Amide Hydrolysis of a Novel Chemical Series of Microsomal Prostaglandin E Synthase-1 Inhibitors Induces Kidney Toxicity in the Rat

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ABSTRACT

A novel microsomal prostaglandin E synthase 1 (mPGES-1) inhibitor induced kidney injury at exposures representing less than 4 times the anticipated efficacious exposure in man during a 7-day toxicity study in rats. The findings consisted mainly of tubular lesions and the presence of crystalline material and increases in plasma urea and creatinine. In vitro and in vivo metabolic profiling generated a working hypothesis that a bis-sulfonamide metabolite (determined M1) formed by amide hydrolysis caused this toxicity. To test this hypothesis, rats were subjected to a 7-day study and were administered the suspected metabolite and two low-potency mPGES-1 inhibitor analogs, where amide hydrolysis was undetectable in rat hepatocyte experiments. The results suggested that compounds with a reduced propensity to undergo amide hydrolysis, thus having less ability to form M1, reduced the risk of inducing kidney toxicity. Rats treated with M1 alone showed no histopathologic change in the kidney, which was likely related to underexposure to M1. To circumvent rat kidney toxicity, we identified a potent mPGES-1 inhibitor with a low propensity for amide hydrolysis and superior rat pharmacokinetic properties. A subsequent 14-day rat toxicity study showed that this compound was associated with kidney toxicity at 42, but not 21, times the anticipated efficacious exposure in humans. In conclusion, by including metabolic profiling and exploratory rat toxicity studies, a new and active mPGES-1 inhibitor with improved margins to chemically induced kidney toxicity in rats has been identified.

Introduction

Deposition of drug-induced crystals within the kidneys can lead to acute and chronic kidney injury due to obstruction (Perazella, 1999; Yarlagadda and Perazella, 2008). A variety of drugs have been shown to precipitate in the renal tubules as a consequence of high local concentrations in the kidney and changes in drug solubility due to pH differences between urine and blood. One group of drugs that has been shown to cause kidney injury by crystalluria is the bacteriostatic antibiotic class of sulfonamides (Perazella, 1999). The sulfonamide drugs such as sulfadiazine and sulfamethoxazole are to a large extent excreted in the urine as parent molecules or metabolites. Because they are weak acids, they are relatively insoluble in acidic urine and have a tendency to precipitate in the tubular lumen when the pH of the urine drops below 5.5 (Hein et al., 1993; Perazella, 1999). High-dose sulfonamide treatment has been associated with an increased risk of sulfah-crystal precipitation in the kidneys (Perazella, 1999).

A new chemical series of sulfonamide-containing compounds was recently identified as potential microsomal prostaglandin E2 synthase-1 (mPGES-1) inhibitors intended for pain treatment (Bylund et al., 2009). This series, which is composed of different bis-sulfonlamino derivatives, has two acidic pKa values around 2.5 and 10.5, CLogP values exceeding 2.5 and molecular weight exceeding 430 Da. Considering their molecular size and lipophilicity, parent compounds of this series and their conjugated metabolites are likely excreted into the bile (Yang et al., 2009), which differs from the antibiotic class of sulfonamides (Fleck et al., 1988; Perazella, 1999).

By assessing possible in vivo safety concerns in relation to anticipated efficacious exposures in humans already within the discovery program, drug candidates with a better chance of success in preclinical testing can be brought forward. mPGES-1 is an enzymatic drug target involved in the production of PGE2 (prostaglandin E2), a mediator of pain and inflammation (Samuelsson et al., 2007). It has been hypothesized that mPGES-1 inhibition might generate pain relieving anti-inflammatory effects similar to cyclooxygenase (COX) inhibitors such as conventional NSAIDs (nonsteroidal anti-inflammatory drugs) and COX-2 inhibitors (Samuelsson et al., 2007; Xu et al., 2008; Iyer et al., 2009). However, mPGES-1 inhibitors differ from COX inhibitors with respect to where in the biosynthetic cascade they inhibit PGE2 production. It has been hypothesized that mPGES-1 inhibitors might exhibit better safety profiles due to their propensity to affect the formation of other eicosanoids than PGE2 in a different way than conventional NSAIDs and COX-2 inhibitors (Samuelsson et al., 2007; Wang and FitzGerald 2010). However, this might also have

ABBREVIATIONS: AUC₀–ṇ₄, area under the curve for 24 hours; AUCₚₖₑₘ, area under plasma concentration time curve needed for therapeutic effect; COX, cyclooxygenase; LC-MS, liquid chromatography mass spectrometry; LC-MS/MS, liquid chromatography-tandem mass spectrometry; mPGES-1, microsomal prostaglandin E synthase-1; NSAID, nonsteroidal anti-inflammatory drug; PGE₂, prostaglandin E₂; PK, pharmacokinetics; TK, toxicokinetics; WBA, whole blood assay.
and [14C]-AZ (phase B). A 7-minute-long gradient starting at 50% and ending at 5% phase A trifluoroacetic acid (phase A) and acetonitrile with 0.3% trifluoroacetic acid in a patent (Bylund et al., 2009). Chemical structures of AZ (Södertälje, Sweden), and the synthesis of such derivatives has been described referred to as M1 and Hyd, originated from AstraZeneca.

In an attempt to develop a novel mPGES-1 inhibitor, we experienced problems associated with chemically induced kidney toxicity in the rat at exposures representing less than 4 times the anticipated efficacious exposure in man. The aim of this study is to describe problem-solving strategies in a drug discovery project encumbered with kidney toxicity. More specifically, we describe the toxicologic kidney findings observed in a 7-day toxicity study, the establishment of a hypothesis for toxicity origination, and the discovery of a new inhibitor with a reduced kidney toxicity risk profile.

Materials and Methods

Chemicals and Reagents. Test compounds, including the metabolites referred to as M1 and Hyd, originated from AstraZeneca’s compound collection (Södertälje, Sweden), and the synthesis of such derivatives has been described in a patent (Bylund et al., 2009). Chemical structures of AZ’7847, AZ’4270, AZ’4284, AZ’0908, and M1 are shown in Fig. 1. Those labeled [14C]-AZ’7847 and [13C]-AZ’4270 were synthesized by the isotope chemistry laboratory at AstraZeneca, Södertälje, Sweden, with a specific activity of 2.1 GBq/mmol and a radiochemical purity exceeding 99.4%. The [14C] label was located, for both compounds, on the carboxyl carbon as depicted in Fig. 5. All other chemicals used were of analytical grade and obtained from commercial suppliers.

Solubility Measurements and pKa Predictions. Solubility samples in rat urine (pH 7.0) were incubated at 37°C for 5 hours and analyzed using a Hewlett Packard 1100 system (Agilent Technologies, Santa Clara, CA) equipped with a Symmetry C18 column (3.5 mm, 4.6 x 50 mm), a photodiode array detector, and a radiochemical purity exceeding 99.4%. The [14C] label was located, for both compounds, on the carboxyl carbon as depicted in Fig. 5. All other chemicals used were of analytical grade and obtained from commercial suppliers.

Liquid Chromatography Mass Spectrometry Analysis. Liquid chromatography–mass spectrometry (LC–MS/MS) analysis for quantifications was performed with a Micromass Quattro Micro triple quadrupole (Waters Corporation, Milford, MA), as previously described elsewhere (Briem et al., 2007) with the exception of the analysis of the M1 metabolite. Total concentration of M1 in plasma was determined by LC-MS/MS using electrospray in the negative ionization mode. Because the analyte was not retained on a conventional C18 column, a porous graphitic carbon column and methanol gradient were used. For quantification of the M1 metabolite, the mass spectrometer was set to select the [M-H]- precursor ion at mass-to-charge ratio 234.9 and for the product ion at mass-to-charge ratio 171.1. Before the LC-MS/MS analysis, the samples were precipitated with acetonitrile.

The LC-MS analysis for metabolic profiling was performed as described here. Liquid chromatography was performed with Schmidazu LC-10AD VP pumps (Schimadzu Deutschland, Duisburg, Germany) and a HTC PAL (CTC Analytics AG, Zwingen, Switzerland). The separations were performed on a Waters Atlantis T3 column (100 x 2.1 mm ID, 3 μm particle size) at a flow rate of 0.25 ml/min. The mobile phase was a binary mixture of 0.1% formic acid in water/acetonitrile (98:2), v/v (solvent A), and a radiochemical purity exceeding 99.4%. The [14C] label was located, for both compounds, on the carboxyl carbon as depicted in Fig. 5. All other chemicals used were of analytical grade and obtained from commercial suppliers.

Animals. Wistar Hannover rats, approximately 8 weeks old, were used for toxicity studies, and male Sprague-Dawley rats were used for pharmacokinetic (PK) studies. All rats were obtained from Taconic M&B A/S (Ry, Denmark). The rats were randomized into groups (n = 2–4/sex) by body weight and were acclimatized for approximately 2 weeks before the start of the study. The animals were housed up to 3 per cage in transparent plastic cages. The bedding material consisted of aspen wood chips. Plastic tunnels and aspen chew blocks were also provided. The animals had free access to pelleted RM1 (E) SQC diet and water from the site drinking water supply, except during urine collection when access to food was temporarily withdrawn. The animal room was illuminated by artificial light from fluorescent tubes on a 12-hour light/dark cycle. The temperature and relative humidity were 17° to 23°C and 40 to 70%, respectively. All experiments were approved by the local animal ethics.

Fig. 1. Structural formula of compounds AZ’7847, M1, AZ’4270, AZ’4284, and AZ’0908 discussed in the text.
committee (Stockholms Södra försöksdjursetiska nämnd) and were conducted in compliance with national guidelines for the care and use of laboratory animals.

**Toxicology Studies.** Three different rat toxicologic studies were performed. A detailed description of the study design is shown in Table 1. Compounds AZ’7847, AZ’4270, and AZ’4284 were each formulated as solutions in 20% (v/v) polyethylene glycol 400 in 0.2 M meglumine (vehicle 1); the M1 metabolite was formulated a solution in 1% (w/v) hydroxypropyl methylcellulose and 0.1% (w/v) Tween 80 (vehicle 2); and AZ’0908 was formulated as solution in 15% (w/v) polyethylene glycol 400 in 0.2 M meglumine (vehicle 3). Animals used for toxicity studies were dosed once daily by oral gavage (10 ml/kg body weight) for 7 or 14 days. Control animals were treated with either vehicle 1 or vehicle 2. Doses were set based on low-dose PK studies where linear kinetics was assumed.

**Toxicokinetics.** Two male and two female satellite rats (animals used for exposure analysis only) were used for toxicokinetic (TK) analysis per dose group in the first study, but only 2 satellite female rats were used per dose group in the second study with AZ’7847 and AZ’4270, and AZ’4284 and the M1 metabolite. In the third toxicity study, five animals per dose group of AZ’0908 were used for TK analysis. All compounds were dosed once daily for 7 consecutive days, and blood samples were taken at 2, 4, 6, 7, and 24 hours on day 1 and on the last day of administration. For satellite animals in toxicity studies 1 and 2, approximately 0.3 ml of blood was taken via the tail vein and was immediately frozen to –70°C. The plasma was transferred to 1.4 ml Matrix vials and was immediately frozen to –70°C. The samples were analyzed for the attainment IC50 value (unpublished data) in a well established whole blood assay IC50 value (unpublished data) in a well established whole blood PGE2 assay (Huntjens et al., 2005) would be satisfactory for pain treatment. Doses aiming to achieve 10 and 20 times the AUCther were set based on low-dose PK studies where linear kinetics was assumed.

**Results**

**Kidney Toxicity of AZ’7847.** Estimations of efficacious therapeutic concentration in humans (AUCther) were based on an assumption that an average steady-state concentration corresponding to 10 times the attained IC50 value (unpublished data) in a well established whole blood PGE2 assay (Huntjens et al., 2005) would be satisfactory for pain treatment. Doses aiming to achieve 10 and 20 times the AUCther

### Table 1

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<tr>
<th>Tox Study</th>
<th>Test Duration</th>
<th>Test Compound</th>
<th>Dose</th>
<th>Sex and No. of Animals</th>
<th>Comments on TK Samples</th>
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<td>AZ’0908</td>
<td>435</td>
<td>10F</td>
<td>5F animals for µ-sampling</td>
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<td></td>
<td></td>
<td>900</td>
<td>10F</td>
<td>5F animals for µ-sampling</td>
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F, female; M, male; TK, toxicokinetic.
* Same vehicle as AZ’7847, AZ’4270, and AZ’4284.
* Same vehicle as for M1.

### Table 2

**Toxicokinetic summary of AZ’7847 in toxicity study 1**

<table>
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<tr>
<th>Dose</th>
<th>Sex and No. of Animals</th>
<th>Anticipated AUCther (µMh)*</th>
<th>Day 1 Cmax</th>
<th>AUC0-24</th>
<th>Day 7 Cmax</th>
<th>AUC0-24</th>
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<td>µMh</td>
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<td>75</td>
<td>1020</td>
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AUC0-24, area under the curve for 24 hours; AUCmax, area under plasma concentration time curve needed for therapeutic effect; F, female; M, male; NA, not available.
* AUCther (area under plasma concentration time curve needed for therapeutic effect) was based on the assumption that a steady-state concentration (Cmax) average corresponding to 10 times the whole blood assay IC50 value was needed.
in humans were used. Rats were orally administered either 500 or 1000 μmol/kg per day for 7 consecutive days. LC-MS and TK analyses revealed that a lower than anticipated exposure on day 7 for low-dose animals was achieved and that no obvious sex difference in AUC was detected (Table 2). No high-dose exposure data at day 7 were available due to premature death or sacrifice.

Toxicologic evaluation showed severe kidney injury in animals from the high-dose group. Necropsy findings included increased kidney weights and pale kidney discoloration. The histopathologic evaluation showed marked tubular degeneration, characterized by the presence of dilated tubules with cellular remnants within the lumen, mixed with basophilic, dilated tubules with hypertrophic nuclei and cytoplasm. The distribution of this finding was cortex and medulla, with emphasis on the medulla. Additional changes in the kidney included multiple pyogranuloma (accumulation of neutrophils/macrophages surrounding amorphous/crystalline material within tubules in the cortex and medulla), inflammation and hemorrhage in the renal pelvis, free crystals in the renal pelvis, transitional cell hyperplasia, and papillitis.

Figure 2 shows the histopathology comparison of a kidney from a control rat and from the kidney of a rat treated with 1000 μmol/kg AZ’7847 per day for 7 days. Analysis of plasma showed extremely high levels of urea and creatinine in rats treated with 1000 μmol/kg per day for 7 days (Fig. 3). At the low dose, 500 μmol/kg, moderate tubular degeneration/regeneration was observed. The characteristic kidney damage and identification of crystal deposits suggested that kidney toxicity was caused by tubular precipitation of AZ’7847-associated drug material.

Amide Hydrolysis of AZ’7847. Metabolic profiling in rat hepatocytes showed that amide hydrolysis of AZ’7847 was a major metabolic pathway in vitro. Comparison of chromatograms detected by mass spectrometry (Fig. 4A) and radiodetection (Fig. 4B) showed that the carboxylic acid metabolite formed (depicted as Hyd in Fig. 5) had a much lower mass spectrometry response than the parent compound, whereas the corresponding bis-sulfonamide metabolite (depicted as M1 in Fig. 5) was not detected by either MS analysis or radiodetection. That amide hydrolysis was a major metabolic pathway in vivo was confirmed by metabolic profiling of the plasma samples from the toxicity study (Fig. 4C). Additional major metabolites tentatively identified by accurate mass LC-MS analysis were a mono-oxidized metabolite (marked +16), a combined oxidized and sulfate-conjugated metabolite.

Fig. 2. Histopathologic changes in the rat kidney papilla. (A) Control animal treated with the same formulation as AZ’7847 for the same time period. (B) Distinct kidney necrosis, inflammation, and crystal deposits were detected in the rats treated with AZ’7847. Kidneys stained with hematoxylin and eosin; magnification, 100×.

Fig. 3. Kidney functional biomarkers. (A) Urea. (B) Creatinine. Mean values generated from two male and two female rats. The standard error of the mean is shown in graphs.
(marked +16+Sulf), a metabolite likely formed by amide hydrolysis in combination with mono-oxidation (Hyd+16), and a metabolite formed by amide hydrolysis in combination with mono-oxidation and sulfate-conjugation (Hyd+16+Sulf). Metabolic in vitro profiling in both cryopreserved and freshly prepared human hepatocytes and cryopreserved dog hepatocytes revealed no indication that AZ’7847 had undergone amide hydrolysis. Metabolic profiling experiments in human hepatocytes suggested that AZ’7847 is highly metabolically stable (Fig. 4D).

The detection of large quantities of the Hyd metabolite in rats obviously suggested formation of large amounts of the corresponding M1 metabolite (Fig. 5). Measurements of the M1 solubility in rat urine at pH 7.0 revealed a moderate solubility of 83 μM, which was similar to the parent molecule (45 μM). The acidic pKa of the M1 metabolite was predicted to be approximately 9.5. Based on the detection of this M1 metabolite that structurally resembles sulfonamide-containing antibacterial agents and the characteristic kidney damage, we hypothesized that a compound with a low degree of hydrolysis would be superior with respect to kidney toxicity risk.

**Evaluation of Kidney Toxicity Hypothesis.** Two compounds, AZ’4270 and AZ’4284 (Fig. 1), containing structural modifications intended to block amide hydrolysis by steric hindrance were identified. No hydrolysis in rat hepatocytes (Fig. 6, A and B) was detected for either of the two compounds, and they were chosen for an exploratory rat toxicity study. Both compounds exhibited IC50 values above 2 μM in the human WBA (unpublished data) and were therefore only considered as tool compounds. A 7-day rat toxicity study focusing on kidney toxicity and M1 exposure was performed with the M1 metabolite and the compounds AZ’4270 and AZ’4284 together with AZ’7847 as a positive control (toxicity study 2 in Table 1). Doses were set to achieve a similar and a significantly higher exposure than what had been achieved by 1000 μmol/kg AZ’7847 in the previous study. LC-MS and TK analyses showed that the exposures of AZ’4270 and AZ’4284 per dose unit were more than 5 times higher than for AZ’7847 (Table 3). TK analysis also showed that the M1 metabolite in the plasma samples of rats administered AZ’4270 and AZ’4284 was much lower than with the positive control AZ’7847. The ratio between the exposure of the M1 metabolite and...
the exposure of the parent molecule at day 7 was more than 30 times lower for AZ\textsuperscript{7847} and more than 75 times lower for AZ\textsuperscript{4270} and AZ\textsuperscript{4284} as compared with AZ\textsuperscript{7847}. The exposure of M1 after M1 administration was significantly lower than for the positive control AZ\textsuperscript{7847}, and approximately the same exposure was achieved after the administration of 500 and 2000 \textmu mol/kg per day (Table 3). M1 accumulation appeared to be low during the administration period, except in rats treated with AZ\textsuperscript{7847} in whom both AUC\textsubscript{0-24} and C\textsubscript{max} increased more than 3-fold.

The histopathologic analysis of kidneys from rats treated with AZ\textsuperscript{7847} was similar to the previous study, and elevated levels of the kidney functional biomarkers urea and creatinine were detected (not shown). Neither pathophysiologic findings nor elevation of kidney functional biomarkers were detected for AZ\textsuperscript{4270} and AZ\textsuperscript{4284} despite exposure levels of parent molecules in the same range or higher than AZ\textsuperscript{7847}. However, crystals were seen in the urine of animals treated with AZ\textsuperscript{4270} (only in high dose) but not with AZ\textsuperscript{4284}. No histopathologic findings or changes in creatinine and urea parameters were detected for rats treated with the M1 metabolite. Crystals were observed in the urine from both dose groups of animals dosed with the M1 metabolite.

Identification and Evaluation of AZ\textsuperscript{0908}. We identified a novel and potent mPGES-1 inhibitor AZ\textsuperscript{0908} (Fig. 1), which exhibited no detectable amide hydrolysis in rat hepatocytes (not shown). A rat PK study using a single dose of 10 \textmu mol/kg (Fig. 7) suggested that the rat exposure per dose unit of AZ\textsuperscript{0908} and the ratio of the plasma concentration of the M1 metabolite and the parent molecule were drastically better than for AZ\textsuperscript{7847} (Table 4). Considering the improved rat PK and possibly also improved redirection of metabolism, AZ\textsuperscript{0908} was chosen for evaluation in a 14-day rat study focusing on kidney toxicity (toxicity study 3 in Table 1).

Doses were set to achieve approximately 20-fold and 40-fold margins to anticipated AUC\textsubscript{ther} in humans. Samples for TK analysis were taken by microsampling, and the M1 metabolite was not monitored in the study. TK analysis showed that the exposure objectives were achieved and that the two doses exhibited dose-proportionality for AUC\textsubscript{0-24} but not for C\textsubscript{max} (Table 3). Toxicologic evaluation showed that the low dose, which generated exposures of 21 times the anticipated AUC\textsubscript{ther} in humans, was not associated with any significant kidney toxicity. However, for 6 out of 10 animals in the high-dose group tubular degeneration was detected, and in two of these animals moderate or severe crystalline deposits were observed. Also, creatinine and urea plasma levels were substantially increased at approximately the same levels as for AZ\textsuperscript{7847}. However, there were no changes in plasma chemistry parameters in the low-dose group with AZ\textsuperscript{0908}. Crystals in the urine were found in both dose groups.

![Fig. 5. Amide hydrolysis of AZ\textsuperscript{7847}. Amide hydrolysis results in the formation of two metabolites: M1 and Hyd. The position of the [\textsuperscript{14}C]-labeling is depicted in the structure.](image)

![Fig. 6. Metabolite profiling of AZ\textsuperscript{4270} and AZ\textsuperscript{4284} in rat hepatocytes. (A) LC-MS chromatogram of a rat hepatocyte incubation of AZ\textsuperscript{4270}. The chromatogram is showing the combined base peak intensities of the molecular ion [M-H]\textsuperscript{-} of the parent molecule m/z (mass-to-charge ratio) 517.071 and the potential metabolites with m/z values of 297.073, 299.089, 315.084, 395.041, 475.121, 515.055, 531.050, 533.066, 549.061, 571.073, 613.022, 709.098, 838.135, and 840.129. The metabolic profiling was confirmed by radio monitoring detection of [\textsuperscript{14}C]-AZ\textsuperscript{4270} (not shown). (B) LC-MS chromatogram of a rat hepatocyte incubation of AZ\textsuperscript{4284}. The chromatogram is showing the combined base peak intensities of the molecular ion [M-H]\textsuperscript{-} of the parent molecule m/z 433.034 and the potential metabolites with m/z values of 213.036, 215.052, 231.047, 311.003, 391.0084, 419.018, 431.018, 433.034, 435.013, 435.049, 447.013, 449.028, 465.028, 467.039, 487.035, 528.985, and 625.061. Putative peak identification was suggested by LC-MS analysis with accurate mass determination. P = parent molecule, and differences in m/z value in relation to parent m/z value are shown for major peaks.](image)
sulfonamide metabolite (M1) associated with amide hydrolysis. To test the hypothesis, rats were given the M1 metabolite and two low potency mPGES-1 inhibitor analogs, where amide hydrolysis was undetectable in rat hepatocyte experiments. The results suggested that compounds with a reduced propensity to undergo amide hydrolysis and thus having less ability to form M1 reduced the risk of inducing kidney toxicity. Additional support for our hypothesis of M1 induced kidney toxicity can be found in a recent methodology study (Nilsson et al., 2012) where it was confirmed that the crystal deposits in rat kidneys formed after AZ’7847 administration were composed of the M1 metabolite. This was realized by using nuclear magnetic resonance analysis and matrix-assisted laser desorption ionization mass spectrometry imaging.

Rats treated with M1 alone surprisingly showed no histopathologic changes in the kidney. However, TK analysis revealed low exposure levels and non-dose-proportionate kinetics for the M1 metabolite. This was likely related to absorption limitations, as the solubility of the M1 molecule, the M1 metabolite. This was realized by using nuclear magnetic resonance analysis and matrix-assisted laser desorption ionization mass spectrometry imaging. For acyclovir, as rapid bolus injections lead to an increased risk of acute renal failure as compared with slow infusions of the same dose (Gnann et al., 1983). However, in this case, when the M1 metabolite is released from the parent molecule, the M1 C_max appears to be directly related to its exposure. The threshold for M1-induced rat kidney injury appeared to be rather high. Exposure to M1 at approximately 1300 μMh and C_max of 80 μM generated visual crystals in the urine, but neither changes in kidney functional biomarkers nor detectable histopathologic changes occurred. A comparison of M1 exposure between day 1 and day 7 clearly occurred. A comparison of M1 exposure between day 1 and day 7 clearly showed an accumulation over time in rats treated with AZ’7847. In rats treated with M1 alone, AZ’4270 or AZ’4284, accumulation was significantly less or not obvious. This is likely related to severely

### Discussion

This study is an example of problem solving in a drug project encumbered with kidney toxicity in rats. The mPGES-1 inhibitor AZ’7847 intended for pain treatment was assessed in a 7-day toxicity study in rats and was found to induce kidney injury at exposures representing less than 4 times the anticipated efficacious exposure in humans. Because AZ’7847 exhibited no activity on the rat mPGES-1 target (not shown), it was unlikely that the observed toxicity was related to target activity. Instead, the characteristic kidney damage and the identification of crystal deposits suggested that the kidney toxicity more likely was caused by tubular precipitation of AZ’7847-associated drug material. Based on metabolic profiling experiments, we hypothesized that the crystal deposits were composed of a bis-

![Fig. 7. Rat PK analysis of AZ’0908 and M1 formation. PK curve for rats administered with a single dose of 10 μmol/kg. Traces marked with triangles represent AZ’0908 and traces marked with circles represent the M1 metabolite.](image-url)
Amide Hydrolysis-Induced Kidney Toxicity

Amphoruthorship

Participated in research design: Bylund, Annas, Hellgren, Andersson, Svanhagen.

Conducted experiments: Bylund, Annas, Hellgren, Bjureström, Andersson, Svanhagen.

Performed data analysis: Bylund, Annas, Hellgren, Bjureström, Andersson, Svanhagen.

Wrote or contributed to the writing of the manuscript: Bylund, Annas, Andersson, Svanhagen.

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