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Efficient Biliary Excretion of Susalimod, Probably via the Bromosulphthalein Carrier, Studied in a Chronic Bile Fistula Model in Dogs

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Abstract

Susalimod is a structural analogue of sulphasalazine, known to be extensively excreted in the bile in various animal species and for inducing bile duct hyperplasia after long-term treatment of the dog with doses exceeding 25 mg kg^{-1}. In this study local concentrations of susalimod in the bile duct were determined after oral administration in dogs.

A chronic bile fistula experimental model was designed to affect the bile duct as little as possible. The dogs received repeated oral doses of 25 – 150 mg kg^{-1} day^{-1} for 5 days; these doses had been used in previous toxicology studies. Extremely high biliary concentrations of unchanged susalimod (20 000 – 43 000 μM) were measured. Biliary excretion approached saturation at the higher doses, resulting in super-proportional increases in peripheral plasma concentrations as the dose was increased. The maximal bile/plasma concentration ratio was 4300. The high biliary clearance was indicative of almost complete first-pass elimination at doses below saturation of the elimination process. Interaction studies with the biliary excretion marker bromosulphthalein (BSP) demonstrated that susalimod and BSP probably share the same carrier transport system in biliary excretion. The elimination of BSP from plasma was prolonged 20 times and the biliary excretion rate was markedly reduced when susalimod was co-administered with BSP.

These results show that susalimod is highly enriched in the bile, in a saturable manner, after oral administration. The compound interacts with the biliary excretion of BSP, suggesting that it shares the same carrier-mediated transport system.

Susalimod (Figure 1), a structural analogue of sulphasalazine, was designed for use in the treatment of rheumatoid arthritis and was therefore intended to retain the immunomodulating properties of sulphasalazine – inhibition of the production of pro-inflammatory cytokines and suppression of the proliferation of lymphocytes (Williams et al 1993). The activity of sulphasalazine, which has been used for decades to treat inflammatory bowel diseases, is derived from the active metabolites 5-aminosalicylic acid and sulphapyridine formed in the colon (Peppercorn 1990). Recently the drug has also been used successfully for treatment of rheumatoid arthritis, where the unchanged compound is believed to exert the therapeutic effect (Rains et al 1995). Therefore, susalimod was designed to be metabolically stable to azoreductases. It was also made more lipophilic to improve its absorption compared with that of the poorly absorbed sulphasalazine. After minor structural modification of sulphasalazine intestinal absorption was increased three to tenfold according to in-situ and in-vivo studies in the rat (unpublished results). After absorption susalimod undergoes extensive biliary excretion as the non-conjugated parent compound, as has been demonstrated in several animal species (unpublished results). The highest bile/plasma concentration ratio of susalimod, approximately 3500, was observed in the anaesthetized dog. At doses >75 mg kg^{-1} in toxicological studies, bile duct hyperplasia appeared after long-term administration in the dog; this has been attributed to high local concentrations of susalimod in the bile duct.

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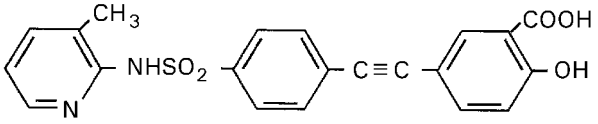

Figure 1. The chemical structure of susalimod.

The current study was designed to determine the local biliary concentrations of susalimod in conscious dogs under experimental conditions mimicking those in the toxicology studies. A bile fistula model was therefore developed in the dog and was designed to affect the bile duct as little as possible while enabling repeated bile sampling. We herein report that extremely high biliary concentrations of susalimod were measured. The model was also useful for demonstrating an interaction between susalimod and bromosulphthalein (BSP) at the biliary excretion site.

Materials and Methods

Materials

Susalimod, 2-hydroxy-5-((4-((3-methyl-2-pyridinyl-amino)sulphonyl)phenyl)ethynyl)benzoic acid, was synthesized at Pharmacia and Upjohn. Isotonic aqueous solutions, 5 and 30 mg L^{-1}, were prepared. Other chemicals were of reagent grade and were obtained commercially.

Animals

Beagle dogs, 2 males and 1 female, 11·8 – 14·7 kg, were used for the bile fistula surgery. They were fed once daily (afternoon) with solid food (H1, Lactamin AB, Sweden), and had free access to tap water. The experiment was approved by the local animal-ethics committee.

Bile fistula surgery

After premedication with a mixture of acepromazine, haloperidol and methadone (0·5 mg L^{-1} each), the dogs were anaesthetized with thiopental sodium (25 mg kg^{-1}) and were intubated for mechanical ventilation. A mixture of oxygen, nitrous oxide and isofluran, 40 : 58 : 2, was given. Laparotomy in linea alba was made from the edge of the breast bone and 25 cm downwards. The model is outlined in Figure 2. The gall bladder, including the cystic duct, was detached from connecting tissue. Diathermy and ligation were used to prevent bleeding from the surrounding tissue. Approximately 1 cm^2 of the skin, fascia and muscles were removed to make a hole to the right of the midline just below the costal arch. The gall bladder was put through that opening and a hole was cut by incising 10 mm at the top, after which the bile was extracted without coming into contact with the abdominal cavity. The bladder was fixed to the abdominal wall by sutures to the peritoneum and to the fascia. A catheter (feeding tube 130·07, Vygon, France) was inserted to measure the individual length to the bile duct. The position at the entrance of the common bile duct was checked by X-ray. A specially designed nipple with a screw to prevent leakage, all made of Teflon, was put into the stomy and was fixed by means of a purse-string suture in the skin. The week after the operation the stomy and gall bladder were rinsed gently with sterile saline. Rehabilitation was monitored repeatedly by serum analyses of alaninaminotransferase, alkaline phosphatase, γ-glutamyltranspeptidase, bilirubin and bile acids. The animals were not used in experiments until serum enzymes, bilirubin and bile acids reached normal levels. The recovery period was approximately 4 weeks (Figure 3).

Biliary excretion

The dogs received three different dose levels of susalimod, 25, 75 and 150 mg kg^{-1} each, as daily repeated oral administration by gavage for 5 days. The wash-out periods between the dose levels were at least one week. One dog was used for two periods at each dose level.

Bile was frequently (9 – 18 occasions) sampled for 1 h at a fixed time of the day in relation to the feeding time and at the expected maximum plasma concentration (C_{max}) of susalimod; both samples were taken before dosing to obtain normal bile flow and on days 1 – 4 during the administration period. After the final dose, bile was sampled for 10-min

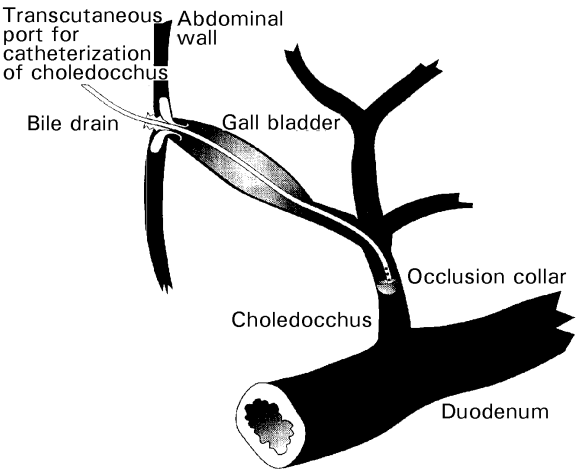

Figure 2. Chronic bile fistula model in the dog.

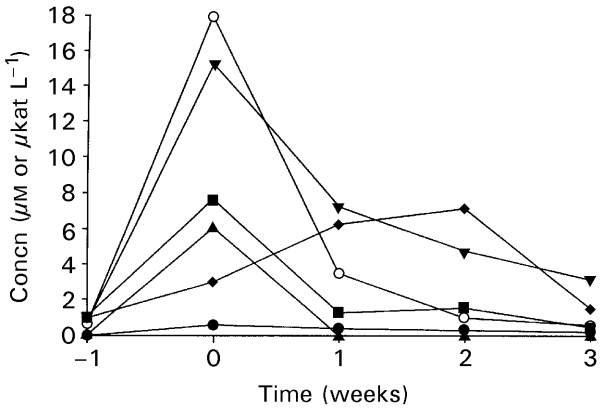

Figure 3. Alaninaminotransferase (○), alkaline phosphatase (▼), γ-glutamyltranspeptidase (●), total (■) and conjugated (▲) bilirubin, and bile acids (◆) in serum before and after bile fistula surgery.

periods on repeated occasions over a 24-h period. The dogs were restrained during bile sampling.

Blood was sampled from a foreleg vein in heparinized tubes each day during the administration period at the middle time point of all the bile sampling periods.

Analyses

Bile and plasma concentrations of susalimod were analysed by reversed-phase liquid chromatography (HPLC) with UV detection (320 nm). Separation was performed on a Kromasil C8 column, 5 μm particles, with a guard column. Methanol–phosphate buffer (0·05 M, pH 7·0) was used as mobile phase. Before analysis proteins were precipitated with methanol. The limit of quantitation was 0·6 μM in both plasma and bile.

Equilibrium dialyses

In bile samples from two dogs receiving 25 mg kg^{-1}, the unbound fraction of susalimod was estimated by equilibrating the samples against pH 7·4 phosphate buffer at 37°C for 5 h. The concentrations of susalimod in buffer and bile were analysed by HPLC. The fraction unbound was calculated from these results taking into consideration the volume shift that occurred during dialysis. The osmolality in bile samples was determined by means of a Wescor vapour-pressure osmometer.

Interaction with bromosulphophthalein (BSP)

BSP (5 mg kg^{-1}) was given intravenously to the bile-fistulated dogs with or without intravenous pre-administration (5 min) of susalimod (25 mg kg^{-1}). Bile was sampled during 15- to 30-min periods up to 4 h after dosing. Blood was collected on repeated occasions during the 10 min after BSP injection, or during the 90 min after the combined administration of BSP and susalimod.

BSP in bile and plasma was determined by a photometric method. A sample (25 μL) was mixed with NaOH (0·1 M, 5 mL) and analysed at 580 nm by means of a Shimadzu UV-160 photometer. Standard curves were prepared in the BSP concentration range 0–2·5 mg mL^{-1} in plasma and 0–5·0 mg mL^{-1} in bile.

Evaluation

The area under the curve (AUC) of profiles of plasma or bile concentration against time were calculated by the linear trapezoidal method to the last sampling time with detectable concentrations. The biliary excretion rate was estimated from the biliary concentrations and bile flow assuming a bile density of one. Half-life and clearance (dose/AUC) of BSP were calculated by non-compartmental analysis using PCNonlin 4.2, model 200.

Results

Plasma and biliary concentrations of susalimod

Maximum concentrations of susalimod in plasma were obtained after approximately 4 h (Table 1). C_{max} and AUC increased much more than in proportion to the increase in dose.

The biliary concentration-time profile of susalimod followed that in plasma (Figure 4), but the levels exceeded the plasma concentrations up to 4000-fold in the low-dose group (Table 1). This bile/plasma concentration ratio decreased as the dose was increased, as a result of saturable biliary excretion. Very high concentrations of susalimod (up to approximately 50 000 μM) were measured in the bile of individual animals. The concentrations of susalimod in the bile substantially exceeded the solubility both in physiological NaCl solution and in bile in-vitro (Table 2). Equilibrium dialysis of bile samples showed that most (80%) of the susalimod was bound to macromolecules in the bile. During the dialysis experiment there was a considerable volume shift for which correction was made in the calculations. The reason for this was probably the lower osmolality of the bile compared with that of the buffer (266 compared with 290 mOsm kg^{-1}).

Table 1. Plasma and biliary concentrations of susalimod on day 5 of daily repeated oral administration to dogs with chronic bile fistula.

Dose (mg kg^{-1})	t_{max} (h)		C_{max} (μM)		AUC (μM h)		C_{max} bile/ C plasma
	Plasma	Bile	Plasma	Bile	Plasma	Bile	
25	4.5 ± 2.5	5.0 ± 2.0	4.6 ± 1.3	20 175 ± 13 892	23.7 ± 9.7	149 414 ± 85 737	4269 ± 2165
75	4.0 ± 1.6	5.0 ± 2.0	72 ± 68	36 075 ± 10 219	668 ± 401	408 325 ± 44 571	859 ± 1139
150	7.0 ± 2.0	4.5 ± 1.0	460 ± 104	42 800 ± 11 469	3483 ± 2529	504 530 ± 27 534	93 ± 13

Results are means ± standard deviation; n = 4. t_{max}, time of maximum plasma concentration; C_{max}, maximum plasma concentration; AUC, area under the plasma-concentration – time curve.

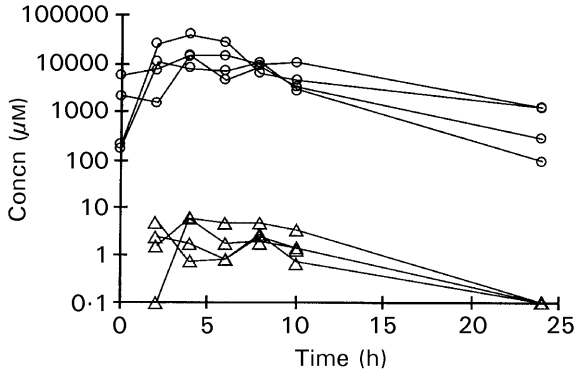

Figure 4. Susalimod concentrations in plasma (△) and bile (○). Dose no. 5 during repeated daily oral administration of 25 mg kg^{-1} day^{-1}.

Bile flow

Susalimod had no significant effect on bile flow (Table 3). The normal bile flow obtained in this model was approximately 0·5 mL h^{-1} kg^{-1}, which is in accordance with literature data (0·5 – 1 mL h^{-1} kg^{-1} (Calabrese 1991; Kararli 1995).

Excretion rate

The biliary excretion rate increased less than in proportion to the dose increase, supporting the idea that the biliary excretion is a saturable process (Table 4). The maximum biliary clearance calculated from the biliary excretion rate and corresponding plasma concentrations were extremely high at the lowest dose, even exceeding the liver blood flow in the dog (30 – 40 mL min^{-1} kg^{-1}; Davies & Morris 1993).

Table 2. In-vivo biliary concentration of susalimod after an oral dose of 150 mg kg^{-1}, compared with the in-vitro solubility of the drug.

C_{max} bile, in-vivo (μM)	43 000
Solubility in 0.9% NaCl (μM)	800
Solubility in bile, in-vitro (μM)	8000
Fraction unbound in bile*	20%

* Estimated by equilibrium dialysis.

Interaction with BSP

After intravenous injection of BSP, 5 mg kg^{-1}, 70 ± 6% of the dose was recovered in the bile during a sampling period of 4 h. The half-life of BSP in plasma was 3·2 ± 1·0 min. When susalimod was injected intravenously 5 min before the BSP dose, the half-life in plasma increased almost 20-fold to 53 ± 29 min (Figure 5). Simultaneously, the rate of biliary elimination of BSP decreased in the presence of susalimod (Figure 5). C_{max} in bile was delayed and decreased from 4·9 ± 0·6 to 2·0 ± 0·4 mg mL^{-1}.

Table 3. Bile flow before and during repeated oral administration of susalimod.

Dose (mg kg^{-1} day^{-1})	Bile flow (g h^{-1})
0	6.5 ± 3.1
25	7.5 ± 3.8
75	7.8 ± 3.1
150	6.6 ± 2.9

Susalimod was measured daily over 1 h at the same time of the day (4 h after dose) for 4 days. Results are means ± standard deviation; n = 16, control (0) = 40.

Table 4. Biliary excretion rate and biliary clearance of susalimod after oral administration.

Dose (mg kg^{-1})	Maximum biliary excretion rate (μmol h^{-1})	Maximum biliary clearance (mL min^{-1} kg^{-1})
25	235 ± 29	186 ± 144
75	443 ± 172	254 ± 289
150	633 ± 109	81 ± 61

Results are means ± standard deviation; n = 4.

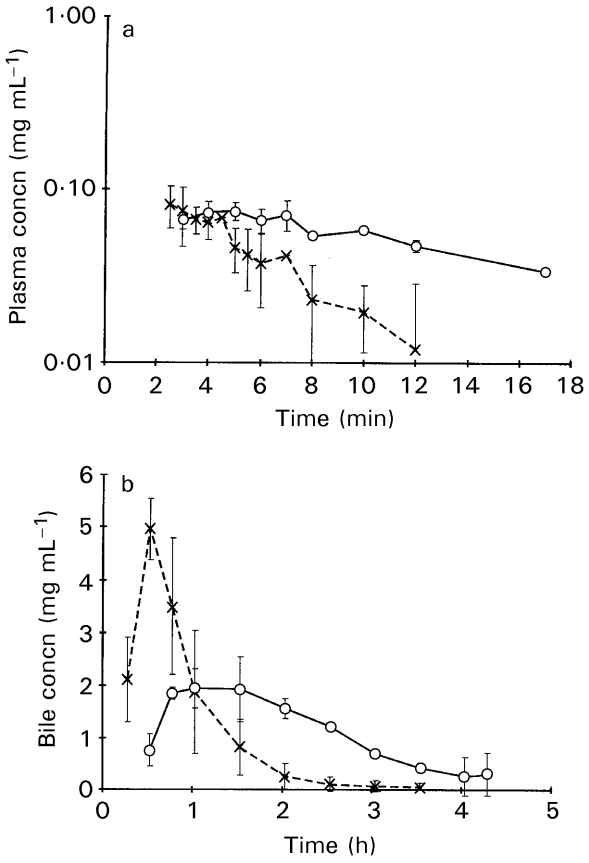

Figure 5. #BSP in plasma (a) and in bile (b) after an intravenous dose, 5 mg kg^{-1}, to dogs with chronic bile fistula, with (○) or without (×) pre-injection of susalimod, 25 mg kg^{-1}. Results are means ± standard deviation, n = 4.

Discussion

Biliary excretion studies in chronically cannulated animals are often associated with a high risk of infection, effect on hepatobiliary function or practical problems such as external parts of implants. In this study foreign material was avoided by using the gall bladder to prepare a fistula, a method which to our knowledge has not been described previously. Bile was sampled by inserting a catheter via the gall bladder fistula into the bile duct at each sampling time. Sampling was believed to be quantitative, because the measured bile flow was in agreement with reported data. Moreover, the recovery of the biliary excretion marker BSP was 70%, similar to that reported by Cantarow et al (1948), 56–74%, and reviewed by Lin (1995), 54–97%. With this simple surgical technique, the quality of life of the dogs was not affected. Several dogs have lived for more than two years with this type of fistula. Because bile was sampled during short repeated periods, 10 min, and not chronically collected, the absorption of orally administered susalimod was thus not expected to be affected. There was no difference between the pharmacokinetic profiles of susalimod for bile fistula dogs and normal dogs. There was a similar disproportionate increase in plasma levels with dose as a result of saturable biliary excretion. Very high intra- and interindividual variation in plasma concentrations was observed in dogs both with and without the fistula. This might be because the extremely high biliary excretion results in only low concentrations in plasma. It is less likely that variation in absorption caused the wide range in plasma levels, because absorption was almost complete, as calculated from AUC in bile when taking the mean bile flow into account. This also means that almost the entire dose was excreted in the bile as unchanged compound.

Biliary concentrations of susalimod were extremely high, >40 000 μM, compared with the low solubility in water, 800 μM. Only 20% of the total amount of susalimod present in bile passed a dialysis membrane, which suggests that susalimod is 80% associated with bile acid micelles or other complexes in the bile. Such association complexes between endogenous or exogenous substances and bile acids are well known. For example, the fraction of conjugated bilirubin or different dyes such as BSP associated with bile varied from 26 to 93%, (Scharschmidt & Schmid 1978). The reason for the discrepancy between the high concentrations found in bile in-vivo and the in-vitro solubility in bile cannot, however, be explained by the results obtained from this experiment.

The high levels of susalimod in the bile duct were obviously not a result of any cholestatic effect of the compound, because the bile flow was unchanged during the period of repeated administration. Dose-related bile duct hyperplasia has been observed in dogs after repeated administration for six months in toxicological studies (unpublished results). Toxicity was observed at a dose of 75 mg kg^{-1} and was more severe at the higher dose, 150 mg kg^{-1}. The phenomena were reversible. In the current study local levels of susalimod in the bile duct increased when the dose range was 25–150 mg kg^{-1}, although the increase was less than proportional to the dose owing to saturation of the transport process. At a dose of 75 mg kg^{-1} the bile duct concentration was 37 000 μM. The biliary concentrations were determined on day five of the daily administration, when it was expected that steady state would have been achieved. The results support the expectation that bile duct hyperplasia was induced by high local concentrations of susalimod. The biliary clearance calculated from excretion rates and corresponding plasma levels in

the current study was 186 mL min^{-1} kg^{-1} at the lowest dose (25 mg kg^{-1}). The corresponding blood clearance was even higher, because the blood/plasma concentration ratio is 0·6 (Påhlman et al 1998). Thus biliary blood clearance was several times higher than liver blood flow (30–40 mL min^{-1} kg^{-1}, Davies & Morris 1993), which might be explained by much higher concentrations in the portal vein compared with the peripheral blood — as a result of extensive first-pass elimination by biliary excretion.

The organic anionic compound BSP, which has a lipophilic structure with two negatively charged SO_3^- groups, has long been used as a marker for biliary excretion (Oude Elferink & Jansen 1994). As shown in the current study, 70% of an intravenous dose of BSP was excreted in the bile. The bile/plasma ratio was approximately 80, in accordance with a previous report in which the ratio varied between 80 and 440 in the dog (Brauer & Pessotti 1950). Thus the concentration gradient between bile and plasma was much higher for susalimod than for BSP, implicating efficient active transport.

Several transport proteins are described as being involved in the sinusoidal uptake and the canalicular excretion both of endogenous compound, and of anionic and cationic xenobiotics (Oude Elferink et al 1995). BSP is taken up into the hepatocytes by a Na^+-independent system in contrast to bile acids which use a separate Na^+-dependent system. The canalicular transport of BSP involves the multispecific organic anion transporter cMOAT. According to the interaction, shown in this study, between susalimod and BSP with regard to biliary elimination, it is likely that cMOAT is also involved in the biliary excretion of susalimod. Plasma elimination and biliary excretion of BSP were both retarded when susalimod was co-administered. Whether this interaction occurs during canalicular transport or sinusoidal uptake cannot be established from these experiments.

In conclusion, we have developed a bile fistula model in the dog which we believe well reflects the biliary disposition of the compounds studied. We used this model to study susalimod and found the compound to be highly enriched in the bile, in a saturable manner, after oral administration. We also demonstrated that susalimod interacts with the biliary excretion of BSP, suggesting that it shares the same carrier-mediated transport system.

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