500 mg after administration of a single dose to six healthy, young, male volunteers (Study 2). Food was ingested 2–2.5 h after dose administration. Note: Data obtained after a 1500 mg dose were chosen to illustrate the profile because of the higher incidence of plasma levels below the LOQ (4 nM) at the lower and therapeutic doses.

approximately 3 h (Figure 3). In most cases, this peak was higher than the first one observed just before food intake. The highest individual C_{max} observed in all studies was 710 nM in an osteoarthritis (OA) patient on 1125 mg bid treatment for 6 weeks (Study 13).

In Study 2, AZD3582 375 mg was given both as an emulsion and a capsule. The C_{max} was 2–3 (mean) to 4–5 (maximum individual) times higher after administration of the emulsion. The median t_{max} was also shorter after dosing of emulsion (0.3 vs 2.2 h).

Nitrate- and sodium-restricted diets appeared to influence the plasma exposure of AZD3582. Fasted volunteers on a normal diet demonstrated C_{max} values three to five times higher (three times for mean C_{max} and five-fold for individual maximum C_{max}) than in fasted volunteers on a nitrate-restricted diet (Study 7). These subjects were not given food at, or within at least 4 h after, dosing. In subjects on sodium-restricted diets (10 mmol day^{-1}; 150 mmol day^{-1} in control group), food intake at 1.5 h after dosing did not lead to an increase in plasma levels, as in volunteers on a normal diet (Study 10). Plasma levels in sodium- and non-sodium-depleted subjects after AZD3582 1500 mg were similar up to the time of food intake. Thirty minutes after food intake, the average AZD3582 level in subjects with normal sodium intake was almost three times higher than in subjects on a sodium-restricted diet.

The C_{max} of AZD3582 increased slightly more than proportionally in relation to dose (the regression coefficient of log dose (β) using the power model was greater than 1 (95% CI 1.094–1.975)). Mean (maximum individual) C_{max} values for AZD3582 doses of 375, 750, 1500 and 2250 mg in a single-dose study in Caucasians (Study 2) were < 4 (5), 12 (21), 42 (88) and 61 (122) nM, respectively. The corresponding values obtained in a similar study in Japanese subjects (Study 11) were < 4 (11), 17 (26), 40 (120) and 32 (50) nM, respectively. The plasma exposure did not appear to be dependent on age, gender, race and time, and no apparent differences were observed between volunteers and OA patients.

Due to the irregular plasma concentration vs time profile and low plasma concentrations (in relation to the LOQ), AUC and $t_{½}$ estimates were generally poor. In cases where it was possible to estimate the terminal $t_{½}$ after administration of therapeutically relevant doses of AZD3582 (375 and 750 mg), it was approximated to 3–4 h. At higher doses where quantifiable levels were found at 12 h after dosing (in very few subjects only), the terminal $t_{½}$ was estimated to be 10 h (Figure 3).

Of the administered [^{3}H]-radioactivity ([^{3}H]-label in the naproxen part of [^{3}H]-[^{15}N]-AZD3582), 94 ± 1.3 and 2.0 ± 1.0% was excreted in urine and faeces after oral dosing, respectively (Study 7). Thus, the f_a of the AZD3582 dose was ≥ 94%. With ≥ 94% absorbed and an F_{rel} of naproxen vs naproxen dosing of 80–85% (see Naproxen section in Results), the f_a of intact AZD3582 was estimated to be at least 9–20% (with the assumption that the metabolites of AZD3582 are smaller and more permeable than AZD3582).

Naproxen

Compared with naproxen administration, AZD3582 administration resulted in a slower and less extensive systemic uptake of naproxen. The C_{max} and AUC after a single dose of AZD3582 375 mg were, on average, 27 and 7% lower, respectively (values corrected for differences in body weight), than after a single dose of naproxen 250 mg (Figure 4). The corresponding unbound C_{max} and AUC ($C_{u,max}$ and AUC_u) were 40–55% and 15–20% lower after AZD3582 administration, respectively. Thus, the F_{rel} vs naproxen dosing was approximately 80–85%. According to [^{3}H]-radioactivity data presented in AZD3582 in the Results section, the f_a of the naproxen content was ≥ 94%. Since the F_{rel} vs naproxen dosing was 80–85%, the metabolic loss of naproxen was 9–20%. The median t_{max} was 1 h later, 3 h vs 2 h, after AZD3582 dosing. The $t_{½}$ (15 to 22 h) was similar after AZD3582 and naproxen administration. AZD3582 and naproxen also demonstrated

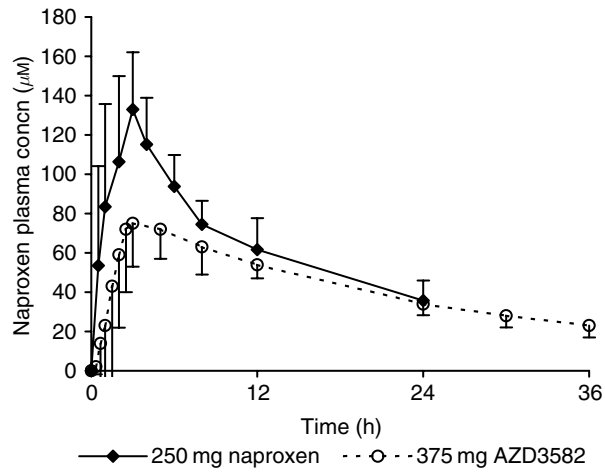

Figure 4 Mean ± s.d. plasma concentrations of naproxen after administration of single doses of AZD3582 375 mg (n = 6) and naproxen 250 mg (n = 8) to fasted, healthy, young, male volunteers (Studies 1 and 2).

similar $C_{ss,min}$ values following repeated dosing, but the $C_{ss,max}$ was 23% lower after AZD3582 dosing (95% CI for the ratio was 0.70–0.86). In Study 2, AZD3582 375 mg was given both as an emulsion and a capsule. The AUCs were similar, while the median t_{max} was shorter (1.5 vs 2.5 h) after administration of the emulsion.

The systemic plasma exposure of naproxen increased less than proportionally in relation to dose within the therapeutic AZD3582 dose and concentration ranges. For AUC and C_{max} the power method gave β estimates equal to 0.83 (95% CI 0.749–0.904) and 0.84 (95% CI 0.769–0.916), respectively. The average f_u was 0.05–0.10% at low plasma concentrations and increased (apparently continuously) up to approximately 0.3–0.6% at the high plasma concentrations obtained after a 2250 mg single dose, and after 1125 and 1500 mg bid. There was quite a large spread in f_u values and a maximum individual value of 1.1% was observed (in Study 6). The plasma exposure of unbound naproxen at trough increased proportionally in relation to dose (Study 14). A linear relationship was also observed for unbound, steady-state plasma concentrations obtained 4 h after dosing for doses up to AZD3582 750 mg bid. At a higher dose (1125 mg bid), however, the 4-h unbound concentration was considerably higher (two-fold) than expected from the linear relationship. In Study 1, the maximum f_u occurred earlier than the C_{max} for total and unbound naproxen (2.5 h vs 3 h). In that study, food was taken 2–2.5 h after dosing. The blood/plasma concentration ratio of naproxen was 0.49–0.54, indicating no or negligible binding to blood cells.

When AZD3582 was taken with food, the rate and variability, but not extent, of systemic naproxen absorption were changed (Study 5, Figure 5). A slower systemic uptake rate was found in many, but not all, subjects in the fed state. The mean plasma concentrations at 0.5, 1 and 2 h after dosing were lower after food intake. The C_{max} and t_{max} were, however, unchanged.

A sodium-restricted diet (10 mmol day^{-1}; 150 mmol day^{-1} in the control group) appeared to influence the plasma exposure of naproxen after both AZD3582 and naproxen administration (Study 10). C_{max} and AUC values were, on average, 10% higher during sodium depletion than during normal sodium intake. Between time of dosing and 2 h post-dose the differences in plasma exposure were more pronounced. Two- to four-fold and 30–40% differences in plasma concentrations were found 0.5 h and 1 h after dosing, respectively.

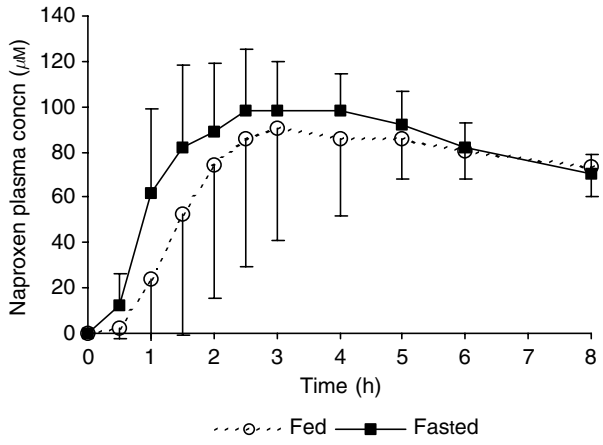

Figure 5 Mean ± s.d. plasma concentrations of naproxen after administration of AZD3582 375 mg to fasted (n = 12) and fed (n = 12) healthy, young, male and female volunteers (Study 5).

There were no apparent differences in the body-weight-corrected C_{max} and AUC values of naproxen between studies, with the exception of a 15–20% higher body-weight-corrected AUC (but not $C_{ss,max}$ and AUC_{ss}) in elderly Caucasians, and 15–20% higher body-weight-corrected AUC_{ss} (but not C_{max} and AUC) in young Japanese volunteers. Sparse and different sampling, and a probable food effect (see above) did not enable an evaluation of age-, race- and gender-related differences of f_u and unbound plasma concentration between studies; available data suggested, however, that there were no apparent differences. The $t_{½}$ of naproxen in young, healthy, male and female volunteers averaged 15–18 h. A slightly longer $t_{½}$ was observed in elderly, healthy, Caucasian male and female volunteers, and young, healthy, Japanese, male volunteers (20–21 and 19–22 h, respectively). Following twice-daily dosing, the $C_{ss,min}$ was approximately 10–15% lower in the evening than in the morning. Thus, the determination of $t_{½}$ was dependent on the terminal sampling points. No other time-dependency was found for naproxen.

The PK model in Study 8 was a one-compartment model with saturable plasma-protein binding and first-order absorption with a lag time. Different absorption rate constants were used for AZD3582 and naproxen administration. An exponential interindividual variability was used for three of the structural model parameters (CL_u, k_a after AZD3582 dosing, and B_{max}), and an exponential interoccasion variability for four of the parameters (CL_u, $V_{c,u}$, k_a after naproxen dosing, and B_{max}). The covariates included were a slope-intercept model for the influence of body weight on $V_{c,u}$ and CL_u. For a subject of median body weight, the average CL_u, $V_{c,u}$, t_{lag}, k_a (after AZD3582 dosing), k_a (after naproxen dosing), B_{max} and K_d values were estimated to be 467 L h^{-1}, 7660 L, 0.5 h, 0.64 h^{-1}, 1.65 h^{-1}, 559 μM and 0.37 μM, respectively. The interindividual variabilities in CL_u, k_a (after AZD3582 dosing) and B_{max} were estimated to be 25, 72 and 14%, respectively. The interoccasion variabilities in CL_u, $V_{c,u}$, k_a after naproxen dosing and B_{max} were 25, 49, 160 and 16%, respectively. The average $V_{c,u}$ increased with body weight at a rate of 1.5% kg^{-1} and CL_u increased at a rate of 0.6% kg^{-1} body weight. The body weight in this study ranged between 50 and 186 kg. In this analysis, the bioavailability of unbound naproxen after AZD3582 administration was estimated to be 84% relative to that after naproxen administration. Age, gender, race, S-albumin and S-creatinine were not shown to influence the PK of naproxen in this study. Nevertheless, this does not exclude the possibility that such relationships could exist in populations in whom the corresponding covariates are more extreme than those included in this study.

Naproxen-related metabolites

Plasma concentrations of naproxen-related metabolites were negligible (trace amounts of sulfate conjugate of 6-O-desmethyl naproxen were found), which was in agreement with the observation that the naproxen and [^{3}H]-activity concentrations in plasma were similar. Naproxen was completely metabolized to, and near-completely excreted in, the urine as various conjugates. The CYP450-dependent pathway was responsible for approximately 40% of the naproxen metabolism. Naproxen was mainly demethylated by CYP2C9, and the main glucuronidating enzyme(s) responsible for the formation of the conjugates of naproxen are being characterized. Metabolites M4 (sulfate conjugate of 6-O-desmethyl naproxen), M5 (acyl glucuronide of 6-O-desmethyl naproxen) and M8 (acyl glucuronide of naproxen) had $t_{½}$ values in urine similar to those of naproxen in plasma (approximately 15–20 h) and peak urine excretion rates a few hours later than t_{max} for naproxen in plasma. Apparently, metabolite M2 (ether glucuronide of 6-O-desmethyl naproxen) peaked in urine earlier than for M4, M5 and M8 (3 vs 5 h), and at the same time point as naproxen peaked in plasma. M2 had a $t_{½}$ in urine (approximately 2 h) that was considerably shorter than for M4, M5, M8 and naproxen. Urine concentrations of M2 were lower and fell below the LOQ more rapidly than for the other related metabolites. Thus, there was greater uncertainty in the M2 data. However, urine concentrations and excretion rates of M2 at 7 to 9 h after dosing were three to six times lower than expected from data obtained for M4, M5 and M8. The total amount of identified metabolites in urine collected up to 48 h after dosing corresponded to 95% of the dose.

Nitrate

The baseline plasma concentration of nitrate ([^{14}N]-nitrate) under nitrate-restricted conditions was $21 \pm 2.5\,\mu$M (Study 7). The C_{max} and $t_{½}$ of [^{15}N]-nitrate following single-dose administration of [^{15}N]-[^{3}H]-AZD3582 750 mg in the study were $66 \pm 14\,\mu$M and 6.2 ± 1.2 h, respectively. The median t_{max} was 4 h. Both this value and the initial rise in plasma concentrations of [^{15}N]-nitrate were in good agreement with those of naproxen. In a study without food and nitrate restrictions (Study 12), baseline concentrations of nitrate ranged between 12 and $43\,\mu$M. After administration of AZD3582 750 mg bid in that study, pre-dose steady-state nitrate levels in plasma increased to between 51 and $90\,\mu$M.

The [^{15}N]/[^{14}N]-nitrate C_{max} and AUC ratios during a nitrate-restricted diet were 2.1 ± 0.45 and 1.1 ± 0.12, respectively. The corresponding values during normal diet conditions were 1.4 ± 0.24 and 0.78 ± 0.12, respectively. Of the administered [^{15}N], 62 ± 5.3% (total amount $1238 \pm 107\,\mu$mol) was recovered in the urine as [^{15}N]-nitrate. This amount was higher than that for [^{14}N]-nitrate in urine during one day of a nitrate-restricted diet ($905 \pm 119\,\mu$mol), but lower than the daily amount recovered in urine following a normal diet ($1630 \pm 362\,\mu$mol). The $t_{½}$ of [^{15}N]-nitrate in urine was similar to that observed in plasma. The baseline renal CL of [^{15}N]-nitrate (and total and [^{14}N]-nitrate) was estimated to be 30–40 mL min^{-1}. It decreased after dosing and the lowest value (–40%) was found at 5 h (a few hours after peak plasma levels of naproxen, [^{15}N]-nitrate and total nitrate). Distinct twin peaks of [^{15}N]-nitrate (and total and [^{14}N]-nitrate) excretion rates in urine were found at 3 and 7 h in most subjects. The average urine excretion rate was higher at 7 h than at 3 h. No food intake was allowed until 4 h after dosing, and during this period (up to 4 h), a slight increase in plasma [^{14}N]-nitrate levels was observed.

Discussion

AZD3582

As expected, the systemic exposure to AZD3582 after administration of a therapeutically relevant dose to fasted humans was higher than in minipigs, and no apparent accumulation occurred following repeated twice-daily dosing. The systemic exposure to AZD3582 was considerably lower than in dogs. In cases where AZD3582 could be quantified for at least 12 h (in a few subjects given high doses), the terminal $t_{½}$ was roughly estimated to be 10 h, which is longer than in minipigs and dogs (approximately 7 h) and in agreement with predictions. A shorter terminal $t_{½}$ (3–4 h) was found after dosing of therapeutically clinically relevant doses. It is possible that these lower values were underestimated because of a terminal phase below the LOQ.

AZD3582 was absorbed more rapidly when given as an emulsion (compared with SEDDS). The t_{max} difference (2 h) was similar to the approximate time for complete in-vitro dissolution of the SEDDS, i.e. 100 min. A higher plasma exposure was also found after administration of the emulsion, indicating that a rapid dissolution (thus a shorter time for degradation in GI fluids) favours the GI uptake of intact AZD3582. The predicted apparent k_a of AZD3582, 0.08 h^{-1}, corresponds to an absorption $t_{½}$ of approximately 9 h. The consistency between the predicted absorption and estimated terminal elimination half-lives suggests the possibility of an absorption-rate-controlled AZD3582 elimination during the terminal phase ('flip-flop'). Thus, the quantifiable plasma concentrations at 12 h might indicate that AZD3582 was still being absorbed (from the colon) at 12 h after dosing and not totally degraded after a 12-h transit in GI fluids. This is supported by its intermediate GI P_e (a GI transit of 30 h is insufficient for complete absorption of intact substance) and degradation rates in GI fluids in vitro. The t_{max} and major part of the AUC occurred within the time of a normal average small intestinal transit (3 h) (Fagerholm et al 1996), suggesting that AZD3582 was mainly absorbed during the first few hours after dosing and from the upper GI tract (especially from the highly permeable and long small intestine). The estimated f_a of intact AZD3582 (at least 9–20%) and dose (at least 94%) agreed well with the predicted values (23–24 and 94–97%, respectively, and values estimated in the minipig ($\geq$ 13 and 98%, respectively)). The f_a of intact AZD3582 in rats was

estimated to be at least 35–43%. The apparently higher value for the lower range in the rat is contradictory to the expected lower GI stability in this species. However, even though the lower range was higher in the rat, the actual (but unknown) value of the fraction absorbed intact is not necessarily higher than in minipigs. The oral bioavailability in minipigs and dogs was only a few per cent and showed large variability. The $t_{½}$ and C_{max} values in humans, minipigs and dogs suggest that the F in humans is higher than in minipigs (mean value 1.4%), but lower than in dogs (mean value 3.9%).

In contrast to naproxen, AZD3582 appears to have negligible binding to plasma proteins. AZD3582 is less permeable than naproxen but still has the potential to permeate from the blood circulation into capillary walls and tissues at a high rate. With these characteristics, AZD3582 has the potential to transport naproxen from the blood circulation more rapidly and extensively than circulating naproxen. The volume of distribution of naproxen might be increased during the initial period when AZD3582 is present in the body. The binding sites of AZD3582 outside plasma are not known. A high V_{ss} (as observed in animals) does not necessarily mean that AZD3582 distributes throughout the body and binds well to body tissues. A high value might instead indicate extensive binding to blood cells and/or blood capillary walls. The esterase sensitivity and metabolic activity in blood capillary walls (Bennet et al 1989) argues against an extensive tissue distribution and redistribution of AZD3582 back to the blood circulation. Degradation of AZD3582 in tissues, blood capillary walls and blood cells will influence the measured V_{ss}. The apparent distribution is therefore metabolism dependent and differences in metabolism between species will cause differences in the V_{ss}.

The AZD3582 ADME/PK was dependent on dose, food intake and diet, but not time. The reason(s) for the non-proportional increase of AZD3582 exposure with dose, and apparently low AZD3582 exposure after a period with a nitrate-restricted diet, are unknown. The reason(s) for the substantial increase in bioavailability of AZD3582 after food intake were probably not enhanced GI solubility, stability and/or P_e. This is because an effect also occurred when food was taken several hours after AZD3582 intake, i.e. when most of the dose had been absorbed and to some extent resided in the lower GI tract. Instead, we hypothesize that food stimulates intestinal and/or liver blood flows, and thereby reduces the first-pass metabolism of the highly extracted AZD3582. The cardiac output, and mesenteric and portal blood flows have been reported to increase approximately 30, 80 and 100%, respectively, after food intake (Muller et al 1992; van Griensven et al 1995). The peak effect was observed at 30 min (Muller et al 1992), which is consistent with the food interaction effect on AZD3582. It is also possible that exercise influences the PK and exposure of AZD3582 (such as decreased oral bioavailability and enhanced systemic CL), since liver blood flow is known to be reduced by exercise (van Griensven et al 1995). The initial (up to 1.5 h) systemic uptake and exposure of AZD3582 in fasted individuals on normal and sodium-restricted diets were similar. However, when food was ingested 1.5 h after dosing, the expected increase of AZD3582 exposure was not found in those on a sodium-restricted diet (10 mmol day^{-1}). The mechanism behind this is unclear. We speculate that this could be due to a reduced responsiveness of blood vessels and therefore a lower potential for food to cause increases of intestinal and/or liver blood flows during sodium depletion. Sodium restriction (75 mmol day^{-1}) significantly reduces the maximal insulin-mediated vasodilation in normotensive and hypertensive subjects (Feldman & Schmidt 1999). In cirrhosis patients on low-sodium diets (20 mmol day^{-1}), a cold pressor stimulus (sympathetic stimulation) results in a significant decrease in forearm blood flow and a significant increase in forearm vascular resistance and mean arterial pressure (Wong et al 1996). Sodium loading leads to a heightened response to reflex sympathetic stimulation in these patients (Wong et al 1996).

Nitric oxide

In order to exert their pharmacological activities, nitric oxide donors (including AZD3582) must be enzymatically metabolized to liberate nitric oxide (Torfgrd & Ahlner 1994). AZD3582 is metabolized to donate nitric oxide via pathways involving CYP450 and mitochondrial aldehyde dehydrogenase, both abundantly available throughout the body (Chen et al 2002; Berndt et al 2005). In-vitro studies have demonstrated the formation of nitric oxide from AZD3582 in intestinal and capillary wall cells (Berndt et al 2005). It has been put forward that esterases (present in plasma, erythrocytes, liver and gut) may be involved in the metabolism of organic nitrate esters (Torfård & Ahlner 1994). It is, however, not known if this occurs for AZD3582. Nitric oxide released into the blood circulation is reported to be rapid (half-life of free nitric oxide in the blood is reported to be 0.05–1.8 ms) and it is extensively degraded to nitrite, which is then rapidly and extensively oxidized to nitrate (Rassaf et al 2002). Other elimination pathways of nitric oxide involve reaction with haem and other haem metalloproteins (Kelm 1999). In the vasculature, a considerable amount of the nitric oxide generated in blood vessel walls binds to haemoglobin in red blood cells to form methaemoglobin and nitrate (Kelm 1999). It has been postulated that haemoglobin might be involved in the systemic transport and delivery of nitric oxide to tissues (Hobbs et al 2002). The lipophilic nature and small size of nitric oxide implies that it can diffuse across (and probably also between) cells very easily and therefore it has good absorption and distribution capabilities. Because of the instability of nitric oxide, the measurement and ADME/PK evaluation of nitric oxide are complicated.

In clinical studies with osteoarthritis (OA) patients, blood pressure was measured up to 6 h after the first dose, at pre-dose after 1 week of dosing, and at non-specified times in relation to dose administration after 2, 4 and 6 weeks of dosing. A nitric-oxide-related acute hypotensive effect (maximum 4 to 7 mm Hg on average) was demonstrated at 1 to 3 h (Schnitzer et al 2005), which is in good agreement with the t_{max} of AZD3582 and the observation that AZD3582 generally reaches plasma levels below the LOQ within 3 to 6 h of dosing. Nitric oxide is

mainly donated within the first few hours and when the amount of AZD3582 in the blood is at its peak. However, we cannot exclude the possibility that nitric oxide might be donated to some extent during the terminal phase of each dosing interval. It has been stated (by Whittle (2004)) that AZD3582 causes a 2–4 mmHg fall in systolic blood pressure, while naproxen and rofecoxib cause increases of 1–2 mmHg. These data include the acute effect and have not been tested statistically, and should therefore not be taken as changes in baseline blood pressure. The baseline blood pressure data obtained at pre-dose after 1 week of dosing showed no apparent differences between AZD3582 (750 mg bid), naproxen (50 mg bid) and rofecoxib (25 mg bid) (Schnitzer et al 2005). The acute blood pressure lowering effect of AZD3582 appeared to diminish with time, which may be an indication of tolerance development (Lohmander et al 2005; Schnitzer et al 2005). Tolerance development is a major limiting factor for nitric oxide donors, and inhibition of catalysing enzymes is believed to be involved. Nitric oxide is capable of inhibiting (both reversibly and irreversibly) the catalytic activities of CYP450 enzymes (Vuppugalla & Mehvar 2004). Thus, CYP450 enzymes may be both a source and a target of nitric oxide (Morgan et al 2001). Whether auto-inhibition of metabolizing enzyme(s) responsible for generation of nitric oxide and/or saturation of the nitric oxide formation was a reason for the time-dependent decrease of the hypotensive effect of AZD3582 is, however, unknown. In the rabbit, inhibition and/or saturation of the formation of nitric oxide appears to be of importance for tolerance development of the blood pressure lowering effect (Adding et al 2005). In this intravenous infusion study with AZD3582, levels of nitric oxide in expired air and hypotensive effect diminished extensively (especially at the highest doses) and rapidly (within minutes) (Adding et al 2005). An alternative explanation for these observations may involve attenuation of mitchondrial aldehyde dehydrogenase (mtALDH) activity (Adding et al 2005). The involvement of mtALDH in the generation of nitric oxide from AZD3582 is, however, not known.

AZD3582 is not dissolved very rapidly and its GI P_e is not very high. This, together with a short residence time in the stomach and duodenum (major sites for NSAID-induced damage and endoscopic measurements), indicates a potential limitation for the mucosal exposure to AZD3582 and nitric oxide in this region during absorption. By utilizing GI P_e, r, t_{res}, area, k_{ge} and k_{diss} data and the f_a prediction approach (see Prediction of fraction absorbed and absorption rate), the uptake of intact AZD3582 from the stomach was roughly approximated to 0.2% of dose. The corresponding rough estimate for naproxen following naproxen dosing was 10%. The ADME/PK properties of AZD3582 and its nitric oxide may possibly explain why there were no significant differences between AZD3582 and naproxen regarding GI safety (30% lower gastroduodenal ulcer incidence with 750 mg AZD3582 bid vs 500 mg naproxen bid in a 6-week OA patient study, $P = 0.07$) and analgesic efficacy in OA patients, and renal safety in volunteers (Huledal et al 2005; Lohmander et al 2005; Schnitzer et al 2005), and contributions by nitric oxide to the GI profile of AZD3582 in humans remains to be proven. Co-therapy of NSAIDs with nitric oxide donators (including long-acting nitric oxide donation therapies such as transdermal nitroglycerin and oral isosorbide mononitrate) has been reported to be compatible with some protection from development of GI ulcers and bleeds compared with NSAID therapy alone (Lanas et al 2000; Rodríguez & Hernández-Díaz 2001). Besides differences in potency and efficacy in nitric oxide donation (AZD3582 was as efficacious but less potent than nitroglycerine during infusion studies in the rabbit) (Adding et al 2005), different ADME/PK properties might explain why different nitric oxide donor therapies differ with regards to GI protection potential. Thus, we do not exclude that other dosing and formulation strategies might enable a demonstrable and pronounced contribution of nitric oxide to the safety of AZD3582.

Nitric oxide donors significantly enhanced the P_e of poorly absorbed compounds in the rat intestine in vitro at concentrations of 0.01–0.1 mM (Yamamoto et al 2001), and increased rectal uptake of insulin (low P_e) has been found in the presence of nitric oxide donors in rabbits (Utoguchi et al 1998). Since substances with intermediate and high P_e (such as AZD3582, naproxen and nitrate) are well absorbed even without absorption enhancers, nitric oxide donation was not expected to cause increased systemic exposure of AZD3582 and its metabolites. Naproxen is well known to cause GI damage and consequently also enhances the P_e. In Study 3 (12 days dosing with AZD3582 750 mg bid; maximum GI levels approximated to 4–9 mM (dose dissolved in 200–500 mL fluid)), the urine excretion of two marker molecules with low P_e (lactulose and L-rhamnose) were not increased (Hawkey et al 2003), indicating that the nitric oxide and/or naproxen content of AZD3582 caused GI damage without an apparent decrease in GI barrier function and enhancement of GI P_e.

Naproxen

As expected from predictions and animal data, both the rate and extent of systemic naproxen absorption were somewhat lower following AZD3582 dosing vs naproxen dosing. This could be explained by the fraction of the dose that was absorbed as the less permeable AZD3582. A slower dissolution rate might also contribute to the slower absorption rate. During the first half hour after dose administration, the systemic absorption rate of naproxen was much slower after AZD3582 (vs naproxen) dosing. Subsequently, increases in naproxen plasma concentrations were similar following the two treatments, indicating that AZD3582 was mainly absorbed as naproxen during this phase (Figure 4). Apparently, AZD3582 and naproxen were absorbed more slowly (lower amount per time and area unit) and distally in the GI tract than after administration of naproxen. The GI mucosal naproxen exposure has been shown to be of importance for the development of GI damage (Somasundaram et al 1997; AstraZeneca data on file). In a study in the rat intestine,

naproxen concentrations of approximately 2–3 mM (probably similar to concentrations of naproxen in upper GI fluids after administration of naproxen 250 or 500 mg to humans, but approximated to be higher than following administration of AZD3582) and above caused significant local damage to enterocyte mitochondria (Somasundaram et al 1997). The differences between apparent k_a values after AZD3582 and naproxen dosing (0.31 vs $\leq 3.2\,h^{-1}$ according to predictions and 0.64 vs $1.65\,h^{-1}$ according to modelling of in-vivo data) indicate at least a two- to three-fold higher flux of naproxen across the upper intestinal epithelium after naproxen (vs AZD3582) dosing. The importance of the local GI naproxen exposure for the development of GI damage has previously also been shown by Hawkey et al (1996), Shanbhag et al (1992), Sheha et al (2002) and Tammara et al (1993). In these repeated dosing (up to 1 week's administration) studies in humans and rodents, masking of the carboxylic acid group or reduction of the dissolution rate of naproxen led to an approximate halving of the GI damage (with maintained systemic naproxen exposure and efficacy). This improvement vs naproxen is similar to what has been observed with AZD3582, and indicates a minor role of nitric oxide in GI protection. In acute studies, the GI damage is to a greater degree caused by the local effects of NSAIDs (vs systemic effects). CINODs and other NSAID pro-drugs may therefore have a more advantageous GI profile vs NSAIDs in acute experiments than in repeated dose studies.

The F_{rel} of AZD3582 (vs naproxen dosing) was expected (from predictions) to be a minimum of 70%. The actual F_{rel} was 80–85%, which is similar to that found in the minipig. The F_{rel} in rats was somewhat lower (55%), which is due to a greater extent of unabsorbed dose and greater metabolic loss of naproxen than in minipigs and humans. When AZD3582 was no longer quantifiable in plasma, the plasma levels (including $C_{ss,min}$) and $t_{½}$ of naproxen were similar after AZD3582 and naproxen administration, which suggests that the naproxen loss occurs mainly during the first-pass through the GI mucosa, portal blood and liver. The similar $t_{½}$ also suggests that elimination is not absorption rate-controlled during this phase. Furthermore, it speaks against a retained increase in the tissue distribution of naproxen. Naproxen is metabolized by intracellular CYPs and conjugating enzymes and therefore we assumed that the metabolic loss of naproxen after AZD3582 (vs naproxen) dosing took place within metabolizing cells rather than in GI fluids. Both AZD3582 and naproxen have to pass through gut wall cells to reach the blood circulation. For these reasons, it seems unlikely that naproxen is more efficiently metabolized in the gut wall when absorbed as AZD3582. The lower naproxen exposure in plasma after AZD3582 administration (vs naproxen administration), especially directly after dosing, could possibly be explained by a greater potential of AZD3582 to distribute out from blood and deliver its naproxen to metabolizing cells (possibly to the greatest extent in the liver). We cannot exclude the possibility that AZD3582 and/or nitric oxide donated within GI mucosal cells stimulates the gut wall metabolism of naproxen and thereby causes a reduced oral bioavailability. However, such a rapid induction seems unlikely. The enhanced CL and metabolic loss vs naproxen dosing following intravenous dosing in animals support our hypothesis (and a temporary increase in the volume of distribution of naproxen).

Naproxen could not be quantified in urine, which indicates the possibility that it might be metabolized in the kidneys and/or completely reabsorbed from the renal tubuli. Naproxen is highly permeable, and passive tubular reabsorption of an acid such as naproxen is favoured by a lower pH in the tubuli than in the blood. Naproxen interacts with renal drug transporters (Khamdang et al 2002) and therefore the possibility that it utilizes active transporter(s) for reabsorption in the renal tubuli cannot be excluded.

The observation that AZD3582 and naproxen gave similar terminal $t_{½}$ values for naproxen and that the oral bioavailability of AZD3582 is probably very small (maximally a few per cent) in relation to that of naproxen (approximately 80% after AZD3582 dosing) speaks against the importance and significance of an enhanced tissue distribution of naproxen after AZD3582 dosing.

Diet, food, time, dose and body weight were found to influence the PK and exposure of naproxen. A low-salt diet has been shown to reduce the gut wall metabolism and enhance the oral bioavailability of quinidine and verapamil in humans (Darbar et al 1998; Fromm et al 1999). Quinidine is a CYP3A4 and P-glycoprotein substrate with low first-pass extraction, and verapamil is a CYP1A2, 2C, 3A and P-glycoprotein substrate with significant gut wall metabolism and high first-pass extraction (Darbar et al 1998; Fromm et al 1999). The data obtained with these two drugs indicate a local (not systemic) alteration of metabolism and/or transport in the intestinal mucosa, and further suggest that β-adrenergic activation by a low-salt diet is one of the components of a signalling pathway whereby intestinal drug disposition is suppressed (Fromm et al 1999). Naproxen is also metabolized by CYP1A2 and CYP2C9 in humans (Davies & Anderson 1997; Swedmark et al 2002). A 10% higher plasma exposure of naproxen was found in volunteers on a sodium-restricted diet. A similar effect was observed after AZD3582 and naproxen dosing, which could imply that naproxen was metabolized to some extent (at least 10% of dose) during passage through the gut wall. Food intake influenced the rate, but not extent, of naproxen absorption. The increased variability and slower uptake observed in some individuals are believed to be mainly due to the influence of food on gastric emptying. $C_{ss,min}$ levels of naproxen were, on average, 10–15% lower in the evening than in the morning, indicating a lower metabolic rate during the night. Thus, the estimation of $t_{½}$ is dependent on terminal sampling times.

In accordance with previous knowledge and our predictions, the systemic exposure of naproxen increased less than proportionally in relation to dose. A dose-proportional increase of unbound naproxen exposure has been demonstrated in the literature (FDA 1997). This was also found for unbound naproxen concentrations obtained pre-dose at steady state in our studies. However, such a

linear relationship was not existent for data obtained just after food intake, especially around the t_{max} at high doses/concentrations. This indicates that food components and/or their degradation products displace naproxen from plasma proteins (particularly albumin). Fatty acids have been reported to bind to an albumin binding site particularly susceptible to conformational changes in the protein, and different from that binding naproxen (Hervé et al 1994). Thus, an occasional conformational change of albumin by food components, with consequent displacement of naproxen from albumin, seems possible. An alternative hypothesis for such a finding could be that naproxen binding may be more saturated in tissues than in plasma (especially at high concentrations) and that this causes a delay in the tissue distribution and longer time to equilibrium of unbound naproxen. This does not, however, explain the observation that f_u peaked just after food intake and before both total and unbound naproxen concentrations. As a consequence of this food effect, relationships between f_u and total concentrations could not be well established. The variability of f_u was quite large, and some unexpectedly high f_u values were measured at both low and high naproxen plasma concentrations. A plotting of f_u and total naproxen concentration data showed, however, an increasing average f_u with total naproxen concentration.

Naproxen-related metabolites

The finding of a naproxen-related metabolite (M2; ether glucuronide of 6-O-desmethyl naproxen) with a $t_{½}$ (in urine) much shorter than for naproxen itself (in plasma) and other naproxen-related metabolites (in urine), and a time of peak excretion rate in urine earlier than for other naproxen-related metabolites and the same as the t_{max} for naproxen in plasma was unexpected. This metabolite has not been identified and quantified after naproxen administration before, although it might have existed. Two hypotheses that might explain this finding are that M2 is formed during the passage through the gut wall (and should therefore be expected to also occur after naproxen dosing) and/or that M2, in contrast to the other naproxen-related metabolites, is formed from AZD3582 via a pathway not including naproxen. The latter hypothesis is supported by identification of a demethylated naproxen with a spacer fragment in rat bile and the metabolic loss of naproxen after AZD3582 dosing. The metabolic loss of naproxen was, however, greater than the fraction of dose that was excreted in urine as M2 (9–20 vs 3%). This finding, together with at least 95% recovery (of dose) of the identified metabolites in urine, indicates that an alternative pathway (if it exists) is less important in explaining the naproxen loss than greater liver distribution and extraction during first-pass metabolism.

Nitrate

Predicted and observed data for naproxen (see above) suggest that the largest portion of naproxen from AZD3582 is already formed in GI fluids. Initially, [^{15}N]-nitrate and naproxen appear to be formed and systemically absorbed at similar and rapid rates. This also indicates that [^{15}N]-nitrate is formed mainly before GI uptake. We cannot, however, exclude the possibility that nitrate is absorbed mainly as nitrooxy-butyl or other possible nitrooxy-compounds. Such compounds are likely to be highly permeable and rapidly absorbed. [^{15}N]-nitrate peaked in plasma slightly later than naproxen despite a shorter $t_{½}$. It is not clear whether this is due to the saturated nitrate CL at this time point and/or formation of [^{15}N]-nitrate from other (possible) circulating nitrogen oxides. Nitrate is a metabolic end-product of many nitrogen oxides, including nitric oxide. Since the $t_{½}$ of [^{15}N]-nitrate is no different to that reported after administration of nitrate, we believe that AZD3582 and nitric oxide-donating metabolites of AZD3582 did not donate nitric oxide with a $t_{½}$ longer than that of [^{15}N]-nitrate, approximately 6 h on average.

The agreement between the urine recovery of [^{15}N]-nitrate in Study 7 and that found after nitrate dosing by others (Schultz et al 1985) indicates that the [^{15}N]-nitrate content is completely or near-completely absorbed (as is the case for [^{3}H]-radioactivity in Study 7), and that the nitrogen oxide part of AZD3582 is not eliminated to a significant extent via pathways other than those known for nitrate. Furthermore, it indicates that AZD3582 dosing would increase the systemic nitrate exposure as predicted from the maximum possible nitrate content in AZD3582. According to predictions, repeated dosing of clinical doses of AZD3582 would add approximately as much nitrate to the body as was already circulating (equivalent to an approximate doubling of the average nitrate exposure), and a single dose of 750 mg AZD3582 would give a nitrate C_{max} in plasma of maximally approximately 60 μM, which is substantially lower than after intake of nitrate-rich food. The predictions agreed quite well with the observations. Following a single dose of 750 mg total nitrate ([^{14}N]-nitrate + [^{15}N]-nitrate) plasma concentrations increased from about 20 to maximally 90 μM. The AUC and urine recovery ratios between [^{15}N]- and [^{14}N]-nitrate during normal feeding conditions were both approximately 0.8 after a 750 mg dose. Thus, AZD3582 375 and 750 mg bid were estimated to enhance the average systemic nitrate level by approximately 80 and 160%, respectively. After repeated administration of AZD3582 750 mg, plasma nitrate baseline levels increased from 12–43 (pre-dose) to 51–90 μM ($C_{ss,min}$). Peak nitrate levels were not obtained after 750 mg bid, but $C_{ss,min}$ levels and increases observed after a 750 mg single dose (+ maximally 70 μM) indicate that (under the assumption that the PK of nitrate is dose-independent) peak levels of around 150 μM can be expected. This is lower than the maximum levels found after intake of food containing 1000 mg nitrate, 205 ± 27 μM (Jungersten et al 1996). Based on dissolution and stability data, it appears that AZD3582 does not release much nitrate directly into the stomach. The daily amount of nitrate excreted in urine decreased from approximately 1600 to 900 μmol when changing from a normal to a nitrate-restricted diet. This suggests that diet normally contributes at

least approximately 40% of the nitrate content in the body.

The reason(s) for the decreased renal (and total) CL of [^{15}N]-nitrate (and total and [^{14}N]-nitrate) are not clear. Possible explanations include inhibition of active tubular secretion and/or stimulation of tubular reabsorption by naproxen and/or its metabolites, alone or in combination with saturated active nitrate secretion. The distinct twin peaks observed for urine excretion rates, but not in plasma, indicate that saturated secretion was not the only reason for the decreased CL, and that naproxen (and/or other AZD3582 metabolites) must have been involved in some way. Naproxen has earlier been shown to inhibit active renal secretion of organic anions and cations (Mulato et al 2000), and active and saturable renal transport has been shown for nitrate (at least in the dog) (Schultz et al 1985). Naproxen reduces the glomerular filtration rate and urine flow rate within a few hours after dosing (Whelton 2001), and a significant decrease in the urine flow rate was also observed after AZD3582 dosing. At 3 h after dosing, the flow rate reached its lowest level, which is about one-third of baseline and lower than the recommended fluid intake per hour. At about 6 h after dosing, the urine flow rate was normalized. One hypothesis is that the decreased urine flow might also be accompanied by increased retention time and nitrate concentrations in the renal tubuli, both of which may favour tubular reabsorption of nitrate. The CL and plasma levels of creatinine were not altered after AZD3582 dosing in that particular study, which suggests that the reason for the decreased renal CL of nitrate is not likely to be due to a decreased glomerular filtration. About 60% of the nitrate content of the dose was excreted unchanged in urine (just as after nitrate intake) and the renal CL decreased by 40% (maximally), indicating, at a maximum, a 25% decrease in total nitrate CL.

Conclusion

AZD3582 has low aqueous solubility, an intermediate and passive intestinal P_e and is metabolized to some extent by intestinal esterases. Ninety-four per cent or more of an AZD3582 dose is absorbed from the GI tract, of which at least 9–20% appears to be absorbed as intact substance. The oral F appears to be maximally a few per cent. The terminal $t_{½}$ of the compound is 3–10 h, and the plasma protein binding degree approximates to 0.1%. AZD3582 does not accumulate after repeated once- and twice-daily dosing, and no apparent time dependencies or demographic differences are observed. Intake of food, both with and after AZD3582 administration, increases the F of AZD3582 by several-fold. It is hypothesized that this might be due to enhanced intestinal and/or liver blood flows. The food effect is not found in sodium-depleted subjects, which might be due to a reduced responsiveness of blood vessels in sodium depletion.

Blood pressure and AZD3582 and nitrate PK data indicate that nitric oxide is mainly donated within 3 h after AZD3582 dosing. AZD3582 does not provide significantly better GI (in patients) and renal (in volunteers) side-effect profiles than naproxen. The considerable GI uptake as naproxen, limited duration and extent of nitric oxide donation in the GI mucosa and circulation, tolerance development (involving auto-inhibition of nitric-oxide-catalysing enzymes) and damage caused by high nitric oxide levels in the GI mucosa are suggested reasons for this.

Naproxen has 40 times higher P_e than AZD3582 and is completely absorbed after oral administration. The GI and systemic uptake of naproxen is less extensive (≥ 94% relative GI uptake and 80–85% relative F) and slightly slower after AZD3582 administration compared with naproxen dosing. A possible explanation for the discrepancy between the relative absorption and F values is a greater potential for AZD3582 to distribute out to metabolizing tissues than the highly protein bound naproxen. Another explanation could be metabolism via a pathway not involving naproxen. The $t_{½}$ (15–22 h) and $C_{ss,min}$ after AZD3582 and naproxen administration are similar. The compounds also show similar analgesic efficacy in patients, which further demonstrates that involvement of nitric oxide is not apparent. Saturable plasma protein binding, apparent and occasional displacement of naproxen from albumin by food components, slower elimination during the night and body-weight-dependent PK are observed for naproxen. Naproxen does not appear to bind to blood cells, could not be quantified in urine and is completely metabolized and excreted in urine as various conjugates. The CYP450-dependent pathway (mainly 1A2 and 2C9) is responsible for approximately 40% of its metabolism. Food intake causes a slight delay and increased variability in the uptake of naproxen into the blood circulation. After a period with sodium-restricted diet, the systemic naproxen exposure increases by 10%. It is suggested that the low-sodium diet might have an inhibiting effect on the gut wall metabolism of naproxen.

The nitrate content of AZD3582 appears to be completely or near-completely bioavailable. The average systemic nitrate exposure is approximately doubled after dosing of 375 to 750 mg AZD3582 twice daily. The renal clearance of nitrate decreases after AZD3582 dosing. Possible explanations are inhibition of active tubular secretion and/or stimulation of tubular reabsorption by AZD3582, naproxen and/or other metabolites, alone or in combination with saturated active nitrate secretion.

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